

Developing water addition/substitution into a
Vitis vinifera cv. Shiraz wine grape must as an
accepted fermentation strategy to lower
ethanol production and maintain wine quality

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Abstract

Compressed, later and hotter vintages due to climate change are evident in grape-growing regions around the world, including Australia. Later vintages present considerable challenges to winemakers as the sugar levels in the grape berries are higher, resulting in wines with higher than typical alcohol concentrations. Winemakers are now faced with the need to produce wines with lower alcohol levels. In 2017, Australia introduced new food regulations permitting water addition into a wine grape must ‘facilitate fermentation’ to a regulated 13.5 °Baumé (Bé) level. This approach was introduced as a response to the increasing problems associated with high ethanol production arising during fermentation, and as one answer to address negative impacts on human health from consumption of higher-alcohol wines, as indicated by the World Health Organization (WHO). The consequences of modified fermentation practices on wine quality have not been settled and continue to be debated by the International Organisation of Vine and Wine (OIV). The use of water in winemaking was recently endorsed by the OIV in May 2022 as an issue to be incorporated into the OIV work program.

I commenced this PhD thesis study by undertaking a literature review, presented in **Chapter 2**, on oenological approaches to reduce alcohol in final wine products. The review demonstrates that limited research is available that adequately establishes the effects of the ‘water treatment of pre-fermentation wine grape must’ (water-treatment) winemaking approach on final wine volatile aroma profiles, chemical compounds and correlated sensory attributes for *Vitis vinifera* cv. Shiraz wines. I also conclude from the literature review (**Chapter 2**, Section 1) that translation of laboratory results for this winemaking technique from previous studies across to commercial applications is limited, given that most studies focused on a single vintage, most relied on only one or two harvest timings, the majority produced only small quantities of wine, and most relied on limited numbers of panellists for sensory analysis.

The next step in the thesis study was to examine wine consumer preferences, especially in relation to wine consumer acceptability of lower-alcohol wines. The mini literature review in **Chapter 3** indicates that there is limited existing research on wine consumer preferences relating to lower-alcohol wines, together with concerns that excessive alcohol

consumption is bad for human health. In this thesis study, the results and analysis of responses from both expert and naïve wine consumers collated from two consumer preference surveys are presented in **Chapter 3**. These survey approaches address the gaps in the literature on the acceptability of lower-alcohol wines by wine consumers (expert and naïve) and, in particular, for those wines produced through the ‘water treatment of pre-fermentation wine grape must’ winemaking technique. The outcomes from the two wine consumer preference surveys of this study reveal that expert wine consumers are less concerned about water adjustments pre-fermentation than naïve wine consumers, where expert wine consumers prioritise quality over other factors. In fact, 80% of expert wine consumer respondents expressed a willingness to purchase wines with alcohol reduced using water dilution. Further, the study outcomes indicate that while alcohol content and health considerations are relevant considerations, these factors do not hold high importance for naïve wine consumers in their preferences. The survey outcomes also indicate that naïve wine consumers prioritise attributes of personal taste preference and value for money when making wine purchasing decisions. The survey results set the scene for the scientific experimental approaches taken in **Chapters 4, 5 and 6**.

Chapters 4 and 5 expand on the outcomes of the consumer preference surveys by presenting the findings of scientific experimental studies for the Shiraz cultivar. These studies investigated the impact on volatile aromas, chemical compound levels and sensory parameters (as determined by expert/and or naïve wine consumers) of wines produced from the Shiraz cultivar for three harvest times (early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé) across two vintages (2020 and 2021), as well as for those wines produced from 15.5 °Bé and middle 14.5 °Bé harvests whose pre-fermentation wine grape musts were subject to dilution or ‘bleed and replace’ water treatments. Shiraz was selected for this study as it is an important grape cultivar, grown in both warm and cool climates, and is recognised as the most-planted red grape variety not only in Australia, but also globally. Accordingly, any winemaking approach taken to reduce final alcohol levels in a Shiraz wine, while still maintaining wine quality as one of the highest objectives, will have important economic impacts for wine producers, wine consumers, the wine industry and export markets.

The combined experimental data results from vintage years 2020 (Year 1) and 2021 (Year 2), presented in **Chapter 4**, demonstrate that harvest timing and alternative water

treatment of wine grape must influences a wine's volatile aromas compositions, and sensory parameters vary. Gas chromatography–mass spectrometry (GC–MS) was used to isolate and analyse free volatile compounds for each treatment group wine. By clustering 43 volatile aromas into 11 compound groups, GC–MS analysis indicates that most fermentation-derived compound groups did not decrease with lower established alcohol levels, regardless of the type of water treatment of wine grape must approach that was implemented. The findings from just about right (JAR) sensory analysis from expert wine consumers, presented in **Chapter 4**, demonstrate that the 15.5 °Bé late harvest diluted to 14.5 °Bé wine was frequently preferred across most sensory attributes, which was correlated to quantitative descriptive analysis (QDA) (expert) mean results. In the **Chapter 4** study, expert wine consumers were targeted to add an extra dimension to the results, given that as ‘experts’ they have undergone continuous prior training in wine tasting, during which they would have sharpened their expertise in discerning fine nuances in taste and smell of wine over many years. The expert sensory panel approach can enhance results for wine producers and distributors who may consider results as more dependable, but also recognise that experts can influence a wine's reputation and, ultimately, its marketability. This is important as economic considerations in winemaking can also be motivators for sustainable wine production approaches.

Continuing the scientific experimental study, **Chapter 5** presents the combined experimental data results from vintage years 2020 (Year 1) and 2021 (Year 2). The results highlight that harvest timing and alternative water treatment of wine grape must influence levels of a number of chemical compounds, and sensory parameter outcomes also vary depending on the treatments implemented. Measures of wine colour density (a.u.), wine colour hue, total phenolics (a.u.), free anthocyanins (m/gL), pigmented tannin (a.u.), pigmented tannin (%), total pigment (a.u.), chemical age 1 and 2, and total tannin (%) were taken by a modified Somers colour assay. While it was observed that there were significant negative impacts on a number of chemical compounds associated with diverse wine grape must water treatments, in particular, decreases in total tannin (%), total phenolics (a.u.), free anthocyanins (m/gL) and total pigment levels (a.u.), JAR sensory analysis for naïve wine consumers indicates that the effect on many sensory parameters is subtler. Interestingly, results show that, while naïve wine consumers rate the 14.5 °Bé middle harvest diluted to 13.5 °Bé wine highest and as the most preferred, none of the

water treatments are found to have an effect on naïve wine consumers' wine purchase likelihood.

The study presented in **Chapter 5** relied on JAR sensory data and analysis collated from a large group of 159 naïve wine consumers from across vintage years 2020 (Year 1) and 2021 (Year 2). The literature review in **Chapter 2** highlights that there are limited existing studies that rely on large sensory panels. The larger sample group in this study provides for a more robust analysis, given the increased statistical power of the results gathered. The sensory results from naïve wine consumers further inform and support winemakers in their quest to produce wines with lower alcohol levels while maintaining favourable chemical and sensory quality attributes of the wines produced, together with purchase likelihood by typical wine consumers. Wine production approaches again will be influenced by economic considerations, that is - by what the general wine consumer is more likely to purchase.

Chapter 6 presents an extension of the scientific experimental study, with experimental data results related to the 2022 (Year 3) vintage. In this study, the Accentuated Cut Edges (ACE) winemaking technique was tested. Previous studies indicate that ACE can be implemented to mechanically break grape skins into small fragments, thereby accelerating phenolic extraction during wine fermentation. However, the literature review in **Chapter 2** indicates that no prior studies have considered how different levels of ACE can effectively address negative chemical and sensory attributes in final wines whose pre-fermentation musts have been subject to water treatment. This extended study was undertaken to test how different levels of ACE would address some of the more significant impacts on chemical compounds and some sensory attributes of 'water-treated pre-fermentation wine grape must' wines, as observed in the **Chapter 5** results. In the **Chapter 6** study, a late 15.5 °Bé 2022 (Year 3) Shiraz harvest pre-fermentation wine grape must was subjected to three levels of ACE treatment: low ACE, moderate ACE and high ACE. The sample treatment groups were also then subjected to water treatment (dilution) of the wine grape must (+ 4.07 L water), implemented to lower wine must sugar concentration from 15.5 °Bé to 13.5 °Bé. The resultant chemical compound levels and JAR sensory (naïve wine consumers) attribute results were compared with two controls: a 15.5 °Bé (non-diluted no ACE) wine, and a late harvest 15.5 °Bé diluted to 13.5 °Bé (no ACE) wine. The results for chemical compounds in **Chapter 6** reveal that reduced

total phenolics (a.u.) in a diluted to 13.5 °Bé (no ACE) wine were addressed by a diluted to 13.5 °Bé high ACE treatment, restoring levels similar to those observed in a 15.5 °Bé (non-diluted no ACE) wine. Total tannins (%) were also able to be restored to a similar level as a 15.5 °Bé (non-diluted no ACE) wine by a diluted to 13.5 °Bé high ACE treatment. Sensory analysis for naïve wine consumers further demonstrates that the diluted to 13.5 °Bé moderate ACE treatment produced wine with the greatest preference for colour intensity, together with increased preference for odour intensity and dark fruit taste. The results in **Chapter 6** show that ACE provides winemakers with an additional mitigation strategy to address any adverse impacts for final wine quality that are associated with ‘water treatment’ winemaking approaches implemented to produce wines with lower alcohol levels.

Linking the outcomes of the two consumer preference surveys (**Chapter 3**) with outcomes and impacts on volatile aromas, chemical compounds and sensory attributes (as tested by expert and naïve wine consumers) (**Chapters 4 and 5**) demonstrates that addition or substitution of water to pre-fermentation high-sugar musts together with ACE mitigation fermentation approaches (**Chapter 6**) can address higher unfavourable ethanol production and maintain final wine quality parameters. These winemaking techniques together can be considered as progressive, effective and commercially viable long-term approaches for the future of winemaking for Australia and for the international wine industry.

Declaration

This is to certify that:

- I. the thesis contains only my original work towards the Doctor of Philosophy – Agriculture, Food and Ecosystem Sciences at the University of Melbourne except where indicated in the Preface;
- II. due acknowledgement has been made in the text to all other material used; and
- III. the thesis contains less than 100,000 words in length excluding tables, bibliographies and appendices.

Billy Xynas

17 May 2024

Preface

The majority of the thesis research was conducted at the School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, at the Dookie Campus, University of Melbourne.

Comprehensive multidimensional gas chromatography–mass spectrometry analysis was conducted at the School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, University of Melbourne. Chemical composition profiling was conducted at Melbourne Mass Spectrometry and Proteomics Facility, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne.

Ethical approval for the consumer preferences surveys (naïve and expert) and sensory analysis (Just About Right and Quantitative Descriptive Analysis) was obtained from the Office of Research Ethics and Integrity, University of Melbourne (Ethics ID: 24275 (**Chapter 3**) and Ethics ID: 11684 (**Chapters 4, 5 and 6**)).

Associate Professor Kate Howell is the Principal Supervisor and Christopher Barnes is the Associate Supervisor of this PhD research project at the University of Melbourne.

All supervisors contributed to the conceptualisation, methodology and writing (revision) of the thesis. The candidate was the primary researcher and contributed to the conceptualisation, methodology, validation, formal analysis, investigation, writing and visualisation of the thesis.

Professional editor Donna Armstrong provided copyediting and formatting assistance, in accordance with university policy and the Institute of Professional Editors' Guidelines for editing research theses.

This thesis is presented with publications. One of the studies of the thesis is published, and two studies have been accepted for publication, all in international journals. Details of the articles resulting from this thesis are provided in the following section.

For the published article, the accepted for publication articles, the candidate contribution was 80%. All co-authors have agreed with the candidate's contribution and have signed the co-author authorisation forms.

Chapter 2 comprises two sections: Chapter 2 Section 1 is a literature review, based on the published article; Chapter 2 Section 1 referencing is consistent with the total thesis referencing approach, noting that there are referencing differences between the publication and thesis, due to the requirements of different publishers. Chapter 2 Section 2 is an extended literature review manuscript.

Chapter 3 is a research paper presenting data and analysis collated from self-administered wine consumer (expert and naïve) preference surveys.

Chapter 4 is a scientific research paper with data results and discussion, together with supplementary confirmation experiments.

Chapter 5 is a scientific research paper with data results and discussion, together with supplementary confirmation experiments (accepted for publication at the time of thesis submission). Chapter 5 referencing is consistent with the total thesis referencing approach, noting that there are referencing differences between the publication and thesis, due to the requirements of different publishers.

Chapter 6 is a scientific research paper with data results and discussion, together with supplementary confirmation experiments (accepted for publication at the time of the thesis submission). Chapter 6 referencing is consistent with the total thesis referencing approach, noting that there are referencing differences between the publication and thesis, due to the requirements of different publishers.

Chapter 7 sets out the conclusions of the thesis as a whole.

Details of published and accepted for publication papers during the PhD candidature

Chapter 2 Section 1

Xynas, B., & Barnes C. (2023). Yeast or water: Producing wine with lower alcohol levels in a warming climate: A review. *Journal of the Science of Food and Agriculture*, 103(7), 3249–3260. <https://doi.org/10.1002/jsfa.12421> (published)

Contribution: 80%. Conceptualisation, analysis, investigation, writing and visualisation.

Chapter 5

Xynas, B., Barnes, C., & Howell, K. (2024). Amending high sugar in *V. vinifera* cv. Shiraz wine musts by pre-fermentation water treatments results in subtle sensory differences for naïve wine consumers. *OENO One* (accepted for publication at the time of thesis submission).

Contribution: 80%. Conceptualisation, methodology, validation, formal analysis, investigation, writing and visualisation.

Chapter 6

Xynas, B., Barnes, C., & Howell, K. (2024). Moderating impacts of water addition in must by the use of accentuated cut edges (ACE) vinification for *Vitis vinifera* cv. Shiraz winemaking. *OENO One* (accepted for publication at the time of thesis submission).

Contribution: 80%. Conceptualisation, methodology, validation, formal analysis, investigation, writing and visualisation.

Awards, and conference presentations during the candidature

Recipient of the Sydney Wine Show Award for 2020.

Poster Presentation at the Australian Wine Industry 17th Annual Technical Conference and Trade Exhibition 2021 (impacted by COVID-19 lockdowns).

Poster Presentation at the Australian Wine Industry 18th Annual Technical Conference and Trade Exhibition 2022.

Conference Attendance Enoforum, May 16-18, 2023 Fiera di Vincenza Congress Center, Italy.

Wine and Spirit Education Trust Level 3 completed December 2022.

Australian Wine Research Institute, Advanced Wine Assessment Course, completed May 2024.

Impact of COVID-19 on progress during candidature

Chemical and statistical analysis of volatile aromas and chemical attributes of wines produced from the 2020 and 2021 vintages were delayed, due to lack of access to the Dookie Campus, University of Melbourne, where treatment wines were located. Gas chromatography–mass spectrometry and chemical and statistical analysis were to be undertaken using equipment located at the Melbourne campus of the University of Melbourne, Parkville, Victoria. However, due to the significant COVID-19 lockdowns in Victoria in 2021–2022, access to the laboratories was also limited for extended periods. For the 2020 wine sample groups, this testing was completed in July 2021, during a break in the COVID-19 lockdowns in Victoria. For the 2021 wine sample groups, testing was completed in August 2022.

Sensory analysis testing was also delayed due to the lack of access to the treatment wines, which were located at Dookie Campus, and limited travel ability for sensory panellists. For the 2020 (Year 1) wines, the sensory analysis testing for just about right (JAR) (naïve), JAR (expert), and quantitative descriptive analysis (QDA) (expert) were completed in July 2021. For the 2021 wines, sensory analysis testing for JAR (naïve), JAR (expert) and QDA (expert) were completed in June 2022.

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Firstly, I would like to express my sincere gratitude to my primary supervisor, Associate Professor Kate Howell. Thank you for guiding me and supporting me throughout my PhD candidature. I appreciate the time taken to review and edit my work and all the feedback provided. I would also like to thank my co-supervisor, Mr Christopher Barnes. I appreciate your assistance in setting up and undertaking my experimental trials in the winery, as well for guiding and supporting me throughout my PhD candidature. Many long days and nights, some good, some great, but always a lot of cleaning up. Thank you also to my committee chair, Dr Dorin Gupta for ensuring that my candidature ran smoothly and that all targets were met. Thank you for all the time and feedback provided. I have been very lucky to have had such a caring supervisory committee who helped support and guide me during my PhD journey.

Special thanks to the academic and professional staff at Dookie college campus – Ros Gall, Ash Stewart, Peter Delaney, Karen Edwards, Jacinta Way, Norma Pelly, Gayle Gall and Stuart Fidge. It takes all of you and many more to keep Dookie Campus operating. I appreciate all your time and dedication to ensuring a fantastic and supportive environment for PhD candidates to thrive and succeed.

To Richard Tallis, thank you for allowing me to utilise your vineyard and fruit to undertake my experiment and your patience with all my small-scale sampling and production. To Richard Smart, thank you for your counsel and ideas to further extend my study utilising ACE methodologies. To Yipeng Cheng, thank you for the time taken out of your own project to assist me in my chemical analysis, and thank you to Cameron Patrick for your consultation and assistance in the statistical analysis of results.

I acknowledge the support from the Australian Government, with the provision of the RTP scholarship, and give an extended thank you to Wine Australia for providing further financial support and backing to undertake the project. As well as this, I appreciate the Royal Sydney Wine Show for their financial contribution.

A heartfelt thank you and appreciation to all my family who supported me through my PhD journey, especially my parents, Arthur Xynas and Professor in Law Dr Lidia Xynas. Without you both, this journey would not have been possible. I appreciate all that you do for me.

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Chapter 1 – General introduction

1. Background

Grape-ripening periods have changed as a result of climate change. Wine grape growing regions across the world, including Australia, are especially affected because premium wine grape production occurs within narrow climate ranges, where even small shifts in climate will affect production (Mozell & Thach, 2014; Webb et al., 2012). Wine grape growers and winemakers have become especially concerned as rising temperatures in a region due to climate change impact their viticultural and oenological practices, as well as the final wine quality and wine quantity being produced (Schultz, 2016). This thesis focuses on selected pre-fermentation oenological practices that can be adopted as an effective mitigation strategy to counter the effects of a warming climate on grape-growing seasons. Compressed vintages due to warmer weather across grape-growing regions, both in Australia and across the globe, presents challenges to winemakers. Winemakers need to balance flavour and phenolic ripeness with potential high sugar levels in the grape berries, resulting in wines with higher than typical alcohol concentrations (Šuklje et al., 2016). This thesis was undertaken following the changes to the Australia New Zealand Food Standards Code (Standard 4.5.1), which now permits the addition of water to must to dilute the sugar under specific conditions to 13.5 °Baumé (Bé), so that high alcohol concentrations in the final wine product can be positively addressed (Australian Government, 2017). This thesis aims to understand wine consumer preferences, with a focus on the acceptability of lower-alcohol wine. The thesis then examines a number of final volatile aromas, chemical compound levels and sensory parameters for wines produced with water added or substituted into their musts, with an additional study that co-implements water addition treated wines with the accentuated cut edges (ACE) vinification approach. An analysis of results is presented so that winemakers can appreciate the effects of water treatment of pre-fermentation wine grape must (including ACE implementation for the 2022 vintage) on the final wine product, in addition to the aim of lowering final alcohol concentrations.

2. Research gaps, significances and objectives

Previous studies have investigated pre- and post-fermentation approaches to reducing alcohol levels in final wines, and the effects that such approaches can have on volatile aromas, chemical and sensory attributes of a final wine product. **Chapter 2** presents an overview of those studies that have tested two of the most common ‘in winery’

approaches implemented to address unfavourable ethanol production during fermentation. These approaches include using yeast and yeast hybrids to reduce ethanol production in fermentations, and water addition or water substitution (bleed and replace) into a high-sugar grape must prior to fermentation, to address ‘fermentation issues’ as well as associated unfavourable high ethanol production. Water addition is where water is added directly to a wine must pre-fermentation so that there is an increase in the volume of the finished wine. Water substitution is where grape juice is run off from the must and is substituted with equal amounts of water (Teng et al., 2020). The new ‘water treatment of pre-fermentation wine grape musts’ approach became legalised in Australia in 2017, following reforms to the Australia New Zealand Food Standards Code to allow the addition of water to ‘facilitate wine fermentation’ to 13.5 °Bé (Australian Government, 2017). This thesis aims to address a gap in the understanding of the consequences of producing wines whose high sugar pre-fermentation wine grape musts have been subject to ‘water treatment’, by focusing not only on the impact on alcohol levels in the final wine, but also the impact on other volatile aromas, chemical levels and sensory parameters that can influence overall final wine quality.

The extended literature review in **Chapter 2** examines green harvest wine substitution winemaking approaches as an alternative to water treatment of high-sugar wine musts (Schelezki et al., 2018). The review also includes ACE winemaking approaches, which use a grape must extraction technique that mechanically breaks grape skins into small fragments. Previous studies have found that the mechanical breaking of berry skins can aid in the extraction of volatile compounds (Kang et al., 2020; Sparrow, Holt, et al., 2016; Sparrow, Smart, et al., 2016; Wang et al., 2022). Further research is required for ACE to be positioned as an effective winemaking approach to negate any adverse wine quality parameters associated with wines that have been subject to water treatment of their pre-fermentation wine grape musts (Sparrow, Holt, et al., 2016; Sparrow, Smart, et al., 2016; Wang et al., 2022).

Chapter 3 of the thesis commences with an introduction discussing how excessive alcohol consumption is bad for human health. One approach put forward to address this is to produce lower strength alcoholic beverages (Australian Government Department of Health, 2019; Lähteenmäki, 2004). A review of previous studies that examine naïve wine consumer and industry perceptions of lower-alcohol wines demonstrates that results are

mixed (Higgins et al., 2015; Wright et al., 2007). Some early studies show that wine consumers can rate lower-alcohol wines as an inferior product from an organoleptic point of view (d’Hauteville, 1993; Stasi et al., 2014); however, these studies are not current, and may not reflect more modern-day attitudes of wine consumers towards lower-alcohol wines.

Chapter 3 then presents experimental results from two wine consumer preference surveys, undertaken as part of this thesis study, which were aimed at addressing this gap in the current understanding of the acceptability and preference for lower-alcohol wines among all wine consumers. One survey was a wine industry survey (expert), distributed to a broad range of Australian expert wine consumers; the other was a survey of naïve wine consumers. Both surveys were used to determine wine consumer understanding, preferences and attitudes towards lower-alcohol wines, especially wines that had been treated through various oenological approaches, with a focus on wine produced through the ‘water treatment of pre-fermentation wine grape musts’ winemaking approach. An additional objective of these wine consumer preference surveys (expert and naïve) was to provide further support for the adoption of the newly legalised fermentation approach in Australia to address high sugar levels in musts, and associated undesirable ethanol yields, through water addition/substitution into a high-sugar pre-fermentation wine grape must, which is the focus of this thesis.

Chapters 4 and 5 present the experimental findings from testing the impact of water addition and water substitution into a high-sugar grape pre-fermentation wine grape must. The thesis brings together data collected from the 2020 and 2021 vintages to examine the effects on ethanol production during fermentation, and impacts on other volatile aroma and chemical compound levels. I also assess the water addition/substitution treated wines’ sensory properties as indicated by both naïve and expert panels, using the ‘just about right’ (JAR) survey approach for both naïve and expert consumers, and the ‘quantitative descriptive analysis’ (QDA) survey approach for the expert wine consumers. While ethanol will contribute to final wine aroma and flavour (Remize et al., 1999), there is a need to balance desired reduction in ethanol production with other desirable wine flavonoids. The results in this thesis are further supported by experimental outcomes of previous research, which indicate that water treatments implemented into a pre-fermentation wine grape must ‘facilitate fermentation’ may have a negative impact on

phenolics concentrations, tannin and non-bleachable pigments, tannin molecular mass, and wine colour density when compared to a late harvest non-treated wine (Teng et al., 2020). Research related to aroma volatile results, combined with the impacts on chemical compounds and sensory analysis for such water-treated wines, is however currently limited. The literature review in **Chapter 2** highlights that the effects related to ‘water-treated’ wines have not yet been extensively explored. Existing research indicates that while the water addition/substitution pre-fermentation approach can be an effective strategy to address high sugar in musts, and to reduce alcohol levels in a final wine, the approach requires additional research (Petrie et al., 2019; Piccardo et al., 2019; Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020).

Chapter 6 presents the experimental findings for the ACE winemaking technique implemented at three grape skin fragmentation levels, together with the addition of water into high-sugar musts. The aims of this extended study were to determine whether the more significant impacts on chemical compounds and sensory parameters of the 2020 and 2021 wines produced from late harvest musts, which were subject pre-fermentation water treatments (as presented in **Chapter 5**) could be addressed by implementing ACE as concurrent winemaking approach. The resulting wines’ sensory properties were assessed using the JAR survey approach (for naïve wine consumers). The research outcomes shown in **Chapter 6** provide an evidence-based approach to adding water to pre-fermentation wine musts to facilitate fermentation, together with ACE implementation at varying levels, so that consumer confidence in Australian wine is supported, domestically and for exports. Research into the use of the ACE winemaking approach at three alternative grape skin fragmentation levels, implemented together with water treatment of a late harvest Shiraz must, has not yet been previously undertaken. The study presented in **Chapter 6** is important, as the majority of existing research has been conducted for ACE-treated Pinot noir wines, with limited studies conducted for ACE plus water addition to pre-fermentation wine grape musts for Shiraz. This thesis also expands on previous ACE studies (Kang et al., 2020; Sparrow, Holt, et al., 2016; Wang et al., 2022) by testing wines which produced from late 15.5 °Bé harvest, whose pre-fermentation must is subject to ACE at three levels of ACE (low ACE at 10 seconds, moderate ACE at 20 seconds and high ACE at 40 seconds). These musts are then subject to water treatment to a 13.5 °Bé, which is more reflective of the impacts of climate change in a hot growing season. Given that water addition into wine musts is now permitted in

Australia to facilitate fermentation, with the aim to reduce ethanol yields during fermentation (Australian Government, 2017; Standard 4.5.1), it is important that winemakers are also provided with additional mitigation strategies to address any resultant negatively perceived and/or actual impacts on final wine organoleptic qualities. ACE is one strategy that may address these impacts.

3. Thesis research questions and hypothesis

3.1 Thesis general aims

The general aims of this thesis research were to demonstrate that water addition/substitution into high-sugar grape musts is an effective, pre-fermentation approach to lowering ethanol production, which will produce wines with lower alcohol levels, without significantly adversely affecting other phenolic, chemical and sensory parameters in final wines. This approach can address the challenges that winemakers face due to compressed, later and hotter vintages (due to climate change) that result in higher sugar levels in grape berries. Without implementing water addition/substitution into high-sugar grape musts, unfavourable wines with higher than typical alcohol concentrations will result. The additional ACE fermentation approach can impact positively on final wine quality parameters for those wines that have been subject to water addition into high-sugar grape must pre-fermentation approaches.

3.2 Thesis research hypothesis

Adding or substituting water into a pre-fermentation wine grape must to lower ethanol production during fermentation, together with ACE to further extract favourable phenolic compounds, provides winemakers with effective tools to produce final wine products with lower, more desirable alcohol levels, without significantly affecting other organoleptic characteristics (volatile aromas, chemical compounds and sensory attributes) of the wine.

3.3 Thesis research questions

1. Do expert or naïve wine consumers accept lower-alcohol wines, produced by the addition/substitution of water to pre-fermentation wine grape must winemaking approach, but only where final wine quality including taste and flavour is unaffected?

2. Will the addition/substitution of water to later harvest wine grape must pre-fermentation winemaking approach affect fermentation-derived volatile aroma compounds?
3. Can the addition/substitution of water to late harvest wine grape must pre-fermentation winemaking approach produce negative outcomes with respect to wine chemistry compounds, when compared to non-treated wines?
4. Will the addition/substitution of water to late harvest wine grape must pre-fermentation winemaking approach lead to unfavourable sensory parameters including colour, olfactory (aroma), olfactory (taste/flavour), taste, mouthfeel and palate sensation, persistence of flavours on the palate, and overall perception, when compared to non-treated wines?
5. Can accentuated cut edges (ACE) together with the water addition/substitution into a high-sugar grape must pre-fermentation winemaking approach positively change the chemical composition and sensory attributes of treated wines, when compared to a diluted to 13.5 °Bé (no ACE) wine?

3.4 Thesis research approaches

A four-stage research approach was undertaken for this thesis. This first involved a literature review evaluation. Surveys of Australian wine consumers (both naïve and experts from the Australian wine industry) were then conducted to test consumer preferences regarding wine quality parameters and real and/or perceived consumer acceptance of wines whose wine grape must had been treated by water addition/substitution, together with wine consumers' general attitudes towards low-alcohol wines. The next approach was to conduct experimental scientific testing for volatile aroma (gas chromatography–mass spectrometry [GC–MS]) analysis, chemical compound analysis and sensory analysis (both naïve and expert) of final wines that had been subject to water addition/substitution into high-sugar wine grape musts pre-fermentation winemaking approaches, and compare that to control wines, across two vintages: 2020 and 2021. An extended experimental study for the 2022 vintage was undertaken with the inclusion of the ACE winemaking technique to address those observed negative impacts for chemical compounds and sensory parameters associated with ‘water treatments’ alone, as observed in the results from Chapter 5.

The details for each stage are as follows:

Stage 1: Literature review (**Chapter 2**) conducted of current evidence for the effects on ethanol production, and on volatile aromas, chemical and sensory characteristics, from both post- and pre-fermentation approaches to producing wines with lower alcohol levels. Scientific literature was evaluated to review the status of current approaches, together with limitations of each approach.

Stage 2: Naïve wine consumer preferences survey and industry (expert) wine consumer preference survey (**Chapter 3**). A mini literature review (also presented in **Chapter 3**) of existing studies of wine consumer perceptions, with respect to consumption of low-alcohol wines, including wines subjected to dealcoholisation processes, showed that current studies related to wine consumer preference are limited. The industry wine consumer preference survey and the naïve wine consumer preference survey for this thesis can be linked to experimental data from **Chapters 4, 5 and 6** regarding acceptance of wine products with lower alcohol levels, and naïve and expert wine consumers' perceptions of sensory parameters and acceptance of those wines that have undergone various oenological treatments. As part of the research for this thesis, I drafted and conducted my own Australian wine consumer survey, together with an industry wine consumer survey, in October 2022. Ethics approval was obtained in October 2022 (HREC No: 24275). The results from these two surveys provide naïve wine consumer and wine industry expert preferences to winemakers. Specifically, information on what consumers expect from water-treated wines with respect to final wine attributes, including alcohol levels, can permit winemakers to adjust their winemaking approaches. The outcomes of this research not only give winemakers an awareness of both expert and naïve consumers' preferences, but also determine what information is important to make available to the broader wine industry, especially in Australia.

Stage 3: Wines were produced across three vintages (2020, 2021 and 2022). For 2020 and 2021, grape berries were picked across three harvest dates for each: at early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé. For 2022, only one late 15.5 °Bé harvest date was implemented. For the 2020 and 2021 harvests, the late 15.5 °Bé and middle 14.5 °Bé higher-sugar musts were subject to a pre-fermentation water addition/substitution winemaking strategy to facilitate fermentation and also to address unfavourable higher ethanol production during fermentation. For the 2022 harvest, the late 15.5 °Bé high-sugar must was subject to the ACE vinification approach at three grape skin

fragmentation levels (low: 10 seconds; moderate: 20 seconds; and high: 40 seconds), together with water addition to lower the Baumé level from 15.5 °Bé to 13.5 °Bé. Results were then compared to two controls – a late 15.5 °Bé harvest wine (no ACE), and a 15.5 °Bé diluted to 13.5 °Bé (no ACE) wine.

The first experimental approach to assess the produced wines was GC–MS analysis via a 7890A gas chromatograph (Agilent, Santa Clara, CA, United States). An Agilent 5975C mass spectrometer was used to identify and quantify analytes. Forty-three commonly identified volatile aromas across both years 2020 (Year 1) and 2021 (Year 2) were classified into 11 compound groups: acids; ethyl esters of odd carbon number fatty acids; ethyl esters of straight-chain fatty acids; higher alcohols; ethyl esters of branched acids; methyl esters of fatty acids; other esters; higher alcohol acetate (HAA) esters from yeast sugar and N Metabolism; HAA esters from grape lipid; isoprenoids; and C6 compounds.

The second experimental approach was to take measures of wine colour density (absorbance units; 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 (%), according to a modified Somers colour assay (Mercurio et al., 2007), for all wine samples tested. Wine pH was adjusted to 3.4 and alcohol concentration to 12% volume by volume (v/v) by using a buffer solution before commencing any analysis. Sodium metabisulphite was incorporated into the buffer solution rather than adding it directly to individual sample groups to minimise preparation time (Mercurio et al., 2007). Modification to the Somers colour assay approach was taken 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 (Mercurio et al., 2007). Tannin measurements were derived from a calibration developed from the methyl cellulose precipitable (MCP) tannin assay (Sarneckis et al., 2006). Each analysis was performed in triplicate.

Stage 4: Wine treatment group samples were then analysed for their sensory parameters by using two separate methods – quantitative descriptive analysis (QDA) and just about right (JAR) methods. Wine sensory properties for the QDA followed the consensus-based approach as set out in previous literature (Lawless & Heymann, 2010), and consisted of two training sessions and one formal session. The QDA was held for expert panellists only (**Chapter 4**), which related to the 2020 and 2021 vintage studies. The training

sessions led to the development of the final attribute list, based on visual, aroma, flavour, mouthfeel and aftertaste sensory attributes of the wines sampled. The attributes tested were: aroma intensity, flavour intensity, red fruit, dark fruit, green, sweet spice, liquorice, chocolate, confectionery, earthy, acidity, sweetness, bitterness, astringency, hotness, and viscosity. The JAR method (both naïve and expert) for the 2020, 2021 and 2022 studies (**Chapters 4, 5 and 6**) evaluated the sensory 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. In addition, the JAR score was combined with overall liking scores. This was implemented to highlight those attributes of a wine that had the greatest impact on liking; a ‘penalty analysis’ was used to analyse the JAR data, to quantify and hierarchise the impact of sensory characteristics on the overall liking of the wine samples (Cadot et al., 2012; Schraidt, 2009). Ethics approval for all the sensory studies was obtained from the University of Melbourne in January 2021 (HREC No: 11684).

4. Conclusion

The aim of the thesis is to examine to what extent water addition/substitution oenological approaches can be used by winemakers in the winery in response to the climatic aspects of the growing season and the timing of the harvest. The quantity of wine produced for the studies in this thesis can be translated to commercial applications, demonstrating scalability of these technological solutions. Recommendations are made based on the experimental outcomes for three vintages, together with the findings of the wine industry and naïve wine consumer preference surveys. The expansion and growth of the wine industry worldwide, and for Australia, demands that modernism and innovation are adopted in light of the effects of climate change. The processes involved in the production of wine have developed over many centuries. The new Australian regulatory development allowing Australian winemakers to add water to wine grape must pre-fermentation to facilitate fermentation necessitates that winemakers are provided with evidence-based outcomes and datasets to further support wine provenance and measures of wine quality. This is especially important for winemakers and distributors, where it can support the ongoing evolution of wine production and supply. While the ‘water treatment of pre-fermentation wine grape must’ winemaking approach is currently limited in other winemaking regions (Christmann et al., 2022), global warming will continue to present challenges for all wine grape growers and winemakers across the globe (Cataldo et al.,

2023), meaning that this additional easy-to-implement winemaking approach may be considered more broadly into the future.

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Chapter 2 – Literature review:
Oenological approaches for reducing alcohol levels
in wine

This thesis commences with a literature review of the most common ‘in winery’ oenological winemaking approaches available to winemakers for producing wines with alcohol levels lower than typical alcohol concentrations from late harvest winemaking. This literature review is presented in two sections.

Section 1 is based on a published article:

Xynas, B., & Barnes, C. (2023). Yeast or water: Producing wine with lower alcohol levels in a warming climate: A review. *Journal of the Science of Food and Agriculture*, 103(7), 3249–3260. <https://doi.org/10.1002/jsfa.12421>.

Contribution: 80%. Conceptualisation, analysis, investigation, writing and visualisation.

The final draft copy submitted to the *Journal of the Science of Food and Agriculture* is included in Section 1 of this chapter, before any final journal typesetting or journal style referencing has been applied.

Section 2 further examines other alternative in winery winemaking approaches that can be taken to produce wines with lower final alcohol levels.

The overall findings indicate significant gaps in the existing scientific knowledge about the ‘water treatment of pre-fermentation wine grape musts’ winemaking approach, and this literature review sets the scene for the thesis overall.

Overall abstract

Compressed, later and hotter vintages (due to climate change) across grape-growing regions around the world, including Australia, present considerable challenges to winemakers as the sugar levels in the grape berries are higher, resulting in wines with higher than typical alcohol concentrations. Higher alcohol consumption has been shown to lead to increased health risks for humans and can affect the chemical and sensory profiles of final wine products due to the altered solubility and volatility of aroma compounds, together with interactions with protein complexes in the wine. Winemakers across the globe have in more recent times sought to produce wine products with lower alcohol levels, implementing a number of oenological approaches, either pre or post fermentation. Section 1 of this chapter is a literature review based on a published review paper that examines two groups of pre-fermentation oenological approaches. Section 2 presents an extended review of additional pre- and post-fermentation oenological approaches. In Section 1, literature demonstrating pre-fermentation approaches that can be implemented to alter alcohol levels in the final wine product, such as using distinctive yeasts (genetically modified (GM), or non-GM yeasts, or yeast hybrids), are reviewed. However, it is noted that experimental results vary depending on the yeast approaches undertaken. Studies related to an additional pre-fermentation approach to reduce alcohol levels in a final wine – water addition/ substitution to a high-sugar grape must – are then reviewed. Previous studies indicate that this approach can effectively reduce alcohol levels in the resultant wine but show that there can be some significant negative effects on the phenolic or sensory characteristics of a final wine. However, research is scant for this winemaking approach. Section 2 of this chapter provides an extended literature review examining additional pre-fermentation approaches including the accentuated cut edges (ACE) vinification approach, which can mitigate against any resultant negative impacts on final wine quality parameters associated with ‘water treatment of pre-fermentation wine grape must’ winemaking alone. By way of contrast, the approach of blending later harvest higher-alcohol wine with green harvest wine is also reviewed, noting that it can produce wines with unfavourable green undertones. The extended review also provides an overview of two common thermal post-fermentation approaches, such as reverse osmosis and spinning cone technologies. It is noted that these post-fermentation approaches to reducing alcohol levels in a final wine have lower consumer acceptance due to associated perceived negative organoleptic attributes in the final treated

wine product. It is concluded that, as a fermentation strategy, the water addition/substitution into high-sugar pre-fermentation wine grape must winemaking approach can address the production of unfavourable higher ethanol in wine grape musts; however, there is limited research on the impacts on wine volatile aromas, chemical compounds and correlated sensory parameters in a final wine. This sets the scene for the PhD study. This winemaking technique, together with the implementation of ACE into pre-fermentation wine grape musts, requires further investigation to improve resource management and sustainability, while supporting informed business decision-making for wine producers. This is especially important as winemakers adapt their winemaking approaches in a changing climate.

Key words: ethanol, water, wine grape musts, fermentation, wine

Section 1 – Yeast or water: Producing wine with lower alcohol levels in a warming climate – a review

Abstract

Climate change effects over the last century have seen grape wine growers being faced with earlier budburst and shorter seasons. One effect is higher sugar levels in the grape berries, resulting in wines with higher than typical alcohol concentrations. Winemakers, both in Australia and globally, need to reassess their wine making approaches to address the challenges associated with warmer drier conditions. This review focuses on two pre-fermentation approaches that address ethanol production during fermentation, so that wine makers can produce a wine with lower final alcohol levels at the same time as addressing any negative impacts on wine quality. First, microbiological yeast approaches are reviewed. This approach can reduce final alcohol levels in a treated wine between 0.2% v/v-3.7% v/v; however, diverse wine quality impacts can result, dependant on the microbiological yeast approach taken. Second, the pre-fermentation approach of water addition/substitution into a high sugar grape must is reviewed. To date, recent studies indicate that this ‘water treatment’ of musts approach is effective with respect to reducing final alcohol levels from between 0.6% v/v-5.9% v/v in the resultant wines, with less variation effects on final wine quality. It is noted that further studies for the ‘water treatment’ of high sugar musts approach are required to provide additional foundational data sets with respect to impacts on chemical, phenolic and sensory attributes of the final wine products, both within and across vintages. This is especially important as winemakers adapt their winemaking approaches in a changing climate.

Key words: ethanol, yeast, water, fermentation, wine

Abbreviations

ABV	alcohol by volume
ATF1	alcohol acetyl transferase 1
BDH1	3-butanediol dehydrogenase 1
Bé	Baumé
CRISPR/Cas9	clustered regularly interspaced short palindromic repeats / CRISPR-associated protein 9
FF	fresh fruit
FPS1	plasma membrane aquaglyceroporin
FRD1	fumarate reductase
g L ⁻¹	grams per litre
GM	genetic modification
GMO	genetically modified organism
GPD1	glycerol-3-phosphate dehydrogenase isozyme 1
GPD2	glycerol-3-phosphate dehydrogenase isozyme 2
MDH2	malate dehydrogenase
MF	mature fruit
mDP	mean degree polymerization
O ₂	oxygen
SO ₂	sulphur dioxide
TCA	tricarboxylic acid
TPA	total phenolic acids
TPS1	trehalose 6-phosphate synthase
v/v	volume by volume
VVM	volume of air sparged (in aerobic cultures) per unit volume of growth medium per minute

1. Introduction

Over the last century grape-ripening periods have changed as a result of global climate change (Petrie & Sadras, 2017; Terral et al., 2010; Webb et al., 2012). Compressed vintages due to warmer weather across grape-growing regions presents challenges to winemakers, both globally and in Australia (Webb et al., 2012). Higher sugar concentrations in the berries due to earlier ripening periods raise the risk of ‘stuck’ fermentations and can produce final wines with undesirable higher alcohol levels when all of the sugars are consumed by the yeasts (Mira de Orduña, 2010). The demand for lower-alcohol wines by consumers, as well as regulatory restrictions on high-alcohol wines, particularly in the export markets, motivate winemakers to explore both viticultural and oenological approaches for reducing alcohol content in final wines (Figure 2.1) while balancing organoleptic factors, wine flavour and quality.

This review addresses two pre-fermentation oenological approaches to lowering ethanol production during fermentation. Microbiological yeast approaches are first reviewed including genetic modification (GM) approaches to creating specific yeast species; non-genetically modified organism (non-GMO) approaches to develop commercial yeast strains; and the use of distinctive yeasts coupled with various fermentation approaches. The microbiological yeast approaches reviewed showed that the ethanol reduction effects ranged from 0.2% v/v to 3.7% v/v when compared to the control wines (Table 2.1). The effects on final wine organoleptic qualities varied depending on the yeast variants and fermentation approaches employed. This review then examines the alternative oenological pre-fermentation approach of water addition/substitution (‘water treatment’) into high-sugar wine musts, which can also reduce ethanol production during fermentation (Australian Government, 2017). Ethanol reduction effects for water treatment approaches were observed to range from 0.6% v/v to 5.9% v/v when compared to the control wines (Table 2.2). The effects on final wine organoleptic qualities also varied, depending on the water treatments employed. This review provides winemakers with additional information to assess the relative merits of these alternative oenological pre-fermentation methods as described in order to assist them in making decisions regarding winemaking approaches for lowering final alcohol levels in wine.

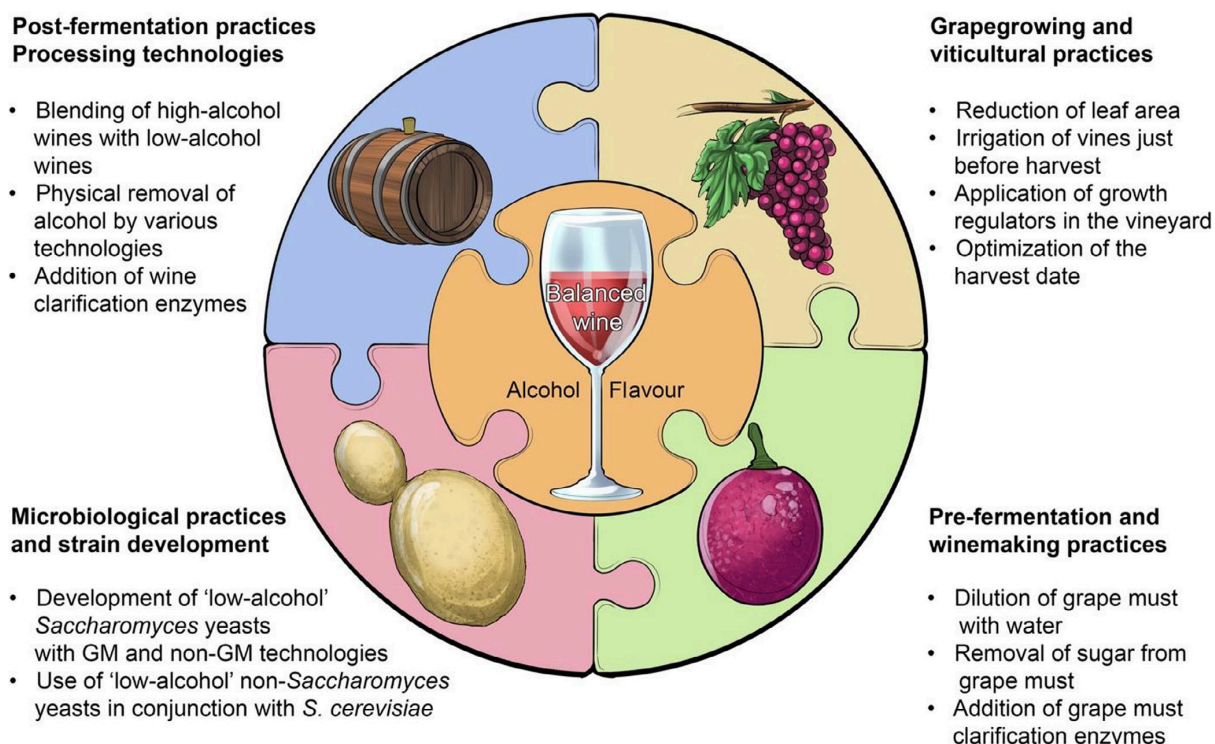


Figure 2.1. Visualisation of the four approaches available to winemakers for producing wines with lower alcohol levels

Source: Goold et al. (2017). (Copyright Licence CC BY 4.0.
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2. Microbiological approaches to address the production of ethanol during fermentation of wine musts

Microbiological approaches to reduce the production of ethanol during wine fermentation has included three major approaches: first, the application of GM techniques to create *Saccharomyces cerevisiae* yeasts or other non-*Saccharomyces* yeasts that can redirect sugar carbon away from ethanol to other compounds such as glycerol; second, the use of non-GMO technologies, such as adaptive evolution approaches, to develop low-alcohol yeast variants of existing wine strains of *S. cerevisiae*; and third, using low-alcohol non-*Saccharomyces* yeasts, typically in conjunction with *S. cerevisiae* in mixed fermentations (Goold et al., 2017).

For all three approaches, especially for GM techniques, glycerol can be used as a carbon sink, where it is relied upon to reduce ethanol yields by diverting carbon flux towards glycerol production (Goold et al., 2017) (Figure 2.2). Glycerol, a colourless polyol, can be a positive element in a wine, where it can have sweet, sometimes oily, attributes, with little effect on wine viscosity (Goold et al., 2017). However, excess production of

glycerol can also have a negative impact on final wine quality and sensory qualities, including elevated concentrations of acetaldehyde, acetic acid, acetoin and 2,3-butanediol where there is an overexpression of *GPD1* or *GPD2* in wine yeast (Goold et al., 2017). For all three approaches, a balance is required between reductions in ethanol production and the production of undesirable higher levels of glycerol (Goold et al., 2017).

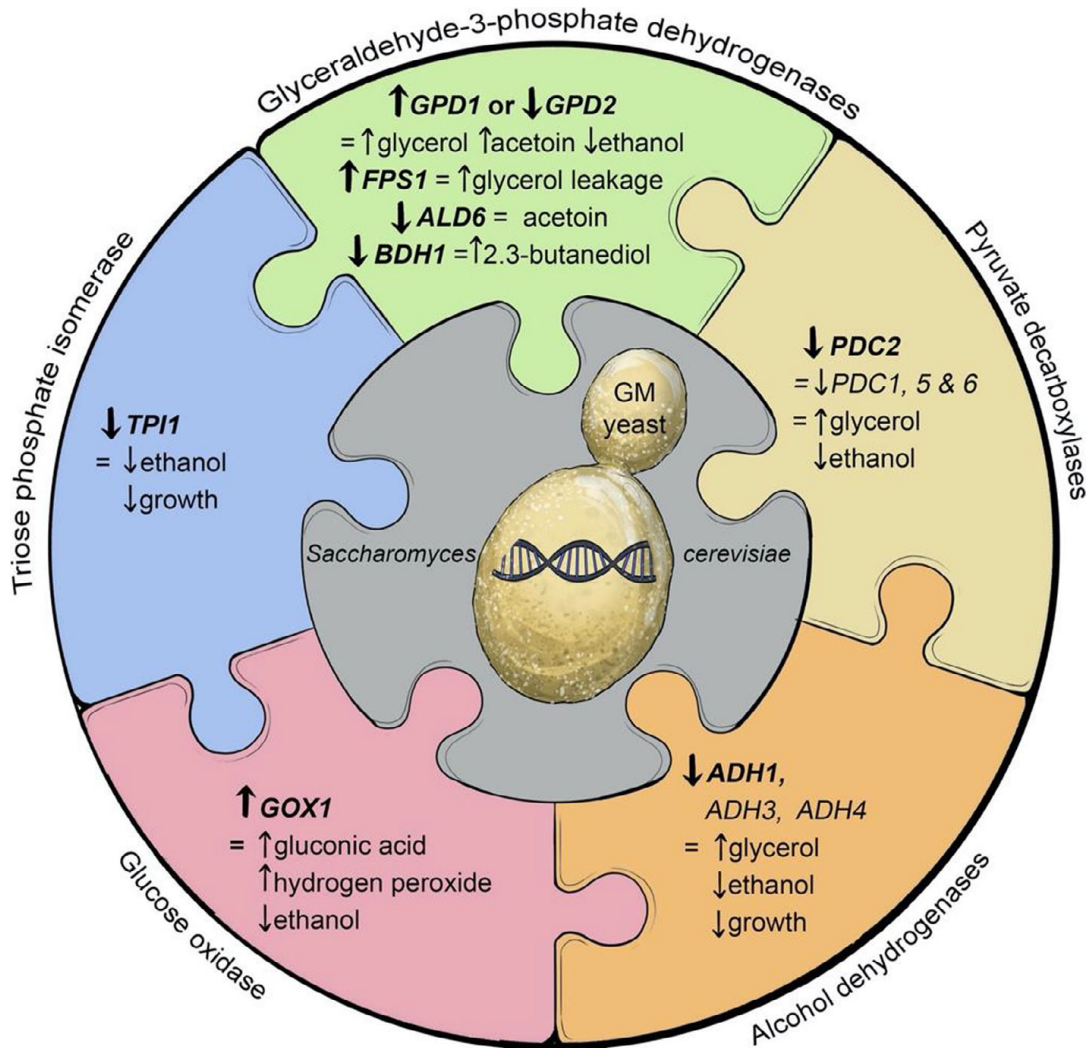


Figure 2.2. Visualisation of genetic modification approaches for reducing ethanol yields by diverting carbon flux towards other end points such as glycerol production

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Five GM strategies are indicated: (i) the overexpression of the yeast's own *GPD1* and/or *GPD2* genes (which encode glycerol-3-phosphate dehydrogenase isozymes); (ii) modification of the glycerol transporter encoded by plasma membrane aquaglyceroporin (*FPS1*); (iii) deletion of the *PDC2* gene encoding pyruvate decarboxylase; (iv) impairment of alcohol dehydrogenases encoded by *ADH1*, *ADH3*, *ADH4* and (v) deletion of *TPI1* (which encodes triosephosphate isomerase). Adapted from Goold et al. (2017).

2.1 GM approaches to generate yeasts with lower ethanol yields

Although GM approaches may not yet be accepted universally (Goold et al., 2017), an overview is provided here because it can offer insights into what a desirable wine yeast can provide (Figure 2.2). By altering a single base pair in the DNA of the host cell or by incorporating multi-generic biosynthetic pathways not present in the host, genetic engineering approaches to yeast gene modification have been shown to more precisely address desirable attributes of a final wine product, including lower final alcohol levels. Michnick et al. (1997) found that by overexpressing *GPD1*, the glycerol response was increased (+28 g L⁻¹) together with a reduction in ethanol production (-15 g L⁻¹). However, increases in acetate and acetoin were also observed, where in higher concentrations, acetoin could have a negative effect on wine aromas (Michnick et al., 1997). Ehsani et al. (2009) addressed the over-production of acetoin by overexpressing *BDHI* (Ehsani et al., 2009). The GM strain they developed was found to produce lower ethanol levels (3% v/v less than the control) during fermentation, together with greater glycerol production, with little impact on sensory parameters in the final wine (Ehsani et al., 2009).

Other GM approaches divert carbon towards the synthesis of intermediates of the tricarboxylic (TCA) cycle, to lift glucose repression from genes encoding enzymes involved in respiration in wine yeast in order to ‘burn’ carbon that would otherwise feed into ethanol production (Varela et al., 2012). This approach has been shown to produce an increased expression of *MDH2* and *FRD1* with a limited 0.3 % v/v reduction in ethanol concentration when compared to the control wine (Varela et al., 2012) (Table 2.1). However, unfavourable high concentrations of acetate, acetaldehyde and acetoin were observed (Varela et al., 2012). Improved results were observed by Rossouw et al. (2013) who diverted carbon away from ethanol formation towards the synthesis of reserve carbohydrates by overexpressing the *TPS1* gene, while also downregulating carbon flux through glycolysis. This resulted in a reduction of ethanol levels by 1.5 % v/v when compared to the control wine (Rossouw et al., 2013) (Table 2.1).

Crabtree-negative strains of *S. cerevisiae* have also been constructed under GM approaches. Otterstedt et al. (2004) substituted all hexose transport genes with one chimeric one. This halted production of ethanol while oxygen was present for respiration. Growth kinetics were, however, found to be very slow for this GM strain (Otterstedt et al.,

2004). The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system has more recently been considered in the development of GM modified *S. cerevisiae* (Di Carlo et al., 2013). The CRISPR/Cas9 system can conduct precise genome editing of any organism by relying on the use of the natural ‘adaptive immunity’ mechanism described in bacteria and archaea (Mans et al., 2015). Van Wyk et al. (2020) used this method to clone *GPD1* and *ATF1* for a haploid wine strain, where they found no discernable differences for ethanol production between the strains (van Wyk et al., 2020). The CRISPR/Cas9 system appears to be an effective GM approach as it can also accelerate wine yeast gene deletions so that any traces of ancillary genetic materials are not present (Mans et al., 2015).

2.2 Non-GMO approaches to develop low-alcohol wine yeast variants of *S. cerevisiae*

An alternative approach has been to use adaptive laboratory evolution (non-GMO approach) to engineer *S. cerevisiae* yeast strains (Tilloy et al., 2014). These strains have also demonstrated decreased ethanol yields by diverting carbon flux towards glycerol production (Tilloy et al., 2014) (Table 2.1). Tilloy et al. (2014) found that this approach could produce a final wine with 0.6% v/v less alcohol when compared to wines produced using the ancestral strain (Table 2.1). By breeding intrastrain hybrids (different isolates of the same strain) and then generating hyperosmotic stress on yeast cells by employing adaptive evolution using potassium chloride, Tilloy et al. (2014) found even more effective reduction in ethanol content of 1.3% v/v and a 41% increase in glycerol yield when compared to the control (Table 2.1). They did not observe any negative impacts on other wine parameters from the increase in glycerol yield for this treatment (Tilloy et al., 2014). This could be explained as being due to the central carbon flux being altered via mutations in genes outside of the canonical high osmolarity glycerol response pathway (Tilloy et al., 2014).

A further approach has been the use of global transcriptional machinery engineering to develop engineered *S. cerevisiae* strains with reduced ethanol-producing capability (Seong et al., 2017). Global transcriptional machinery engineering can be implemented to mutate the main DNA-building protein that regulates promoter specific in yeast, the TATA-binding protein, encoded by *SPT15* (Alper et al., 2006). Du et al. (2020) found that the engineered *S. cerevisiae* YS59-409 strain demonstrated a reduced ethanol yield

of 34.9% during fermentation in a Triple M medium when compared to the host control strain *S. cerevisiae* YS59 (Du et al., 2020). In addition, they observed glycerol synthesis to be 46% higher than the control strain. While no significant differences were observed for acetic acid production for this study, it was noted that further studies are required to examine impacts on other wine by-products (Du et al., 2020).

2.3 Non-*Saccharomyces* yeasts in alcoholic fermentations: how they can affect ethanol yields in fermentations

Crossbreeding yeasts to create interspecific yeast hybrids which are then used in fermentations is another approach in reducing ethanol yields during fermentation. Arroyo-Lopez et al. (2010) found that under micro-vinification conditions, higher glycerol levels together with lower ethanol yields could be effected from fermentations with the hybrid *S. cerevisiae* × *S. kudriavzevii* W27 strain when compared with wines produced solely from *S. cerevisiae* or the non-wine yeast *S. kudriavzevii* IFO 1802T (Arroyo-Lopez et al., 2010). They also observed that the impact on ethanol yields was also affected by temperature, where the hybrid *S. cerevisiae* × *S. kudriavzevii* W27 strain ferment exhibited more glycerol, with potentially reduced ethanol yield at low temperatures around 14 °C (Arroyo-Lopez et al., 2010) (Table 2.1).

The approach of using co-fermentation and sequential inoculation approaches utilising various non-*Saccharomyces* yeasts strains with selected *S. cerevisiae* strains have also demonstrated varied results in reducing ethanol yields. Rolle et al. (2018) tested two *Starmerella bacillaris* strains in mixed fermentations with a *S. cerevisiae* strain. *Starm. bacillaris* has been shown to have low ethanol tolerance, and high glycerol and moderate volatile acidity production (Englezos et al., 2015). For the mixed ferments with *S. cerevisiae*, it was found that the treated wines exhibited a reduction of alcohol concentrations by 0.2% v/v to 0.3% v/v when compared to the *S. cerevisiae* control wine (Rolle et al., 2018) (Table 2.1). Lemos Junior et al. (2019) found ethanol concentrations for mixed sequential fermentations with strains of *Starm. bacillaris* and *S. cerevisiae*, which ranged from 4.28% v/v to 4.94% v/v, were not significantly different from those measured in an *S. cerevisiae* single-strain fermentation of 4.9% v/v (Lemos Junior et al., 2019) (Table 2.1). Limited results have also been observed for *Lachancea (Kluyveromyces) thermotolerans* and *S. cerevisiae* in sequential fermentations at lower

temperatures where ethanol concentrations were reduced by 0.7% v/v when compared to the *S. cerevisiae* controls (Gobbi et al., 2013) (Table 2.1).

Improved results have been observed for other yeast species and inoculation approaches. Contreras et al. (2014) found that a *Metschnikowia pulcherrima* strain, in sequential inoculations with *S. cerevisiae*, produced the lowest ethanol yields from 0.9% v/v to 1.6% v/v when compared to the *S. cerevisiae* control ferment (Contreras et al., 2014) (Table 2.1). Enhanced results were observed by Canonico et al. (2016) who found that *Hanseniaspora osmophila*, *Hanseniaspora uvarum*, *Starmerella bombicola* and *M. pulcherrima* strains, when sequentially inoculated with free *S. cerevisiae* cells, produced 1.00% v/v, 1.21% v/v, 1.64% v/v and 1.46% v/v reductions in ethanol levels, respectively, when compared to the *S. cerevisiae* control ferment (Canonico et al., 2016) (Table 2.1). Contreras, Curtin, and Varela (2015) observed limited ethanol reductions for an *M. pulcherrima* strain in a sequential inoculation with an *S. cerevisiae* strain at 0.9% v/v less ethanol when compared to wines produced from the *S. cerevisiae* control ferment. Improved results from the mixed culture of an *M. pulcherrima* strain and an *S. uvarum* strain, in sequential inoculation with *S. cerevisiae*, demonstrated 1.7% v/v lower ethanol concentrations compared to wines produced from the *S. cerevisiae* control ferment (Contreras, Curtin, & Varela, 2015) (Table 2.1).

2.4 Yeasts and aerobic fermentation approaches for affecting ethanol yields in fermentations

The role of oxygen (O₂) together with the respiratory metabolism of different yeast species that consume sugars by respiration and not fermentation in a wine must can lead to a reduction of alcohol levels in the final wine. The addition of extra O₂ to musts that have been inoculated with non-*Saccharomyces* yeasts and *S. cerevisiae* yeast strains enables reduced ethanol-producing non-*Saccharomyces* yeasts to survive longer during fermentation. The potential of sugar respiration by non-*Saccharomyces* yeasts can also lower alcohol levels in a final wine product (Contreras et al., 2014) (Table 2.1). For single inoculum *S. cerevisiae* strain ferments, however, because this is a Crabtree-positive yeast (where aerobic production of ethanol occurs in the presence of repressing levels of glucose), it will generally ferment sugars in the wine must and produce alcohol despite the presence of O₂; thus using strains of this yeast on its own has its limitations under this approach (Erten & Campbell, 2001).

Gassing conditions will also affect ethanol yields. Gassing conditions of 25% air has been shown to produce an alcohol level reduction of 2.2% v/v (Holm Hansen et al., 2001). For ferments with *M. pulcherrima* sequentially inoculated in musts with *S. cerevisiae*, reduced ethanol yields of up to 3.7% v/v have been observed in aerobic treated cultures when compared to anaerobic cultures. This is related to the growth of *S. cerevisiae* under aerobic conditions, at less than 0.35 g L⁻¹, and levels of dissolved oxygen (Morales et al., 2015) (Table 2.1). Ferments with non-*Saccharomyces* yeasts *Torulaspora delbrueckii* and *Zygosaccharomyces bailii*, when sequentially inoculated in musts with *S. cerevisiae* and subjected to agitation and VVM aeration, have also been observed to produce wine with reduced ethanol levels in the laboratory (reduced by 1.5% v/v and 2.0% v/v, respectively) when compared to the *S. cerevisiae* anaerobic control (Contreras, Curtin, & Varela, 2015) (Table 2.1).

2.5 The impacts on other phenolic attributes of wines produced from yeast approaches implemented to affect ethanol yields in fermentations

The studies as noted above demonstrate varied effects on reducing ethanol production during fermentation (Table 2.1). However, winemakers also need to balance the effects on other phenolic attributes (Ciani et al., 2016). Rolle et al. (2018) found that anthocyanin and some esters of fatty acids (ethyl hexanoate and ethyl dodecanoate), which can contribute positively to wine aromas (Ferreira et al., 2000), were present in the final treated wines. However, other studies found that other negative organoleptic attributes were observed, including the production of additional esters and high ethyl acetate levels (Rosenfeld et al., 2003). Esters can impart pear or banana sensory descriptors, and high levels of ethyl acetate can impart an undesirable nail polish sensory aspect to a wine which is considered detrimental to wine aromas (Jackson, 2009). Contreras et al. (2014) found that for Chardonnay wines produced from the *M. pulcherrima* with *S. cerevisiae* sequential inoculation, undesirable higher ethyl acetate levels were produced when compared to the control. Canonico et al. (2016) also found that for the sequential fermentation of *H. uvarum* with *S. cerevisiae*, higher levels of ethyl acetate were produced.

The production of acetic acid, which can impart sour and bitter taste in the final wine, has also been shown to increase when sequential inoculation fermentation approaches are

coupled with oxygenation and aeration of the musts (Contreras, Hidalgo, et al., 2015; Morales et al., 2015). Winemakers must also balance the amount of oxygen addition and the timing, as this approach can contribute to higher levels of esters and volatility acidity levels by the *S. cerevisiae* yeasts present in the wine must (Contreras, Hidalgo, et al., 2015). Few studies, however, have evaluated analytical and sensorial profiles or oxygen modulation in sequential inoculation fermentation approaches, which indicates that the aeration of grape must approach needs further study (Contreras, Hidalgo, et al., 2015; Morales et al., 2015).

Table 2.1. Parameters including reduction of ethanol levels for wines produced from alternative microbiological fermentation strategies

Fermentation approach reviewed	Fermentation medium	Initial sugars (g L ⁻¹)	Sugar consumption % consumed or residual sugars (g L ⁻¹)	Sensory analysis	Reduction in ethanol levels	Reference
GM yeast gene modification of <i>S. cerevisiae</i> strain modulated with TPS1	Synthetic wine must MS300	125	No impairment to fermentation	No	1.5% v/v	Rossouw et al. (2013)
GM yeast gene modification of <i>S. cerevisiae</i> strain modulated with GPD1	Synthetic medium	200	No impairment to fermentation	No	Ethanol yield was reduced by 20% for V5/GPD1 compared with control V5	Michnick et al. (1997)
GM yeast gene modification of <i>S. cerevisiae</i> strain V5 (<i>MATa ura3</i>) to produce GM strain V5 <i>ald6 GPD1 BDH1</i>	MS synthetic medium	200–240	All sugars consumed in 60-hr ferment	Yes No negative sensory contribution to wine quality observed	3.0% v/v	Ehsani et al. (2009)
GM yeast gene modification <i>S. cerevisiae</i> strain to produce GM strain AWRI2531	Chardonnay	246	All sugars consumed	No	2.4% v/v	Varela et al. (2012)
GM yeast gene modification <i>S. cerevisiae</i> strain to produce GM strain AWRI2532	Cabernet Sauvignon	246	All sugars consumed	No	3.6% v/v	Varela et al. (2012)
Sequential <i>Starmerella bacillaris</i> strains C54 and C.z03/ <i>S. cerevisiae</i>	Barbera	140	All sugars consumed	No	0.20% – 0.30% v/v	Rolle et al. (2018)

Fermentation approach reviewed	Fermentation medium	Initial sugars (g L ⁻¹)	Sugar consumption % consumed or residual sugars (g L ⁻¹)	Sensory analysis	Reduction in ethanol levels	Reference
Sequential <i>L. thermotolerans</i> / <i>S. cerevisiae</i>	Sangiovese and Cabernet Sauvignon	224	Residual sugars Control <i>S. cerevisiae</i> 5.93 ± 0.18 g L ⁻¹ Residual sugars Sequential 24-hr fermentation 27.87 ± 8.61 g L ⁻¹ Residual sugars Sequential 48-hr fermentation 18.0 ± 1.47 g L ⁻¹	Yes Increases in spicy notes and in total acidity observed	0.70% – 0.90% v/v	Gobbi et al. (2013)
Sequential <i>M. pulcherrima</i> ARWI1149/ <i>S. cerevisiae</i>	Chardonnay	150	Consumption at 100% ± 0.00	No	0.90% v/v	Contreras et al. (2014)
Sequential <i>M. pulcherrima</i> ARWI1149/ <i>S. cerevisiae</i>	Shiraz	150	Consumption at 99.8% ± 0.00	No	1.60% v/v	Contreras et al. (2014)
Cross breeding <i>S. cerevisiae</i> × <i>S. kudriavzevii</i> W27	Complex synthetic medium	200	Consumption at 100% ± 0.00	No	Exact levels not presented; however, lower than <i>S. cerevisiae</i> control	Arroyo-Lopez et al. (2010)
Adaptive laboratory evolution and inoculation treatments with <i>S. cerevisiae</i> K300.1(b)	Shiraz	260	Consumption at 99.9%	No	0.6% v/v – 1.3% v/v	Tilloy et al. (2014)

Fermentation approach reviewed	Fermentation medium	Initial sugars (g L ⁻¹)	Sugar consumption % consumed or residual sugars (g L ⁻¹)	Sensory analysis	Reduction in ethanol levels	Reference
Sequential <i>M. pulcherrima</i> ARWI1149/ <i>S. cerevisiae</i> ARWI1631	Shiraz (sterile must)	240–260	Residual sugars Control <i>S. cerevisiae</i> ARWI1631 0.41 ± 0.05 g L ⁻¹ Residual sugars ARWI1149/ARWI1631 0.56 ± 1.0 g L ⁻¹	No	0.90% v/v	Contreras, Curtin, & Varela (2015)
Sequential <i>M. pulcherrima</i> ARWI1149/ <i>S. uvarum</i> ARWI12846/ <i>S. cerevisiae</i> ARWI1631	Shiraz (sterile must)	240–260	Residual sugars Control <i>S. cerevisiae</i> 5.93 ± 0.18 g L ⁻¹ Residual sugars ARWI1149/ ARWI12846/ARWI1631 Ranges from 0.40 ± 0.24 g L ⁻¹ to 0.54 ± 0.05 g L ⁻¹	No	1.70% v/v	Contreras, Curtin, & Varela (2015)
Sequential <i>Torulaspora delbrueckii</i> / <i>S. cerevisiae</i> with agitation and VVM aeration	Laboratory chemically defined grape juice medium	200	Consumption ranges from 99.8 % ± 0.10, 99.8 % ± 0.10, 99.7 ± 0.00, and 99.7 % ± 0.10	No	1.5% v/v	Contreras, Hidalgo, et al. (2015)
Sequential <i>Zygosaccharomyces bailii</i> / <i>S. cerevisiae</i> with agitation and VVM aeration	Laboratory chemically defined grape juice medium	200	Consumption ranges from 99.7 % ± 0.10, 99.7 % ± 0.10, 99.9 ± 0.00, and 99.9 % ± 0.10	No	Over 2.0% v/v	Contreras, Hidalgo, et al. (2015)

Fermentation approach reviewed	Fermentation medium	Initial sugars (g L ⁻¹)	Sugar consumption % consumed or residual sugars (g L ⁻¹)	Sensory analysis	Reduction in ethanol levels	Reference
Sequential <i>M. pulcherrima</i> / <i>S. cerevisiae</i> under aerobic conditions (below 0.35 g L ⁻¹), with oxygenation	Mixture of natural wine juice from Malvasia and Viura	262–265	Residual sugars After 48-hr fermentation <i>S. cerevisiae</i> control (air) 12.9 ± 0.70 (% w/v) <i>M. pulcherrima</i> / <i>S. cerevisiae</i> (air) 9.6 ± 0.80 (% w/v)	No	3.7% v/v under high aeration to 2.2% v/v under optimised conditions	Morales et al. (2015)
Sequential <i>Starm. bombicola</i> / <i>S. cerevisiae</i>	Synthetic grape juice medium/Verdiccio	220	After 79-hr fermentation: Sugars consumed 216.74 ± 0.14 g L ⁻¹ or 98%	No	1.64% v/v	Canonico et al. (2016)
Sequential <i>M. pulcherrima</i> / <i>S. cerevisiae</i>	Synthetic grape juice medium/Verdiccio	220	After 79-hr fermentation: Sugars consumed 217.79 ± 0.04 g L ⁻¹ or 99%	No	1.46% v/v	Canonico et al. (2016)
Sequential <i>H. uvarum</i> / <i>S. cerevisiae</i>	Synthetic grape juice medium/Verdiccio	220	After 79-hr fermentation: Sugars consumed 215.86 ± 0.37 g L ⁻¹ or 98.1%	No	1.21% v/v	Canonico et al. (2016)
Sequential <i>H. osmophila</i> / <i>S. cerevisiae</i>	Synthetic grape medium/Verdiccio	220	After 79-hr fermentation: Sugars consumed 216.90 ± 0.28 g L ⁻¹ or 98.6%	No	1.00% v/v	Canonico et al., (2016)

Fermentation approach reviewed	Fermentation medium	Initial sugars (g L ⁻¹)	Sugar consumption % consumed or residual sugars (g L ⁻¹)	Sensory analysis	Reduction in ethanol levels	Reference
Sequential <i>S. bacillaris</i> / <i>S. cerevisiae</i>	Synthetic must	40 g L ⁻¹ glucose, 40 g L ⁻¹ fructose and 12 g L ⁻¹ malic acid (80–12)	All sugars consumed after 290 hours	No	Ethanol yields not statistically different to control	Lemnos Junior et al. (2019)
Non-GMO-engineered <i>S. cerevisiae</i> YS59-409	Synthetic medium similar to a 'Triple M medium'	150	All sugars consumed	No	Ethanol yield was reduced by 34.9% when compared to the host <i>S. cerevisiae</i> YS59	Du et al. (2020)

3. Water addition and water substitution approaches (water treatment) into high-sugar wine musts to lower final alcohol levels in a wine

An alternative pre-fermentation approach to addressing ethanol levels produced in a wine must is the ‘water treatment’ of the must. Water treatment of wine musts is not a new approach. Indeed, water addition to wine musts was an accepted practice in winemaking processes in ancient Greece (Henderson, 2014). Several countries permit water addition to a wine must, which can have an effect (intended or otherwise) on ethanol production during fermentation. For example, at the federal level in the United States (US Code of Federal Regulations), water addition into a wine must is permitted in order to reduce the acid level of the must, which can impact wine flavour (Alcohol, Tobacco Products and Firearms, 2010). In the European Union, there is a prohibition on water addition approaches in winemaking ‘except where required on account of a specific technical necessity’ and where it does not modify the characteristics of the wine (European Parliament, 2013). Furthermore, in South Africa, water addition winemaking approaches are only permitted where they are deemed to be necessary for the incorporation of additives (Liquor Products Act 60 of 1989 [SA], 1990). Since 2017, Australia has permitted water addition into a wine must to ‘facilitate fermentation’ to 13.5 °Baumé (Bé), under the Australia New Zealand Food Standards Code (Standard 4.5.1) (Australian Government, 2017). The Australian approach was as a response to the problems associated with high ethanol production arising during fermentation (Bindon et al., 2019) and as one answer to address negative impacts on human health from consumption of higher-alcohol wines (Saliba et al., 2013).

3.1 Water treatment of distinctive *Vitis vinifera* grape cultivar musts, and the impacts on final wine alcohol levels

Water treatment of wine musts can include ‘addition’ or ‘substitution’ approaches. ‘Water addition’ into a wine must is where water is added by direct addition to a wine must pre fermentation so that there is an increase in the volume of the finished wine (Teng et al., 2020). ‘Water substitution’ is where grape juice is run off from the wine must and is substituted with equal amounts of water (Teng et al., 2020). The water substitution into must winemaking approach is similar to the saignée winemaking technique, which is sometimes used in red winemaking. Under the saignée approach, juice is bled off from a

fermentation, concentrating the must, which in turn can increase final wine colour and tannins (Cassasa et al., 2021). For the water substitution treatment of wine musts, the saignée approach is extended upon by replacing the removed juice with water in order to affect ethanol production during fermentation (Harbertson et al., 2009).

For both water treatments of wine must, outcomes for final wine alcohol levels and impacts on other wine parameters will be dependent on a number of factors, including selection of a grape cultivar. This is because each grape cultivar varies in levels and concentrations of relevant grape constituents such as acid, sugar, anthocyanins, volatile precursors and nutrients (Gutiérrez-Escobar et al., 2021; Teng et al., 2020). Water addition and substitution levels will also have an impact (Table 2.2). Schelezki, Antalick, et al. (2020) found that for the Cabernet Sauvignon wines treated with pre-fermentation water substitution to 13.5 °Bé, at different levels of water substitution treatments, significant decreases in the alcohol levels of between 1.0% and 3.0% ABV were achieved (Schelezki, Antalick, et al., 2020). These decreases did not appear to be as high when compared to outcomes for similarly treated Shiraz wines (Schelezki, Antalick, et al., 2020). Teng et al. (2020) tested both modes of water treatment to a Shiraz must directly. They observed that both water treatments of wine musts (taken from three harvest dates in one vintage), demonstrated higher reductions in ethanol levels for the water-treated wine musts from mature fruit, when compared to the untreated wine from a later 15.5 °Bé harvest occasion (control), from 0.90% v/v to 2.1% v/v (Teng et al., 2020) (Table 2.2). Similar results were observed by Schelezki, Deloire, and Jeffrey (2020) for comparably treated Shiraz musts from two different harvest times within one vintage, where one was picked early (fresh fruit [FF]) and one picked later in the harvest season (mature fruit [MF]). After vinification, the FF control wine had a 13.6% ABV, while the MF control wine had 15.5% ABV (Schelezki, Deloire, & Jeffrey, 2020). When compared to the control FF wine, alcohol levels in the final wines from the FF wine musts treated with water addition decreased for all three samples by 4.6% ABV, 2.8% ABV and 1.0% ABV for different water addition levels, respectively (Schelezki, Deloire, & Jeffrey, 2020) (Table 2.2). For the FF wines treated under the water substitution approach, the alcohol levels reduced by 4.0% ABV, 2.6% ABV and 1.0% ABV, respectively, for different water substitution levels (Schelezki, Deloire, & Jeffrey, 2020) (Table 2.2). The decrease in alcohol levels were more pronounced for treated wines from the MF harvest, which appears to support the outcomes of Teng et al. (2020). When compared to the control MF

wine, alcohol levels for the wines from the MF musts treated with water addition decreased for all three samples by 5.9% ABV, 2.8% ABV and 1.1% ABV, respectively, for different water addition levels (Schelezki, Deloire, & Jeffrey, 2020) (Table 2.2). Where the MF musts were treated under the substitution approach, the alcohol levels in the final wines reduced by 4.9% ABV, 3.5% ABV and 1.5% ABV, respectively, for different substitution levels (Schelezki, Deloire, & Jeffrey, 2020) (Table 2.2). Other studies for Shiraz wines that have been treated with pre-fermentation water substitution treatment to 13.5 °Bé have also demonstrated that alcohol levels can decrease by between 0.50% ABV and 2.00% ABV (Schelezki, Antalick, et al., 2020) (Table 2.2).

For Merlot noir and Tempranillo tinto grape varieties, high-sugar wine musts treated with acidified water addition and/or substitution, or acidified musts treatments have also been found to produce varied reduced ethanol levels when compared to control wines (Piccardo, Favre, et al., 2019) (Table 2.2). Piccardo, Favre, et al. (2019) observed that for those wines produced from a late harvest of Merlot noir, where the musts had been treated with the addition or substitution of acidified water, ethanol levels decreased by 1.0% v/v and 0.7% v/v, respectively, when compared to the late harvest control wine. More-favourable results were observed for Merlot noir musts that were treated with addition or substitution of acidified musts, with decreases in ethanol levels of 1.1% v/v and 0.9% v/v observed, respectively, when compared to the late harvest control wine (Piccardo, Favre, et al., 2019). Piccardo, Favre, et al. (2019) also observed that for the wines produced from the late harvest of Tempranillo Tinto grapes, where the musts had been treated with the addition or substitution of acidified water, ethanol levels decreased by 1.1% v/v and 0.6% v/v, respectively, when compared to the late harvest non-treated control wine. For late harvest Tempranillo Tinto musts that had been treated with addition or substitution of acidified musts, the ethanol levels decreased by 1.0% v/v and 0.7% v/v, respectively (Piccardo, Favre, et al., 2019). These results indicate that the reduction of ethanol levels can be dependent not only on fermentation approaches, but also on grape cultivar and different water addition and substitution levels (Piccardo, Favre, et al., 2019) (Table 2.2).

Table 2.2. Parameters including reduction of ethanol levels for wines produced from water treatments of pre-fermentation wine musts

Treatment	Fermentation medium	Wine must water treatment	Water addition/substitution levels	Control wine: Potential alcohol level % v/v or ABV	Reductions in ethanol: % v/v or ABV levels when compared to control wine	Sensory analysis	Reference
Water addition and substitution approaches to control wine musts	Shiraz	Water addition	Add 7.5% v/v water (from 15.5 °Bé to 14.5 °Bé)	16.5% v/v	0.9% v/v	Yes	Petrie et al. (2019)
		Water substitution	Run off juice and replace with 7.5% v/v water (from 15.5 °Bé to 14.5 °Bé)	16.5% v/v	0.9% v/v	No significant difference in tannin concentration for either water treatment	Teng et al. (2020)
		Water addition	Add 14% v/v water (from 15.5 °Bé to 13.5 °Bé)	16.5% v/v	2.1% v/v	Dilution of other sensory characteristics were observed for all treatments	
		Water substitution	Run off juice and replace with 14% v/v water (from 15.5 °Bé to 13.5 °Bé)	16.5% v/v	1.6% v/v		

Treatment	Fermentation medium	Wine must water treatment	Water addition/ substitution levels	Control wine: Potential alcohol level % v/v or ABV	Reductions in ethanol: % v/v or ABV levels when compared to control wine	Sensory analysis	Reference
Water addition and substitution approaches to late harvest control wine musts	Merlot noir	Acidified water addition	Add approx. 8.5% v/v acidified water	Late harvest 15.4% $\pm$ 0.1% v/v	1.1% v/v	Yes	Piccardo, Favre, et al. (2019)
		Acidified water substitution	Run off juice and replace with approx. 8.6% v/v acidified water	Late harvest 15.4% $\pm$ 0.1% v/v	0.7% v/v	When compared to the control wine for both treatments, it was observed that the resultant water-treated wines were more vegetal and acidic. They were also observed to be less fruity and structured.	
Water addition and substitution approaches to late harvest control wine musts	Tempranillo	Acidified water addition	Add approx. 8.0% v/v acidified water	15.3% $\pm$ 0.1% v/v	1.1% v/v	Yes	Piccardo, Favre, et al. (2019)
		Acidified water substitution	Run off juice and replace with approx. 8.1% v/v acidified water	15.4% $\pm$ 0.1% v/v	0.6% v/v	When compared to the control wine, both water-treated wines were observed to be more vegetal and acidic. They were also observed to be less spicy.	

Treatment	Fermentation medium	Wine must water treatment	Water addition/ substitution levels	Control wine: Potential alcohol level % v/v or ABV	Reductions in ethanol: % v/v or ABV levels when compared to control wine	Sensory analysis	Reference
Water addition and substitution approach of an acidified must into late harvest control wine musts	Merlot Noir	Acidified must addition	Add approx. 25.0% v/v acidified must	15.4% $\pm$ 0.1% v/v	1.1% v/v	Yes	Piccardo, Favre, et al. (2019)
		Acidified must substitution	Run off juice and replace with approx. 21.5% v/v acidified must	15.4% $\pm$ 0.1% v/v	0.9% v/v	Sensory parameters observed to be similar to control wine	
Water addition and substitution approach of an acidified must into late harvest control wine musts	Tempranillo	Acidified must addition	Add approx. 20.8% v/v acidified must	Late harvest 15.4% $\pm$ 0.1% v/v	1.0% v/v	Yes	Piccardo, Favre, et al. (2019)
		Acidified must substitution	Run off juice and replace with approx. 21.2% v/v acidified must	Late harvest 15.4% $\pm$ 0.1% v/v	0.7% v/v	Sensory parameters observed to be similar to control wine	

Treatment	Fermentation medium	Wine must water treatment	Water addition/ substitution levels	Control wine: Potential alcohol level % v/v or ABV	Reductions in ethanol: % v/v or ABV levels when compared to control wine	Sensory analysis	Reference
Water treatment approach to pre-fermentation control wine musts utilising dilution methods	Shiraz Early harvest Fresh fruit (FF)	Water addition	Add water 41.0% v/v 26.6% v/v 11.6% v/v	13.6% ABV	4.6% ABV 2.8% ABV 1.0% ABV	Yes For FF dilution treatment, a decline in flavour intensity and body were noted	Schelezki, Deloire, & Jeffrey (2020)
	Shiraz Late harvest Mature fruit (MF)	Water addition	Add water 47.2% v/v 34.0% v/v 10.2% v/v	15.5% ABV	4.0% ABV 2.6% ABV 1.0% ABV	For MF dilution treatment, at 10.2% wine colour properties were decreased. Tannin concentrations remained stable.	
Water treatment of pre-fermentation control wine musts utilising run off and substitution methods	Shiraz Early harvest Fresh fruit (FF)	Water substitution	Run off juice and replace with water 41.0% v/v 26.6% v/v 11.6% v/v	13.6% ABV	5.9% ABV 2.8% ABV 1.1% ABV	Yes For FF treatment of 11.6% v/v, colour property changes were more significant than dilution treatments	Schelezki, Deloire, & Jeffrey (2020)
	Shiraz Late harvest Mature fruit (MF)	Water substitution	Run off juice and replace with water 47.2% v/v 34.0% v/v 10.2% v/v	15.5% ABV	4.9% ABV 3.5% ABV 1.5% ABV	For MF treatment of 10.2% v/v, decreased wine colour was observed	

Treatment	Fermentation medium	Wine must water treatment	Water addition/ substitution levels	Control wine: Potential alcohol level % v/v or ABV	Reductions in ethanol: % v/v or ABV levels when compared to control wine	Sensory analysis	Reference
Water treatment of pre-fermentation high-sugar control wine musts utilising run off and substitution methods	Cabernet Sauvignon	Water substitution	CSBw1: 25% run off juice and replace with water CSBw2: 16% run off juice and replace with water CSBw3: 10% run off juice and replace with water	15.5% ABV	1.0% to 3.0% ABV	Yes CSBw1 observed to have diminished colour stability	Schelezki, Antalick, et al. (2020)
Water treatment of pre-fermentation high-sugar control wine musts utilising run off and substitution methods	Shiraz	Water substitution	SHBw1: 25% run off juice and replace with water SHBw2: 12% run off juice and replace with water SHBw3: 6% run off juice and replace with water	15.4% ABV	0.5% to 2.0% ABV	Yes Dark fruit tended to diminish with decreasing alcohol levels Implementation of water (up to 25%) diminished colour stability	Schelezki, Antalick, et al. (2020)

3.2 Water treatment of wine musts and the impacts on wine phenolics and other wine quality parameters

Winemakers also need to balance desired reduction in ethanol production with other desirable wine flavonoids. Winemakers should also appreciate that ethanol will contribute to final wine aroma and flavour (Remize et al., 1999). It has been observed that impacts on other wine attributes may not be too dissimilar where reduction of alcohol for both water treatments of wine musts are at equivalent levels (Teng et al., 2020). However, there can be significant decreases in phenolics concentrations, tannin and non-bleachable pigments, tannin molecular mass, and wine colour density when compared to a late harvest wine (Teng et al., 2020).

Water treatment of wine musts can negatively affect tannin levels in the final wine. Tannins are important flavonoids in wine production. Tannins will come from the grape skins, pips (seeds) and stems, and the wood barrels used during aging (if used). Tannins provide texture and mouthfeel to wine as well as a sense of weight and structure (Rousserie et al., 2019). One study found that reducing the sugar concentration of the must with a 15.5 °Bé to a 13.3 °Bé, by water treatment of wine musts, could have an effect on tannin concentrations at pressing (Harbertson et al., 2009). Diverse outcomes have also been found for different *Vitis vinifera* grape cultivars. The study by Casassa et al. (2013) indicated little impact to wine tannin concentration or colour for equal water substitution approaches in wine must for Cabernet Sauvignon and Merlot. However, for Shiraz, tannin levels have been observed to be more adversely affected with the water addition treatments into wine must for early harvest Shiraz wines (Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020).

Water treatment of pre-fermentation wine musts can also have an effect on other phenolic aspects in the resultant wines. Phenolic compounds are important quality factors of wines as they contribute to the organoleptic characteristics of wine. They will have impacts on colour, astringency and bitterness characteristics in the final wine, all of which are related to the grade and market value of a wine (Kassara & Kennedy, 2011; Rousserie et al., 2019). Phenolics in a final wine product can be impacted by grape phenolics composition (which will be different depending on the harvest time) as well as oenological winemaking approaches (Setford et al., 2017; Varela et al., 2015). Piccardo, Favre, et al. (2019) observed that for the Tempranillo tinto and Merlot noir grape cultivars, adding or

substituting overripe wine juice with acidified water treatments caused an increase in wine pH, together with additional negative effects on other wine phenolics. Further, they found that sensory analysis for wines subjected to both treatments indicated that wines were more vegetal and acidic for both grape cultivars. By contrast, they also observed that the substitution of acidified Tempranillo Tinto must approach demonstrated less impacts on other wine parameters, while still reducing ethanol production during fermentation (Piccardo, Favre, et al., 2019).

Other important compounds in wines are polysaccharides, wine anthocyanin and colour properties, which can also be negatively affected by water treatment of pre-fermentation wine musts. Polysaccharides are a chemically diverse and abundant group of compounds present in wine, which will influence wine taste and mouthfeel, where the level of effects will depend on different polysaccharide types and will be influenced by wine pH and alcohol level (Gawel et al., 2016; Petrie et al., 2019). In their sensory analysis of wines for which musts were subject to water treatments, Petrie et al. (2019) observed that the Shiraz wines produced from a control (non-treated) 15.5 °Bé occasion displayed a higher darker fruit aroma, together with ‘hotness’, viscosity, astringency and opacity (Petrie et al., 2019). Where water was added to the 15.5 °Bé wine must occasion, the effect on sensory attributes were observed to be higher than the impact on the phenolics, where the highest level of water addition into a wine must produced ‘cooked vegetable’, drain and/or savoury characteristics (Petrie et al., 2019).

Changes in solvent composition in a high-sugar wine must that has been subject to water treatment may also have a significant negative impact on wine phenolics. With respect to wine anthocyanin and colour properties, reductions in anthocyanin over time have been demonstrated to be less marked for wines whose musts were subjected to water treatment than in late harvest control wines (Teng et al., 2020). Wines produced from late harvest musts that have been treated with water addition have been observed to display higher wine phenolics, tannin, non-bleachable pigment and polymeric pigment, when compared to those wines produced from non-treated musts from earlier harvests in the same vintage year (Teng et al., 2020).

These outcomes indicate that a later harvest may be preferable to early ones, so that wine phenolics and tannins can be maximised. The later harvest must can then be subjected to water treatment pre-fermentation winemaking approaches, to dilute the high sugars so as

to address high ethanol production. Winemakers, however, also need to balance harvesting late and water treatment of musts with other potential impacts: the possible loss of berries and yield loss, possible increases in disease, and possible increases in other negative sensory aspects associated with the wines produced from a late harvest (Teng et al., 2020). For example, wines produced from late harvests have been shown to impart an increase in a ‘port-like’ sensory attribute, where this attribute can still be present even in wines whose high-sugar musts have been subject to water treatment (Longo et al., 2018; Schelezki, Smith, et al., 2018).

3.3 Limitations of studies reviewed for water treatment of wine must approaches to reducing alcohol levels in a final wine

A number of the water treatment studies reviewed relied on small samples of fruit tested per each occasion (Petrie et al., 2019; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020), which could potentially limit the transferability of the laboratory results into commercial applications. Further research is also required given the growing climate change effects on grape maturity. For example, the 2017 harvest year, which was the subject of the Australian study by Schelezki, Deloire, and Jeffrey (2020) was a cooler one when compared to the previous year; thus, the results from this study may have some limitations given that climate change science indicates future years’ harvests are being shortened due to global warming (Holland & Smit, 2010; Webb et al., 2012).

4. Conclusions

This review examined two pre-fermentation oenological tools available to winemakers to reduce the ethanol levels produced from high-sugar musts. First, a number of microbiological pre-fermentation approaches utilising GM and non-GMO approaches were reviewed. Distinctive yeasts implemented with various inoculation and fermentation approaches (including aeration) to address ethanol production in fermentations were then examined. Some limitations were found, in particular the variability of the resultant alcohol levels in the final wines produced (Table 2.1). This review then examined an alternative pre-fermentation approach to reducing alcohol levels in a final wine: ‘water treatment’ of a high-sugar grape must. It was observed that water treatment of must approaches can effectively reduce ethanol production during fermentation (Table 2.2). It is concluded that further research is required, especially for water treatment of must approaches, in order to provide winemakers with better information datasets on the effects

on final wine quality parameters, so that they can futureproof wine production. Additional research in this area can improve the wine industry's resilience in the face of climate change and drought, and improve productivity. It will also enhance consumer confidence, which is especially important for local consumers as well as for export markets.

Section 2 – Extended literature review

5. Introduction

Section 2 of this literature review further examines a number of other common additional oenological approaches that can also address final alcohol levels in wine, while considering the impact on final wine quality parameters. The extended literature review examines those limited studies to date that test the water addition treatments of musts together with the accentuated cut edges (ACE) vinification approach, including observed impacts for final alcohol levels and wine quality parameters. The review then examines blending or substitution approaches of a green harvest wine (GHW) into later harvest high-alcohol wine which can also be implemented to lower alcohol levels in final treated wine. However, this GHW blending or substitution vinification approach is not further explored in this thesis; it is provided only as a comparative vinification approach to producing wines with lower alcohol levels. An overview of common, industrially conventional, thermal oenological approaches to removing alcohol from final wine products post fermentation, including reverse osmosis and spinning cone technologies, is then also provided. Again, these ‘in winery’ vinification approaches are not further explored in the thesis; the review provides these post-fermentation approaches as one possible comparative approach to producing final wines with lower alcohol levels than would be produced without the implementation of the technology. The ACE vinification technique is, however, further addressed in the thesis (in **Chapter 6**), as research demonstrates that it can further extract phenolic attributes in wine when applied in a wine must, and thus ACE may be able to address some of the negative consequences for final wine quality for those wines that have been subject to water treatment of their high-sugar pre-fermentation wine grape musts (as discussed in Section 1 of this literature review above).

6. Accentuated cut edges (ACE) vinification together with water addition treatments of high-sugar musts

ACE is the process in which grape skins are mechanically cut into smaller fragments, with the aim of assisting the extraction of phenolic components from the grape skin of the floating pomace cap in the early stages of fermentation. During early fermentation, the extraction of phenolic compounds from the grape skins is greater due to more broken

edges achieved through ACE (Sparrow et al., 2016). Previous studies have found that ACE can increase colour density and hue, total tannin, total phenolics, and stable pigments for Shiraz and Pinot noir wines when compared to wines with no ACE implementation into their pre-fermentation wine grape musts (Kang et al., 2020; Sparrow et al., 2016).

As noted in Section 1 above, water treatment of high-sugar wine musts can have an impact on final wine organoleptic qualities. Limited studies to date have considered whether the ACE vinification strategy can be implemented together with water treatments of high-sugar wine grape musts to effectively address perceived or actual negative impacts on phenolic, chemical and sensory parameters associated with water-treated wines. Kang et al. (2020) implemented the ACE approach (at one skin fragmentation level only) for the Shiraz variety, and observed ACE impacts on basic chemical compositions, colour, phenolic compounds and polysaccharides, as well as sensory aspects of the final wines. One aspect of their study was to test the impact of ACE (at one skin fragmentation level) on Shiraz wines that had also been treated with water addition from a 14.0 °Bé to a 13.5 °Bé (now permitted under Food Standards Australia New Zealand Standard 4.5.1, 2017, as noted above), to further determine whether ACE could positively address the perceived dilution of wine aromas, flavours and astringency parameters associated with water-treated wines. They found that when subject to an expert sensory panel, the water-treated wines (without ACE) displayed an impact on the astringency profile. Combining water addition treatment with the ACE technique addressed the astringency issues, with the resultant wine displaying an increase in intensity of perception of adhesive and graininess aspects for the trained panel, but not the consumer panel (Kang et al., 2020).

The impact of ACE treatment on the wine aroma quality for Shiraz is underpinned in a later study by Wang et al. (2022). They examined concentrations of varietal thiol precursors in Shiraz grape must which had been treated with ACE (again implemented at only one level), as well as the concentrations of thiols present in the final wines produced. Varietal thiols are polyfunctional compounds that can significantly influence wine aroma as a result of their very low odour detection thresholds (Roland et al., 2011). While they are not as abundant in red wines, thiols have been shown to have an impact on aroma characteristics for both red wines and rosé (Ferreira et al., 2000; Rigou et al., 2014). This study also included a limited study of water addition treated wine, to the regulated limit

of 13.5 °Bé, and varied skin contact time (short – 3 days, or long – 6 days) during fermentation in conjunction with ACE treatment. The results were compared with wines produced without ACE (NOACE treatments) (Wang et al., 2022). Wang et al. (2022) found that for both ACE and NOACE treatments, including for those subjected to water addition treatment, there were no differences in the precursors of thiols in the musts immediately after crushing. However, ACE treatment wines demonstrated a significant consumption of 3-*S*-glutathionylhexan-1-ol (GSH-3SH) (43%) when compared to NOACE treatment wines. Wang et al. (2022) also observed that the crushing methods and the length of skin contact time impacted levels of 3-sulfanylhexas-1-ol and ethyl esters, where NOACE or shorter skin contact time produced higher concentrations of these compounds. They also observed that for other volatiles, including acetates, higher alcohols, fatty acids and isoprenoids, the results were varied, depending on the crushing methods employed as well as skin contact time. ACE short treatment (3-day racked off grape skins) demonstrated the highest potential for improvements to all groups of volatiles ($p = 0.12$), apart from acetates. Wang et al. (2022) also applied RATA (rate all that apply) sensory analysis (Danner et al., 2018), based on a panel of 61 untrained regular wine consumers. Twenty-two aroma and corresponding flavour attributes were subject to evaluation. Wang et al. (2022) found that it was only the ‘red fruits’, ‘earthy/dusty’, ‘floral/ perfume/musk’, ‘herbaceous’ and ‘vanilla’ attributes that were significantly different ($p < 0.1$).

6.1 Limitations of previous ACE approaches

The study by Kang et al. (2020) found that ACE could be an additional strategy to address impacts on final wine quality parameters for water addition treated Shiraz wines. However, it is noted that the conclusions reached in this study are limited as the researchers focused on water addition treated wine from a 14.0 °Bé to a 13.5 °Bé occasion, which may not sufficiently consider the impacts of climate change generally. Further research is required to test water addition treated wines from a 15.5 °Bé to a 13.5 °Bé occasion, which may be more reflective of the impacts of climate change in a hot growing season. The study by Wang et al. (2022) focused on an examination of thiols and other volatiles. While thiols have been shown to have an impact on aroma characteristics for both red wines and rosé, they are not as abundant in red wines (Rigou et al., 2014); further investigation for impacts on other chemical compounds is therefore required.

Further, both the Kang et al. (2020) and Wang et al. (2022) studies utilised the Della Toffola Maceration Accelerator (DTMA) machine (Della Toffola, Italy) for ACE implementations. Kang et al. (2020) and Wang et al. (2022) acknowledged that by using this ACE crushing method, they were able to reduce the dimensions of the crushed grape skins, thereby increasing the surface area of the grape film in contact with the must. For both studies, however, the DTMA machine reduced the dimensions of the crushed grape skins to one predetermined level only. Anecdotally, some winemakers have been advised by the manufacturers of this machinery to run the ‘crush’ through the DTMA twice to achieve better results. Neither of the studies reviewed indicated that this was done. Additional research for the ACE winemaking approach is therefore required, to further investigate alternatives to this common skin fragmentation mechanical approach. In addition, exploring the results from ACE implementation into pre-fermentation wine grape musts at different skin fragmentation levels may provide additional foundational datasets for winemakers. **Chapter 6** of this thesis describes the additional study I undertook with alternative levels of ACE approaches, so that winemakers can make supported and informed decisions when implementing the ‘water treatment into a pre-fermentation wine must’ winemaking approach to mitigate the high production of ethanol, where the addition of ACE may further enhance desirable wine quality parameters in final wines.

7. Green harvest or early harvest wines: Blending or substitution vinification approaches to address final alcohol levels in the resultant wine

Even where an early harvest exhibits less alcohol in the finished wine, astringent and acid characters can be negatively affected (Longo et al., 2016). This is because after veraison, less-desirable compounds like methoxypyrazine will decrease during the ripening of the fruit, while anthocyanin, tannin and other desirable volatile compounds will continue to increase. Studies show that producing wines from earlier ‘green harvests’ to yield wines with lower than typical alcohol levels is to be avoided as it has been shown to produce unfavourable green or vegetative characteristics in a final wine (Bindon et al., 2014).

7.1 Blending early harvest wines with later harvest wines

However, blending high-alcohol wines with wines with lower alcohol levels is an alternative approach that can be implemented to reduce the alcohol level in a final wine

product. The study by Kontoudakis et al. (2011) added charcoal and bentonite to highly acidic, low-alcohol wines, which were then blended with wines from a later harvest. Each final blended wine reduced alcohol levels (by 0.9% v/v, 1.7% v/v and 3% v/v, respectively) when compared to the control wines that had not been blended (Kontoudakis et al., 2011) (Table 2.3). Longo et al. (2018) expanded on this approach by examining the effectiveness of a monovarietal blend to reduce ethanol in the final wine. The resultant wines were made from a blend of wine that had been vinified from less-ripe grapes together with a wine that had been vinified from riper fruit. For wines produced from *V. vinifera* L. Verdelho and Petit Verdot grapes, alcohol levels decreased in a linear fashion. For instance, for Verdelho, early harvest wine with an alcohol level of 7.2% v/v $\pm$ 0.1, when blended with a later harvest wine with an alcohol level of 10.3% v/v $\pm$ 0.1 could produce a final wine with an alcohol level of 8.8% v/v $\pm$ 0.1. For Petit Verdot, blending early harvest wine with a alcohol level of 9.3% v/v $\pm$ 0.1, with a later harvest wine with an alcohol level of 12.6% v/v $\pm$ 0.1 could produce a final wine with an alcohol level of 11.0% v/v $\pm$ 0.2 (Longo et al., 2018) (Table 2.3).

7.2 Green harvest wine (GHW) substitution into wine musts

Substituting green harvest wine (GHW) into a high-sugar wine must, while removing juice, is another alternative approach to producing lower alcohol levels in a resultant wine. GHW is wine that has been produced from an earlier harvest, usually from grapes at the veraison stage. GHWs on their own in wine production can produce lower alcohol levels but with other unfavourable sensory results for final wines (Schelezki, Antalick, et al., 2020) (Table 2.3).

GHW when used in a substitution (run off and replacement) strategy into a later harvest high-sugar has also been shown to address ethanol production during fermentation, while impacts on other final wine parameters will vary depending on the wine cultivar. Schelezki, Antalick, et al. (2020) found that for Cabernet Sauvignon wines, the lowest addition of the GHW treatment (16% v/v juice substitution with GHW) giving 14.5% ABV had the most resemblance to the untreated late harvest control wine of 15.5% ABV. This treatment impacted just three compound groups – acids, methyl esters of fatty acids, and higher alcohol acetate (HAA) esters from yeast sugar and N metabolism – when compared to the control wine (Schelezki, Antalick, et al., 2020). It was further observed that the 16% v/v GHW substitution treatment was able to the lower

the perception of ‘hotness’ compared to the control without significantly changing ‘fruity’ attributes. However, this treatment wine was significantly less astringent, as were all other GHW-treated wines (16%, 23% and 40%), when compared to the control and all other concurrent water into must substitution treatments tested. This could be related to lower tannin concentrations in these wine treatments, which may negatively affect perceived mouthfeel qualities (Ma et al., 2014). Wines with higher juice substitution of GHW rates (46% and 26%) displayed markedly increased levels of isoprenoids, together with decreased sensory attributes of ‘flavour intensity’, and ‘red fruit’ and ‘dark fruit’ flavour. For Shiraz water-treated wines, the effects were found to be more pronounced (Schelezki, Antalick, et al., 2020) (Table 2.3). These studies indicate that GHW treatment can be another strategy to manage alcohol levels for the Shiraz cultivar. However, this winemaking approach can produce pronounced changes in chemical composition and sensory qualities of the final wine and can attract higher costs due to additional winemaking undertakings (Schelezki, Šuklje, et al., 2018).

Table 2.3. Reduction of ethanol levels for resultant wines related to blending approaches and green harvest wine treatments pre and post fermentation

Blending approach	Fermentation medium	Reductions in ethanol level (% v/v) compared with control wine not blended	Reference
Post fermentation – adding charcoal and bentonite to highly acidic, low-alcohol wines with later harvest wines	Cabernet Sauvignon	0.9	Kontoudakis et al. (2011)
Post fermentation – adding charcoal and bentonite to highly acidic, low-alcohol wines with later harvest wines	Merlot	1.7	Kontoudakis et al. (2011)
Post fermentation – adding charcoal and bentonite to highly acidic, low-alcohol wines with later harvest wines	Bobal	3.0	Kontoudakis et al. (2011)
Post fermentation – monovarietal blend made with less-ripe grapes with later harvest wines	Verdelho	1.5	Longo et al. (2018)
Post fermentation – monovarietal blend made with less-ripe grapes with later harvest wines	Petit Verdot	1.6	Longo et al. (2018)
Pre fermentation – green harvest wine (GHW) substitution into later harvest musts	Cabernet Sauvignon 43.7% v/v 27.3% v/v 13.6% v/v	3.8 2.4 1.2	Schelezki, Smith, et al. (2018), Schelezki, Šuklje, et al. (2018)
Pre fermentation – GHW addition to later harvest musts	Shiraz Cabernet Sauvignon	1.0 to 3.0 0.5 to 2.0	Schelezki, Antalick, et al. (2020)

8. Post-fermentation approaches to removing alcohol from the final wine product

A number of technologies can be used post fermentation to produce wines with reduced final alcohol levels, such as distillation under vacuum or atmospheric pressure, membrane filtrations, spinning cone columns, adsorption, freeze concentration, and evaporation and extraction (Pickering, 2000). The common post-fermentation oenological approaches to removing alcohol are reviewed in this extended literature review, including reverse osmosis membrane filtration techniques and the implementation of spinning cone technologies, as a comparative approach to the pre-fermentation approaches reviewed above.

8.1 Reverse osmosis for removing alcohol from a final wine product

Reverse osmosis is a thermal distillation process that can be used to remove alcohol from a final wine product. This commercial process relies on membrane technology to remove alcohol from beverages including wine. High pressure is passed through a semipermeable membrane that will retain molecules that are larger than a certain size. What remains in the permeate are smaller molecules such as water, mineral salts and ethanol, while all other parts of the wine product, such as aromas, polysaccharides and phenolic compounds, are left in the retentate. Ethanol has a higher molecular weight than water, so water will permeate faster, resulting in a permeate that has a lower ethanol content than the original wine, by around 0.7–1.5% v/v (Massot et al., 2008). The components left in the retentate are at a higher concentration, including alcohol. To counter this, the alcohol needs to be separated from the water by thermal distillation, using a membrane contactor or by pervaporative extraction. The original water is added back into the retentate, while a high-ethanol by-product is produced. The permeate starts off with a low ethanol level, so the wine needs to be recirculated a few times to reach the desired end alcohol level in the final wine product. The effects of removing ethanol by reverse osmosis on the final wine product can be varied. It has been noted that because volatile compounds are stripped during the process of alcohol removal, the reverse osmosis approach can produce wines that are organoleptically unacceptable (Gómez-Plaza et al., 1999). However, later studies have shown different effects for chemical compounds and sensory parameters related to reverse osmosis winemaking (Gil et al., 2013). For instance, wines produced from Cabernet Sauvignon (AOC Penedes) and Grenache and Carignan

(AOC Priorat) that were de-alcoholised by reverse osmosis, to decrease final alcohol levels by 1% v/v or 2% v/v, demonstrated insignificant changes to other analytical parameters, including titratable acidity, colour intensity, hue, anthocyanins, total phenolic acids (TPA), proanthocyanins and the mean degree polymerization (mDP) (Gil et al., 2013). Sensory panel tests found that when comparing the control wines with -1% decreased alcohol level wines, 7 of the 11 tasters preferred the control wine, and 2 tasters preferred the -1% decreased alcohol wine. However, the preferences were not statistically significant ($p = 0.703$) (Gil et al., 2013). The outcomes from this particular study support reverse osmosis as one option for lowering alcohol levels in a final wine product; however, consumer aversion to such an invasive thermal process of alcohol removal requires further addressing (Schmidtke et al., 2012).

8.2 Spinning cone column for removing alcohol from a final wine product

Another common thermal distillation approach to alcohol removal in wine is the use of the spinning cone column (SCC). The SCC is a device that will extract volatile flavour components from a slurry or liquid. The column itself operates at a negative pressure, where volatile components are evaporated at a reduced temperature (Belisario-Sánchez et al., 2009). One advantage of the SCC approach is that flavours can be retained because the SCC can operate at a low temperature. Removing alcohol from a final wine product requires a two-step process under an SCC approach. Generally, first, the wine will pass through the SCC at low temperatures of 26–28 °C and a vacuum, so that aroma and flavour can be retained in approximately 1% of the total wine volume. The second step requires that the wine passes through the SCC at a higher temperature of 30–38 °C, under vacuum conditions so as to remove the alcohol. Once both steps are completed, the final wine is then blended back with the wine collected during the first step, to add back in the flavour and aroma collected (Belisario-Sánchez et al., 2009).

Belisario-Sánchez et al. (2009) conducted a study on the effects of SCC when used to remove alcohol, focusing on the phenolic composition for a number of Spanish red and white wines. The researchers examined the effects of SCC for 19 different raw wines by comparing them pre and post dealcoholisation. By relying on spectrophotometry, they were able to test total phenolic content, flavanols, tartaric esters and anthocyanins in both the control and the dealcoholised wine products. High-performance liquid chromatography (HPLC) was then relied on to test the content of phenolic compounds

such as stilbenes (trans- and cis-resveratrol), flavanols (rutin, quercetin and myricetin), flavan-3-ols [(p)-catechin and (-)-epicatechin] and anthocyanins (malvidin 3-glucoside), as well as non-flavonoids (gallic, caffeic and *p*-coumaric acids). The study found that for the red wines, the resveratrol levels ranged from 1.81 mg/L to 34.01 mg/L in the controls, and 2.12 mg/L to 39.57 mg/L in the dealcoholized wines. However, the results did not demonstrate any significant differences in phenolic compounds, with any slight increases in the dealcoholized wines being attributed to the concentration effect following the removal of ethanol. The study also relied on the organic radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a standard reagent for the measurement of the antioxidant capacity of wines. The DPPH level was found to be very similar in the control wines and the dealcoholized wines. The researchers surmised that this may have been because of the sulphur dioxide (SO₂) removal from the control wines during distillation (Belisario-Sánchez et al., 2009). The results from this study were therefore inconclusive, given the concentration effects, and the impact of SO₂ removal.

External limitations of both the reverse osmosis processes and the SCC approaches to removing alcohol from a wine post fermentation are that they can be very costly and energy reliant (Pilipovik & Riverol, 2005). SCC is very expensive, due to equipment hire and operating costs. Thus, SCC has been mostly restricted to high-volume operations (Saha, et al., 2013). The results as indicated above also demonstrate that the effect on sensory attributes from such post-fermentation approaches can be inconclusive, with respect to the phenolic attributes of the dealcoholized wine when compared to the control wine (Belisario-Sánchez et al., 2009; Gil et al., 2013). Further, the studies do not provide adequate responses to actual or perceived consumer aversions to low-alcohol wines produced through post-fermentation processes (Schmidtke et al., 2012).

9. Overall conclusion

Compressed, later and hotter vintages (due to climate change) across grape-growing regions around the world, including Australia, present considerable challenges to winemakers as the sugar levels in the grape berries are higher, resulting in wines with higher than typical alcohol concentrations. To respond to consumer demands for lower-alcohol wine, concerns regarding alcohol being bad for human health, the need for wine quality steadiness across vintages, and regulatory requirements, winemakers are now faced with the requirement to produce wines with lower alcohol levels. As a result, many

are already implementing a number of oenological approaches, either pre or post fermentation, to achieve more-favourable lower alcohol levels in the final wines.

Section 1 presented a preliminary literature review comparing two groups of pre-fermentation approaches to addressing ethanol production during fermentation, with the overall goal being to produce a final wine with lower alcohol while minimising any negative impacts on final wine quality parameters (both chemical and sensory). First, a number of microbiological pre-fermentation approaches using GM and non-GMO approaches were reviewed. Distinctive yeasts implemented with various inoculation and fermentation approaches (including aeration) to address ethanol production in fermentations were then examined. It was noted that the use of distinctive yeasts and yeast hybrids to address ethanol levels in the final wine product can have major limitations given the variability of the outcomes in ethanol levels in wine production (Arroyo-Lopez et al., 2010; Contreras, Hidalgo, et al., 2015; Gobbi et al., 2013; Morales et al., 2015; Tilloy et al., 2014). These yeast approaches were then compared to an alternative pre-fermentation approach to reduce alcohol levels in a final wine: water addition/substitution into a high-sugar grape must. The Food Standards Australia New Zealand now permit this water treatment approach to yield a minimum of 13.5 °Bé, without the need to remove any juice (Australian Government, 2017). It was found that these water approaches effectively reduce alcohol levels in a final wine product, and in many instances minimise the negative effects on phenolics and sensory aspects of the resultant wine product (Piccardo, Favre, et al., 2019; Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020). However, it was also noted that existing research for the cultivar Shiraz is limited. Given that Shiraz is one of the most widely grown grape varieties in both Australia and in other growing regions because it can be grown in varied climates, further investigations into Shiraz wines produced from this winemaking technique are required. This thesis addresses this gap in the scientific knowledge, in **Chapters 4, 5 and 6**.

Section 2 presented an extended literature review by first examining the ACE vinification winemaking approach, which has been shown to have positive impacts on the phenolic composition of the final wine (Sparrow et al., 2016). ACE may address negative impacts on final chemical and sensory outcomes related to ‘water treated’ wines, by producing higher levels of anthocyanidin and tannin, while maintaining wine aroma and colour when compared to a ‘water treated’ NO ACE wine (Kang et al., 2020; Wang et al., 2022).

It was noted that further research in this area is required, as limited studies for Shiraz cultivar have been conducted for combined water addition/ACE winemaking approaches to date. In addition, there are no studies to date that test ACE implementation at varying fragmentation levels. This thesis examines this gap in scientific knowledge in **Chapter 6**. The extended literature review also provides by way of comparison other more common alternative winemaking approaches available ‘in winery’ that winemakers are already implementing to produce wines with lower than typical alcohol levels. First, a number of previous studies have indicated that blending approaches with low-alcohol wines can reduce alcohol levels in the final wine product; however, this can produce varied compositional and sensory qualities of the final wine, and can attract higher costs and additional winemaking undertakings (Schelezki, Šuklje, et al., 2018). Substituting green harvest wine (GHW) into a high-sugar wine must was another alternative approach reviewed. This approach was shown to produce lower alcohol levels in the resultant wine, but it was found that outcomes from this approach could also vary, depending on the wine cultivar (Schelezki, Smith, et al., 2020). The extended literature review also examined the more common post-fermentation thermal approaches to reduce alcohol levels in a final wine product, including reverse osmosis and the implementation of spinning cone column (SCC) technologies. A limitation identified with such approaches was that they can be costly and energy reliant (Pilipovik & Riverol, 2005). In addition, it was noted that the effects on sensory attributes of the resultant wines produced from these approaches were inconclusive (Gil et al., 2013), with further studies required.

The expansion and growth of the wine Industry and the challenges it faces due to climate change demand that innovation in wine production is adopted. The approach to water addition/substitution into a high-sugar must, designed to facilitate fermentation, can be the preferred methodology as it is cheap, can have a limited negative impact on sensory attributes, and is less invasive than thermal post-fermentation options for alcohol removal. Coupling ‘water treatment of pre-fermentation wine grape musts’ with ACE may also have further significant positive impacts for phenolic, chemical and sensory compositions of the final wines. ACE may be able to tackle some of the negative impacts on the final chemical and sensory outcomes related to ‘water treatment of wine grape must’ winemaking. It is concluded that further studies for these two winemaking approaches are required to provide winemakers with better datasets on the effects for wine quality parameters. Further research can improve the wine industry’s resilience in

the face of climate change and drought, by improving productivity while maintaining wine quality.

Note: Use of images or other material from Goold, H. D., Kroukamp, H., Williams, T. C., Paulsen, I. T., Varela, C., & Pretorius, I. S. (2017). Yeast's balancing act between ethanol and glycerol production in low-alcohol wines. *Microbial Biotechnology*, 10(2), 264–278 is permitted as it is an open access article ([Creative Commons license CC by 4.0 Deed](#)), and the original work is properly cited.

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Chapter 3 – Are winemaking adjustments to reduce alcohol in wine accepted? A survey of Australian naïve and expert wine consumers

This chapter presents an examination of the results and analysis of two self-administered wine consumer preference surveys. The outcomes of these two surveys offer additional insights into the behaviours and preferences of both expert and naïve wine consumers, enabling wine producers to tailor their production approaches to meet consumer demand more effectively. The two consumer preference surveys from this study set the scene for the quantitative descriptive analysis (QDA) and just about right (JAR) sensory analysis surveys conducted for the later scientific experimental studies of the thesis (presented in **Chapters 4, 5 and 6**).

Abstract

The warming climate across the globe over the past few decades has caused a shift in grape-growing seasons, including in Australia. Grape growers and winemakers are facing challenges as a result, such as shortened grape-growing periods leading to berries with high sugar content. Resultant wines contain higher alcohol levels, together with negative impacts on sensory parameters. One solution to address these challenges in the winery is the recent Australian approach that allows water treatment directly into pre-fermentation musts to facilitate fermentation, resulting in the production of lower-alcohol wines. However, limited research exists on the acceptability among wine consumers of the lower-alcohol wines produced through this winemaking approach. I explore this gap by conducting a brief literature review on the adverse health effects linked to excessive alcohol consumption, and by reviewing previous studies that have examined consumer perceptions of lower-alcohol wines. I then present the results from two self-administered wine consumer preference surveys conducted as part of this study. The study outcomes show that expert wine consumers prioritise quality over other factors and are less concerned about water adjustments during fermentation than naïve wine consumers. In fact, 80% of expert wine consumer participants expressed a willingness to purchase wines that had their alcohol reduced using water dilution. The study also found that while alcohol content and health considerations do affect buying decisions, they do not hold the utmost importance for naïve wine consumers in their preferences. Naïve wine consumers prioritise attributes of personal taste preference and value for money when making wine purchasing decisions. For both naïve and expert wine consumers, the perception of different alcohol reduction techniques indicates a preference for natural early harvest winemaking approaches. The results also indicate that transparency in winemaking practices and clear labelling are integral considerations for naïve wine consumers, reflecting their desire for informed and empowered choices. The outcomes of these two wine consumer preference surveys offer additional insights into the behaviours and preferences of wine consumers, enabling wine producers to tailor their production approaches to meet consumer demand more effectively.

Abbreviations

AU/NZ	Australia and New Zealand
E_i	Expected value
km	kilometres
O_i	Observed value
p	p-value
supp	supplementary

1. Introduction

Overconsumption of alcohol is bad for human health. Misuse of alcohol is the third highest contributor to the global burden of diseases, and the seventh leading risk for premature death (GBD 2016 Alcohol Collaborators, 2018). As early as 2010, the World Health Organization (WHO) implemented a global strategy to reduce the harmful use of alcohol (WHO, 2010). In Australia, one of the leading causes of preventable death has also been related to the misuse of alcohol (AIHW, 2023). More recently, the Australian Institute of Health and Welfare (AIHW) reported that alcohol-induced deaths in Australia increased on average 8.2% per year from 2010–11 to 2019–20 (AIHW, 2023). A few options to deal with these impacts have been implemented across the globe over the last two decades. Options put forward have included strategies to reduce the negative consequences of drinking and alcohol intoxication, as well as the implementation of alcohol policies targeting the overall availability, marketing and pricing of alcohol. In this regard, WHO recognised that ‘reducing the alcoholic strength inside different beverage categories’ was one positive approach that could be implemented by policymakers (Chick, 2011; WHO, 2010, p. 17). The Australian Government’s National Alcohol Strategy 2019–2028 is currently targeting a 10% decrease in alcohol consumption over this period, by focusing on four areas: ‘promoting healthier communities’, ‘improving community safety and amenities’, ‘managing availability price and promotion’, and ‘supporting individuals to obtain help and systems to respond’ (Australian Government Department of Health, 2019) (Figure 3.1).

As part of the managing availability, price and promotion focus area, one option is supply reduction, where lower strength alcohol sale requirements are one of the recommendations put forward (Australian Government Department of Health, 2019).

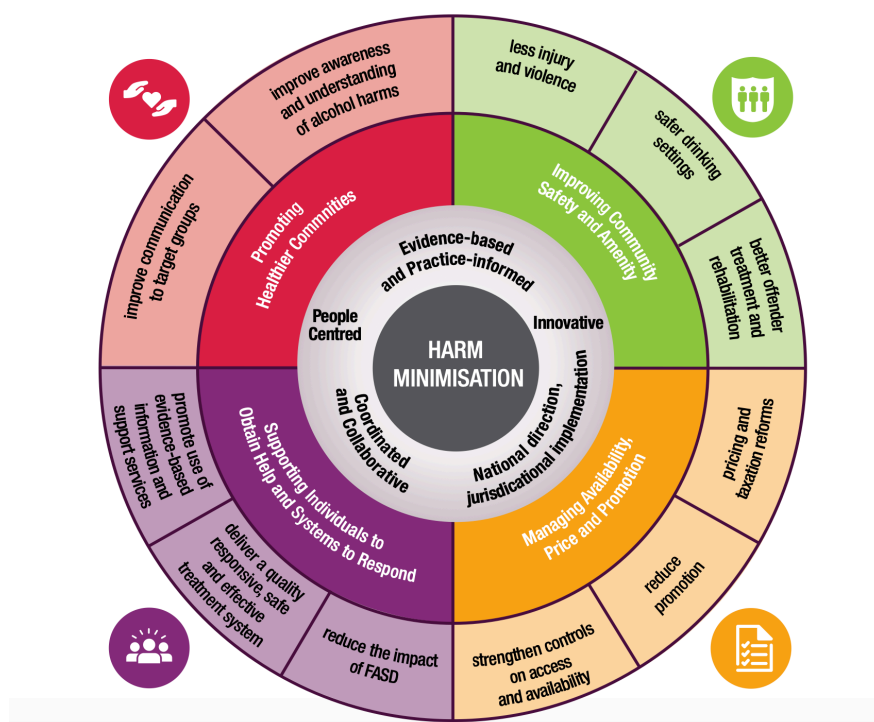


Figure 3.1. The Australian National Alcohol Strategy 2019–2028 at a glance

Source: Australian Government Department of Health, 2019 ([Creative Commons license CC by 4.0 Deed](#)).

The production of and the corresponding consumption of lower-alcohol wines is one strategy that may therefore address the health risks associated with high and harmful alcohol consumption. Indeed, a number of studies collated by the National Health and Medical Research Council [Australia] (NHMRC) illustrate and support the notion that lower alcohol consumption rates can have positive health benefits and reduce mortality rates (Fernández-Solà, 2015; Mumenthaler et al., 1999; NHMRC, 2020; Stockwell et al., 2016). Such a strategy for wine production has been previously supported, where it has been noted that the consumption of low-alcohol wines, or lower alcohol consumption, can actually improve human health (Lähteenmäki, 2004).

The recent recommendations related to positively addressing human health, together with corresponding consumer demand for lower-alcohol wines and regulatory restrictions on high-alcohol wines in export markets, drive winemakers to investigate viticultural and oenological methods to decrease alcohol content in final wines. In addition, the concerns that wine consumption is declining globally drives winemakers to explore the production of alternative wine products that meet current wine consumer demand (Wine Australia, 2023). One ‘easy-to-implement’ approach to producing wines with lower final alcohol

levels is the oenological practice of adding or substituting water in high-sugar musts before fermentation. Water addition treatment of a pre-fermentation wine must is a winemaking process where water is added to must before fermentation, increasing the volume of the finished wine. Water substitution treatment of a pre-fermentation wine must require grape juice being run off from the must, which is then substituted with equal amounts of water (Teng et al., 2020). Of concern, however, is how accepting wine consumers will be of the wines produced, which are lower in alcohol content than those customarily consumed. This is because it can be difficult to decrease alcohol content while maintaining a balance with organoleptic factors, wine flavour and quality (Petrie et al., 2019; Piccardo et al., 2019; Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020), and there is also a perceived aversion by wine consumers to wines that are not naturally produced (Bucher et al., 2020).

Notably, very few studies to date have been conducted to test how consumers perceive and rate low-alcohol wines (Bucher et al., 2018; Bucher et al., 2019). Further, mixed results have been observed in studies conducted to test consumers' willingness to pay for non-standard wines with health benefits. For instance, one study found that healthfulness did not rate highly as a main purchase criterion (Wright et al., 2007). Another study found that wine labels that included health risks on the wine bottles did not have as high an impact on consumer purchasing decisions as price and taste (Higgins & Llanos, 2015). These factors are important to consider when testing low-alcohol wine consumption behaviour.

An additional concern has been how to adequately address consumer perceptions that low-alcohol wines are an inferior wine product, especially for those wines that have had their alcohol content deliberately lowered, regardless of the winemaking methods employed. The early study by d'Hauteville (1993) found that there was a significant consumer perception that lower-alcohol wines (regardless of the methods employed to produce such wines) were an inferior product from an organoleptic point of view. In this early study, modelling samples of naïve consumer preferences with respect to low-alcohol wine consumption with meals were undertaken in the UK, Germany and France. It was found that lower-alcohol wines were perceived to lack body and flavour, with the researchers noting that consumers preferred lower-alcohol wines only where the flavour was appropriate (d'Hauteville, 1993). A later study by Stasi et al. (2014) found that

completely alcohol-free wine generated the most aversion in consumers, whereas other dealcoholisation intensities were less significant. For their study, they evaluated Italian wine consumer preferences, using choice modelling techniques, by reference to dealcoholisation levels, price and wine attributes. Overall, their survey results indicated that only 10% of those surveyed would buy dealcoholised wines, regardless of the final reduced alcohol levels (Stasi et al., 2014).

It is these concerns that this study addresses, by presenting the feedback outcomes and data analysis of two self-administered wine consumer (naïve and expert) preference surveys. The objectives of both surveys were to: ascertain the level of knowledge of wine consumers of the practice of implementing water treatments into a high-sugar wine grape must to affect alcohol levels in a final wine; understand if this winemaking practice leads to the perception of higher or lower quality wines by wine consumers; and to determine what people like in wines. Overall, the surveys aimed to highlight the preferable characteristics of wine quality parameters in lower-alcohol wines for both naïve and expert wine consumers in Australia.

The survey outcomes are particularly important to consider for winemakers, as wine consumers may make alternative choices about what they ultimately consume depending on the variety and treatment of the wine. The wine consumer preference survey data collected from both naïve and expert wine consumers can inform winemakers and their distributors, so that they can adjust their supply and marketing strategies and therefore adopt a systematic approach that considers the heterogeneity of consumer preferences (Thiene et al., 2013).

2. Methods

2.1 Survey methods

Ethics approval for the two consumer preference surveys for this study was provided by the University of Melbourne's Human Research Ethics Committee (No. 24275). A broad selection of both naïve and expert wine consumers across Victoria, Australia, was undertaken. Naïve wine consumers consisted of university wine studies students, and general wine consumers as identified through retail wine outlets in Victoria. Expert wine consumers included wine educators, winemakers, oenologists, wine traders, wine show

judges, viticulturists, or wine industry professionals with 5 years or more of industry experience in Australia.

In this study, power analysis was employed to determine the optimal sample sizes for the two surveys (Hair et al., 2018). Using the G*Power program (Faul et al., 2009) to choose the sample size, the 'F tests' analysis was selected, followed by selecting the statistical test – 'Linear multiple regression: Fixed model, R^2 deviation from zero'. The type of power analysis was then set to 'A-priori: Compute required sample size – given α , power, and effect size'. In the G*Power program, the effect size was selected at 0.15 (medium effect), the significance level α was selected at 0.05, and the power was selected at 0.80. These levels were chosen as they are the most common levels used (Hair et al., 2010). The number of predictors chosen related to the questions posed that did not relate to general information or open-ended questions with free text. In this study, this was 10 for the naïve survey and 9 for the expert survey.

The Qualtrics software tool was used to design, send and analyse surveys online. In this context, the Qualtrics program was used to formulate survey questions and to gather survey participants' feedback via direct email. These surveys were run via email over 4 weeks (early January 2022 – early February 2022), to both groups of survey participants, to enable the maximum number of responses required to be collected. Together, the surveys obtained 104 survey responses overall.

The surveys in this study were used as tools to obtain information from the survey participants. The surveys first asked demographic questions of participants (age, gender, professional level, etc.), so that researchers could be better equipped to understand who the main consumers of the wine products in question were (Barber, et al., 2006). For the naïve wine consumers, the information/data sought covered specific consumer demographics, their tastes, their preferences regarding wines that had their grape musts treated with water, and other responses regarding reduced-alcohol wines. For the expert wine consumers, initial information/data sought included occupation and years in the industry, and for those who are winemakers, information on Australian regulations and practice in the wine industry in relation to water treatments of high-sugar musts. The survey questions were diverse so as to elicit honest, direct, unbiased and efficient answers. The questionnaires for both surveys are included in Appendix 1.

2.2 Statistical approaches

For both surveys, the content-analysed responses from the open-ended questions were added to all other responses in the Qualtrics surveys. All feedback was then transformed into a statistical output to determine which answers were most commonly chosen. Frequency tests were undertaken to determine which answer to each question was most commonly chosen; open-ended data converted to content analysis was also tested for frequency.

Where the same question was asked in both surveys (naïve and expert), responses were subjected to crosstabs chi-square tests to determine whether there were any significant differences between the two groups of survey participants (Rakesh & Singhal, 2015). Converting open-ended data to content analysis is part of this approach. In the tables presenting chi-square results and analysis, the first number in each cell is the observed cell total, that is, the total number of participants in that category. The number in the () brackets is ‘the expected cell total’. The expected counts refer to the projected frequencies in each cell if the null hypothesis is true (i.e. no association between the variables); the expected count for each cell is the product of the corresponding row and column totals, divided by the sample size. The number appearing in [] brackets represents ‘the chi-square statistic for each cell’. The chi-square statistic compares the observed values to the expected values.

The formula relied on for the chi-square statistic is expressed as:

$$\chi^2 = \sum (O_i - E_i)^2 / E_i$$

where O_i = observed value (actual value) and E_i = expected value (Rakesh & Singhal, 2015).

3. Results

3.1 Power analysis and the G*Power program to determine optimal sample size

The G*Power program (Faul et al., 2009) determined that the optimal sample size for the naïve consumer survey was 118 (Supplementary materials: Supp. Figures S3.1 and S3.2) and for the expert consumer survey the optimal sample size was 114 (Supplementary materials: Supp. Figures S3.3 and S3.4). In order to gain the maximum number of

responses to meet the sample size thresholds as set by the power analysis using the G*Power program, 300 emails were sent to targeted naïve wine consumers, and 200 emails were sent to targeted expert wine consumers. Of these, complete survey responses were received from 67 naïve wine consumers (22% response rate) and 37 expert wine consumers (18.5% response rate).

3.2 Survey demographics

The surveys employed in this study determined the preferences and entrenched positions of naïve and expert wine consumers through two steps. The first step was a survey distribution based on the number of optimal survey participants as determined through the G*Power program. Surveys were used to collect primary data from naïve wine consumers throughout Melbourne and wider regional Victoria, and from expert wine consumers working in boutique retail wine outlets, wineries and wine industry organisations across Melbourne and wider regional Victoria.

Table 3.1 indicates where the naïve and expert wine consumer surveys were distributed and the relative populations of Melbourne and regional areas. The number of surveys distributed in each location were relative to the percentage of population (Table 3.1). Of the 67 naïve wine consumer surveys distributed, 45 were in Melbourne, with a further 22 in regional towns. The same proportion applied to expert consumer preference surveys; of the 37 expert wine survey participants, 25 were in Melbourne and 12 were in regional towns (Table 3.2).

Table 3.1. Distribution of the 300 naïve wine consumer surveys and 200 expert wine consumer surveys, according to relative population percentages for Victoria, Australia (Australian Bureau of Statistics, 2021)

Location	Overall Victorian population %	% of naïve surveys distributed	% of expert surveys distributed
Melbourne	5,061,000/6,643,100 = 76.2%	76%	76%
Regional	1,582,100/6,643,100 = 23.8%	24%	24%

Table 3.2. Location of and number of completed surveys collected

Location	Number of surveys collected	Location of respondent
Melbourne	45 naïve; 25 expert	Within 100 km of CBD
Regional	22 naïve; 12 expert	External to 100 km of CBD, in Victoria

3.3 The survey questions and collecting survey answers

The survey questions were designed as both closed and open-ended questions for both naïve and expert wine consumers. Open-ended questions were qualitative, where the answers were categorised into themes so a content analysis could be carried out.

3.3.1 Expert wine consumers are aware of ‘water treatment winemaking’ and are willing to purchase ‘water-treated’ final wines

Experts included representatives from wineries working in the industry, from both Melbourne and regional Victoria. Winemakers from a selection of Victorian wineries were also targeted. For the expert wine consumer survey, initial questions related to the participant’s occupation details and number of years in the industry to determine their level of expertise in wine and the wine industry. If the response indicated less than 5 years expertise, the survey was automatically terminated. There were a final 37 expert wine consumers who undertook the ‘wine industry survey’. Further questions related to their knowledge of wine regulations and their knowledge of water treatment of wines in Australia. These questions were posited to gauge their level of acceptance of this practice and whether they thought it was a positive development for the Australian wine industry. Interestingly, 60% of the survey participants were female, and on average, all consumed wine at least 2 times per week. In response to Question 7 about their awareness of new Australian regulations relating to water addition to wine must pre fermentation as one strategy to produce wine with lower alcohol levels, 57.14% of the expert wine consumer participants indicated that they were aware of the regulations.

Question 8 was then an open-ended question asking: ‘If yes, what is your understanding of the change?’ Content analysis indicated that 11.76% of those who were aware that a change in regulations had occurred understood that it was to facilitate the reduction of alcohol content in the final wines produced, and 35.29% of participants said the change was implemented to lower Baumé levels.

In answer to Question 9, which asked participants their thoughts on whether the addition of water to grape musts can have a positive contribution to final wine products, 60% of participants answered 'yes', 8.57% said 'no' and 31.43% said they were 'unsure' (Figure 3.2).

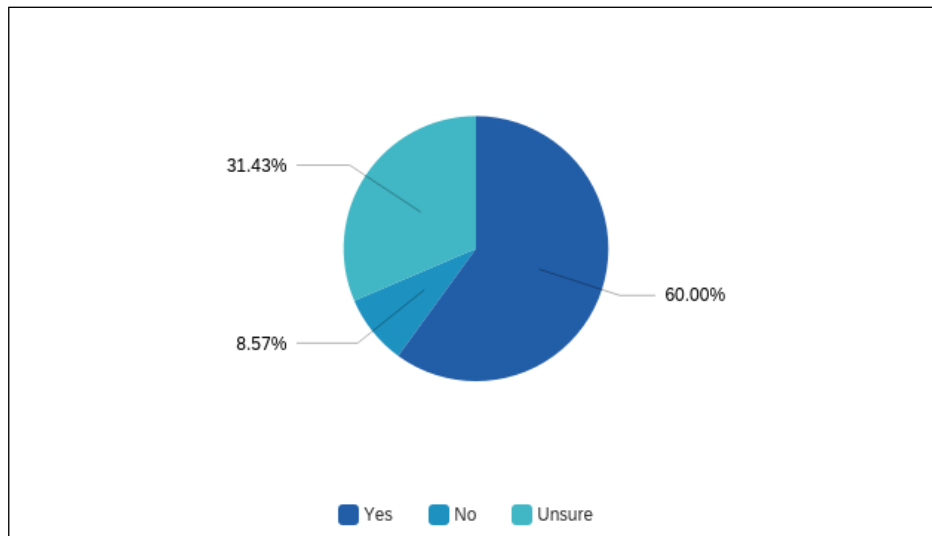


Figure 3.2. Percentage of expert wine consumer participants indicating their position on water addition to grape musts having a positive effect for final wine products

An open-ended question followed, which asked 'Based on your answer to Question 9, explain your reasons'. Content analysis indicated that among those who believed that adding water to grape must could enhance final wine products 10 out of 27 participants thought that this practice could positively influence wines by mitigating alcohol levels in the finished product. 5 of the 27 participants indicated that a more balanced wine could be achieved through this process. Furthermore, 3 of the 27 participants contended that it granted better control over the final product during the winemaking process. One respondent from the same group indicated uncertainty about the effects on colour and dilution.

Of the participants who marked themselves as 'unsure' (4 of the 27 participants who answered this question), these 4 all voiced concerns about the potential misuse of this measure within the industry. They indicated that this practice might lead to the abuse of

dilution to inflate volumes in larger-scale commercial winemaking. Two respondent's comments were particularly illustrative of this viewpoint:

It can be used for its intended purpose of reducing higher alcohol ferments, especially in warmer regions. However, it can also be abused to produce more volume from the same amount of grapes. Legalising it might help control extreme cases of abuse.

And

Large company's exploit this by forcing growers to not irrigate their fruit, threatening to break contract. Winery then will then water fruit back from anything from 18 Baume down to an acceptable level.

Two participants who believed that the addition of water would not contribute positively to the final wine provided detailed explanations. These individuals expressed concerns about the impact of dilution on flavour and aromatics, and one respondent expressed a concern about the process being 'unnatural'.

Sentiment analysis of responses to Question 9 ('Do you think that the addition of water to grape must can have a positive contribution to final wine products?') revealed a wide range of perspectives among the participants, with varying degrees of positivity, negativity, neutrality and mixed viewpoints regarding the potential benefits of incorporating water into grape must for the improvement of final wine products: 25.93% of responses were very positive, 33.33% were positive, 7.41% were neutral, 14.81% were negative, 7.41% were very negative and 11.11% were a mix of both (Figure 3.3).

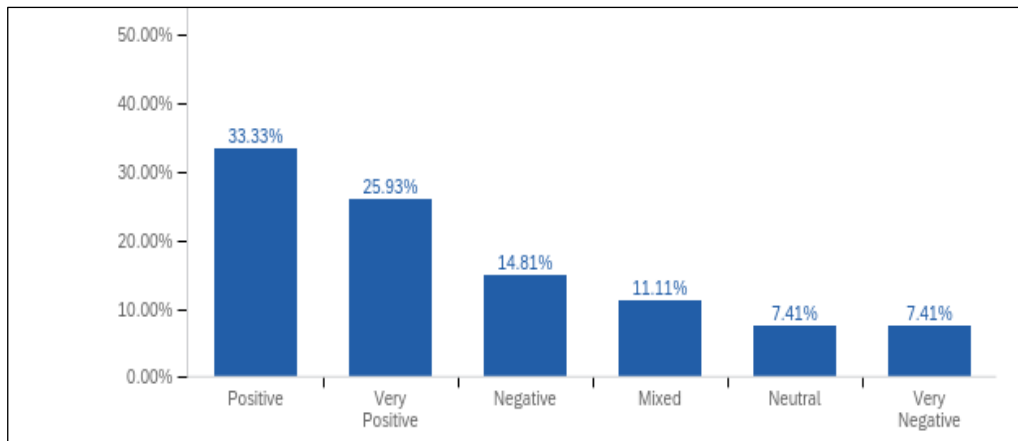


Figure 3.3. Sentiment analysis for expert wine consumers for the question ‘Do you think that the addition of water to grape must can have a positive contribution to final wine products?’

When asked if they had performed or been made aware of any water additions into grape must (Question 11), 71.43% of participants responded, ‘yes’ and 28.57% responded ‘no’ (Figure 3.4). Question 11 was then reposed in Question 12 to ask directly if the respondent themselves had added water to grape must before, with 60% responding ‘yes’ and 40% responding ‘no’ (Figure 3.5).

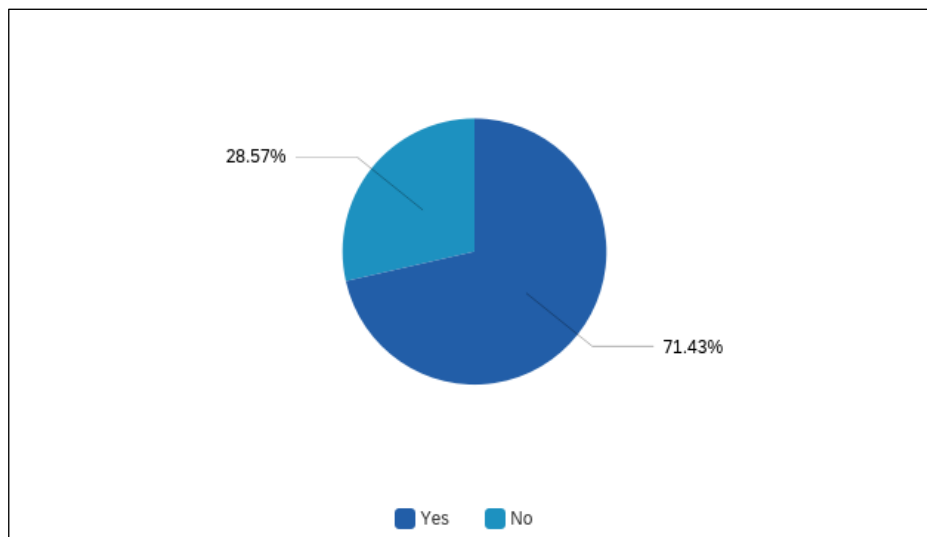


Figure 3.4. Responses to awareness of water additions into grape must for expert wine consumers

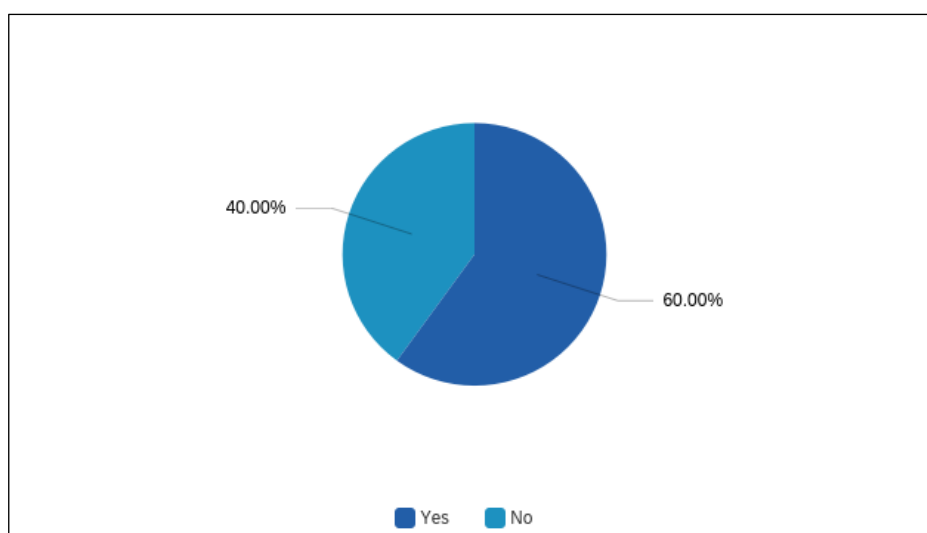


Figure 3.5. Responses for expert wine consumers who have or have not added water to grape must

Another question asked participants ‘Would you purchase a wine that has had its alcohol reduced during the winemaking process using water dilution?’ Here, 80% of all participants replied, ‘yes’ and 20% replied ‘no’ (Figure 3.6).

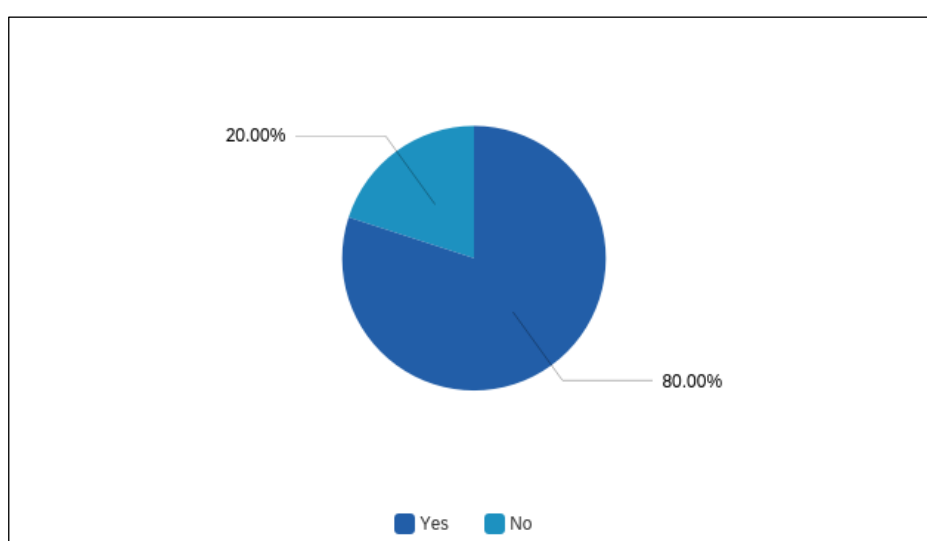


Figure 3.6. Percentage of expert wine consumers who would/would not purchase wine that has had alcohol reduced by water treatment of grape must approaches

Participants were also asked about the significance of regulation in food packaging with regards to the mandatory disclosure of water additions into wine, using a scale from ‘not important at all’ to ‘extremely important’. Here, 45.71% of participants indicated that it was ‘not important at all’, while 14.29% of participants indicated that it was ‘extremely

important’. The remaining 40.01% of participants distributed their responses evenly across the intermediate categories on the importance scale (Figure 3.7).

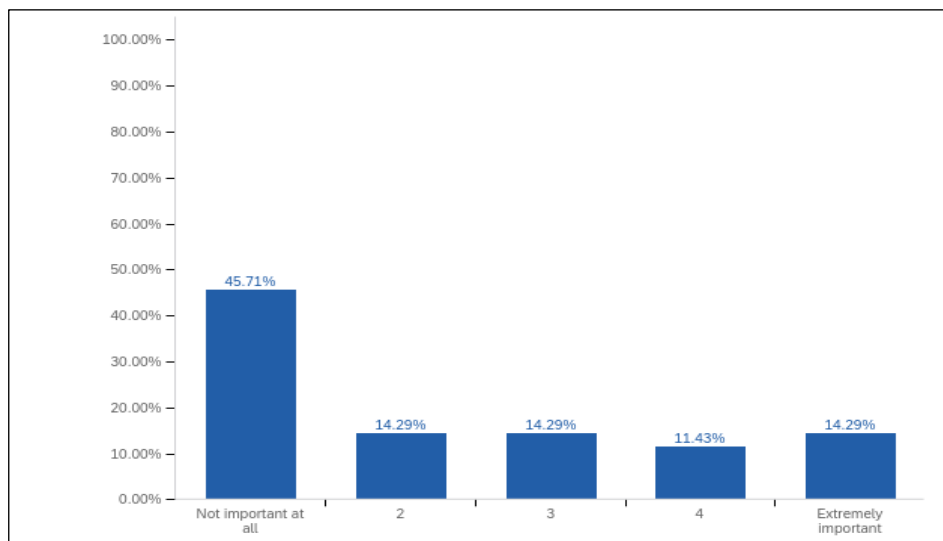


Figure 3.7. Importance of food packaging regulations and wine labelling, including the disclosure of water into grape must winemaking practices, among expert wine consumers

3.3.2 Naïve wine consumers indicate that personal taste and value for money rate highly, while alcohol content and health considerations are less significant

Once a naïve wine consumer commenced the Qualtrics survey, the first question determined if the participant was aged over 18; if they were not, the survey was automatically terminated. A total of 61 final survey participants took part in the naïve wine consumer survey. To begin the survey, several preliminary questions were asked, including the participant’s age and gender, and then more specific questions about how often they drank wine, how much they would spend on average on a bottle of wine, what style of wine they drank, and if there was a region in Australia that they liked to drink wine from. These questions were set up in a manner to determine the participants’ general preferences for wine. Among these participants, the majority fell within the age range of 18 to 24, and a predominant percentage (51.67%) identified as female. The survey revealed that, on average, participants reported a weekly frequency of wine consumption, though the exact quantity consumed was not specified.

Naïve wine consumer participants were asked whether the percentage of alcohol in a wine would affect their purchasing decisions. Among the participants, 58.33% stated that the

alcohol percentage of a wine did not influence their purchasing decision, while 41.6% indicated that it did play a role (Figure 3.8).

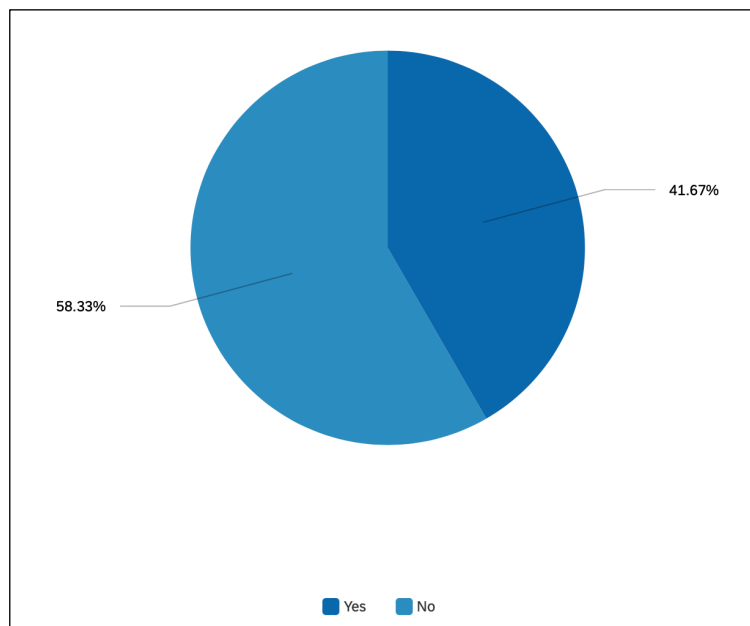


Figure 3.8. Responses to whether the alcohol percentage levels in wine would impact purchasing decision-making for naïve wine consumers

Notably, when asked about whether they believed Australian wines had excessively high alcohol content, an overwhelming majority of 96.67% responded with a negative answer (Figure 3.9).

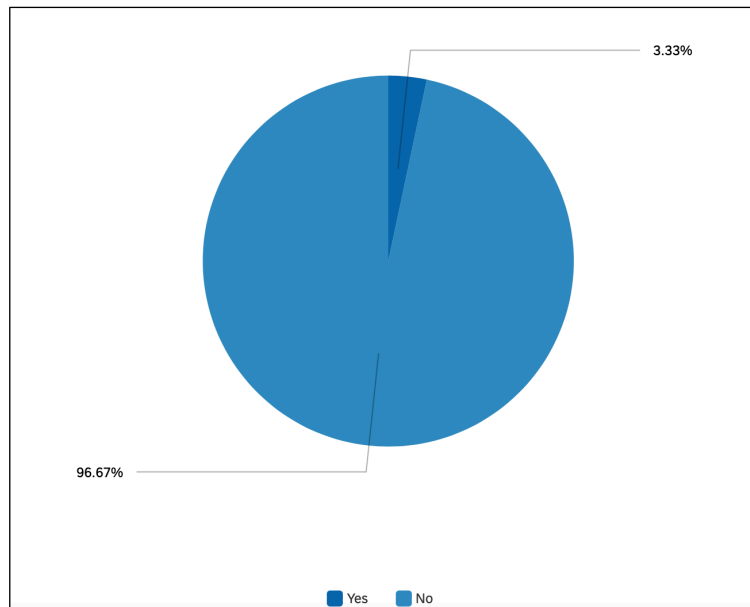


Figure 3.9. Percentage indicator of naïve wine consumers' perceptions of whether wines in Australia have excessively high alcohol content

When asked 'Are you aware that water is a permitted addition into grape juice during the winemaking process since 2017 as per the change in the AU/NZ food standards act?', 38.33% of the participants demonstrated awareness of the fact that water addition is now permitted during the winemaking process. The larger portion of survey participants, comprising 61.67%, were not aware of this change (Figure 3.10).

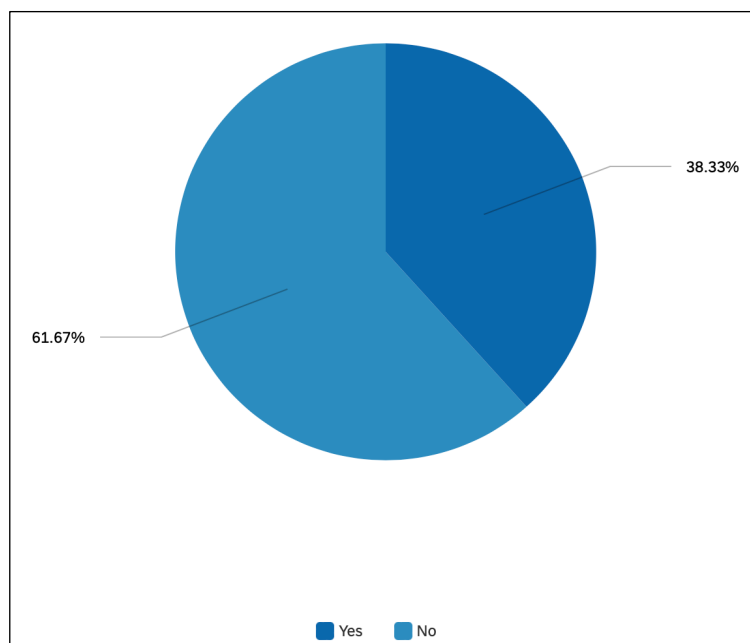


Figure 3.10. Percentage indicator of naïve wine consumers' recognition of the Food Standards Australia New Zealand 2017 regulatory changes for wine production

When questioned about their willingness to purchase a wine that underwent alcohol reduction through the process of water dilution, 53.33% of survey participants responded affirmatively, while 46.67% responded negatively (Figure 3.11). The survey participants were then prompted by an open-ended question to provide reasons for their response. Among those who were inclined to buy wines with diluted alcohol content, 27.05% expressed willingness, provided that the addition of water did not adversely affect the taste or flavour of the wine. In addition, 14.56% of participants expressed an openness to trying this style of wine. Another 6.24% cited trust in the manufacturing process, with one respondent highlighting that the dilution of beverages is already commonplace in the spirits industry. A smaller fraction of participants (2.08%) indicated indifference towards the practice, while the same percentage referenced responsible alcohol consumption. An additional 2.08% of participants perceived the water dilution approach as more natural. Among those not in favour of purchasing wines with diluted alcohol, the concerns were as follows: 27.05% believed that adding water would weaken taste and flavour by diluting the wine; 9.98% indicated concern about the wine's overall quality being compromised; and 7.98% expressed a preference for wines with higher alcohol content.

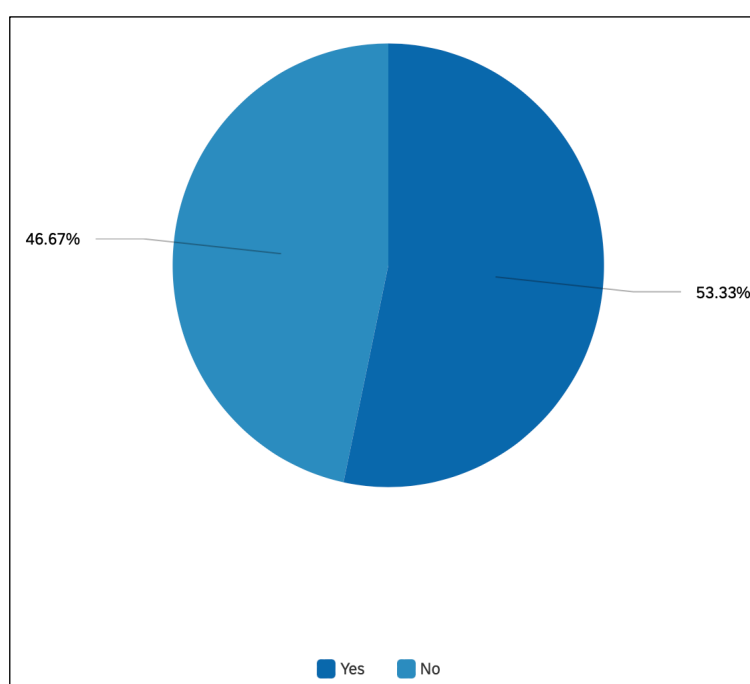


Figure 3.11. Percentage indicator of naïve wine consumers' preference to purchase wine that has had its alcohol levels reduced by the water dilution winemaking approach

The next question required naïve wine consumer participants to categorise their purchase preferences for lower-alcohol wines that have been produced by varying techniques in the winery (Figure 3.12). The most significant preference for purchasing wines that had undergone alcohol reduction was attributed to the ‘early harvest wine’ technique, with 58.62% of participants ranking it as their top choice. The water addition and chemical addition techniques garnered much lower preference, with 18.97% and 22.41% of participants, respectively, ranking these methods as their top choice, indicating these methods as overall participants’ least preferred choices when purchasing alcohol-reduced wines. This pattern of preference was consistent when considering participants’ least favoured options for alcohol-reduced wines. The chemical techniques category was the least preferred, with 46.55% of participants indicating it as their last choice, while 39.66% indicated water addition as their last choice. In contrast, the early harvest wine technique maintained a much higher level of preference, with only 13.79% ranking it as their least preferred option (Figure 3.12).

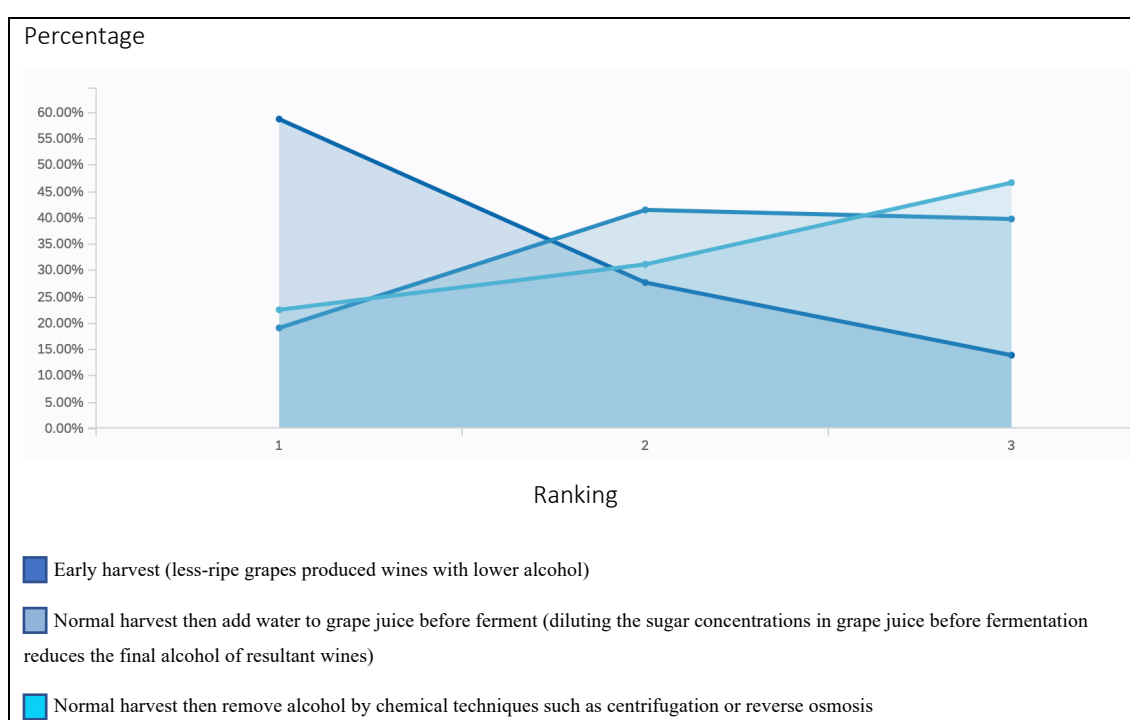


Figure 3.12. Representation of naïve wine consumers’ purchase preference rankings, for lower-alcohol wines that have been produced by varying techniques in the winery

Naïve wine consumer participants were also asked to rate potential attributes – such as health benefits, health risks, alcohol content and calorie intake – on a scale of 1 to 10, where 1 represented the least desirable and 10 the most desirable. The attribute with the

highest mean value was alcohol content, at a mean value of 6.45. Following closely was health benefits, ranked second with a mean value of 5.75. Calorie intake took the third position, with a mean value of 4.78. Lastly, health risks were ranked the lowest, with a mean value of 4.23 (Figure 3.13; Supplementary materials: Supp. Figure S3.5).

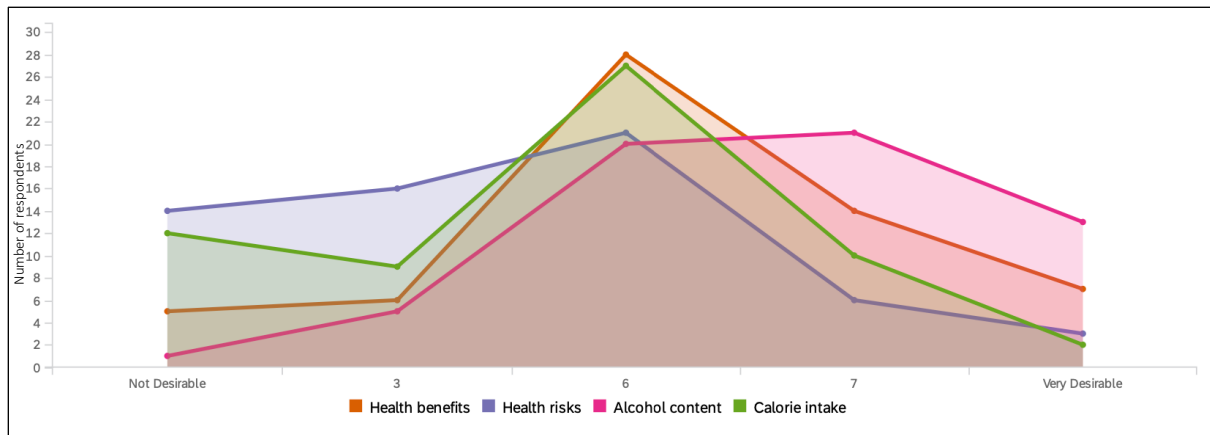


Figure 3.13. Representation of naïve wine consumers' ranking of potential attributes of wine in purchase decision-making

The survey also informed naïve wine consumer survey participants that water can be added during the winemaking process in Australia without any labelling indication, and participants were then asked to consider how important it was for regulation in food packaging regarding the mandatory disclosure of water additions into wine. Overall, 3.33% of participants considered this to be extremely important, 31.67% regarded it as very important, 36.67% deemed it somewhat important, while 15% found it slightly important; 13.33% of participants indicated that it was not important at all (Figure 3.14).

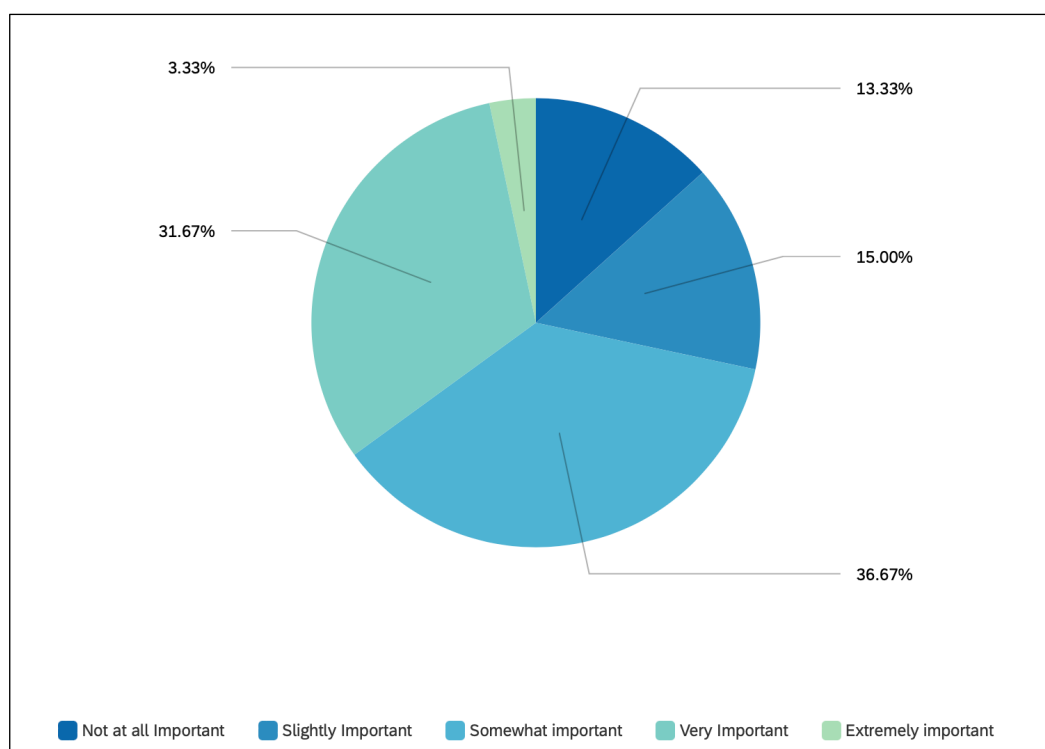


Figure 3.14. Naïve wine consumer perceptions of the importance of regulation in food packaging requiring mandatory disclosure of water additions into wine

The survey also required naïve wine consumer participants to consider a list of wine features that people generally might consider when making purchasing decisions. Participants were asked to rate the desirability of each feature on a scale of 1 to 5, where 1 represented not at all desirable and 5 was extremely desirable (Figure 3.15; Supplementary materials: Supp. Figure S3.6). The results indicated that the highest ranked feature was ‘varietal I like’, with a mean desirability value of 4.10. This was closely followed by ‘good value for money’, which had a mean desirability value of 4.08. These two attributes emerged as the two most important features for naïve wine consumers when making wine purchasing decisions (Figure 3.15; Supplementary materials: Supp. Figure S3.6). In addition, the lowest ranked feature was ‘additives’, with a mean desirability value of 2.22. Similarly, the next lowest ranked feature was ‘alcohol level’, which had a mean desirability value of 2.88 (Figure 3.15; Supplementary materials: Supp. Figure S3.6).

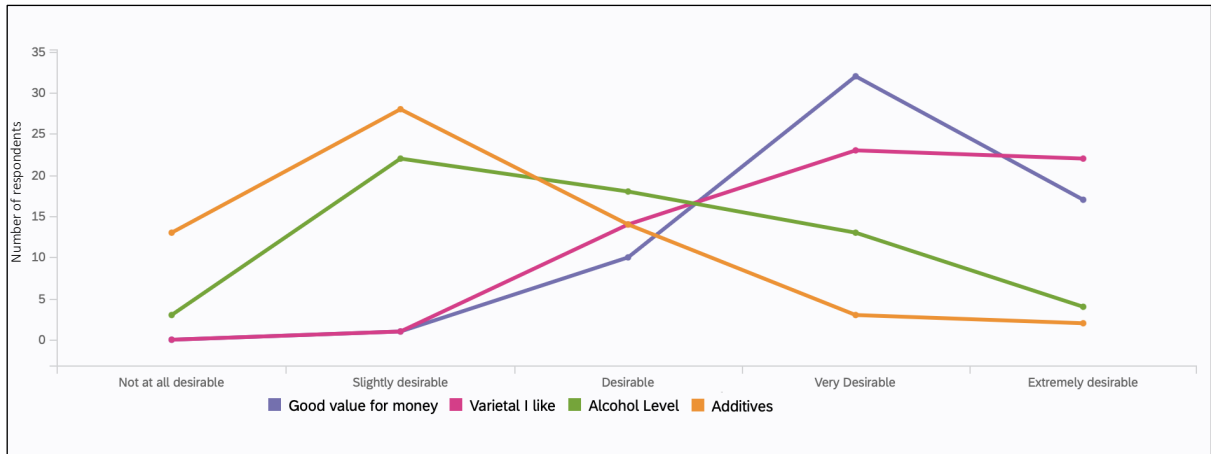


Figure 3.15. Representation of desirability of features of wine naïve wine consumers may look for wine when making a wine purchasing decision

3.3.3 Outcomes of Chi-square tests of independence for the equivalent expert and naïve consumer survey questions

A chi-square test of independence was performed to examine the relationship between the test groups (naïve and expert wine consumer survey participants) and their awareness of the changes to the Australia New Zealand Food Standards Code in 2017 (Australian Government, 2017). A chi-square test of independence showed that there was no significant association between wine expertise and knowledge of the change ($X^2(1, N = 96) = 3.40, p = 0.065$) (Table 3.3).

Table 3.3. Chi-square analysis of expert and naïve consumer preference survey outcomes for the question: ‘Are you aware of the changes in 2017 to the AU/NZ Food Standards Act that allow the addition of water to grape juice pre-fermentation?’

	Yes	No	Row totals
Consumer	23 (27.32) [0.68]	38 (33.68) [0.55]	61
Expert	20 (15.68) [1.19]	15 (19.32) [0.97]	35
Column totals	43	53	96 (grand total)

The chi-square statistic is 3.398. The p-value is 0.065. The result is not statistically significant at $p < 0.05$.

A chi-square test of independence was also performed to examine the relationship between the test groups (naïve and expert wine consumer survey participants) in their willingness to purchase wine that has had its alcohol reduced by water addition. Results showed that there was a significant relationship between the two variables, where naïve wine consumers were less likely than expert consumers to buy wines that have been subject to water dilution, $X^2(1, N = 96) = 6.44, p = 0.011$ (Table 3.4).

Table 3.4. Chi-square analysis of expert and naïve consumer preference survey outcomes for the question: ‘Would you purchase a wine that has had its alcohol reduced during the winemaking process using water dilution?’

	Yes	No	Row totals
Consumer	33 (38.76) [0.86]	28 (22.24) [1.49]	61
Expert	28 (22.24) [1.49]	7 (12.76) [2.60]	35
Column Totals	61	35	96 (grand total)

The chi-square statistic is 6.4406. The p-value is 0.011. The result is statistically significant at $p < 0.05$.

A chi-square test of independence was further performed to examine the relationship between the test groups (naïve and expert wine consumer survey participants) in their sentiment regarding mandatory disclosure of water addition into wine. Results showed that there was a statistically significant relationship between the two variables, where naïve wine consumers attributed more importance to mandatory disclosure than did expert consumers ($\chi^2 (1, N = 96) = 20.94, p = 0.0003$) (Table 3.5).

Table 3.5. Chi-square analysis of expert and naïve consumer preference survey outcomes for the question: ‘Water can be added during the process of making wine in Australia without any indication on the labelling. How important do you feel it is that there should be regulation in food packaging regarding the mandatory disclosure of water additions into wine?’

	Not at all i mportant	Slightly important	Somewhat important	Very important	Extremely important	Row totals
Consumer	8 (15.25) [3.45]	9 (8.90) [0.00]	23 (17.79) [1.52]	19 (14.61) [1.32]	2 (4.45) [1.35]	61
Expert	16 (8.75) [6.01]	5 (5.10) [0.00]	5 (10.21) [2.66]	4 (8.39) [2.29]	5 (2.55) [2.35]	35
Column totals	24	14	28	23	7	96 (grand total)

The chi-square statistic is 20.9439. The p-value is 0.0003. The result is statistically significant at $p < 0.05$.

4. Discussion

The survey approach that was chosen in this study was based on a review of alternative survey methods that could have been employed. Early surveys conducted over the telephone have generally been found not to be representative of the population in question, while self-administered surveys, in which the interviewer is left out of the process, were found to be the best approach (Dillman, 1999). Studies have shown that

this approach can provide for reasoned, thought-out responses, and encourages honesty and minimises bias. In addition, different types of self-administered surveys have been shown not to produce any significant differences in outcomes (Knapp & Kirk, 2003). Ultimately, the most important aspect of self-administered surveys is the questions that are posed to prospective participants, which should aim for spontaneous answers. It was on this basis that self-administered surveys were used for this study and distributed via email to naïve and expert wine consumer participants.

In this study, it was important to first specify a certain location subject of the surveys before testing both naïve and expert wine consumer preferences. It has been noted that the broader the location, the more accurate the results of any survey will be (Kelley et al., 2003). In addition, depending on geography and demographics (including culture, lifestyle and expectations of a wine style), consumer behaviours and preferences will differ across countries, states and regions (Barber et al., 2006). For example, wine is regarded by many French wine consumers as part of their national heritage and has little to do with actual wine consumption (Demossier, 2010). For this study, the demographic area chosen for the consumer preference surveys was Victoria, Australia (population 6.74 million) (Population Australia, 2022). Arguably, this choice was broad enough to provide for reliable survey result datasets. This is because Victoria is a significant Australian wine-producing state, spanning 22 diverse regions, and is Australia's third largest wine grapes producer behind South Australia (SA) and New South Wales (NSW). In 2019–2020, Victoria accounted for 13% of Australia's wine exports, valued at \$2.9 billion, behind SA (64%) and NSW (19%) (Agriculture Victoria, 2021). The size of the population of Victoria provides enough diversity to provide varietal results, and still has an average common interest in wine to provide enough useful information from the survey data collected.

The next important considerations were the sample size and target groups. In this regard, sample strength can be related to an accurate selection of the target groups, rather than just based on size. The range of participants targeted should be broad, while representing an efficient range of the population (Mooi et al., 2018). Collecting sample data is important as it is not possible to collect data from the whole of a population (Kumar et al., 2013). Determining the optimal sample sizes for each of the surveys was therefore important in this study. Prior studies show that the determination of sample size is an

important factor to consider so that the findings of the surveys employed can support realistic conclusions from the research findings (Memon et al., 2020; Sekaran & Bougie, 2010). However, choosing the right sample size and sample selection is a complex challenge (Dattalo, 2008). Indeed, it has been shown that one of the most limiting aspects of many empirical studies is the sample size employed (Uttley, 2019). Based on this approach, survey participants in this current study were carefully selected, and while the number of survey participants may have been considered a small sample of the total population, it can be argued that it could still produce more meaningful data than a blindly selected larger sample of the total population (Hickey et al., 2018).

4.1 Most expert wine consumers will purchase ‘water-treated’ wine, but naïve wine consumers are less likely to do so, preferring early harvest lower-alcohol wine

The general problem statement for both survey studies was the extent to which wine consumers will purchase and/or consume lower-alcohol wines that have been treated by addition/substitution of water to a grape must pre fermentation. The results from the expert wine consumer survey indicated that 80% of participants would purchase such wines (Figure 3.6). For the naïve wine consumer participants, just over half (53.33%) indicated that they would purchase such wines, with 50% of this group highlighting that they would only purchase if wine taste could be maintained (Figure 3.11). A chi-square test of independence indicated that there is a statistically significant relationship between the two variables, where naïve wine consumers are less likely than expert consumers to purchase wines that have been subject to water treatment of their musts (Table 3.4).

There was a diverse range of attitudes from expert wine consumer participants about whether the addition of water to pre-fermentation grape can have a positive contribution to final wine products. Sentiment analysis indicated that approximately 60% of these expert wine participants were either very positive or positive towards the practice (Figure 3.3). A smaller percentage of expert wine consumer participants (22.22%) indicated that did not think it would have a positive contribution to final wine products (negative and very negative) (Figure 3.3). This particular concern has been recognised over the years by many regulating wine bodies from a number of grape-growing regions, where wine standards have been introduced to arguably ‘protect consumer benefits’. Most regulations, however, actually protect producer interests (Swinnen, 2016, 2017). For

example, wine regulations were first introduced in France as a protectionist reaction to international trade and to protect the interests of rich wine merchants and vineyard owners (Meloni et al., 2019). However, many other wine-producing countries have implemented regulations that restrict the use of additives in wine, with the aim of preserving the quality of the final product and to address fraudulent practices such as diluting wine with water to increase production (Galpin, 2006). While this stance may be one foundation for many wine regulations worldwide, the findings of this thesis study suggest that expert wine consumers do not view this issue as a major concern.

Among naïve wine consumers, the ‘early harvest’ winemaking technique emerged as the most favoured, with 58.62% ranking it as their top choice for purchasing alcohol-reduced wines, when compared to wines produced from ‘water treatment’ or removal of alcohol by ‘chemical techniques’ (Figure 3.12). This may be related to only 38.33% of naïve wine consumer participants indicating that they knew about the new changes to wine regulations, which now permit addition of water to grape juice during the winemaking process (Figure 3.10). However, this awareness was significantly higher for expert wine consumer participants (71.43%) who indicated that they had either added water to grape must or were aware of such practices (Figure 3.4), with 60% indicating that they themselves had added water to grape must (Figure 3.5). Though there is a large difference between the two groups in percentage analysis, a chi-square test of independence showed that there was no significantly significant association between wine expertise and knowledge of the ‘water treatment’ winemaking regulatory change (Table 3.3). Notably, the aversion of wine consumers to post-fermentation chemical treatments to remove alcohol has been documented in previous studies (Schmidtke et al., 2012).

Interestingly, the two factors that emerged from the results as the major influences shaping naïve wine consumer choices were ‘varietal I like’ (highest ranked, mean desirability value of 4.10), closely followed by ‘good value for money’ (mean desirability value of 4.0). Interestingly the lowest ranked attribute was ‘alcohol level’ (mean desirability value of 2.88) (Figure 3.15; Supplementary materials: Supp. Figure S3.6). This suggests that while naïve wine consumers do consider alcohol content, it is not the sole or primary determinant of their wine choices. This sentiment is confirmed by the 58.33% of naïve wine consumers who indicated that the alcohol percentage of a wine did not influence their purchasing decision (Figure 3.8), while an overwhelming 96.67% felt

that wines made in Australia did not have alcohol concentrations that were too high (Figure 3.9).

Mandatory nutrition and ingredient labelling for wine has also changed over the years. In 2017, the European Union declared that food and beverage products with less than 1.2% alcohol levels would no longer be exempt from having to provide nutritional and ingredient labelling (European Commission, 2017, p. 58). This new approach is interesting for the wine industry because it has been found that wine consumers generally have little knowledge of what is contained in wine (Grunert et al., 2018). A more recent study by Pabst et al. (2021) found that wine consumers would only be interested in wine ingredient lists if they were short. Italian wine consumers rated attribute importance for listing ingredients on wine labels at 4%, with Australian and German wine consumers rating it at less than 1% importance (Pabst et al., 2021). Interestingly, the findings in our study also show that over 40% of expert wine consumers consider that regulation in food packaging with regard to the mandatory disclosure of water additions into wine is not important at all to them, with only 14.29% indicating that it was extremely important (Figure 3.7). However, 35% of naïve wine consumers felt disclosure was either very important or extremely important (Figure 3.14). These observations were confirmed by the chi-square test of independence undertaken to examine the relationship between test groups (naïve and expert wine consumer survey participants) in their sentiment regarding mandatory disclosure of water addition into wine. This test showed that there was a significant relationship between the two variables, where naïve wine consumers attributed more importance to mandatory disclosure than the expert wine consumers (Table 3.5).

5. Conclusion

Adaptive oenological approaches mean that winemakers can produce wines with lower alcohol levels in the winery. These wines not only can address consumer preferences for lower-alcohol wines, but also can have positive health benefits overall. The purpose of this study was two-fold: first, to explore survey participants' perceptions of low-alcohol wines, and second, to investigate participants' willingness to purchase and consume low-alcohol wine. Interestingly, the findings highlight that for naïve wine consumers, while alcohol content and health considerations do play roles in their preferences and willingness to purchase a wine, these features do not hold the utmost importance. Importantly it was found that naïve wine consumers prioritise attributes like personal taste

preference and value for money when making wine purchasing decisions. These two attributes were consistently rated as highly desirable, underscoring their pivotal roles in shaping consumer choices. In addition, for naïve wine consumers, the negative perception of different alcohol reduction techniques indicates a preference for natural and transparent approaches. This finding was also in line with expert wine consumers, who indicated that they were less trusting of the more invasive alcohol thermal treatments, favouring the less-invasive pre-fermentation approaches to lowering final wine alcohol levels in the final wine product. Transparency in winemaking practices and clear labelling is integral for naïve wine consumers, reflecting their desire for informed and empowered choices. Knowing what consumers expect from water-treated wines with respect to final wine attributes, including alcohol levels, can provide winemakers with the justifications required to adjust their winemaking approaches. In addition, not only will this research give winemakers an appreciation of consumer preferences, it will also determine what information is important to have made available to the broader global wine industry.

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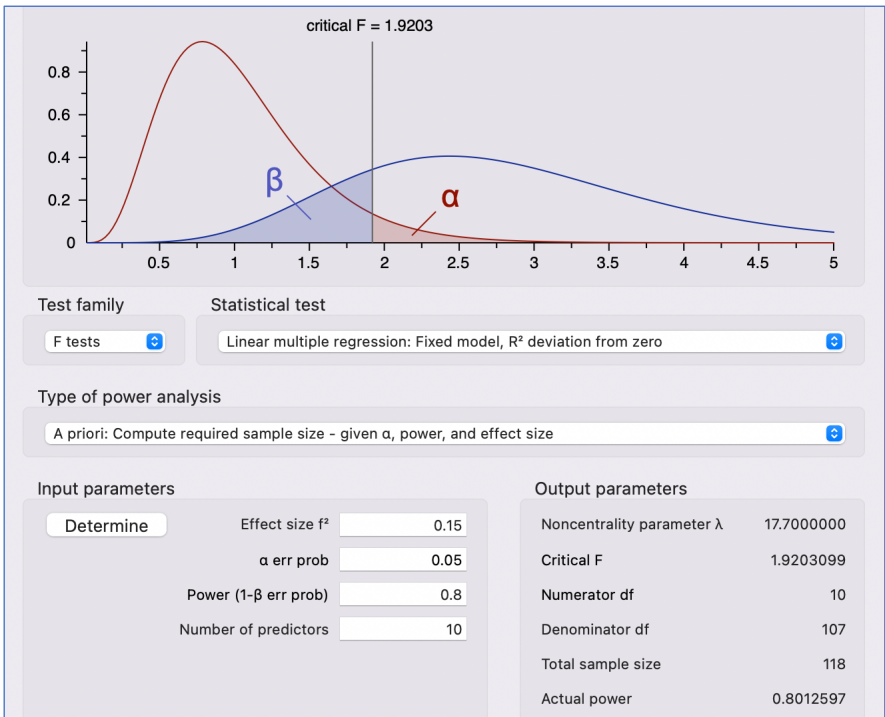
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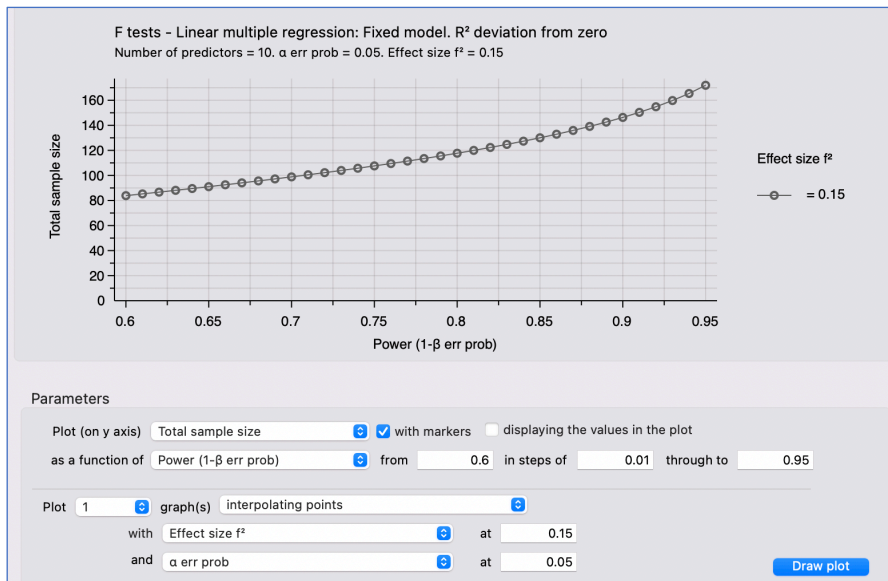
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Chapter 3 Supplementary materials

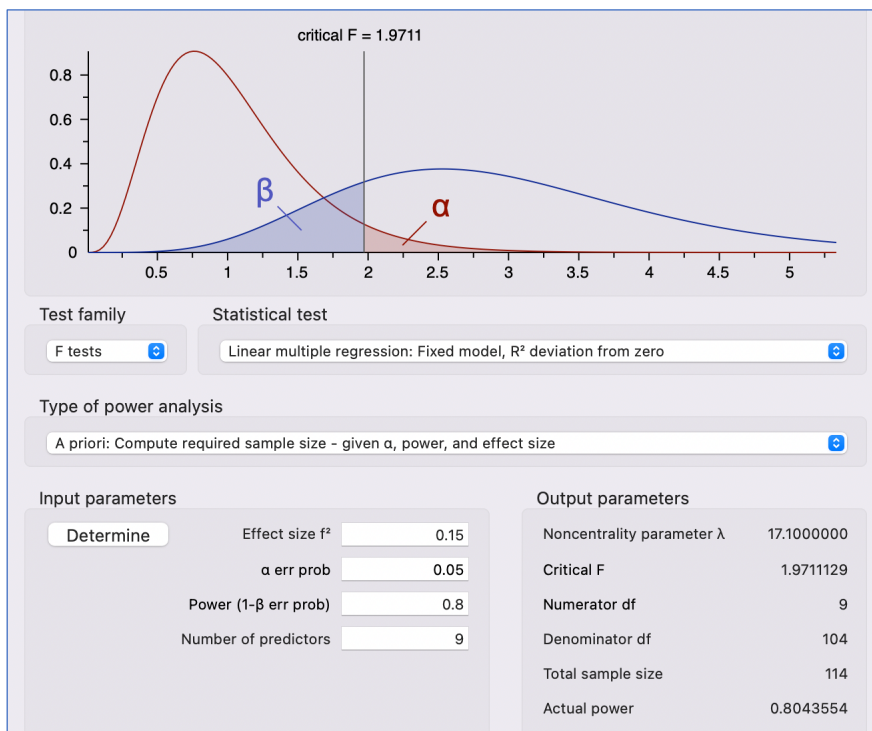


Supp. Figure S3.1. Naïve consumer sample size G*Power outcome

Power analysis, using a linear multiple regression model, Fixed model R² deviation from zero, to compute required sample size – given α (err prob), power ($1-\beta$ err prob) and effect size. (Faul et al., 2009).

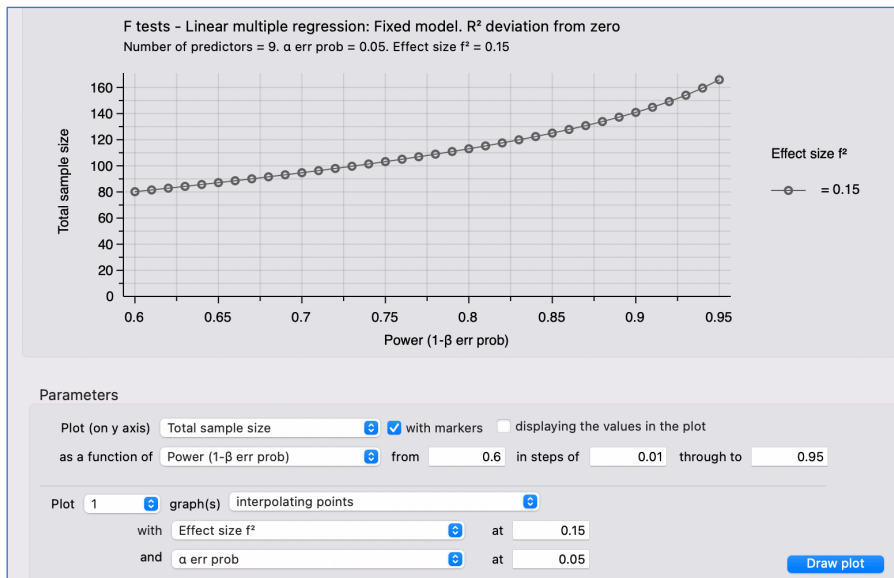


Supp. Figure S3.2. Linear representation of naïve consumer sample size at 80% power. (Faul et al., 2009).



Supp. Figure S3.3. Expert consumer sample size G*Power outcome.

Power analysis, using a linear multiple regression model, Fixed model R^2 deviation from zero, to compute required sample size – given α (err prob), power (1- β err pro) and effect size. (Faul et al., 2009).



Supp. Figure S3.4. Linear representation of expert consumer sample size at 80% power (Faul et al., 2009).

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	Health benefits	1.00	8.00	5.75	1.90	3.62	60
2	Health risks	1.00	8.00	4.23	2.33	5.41	60
3	Alcohol content	1.00	8.00	6.45	1.48	2.18	60
4	Calorie intake	1.00	8.00	4.78	2.27	5.17	60

Supp. Figure S3.5. Naïve wine consumer ranking of potential attributes of wine in purchase decision-making

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	Brand I have tasted before	1.00	5.00	3.55	0.96	0.91	60
2	Good value for money	2.00	5.00	4.08	0.71	0.51	60
3	Varietal I like	2.00	5.00	4.10	0.81	0.66	60
4	Alcohol Level	1.00	5.00	2.88	1.02	1.04	60
5	A complement to food	1.00	5.00	3.27	0.93	0.86	60
6	Attractive label	1.00	5.00	3.07	1.01	1.03	60
7	Region of origin	1.00	5.00	3.18	0.97	0.95	60
8	Produced sustainably	1.00	5.00	3.32	1.02	1.05	60
9	Organically grown	1.00	5.00	2.62	0.98	0.97	60
10	Additives	1.00	5.00	2.22	0.95	0.90	60

Supp. Figure S3.6. Indicators of wine features that naïve wine consumers may consider when making wine purchasing decisions

Chapter 4 – Lower levels of water addition to a late harvest Shiraz wine grape must can maintain favourable ‘fruity’ sensory characteristics in a final wine

The scientific experimental part of this thesis study begins in this chapter. It commences with the investigation of *Vitis vinifera* L. Shiraz wines produced from grapes harvested early 13.5 °Baumé (Bé), middle 14.5 °Bé and late 15.5 °Bé for the vintage years of 2020 (Year 1) and 2021 (Year 2). Volatile aroma profiles and sensory outcome measures for these wines are compared with those of water-treated wines that have had their later Bé harvest musts treated with water addition or substitution of juice with water (bleed and replace). Sensory analysis is conducted using two methods, just about right (JAR) and quantitative descriptive analysis (QDA), both for expert wine consumers. The ‘experts’ add an extra dimension to the results, given that they have undergone continuous prior training in wine tasting, during which they have sharpened their expertise in discerning fine nuances in taste and smell of wine over many years. The JAR and QDA survey outcomes complement each other and together they can give more nuanced outcomes. There is a lack of current research that explores wine aroma profiling together with QDA and JAR sensory analysis to determine final wine quality attributes for the Shiraz cultivar whose pre-fermentation wine grape musts have been subject to water treatment predominately to produce wines with lower final alcohol levels. This chapter’s findings address these gaps and contribute further to the existing scientific knowledge in this area.

Abstract

Water treatment is an easy-to-implement winemaking approach to manage wine alcohol levels. However, there is limited research on how pre-fermentation water addition affects volatile aroma profiles and how the changes in the concentrations of these compounds correlate with sensory attributes of wines. In this study, the volatile aroma profiles of Shiraz wines produced from grapes harvested early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé for the vintage years of 2020 and 2021 were first tested. The volatile aroma profiles and sensory outcome measures of these wines were then compared with those of water-treated wines that had their later Bé harvest musts treated with water addition or substitution of juice with water (bleed and replace). Gas chromatography–mass spectrometry (GC–MS) were used to isolate and analyse free volatile compounds for each treatment group wine. By clustering 43 volatile aromas into 11 compound groups we show that both harvest timing and alternative water treatments yield lower wine alcohol concentrations and influence the volatile composition of the resultant wine. We linked these results to sensory data gathered from expert wine consumer panellists through quantitative descriptive analysis (QDA) and just about right (JAR) surveys to define the sensory aroma profiles of each tested wine. The JAR sensory results indicate that many fruity aromas, flavours and mouthfeel profiles were least preferred for early 13.5 °Bé wine. This is confirmed by the QDA outcomes. This preference is attributed to lower concentrations of volatiles, such as ‘higher alcohols’, ethyl esters of branched acids, isoprenoids and C6 compounds in this early harvest wine. JAR outcomes also show that the late 15.5 °Bé wine had the highest rating for dark fruit smell, which may be attributable to higher concentrations of esters and higher alcohols. However, this wine was least preferred for flavour intensity, which was considered ‘too high’ by our panellists. This may be attributed to lower concentrations of acids, in particular octanoic acid, which can limit the addition of fruity notes. Interestingly, we show that most fermentation-derived compound groups do not decrease with lower established alcohol levels, regardless of the water treatment of must approach implemented. Notably, the approach to reducing ethanol production during fermentation by adding less water to the late 15.5 °Bé harvest must, to produce a wine with a middle of the range 14.5% alcohol by volume (ABV), was most effective in maintaining many positive flavonoid parameters associated with ethanol. Sensory attributes were also less affected by lower levels of water treatment in a high-sugar must. JAR sensory outcomes show that the late diluted 14.5 °Bé

wine was most preferred for most sensory parameters, including colour intensity, odour intensity, red fruit taste, dark fruit taste, liquorice and chocolate flavours, and flavour intensity. These preferences correspond to QDA sensory observations. While isoprenoid group concentrations increased to similar levels for all water treatments, higher concentrations of the isoprenoid beta(β)-damascenone in the late diluted 14.5 °Bé wine may contribute to its higher ‘fruity’ characteristics. This study provides winemakers with additional knowledge to make informed decisions about water treatment of pre-fermentation must techniques to produce wines that are favoured by wine consumers.

Key words: fermentation, water treatment, volatile aroma compounds, expert wine consumer panellists, QDA, JAR, wine quality

Abbreviations

ABV	alcohol by volume
Bé	Baumé
cm sec ⁻¹	centimetres per second
GC-MS	gas chromatography-mass spectrometry
g	gram
HAA	higher alcohol acetate
hL	hectolitre
JAR	just about right
kg	kilogram
K ₂ O ₅ S ₂	potassium metabisulphite
L	litre
mg	milligram
min	minutes
mL	millilitre
mL min ⁻¹	millilitres per minute
mm	millimetre
m/z	mass divided by charge number
ppm	parts per million
QDA	quantitative descriptive analysis
SO ₂	sulphur dioxide
v/v	volume by volume
μL	microlitre
μm	micrometre

1. Introduction

In the last two decades, global warming and rising temperatures have caused wine to be produced with higher alcohol and pH levels due to higher sugar levels in wine musts (Petrie & Sadras, 2017; Schultz, 2010). Higher growing season temperatures decrease the intervals between veraison and grape maturity, making earlier harvest dates more common, and consequently leading to a more frequent production of higher-alcohol and unbalanced wine (Zamora, 2014). Higher levels of ethanol in a wine must can delay fermentation, increase volatile acidity and alter various sensory attributes in the final wine (Bindon et al., 2019; Teng et al., 2020). Additionally, there has been an increase in the demand for lower-alcohol wine due to the adverse effects that high alcohol consumption can have on human health (Saliba et al., 2013). Alcohol is ranked as the seventh leading risk for premature death, with no amount of alcohol consumption being deemed safe (GBD 2016 Alcohol Collaborators, 2018). It is these concerns that face winemakers, who need to find adaptive and alternative winemaking approaches to reduce alcohol content while still maintaining organoleptic factors, wine flavour and wine quality in wines produced.

As one approach available in the winery, Australia introduced an approved regulatory measure in 2017 that now allows for ‘water treatment’ of high-sugar musts to a regulated 13.5 °Bé level (Australian Government, 2017). As previously noted, water treatment of wine musts can involve water addition or substitution. Water addition (dilution) refers to directly adding water to the wine must pre fermentation, increasing the volume of the finished wine (Teng et al., 2020). Alternatively, water substitution (bleed and replace) involves removing grape juice from the must pre fermentation and substituting it with an equal amount of water (Teng et al., 2020). While reducing final alcohol levels in wine has been shown to be successful for both these techniques, the impacts on wine volatile aromas and correlated sensory attributes of final treated wine produced is lacking, especially for Shiraz.

Limited recent studies for Shiraz demonstrate that final wine alcohol levels, wine aroma volatile concentrations and sensory parameters for water-treated Shiraz wine are dependent on harvest time and the type of water treatment involved (Schelezki, Antalick, et al., 2020; Teng et al., 2020). While the water addition/substitution into pre-fermentation musts approaches can be an effective strategy to address high sugar in musts,

and to reduce alcohol levels in a final wine, further research is required that can be more readily translated into commercial applications. This study expands on previous studies by testing Shiraz wine produced from three different harvest times – early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé – across two vintages (2020 [Year 1] and 2021 [Year 2]). Testing was also performed for Shiraz wine produced from the later harvests, subjecting them to varied water treatments of their musts before fermentation. The final wines were then evaluated (in triplicate) for their chemical aroma compositions and sensory characteristics.

This thesis study focuses on the Shiraz cultivar, as it is recognised as the most-planted red grape variety in Australia and is grown in both warm and cool climates (Schelezi, Deloire, & Jeffrey, 2020; Wine Australia, 2023). In 2023, Shiraz continued to be the largest red wine grape variety grown in Australia, making up 26% of all red wine grape cultivars grown (Wine Australia, 2023). Any winemaking approach taken to reduce final alcohol levels in Shiraz wine, while still maintaining Shiraz wine quality, thus has a high economic impact for both winemakers, wine consumers and export markets. The results from this study can allow for winemakers to make informed decisions as to which water treatment approach is most preferred by expert wine consumers in the wine industry. This is important as expert wine consumer judgements can influence the reputation of a wine, and ultimately its commerciality.

2. Materials and methods

2.1 Vineyard and vintage conditions

2.1.1 *Vintage conditions*

The study was undertaken across the 2020 and 2021 vintage years. Temperatures and rainfall data from Yarrawonga weather station (located 45 km from Dookie, Victoria; Station number: 081124, Victoria; latitude: 36.03°S; longitude: 146.03°E; elevation: 129 m) and Numurkah weather station (located 33.7 km from Dookie, Victoria; Station number: 80101, Victoria; latitude: 36.09°S; longitude: 145.45°E; elevation: 105 m) (Australian Bureau of Meteorology, 2023a, 2023b) were relied upon and used to calculate the Heliothermal Index of Huglin (HI) (Huglin, 1978) according to standard measures (Tonietto & Carbonneau, 2004; Supplementary materials: Supp. Tables S4.1–S4.5).

2.1.2 Grape sampling

The Shiraz grapes for 2020 and 2021 vintages were sourced from the Tallis Winery (195 Major Plains Road, Dookie, North East Victoria; 36°21'08.2"S 145°45'37.0"E). For each vintage and for the three harvest times – early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé – fruit was selected at random from panels along the length of each row, and hand-harvesting of 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 sampling of 1.0 kg of 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.

2.2 Winemaking and fermentation

Identical winemaking approaches were implemented for both 2020 (Year 1) and 2021 (Year 2) vintage years. The vines were irrigated in both growing seasons, which supplemented rainfall to about 30% for both vintages. The ‘drip/trickle’ system was used for irrigation, 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 mitigate fungal diseases, including Botrytis rot or grey mould, and spraying for grapevine rust mites were implemented in both vintages (Bernard et al., 2005; Richard Tallis, personal communication, 2021 January 11).

For each vintage, there were seven sample groups (in triplicate) of 100 kg of fruit (21 samples in total), which each produced approximately 65 L of wine; therefore, a total of 1365 L of wine (more due to dilution of ferments) was produced for each year. All grape must treatment sample groups were subjected to crushing through rotary roller crushers set at 8 mm spacing before being separated into their own fermentation tanks/treatment group. Each of the seven treatment groups (in triplicate) were fermented in a 225 L plastic food-grade barrel (21 in total), and were assigned their own identifiers. The first three sample groups were the early 13.5 °Bé harvest (early 13.5 °Bé), the middle 14.5 °Bé harvest (middle 14.5 °Bé) and the late 15.5 °Bé harvest (late 15.5 °Bé) ferments (all in triplicate).

The middle 14.5 °Bé and late 15.5 °Bé harvest musts were also subject to varied water treatments pre fermentation. Two water treatment approaches were taken for the middle 14.5 °Bé and late 15.5 °Bé harvest musts: (i) water was directly added to the must, which altered the solids to liquid ratio (dilution); and (ii) juice from the must was substituted for water, with no change to the solid to liquid ratio (bleed and replace) (Figure 4.1). Deionised water was used for all water treatments. The additional four sample treatment groups (in triplicate) were: the middle 14.5 °Bé harvest subject to water dilution treatment to lower 14.5 °Bé to 13.5 °Bé (+ 5.3 L water) (middle diluted 13.5 °Bé); the late 15.5 °Bé harvest subject to water dilution treatment to lower 15.5 °Bé to 14.5 °Bé (+ 3.49 L water) (late diluted 14.5 °Bé); the late 15.5 °Bé harvest subject to water dilution treatment 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 (− 9.13 L juice and + 9.13 L water) (late ‘bleed and replace’ 13.5 °Bé). The water addition to must winemaking approach was undertaken according to Australian regulations (Standard 4.5.1; Australian Government, 2017). The volume of water added was calculated using the Australian Wine Research Institute (AWRI) online water addition calculator (AWRI, 2023). Final alcohol levels were measured by standard measures, where Baumé = 1.8 Brix = 18 g/L sugar = 1% potential alcohol (AWRI, 2021; Iland et al., 2004).

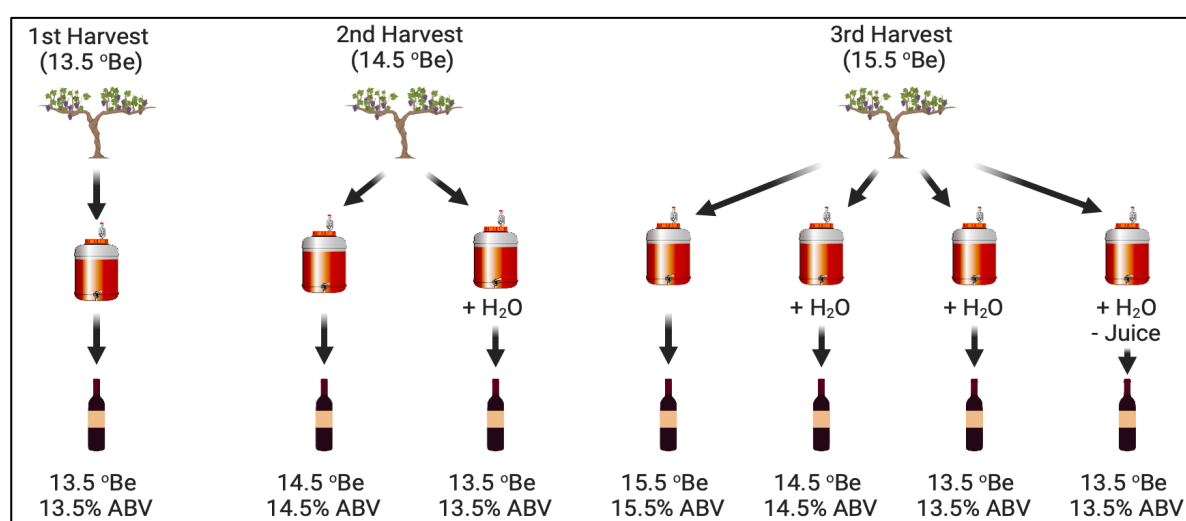


Figure 4.1. Winemaking treatments implemented for each harvest. Source: Xynas et al., (2024) accepted for publication *OENO One*, also presented in Chapter 5.

Bé levels are correlated to final alcohol by volume levels (% ABV) (Iland et al., 2004).

A standard 50 ppm Sulphur Dioxide (SO₂) (as *Potassium metabisulphite*, K₂O₅S₂, Australian Home Brewing, Victoria, Australia) was added to all samples at crush, and after water treatment for the latter four sample groups (in triplicate). Inoculation with Vitilevure Syrah YSEO™ (*Saccharomyces cerevisiae* var. *cerevisiae*; Danstar, Rhone Valley, France), a commercial culture of yeast, was then applied at 30 g/hL. After 24 hours, all fermentations were further inoculated with Martin Vialatte Reflex Malo® 360 (*Oenococcus oeni*; Martin Vialatte, Magenta, France) lactic acid bacteria at 1 g/hL, to carry out co-malolactic fermentation. All fermentations were maintained on skins for 5 days, with the use of refrigeration to maintain temperatures between 20 °C and 30 °C, before being pressed. Punch downs were implemented twice daily to homogenise fermentations. Musts all finished primary alcoholic fermentation on day 5. Acid additions were made post pressing on day 5 to target a titratable acidity of 6.5 g/L tartaric acid (Australian Tartaric Products, Victoria, Australia). Twice-daily samples of 100 mL from each treatment group were taken during the fermentation process, which tracked temperature and Baumé (Supplementary materials: Supp. Tables S4.6, S4.7, S4.8 and S4.9). An additional 50 ppm SO₂ (as *Potassium metabisulphite*, K₂O₅S₂, Australian Home Brewing, Victoria, Australia) was added 21 days after fermentation prior to bottling wines in 750 mL glass wine bottles. All wines were labelled with their identifiers and matured at an average temperature of 18 °C for approximately 18 months.

2.3 Wine volatiles using gas chromatography–mass spectrometry

In July of 2021 and August of 2022, approximately 18 months after bottling for the 2020 and 2021 vintages, respectively, samples of wines were taken from each treatment group. For each vintage, there were seven treatment groups in triplicate, resulting in a total of 21 individual samples. To ensure minimal compositional variations between the sensory and analytical replicates, wine samples were assessed, extracted and analysed in random order during the same period in each testing year.

For both testing years, gas chromatography–mass spectrometry (GC–MS) analysis was performed using a 7890A gas chromatograph (Agilent, Santa Clara, CA, USA). An Agilent 5975C mass spectrometer was used for the identification and quantification of analytes. According to standard protocols as outlined in previous studies (Schelezki, Deloire, & Jeffrey, 2020), wine sample assays were prepared with a 1:2 dilution with MilliQ water to a final volume of 10 mL for each sample. Sodium chloride (3 g; Hanna

Instruments, Australia) was added to each SPME sampling vial (20 mL), to which the diluted wine samples were then added. Following this, 10 μ L of a standard solution was added. The standard solution had been prepared with d13-hexanol (920 mg/L), d11-hexanoic acid (930 mg/L), d16-octanal (82.1 mg/L), d5-ethyl nonanoate (9.2 mg/L) and d3-linalool (1.73 mg/L).

For GC–MS analysis, 1 μ L of extract from each sample was injected into the Agilent 7890A gas chromatograph fitted with a ZBWax column (30 m $\times$ 0.25 mm i.d. and film thickness 0.25 μ m; Phenomenex, Sydney, Australia) using a Gerstel MPX autosampler (Linthicum, DA, USA) with a Peltier tray cooler at 4 °C. The injector temperature was 240 °C, septum purge flow was 3 mL min⁻¹, and the split ratio was 10:1. Using helium as a carrier gas (Ultrahigh Purity; Air Liquide, Adelaide, Australia), the helium flow rate through the column was 1.5 mL min⁻¹, with an average velocity of 31 cm sec⁻¹; the column temperature was held at 40 °C for 5 minutes, then increased at 2 °C per minute to 210 °C and held for 20 minutes. Using an Agilent 5975C mass detector operating in electron ionisation mode, scanning from 35 to 350 m/z with a detection threshold of 100, mass spectra were collected at a scan rate of 4.4 per second. The detector was switched off between 6.50 and 8.70 minutes during efflux of solvent. Source temperature was 240 °C, transfer line temperature was set to 220 °C, and quadrupole temperature was 140 °C. Total elution time during which mass spectra were collected for each sample was 120 minutes.

2.4 Just about right (JAR) and quantitative descriptive analysis (QDA) surveys

Ethics approval was provided by the University of Melbourne's Human Research Ethics Committee (HREC No. 11684) for the JAR and QDA expert wine consumer sensory testing surveys. Sensory testing was conducted for all the final wines produced for both 2020 (Year 1) and 2021 (Year 2) vintages. Panellists were first asked for general background information; all had least 5 years of experience working in the wine industry, and those that participated were considered experts based on previously determined criteria, including superior olfactory recognition and perceptual skill (Parr et al., 2004). The expert wine consumers chosen to participate were drawn from wine educators, winemakers, oenologists, wine traders, wine show judges, viticulturists and a number of expert wine lovers.

For the QDA and JAR surveys, data was collected from sensory panels of expert wine consumers in July 2021 (25 panellists for the 2020 study) and in June 2022 (16 panellists for the 2021 study). The QDA and JAR sensory analyses followed the consensus-based approach as set out in previous literature (Lawless & Heymann, 2010) and consisted of two training sessions and one formal session.

Panellists were provided with an introductory baseline session to inform them of the process, requirements and risks associated with the QDA and JAR surveys prior to commencing the combined surveys. The training sessions led to the development of the final attribute list, based on visual, aroma, flavour, mouthfeel and aftertaste sensory attributes of the wines sampled. For purple, red and colour intensity parameters, panellists were provided with a chart of red colours, which displayed reds from light to dark red to purple. For opacity, aroma intensity, odour complexity, flavour intensity, length, fruit aftertaste and sour aftertaste, panellists were engaged in repetitive training sessions. Other sensory attributes were measured against a reference sample standard of 30 mL of Shiraz wine (Table 4.1).

Samples of wines were presented to panellists in 30 mL aliquots in coded ISO standard wine glasses, during daylight hours. Each panellist was presented with seven wine sample groups (approximately 18 months after bottling), all presented at once in a randomised order. The wine sample groups were evaluated in one sitting of each panel (June/July 2021 and June 2022). Paper scoring sheets for each wine sample group were given to each panellist and collected by the researchers at the end of the sessions.

For the QDA survey, questionnaires relied on panellists scoring wines on a 100-point rating scale, as set out in previous studies (International Organisation of Vine and Wine, 2009; Schelezk, Šuklje, et al., 2018). Visual, olfactory, taste, mouthfeel and palate sensation, presence of flavours on the palate, and overall perception (overall liking of the wine) were measured on a quantitative basis. For the JAR survey, visual, olfactory and mouthfeel sensory attributes were tested on a qualitative basis. Data from the JAR survey was collected on a non-structured linear scale, where the middle of the scale for each attribute corresponded to just about right scores for the expert panellists (Likert, 1932). For each sensory attribute, panellists ranked the wine sample groups on a 5-level Likert scale; for example, too weak/pale/short; weak/pale/short; just about right; strong/a little dark/long; too strong/too dark/too long. For overall perception, overall liking was scored

on a 9-point line scale ranging from dislike extremely, to neutral, to like extremely; and likelihood to purchase was scored on a 5-point line scale ranging from extremely unlikely, to neutral, to extremely likely.

JAR rankings were also combined with overall liking scores to highlight those attributes of a wine that had the greatest impact on liking, and a penalty analysis was undertaken (Cadot et al., 2010; Schraidt, 2009). The purpose of the penalty analysis was to evaluate the impact of a non-JAR category on a given attribute by assessing a ‘penalty’ that represents the overall consumer preference for a wine. Refer to Appendix 2 for the JAR and QDA expert wine consumer survey questionnaires.

Table 4.1. QDA and JAR combined sensory attributes

1	2	3	4
Descriptor	Type	Definition	Standard mixed with 30 ml of Shiraz wine
Opacity	Visual	The overall clearness of the wine	Repetitive training sessions
Purple Colour	Visual	The perception of blue and red influences in the final wine	Provided a chart of red colours displayed from light to dark red to purple.
Red Colour	Visual	The perception of red influences in the final wine	Provided a chart of red colours displayed from light to dark red to purple.
Colour intensity	Visual	The overall intensity of the sum of all visual attributes perceived in the wine	Provided a chart of red colours displayed from light to dark red to purple.
Red fruit	Aroma/flavour	The smell/flavour of cherry, raspberry and strawberry	2 strawberries, 2 cherries and 4 raspberries
Dark fruit	Aroma/flavour	The smell/flavour of cassis, black berries	4 blackberries and 4 blueberries
Ripe Fruit	Aroma/taste/flavour	The smell, taste and flavour that suggest ripe fruits, candied fruits	8 raisins crushed
Vegetal	Aroma/taste/flavour	The smell/flavour of green capsicum and/or the smell of fresh cut grass	Teaspoon minced green capsicum
Sweet spice	Aroma/flavour	The smell/flavour of cloves, cinnamon, cardamon, mixed oriental spice	Pinch of mixed spice
Liquorice	Aroma/flavour	The smell/flavour of liquorice/ aniseed	½ teaspoon of soft liquorice
Chocolate	Aroma/flavour	The smell/flavour of dark chocolate	1 teaspoon of ground dark chocolate

1	2	3	4
Descriptor	Type	Definition	Standard mixed with 30 ml of Shiraz wine
Confectionary	Aroma	Smell of confectionery	4 sweet soft lollies/ bubble-gum
Earthy	Aroma/flavour	The smell/flavour of soil, dust	½ teaspoon of clean sifted dirt
Aroma intensity	Aroma	The overall intensity of the sum of all aroma attributes perceived in the wine	Repetitive training sessions
Odour complexity	Aroma	The overall intensity of the sum of all aroma attributes, including smell, complexity and richness perceived in the wine	Repetitive training sessions
Flavour intensity	Flavour	The overall intensity of the sum of all flavour attributes perceived in the wine	Repetitive training sessions
Acidity	Taste/ mouthfeel	The perceived acidity on the tongue	1 teaspoon of tartaric acid
Sweetness	Taste/mouthfeel	The perceived sweetness on the tongue	1 teaspoon of white sugar
Bitterness	Taste/ mouthfeel	The perceived bitterness on the tongue	1 teaspoon of quinine concentrate
Astringency	Palate sensation/ mouthfeel	The astringency, perceived as puckering and drying sensation on the oral mucosa	Add 0.2gm of powdered white tannin
Hotness	Palate sensation/mouthfeel	The perceived hotness that is caused by alcohol	Add food grade ethanol to raise alcohol level by 2%
Viscosity	Mouthfeel	Thickness on the mouth	Add 0.02gm/L food grade soluble Xanthan gum
Body	Palate sensation/ mouthfeel	The perception of the body, richness, fullness	Add 0.2gm powdered white tannin and food grade ethanol to raise alcohol level by 2%
Length	Palate sensation/ mouthfeel	The time that overall aftertaste persists in the mouth	Repetitive training sessions
Fruit aftertaste	Palate sensation	The time that fruit aftertaste persists in the mouth	Repetitive training sessions
Sour aftertaste	Palate sensation	The time that sour aftertaste persists in the mouth	Repetitive training sessions
Overall Perception	Overall liking and how likely to purchase	Balance between aromas, flavour, structure and mouthfeel, based on all aspects of a wine	Repetitive training sessions

2.5 Statistical analysis

All GC–MS and sensory analysis data collected from the 2020 (Year 1) and 2021 (Year 2) vintages were combined to increase the sample sizes and statistical power of the data, given the larger datasets produced (Riffle et al., 2022; Serdar et al., 2021).

For sensory analysis, the approach included 41 sensory analysis expert panellist responses across both vintages, which produced a significantly larger dataset when compared to earlier studies for Shiraz (Schelezki, Antalick, et al., 2020; Teng et al., 2020). For sensory data, differences in sensory attributes were determined by mixed model analysis of variance (ANOVA), using independent sample group t-test and two-way ANOVA, followed by Tukey’s HSD multiple comparison test ($\alpha = 0.05$). All sensory data are expressed as the arithmetic mean of scores from 41 panellists combined from both vintages.

For GC–MS aroma volatile data, relative concentration differences of volatile compounds were calculated for all wines produced from early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé harvest times, and for those wines subjected to water treatment of their pre-fermentation wine grape must. Results are presented in heatmaps as averages per compound group. Results are presented for each vintage and combined. Analytical data is 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.

3. Results

3.1 Vintage conditions

Vintage growing and wine grape berry health conditions were similar for both years (Richard Tallis, personal communication, 2021 January 11); both seasons were similarly classed as ‘temperate warm’ under the HI (Huglin, 1978; Supplementary materials: Supp. Tables S4.1–S4.5).

3.2 Volatile aroma parameters in wines are slightly impacted by alternative harvest times and water treatment of musts

Forty-three chemical volatile aromas were classified into 11 compound groups: including acids; ethyl esters of odd carbon number fatty acids; ethyl esters of straight-chain fatty acids; higher alcohols; ethyl esters of branched acids; methyl esters of fatty acids; other esters; higher alcohol acetate (HAA) esters from yeast sugar and N metabolism; HAA esters from grape lipid; isoprenoids; and C6 compounds (Supplementary materials: Supp. Table S4.10, Supp. Figure S4.1). Concentrations of these groups of compounds were compared for each of the treatment (process) sample groups, which represent harvest timings and varying water treatments implemented (Figures 4.2, 4.3 and 4.4). When comparing results between vintages, different results are seen in terms of acids, with varying statistical disparities observed between the treatment groups. Notably, consistent results for both vintages are observed for higher alcohols and C6 compounds, with similar statistical differences between treatment groups (Figures 4.2 and 4.3). Although the concentration of higher alcohols varied across the two vintages, the statistical differences between treatment groups in each vintage closely follow each other. For the 2020 vintage study, no discernible statistical distinctions are evident among treatment groups regarding ethyl esters of branched acids or isoprenoids (Figure 4.2).

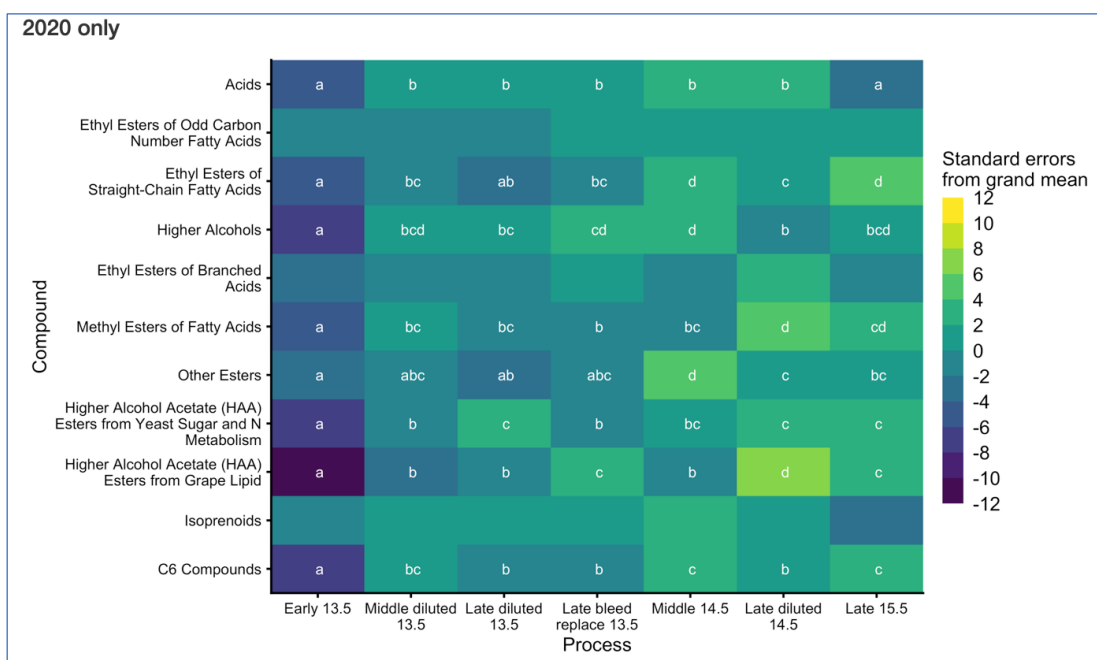


Figure 4.2. Volatile compound groups in Shiraz wines, based on average relative concentration differences of compounds in treatments: heat map for Year 1 (2020)

Map highlights changes of semi-quantitative data for volatile compound groups. Increasing concentrations are indicated by darker green, blue and purple fill colours, whereas concentrations decrease with lighter green and yellow fill colours according to the legends. Mean results for triplicate samples are presented.

Conversely, for the 2021 vintage study, statistical differences between treatment groups are apparent (Figure 4.3).

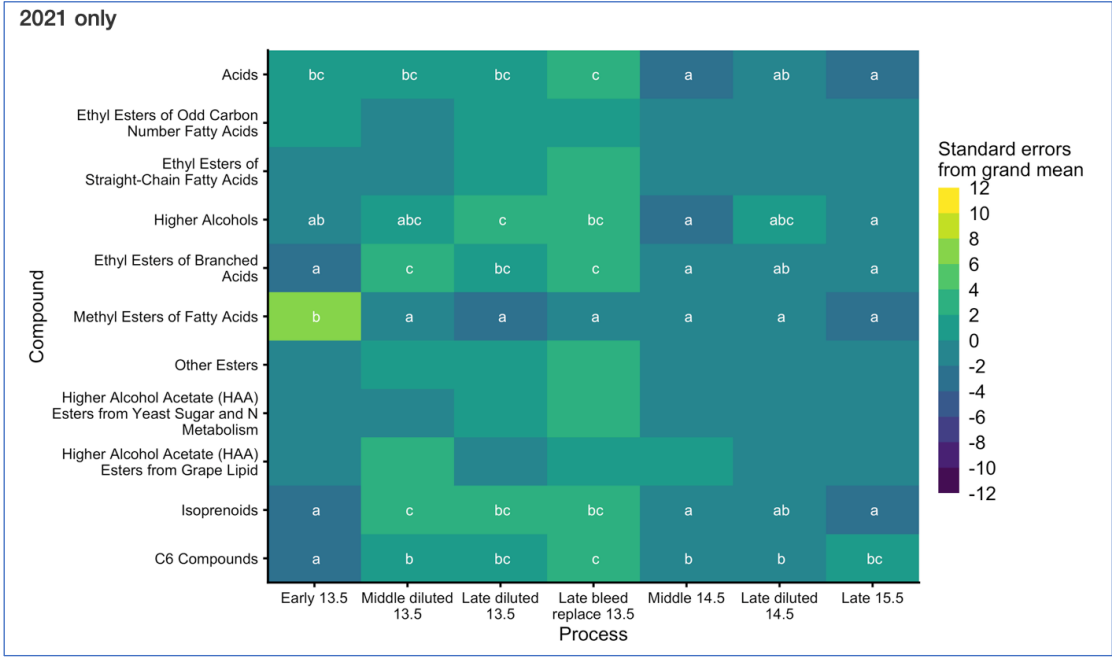


Figure 4.3. Volatile compound groups in Shiraz wines, based on average relative concentration differences of compounds in treatments: heat map for Year 2 (2021)

Map highlights changes of semi-quantitative data for volatile compound groups. Increasing concentrations are indicated by darker green, blue and purple fill colours, whereas concentrations decrease with lighter green and yellow fill colours according to the legends. Mean results for triplicate samples are presented.

The consolidation of datasets increases collective statistical power, showing discernible statistical disparities between treatment groups, as detailed below (Figure 4.4). Combined data shows that the impacts on wine volatiles in the compound groups are varied, and dependent on harvest timings and water treatments implemented (Figure 4.4; Supplementary materials: Supp. Figure S4.2).

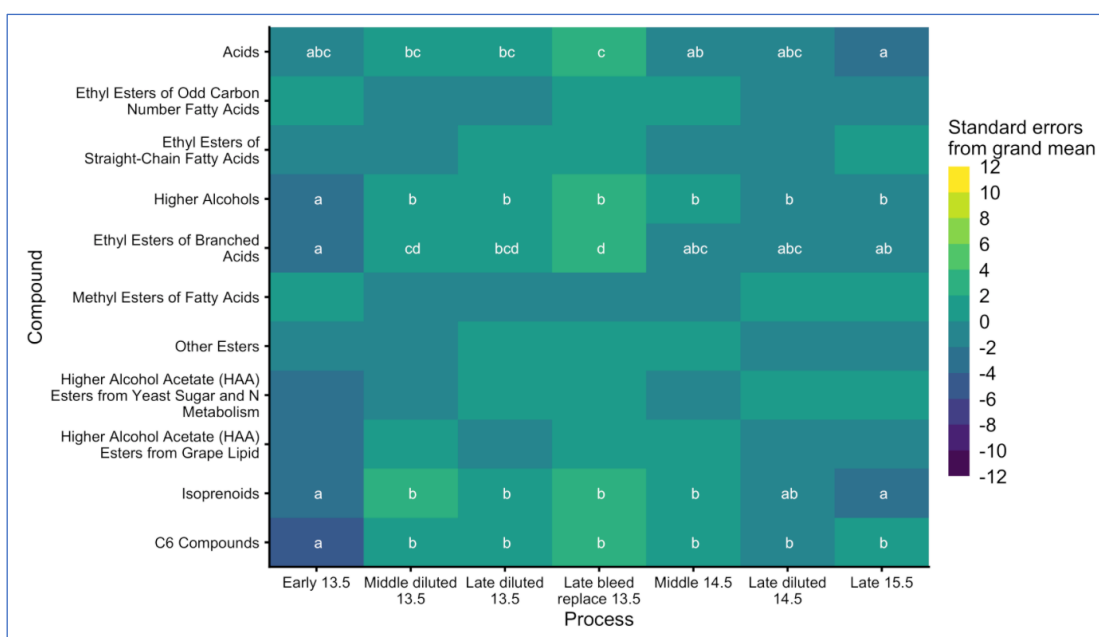


Figure 4.4. Volatile compound groups in Shiraz wines, based on average relative concentration differences of compounds in treatments: heat map for Years 1 (2020) and Year 2 (2021) combined

Map highlights changes of semi-quantitative data for volatile compound groups. Increasing concentrations are indicated by darker green, blue and purple fill colours, whereas concentrations decrease with lighter green and yellow fill colours according to the legends. Mean results for triplicate samples are presented.

3.2.1 All water treatments maintained concentrations of higher alcohols and C6 compounds when compared to late 15.5 °Bé wine

Higher alcohols and C6 compounds were maintained by all water treatments to statistically similar levels as observed for late 15.5 °Bé wine, and all were at statistically significantly higher levels when compared to early 13.5 °Bé wine (Figure 4.4; Supplementary materials: Supp. Figure S4.2).

3.2.2 All water treatments increased concentrations of acids and ethyl esters of branched acids, with highest levels for both observed in ‘bleed and replace’ 13.5 °Bé wine

Late 15.5 °Bé wine had the lowest observed concentration of acids but was still statistically similar to middle diluted 13.5 °Bé, middle 14.5 °Bé and early 13.5 °Bé wines. Conversely, the late ‘bleed and replace’ 13.5 °Bé wine displayed the highest acids concentration, but this was statistically similar to early 13.5 °Bé, middle diluted 13.5 °Bé, late diluted 13.5 °Bé and late diluted 14.5 °Bé wines (Figure 4.4; Supplementary materials: Supp. Figure S4.2).

Similar to acids, the concentration of ethyl esters of branched acids was higher in all water-treated wines. Late ‘bleed and replace’ 13.5 °Bé wine demonstrated the highest observed concentration of ethyl esters of branched acids, which was statistically similar to middle diluted 13.5 °Bé and late diluted 13.5 °Bé wines. In contrast, early 13.5 °Bé wine had the lowest concentration, which was statistically similar to middle 14.5 °Bé, late diluted 14.5 °Bé, and late 15.5 °Bé wines (Figure 4.4; Supplementary materials: Supp. Figure S4.2).

3.2.3 All water treatments increased isoprenoid levels to varying degrees

All water-treated wines had elevated concentrations of isoprenoids; middle diluted 13.5 °Bé wine had the highest concentrations, which was statistically significantly higher than both early 13.5 °Bé and late 15.5 °Bé wines (Figure 4.4; Supplementary materials: Supp. Figure S4.2).

3.2.4 Water treatments had no statistically significant influences for many other chemical compounds

The impact of water treatment of pre-fermentation musts on the remaining compound groups (ethyl esters of odd carbon number fatty acids, ethyl esters of straight-chain fatty acids, methyl esters fatty acids, other esters, HAA esters from yeast sugar and N metabolism, HAA esters from grape lipid) demonstrated no statistical differences between treatment groups (Figure 4.4; Supplementary materials: Supp. Figure S4.2). While levels for all these compounds were generally higher at later harvest dates, no particular patterns were observed.

3.3 Just about right (JAR) and quantitative descriptive analysis (QDA) of sensory attributes

The JAR approach is a direct approach used to measure the deviation from ideal levels of each sensory attribute in each wine sample (Rothman & Parker, 2009). The JAR for the expert wine consumer panellists implemented in the 2020 (Year 1) and 2021 (Year 2) vintage studies measured the perceived attribute intensities, but did not directly quantify them (Cadot et al., 2010). The QDA approach, which was taken concurrently with the JAR survey by the expert wine consumer panellists, measured wines on a quantitative basis, using a 100-point rating scale (Schelezi et al., 2018). The sensory attributes of

each wine sample were scored by the wine consumer expert panellists according to the sensory standards as developed and set out in Table 4.1.

Combined JAR results for both years indicate that later harvests correlated with higher mean values for sensory attributes, and water treatments implemented into later harvest pre-fermentation musts generally resulted in decreased mean values for most sensory attributes, usually correlated with increased preference (Figure 4.5). Combined results for QDA show that mean values for the sensory parameters of opacity, red colour, sweet spice smell, liquorice flavour, chocolate flavour, flavour intensity, bitterness, astringency, hotness, viscosity, body and length all increased with later harvest timing. Conversely, mean values for purple colour, red fruit taste and acidity decreased with later harvest timing. Further, none of the water treatments implemented into later harvest pre-fermentation musts resulted in statistically significant differences in concentration levels for red fruit smell, ripe fruit smell, liquorice smell, chocolate smell, confectionery smell, odour complexity, vegetal taste, ripe fruit taste, sweet spice flavour, sweetness, sour aftertaste or balance (Figures 4.6 and 4.7)

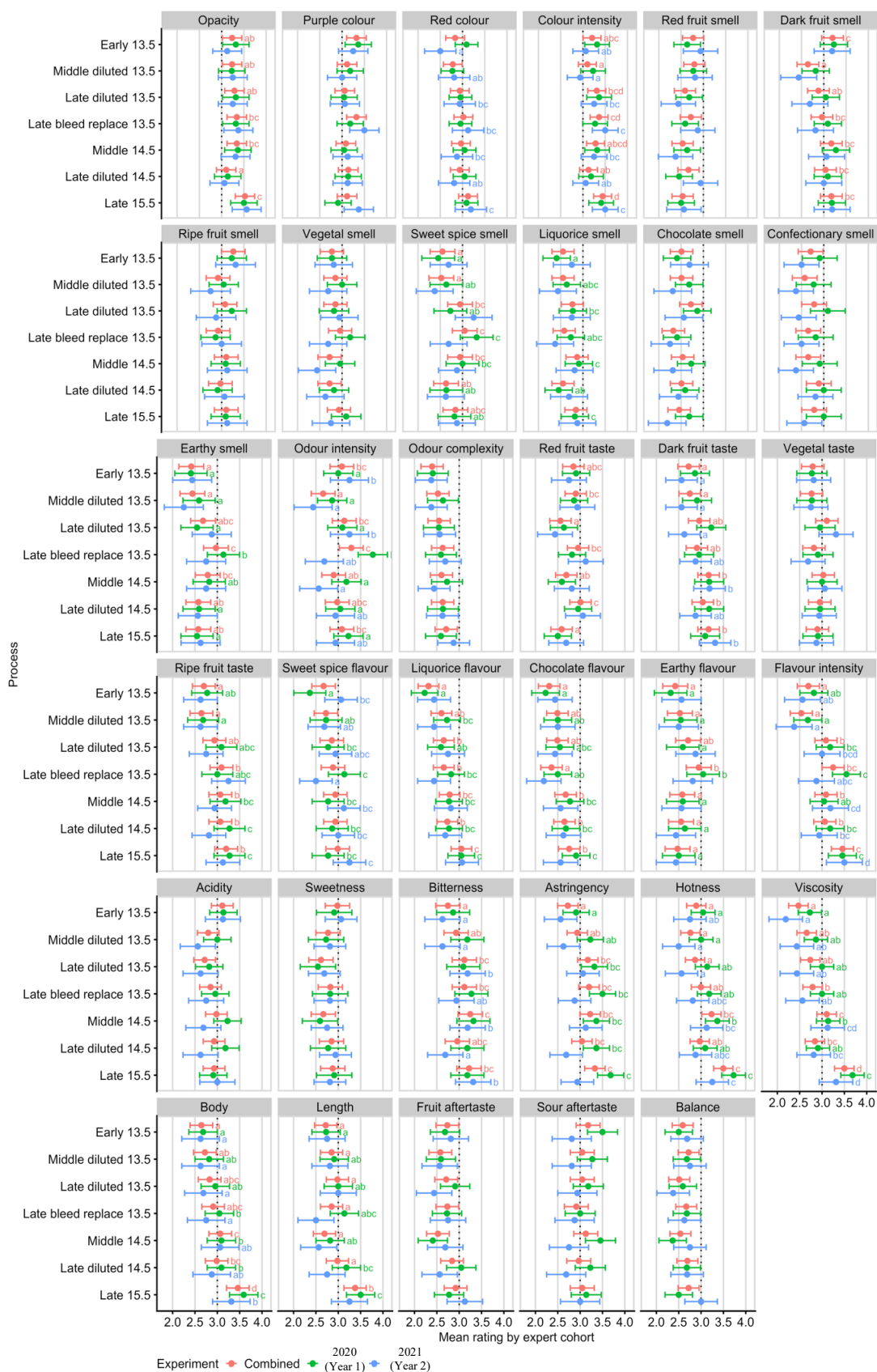


Figure 4.5. Just about right (JAR) combined data for both vintages: sensory parameters of the final wine, by process (treatment group) and mean rating

All represent mean figures of triplicate samples. Error bars show 95% confidence intervals for the mean. Treatment groups not sharing a letter are statistically significantly different ($p < 0.05$).

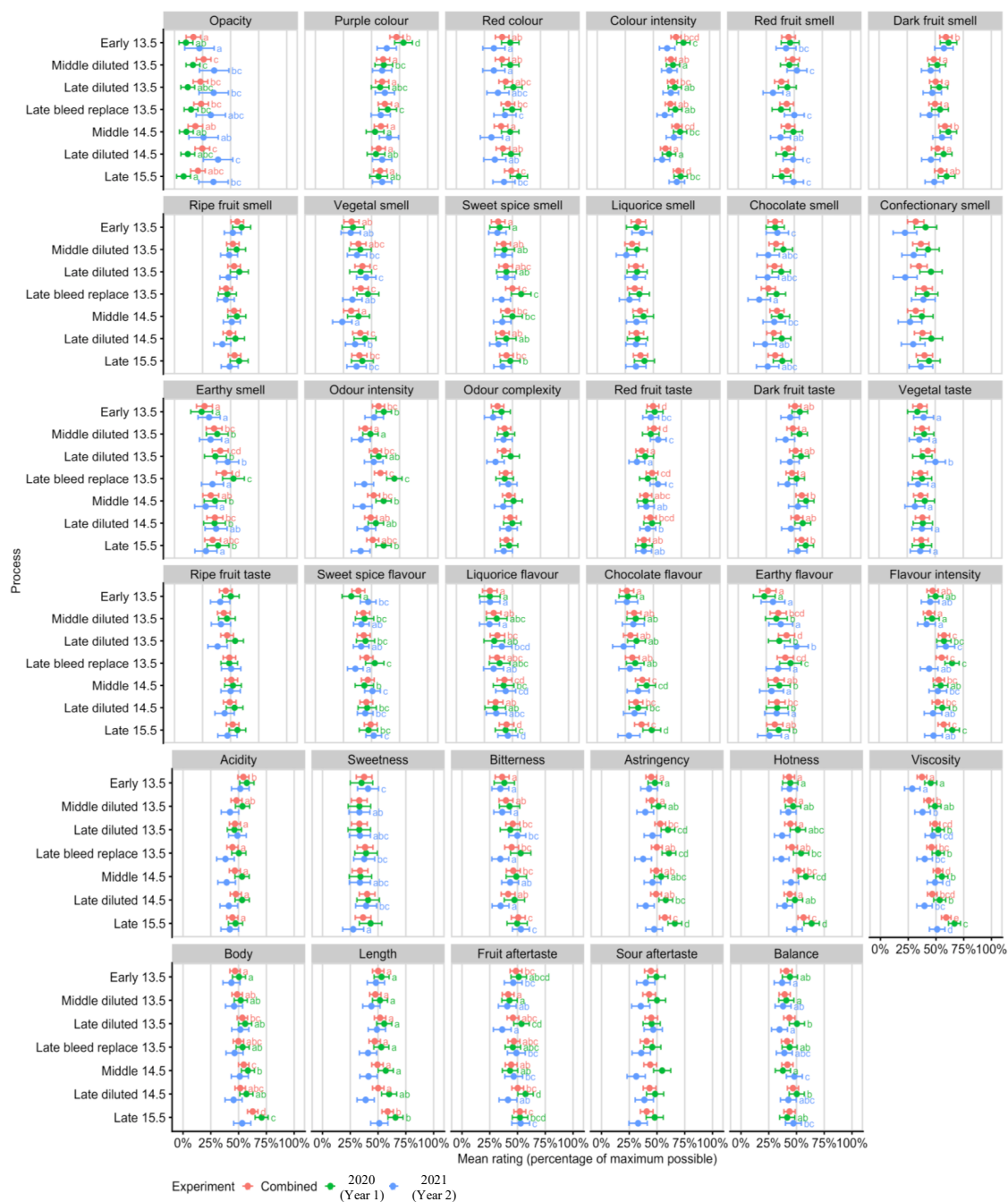


Figure 4.6. Quantitative descriptive analysis (QDA) combined data for both vintages: sensory parameters of the final wine, by process (treatment group) and mean rating

All represent mean figures of triplicate samples. Error bars show 95% confidence intervals for the mean. Treatment groups not sharing a letter are statistically significantly different ($p < 0.05$).

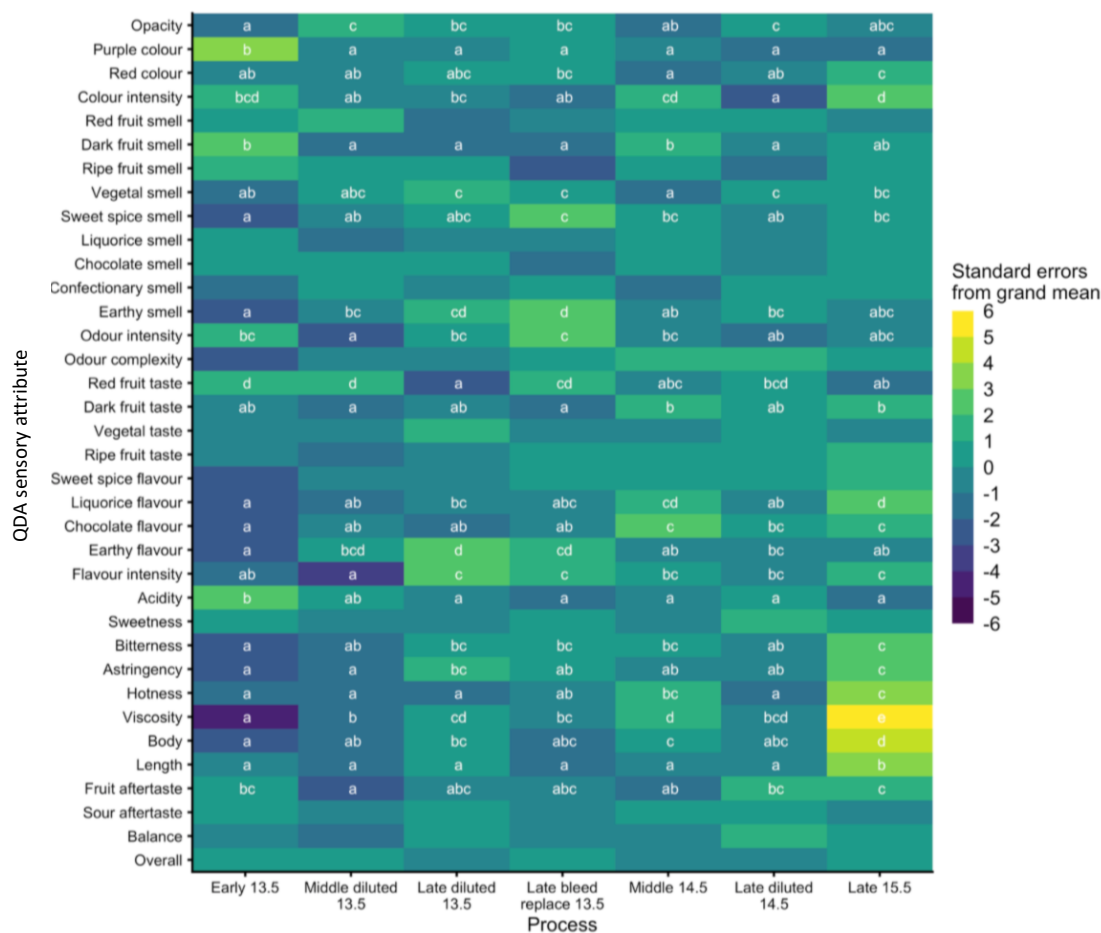


Figure 4.7. Heat map combined data for both vintages, highlighting QDA mean values for sensory attributes in Shiraz wines, based on process/treatments implemented

Year 1 (2020) and Year 2 (2021) combined data. Increasing mean values are indicated by darker green, blue and purple fill colours, whereas mean values decrease with lighter green and yellow fill colours according to the legend. Treatment groups not sharing a letter are statistically significantly different ($p < 0.05$).

3.3.1 Early 13.5 °Bé and middle diluted 13.5 °Bé wines were least preferred for many sensory attributes

JAR results show early 13.5 °Bé wine had the lowest mean rating (2.31) for chocolate flavour, which was considered ‘too low’ by expert panellists. This was also the case for bitterness, astringency, hotness, body and length. Early 13.5 °Bé wine had the highest mean score for dark fruit smell (3.20), but this was considered as ‘too high’. In addition, early 13.5 °Bé wine and middle diluted 13.5 °Bé wines had the lowest preference ratings for sweet spice smell (2.55 and 2.52, respectively), earthy flavour (2.42 and 2.44, respectively), dark fruit taste (2.72 and 2.75, respectively), and ripe fruit taste (2.70 and 2.40, respectively); expert panellists considered the levels for these sensory parameters to be ‘too low’ (Figure 4.5).

QDA outcomes show that early 13.5 °Bé wine had the lowest mean value for opacity (0.17), vegetal smell (0.32), sweet spice smell (0.37), earthy flavour (0.27), flavour intensity (0.44), bitterness (0.36), astringency (0.45), body (0.50) and viscosity (0.45), but the highest mean value for purple colour (0.72), red fruit taste (0.50) and acidity (0.54) (Figure 4.6). Late 15.5 °Bé wine had the highest preference for liquorice and chocolate flavour

JAR results show late 15.5 °Bé wine had the greatest preference rating for liquorice and chocolate flavours (3.05 and 2.75, respectively); however, these outcomes were statistically similar to middle 14.5 °Bé wine (2.79). Notably, late 15.5 °Bé wine was least preferred for flavour intensity, opacity and colour intensity (3.46, 3.74 and 3.44, respectively), where these attributes were all considered ‘too high’. Similarly, for bitterness, astringency, hotness, body and length attributes, late 15.5 °Bé wine had the highest mean values, but these were also observed to be ‘too high’ by panellists and were least preferred (Figure 4.5).

QDA results indicate late 15.5 °Bé wine had the highest mean value for red colour, bitterness, astringency, hotness and length (0.49, 0.51, 0.57, 0.56 and 0.59, respectively). For dark fruit taste, liquorice and chocolate flavour, late 15.5 °Bé (0.56, 0.49 and 0.44, respectively respectfully) and middle 14.5 °Bé wines (0.57, 0.50 and 0.43, respectively) had the highest mean values of all treatment groups (Figure 4.6).

3.3.2 Late diluted 14.5 °Bé wine was most preferred for many sensory attributes

JAR results show late diluted 14.5 °Bé wine had the highest preference rating for opacity (3.10) when compared to all other treatment groups. Preferences ratings for odour intensity, red fruit taste, dark fruit taste and ripe fruit taste were also high for this treatment wine (2.98, 3.01, 3.04 and 2.98, respectively); however, these outcomes were not statistically significantly different from all other treatment wines, which were all clustered around the ideal mean of 3.0 (Figure 4.5).

QDA outcomes for late diluted 14.5 °Bé wine indicate that chocolate flavour was able to be maintained at similar levels (0.39) to that of late 15.5 °Bé wine (0.47). However, QDA results also show a significant decrease in red colour (0.42), colour intensity (0.61) and bitterness (0.44) when compared to the late 15.5 °Bé wine (0.49, 0.72 and 0.51,

respectively) (Figure 4.6). Late 'bleed and replace' 13.5 °Bé wine was most preferred for earthy flavour, with late diluted 13.5 °Bé wine displaying lowest flavour intensity concentrations.

JAR sensory results show 'bleed and replace' 13.5 °Bé wine had the greatest preference rating for earthy flavour (2.95), which was statistically similar to late diluted 13.5 °Bé wine (2.71). Notably, this treatment wine had the highest preference rating for sweet spice smell (2.89). This was also the case for earthy smell (2.97); however, this was a statistically similar preference rating to late diluted 13.5 °Bé (2.68) and middle 13.5 °Bé (2.79) wines (Figure 4.5).

QDA outcomes show correlated results for earthy smell, with the most significant increases in mean rating observed for late 15.5 °Bé wine (0.34) to late 'bleed and replace' 13.5 °Bé wine (0.44). In addition, this treatment was able to maintain higher mean values of odour intensity (0.58) when compared to other treatment groups. Further, the late 'bleed and replace' 13.5 °Bé wine and the middle diluted 13.5 °Bé wine were able to increase perceptions of red fruit taste to a similar level (0.49) as early 13.5 °Bé wine (0.50). Conversely, dark fruit taste was perceived in lowest concentration in the late 'bleed and replace' 13.5 °Bé wine (0.48) and the middle diluted 13.5 °Bé wine (0.49) (Figures 4.6 and 4.7).

JAR results show that for red fruit taste and liquorice flavour, late diluted 13.5 °Bé wine had the lowest mean ratings (2.56 and 2.31, respectively) when compared to all other treatment groups; these levels were considered 'too low' by expert panellists (Figure 4.5). QDA results show flavour intensity was perceived to be lowest in late diluted 13.5 °Bé wine (0.44) and early 13.5 °Bé wine (0.48). Highest concentration of earthy flavour, however, were seen in the late diluted 13.5 °Bé wine (0.43). Notably, late diluted 13.5 °Bé wine and late diluted 14.5 °Bé wine showed significantly decreased bitterness when compared to late 15.5 °Bé wine (0.46, 0.47 and 0.52, respectively). However, for astringency, the late 15.5 °Bé wine (0.57) and late diluted 13.5 °Bé wine (0.53) displayed the highest perceived concentration levels, when compared to all other treatment groups (Figures 4.6 and 4.7).

3.3.3 Overall sensory assessment and purchase likelihood of wines

For overall assessment and purchase likelihood of wine, when data is separated by year, there are no statistical differences between any of the treatment groups in either the 2020 (Year 1) or 2021 (Year 2) studies in isolation. Overall assessment and purchase likelihood of wines did not change when pooling the data from each year together, despite a greater sample size, with associated reduced confidence intervals (Supplementary materials: Supp. Figures S4.3 and S4.4)

3.3.4 Penalty analysis

Penalty analysis shows that too much bitterness, sour aftertaste, vegetal taste and astringency are all wine attributes that fall into the critical corner. ‘Too much’ of these attributes in a wine was observed to have the most negative impact on overall liking when observed in the JAR scale. Late 15.5 °Bé wine had the highest mean value for bitterness and was least preferred in most attributes. All water treatments of the late 15.5 °Bé harvest had generally lower perception of these negative attributes, correlating with increased preference. While the highest mean value for sour aftertaste on the JAR scale was attributed to early Bé wine, sour aftertaste was not observed to be statistically significantly different between any of the treatment groups. Similarly, for vegetal taste there were no statistically significant differences between any of the treatment groups. Not enough balance, fruit aftertaste, red fruit taste, flavour intensity, body, ripe fruit taste, dark fruit taste and viscosity also fall into the critical corner. Here ‘not enough’ of these attributes in a wine was observed to have the most negative impact on overall liking when observed in the JAR scale. For red fruit taste, late 15.5 °Bé wine and late diluted 13.5 °Bé wine had the lowest overall rating and perception of this attribute, with highest preference in the late diluted 14.5 °Bé wine (Figure 4.8).

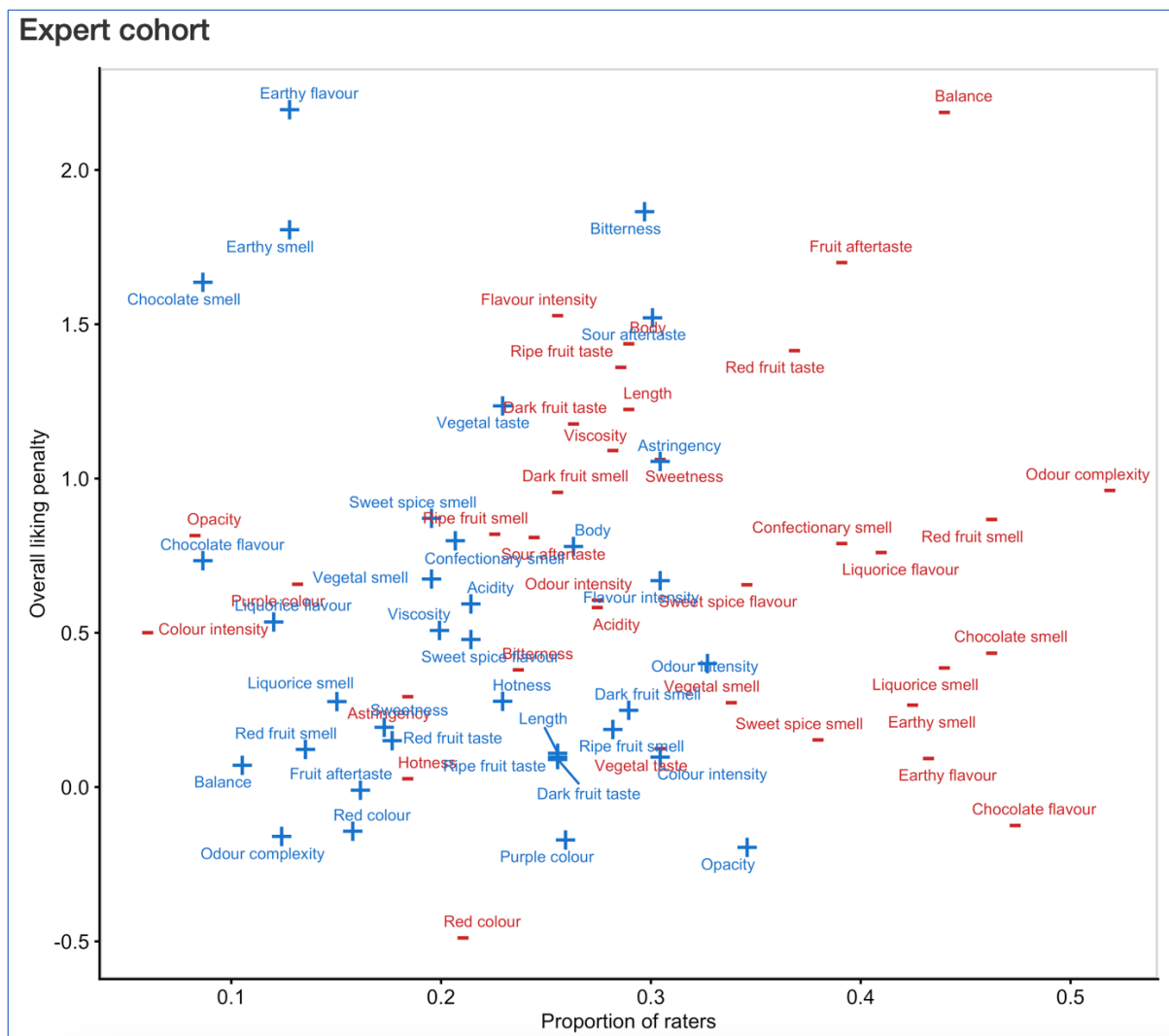


Figure 4.8. Overall liking penalty and proportion of raters for sensory attributes

Too much bitterness, sour aftertaste, vegetal taste and astringency fall into the critical corner. Not enough balance, fruit aftertaste, red fruit taste, flavour intensity, body, ripe fruit taste, dark fruit taste and viscosity also fall into the critical corner. ‘+’ denotes too much of an attribute, and ‘-’ denotes too little of an attribute.

4. Discussion

Water addition or water substitution into pre-fermentation high-sugar musts to a regulated 13.5 °Bé (Standard 4.5.1; Australian Government, 2017) can address the issue of resultant stuck fermentations, and associated negative impacts on chemical and sensory characteristics of the final wines produced from a late harvest (Gawel et al., 2007). Importantly, this winemaking approach will also produce more-favourable lower-alcohol wine. However, recent studies have found that negative impacts on phenolic concentrations and sensory characteristics from wine must water treatments can also eventuate (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng

et al., 2020). Different levels and types of water treatments into musts from varying harvest times will produce fluctuating results. It has been shown that olfactory and mouthfeel sensations can be correlated to specific chemical components of wine, including the interaction of sugars, acids, alcohols and phenolic compounds (Capone et al., 2013). Volatile aroma compounds can also contribute to the overall composition and sensory perception of the water-treated wines (Schüttler et al., 2015; Teng et al., 2020).

Importantly, the desired reduction in ethanol production from the ‘water treatment of pre-fermentation wine grape must’ winemaking approach must be balanced with other desirable wine flavonoids (Remize et al., 1999). This is because ethanol impacts the concentrations of wine volatiles that contribute to final sensory attributes such as wine aroma and flavour (Carpena et al., 2021). Striking this balance can be difficult. The aim of this study is to further address this challenge.

In this chapter, the characterisation of how a wine’s aroma compositions were impacted upon by different harvest timings and varied water treatments implemented into later harvest pre-fermentation wine musts was presented first. Wine aroma is formed by more than 800 volatile compounds and is characteristic of each product (Sagratini et al., 2012). These compounds can be represented in different chemical classes including ethyl methyl esters, lactones, monoterpenes, norisoprenoids, methoxypyrazines, thiol compounds, as well as many others (Mihnea et al., 2015). By implementing stable isotope dilution analysis in conjunction with GC–MS, a range of compounds were identified and categorised into 11 groups: acids, ethyl esters of odd carbon number fatty acids, ethyl esters of straight-chain fatty acids, higher alcohols, ethyl esters of branched acids, methyl esters of fatty acids, other esters, HAA esters from yeast sugar and N metabolism, HAA esters from grape lipid, isoprenoids, and C6 compounds (Supplementary materials: Supp. Table S4.10, Supp. Figure S4.2).

The results from two sensory surveys of expert wine consumers – a just about right (JAR) survey and quantitative descriptive analysis (QDA) – were then presented. The aims of these surveys were to test which wine was most preferred (JAR) and which had more favourable mean ratings of sensory parameters (QDA). The sensory analysis of wine is based on visual perceptions, olfactory sensations, and taste and mouthfeel sensations (Jackson, 2022). A wine’s final quality in terms of its sensory parameters will be judged

by three types of wine assessors: naïve wine consumers, specially trained wine assessors and expert wine consumers. While no one wine assessor group is better than another, given that each group performs different tasks, expert wine consumers are nevertheless to be distinguished from naïve wine consumers and trained wine assessors (Schelezki, Antalick, et al., 2020; Teng et al., 2020). Naïve wine consumers will rate a wine based on their preferences, regardless of the quality of the given wine (Schelezki, Antalick, et al., 2020), which means that even a poor-quality wine may be assessed as a preferred wine, depending on consumer 'likings'. Trained wine assessors serve as the 'standard' for establishing sensory properties of a given food item (Ares & Varela, 2017), having undergone significant prior training to equip them for the sensory tasks ahead (Savela-Huovinen et al., 2018). However, one limitation of using trained wine assessors is that it can be expensive and time-consuming because of the extensive prior training they need (Sáenz-Navajas et al., 2016). The expert wine consumers relied on in this study for sensory testing are acknowledged as 'experts', having undergone continuous prior training in wine tasting, during which they have sharpened their expertise in discerning fine nuances in taste and smell of wine over many years (Franco-Luesma et al., 2019). The use of expert wine consumers in this study can also add an extra dimension to the results, as the perceptions of such experts can ultimately affect a wine's reputation and marketability (Hendrickx et al., 2016).

The results show that the JAR and QDA survey outcomes complement each other, which agrees with previous studies showing that this combination of sensory testing can give a better understanding of the descriptions of a wine, by expert wine professionals (Cadot et al., 2010). Further, despite the widespread use and familiarity of both sensory methods, there is a lack of current research that explores wine aroma profiling with QDA and JAR sensory analysis to determine final wine quality attributes. This study aims to fill some of these gaps and contribute further to the existing knowledge in this area. Generally, wine aroma fingerprinting results in this study show that harvest timings and alternative water treatments of wine grape pre-fermentation musts to yield lower wine alcohol concentrations can have influences on many volatile aroma compositions in a final wine, with some significant correlations to JAR and QDA sensory attributes.

4.1 Final wine quality is not effectively addressed by early 13.5 °Bé winemaking

Early harvests will generally produce wine with lower final alcohol levels; however, wine sensory attributes can be negatively affected, together with impacts on many volatile compounds (Schelezkki, Antalick, et al., 2020). In this study, JAR results for early 13.5 °Bé wine (together with middle diluted 13.5 °Bé wine) indicate the least preference for dark fruit taste, sweet spice smell, earthy smell, ripe fruit taste, chocolate flavour and earthy flavour, which were all observed as being ‘too low’. Similar results were observed in regards to bitterness, astringency, hotness, body and length, with early 13.5 °Bé wine having the lowest JAR preference scores, and all these attributes also observed as being ‘too low’. Correlated QDA results support these JAR outcomes. Volatile aroma profiling indicates the lowest concentrations of acids, higher alcohols, ethyl esters of branched acids, isoprenoids and C6 compounds were observed in the early 13.5 °Bé wine. These findings are in line with previous studies that also show that early harvest wine will have associated unfavourable green or vegetative characteristics (Bindon, Kassara, et al., 2014). On balance, the early harvest 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 some later harvest more-favourable volatile aroma compounds is not possible, and early harvest winemaking can have significant negative impacts on final wine sensory characteristics.

4.2 Final wine quality is not effectively addressed by late 15.5 °Bé winemaking

Harvesting late can produce robust wine with specific aroma profiles, and for red wine such Shiraz, there can be more-favourable dark fruit attributes produced with less-favourable green attributes which are generally associated with earlier harvest wine (Bindon, Kassara, et al., 2014). However, late harvest wine has also been shown to have unfavourable higher alcohol levels, with other less-preferred sensory attributes (Bindon, Varela, et al., 2014; Pérez-Magariño & González-San José, 2006; Teng et al., 2020).

In this study, JAR results show that for late 15.5 °Bé wine, there were intense preferences for dark fruit attributes, including dark fruit smell (although not statistically significantly different to any other treatments, except for middle diluted 13.5 °Bé wine); this result also correlated to the QDA mean level for this attribute. The dark fruit smell or aroma attribute

can be related to higher concentrations of esters and higher alcohols in the late 15.5 °Bé wine. Esters that are produced during fermentation can contribute to the fruity notes of wine (Ilc et al., 2016). Previous research has also found that HAA esters from grape lipid (hexyl acetate) can contribute to red or dark fruit smell with sweet and perfume-like attributes (Bindon, Holt, et al., 2014). However, while these compounds were at higher concentration levels in the late 15.5 °Bé wine, there were no statistically significant differences observed for this volatile between any of the harvest timing wines or water treatment wines.

Interestingly, the preference for dark fruit smell and its associated QDA higher mean value may be related to the late harvest Shiraz wine having more bold red fruit notes, which can sometimes be seen to be a positive aspect in a full-bodied red wine such as Shiraz (Teng et al., 2020). In this study, favourable sensory attributes generally associated with later harvests, such as chocolate and liquorice, were also most preferred by our panellists, which is also consistent with QDA higher mean values (Teng et al., 2020). Dark fruit notes can be very pleasant attributes when balanced with other sensory attributes. However, the late 15.5 °Bé wine showed the lowest observed concentration of acids (hexanoic acid, octanoic acid), although statistically similar to middle diluted 13.5 °Bé, middle 14.5 °Bé and early 13.5 °Bé wines (Supplementary materials: Supp. Figure S4.1). Octanoic acid is an important volatile that can also contribute to a fruity odour, but it can sometimes have negative rancid and harsh effects (Siebert et al., 2005); this may indicate why many other JAR sensory attributes of the late 15.5 °Bé wine were least preferred. In particular, the late 15.5 °Bé wine in this study was least preferred for flavour intensity, where it was considered ‘too high’, also correlating with its higher QDA mean rating.

Notably, the findings indicate that if winemakers seek to address high alcohol levels and avoid many other associated unfavourable wine quality features, they should avoid harvesting late due to the detrimental impact on many volatiles and sensory parameters.

4.3 Final wine quality can be effectively addressed by late diluted 14.5 °Bé winemaking

Harvesting later and reducing Bé to regulated levels by water treatment into a pre-fermentative wine grape must, will produce wines with lower alcohol levels (Petrie et al., 2019; Schelezki, Antalick, et al., 2020; Teng et al., 2020). The impacts on volatile

aroma profiles of treated wines, coupled with sensory data outcomes, can vary depending on the type of water treatment implemented into a wine grape must (Schelezi, Antalick, et al., 2020). The outcomes in this study agree with these previous findings.

One approach to reducing ethanol production during fermentation is to add lower levels of water to the late harvest must, so as to produce a wine with a middle of the range 14.5% alcohol by volume (ABV). This approach can be taken to minimise some of the negative impacts on wine volatile compounds that result from higher water addition levels, while still maintaining some of the positive flavonoid parameters associated with ethanol. Sensory attributes may also be less affected.

The results from this study show that this winemaking approach can have less impact on intensity of colour in a wine and its opacity. Deeply opaque red wines such as Shiraz have been noted for having more pigment and phenolics than more translucent red wines (Teng et al., 2020). Interestingly, for opacity, this study's JAR results indicate late diluted 14.5 °Bé wine had the highest preference, correlating with the high mean value for opacity in the QDA results. JAR results also show that late diluted 14.5 °Bé wine (together with middle diluted 13.5 °Bé wine and early 13.5 °Bé wine) was most preferred for colour intensity, also aligning with higher mean scores for colour intensity in QDA. Other sensory attributes in the late diluted 14.5 °Bé wine, such as odour intensity, red fruit taste, dark fruit taste, liquorice and chocolate flavours, flavour intensity, bitterness, astringency, viscosity, body and length, all had higher preferences under JAR analysis, and all correlated closely to their QDA mean value outcomes. For hotness, JAR results indicate the highest preference was in late diluted 14.5 °Bé wine, also correlating with its lower mean value under QDA.

Interestingly, concentrations of most fermentation-derived compound groups did not decrease with lower established alcohol levels, regardless of the water treatment of must approach implemented. In particular, for all water treatments into pre-fermentative musts, no statistically significant change was observed in the volatile compound groups of ethyl esters of odd carbon number fatty acids, ethyl esters of straight-chain fatty acids, HAA esters from yeast sugar and N metabolism, HAA esters from grape lipid, methyl esters of fatty acids, other esters, or higher alcohols. However, when observing singular compounds from the 11 compound groups, results show that there was a positive correlation between the water treatment implemented and the increase in the mean

concentration of the isoprenoid β -damascenone. Water treatments into the late harvest increased β -damascenone concentration to a statistically significant degree in the late diluted 14.5 °Bé wine when compared to the late 15.5 °Bé wine (although this was not statistically significantly different to any other water treatment wines). Importantly β -damascenone can enhance ‘fruity’ attributes in wines (Pineau et al., 2007).

5. Conclusion

Findings of this study show that levels in aroma compositions for Shiraz wines can vary depending on harvest timing and/or type of water treatment of pre-fermentation wine grape musts. For instance, late 15.5 °Bé harvest wine displayed lower levels for many fermentation volatiles, including the lowest observed concentration of acids that can contribute to fruity odour. This wine also had the lowest preferences for most sensory attributes, with correlated higher QDA mean values. It also had the highest alcohol levels at 15.5% ABV. By comparison, while early 13.5 °Bé harvest wine had a more-favourable 13.5% ABV level, this wine demonstrated adverse ‘green’ aroma profiling, and negative impacts were also observed for many sensory attributes. On balance, the early harvest winemaking approach is thus to be avoided due to the detrimental impact on many volatile aromas and sensory parameters. Notably, the concentration of most fermentation-derived volatile aroma compounds (in 11 distinct groups) did not decrease with lower established alcohol levels, regardless of the water treatment of must approach implemented. Further, water treatments into later harvest musts across all treatment groups generally resulted in increased preferences for many sensory attributes under JAR, with correlating decreased QDA mean values. In particular, JAR sensory findings demonstrated that late diluted 14.5 °Bé wine was frequently preferred across most sensory attributes, with correlated QDA mean results. However, none of the water treatments affected expert wine consumers overall assessment or purchase likelihood of any of the wines tested. This study is important for winemakers because it provides them with reliable data to evaluate optimal harvest times and the suitability of different water treatment into high-sugar wine must winemaking approaches. While the primary objective of this winemaking method is to produce wine with lower, more desirable alcohol levels, the acceptability and preference for water-treated wines is dependent on maintaining or achieving satisfactory final wine quality parameters, as indicated in this study by the ratings of expert wine panellists and levels of GC–MS highlighted chemical aroma analytes. These outcomes

are important to maintain Australian Shiraz wine quality and reputation in the domestic and export markets.

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Chapter 4 Supplementary materials

Supp. Table S4.1. The coefficient length of day (d) by latitude for the heliothermal index

Latitude	Length of day coefficient (d)
<40°00'	1.00
40°01' – 42°00'	1.02
42°01' – 44°00'	1.03
44°01' – 46°00'	1.04
46°01' – 48°00'	1.05
48°01' – 50°00'	1.06

Source: Tonietto and Carbonneau, 2004.

Supp. Table S4.2. Classes of viticultural climate for the heliothermal index of the grape-growing regions

Index	Class of viticultural climate	Acronym	Class interval
Heliothermal index, HI	Very warm	HI + 3	>3000
	Warm	HI + 2	>2400 ≤ 3000
	Temperate warm	HI + 1	>2100 ≤ 2400
	Temperate	HI – 1	>1800 ≤ 2100
	Cool	HI – 2	>1500 ≤ 1800
	Very cool	HI – 3	≤1500

Source: Tonietto and Carbonneau, 2004.

Supp. Table S4.3. Monthly mean maximum temperature for Yarrawonga weather station (located 45 km from Dookie, Victoria) – Station number: 081124, Victoria; latitude: 36.03°S; longitude: 146.03°E; elevation: 129 m

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2018	35.3	32.8	29.4	27.2	18.9	14.6	14.3	14.9	19.4	25.2	27.4	32.8	24.3
2019	37.2	32.0	29.3	24.9	17.4	14.7	13.8	14.3	18.7	25.7	27.1	33.1	24.0
2020	33.2	31.1	26.6	21.3	16.7	13.8	13.8	14.5	19.1	23.3	30.7	29.9	22.8
2021	32.4	30.0	26.0	23.0	18.9	13.7	12.8	15.5	18.5	21.0	24.0	30.5	22.2

Source: Australian Bureau of Meteorology (2023a).

Supp. Table S4.4. Monthly mean minimum temperature for Yarrawonga weather station (located 45 km from Dookie, Victoria) – Station number: 081124, Victoria; latitude: 36.03°S; longitude: 146.03°E; elevation: 129 m

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2018	17.2	15.5	12.3	10.1	6.0	3.3	2.7	2.7	2.9	8.0	11.3	16.7	9.1
2019	19.7	15.7	14.6	10.1	7.2	3.5	4.5	1.8	3.7	7.1	10.3	14.1	9.4
2020	15.7	16.4	12.8	9.6	5.0	3.5	2.7	3.5	6.1	9.0	12.6	12.3	9.1
2021	15.5	14.3	11.6	7.5	5.8	4.8	4.2	3.7	5.2	6.6	10.3	12.4	8.5
2022	17.7	15.0	14.0	11.2	7.0	4.3	1.0	4.5	5.6	9.4	10.1	12.3	9.3

Source: Australian Bureau of Meteorology (2023b).

Supp. Table S4.5. Monthly rainfall (mm) for Numurkah weather station (located 33.7 km from Dookie, Victoria) –Station number: 80101, Victoria; latitude: 36.09°S; longitude: 145.45°E; elevation: 105 m

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2019		5.4		9.4	49.4	42.4	54.3	20.6	11.2	7.1	43.1	0.0	
2020	67.9	20.0	69.6	105.2	28.8	22.8	30.8	50.6	16.1	50.6	21.0	12.7	496.1
2021	69.6	10.6	70.7	4.2	21.0	64.7	46.0	27.0	79.2	19.2	59.6	9.0	480.8
2022	78.2	9.4	45.2	70.6		38.2	20.2	63.8	90.3	175.8	77.4	74.8	

Source: Australian Bureau of Meteorology (2023c).

Supp. Table S4.6. Fermentation tracking 2020 (Year 1)

		DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
		PM		AM	PM	AM	PM	AM	PM	AM	PM
Early 13.5	Temp (oC)		21.0	22.5	25.5	28.8	27.3	24.5	24.5	23.2	22.2
	Baume (Be)		13.5	12.6	9.3	7.3	5.1	1.9	1.1	-0.4	-0.4
Middle 14.5	Temp (oC)		16.5	18.3	20.0	22.3	24.5	26.0	29.5	21.0	20.0
	Baume (Be)		14.5	14.3	13.9	12.6	11.3	6.3	4.1	1.8	-0.1
Middle diluted 13.5	Temp (oC)		16.0	18.5	19.8	22.2	24.5	26.8	28.8	21.2	20.3
	Baume (Be)		14.5	13.1	12.4	11.3	10.5	5.3	3.5	1.1	-0.2
Late	Temp (oC)		18.0	19.0	19.3	21.2	22.0	25.7	28.7	21.7	21.0
	Baume (Be)		15.3	15.3	15.3	13.6	13.0	8.5	6.7	2.5	-0.1
Late diluted 14.5	Temp (oC)		18.0	18.5	18.8	20.3	21.7	24.7	27.0	22.3	21.3
	Baume (Be)		15.2	14.5	14.2	13.1	11.9	8.4	6.5	2.6	-0.1
Late diluted 13.5	Temp (oC)		18.0	18.8	19.0	21.2	21.5	24.5	27.2	23.3	21.0
	Baume (Be)		15.3	13.4	13.1	12.2	11.3	7.2	5.7	1.5	-0.2
Late bleed and replace 13.5	Temp (oC)		18.0	19.0	19.0	20.7	22.2	24.3	26.7	23.0	21.0
	Baume (Be)		15.1	13.4	12.9	11.7	10.5	6.8	5.1	1.6	-0.2

Mean measurements for triplicate samples are presented.

Supp. Table S4.7. Fermentation tracking 2021 (Year 2)

		DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
		PM		AM	PM	AM	PM	AM	PM	AM	PM
Early 13.5	Temp (oC)		22.0	24.5	27.8	27.5	27.3	23.5	23.3	23.1	21.1
	Baume (Be)		13.5	12.7	9.4	7.4	5.2	3.4	2.2	-0.2	-0.4
Middle 14.5	Temp (oC)		18.0	18.6	21.0	22.0	24.5	25.5	24.5	22.5	21.2
	Baume (Be)		14.5	14.2	13.8	12.5	11.3	6.4	3.9	1.0	-0.1
Middle diluted 13.5	Temp (oC)		18.0	18.4	20.0	22.0	24.5	25.4	27.4	21.6	21.3
	Baume (Be)		14.5	13.2	12.5	11.4	10.5	6.5	3.6	1.0	-0.1
Late	Temp (oC)		18.5	18.6	19.3	21.4	22.0	25.4	26.8	22.5	22.0
	Baume (Be)		15.5	15.2	14.8	13.6	13.0	6.8	3.9	1.9	-0.1
Late diluted 14.5	Temp (oC)		18.5	18.4	19.0	21.5	21.7	25.8	26.5	23.5	22.1
	Baume (Be)		15.5	14.6	14.0	13.5	11.9	8.5	4.9	1.8	-0.1
Late diluted 13.5	Temp (oC)		18.6	19.1	19.0	23.5	21.5	26.5	27.5	23.5	23.1
	Baume (Be)		15.4	13.6	13.0	11.9	11.3	6.9	3.5	1.6	-0.1
Late bleed and replace 13.5	Temp (oC)		19.0	19.1	19.5	21.5	22.2	23.9	27.4	23.0	23.1
	Baume (Be)		15.1	13.5	12.8	11.6	10.5	6.8	3.6	1.6	-0.2

Mean measurements for triplicate samples are presented.

Supp. Table S4.8. Analysis of wine treatments post fermentation (day 5) 2020 (Year 1)

Trial	pH	VA g/L	Lactic Acid g/L	Total sugar g/L	TA g/L	Malic Acid g/L
Early 13.5	3.61	0.48	2.10	1.93	7.01	0.82
Middle 14.5	3.73	0.37	1.86	2.42	6.32	0.96
Middle diluted 13.5	3.67	0.44	1.91	2.21	6.35	0.83
Late	3.71	0.38	1.53	2.32	6.35	1.07
Late diluted 14.5	3.74	0.36	1.56	2.04	6.55	0.96
Late diluted 13.5	3.73	0.37	1.62	1.95	6.34	0.88
Late bleed and replace 13.5	3.72	0.39	1.70	1.63	6.37	0.78

Mean measurements for triplicate samples are presented.

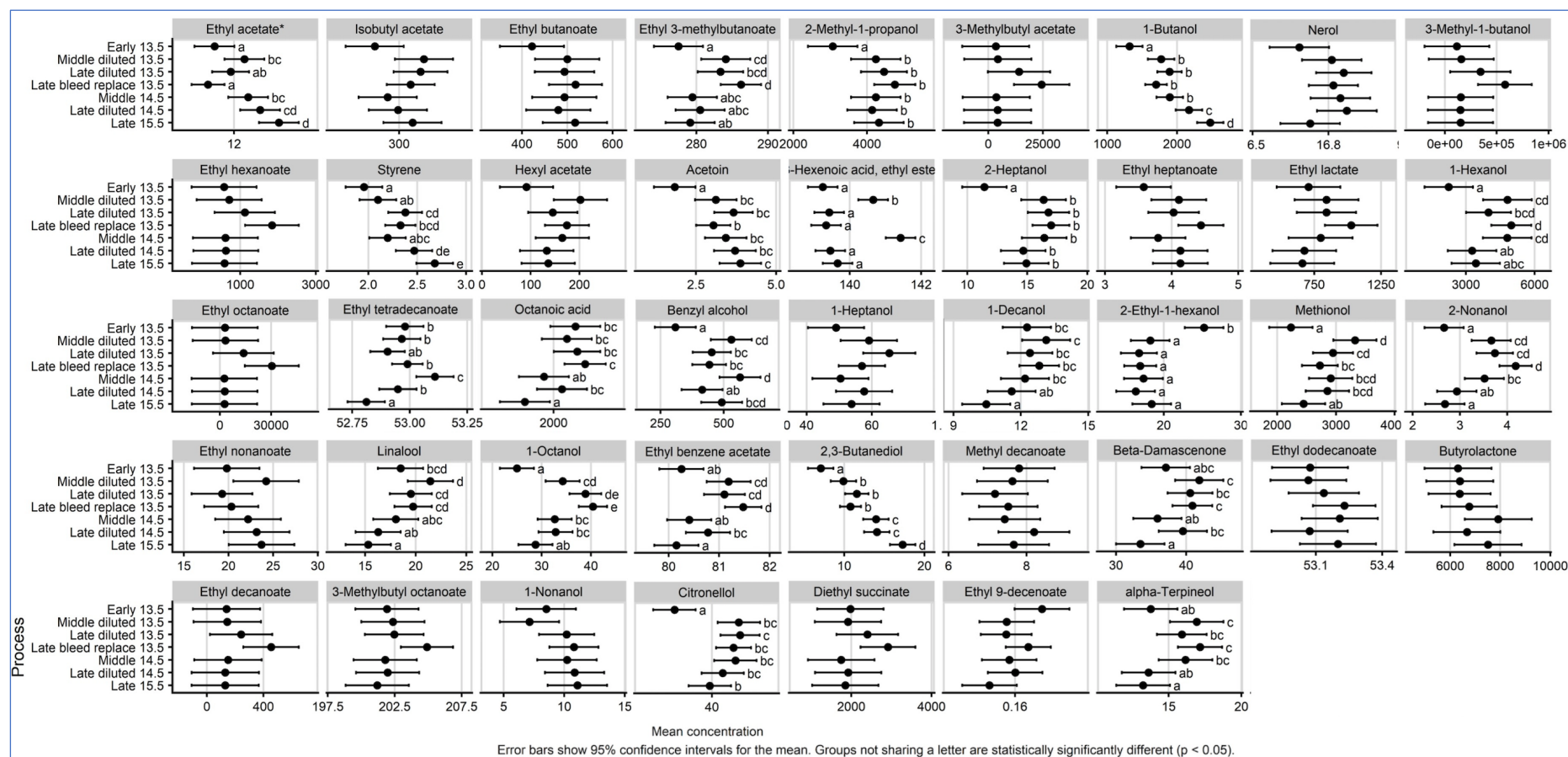
Supp. Table S4.9. Analysis of wine treatments post fermentation (day 5) 2021 (Year 2)

Trial	pH	VA g/L	Lactic Acid g/L	Total sugar g/L	TA g/L	Malic Acid g/L
Early 13.5	3.62	0.42	2.12	1.93	6.75	0.80
Middle 14.5	3.71	0.36	1.93	2.10	6.30	0.90
Middle diluted 13.5	3.70	0.38	1.91	1.96	6.29	0.82
Late	3.71	0.40	1.81	2.07	6.25	1.00
Late diluted 14.5	3.74	0.31	1.68	2.00	6.36	1.01
Late diluted 13.5	3.73	0.39	1.63	1.99	6.37	0.93
Late bleed and replace 13.5	3.72	0.36	1.66	1.90	6.40	0.86

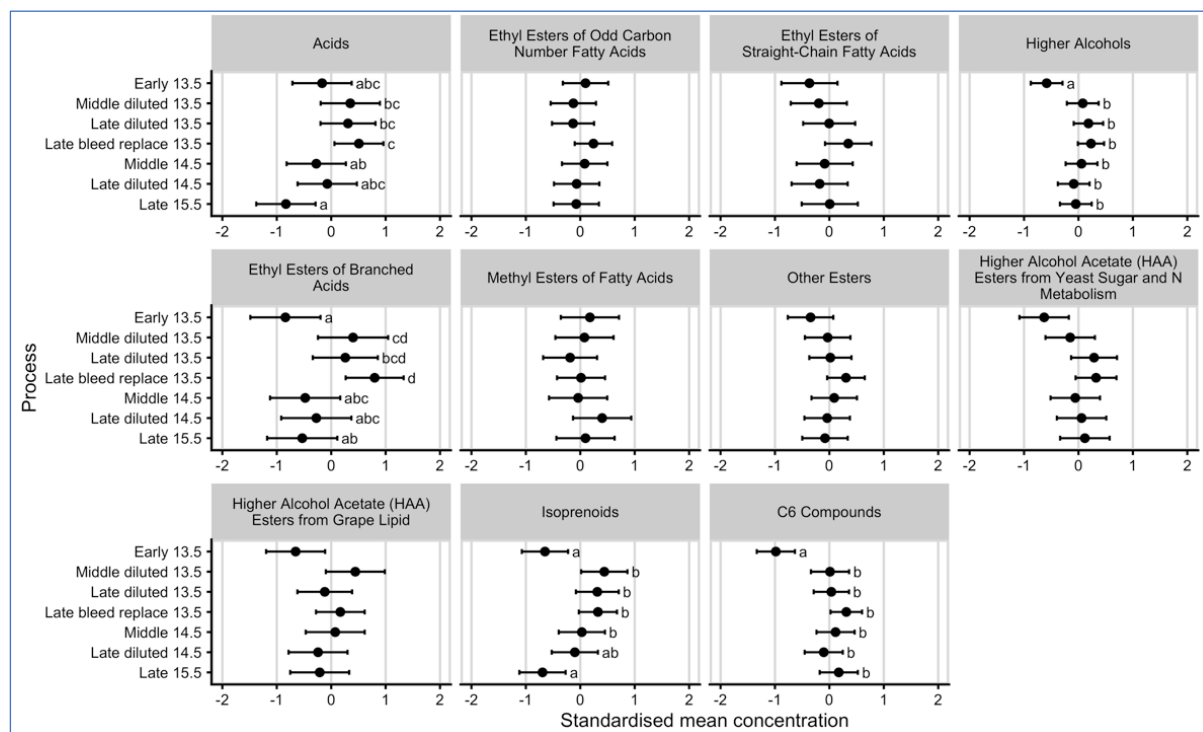
Mean measurements for triplicate samples are presented.

Supp. Table S4.10. Categorisation of volatile aroma groups by GC–MS groupings

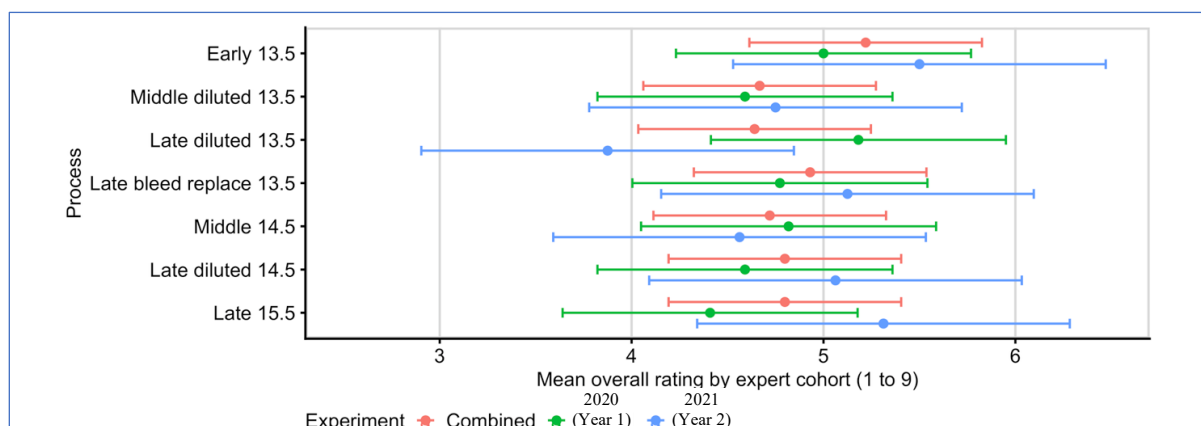
Volatile aroma compound group	Volatile compounds
Acids	Hexanoic acid Octanoic acid
Ethyl esters of odd carbon number fatty acids	Ethyl 9-decenoate Ethyl heptanoate Ethyl nonanoate
Ethyl esters of straight-chain fatty acids	Ethyl decanoate Ethyl hexanoate Ethyl octanoate Ethyl butanoate Ethyl acetate Ethyl dodecanoate
Higher alcohols	1-Butanol 1-Decanol 1-Heptanol 1-Nonanol 1-Octanol 2-Ethyl-1-hexanol 2-Heptanol 2-Methyl-1-propanol 2-Nonanol 3-Methyl-1-butanol Methionol Benzyl alcohol
Ethyl esters of branched acids	Ethyl 3-methylbutanoate
Methyl esters of fatty acids	Methyl decanoate
Other esters	2,3-Butanediol 3-Methylbutyl octanoate Butyrolactone Diethyl succinate Ethyl benzeneacetate Ethyl lactate Ethyl tetradecanoate Isobutyl acetate
Higher alcohol acetate (HAA) esters from yeast sugar and N metabolism	Acetoin 3-Methylbutyl acetate
Higher alcohol acetate (HAA) esters from grape lipid	Hexyl acetate
Isoprenoids	alpha-Terpineol Nerol Linalool Citronellol beta-Damascenone
C6 compounds	Styrene 1-Hexanol



Supp. Figure S4.1. GC–MS volatile aroma compounds of all compounds identified

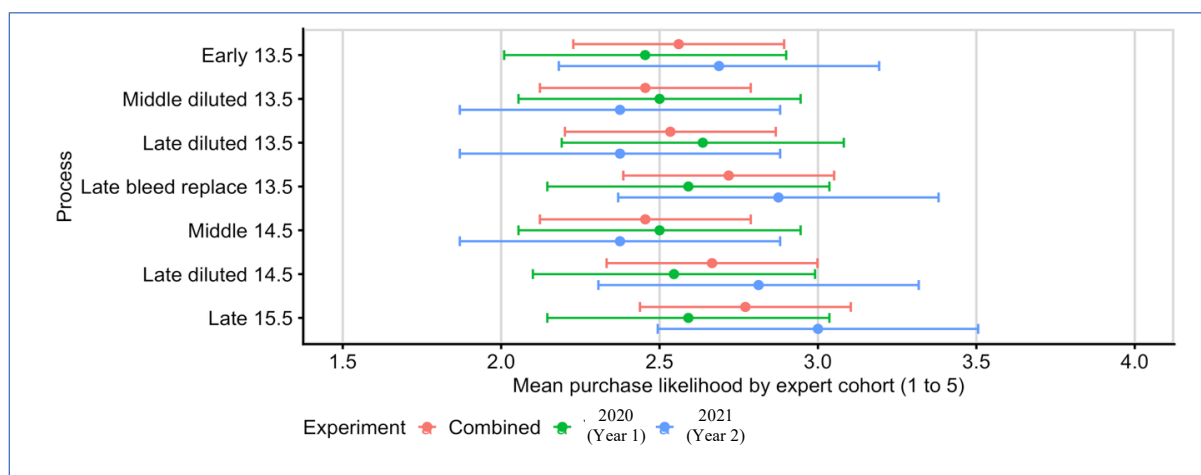


Supp. Figure S4.2. GC–MS volatile aroma compounds in 11 groups for: acids; ethyl esters of odd carbon number fatty acids; ethyl esters of straight-chain fatty acids; higher alcohols; ethyl esters of branched acids; methyl esters of fatty acids; other esters; higher alcohol acetate (HAA) esters from yeast sugar and N metabolism; HAA esters from grape lipid; isoprenoid; and C6 compounds



Supp. Figure S4.3. Overall 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. There are no statistically significant differences between groups ($p < 0.05$).



Supp. Figure S4.4. Purchase likelihood of wines by process (harvest timing and water treatment) and mean purchase likelihood – 2020 (Year 1), 2021 (Year 2) and combined

Error bars show 95% confidence intervals for the mean. There are no statistically significant differences between groups ($p < 0.05$).

Chapter 5 – Amending high sugar in *Vitis vinifera* cv.
Shiraz wine musts by pre-fermentation water
treatments results in subtle sensory differences for
naïve wine consumers

This chapter continues the scientific experimental analysis from Chapter 4, drawing from the same materials and methods for winemaking, for wines produced from the 2020 (Year 1) and 2021 (Year 2) vintage years. In this chapter, I expand on the scientific experiments to further investigate the chemical profiles together naïve wine consumer sensory outcomes for all treatment wines. Just about right (JAR) sensory analysis from a large group of 159 naïve wine consumers (across both vintage years) is relied upon. Measurements and results of concentrations of chemical compounds in the treatment wines is undertaken using analysis of variance (ANOVA) and principal component analysis laboratory techniques. There are limited studies that rely on large sample numbers for sensory panels. The larger sensory panel group in this study provides for a more robust analysis as it increases the statistical power of data collected. The observed outcomes for both chemical levels and correlated sensory results can support winemakers in their quest to produce wines with lower alcohol levels while maintaining favourable chemical and sensory quality attributes, together with the knowledge of purchase likelihood of a wine by typical wine consumers.

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Xynas, B., Barnes, C., & Howell, K. (2024). Amending high sugar in *V. vinifera* cv. Shiraz wine musts by pre-fermentation water treatments results in subtle sensory differences for naïve wine consumers. Accepted for publication in *OENO One*, together with supplementary materials.

Contribution: 80%. Conceptualisation, analysis, investigation, writing and visualisation.

Abstract

Global warming presents challenges to all wine grape growers and winemakers across the globe. Increasing growing season temperatures mean 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 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 [Year 1] and 2021 [Year 2]). Just about right sensory analysis assesses 159 naïve wine consumer panellists' feedback from across both vintage years 2020 (Year 1) and 2021 (Year 2). We show that late 15.5 °Bé and middle 14.5 °Bé wines had the highest levels for chemical compounds of colour density (absorbance units; a.u.), total tannin (%), total pigment (a.u.), total phenolics (a.u.) and free anthocyanins (mg/L), as confirmed by principal component analysis outcomes. Just about right (JAR) sensory analysis shows least preference for vegetal smell (2.50) in 13.5 °Bé wine, with highest preference for colour intensity (3.06) in early 13.5 °Bé wine and for body in late 15.5 °Bé wine. We also tested both chemical and sensory attributes for wines produced from 15.5 °Bé and middle 14.5 °Bé harvests whose pre-fermentation musts were subjected to water dilution or bleed and replace treatments. These wines showed lower final alcohol levels, with decreases in most chemical compound levels; however, the impacts on sensory profiles were less pronounced. For instance, there was 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) was most preferred in late 'bleed and replace' 13.5 °Bé wine. All water treatments had minimal impact for dark fruit smell, ripe fruit smell, odour intensity, odour complexity, red fruit taste and dark fruit taste. Middle diluted 13.5 °Bé wine had the highest overall rating and was 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 a number of chemical compounds, many favourable sensory properties for naïve wine consumers can still be preserved. This is a crucial consideration

for winemakers when addressing wine quality and general wine consumer preferences for wines produced from these winemaking techniques.

Key words: harvest timing, fermentation, water treatment, chemical compounds, sensory attributes, wine quality

Abbreviations

ABV	alcohol by volume
a.u.	absorbance units
Bé	Baumé
g	gram
hL	hectolitre
JAR	just about right
kg	kilogram
K ₂ O ₅ S ₂	potassium metabisulphite
L	litre
MCP	methyl cellulose precipitable
mg	milligram
mL	millilitre
PCA	principal component analysis
ppm	parts per million
QDA	quantitative descriptive analysis
SD	standard deviation
SO ₂	sulphur dioxide
Supp.	supplementary
TL	too little
TM	too much
UV-Vis	ultraviolet–visible spectroscopy
var	variance
v/v	volume by volume

1. Introduction

1.1 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 a number of winemaking approaches available to them to produce wines with lower, more-favourable alcohol levels. These include microbiological yeast approaches (Lemos Junior et al., 2019; Tilloy et al., 2015), blending later harvest wines with green harvest wines (Kontoudakis et al., 2011; Longo et al., 2017), and thermal approaches to removing alcohol post fermentation (Belisario-Sánchez et al., 2009; Gómez-Plaza et al., 1999 Margallo et al., 2015; Massot et al., 2008). Each of these approaches can have both positive and negative outcomes for final wine volatile aromas, 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 wine grape musts before fermentation to reduce ethanol yield during fermentation (Australian Government, 2017; Teng et al., 2020). Early studies showed that this winemaking approach can have varying impacts on final wine volatile aromas, chemical and sensory parameters, depending on grape variety, harvest timings and the type of water treatment implemented. For instance, Piccardo et al. (2019) showed that for *V. vinifera* cv. Merlot noir and cv. Tempranillo Tinto, water treatments reduced ethanol levels, but resulted in significant differences in flavour profiles for each variety (Piccardo et al., 2019). Schelezki, Antalick, et al. (2020) demonstrated that for *V. vinifera* cv. Cabernet Sauvignon, water substitution treatments (at varying levels) of a late harvest 15.5 °Bé high-sugar must reduced alcohol levels in

the final wine by 1.0% to 3.0% alcohol by volume (ABV) compared to a late harvest control wine. For this variety, wine volatile profiles were shown to be markedly affected, decreasing as water levels increased, while wine sensory profiles were observed to be similar to those of an early harvest wine (Schelezki, Antalick, et al., 2020). For *V. vinifera* cv. Shiraz, Schelezki, Deloire, and Jeffrey (2020) demonstrated that implementation rates of 47.2%, 34.0% and 10.2% volume by volume (v/v) of water into a mature fruit harvest must produced lower-alcohol wines with 10.6%/9.6%, 12.0%/11.7% and 14.5%/14.4% ABV levels, respectively. They also showed that for the lowest water addition treatment (+10.2% v/v), wine colour properties were significantly decreased, but tannin concentrations remained stable (Schelezki, Deloire, & Jeffrey, 2020). Schelezki, Antalick, et al. (2020) also demonstrated that water substitution treatments (at varying levels) of a late harvest 15.4 °Bé high-sugar Shiraz must decreased the alcohol levels in the final wines by 0.5% to 2.0% ABV compared to the late harvest control wine. In this study, they observed that tannin levels actually decreased with higher water substitution rates, together with dark fruit attributes tending to diminish with decreasing alcohol levels (Schelezki, Antalick, et al., 2020). Teng et al. (2020) also demonstrated that for Shiraz, water treatments of late harvest high-sugar musts to a 13.5 °Bé produced reductions in ethanol levels of 1.6% v/v to 2.1% v/v compared to the late harvest control wine, but all final water-treated wines displayed unfavourable cooked vegetable and drain attributes. Outcomes from many of the previous studies indicate high variability in results, depending on a number of factors including cultivar, harvest timings, vintage climatic conditions and type of water treatments involved. This indicates that winemakers require additional experimental studies that they can refer to, that address some of the current limitations in the field. Our study further expands on the existing level of scientific knowledge for pre-fermentation water treatment winemaking techniques.

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

Shiraz is an important grape cultivar, recognised as the most-planted red grape variety in Australia (Schelezki, Deloire, & Jeffrey, 2020; Wine Australia, 2023b), but also grown in abundant quantities in many other wine grape growing regions globally, where it is placed as the fourth most planted grapevine cultivar across the grape-growing world. Shiraz has the largest plantings in France, and is France's third most commonly planted wine grape. Notably, Australia ranks second to France, and is home to many of the oldest

Shiraz vineyards in the world, given that many existing Australian Shiraz vines predate the phylloxera epidemic that decimated many European vineyards in the 1800s (Onetto et al., 2023).

Any winemaking approach taken to reduce final alcohol levels in a Shiraz wine while still maintaining wine quality will therefore 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 wines that were subject to varied water treatments of their musts pre fermentation. In this study, we expand on the testing undertaken and results analysed as presented in Chapter 4 of the thesis. In this additional study, all wine treatment groups (in triplicate) are assessed for their chemical compositions, and an additional expanded sensory analysis is undertaken from 159 naïve wine consumer panellists. This approach was taken to further evaluate optimal harvest times and the suitability of different water treatment winemaking approaches into high-sugar Shiraz wine musts. The results can provide further insights for impacts on chemical compounds and the effects on sensory attributes as judged by naïve wine consumers, which can further assist winemakers to determine which wine will maintain or improve final wine quality parameters.

1.3 Global approaches for ‘water-treated wine grape musts’ winemaking to produce wines with lower final alcohol levels

This study predominantly focuses on the Australian approach, where water addition into a wine must is now permitted to ‘facilitate fermentation’ to 13.5 °Bé (Australia New Zealand Food Standards Code – Standard 4.5.1) (Australian Government, 2017). This recently introduced Australian approach was as a response to the problems associated with high ethanol production arising during fermentation (Bindon et al., 2019), and as one answer to address 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 Regulation §24.176) (Alcohol, Tobacco Products and Firearms, 2010). Further, in South Africa, water addition winemaking approaches are permitted, but only where they are deemed to be necessary for the

incorporation of additives (Liquor Products Act 60 of 1989 [SA], 1990). Use of water into pre-fermentation musts is limited in the European Union, where water addition can only be for a ‘specific technical necessity’ and it does not modify the characteristics of the wine (EU Regulation No. 1308/2013) (Christmann et al., 2022; European Parliament, 2013). While the outcomes of this study are relevant for Australian winemakers, should water treatment of pre-fermentation must winemaking techniques become more generally recognised on a global scale, it is important that all winemakers are provided with additional information and supported datasets to better address any related impacts for final wine quality.

2. Materials and methods

2.1 Vintage and vineyard conditions

The study was undertaken across the 2020 and 2021 vintage years. Temperatures and rainfall data from Yarrawonga weather station (located 45 km from Dookie, Victoria; Station number: 081124, Victoria; latitude: 36.03°S; longitude: 146.03°E; elevation: 129 m) and Numurkah weather station (located 33.7 km from Dookie, Victoria; Station number: 80101, Victoria; latitude: 36.09°S; longitude: 145.45°E; elevation: 105 m) (Australian Bureau of Meteorology [BOM], 2023a, 2023b, 2023c) were relied upon and used to calculate the Heliothermal Index of Huglin (HI) (Huglin, 1978) using standard measures (Tonietto & Carbonneau, 2004; Supplementary materials: Supp. Tables S5.1–S5.5).

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, personal communication, 2021 January 11). 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* spp. and *Greenaria* spp. were implemented for both vintages. Spraying for grapevine rust mites was also applied at the woolly bud stage for both vintages (Bernard et al., 2005; Richard Tallis, personal communication, 2021 January 11).

For each vintage, and for the three harvest times – early 13.5 °Bé, middle 14.5 °Bé and late 15.5 °Bé – 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 sampling of 1.0 kg of 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.

2.2 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 groups. Each of the sample groups (in triplicate) were fermented in a 225 L plastic food-grade barrel and were assigned their own identifiers. The first three sample groups were 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 5.1).

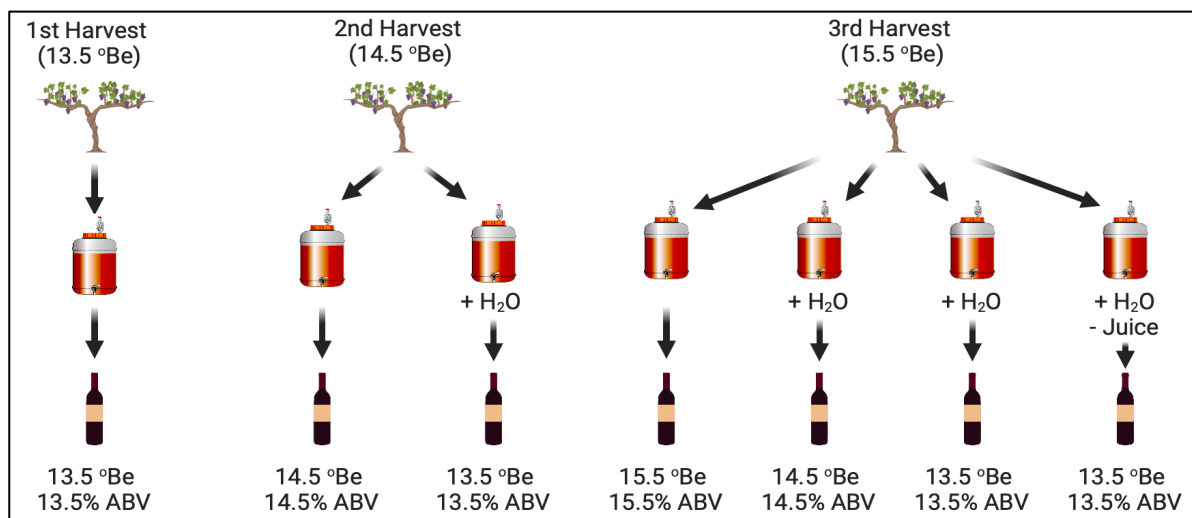


Figure 5.1. Vinification approaches taken in both 2020 (Year 1) and 2021 (Year 2) vintages. Source: Xynas et al., (2024). Accepted for publication at time of PhD Submission.

Two water treatment approaches were taken for the late 15.5 °Baumé (Bé) and middle 14.5 °Bé harvest dates in each year: (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).

Deionised water was used for all water treatments. The additional four 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 (+ 5.3 L 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 (− 9.13 L juice and + 9.13 L water) (late ‘bleed and replace’ 13.5 °Bé). For each vintage, 2100 kg of fruit was picked. Of this, there were seven sample groups (in triplicate) of 100 kg of fruit (21 samples in total), which each produced approximately 65 L of wine; a total of 1365 L of wine was therefore produced for each year.

A standard 50 ppm Sulphur Dioxide (SO₂) (as *Potassium metabisulphite*, K₂O₅S₂, Australian Home Brewing, Victoria, Australia) was added to all samples at crush, and after water treatment for the latter four sample groups, followed by inoculation with Vitilevure Syrah YSEO™ (*Saccharomyces cerevisiae*; Danstar, Rhone Valley, France), a commercial culture of yeast, at 30 g/hL. After 24 hours, all fermentations were inoculated with Martin Vialatte Reflex Malo® 360 (*Oenococcus oeni*; Martin Vialatte, Magenta, France), lactic acid bacteria at 1g/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 and 30 °C, before being pressed. Punch downs were implemented twice daily to homogenise fermentations. Sampling twice daily of 100 mL from each sample group was taken during the fermentation process, which tracked temperature and Bé (Supplementary materials: Supp. Tables S5.8-S5.11). Musts all finished primary alcoholic fermentation on day 5. Acid additions were made post pressing on day 5 to target a titratable acidity of 6.5 g/L tartaric acid (Australian Tartaric Products, Victoria, Australia, 2020). An additional 50 ppm SO₂ (as *Potassium metabisulphite*, K₂O₅S₂, Australian Home Brewing, Victoria, Australia) 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 approximately 18 months.

2.3 Measuring chemical parameters of all sample wines approach

Samples of wines approximately 18 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 & 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 (absorbance units; 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 (AWRI, 2023; Mercurio et al., 2007). 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 this 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.

2.4 Just about right (JAR) sensory analysis approach

Ethics approval was provided from the University of Melbourne's Human Research Ethics Committee (HREC No. 11684) for the just about right (JAR) naïve wine 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 wine consumer sensory analysis data was collected from panels of undergraduate wine students in July 2021 (for the 2020 study) and June 2022 (for the 2021 study). Participants were recruited from students in concurrent winemaking studies at the University of Melbourne, Dookie Campus, during the winter study period 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 between 18 and 21 years of age. Visual, olfactory and mouthfeel sensory attributes were tested. For all wine samples, 80 from 2020 (Year 1) and 79 from

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 just about right (JAR) scores for the panellists (Likert, 1932). For each sensory attribute, panellists ranked the wine samples on a 5-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 likelihood to purchase on a 5-point line scale ranging from extremely unlikely, to neutral, to extremely likely. We combined just about right (JAR) sensory results 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 with the aim of assessing the influence of a JAR category for a given attribute by applying a ‘penalty’ to reflect consumer overall liking of a wine.

2.4.1 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 survey 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 fruits attributes, as well as for vegetal, hotness, astringency, body, viscosity, length and balance sensory parameters (Table 5.1).

Panellists were presented with samples of wines in 30 mL aliquots in coded ISO standard wine glasses, during daylight hours. For all 2020 (Year 1) and 2021 (Year 2) vintage samples, we presented each panellist with seven wine samples all 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.

Table 5.1. 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)	Wine 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	Powdered white tannin
Body	Mouthfeel/Texture	Wine marked by richness, fullness; full-bodied	Powdered 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 0.02gm/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

2.5 Statistical analysis

2.5.1 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, which were measured in both years, experiment year was also included as a blocking factor. Our analytical data is 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 was performed with a full cross validation, plotted by ggbiplot.

2.5.2 Sensory analysis

For sensory data, differences in sensory attributes were determined by linear mixed effects ANOVA, using independent sample group t-test, two-way ANOVA followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$). A separate model was used for each attribute, including overall wine preference. Process and experiment year were included as fixed effects, and taster was 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 that do not share a letter have statistically significant differences between means for that compound.

Penalty analysis was implemented in both years' 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 were the 'just about right' (JAR) scales.

In addition, 'overall liking' data was collected, where the 9-point hedonic scale was used. Panellists were grouped in 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 three categories was 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 were estimated.

Penalties were 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 results are represented graphically. A penalty was not considered significant if the percentage of consumers in the TL or TM categories was less than 20%. This forms the bounds of the critical corner in Figure 5.7 in the results below (>20% of consumers and >1.0-point mean liking drop).

3. Results

3.1 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, BOM, 2023c). Measurements under the HI index, however, classed both seasons as 'temperate warm' (Huglin, 1978; Tonietto & Carbonneau, 2004; Supplementary materials: Supp. Tables S5.1–S5.5). 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, personal communication, 2021 January 11).

3.2 Harvest timings, water treatments and the impacts on chemical compounds in wines

Wine chemical compositions were 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 (%) demonstrated higher average values across all treatment groups in 2021 (Year 2). No discernible differences were 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 were 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 that most disparities between the two years' studies for chemical compounds are mitigated through the amalgamation of the datasets (Figure 5.2; Supplementary materials: Supp. Table S5.6 (2020 [Year 1] and 2021 [Year 2] combined raw data for chemical compounds)).

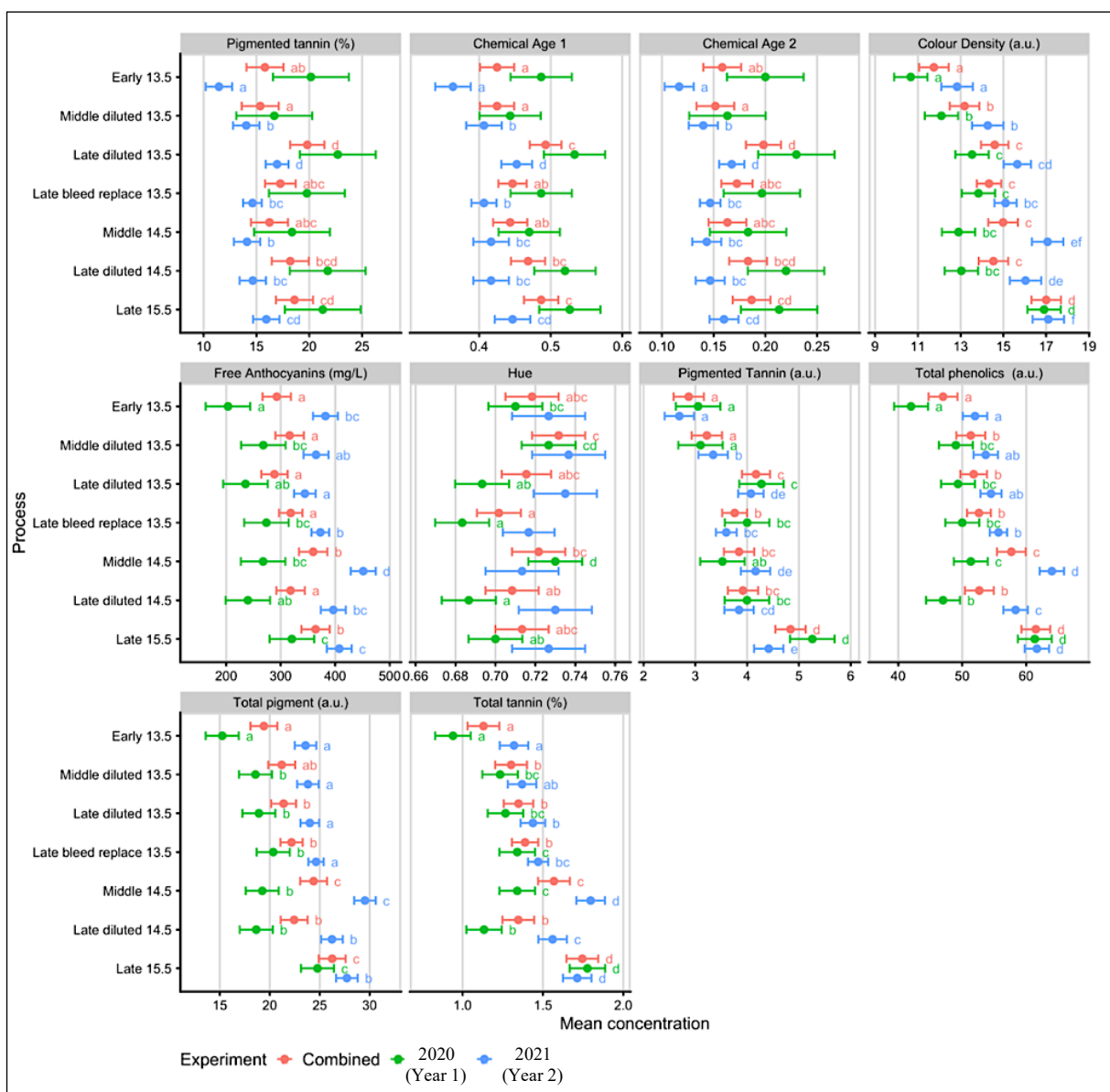


Figure 5.2. Chemical compounds by process and mean concentrations for all wines for 2020 (Year 1) and 2021 (Year 2) and combined

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 ($p < 0.05$).

To further demonstrate the differences in wine chemical analysis, combined chemical data collected for 2020 (Year 1) and 2021 (Year 2) of the study were also analysed by PCA as illustrated in Figure 5.3, where 90.7% of the total variance is explained by the first two dimensions.

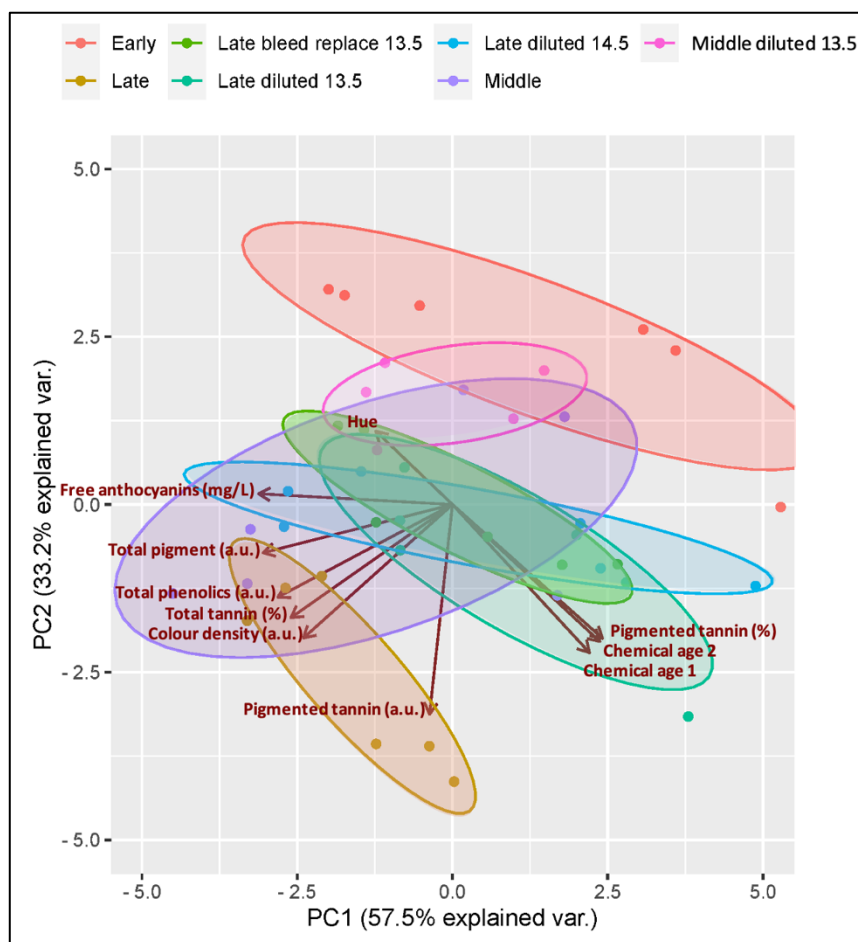


Figure 5.3. Principal component analysis of chemical compound components of Shiraz wine, including: pigmented tannin (%), chemical age 1, chemical age 2, colour density (a.u.), free anthocyanins (mg/L), hue, pigmented tannin (a.u.), total phenolics (a.u.), total pigment (a.u.) and total tannin (%) by treatment group

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, different colours represent different groups. Ellipses represent 68% confidence intervals of core regions. Arrows represent original variables, the directions of arrows represent correlation between original variable and principle components, and lengths represent devotion of original data to principle components.

3.2.1 Late diluted 13.5 °Bé and late diluted 14.5 °Bé wines were 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) 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 5.2).

Notably, while all other water treatments resulted in decreases in pigmented tannin (%), the late diluted 13.5 °Bé treatment significantly increased pigmented tannin (%) levels compared to other water dilution treatments (Figure 5.2). Interestingly, water treatments into both the late 15.5 °Bé and middle 14.5 °Bé harvest pre-fermentation musts did not reduce chemical age 1 or 2 to a statistically significant degree in any of the water treatment groups (Figure 5.2).

PCA results also shows that late diluted 13.5 °Bé and late diluted 14.5 °Bé wines were 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 5.3).

3.2.2 Middle diluted 13.5 °Bé wine had increased wine hue levels, when compared to other treatment groups

Combined chemical data results from 2020 (Year 1) and 2021 (Year 2) show harvest timing had no statistically significant effect on wine hue, but there were differences observed between different water treatments from different harvests. The highest value for wine hue was observed in the middle diluted 13.5 °Bé wine, with the lowest value for wine hue observed in the late ‘bleed and replace’ 13.5 °Bé wine (Figure 5.2). These observations are in line with levels observed in the early 13.5 °Bé wines. Further, PCA indicated that in the space defined by negative Dim 1 and positive Dim 2, middle diluted 13.5 °Bé wine was associated with increased hue (Figure 5.3).

3.2.3 Late 15.5 °Bé and middle 14.5 °Bé wines had highest levels for colour density (a.u.), total tannin (%), total pigment (a.u.) and total phenolics (a.u.)

Combined chemical data results from 2020 (Year 1) and 2021 (Year 2) show show that colour density (a.u.), total tannin (%), total phenolics (a.u.) and total pigment (a.u.) increased significantly from early, to middle and to late harvests. The highest value for all these compounds was observed in the late 15.5 °Bé wine, with the lowest value observed in the early 13.5 °Bé wine (Figure 5.2).

All water treatments reduced 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 5.2). There were no statistical differences in concentration of these compounds between the water treatment groups. The exception to this was colour density (a.u.), where middle diluted 13.5 °Bé wine had a statistically significant decreased colour density (a.u.) when compared to all other water treatment groups (Figure 5.2). It is noted that concentrations of all the above compounds in all treatment groups were still higher than those observed for early 13.5 °Bé wine (Figure 5.2).

Results also show that all water treatments of late 15.5 °Bé and middle 14.5 °Bé harvests decreased free anthocyanin (mg/L) concentrations significantly, where all treatment groups had statistically similar reduced values. Notably, the late diluted 13.5 °Bé wine's free anthocyanin concentrations were observed to be lower than those for the early 13.5 °Bé wine (Figure 5.2). PCA confirmed these observations, where late 15.5 °Bé and middle 14.5 °Bé wines were found in the space defined by negative Dim 1 and negative Dim 2, which was associated with increased colour density (a.u.), total tannin (%), total pigment (a.u.) and total phenolics (a.u.) (Figure 5.3).

3.2.4 Late 15.5 °Bé wines had highest levels for pigmented tannin (a.u.)

Combined chemical data results from 2020 (Year 1) and 2021 (Year 2) show that pigmented tannin (a.u.) levels increased significantly from early, to middle and to late harvests. All water treatments reduced pigmented tannin (a.u.) concentrations to a statistically significant degree when compared to non-treated wines (Figure 5.2). The highest value for wine pigmented tannin (a.u.) was 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 5.2). PCA confirmed these observations, where late 15.5 °Bé wine was found in the space defined by negative Dim 1 and negative Dim 2, which was associated with pigmented tannin (a.u.). Early 13.5 °Bé wines were distributed in the opposite space (Figure 5.3).

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) were observed to be generally consistent across most sensory parameters (Figure 5.4). 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 significant different results (Figure 5.4).

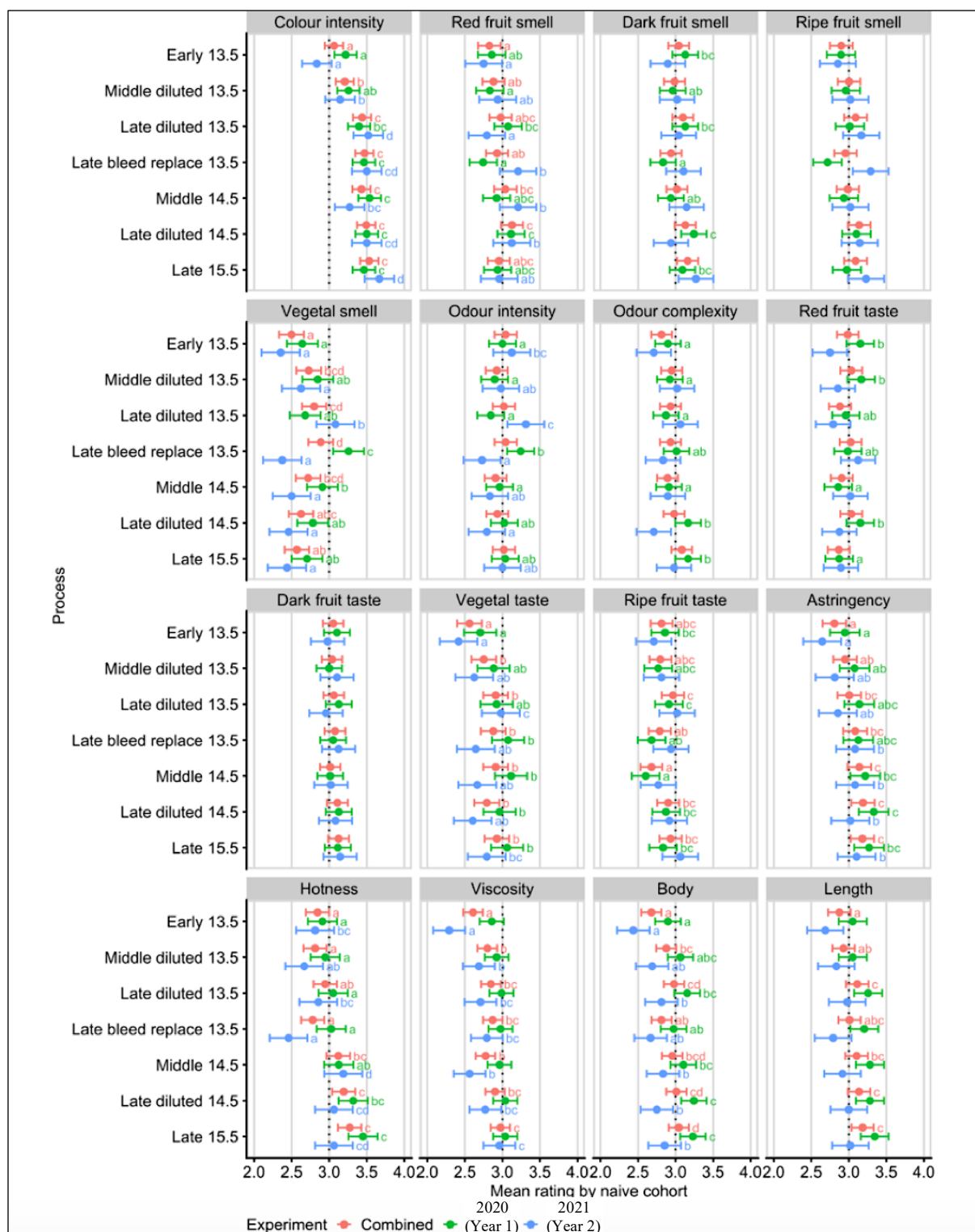


Figure 5.4. Just about right sensory parameters by process (harvest timing or water treatment) and mean rating – 2020 (Year 1) and 2021 (Year 2) combined

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

3.3.1 Wines subject to water treatment of pre-fermentation wine grape must showed less significant impacts for many JAR sensory parameters in wine

JAR analysis outcomes for combined data from 2020 (Year 1) and 2021 (Year 2) 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 or dark fruit taste (Figures 5.4 and 5.5). 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 that for red fruit smell, late 15.5 °Bé, middle 14.5 °Bé and late diluted 13.5 °Bé wines had the greatest preference. However, all treatment groups are clustered closely around the ideal mean value of 3.0, suggesting that panellists generally favoured 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 had the lowest observed vegetal smell preferences, with lowest mean values, where panellists rated the vegetal smell as ‘too low’ for these treatment wines. The highest preference for vegetal smell was observed in the late ‘bleed and replace’ 13.5 °Bé and middle 14.5 °Bé wines, closely followed by middle diluted 13.5 °Bé and late diluted 13.5 °Bé wines, although the differences between all treatment groups were not statistically significant.

For ripe fruit taste and astringency, JAR results show that the late diluted 13.5 °Bé wine had the highest mean values, with panellists indicating highest preference for these two sensory parameters related to this treatment. However, results were not statistically significantly different to other wine treatments (Figures 5.4 and 5.5).

For viscosity, JAR results show statistically significant increases between different harvest treatment groups, with increasing mean values in later harvests. All water treatments resulted in reductions in viscosity; however, none of the reductions observed were statistically significantly different between any of the treatment groups (Figures 5.4 and 5.5).

For the sensory parameter of length, JAR results show statistically significant increases between different harvest treatment groups, with late 15.5 °Bé wine having the highest mean value for length, but this was perceived as ‘too long’ by panellists. All water treatments of the late 15.5 °Bé harvest were effectively able to increase preference for

length, where the late ‘bleed and replace’ 13.5 °Bé wine had the highest preference for length, although these changes were not statistically significant (Figures 5.4 and 5.5). Notably, for vegetal taste, while JAR results show that late 15.5 °Bé wine had the highest preference, this was not significantly different to any other wines (including all water treatment wines), other than the early 13.5 °Bé wine (Figures 5.4 and 5.5).

For the sensory parameter of body, JAR results show that the late 15.5 °Bé wine had the highest preference (Figures 5.4 and 5.5). Water treatments of the late 15.5 °Bé harvest did 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. The late ‘bleed and replace’ 13.5 °Bé wine demonstrated a statistically significant reduction in body preference perception; however, panellists found this ‘too low’ (Figures 5.4 and 5.5).

Further, JAR results show that the early 13.5 °Bé wine had prominent preference for colour intensity, with a mean value of 3.06. In contrast, all other treatment groups exhibited 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 had a lower preference than that of early 13.5 °Bé wine. These findings suggest that all harvests conducted after the early 13.5 °Bé harvest led to an increase in colour intensity, which was not preferred by the panellists (Figures 5.4 and 5.5).

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 had the highest mean ratings for hotness; however, this was 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 showed the lowest mean ratings for hotness, but this was perceived as ‘too low’ by panellists. The late diluted 13.5 °Bé wine had 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, which had a mean value of 3.27 (Figures 5.4 and 5.5).

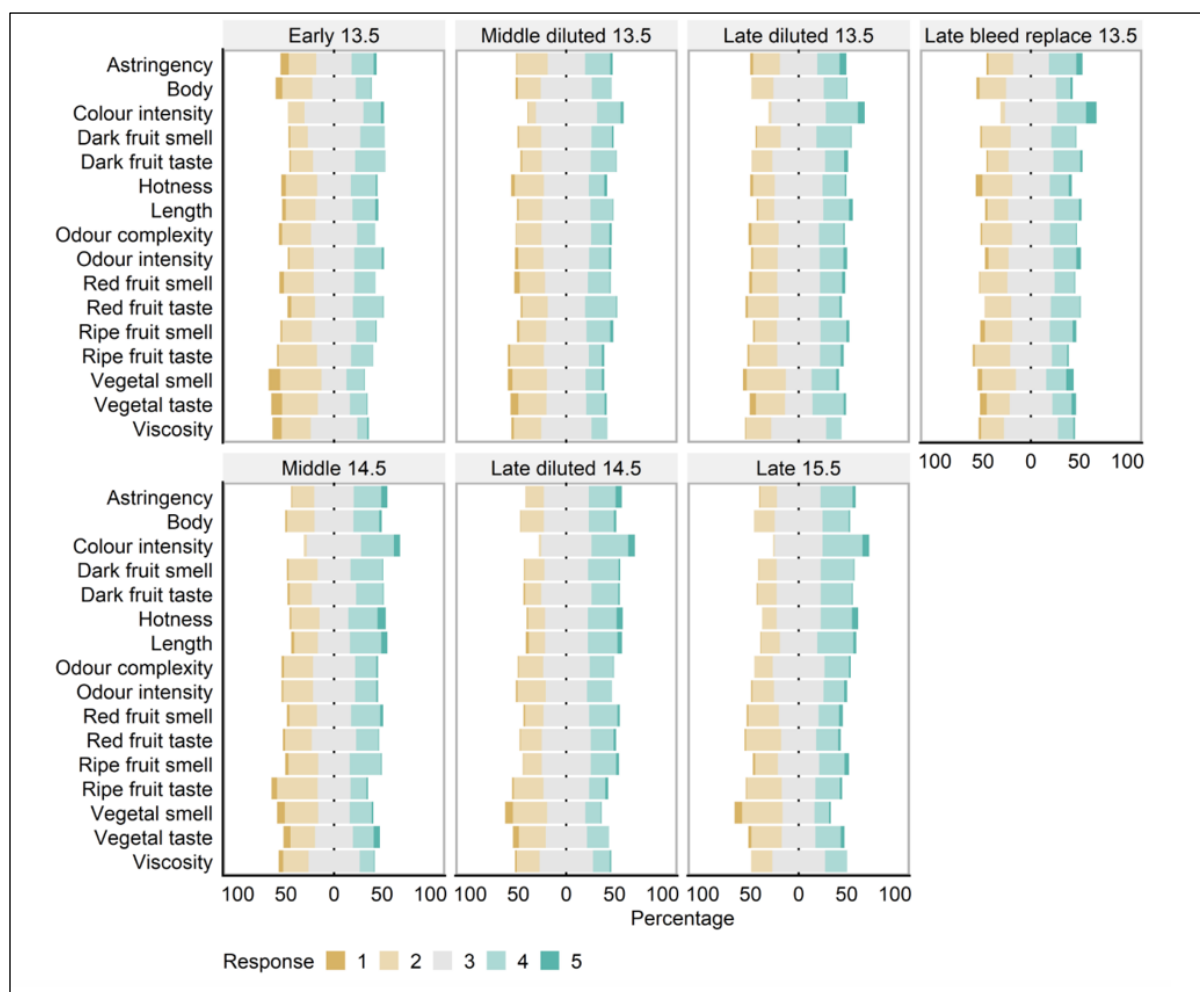


Figure 5.5. Likert plot for JAR sensory parameters and % preference levels – 2020 (Year 1) and 2021 (Year 2) combined

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 S5.7) for the raw JAR 2020 (Year 1) and 2021 (Year 2) combined data to support the result outcomes in Figure 5.5, together with Supp. Figure S5.1, which provides further visual representation of the JAR sensory 2020 (Year 1) and 2021 (Year 2) combined data results as a spider plot.

3.4 Middle diluted 13.5 °Bé wine rated highest in overall assessment

The study also evaluated 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 were 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 varied across years, the statistical

differences between treatments in each year's study closely follow in each other (Figure 5.6).

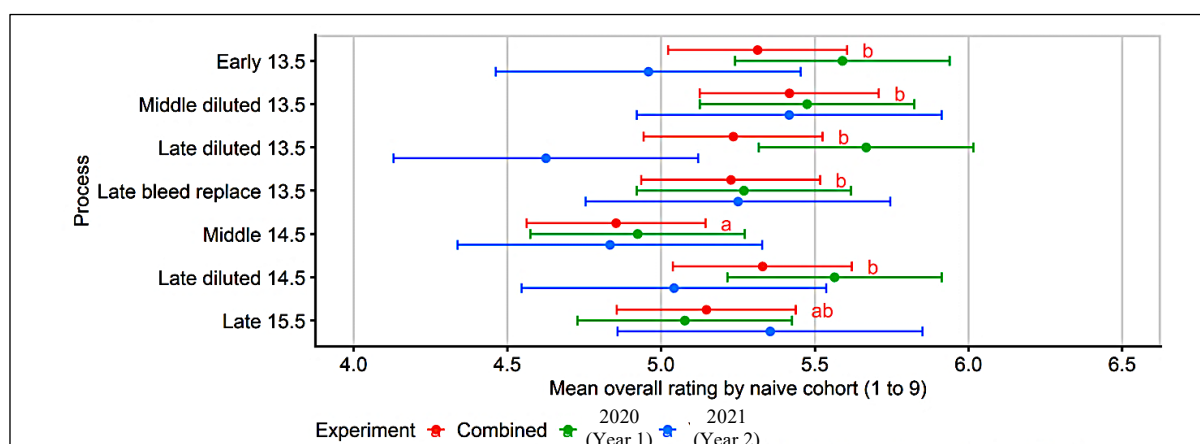


Figure 5.6. Overall preference assessment of wines by process (harvest timing and water treatment) and overall mean rating – 2020 (Year 1), 2021 (Year 2) and combined

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

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 had higher preference ratings compared to the middle 14.5 °Bé wine. In the late 15.5 °Bé harvest, all water treatments demonstrated an increase in preference for overall assessment of wines. However, these increases were not statistically significant. There was a statistically significant increase in overall assessment preference from middle 14.5 °Bé wine to the middle diluted 13.5 °Bé wine, which was the highest-rated wine overall (Figure 5.6).

3.5 Penalty analysis – ‘too much’ hotness falls in the critical corner, where ‘hotness’ was most preferred for late diluted 13.5 °Bé wine

Combined data results from 2020 (Year 1) and 2021 (Year 2) indicate that ‘too much’ hotness is the only wine sensory attribute that falls into the critical corner (Figure 5.7). ‘Too much’ of this attribute in a wine had the most negative impact on overall liking when observed in the JAR scale. The highest mean ratings for hotness were observed in late 15.5 °Bé, late diluted 14.5 °Bé and middle 14.5 °Bé wines. This represents all wines with final percentage alcohol of 14.5% ABV or 15.5% ABV. For these wines, panellists perceived the hotness as ‘too high’. Conversely, lowest mean ratings for hotness were 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 had a final percentage alcohol of

13.5% ABV. The highest preference for hotness was observed in the late diluted 13.5 °Bé wine. While this treatment brought the preference rating closer to the ideal mean value of 3.0, the result shifted from ‘too high’ to ‘too low’, indicating that the reduction in hotness by this water treatment was too significant. The late diluted 14.5 °Bé wine moved the mean closer to 3.0; however, this reduction was not statistically significant. Furthermore, the perception of hotness in the middle diluted 13.5 °Bé wine was also reduced; however, this reduction shifted the preference rating further from the ideal mean value where it was still considered as ‘too low’ (Figure 5.7).

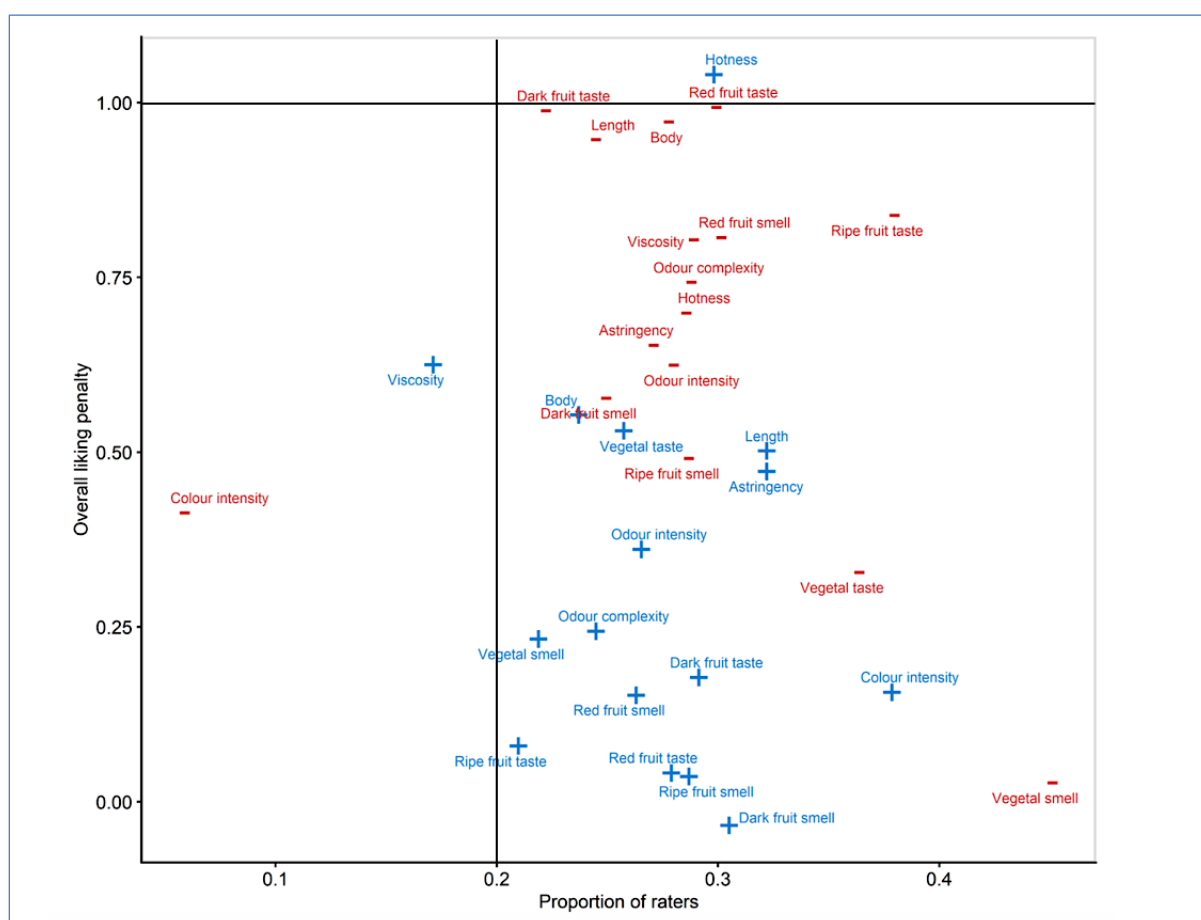


Figure 5.7. Overall liking penalty and proportion of raters for sensory attributes – 2020 (Year 1) and 2021 (Year 2) combined

‘+’ 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.

4. 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 single-year datasets (Riffle et al., 2022; Serdar et al., 2021). This combination is appropriate as there were only slight variabilities between grape-growing seasons of 2020 and 2021, with both classified as ‘temperate warm’ under the HI (Huglin, 1978). Importantly too, grape quality, health and vineyard management between these years showed little variance between these years. Moreover, we used identical winemaking and fermentation approaches for both years.

Our findings are supported by previous studies which indicated that varying harvest times in a vintage can affect final alcohol levels in a wine and impact the chemical compounds and sensory attributes (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020). Additionally, our results are further supported by earlier studies which demonstrated that water treatment of high-sugar musts before fermentation can produce wines with lower final alcohol levels (Piccardo et al., 2019; Teng et al., 2020). However, it has been noted that this winemaking technique is often accompanied by a negative perception that an inferior wine will be produced (Schelezki, Deloire, & Jeffrey, 2020). Our results are further supported by earlier study outcomes regarding some observed negative effects on chemical compounds for water-treated wines (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020). However, our results expand on these earlier studies with an extensive sensory analysis approach undertaken, which demonstrates that the impacts on several crucial sensory attributes are not as significant as indicated by many of the chemical compound changes observed. In this regard, sensory attributes of a final wine are important as they provide for its perceived quality, preference and purchase likelihood (Teng et al., 2020). Given the larger than typical wine treatment sample group volume and the larger than typical sensory panellist size, our results may be more readily transferable from the laboratory to commercial applications, where previous studies may be limited in this regard (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020).

4.1 Early 13.5 °Bé winemaking adversely affected 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; we found that all chemical attributes were at their lowest levels in these wines, except for wine hue (which was consistent across all different harvest timings) (Figure 5.2). PCA results confirm these observations (Figure 5.3). For the sensory parameters of red fruit smell, vegetal smell, vegetal taste, astringency, viscosity, length and body, lowest mean ratings were observed for the early 13.5 °Bé wines (Figure 5.4). 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 found that only one sensory parameter, colour intensity, was 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 5.4). This result may be attributed to Shiraz being a full-bodied red wine, which can have high colour intensity present even in early harvest wines. While flavonoid compounds in grape berries, which can contribute to colour intensity, may be influenced by viticultural and environmental factors and/or the degree of berry ripeness, they can also be determined by genetic factors (variety) (Gómez-Míguez et al., 2007). 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 that are associated with a later harvest is not possible, together with associated negative impacts from early harvest winemaking on a higher number of final wine sensory characteristics.

4.2 Late 15.5 °Bé winemaking produced wines with 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 study findings which demonstrate that harvesting late will produce wines with higher alcohol levels and higher levels of most chemical compounds (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020) (Figure 5.2). Our PCA results also show that late 15.5 °Bé wines and middle 14.5 °Bé wines were 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 had many less-preferred sensory attributes, except for vegetal taste and red fruit taste (Figure 5.4). 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 5.4), together with the production of higher levels of less-favourable chemical compounds (Figures 5.2 and 5.3), 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.

4.3 Water treatments of late 15.5 °Bé harvest must reduced alcohol levels and adversely affected many chemical compounds, while retaining many positive sensory attributes in final wines

The findings are supported by previous studies that show water treatments of late 15.5 °Bé must can produce wines with lower alcohol levels, but with many chemical compounds being adversely affected (Petrie et al., 2019; Schelezki, Smith, et al., 2018; Schelezki, Šuklje, et al., 2018; Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020) (Figures 5.2 and 5.3). Notably, we show that adverse impacts on many more sensory attributes are not as pronounced as some impacts on chemical compounds would suggest (Figures 5.4 and 5.5).

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

For late diluted 13.5 °Bé wine, reductions in several chemical compounds were observed (Figures 5.2 and 5.3). Importantly, our findings agree with previous studies that show that total phenolics will decrease in wines that have been subjected to water treatment of their musts pre fermentation (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020). The lowest level for total phenolics was observed in the late diluted 13.5 °Bé wine, although this was not statistically significantly 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 (Aleixandre-Tudó et al., 2016). Interestingly, JAR sensory results indicate that late diluted 13.5 °Bé wine had the highest preference for correlated ripe fruit taste and red fruit smell (Figures 5.4 and 5.5), 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, Smith, et al., 2018; Schelezki, Šuklje, et al., 2018). 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 late diluted 13.5 °Bé 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 (Cadot et al., 2012; Schraidt, 2009). Interestingly, penalty analysis shows that the late diluted 13.5 °Bé wine had a preference rating for hotness closer to the ideal mean value of 3.0; however, the result shifted from ‘too high’ to ‘too low’, indicating that the reduction in hotness by this water treatment into a late 15.5 °Bé harvest was 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 study findings (Teng et al., 2020), lower levels of free anthocyanins for young wines such as ours should not be of significant concern for winemakers. This is because free anthocyanins, which are highly coloured compounds responsible for the colour of red grapes, will play less of a role in producing red colour in the wines as they age (Heredia et al., 1998; Somers, 1971).

The results also show that chemical age levels are similar in both chemical age 1 and chemical age 2 across all harvest wines and water treatments (Figures 5.2 and 5.3). Chemical age can be defined as the ratio of wine aging, 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 sulphur dioxide (SO₂) 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 occurred for these chemical attributes regardless of treatments implemented.

Interestingly, the late diluted 13.5 °Bé wine had significantly increased pigmented tannin (%) levels compared to other water treatment wines (Figures 5.2 and 5.3). 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 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 had 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 wine grape 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.

4.3.2 Late diluted 14.5 °Bé and late 'bleed and replace' 13.5 °Bé winemaking maintained fewer favourable sensory quality attributes in wine

In order to produce a wine with a middle of the range 14.5% ABV, less quantities of water is added to the late harvest must. We found that this winemaking approach resulted in lower levels of most chemical parameters when compared to a late 15.5 °Bé wine; however, the reductions were not significantly different between all water treatments of the late 15.5 °Bé harvest (Figure 5.2). PCA results also confirm 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 5.3). Notably for the late diluted 14.5 °Bé wine, the JAR sensory parameter of hotness was still rated ‘too high’ (Figures 5.4 and 5.5). Thus, we find that this winemaking approach cannot effectively address unfavourable higher hotness attributes generally associated with wines produced from late harvests.

We also implemented the alternative late ‘bleed and replace’ 13.5 °Bé saignée winemaking approach, where juice is bled off from the fermentation, concentrating the must (Cassasa et al., 2021), and is then replaced with equal amounts of water to affect ethanol production during fermentation (Harbertson et al., 2009). Chemical results show that 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 5.2). This is an important consideration for winemakers because wine colour and associated wine quality parameters can be attributed to pigmented tannins (a.u.), which are 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 wine grape must juice will result in less tannins left in the wine must to react with anthocyanins. Impacts from this winemaking approach on JAR sensory parameters were also varied, with a statistically significant reduction in body preference perception observed; however, this was rated by panellists as ‘too low’ (Figures 5.4 and 5.5). However, panellists rated this treatment wine as having the highest preference for length, although it was 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 wine grape musts of late harvests.

4.4 Middle diluted 13.5 °Bé winemaking produced wines that were most preferred overall

Wines produced from a middle 14.5 °Bé harvest and a middle diluted 13.5 °Bé treatment represent two additional alternative winemaking approaches to producing a final wine with lower alcohol levels. These winemaking approaches may be more effective in

preserving many chemical compounds and favourable sensory attributes in a final wine. This can be related to middle 14.5 °Bé wine having a lower starting Bé than a late harvest, but a higher Bé than an early harvest, and also to less water added into the pre-fermentation must for the middle diluted 13.5 °Bé wine. 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.

Results from our study show that unfavourable impacts on chemical compounds were 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 were less adversely affected when compared to those observed in late diluted 13.5 °Bé wines (Figure 5.2). In addition, the highest value for wine hue was observed in the middle diluted 13.5 °Bé wine (Figures 5.2 and 5.3). These observations are in line with those for early 13.5 °Bé wine.

In addition, chemical results show middle diluted 13.5 °Bé wine had 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’ was noted for this treatment; however, panellists indicated a lower preference for this attribute when compared to early 13.5 °Bé wine (Figures 5.4 and 5.5). 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, Antalick, et al., 2020). 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 consumer overall assessment preference of wines. We found that middle diluted 13.5 °Bé wine had the highest preference ratings (Figure 5.6).

These observed outcomes can assist winemakers in assessing whether the water treatment of a middle 14.5 °Bé harvest winemaking approach can be an effective winemaking approach which can 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.

4.5 Many consistent sensory attributes were found across all harvest timings and water treatments implemented

Importantly we also found that there were 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 5.4). 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.

5. Limitations

The study was an Australian one, where wine regulations now permit water treatment of pre-fermentation wine grape musts to facilitate fermentation to regulated levels (Australian Government, 2017). It is acknowledged that this winemaking approach is currently limited in other winemaking 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 have an impact on 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 (Schelezkki, Antalick, et al., 2020), which means that even a poor-quality wine can be assessed as a preferred wine, depending on consumer ‘likings’. Nonetheless, naïve wine consumers can provide winemakers with an informed perspective as to what typical wine consumers expect from a wine, so that they can adjust their winemaking and distribution approaches accordingly, based on economic, social and health considerations.

6. Conclusion

Water treatment into late 15.5 °Bé and middle 14.5 °Bé harvests winemaking can produce wines with preferred lower final alcohol levels. We demonstrate that these winemaking techniques can address some of the unfavourable higher levels of 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 found that the impacts on wine sensory profiles were not as striking as the wine chemical compositional modifications would seem to indicate for a number of the water treatments implemented. While water dilution treatment into a late 15.5 °Bé harvest had the most impact on chemical and sensory attributes in the final wines, many of the wine sensory qualities as determined by the harvest date appeared to be maintained. Overall, naïve wine sensory panellists in this study indicated 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 that are favoured by mainstream wine consumers.

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Chapter 5 Supplementary materials

Supp. Table S5.1. The coefficient length of day (d) by latitude for the HI Index

Latitude	Length of day coefficient (d)
<40°00'	1.00
40°01' – 42°00'	1.02
42°01' – 44°00'	1.03
44°01' – 46°00'	1.04
46°01' – 48°00'	1.05
48°01' – 50°00'	1.06

Source: Tonietto and Carbonneau, 2004.

Supp. Table S5.2. Classes of viticultural climate for the heliothermal index of the grape-growing regions

Index	Class of viticultural climate	Acronym	Class interval
Heliothermal index, HI	Very warm	HI + 3	>3000
	Warm	HI + 2	>2400 ≤ 3000
	Temperate warm	HI + 1	>2100 ≤ 2400
	Temperate	HI – 1	>1800 ≤ 2100
	Cool	HI – 2	>1500 ≤ 1800
	Very cool	HI – 3	≤1500

Source: Tonietto and Carbonneau, 2004.

Supp. Table S5.3: Monthly mean maximum temperature for Yarrawonga weather station (located 45 km from Dookie, Victoria) – Station number: 081124, Victoria; latitude: 36.03°S; longitude: 146.03°E; elevation: 129 m

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2018	35.3	32.8	29.4	27.2	18.9	14.6	14.3	14.9	19.4	25.2	27.4	32.8	24.3
2019	37.2	32.0	29.3	24.9	17.4	14.7	13.8	14.3	18.7	25.7	27.1	33.1	24.0
2020	33.2	31.1	26.6	21.3	16.7	13.8	13.8	14.5	19.1	23.3	30.7	29.9	22.8
2021	32.4	30.0	26.0	23.0	18.9	13.7	12.8	15.5	18.5	21.0	24.0	30.5	22.2

Source: Australian Bureau of Meteorology (2023a)

Supp. Table S5.4. Monthly mean minimum temperature for Yarrawonga weather station (located 45 km from Dookie, Victoria) – Station number: 081124, Victoria; latitude: 36.03°S; longitude: 146.03°E; elevation: 129 m

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2018	17.2	15.5	12.3	10.1	6.0	3.3	2.7	2.7	2.9	8.0	11.3	16.7	9.1
2019	19.7	15.7	14.6	10.1	7.2	3.5	4.5	1.8	3.7	7.1	10.3	14.1	9.4
2020	15.7	16.4	12.8	9.6	5.0	3.5	2.7	3.5	6.1	9.0	12.6	12.3	9.1
2021	15.5	14.3	11.6	7.5	5.8	4.8	4.2	3.7	5.2	6.6	10.3	12.4	8.5
2022	17.7	15.0	14.0	11.2	7.0	4.3	1.0	4.5	5.6	9.4	10.1	12.3	9.3

Source: Australian Bureau of Meteorology (2023b)

Supp. Table S5.5. Monthly rainfall (mm) for Numurkah weather station (located 33.7 km from Dookie, Victoria) – Station number: 80101, Victoria; latitude: 36.09°S; longitude: 145.45°E; elevation: 105 m

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2019		5.4		9.4	49.4	42.4	54.3	20.6	11.2	7.1	43.1	0.0	
2020	67.9	20.0	69.6	105.2	28.8	22.8	30.8	50.6	16.1	50.6	21.0	12.7	496.1
2021	69.6	10.6	70.7	4.2	21.0	64.7	46.0	27.0	79.2	19.2	59.6	9.0	480.8
2022	78.2	9.4	45.2	70.6		38.2	20.2	63.8	90.3	175.8	77.4	74.8	

Source: Australian Bureau of Meteorology (2023c)

Supp. Table S5.6. Harvest time and water must treatment effects on measures of pigmented tannin (%), chemical age 1 and 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 (%) – combined data results for 2020 (Year 1) and 2021 (Year 2)

Phenolic compound Treatment	Early 13.5 °Bé			Middle 14.5 °Bé			14.5 °Bé middle diluted 13.5 °Bé			Late 15.5 °Bé			15.5 °Bé late diluted 13.5 °Bé			15.5 °Bé late diluted 14.5 °Bé			15.5 °Bé late bleed and replace 13.5 °Bé		
	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI
Pigmented tannin (%)	15.80	0.86	14.05, 17.55 ab	16.23	0.86	14.48, 17.98 abc	15.36	0.86	13.61, 17.11 a	18.60	0.86	16.85, 20.35 cd	19.81	0.80	18.19, 21.43 d	18.20	0.86	16.45, 19.95 bcd	17.26	0.71	15.82, 18.71 abc
Chemical age 1	0.43	0.01	0.40, 0.45 a	0.44	0.01	0.42, 0.47 ab	0.43	0.01	0.40, 0.45 a	0.49	0.01	0.46, 0.51	0.49	0.01	0.47, 0.52 c	0.47	0.01	0.44, 0.49 bc	0.45	0.01	0.43, 0.47 ab
Chemical age 2	0.16	0.01	0.14, 0.18 ab	0.16	0.01	0.15, 0.18 abc	0.15	0.01	0.13, 0.17 a	0.19	0.01	0.17, 0.20 cd	0.20	0.01	0.18, 0.22 d	0.18	0.01	0.17, 0.20 bcd	0.17	0.01	0.16, 0.19 abc
Colour density (a.u.)	11.75	0.34	11.06, 12.44 a	14.98	0.34	14.29, 15.67	13.18	0.34	12.49, 13.87 b	17.00	0.34	16.31, 17.69 d	14.59	0.32	13.95, 15.23 c	14.53	0.34	13.84, 15.22 c	14.33	0.28	13.75, 14.90 c
Free anthocyanins (mg/L)	292.67	12.85	266.65, 318.68 a	359.50	12.85	333.49, 385.51 b	316.33	12.85	290.32, 342.35 a	363.83	12.85	337.82, 389.85 b	288.41	11.92	264.29, 312.53 a	318.00	12.84	291.99, 344.01 a	318.28	10.61	296.81, 339.76 a
Hue	0.72	0.01	0.70, 0.73 abc	0.72	0.01	0.71, 0.74 bc	0.73	0.01	0.72, 0.75 c	0.71	0.01	0.70, 0.73 abc	0.72	0.01	0.70, 0.73 abc	0.71	0.01	0.69, 0.72 ab	0.70	0.01	0.69, 0.71 a
Pigmented tannin (a.u.)	2.87	0.14	2.58, 3.16 a	3.84	0.14	3.55, 4.14 bc	3.22	0.14	2.93, 3.51 a	4.84	0.14	4.55, 5.13 d	4.17	0.13	3.90, 4.44 c	3.92	0.14	3.63, 4.21 bc	3.67	0.12	3.52, 4.00 b
Total phenolics (a.u.)	47.00	1.11	44.75, 49.25 a	57.67	1.11	55.41, 59.92 c	51.33	1.11	49.08, 53.59 b	61.50	1.11	59.25, 63.75 d	51.78	1.03	49.70, 53.87 b	52.67	1.11	50.92, 54.92 b	52.61	0.92	50.75, 54.47 b
Total pigment (a.u.)	19.41	0.66	18.07, 20.75	24.39	0.66	23.04, 25.73	21.19	0.66	19.85, 22.53 ab	26.25	0.66	24.90, 27.59 c	21.38	0.62	20.14, 22.63 b	22.44	0.66	21.09, 23.78 b	22.18	0.55	21.07, 23.29 b
Total tannin (%)	1.13	0.05	1.03, 1.23 a	1.57	0.05	1.47, 1.67 c	1.30	0.05	1.20, 1.40 b	1.74	0.05	1.65, 1.84 d	1.35	0.05	1.26, 1.44 b	1.35	0.05	1.25, 1.45 b	1.39	0.04	1.31, 1.47 b

Mean measurements for triplicate samples are presented. Treatment groups that do not have statistically significant differences ($p < 0.05$) in these plots will share the same letter (95% CI); those processes that are statistically significantly different will have different letters. For the cells where no letters are present, this indicates no overall difference between any treatment groups/processes for that attribute.

Supp. Table S5.7. Just about right naïve wine consumer sensory analysis raw data, by process (treatment group) and sensory attributes, combined results for 2020 (Year 1) and 2021 (Year 2)

Process	Mean	SE	df	95 % CI	group	Process	Mean	SE	df	95 % CI	group
Colour intensity						Vegetal taste					
Early 13.5	3.06	0.06	505	2.94, 3.19	a	Early 13.5	2.56	0.08	512	2.40, 2.73	a
Middle diluted 13.5	3.21	0.06	505	3.09, 3.33	b	Middle diluted 13.5	2.75	0.08	512	2.59, 2.92	b
Late diluted 13.5	3.44	0.06	505	3.32, 3.56	c	Late diluted 13.5	2.91	0.08	512	2.74, 3.08	b
Late bleed replace 13.5	3.47	0.06	505	3.35, 3.59	c	Late bleed replace 13.5	2.88	0.08	512	2.71, 3.04	b
Middle 14.5	3.43	0.06	505	3.31, 3.55	c	Middle 14.5	2.91	0.08	512	2.74, 3.08	b
Late diluted 14.5	3.49	0.06	505	3.37, 3.61	c	Late diluted 14.5	2.79	0.08	512	2.63, 2.96	b
Late 15.5	3.53	0.06	505	3.41, 3.65	c	Late 15.5	2.93	0.08	512	2.76, 3.09	b
Red fruit smell						Ripe fruit taste					
Early 13.5	2.83	0.08	726	2.68, 2.97	a	Early 13.5	2.81	0.07	558	2.67, 2.96	abc
Middle diluted 13.5	2.88	0.08	726	2.73, 3.03	ab	Middle diluted 13.5	2.80	0.07	558	2.65, 2.94	abc
Late diluted 13.5	2.98	0.08	726	2.83, 3.12	abc	Late diluted 13.5	2.96	0.07	558	2.82, 3.11	c
Late bleed replace 13.5	2.93	0.08	726	2.78, 3.08	ab	Late bleed replace 13.5	2.79	0.07	558	2.64, 2.94	ab
Middle 14.5	3.04	0.08	726	2.89, 3.19	bc	Middle 14.5	2.68	0.07	558	2.53, 2.83	a
Late diluted 14.5	3.13	0.08	726	2.98, 3.28	c	Late diluted 14.5	2.90	0.07	558	2.75, 3.05	bc
Late 15.5	2.95	0.08	726	2.80, 3.10	abc	Late 15.5	2.93	0.07	558	2.79, 3.08	bc
Dark fruit smell						Astringency					
Early 13.5	3.04	0.07	694	2.90, 3.18		Early 13.5	2.81	0.08	552	2.65, 2.96	a
Middle diluted 13.5	2.99	0.07	694	2.85, 3.12		Middle diluted 13.5	2.95	0.08	552	2.79, 3.11	ab
Late diluted 13.5	3.10	0.07	694	2.96, 3.24		Late diluted 13.5	3.01	0.08	552	2.85, 3.16	bc
Late bleed replace 13.5	2.94	0.07	694	2.80, 3.08		Late bleed replace 13.5	3.09	0.08	552	2.93, 3.24	bc
Middle 14.5	3.02	0.07	694	2.88, 3.16		Middle 14.5	3.14	0.08	552	2.98, 3.30	c
Late diluted 14.5	3.13	0.07	694	2.99, 3.27		Late diluted 14.5	3.19	0.08	552	3.03, 3.35	c
Late 15.5	3.16	0.07	694	3.02, 3.30		Late 15.5	3.18	0.08	552	3.02, 3.34	c
Ripe fruit smell						Hotness					
Early 13.5	2.90	0.08	682	2.75, 3.05		Early 13.5	2.84	0.08	624	2.69, 3.00	a
Middle diluted 13.5	3.00	0.08	682	2.85, 3.15		Middle diluted 13.5	2.81	0.08	624	2.66, 2.97	a
Late diluted 13.5	3.09	0.08	682	2.94, 3.24		Late diluted 13.5	2.95	0.08	624	2.79, 3.10	ab
Late bleed replace 13.5	2.96	0.08	682	2.81, 3.11		Late bleed replace 13.5	2.78	0.08	624	2.62, 2.94	a
Middle 14.5	2.99	0.08	682	2.84, 3.14		Middle 14.5	3.12	0.08	624	2.97, 3.28	bc
Late diluted 14.5	3.14	0.08	682	2.99, 3.29		Late diluted 14.5	3.19	0.08	624	3.04, 3.35	c
Late 15.5	3.09	0.08	682	2.94, 3.24		Late 15.5	3.27	0.08	624	3.12, 3.43	c
Vegetal smell						Viscosity					
Early 13.5	2.50	0.08	638	2.33, 2.66	a	Early 13.5	2.61	0.07	559	2.48, 2.74	a
Middle diluted 13.5	2.73	0.08	638	2.56, 2.89	bcd	Middle diluted 13.5	2.80	0.07	559	2.67, 2.93	b
Late diluted 13.5	2.80	0.08	638	2.64, 2.96	cd	Late diluted 13.5	2.85	0.07	559	2.72, 2.98	bc
Late bleed replace 13.5	2.89	0.08	638	2.72, 3.05	d	Late bleed replace 13.5	2.87	0.07	559	2.74, 3.00	bc
Middle 14.5	2.72	0.08	638	2.56, 2.88	bcd	Middle 14.5	2.78	0.07	559	2.65, 2.90	b
Late diluted 14.5	2.62	0.08	638	2.46, 2.79	abc	Late diluted 14.5	2.90	0.07	559	2.77, 3.03	bc
Late 15.5	2.57	0.08	638	2.41, 2.73	ab	Late 15.5	2.97	0.07	559	2.84, 3.10	c
Odour intensity						Body					
Early 13.5	3.04	0.07	686	2.90, 3.19		Early 13.5	2.68	0.07	499	2.54, 2.81	a
Middle diluted 13.5	2.92	0.07	686	2.78, 3.07		Middle diluted 13.5	2.88	0.07	499	2.74, 3.01	bc
Late diluted 13.5	3.02	0.07	686	2.87, 3.17		Late diluted 13.5	2.98	0.07	499	2.84, 3.11	cd
Late bleed replace 13.5	3.04	0.07	686	2.90, 3.19		Late bleed replace 13.5	2.81	0.07	499	2.68, 2.95	ab
Middle 14.5	2.91	0.07	686	2.76, 3.06		Middle 14.5	2.96	0.07	499	2.82, 3.09	bcd
Late diluted 14.5	2.93	0.07	686	2.79, 3.08		Late diluted 14.5	3.01	0.07	499	2.88, 3.15	cd
Late 15.5	3.02	0.07	686	2.87, 3.17		Late 15.5	3.04	0.07	499	2.91, 3.18	d
Odour complexity						Length					
Early 13.5	2.81	0.07	703	2.67, 2.95		Early 13.5	2.87	0.08	593	2.73, 3.02	a
Middle diluted 13.5	2.95	0.07	703	2.81, 3.09		Middle diluted 13.5	2.93	0.08	593	2.78, 3.08	ab
Late diluted 13.5	2.93	0.07	703	2.79, 3.07		Late diluted 13.5	3.11	0.08	593	2.96, 3.26	c
Late bleed replace 13.5	2.93	0.07	703	2.79, 3.07		Late bleed replace 13.5	3.01	0.08	593	2.86, 3.16	abc
Middle 14.5	2.89	0.07	703	2.75, 3.03		Middle 14.5	3.10	0.08	593	2.96, 3.25	bc
Late diluted 14.5	2.98	0.07	703	2.84, 3.12		Late diluted 14.5	3.14	0.08	593	2.99, 3.29	c
Late 15.5	3.08	0.07	703	2.94, 3.22		Late 15.5	3.18	0.08	593	3.04, 3.33	c
Red fruit taste						Overall					
Early 13.5	2.99	0.07	694	2.84, 3.13		Early 13.5	5.31	0.15	555	5.02, 5.60	b
Middle diluted 13.5	3.03	0.07	694	2.89, 3.18		Middle diluted 13.5	5.42	0.15	555	5.13, 5.71	b
Late diluted 13.5	2.88	0.07	694	2.74, 3.03		Late diluted 13.5	5.23	0.15	555	4.94, 5.53	b
Late bleed replace 13.5	3.03	0.07	694	2.88, 3.17		Late bleed replace 13.5	5.23	0.15	555	4.94, 5.52	b
Middle 14.5	2.91	0.07	694	2.76, 3.05		Middle 14.5	4.85	0.15	555	4.56, 5.14	a
Late diluted 14.5	3.03	0.07	694	2.89, 3.18		Late diluted 14.5	5.33	0.15	555	5.04, 5.62	b
Late 15.5	2.87	0.07	694	2.72, 3.01		Late 15.5	5.15	0.15	555	4.86, 5.44	ab
Dark fruit taste						Purchase likelihood					
Early 13.5	3.05	0.07	600	2.92, 3.19		Early 13.5	2.79	0.09	531	2.62, 2.96	
Middle diluted 13.5	3.04	0.07	600	2.90, 3.17		Middle diluted 13.5	2.77	0.09	531	2.60, 2.94	
Late diluted 13.5	3.06	0.07	600	2.92, 3.20		Late diluted 13.5	2.80	0.09	531	2.63, 2.97	
Late bleed replace 13.5	3.08	0.07	600	2.94, 3.21		Late bleed replace 13.5	2.85	0.09	531	2.68, 3.01	
Middle 14.5	3.01	0.07	600	2.88, 3.15		Middle 14.5	2.56	0.09	531	2.39, 2.73	
Late diluted 14.5	3.11	0.07	600	2.97, 3.25		Late diluted 14.5	2.71	0.09	531	2.54, 2.88	
Late 15.5	3.12	0.07	600	2.99, 3.26		Late 15.5	2.66	0.09	531	2.49, 2.83	

Supp. Table S5.8. Fermentation tracking 2020 (Year 1)

		DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
			PM	AM	PM	AM	PM	AM	PM	AM	PM
Early 13.5	Temp (oC)		21.0	22.5	25.5	28.8	27.3	24.5	24.5	23.2	22.2
	Baume (Be)		13.5	12.6	9.3	7.3	5.1	1.9	1.1	-0.4	-0.4
Middle 14.5	Temp (oC)		16.5	18.3	20.0	22.3	24.5	26.0	29.5	21.0	20.0
	Baume (Be)		14.5	14.3	13.9	12.6	11.3	6.3	4.1	1.8	-0.1
Middle diluted 13.5	Temp (oC)		16.0	18.5	19.8	22.2	24.5	26.8	28.8	21.2	20.3
	Baume (Be)		14.5	13.1	12.4	11.3	10.5	5.3	3.5	1.1	-0.2
Late	Temp (oC)		18.0	19.0	19.3	21.2	22.0	25.7	28.7	21.7	21.0
	Baume (Be)		15.3	15.3	15.3	13.6	13.0	8.5	6.7	2.5	-0.1
Late diluted 14.5	Temp (oC)		18.0	18.5	18.8	20.3	21.7	24.7	27.0	22.3	21.3
	Baume (Be)		15.2	14.5	14.2	13.1	11.9	8.4	6.5	2.6	-0.1
Late diluted 13.5	Temp (oC)		18.0	18.8	19.0	21.2	21.5	24.5	27.2	23.3	21.0
	Baume (Be)		15.3	13.4	13.1	12.2	11.3	7.2	5.7	1.5	-0.2
Late bleed and replace 13.5	Temp (oC)		18.0	19.0	19.0	20.7	22.2	24.3	26.7	23.0	21.0
	Baume (Be)		15.1	13.4	12.9	11.7	10.5	6.8	5.1	1.6	-0.2

Mean measurements for triplicate samples are presented

Supp. Table S5.9. Fermentation tracking 2021 (Year 2)

		DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
			PM	AM	PM	AM	PM	AM	PM	AM	PM
Early 13.5	Temp (oC)		22.0	24.5	27.8	27.5	27.3	23.5	23.3	23.1	21.1
	Baume (Be)		13.5	12.7	9.4	7.4	5.2	3.4	2.2	-0.2	-0.4
Middle 14.5	Temp (oC)		18.0	18.6	21.0	22.0	24.5	25.5	24.5	22.5	21.2
	Baume (Be)		14.5	14.2	13.8	12.5	11.3	6.4	3.9	1.0	-0.1
Middle diluted 13.5	Temp (oC)		18.0	18.4	20.0	22.0	24.5	25.4	27.4	21.6	21.3
	Baume (Be)		14.5	13.2	12.5	11.4	10.5	6.5	3.6	1.0	-0.1
Late	Temp (oC)		18.5	18.6	19.3	21.4	22.0	25.4	26.8	22.5	22.0
	Baume (Be)		15.5	15.2	14.8	13.6	13.0	6.8	3.9	1.9	-0.1
Late diluted 14.5	Temp (oC)		18.5	18.4	19.0	21.5	21.7	25.8	26.5	23.5	22.1
	Baume (Be)		15.5	14.6	14.0	13.5	11.9	8.5	4.9	1.8	-0.1
Late diluted 13.5	Temp (oC)		18.6	19.1	19.0	23.5	21.5	26.5	27.5	23.5	23.1
	Baume (Be)		15.4	13.6	13.0	11.9	11.3	6.9	3.5	1.6	-0.1
Late bleed and replace 13.5	Temp (oC)		19.0	19.1	19.5	21.5	22.2	23.9	27.4	23.0	23.1
	Baume (Be)		15.1	13.5	12.8	11.6	10.5	6.8	3.6	1.6	-0.2

Mean measurements for triplicate samples are presented

Supp. Table S5.10. Analysis of wine treatments post fermentation (day 5) 2020 (Year 1)

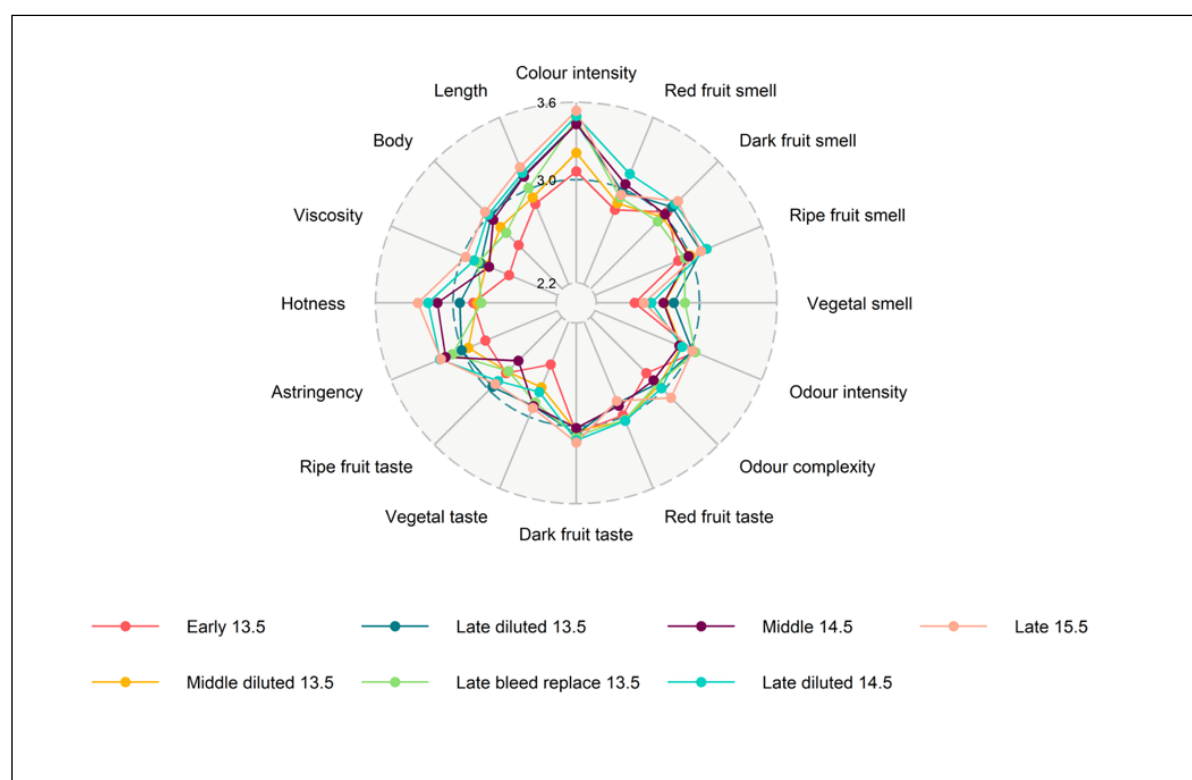
Trial	pH	VA g/L	Lactic Acid g/L	Total sugar g/L	TA g/L	Malic Acid g/L
Early 13.5	3.61	0.48	2.10	1.93	7.01	0.82
Middle 14.5	3.73	0.37	1.86	2.42	6.32	0.96
Middle diluted 13.5	3.67	0.44	1.91	2.21	6.35	0.83
Late	3.71	0.38	1.53	2.32	6.35	1.07
Late diluted 14.5	3.74	0.36	1.56	2.04	6.55	0.96
Late diluted 13.5	3.73	0.37	1.62	1.95	6.34	0.88
Late bleed and replace 13.5	3.72	0.39	1.70	1.63	6.37	0.78

Mean measurements for triplicate samples are presented.

Supp. Table S5.11. Analysis of wine treatments post fermentation (day 5) 2021 (Year 2)

Trial	pH	VA g/L	Lactic Acid g/L	Total sugar g/L	TA g/L	Malic Acid g/L
Early 13.5	3.62	0.42	2.12	1.93	6.75	0.80
Middle 14.5	3.71	0.36	1.93	2.10	6.30	0.90
Middle diluted 13.5	3.70	0.38	1.91	1.96	6.29	0.82
Late	3.71	0.40	1.81	2.07	6.25	1.00
Late diluted 14.5	3.74	0.31	1.68	2.00	6.36	1.01
Late diluted 13.5	3.73	0.39	1.63	1.99	6.37	0.93
Late bleed and replace 13.5	3.72	0.36	1.66	1.90	6.40	0.86

Mean measurements for triplicate samples are presented.



Supp. Figure S5.1. Just about right analysis for all sensory parameters – for 2020 (Year 1) and 2021 (Year 2)

Combined naïve cohort sensory data analysis shows that there is a difference in consumer perception of different wine attributes between wines at early, middle and late harvest times and between different water treatment groups.

Chapter 6 – Moderating impacts of water addition
in must by the use of accentuated cut edges (ACE)
vinification for *Vitis vinifera* cv. Shiraz winemaking

In this chapter, the scientific experimental sections of the thesis conclude by investigating the implementation of the accentuated cut edges (ACE) winemaking technique, for *Vitis vinifera* cv. Shiraz L. wines produced from the late 15.5 °Bé harvest of the 2022 vintage (Year 3). Identical winemaking methods and fermentation approaches as for the 2020 (Year 1) and 2021 (Year 2) vintages were implemented. ACE was introduced at three grape skin fragmentation levels into pre-fermentation 15.5 °Bé wine grape must samples (in triplicate), which were then diluted with water to 13.5 °Bé. The aim of this study was to use ACE in winemaking to address the negative impacts for chemical compounds and sensory attributes associated with wines produced from the ‘water treatment’ winemaking technique, as observed in **Chapter 5** of the thesis. In this current study, treatment wines were assessed for both their chemical compound levels and ‘just about right’ sensory attributes, again using naïve wine consumers to allow close comparisons with **Chapter 5** results. There are no existing studies that test ‘water treatment of pre-fermentation wine grape musts’ winemaking approaches together with ACE implemented at different maceration levels in one vintage. ACE can provide winemakers with an additional mitigation strategy to address any adverse impacts for final wine quality associated with ‘water treatment’ winemaking implemented to produce wines with lower alcohol levels.

This chapter manuscript is based on the draft version of an article which was accepted for publication at the time of the PhD submission, and before typesetting by the journal.

Xynas, B., Barnes, C., & Howell, K. (2024). Moderating impacts of water addition in must by the use of accentuated cut edges (ACE) vinification for *Vitis vinifera* cv. Shiraz winemaking. *OENO One*. Accepted for publication, together with supplementary materials.

Contribution: 80%. Conceptualisation, analysis, investigation, writing, and visualisation.

Abstract

In a warming climate, high-quality wine production is challenging due to grapes having higher sugar concentration, with wines being produced with higher alcohol levels, which can negatively impact final wine quality. Australian regulations now permit water treatment of pre-fermentation musts, which can ameliorate these effects, but final wines often lack colour and flavour. Accentuated cut edges (ACE) can overcome these drawbacks. ACE mechanically breaks grape skins into small fragments and accelerates phenolic extraction during wine fermentation, yet we do not know to what extent by reference to different applied levels of ACE. Expanding on previous studies, we implement ACE at three grape skin fragmentation levels – 10 second ACE treatment (low), 20 second ACE treatment (moderate) and 40 second ACE treatment (high) – into pre-fermentation 15.5 °Bé musts for *Vitis vinifera* cv. Shiraz, which are then diluted with water to 13.5 °Bé. For chemical compounds, reduced total phenolics (a.u.) in diluted to 13.5 °Bé (no ACE) treatment wine (53.00 a.u.) can be addressed by diluted to 13.5 °Bé high ACE treatment, restoring levels similar to 15.5 °Bé (non-diluted no ACE) treatment wine (54.33 a.u.). Total tannins (%) are restored to a similar level as 15.5 °Bé (non-diluted no ACE) treatment wine (1.19%) by diluted to 13.5 °Bé high ACE treatment (1.20%). Sensory analysis for naïve wine consumers shows that diluted to 13.5 °Bé moderate ACE treatment produces wine with the greatest preference for colour intensity, and increased preference for odour intensity and dark fruit taste. Further, 13.5 °Bé high ACE treatment produces a wine with greatest preference for hotness and vegetal smell, and an increase in preference for dark fruit taste. Both diluted to 13.5 °Bé high ACE and diluted to 13.5 °Bé moderate ACE treatment wines demonstrate a statistically significant increase in purchase likelihood when compared to 15.5 °Bé (non-diluted no ACE) and diluted to 13.5 °Bé (no ACE) treatment wines. If and when water treatment of pre-fermentative musts winemaking approaches become more widely accepted globally, it is important that all winemakers are provided with additional mitigation strategies, such as ACE, which can further address any consequential impacts for final wine quality outcomes.

Key words: accentuated cut edges (ACE), water dilution, chemical compounds, sensory parameters, wine quality

Abbreviations

ACE	accentuated cut edges (ACE)
alc/vol	alcohol by volume
Bé	Baumé
cm	centimetre
Dilution	water dilution treatment into a grape must pre fermentation
FSANZ	Food Standards Australia and New Zealand
g	gram
high ACE	high ACE implementation at 40 seconds
H ₂ O	water
JAR	just about right
kg	kilogram
K ₂ S ₂ O ₅	potassium metabisulphite
L	litres
low ACE	low ACE implementation at 10 seconds
mm	millimetre
mod ACE	moderate ACE implementation at 20 seconds
no ACE	no ACE implementation
PEACE	pressed early accentuated cut edges
ppm	parts per million
RATA	rate all that apply
SA	surface area
SO ₂	sulphur dioxide

1. Introduction

1.1 Climate change, wine quality and water treatment of pre-fermentation grape musts

Compressed vintages due to warmer weather across Australia's grape-growing regions are presenting significant challenges to winemakers (Petrie & Sadras, 2017; Terral et al., 2010; Webb et al., 2023). Increases in atmospheric carbon dioxide can lead to faster growth of wine grapes, with resultant higher sugar concentrations in the berries (Webb et al., 2023). These developments can negatively affect a final wine's quality, where shifts in relative humidity and hotter temperatures can lead to an increase in biomass, an increase in sugar and a decrease in acid levels in wine grapes at harvest, all impacting on a final wine's alcohol levels, flavour and aroma (Schultz, 2010). These challenges are also relevant to winemakers globally (Cataldo et al., 2023).

Water treatment of pre-fermentation must is one winemaking technique that can be readily implemented to address fermentation issues associated with high-sugar musts, as well as addressing associated production of unfavourable alcohol levels in a final wine. However, this winemaking approach is currently not universally accepted. Importantly, the introduction of water into pre-fermentation musts is currently limited in the European Union, where water can only be used in those circumstances where it is for a 'specific technical necessity' and it does not modify the characteristics of wine (EU Regulation No. 1308/2013) (Christmann et al., 2022; European Parliament, 2013). In the United States, however, water treatment of musts is an accepted practice that can be employed to facilitate fermentation, but the density of the juice cannot be reduced below 22 degrees Brix (US Regulation §24.176) (Alcohol, Tobacco Products and Firearms, 2010).

In 2017, Australia implemented further regulatory changes where water addition into a pre-fermentation grape must is now permitted in order to 'facilitate fermentation' to 13.5 °Baumé (Bé) (the measurement of grape sugar content) (Australia New Zealand Food Standards Code – Standard 4.5.1) (Australian Government, 2017). The Australian approach was as a response to the problems associated with high ethanol production arising during fermentation (Bindon et al., 2019) and as one answer to address negative impacts on human health from consumption of higher-alcohol wines (Saliba et al., 2013). This study focuses on *Vitis vinifera* cv. Shiraz, which is an important grape cultivar, as it is the most-planted red grape variety globally, including Australia (Onetto et al., 2023;

Schelezki, Deloire, & Jeffrey, 2020; Wine Australia, 2023), therefore vinicultural approaches to maintain wine quality will have a high economic impact for wine consumers, wine producers and wine distributors across the globe.

Recent studies have demonstrated that while water treatment of high-sugar must can facilitate the reduction of unfavourable ethanol yields during fermentation, it can also negatively impact final wine flavour and colour (Harbertson et al., 2009; Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020). 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, it is important that all winemakers are provided with additional mitigation strategies to address any consequential adverse impacts for final wine quality.

1.2 Accentuated cut edges (ACE) as an emerging winemaking approach

Accentuated cut edges (ACE) is one winemaking strategy that may address these impacts. ACE is a winemaking process in which the grape skins are mechanically cut into smaller fragments, to assist the extraction of phenolic components from the grape skin of the floating pomace cap in the early stages of fermentation (Sparrow, Smart, et al., 2016). During early fermentation, the extraction of phenolic compounds from the grape skins is greater due to more broken edges achieved through ACE. ACE has been shown to positively impact the phenolic composition of the final wine (Sparrow, Smart, et al., 2016). In addition, it has been shown that ACE implemented by an industrial handheld mixer rather than by implementation with an ACE machine-assisted approach can minimise seed damage, therefore limiting extraction of associated bitter compounds (Sparrow, Smart, et al., 2016).

Early studies for ACE processes focused on *Vitis vinifera* cv. Pinot noir as this cultivar can demonstrate poor colour development and low pigment stability, which can be negatively viewed by consumers who tend to prefer a more intense colour in red wines (Parpinello et al., 2009). ACE, when implemented to reduce grape skins to 6% of their original size, was shown to produce Pinot noir wines with 50% greater wine colour density and a 95% higher stable pigment concentration when compared to the control no ACE wine (Sparrow, Smart, et al., 2016). Tannin concentrations also increased for ACE-treated Pinot noir wines, together with higher intensities of fruity components including banana, peach, black currant and dark fruit when compared to the control wine

(Sparrow, Holt, et al., 2016; Sparrow, Smart, et al., 2016). An extended pressed early accentuated cut edges (PEACE) vinification approach has also been shown to improve anthocyanidin and tannin levels for Pinot noir when compared to a no ACE control wine (Sparrow & Smart, 2017).

More recently, the ACE winemaking approach was applied to Shiraz, where Kang et al. (2020) found that for water-diluted treatments from 14.0 °Bé to a 13.5 °Bé, which were also subjected to an ACE machine-assisted approach, the resultant accelerated release of wine components added to the flavour of earthy characters in the final wine. They also found that the impact on tannins and phenolic attributes of the final wine were not significantly affected when compared to a control wine, however, there were still significant negative dilution impacts on consumer-perceived wine aromas and flavours (Kang et al., 2020). When subject to an expert sensory panel, the water-diluted wines alone had a different astringency profile, but by combining water treatment with the ACE winemaking technique, this could address astringency, in particular the perception of adhesive and graininess aspects to the wine for the trained panel, but not the consumer panel (Kang et al., 2020). The later study by Wang et al. (2022) also investigated ACE impacts for water-treated must Shiraz wines. They observed that for volatiles, including acetates, higher alcohols, fatty acids and isoprenoids, the results were varied, depending on the crushing methods employed as well as skin contact time. Apart from acetates, ACE short treatment (3-day racked off grape skins) demonstrated the highest potential for improvements to all groups of volatiles (Wang et al., 2022). Wang et al. (2022) also applied RATA (rate all that apply) sensory analysis (Danner et al., 2018), and found that it was only the 'red fruits', 'earthy/dusty', 'floral/perfume/musk', 'herbaceous' and 'vanilla' attributes that were significantly different ($p < 0.1$) (Wang et al., 2022). Overall, Wang et al. (2022) found that ACE plus skin contact time had potential to improve wine volatile attributes as well as some sensory aspects of a final wine.

The aim of this study is to expand on previous ACE studies in order to identify any changes in the chemical composition and sensory profiles for Shiraz wines that have been also subject to water dilution treatment of musts before fermentation, together with the ACE winemaking approach at three grape skin fragmentation levels. We test whether ACE implemented at varying levels can change the chemical composition for water-diluted wines when compared to no ACE treated wines (non-diluted and diluted). We also

test if ACE implemented at varying levels will influence consumer perceptions of different wine attributes between water-diluted wines when compared to the no ACE treated wines (non-diluted and diluted), and if there is a difference in preference amongst consumers for each of the wine samples tested. Finally, we also test if different wine attributes impact consumer preferences more than others.

The outcomes of our study can be relevant for Shiraz wine producers in Australia and internationally as global warming continues to present winemakers with many challenges. This paper's aim is to provide advice to winemakers, so that they can make supported and informed decisions when implementing a water dilution fermentation winemaking approach into high-sugar musts in order to mitigate the high production of ethanol during fermentation, together with alternative levels of ACE approaches implemented to further address desirable final phenolic and sensory wine quality parameters.

2. Materials and methods

2.1 Grape sample groups and fermentation

This Australian study relied on Shiraz grapes which were sourced from the Richard Tallis Winery (195 Major Plains Road, Dookie, North East Victoria, Victoria). Rows 85–91 were selected. These rows were all from the north-eastern block on Richard Tallis's property (36°21'08.2"S 145°45'37.0"E). The block consisted of 18 rows of Shiraz grapes. These rows were selected for their relatively middle position within the block to reduce end-row influences. The rows selected consisted of 54 panels of 200 vines, with approximately 50 vines contributing to the grapes taken in each season. Row spacing was approximately 2.8 metres. Vines were planted approximately 1.2 metres apart. Only one late harvest at 15.5 °Bé was conducted for a total harvest of 900 kg of fruit. At harvest, fruit was selected at random from panels along the length of each row, taking fruit from both sides of the vine. Each panel was assigned a number and, using a randomiser, selected at random for harvest. All fruit was hand harvested.

There were 15 triplicate sample groups of 60 kg, where each produced approximately 36 litres of wine; therefore, a total 540 litres of wine (more due to dilution of ferments) was produced. All grape must treatment sample groups were subjected to crushing through rotary roller crushers set at 8 mm spacing before being separated into their own fermentation tanks/treatment groups (15 in total). Three triplicate sample groups were

then subject to three levels of accentuated cut edges (ACE) skin fragmentation: low ACE, moderate (mod) ACE and high ACE treatments (as detailed below). All sample groups except for the triplicate 15.5 °Bé (non-diluted no ACE) sample group were then subject to diluted must treatment (+ 4.07 L water), so as to lower 15.5 °Bé to 13.5 °Bé (Figure 6.1). Deionised water was used for water treatments so as to minimise chlorine/chloramines that may impede fermentation and stain the flavour of the wine. The water addition to must winemaking approach is permitted under Australian regulations (Australian Government, 2017). The volume of water added was calculated using the Australian Wine Research Institute online water addition calculator (AWRI, 2023). The two no ACE sample groups consisted of a 15.5 °Bé (non-diluted no ACE) treatment and a diluted to 13.5 °Bé (no ACE) treatment (Figure 6.1).

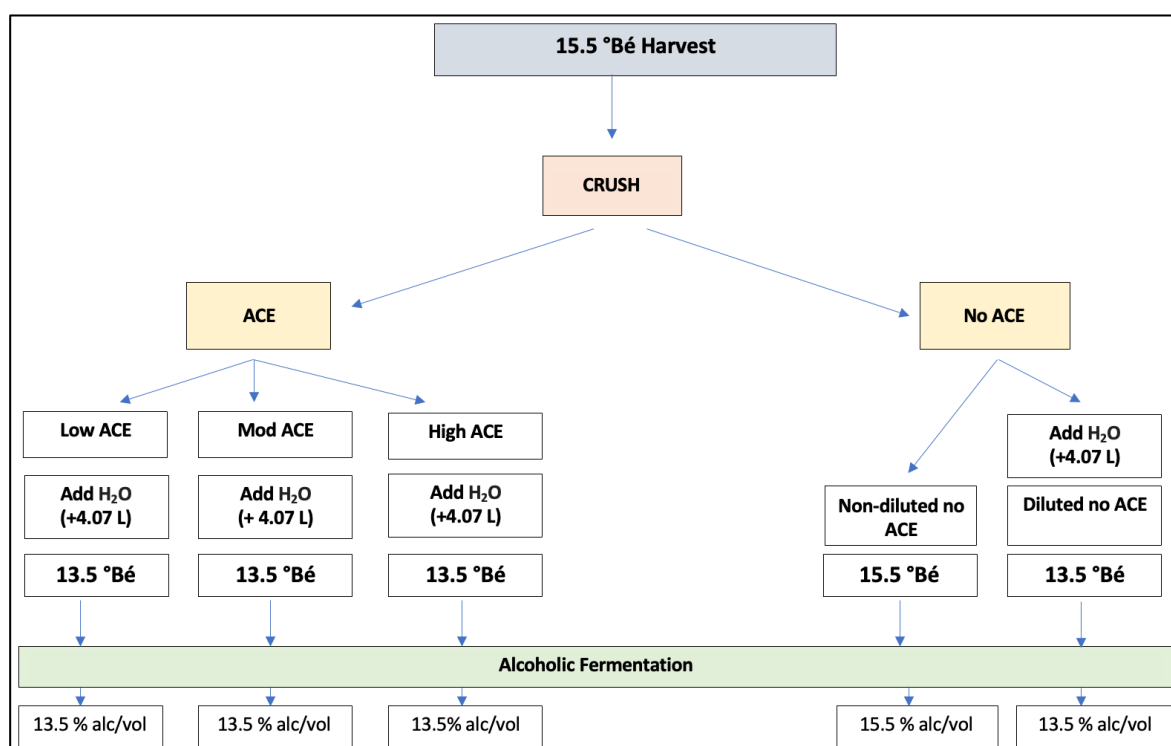


Figure 6.1. Pictorial representation of the ACE experimental design outline. Xynas et al (2024), Accepted for publication at time of PhD submission.

A standard 50 ppm Sulphur Dioxide (SO₂) (as *Potassium metabisulphite*, K₂O₅S₂, Australian Home Brewing, Victoria, Australia) was added to all sample groups at crush followed by inoculation with Vitilevure Syrah YSEO™ (*Saccharomyces cerevisiae* var. *cerevisiae*; Danstar, Rhone Valley, France, 2020) commercial culture of yeast (at 30 g/hL). After 24 hours, all fermentations were then inoculated with Martin Vialatte

Reflex Malo[®] 360 (*Oenococcus oeni*; Martin Vialatte, Magenta, France, 2020) lactic acid bacteria (1 g/hL) to carry out malolactic fermentation. All fermentations were maintained on skins for 5 days, with the use of refrigeration to maintain temperatures between 20 °C and 30 °C, before being pressed. Sampling daily of 100 mL from each treatment group was taken during the fermentation process, which tracked temperature and Bé. Musts all finished 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 (2 g/L tartaric acid addition) (Australian Tartaric Products, Victoria, Australia, 2020). An additional 50 ppm SO₂ (as *Potassium metabisulphite*, K₂O₅S₂, Australian Home Brewing, Victoria, Australia) was added 21 days after fermentation, prior to bottling wines in 750 mL glass wine bottles with wines maturing at an average temperature of 18 °C for 12 months.

2.2 Accentuated cut edges (ACE) treatment approach

Accentuated cut edges (ACE) skin fragmentation approach using an industrial handheld mixer machine (Robot Coupe MP600 Ultra [B2B], with a 600 mm shaft) was implemented, as this ACE approach has been shown to minimise seed damage (Sparrow, Smart, et al., 2016) and to also further provide for different fragmentation levels. Three groups of must samples in triplicate were subject to skin fragmentation treatment, each at a different level of ACE. Low ACE involved holding the handheld mixer into fermenters for 10 seconds at low speed (set at speed level 1, where the highest speed is level 10). Moderate ACE used 20 seconds at low speed. High ACE used 40 seconds at low speed. These three sample groups were then all subject to water-diluted must treatment (+ 4.07 L water) in order to lower 15.5 °Bé to 13.5 °Bé (Figure 6.1).

2.3 Chemical analysis approach

Sample groups of wines 12 months after bottling for this study were taken from each treatment group. Wine colour absorbances were measured using Agilent Cary 60 UV-Vis (Agilent Technologies Inc., PaloAlto, CA, USA), with a 1 mm path length quartz cuvette (Somers & Evans, 1974). Deionised water was set as the blank reference. Each analysis was performed in triplicate. Measures of wine colour density, wine colour hue, total phenolics, total anthocyanins, degree of ionisation of anthocyanins, chemical age 1 and chemical age 2 were undertaken for each of the must treatment sample groups. The Somers colour assay was modified 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 (Mercurio et al., 2007). The main modification taken was to standardise wine pH to pH 3.4 and the alcohol concentration to 12% v/v using a buffer solution before any analysis being undertaken. Sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) was incorporated into the buffer solution rather than being added directly to individual sample groups, thus minimising preparation time (Mercurio et al., 2007). Tannin measurements were derived from a calibration developed from the methyl cellulose precipitable (MCP) tannin assay (Sarneckis et al., 2006).

2.4 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 for the final wines, utilising a Likert scale (Likert, 1932).

The naïve sensory analysis data was collected from sensory panels of wine students in June 2023. Testing was taken after students had some level of preliminary training in sensory analysis experiences through their concurrent studies. Visual, olfactory and mouthfeel sensory attributes were tested. Samples of wines 12 months after bottling were taken from each treatment group. Sample groups were evaluated by 79 naïve wine consumers to evaluate 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. Data was collected on a non-structured linear scale, where the middle of the scale for each attribute corresponded to 'just about right' for the naïve sensory panellists (Likert, 1932). For each sensory attribute, panellists ranked the wine sample group on a 5-level Likert scale: too weak/pale; weak/pale; just about right; strong/a little dark; or too strong/too dark. Overall liking was scored on a 9-point line scale ranging from dislike extremely, to neutral, to like extremely. Likelihood to purchase was scored on a 5-point line scale ranging from extremely unlikely, to neutral, to extremely likely. In addition, the JAR was combined with overall liking scores to highlight those attributes of a wine that have the greatest impact on liking, where a 'penalty analysis' was taken (Cadot et al., 2012; Schraidt, 2009). The aim of penalty analysis generally is to assess the influence of a JAR category for a given attribute by assessing a 'penalty' to reflect consumer overall liking of a wine.

Panellists were provided with an introductory baseline session to inform them of the process, and the requirements and risks associated with the JAR survey prior to commencing the survey. Reference standards were agreed upon by the panellists to familiarise them with the colour and odour intensities and complexities, red, ripe and dark fruits attributes, as well as for vegetal, hotness, astringency, body, viscosity, length and balance. For colour intensity, panellists were provided with a chart of red colours, which displayed red from light to dark red. For odour complexity and intensity, and length and balance panellists were engaged in repetitive training sessions. Other sensory attributes were measured against a reference sample standard of 30 mL of Shiraz wine. For red fruits 'aroma and taste', this consisted of mixing in two strawberries, two cherries and four raspberries. For dark fruits 'aroma and taste', this consisted of mixing in four blackberries and four blueberries. For ripe fruits 'aroma and taste', this consisted of mixing in eight crushed raisins. For vegetal 'aroma and taste', this consisted of mixing in 1 teaspoon of grated capsicum. For hotness 'mouthfeel and texture', this consisted of mixing in food-grade ethanol to raise the alcohol level by 2%. For astringency 'mouthfeel and texture', this consisted of adding 0.2 g of powdered white tannin. For body 'mouthfeel and texture', this consisted of adding 0.2 g of powdered tannin and food-grade ethanol to the raise alcohol level by 2%. For viscosity 'mouthfeel and texture', this consisted of adding 0.02 g/L of food-grade soluble xanthan gum (Table 6.1). The five sample groups were then presented to panellists in 30 mL aliquots in coded ISO standard wine glasses, during daylight hours. Each panellist was provided with the five sample groups, all presented at once in a randomised order. The five sample groups were evaluated in one sitting. Paper scoring sheets for each wine sample group were given to each panellist and collected by the researchers at the end of the sessions.

2.5 Statistical analysis approach – chemical compounds

Statistical analysis for chemical compounds (mean values and standard deviation [SD]) was undertaken by using independent sample group t-test, two-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$) was conducted with R Project for Statistical Computing. For individual phenolic components in each of the wine sample groups, data was analysed using repeated measures of variance (ANOVA) with treatment (process) approaches as fixed factors.

2.6 Statistical analysis approach – sensory parameters

A linear mixed effects model (LMM) approach was applied, where linear mixed effects models were fitted to the ratings of each attribute (Zuur et al., 2009). A separate model was used for each attribute: 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, and overall liking and purchase likelihood. The linear mixed effects model allowed for nested random effects for each attribute to be assessed. Wine treatment (process) was considered a fixed effect, while panellist was considered a random effect. Where the p-value was equal to or less than the significance level (≤ 0.05), then it could be assumed that process (wine treatment) significantly affected the response received.

3. Results

3.1 ACE treatments increase fragment size, particle number, total perimeter and surface area of grape berries

ACE was applied at three different levels of low, moderate and high so as to influence the fragment size, particle number, total perimeter (cm) and surface area (cm²) in the grape berries. These three sample groups were repeated in triplicate, where the mean results are shown (Figures 6.2A and 6.2B). Following ACE application, the three ACE-treated sample groups were subject to diluted must treatment through the addition of deionised water (H₂O) (+ 4.07 L) in order to lower 15.5 °Bé to 13.5 °Bé. A further two sample groups consisted of 15.5 °Bé (non-diluted no ACE) (in triplicate) and diluted to 13.5 °Bé (no ACE) (in triplicate). Again, mean results are shown (Figures 6.2A and 6.2B). A 5 g sample was taken from each fermentation and placed into 90 mm petri dishes. The fragments were floated in 15 mL of water, pressed on a piece of graph (5 mm × 5 mm grid) paper and photographed. The number of fragments per berry from their surface area and perimeter were calculated using open-source image analysis software ImageJ (Version 1.51a) (Rasband, 2016) (Figures 6.2A and 6.2B).

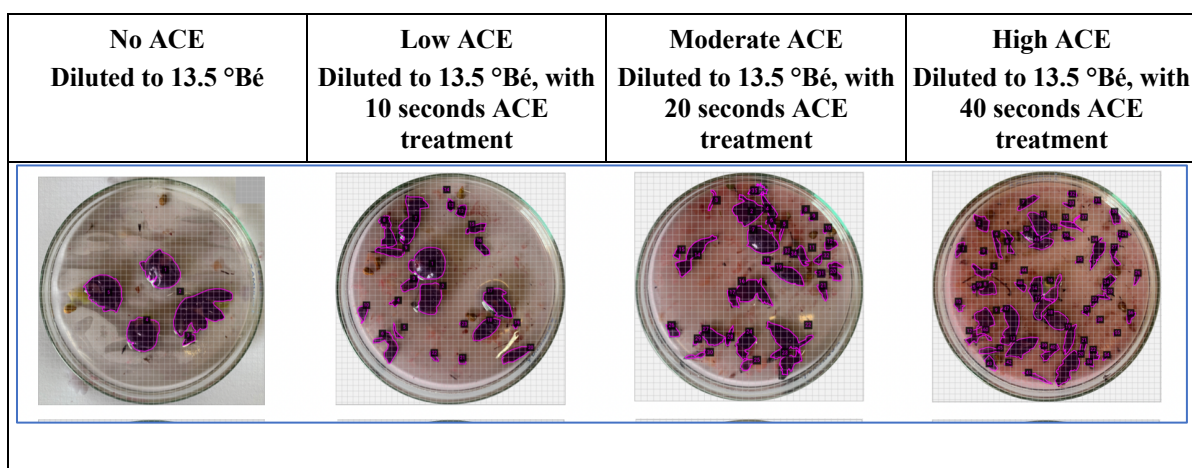


Figure 6.2A. Grape skin pulp and skin fragments based on time of ACE treatment

Representation of the skins after ACE treatment but before any water dilution or fermentation commences. A representative image of each triplicate group is shown.

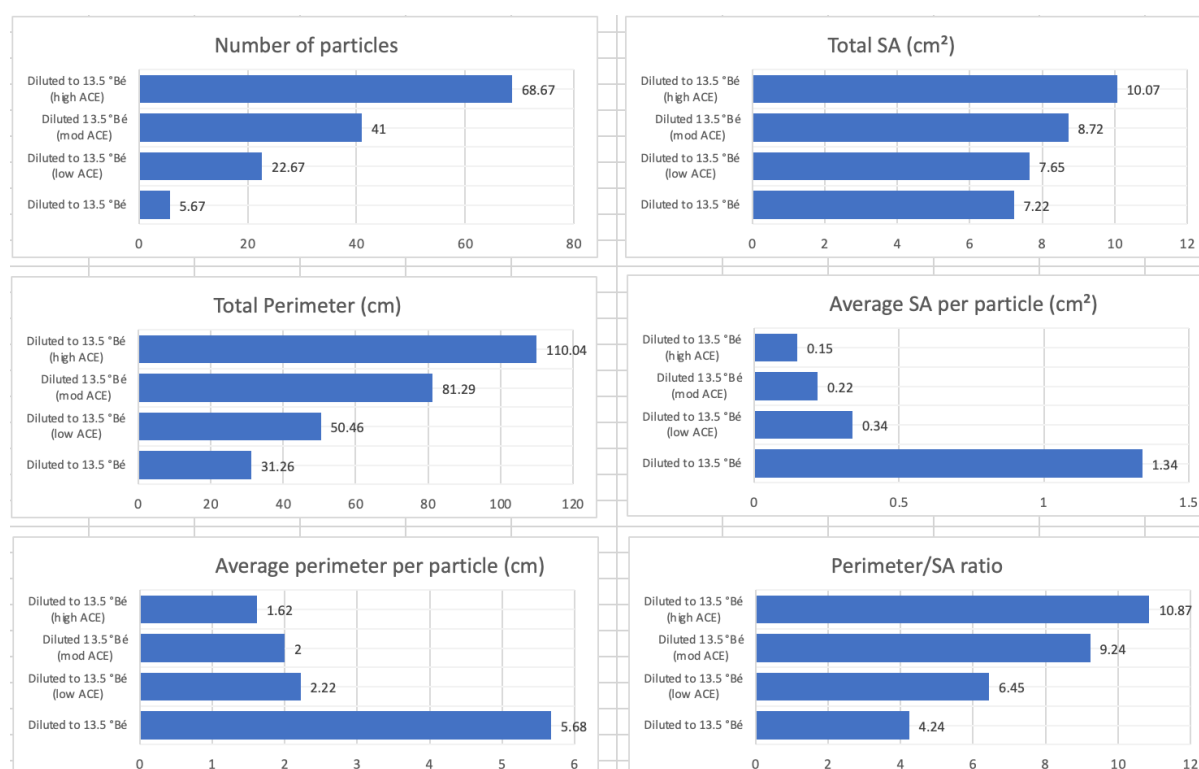


Figure 6.2B. Quantification of ACE skin fragmentation

Mean results for triplicate samples are presented. SA refers to surface area.

The number of particles (fragment size) as presented in the sample group petri dishes are at the highest levels with the implementation of a high ACE treatment (Figures 6.2A and 6.2B). When compared to the diluted to 13.5 °Bé (no ACE) sample group with 5.67 berry particles, the number of berry particles for the diluted to 13.5 °Bé high ACE sample group increased to 68.67. The total surface area (cm²) remained stable for all ACE

treatments, and increased only moderately from the diluted to 13.5 °Bé (no ACE) sample group with a surface area of 7.22 cm² up to 10.07 cm² for the diluted to 13.5 °Bé high ACE sample group. The total perimeter (cm) increased progressively as the duration of ACE treatment undertaken increased, from low, to moderate and to high ACE implementation. For the diluted to 13.5 °Bé (no ACE) sample group, the total perimeter was 31.26 cm, which increased to 110.04 cm for the diluted to 13.5 °Bé high ACE sample group. The average surface area per particle (cm²) decreased as the duration of ACE treatment undertaken increased. For the diluted to 13.5 °Bé high ACE sample group, the average surface area was 0.15 cm², compared to 1.34 cm² for the diluted to 13.5 °Bé (no ACE) sample group. The average perimeter per particle (cm) also decreased as the duration of ACE treatment undertaken increased. For the diluted to 13.5 °Bé high ACE sample group, the average perimeter per particle was 1.62 cm, compared to 5.68 cm for the diluted to 13.5 °Bé (no ACE) sample group. The perimeter/surface area ratio increased as the duration of ACE treatment undertaken increased, where the perimeter/surface area ratio for the diluted to 13.5 °Bé (no ACE) sample group was at a 4.24 ratio, increasing to a 6.45 ratio for the diluted to 13.5 °Bé low ACE sample group, up to a 9.24 ratio for the diluted to 13.5 °Bé moderate ACE sample group, increasing to a 10.87 ratio for the diluted to 13.5 °Bé high ACE sample group. Also refer to Supplementary materials (Supp. Table S6.1) for supporting data.

3.2 ACE treatment can have statistically different impacts on some but not all chemical compounds in wine

ACE applied at the three different levels of low, moderate and high can influence chemical parameter outcomes for the treated wines at statistically different levels (Figure 6.3).

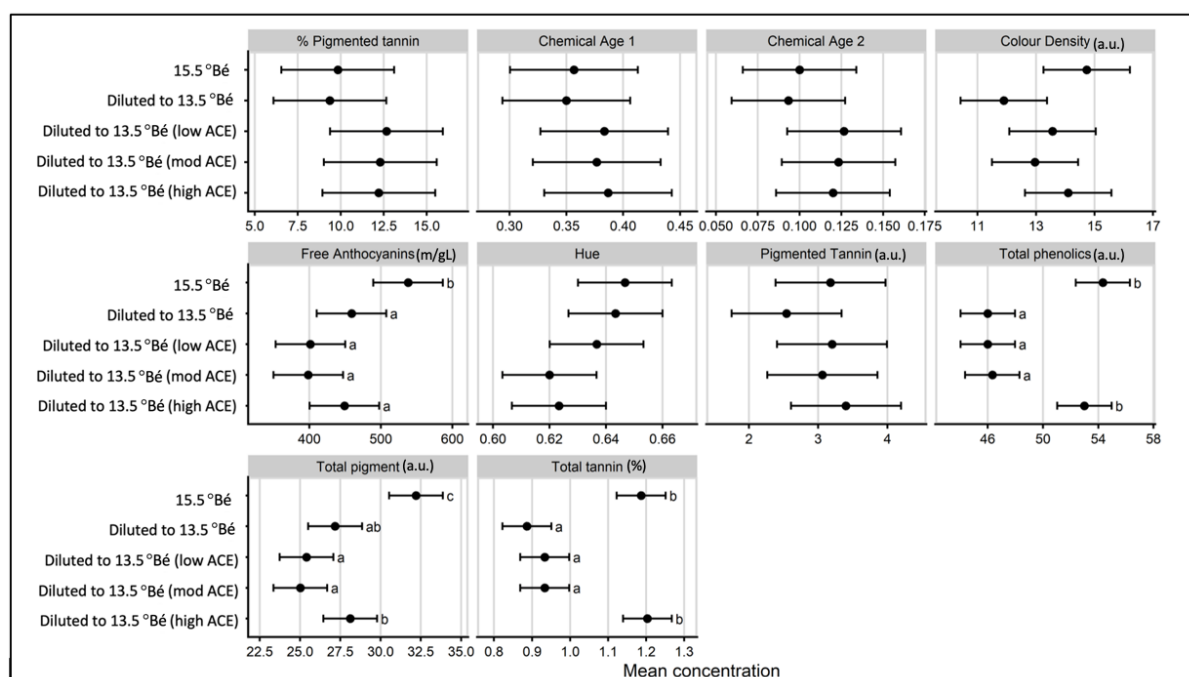


Figure 6.3. Chemical compound concentrations differ by ACE treatment and dilution treatments

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

Figure 6.3 sets out ACE treatment effects on the chemical compounds of pigmented tannin (%), chemical age 1 and 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 (%), as compared to the 15.5 °Bé (non-diluted no ACE) and diluted to 13.5 °Bé (no ACE) treatment groups. Also refer to Supplementary materials (Supp. Table S6.2) for raw data that supports the representations in Figure 6.3.

Free anthocyanins are measured in highest concentrations in the 15.5 °Bé (non-diluted no ACE) treatment group (538.00 mg/L), with all other treatments having statistically significantly lower results for free anthocyanin concentration. The lowest observed free anthocyanin concentration is in the diluted to 13.5 °Bé low ACE and in the diluted to 13.5 °Bé mod ACE treatment groups (401.33 mg/L; 398.33 mg/L). The diluted to 13.5 °Bé high ACE treatment group shows higher free anthocyanin concentration (449.00 mg/L) than the diluted to 13.5 °Bé low ACE treatment group (410.33 mg/L) and in the diluted to 13.5 °Bé mod ACE treatment group (398.33 mg/L); however, this is not statistically significant. Total phenolics concentrations are highest in the 15.5 °Bé (non-diluted no ACE) treatment group (54.33 a.u.) and in the diluted to 13.5 °Bé high

ACE treatment group (53.00 a.u.). All other treatments have statistically significantly lower results for total phenolics. The highest concentration of total pigment is shown in the 15.5 °Bé (non-diluted no ACE) treatment group (32.20 a.u.). All other treatment groups have statistically significantly lower results for total pigment. Total tannin is shown to be highest in the 15.5 °Bé (non-diluted no ACE) treatment group (1.19%) and in the diluted to 13.5 °Bé high ACE (1.20%) treatment group. All other treatments have statistically significantly lower results for total tannin. For chemical age 1 and 2, values are similar across all treatment groups, where none are statistically significantly different to each other (Figure 6.3; Supplementary materials: Supp. Table S6.2).

3.3 ACE treatment affects sensory attributes including colour intensity, dark fruit taste, astringency, hotness and length

A just about right (JAR) sensory survey approach was a direct approach to measure the deviation from ideal levels per sensory attribute for each wine sample (Rothman & Parker, 2009). The JAR measured the perceived attribute intensities, but did not directly quantify them (Cadot et al., 2010). The sensory attributes of each wine sample were scored by the consumer naïve consumer panellists according to the sensory standards developed (Table 6.1).

Table 6.1. Sensory standards given to panellists for wine attributes for type, definitions, and reference standards

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)	Wine 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	Add 0.2 g of powdered white tannin
Body	Mouthfeel/texture	Wine marked by richness, fullness; full-bodied	Add 0.2 g powdered 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	Add 0.02 g/L food-grade soluble xanthan gum
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

The JAR sensory outcomes show that the diluted to 13.5 °Bé high ACE treatment group has the highest colour intensity; however, this is observed as being ‘too high’. All treatments without ACE show the lowest preference relating to vegetal smell. The 15.5 °Bé (non-diluted no ACE) treatment group also shows high odour intensity; however, this is rated as ‘too high’. All ACE treatment groups show highest overall preference for dark fruit taste, with the diluted to 13.5 °Bé mod ACE being the highest preference treatment. The diluted to 13.5 °Bé (no ACE) treatment group shows the lowest observed dark fruit taste. All ACE treatment groups show an increase in astringency; however, this is not demonstrated to be a positive preference by panellists. The 15.5 °Bé (non-diluted no ACE) and diluted to 13.5 °Bé high ACE treatment groups both show greatest overall preference for hotness. The diluted to 13.5 °Bé high ACE treatment group also shows greatest overall observed length; however, this is demonstrated to be ‘too high’, with the lowest preference of all other treatment groups, including diluted to 13.5 °Bé (no ACE) and 15.5 °Bé (non-diluted no ACE) treatment groups (Figure 6.4, Figures 6.7A and 6.7B). Also refer to supplementary materials (Supp. Table S6.3) for supporting data.

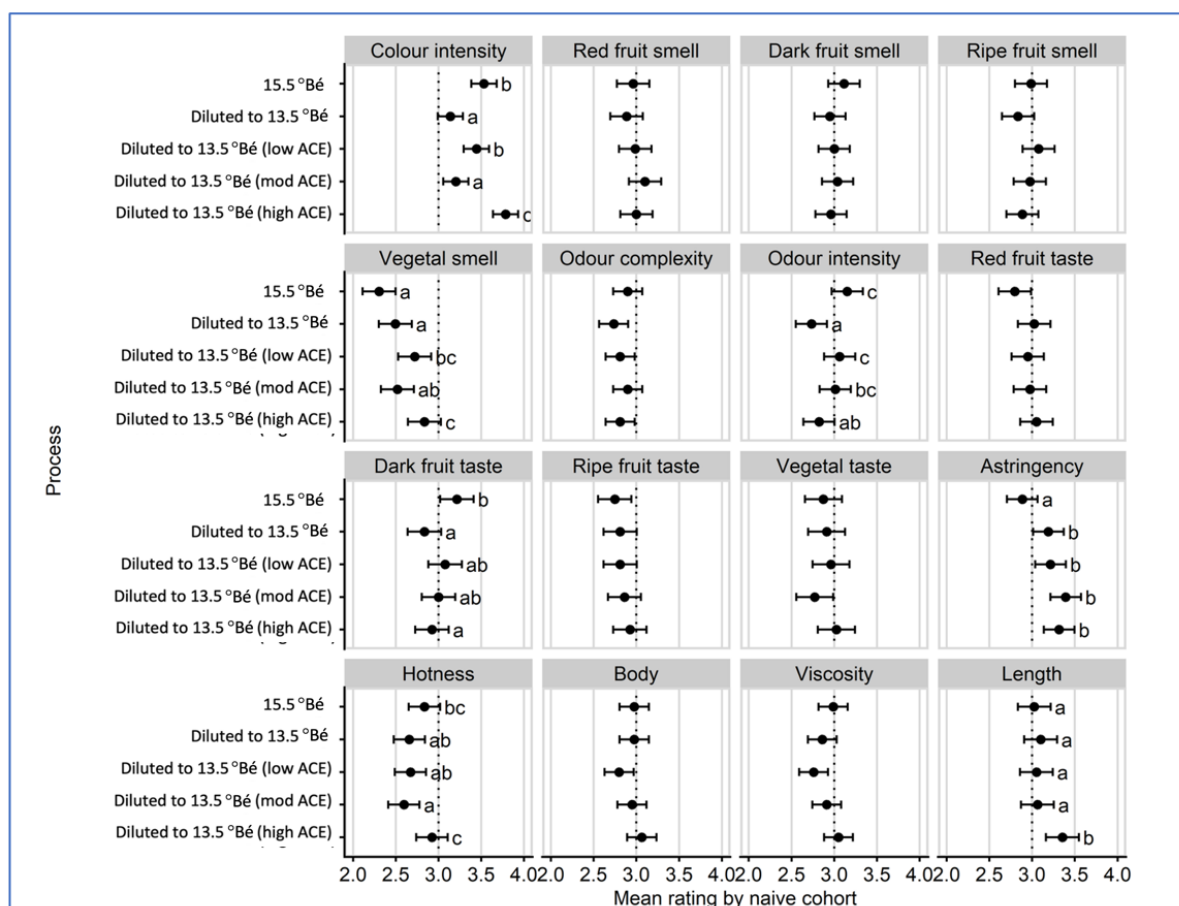


Figure 6.4. ACE impact on sensory parameters of the final wine for the naïve wine consumer panellists

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

3.4 Assessment and purchase likelihood are increased in ACE-treated wines

The diluted to 13.5 °Bé high ACE and the diluted 13.5 °Bé mod ACE treatment groups are highest rated in overall assessment when compared to all other treatment groups; however, there is no statistically significant difference shown between treatments groups and consumer overall liking of the wines (Figure 6.5). Purchase likelihood shows a statistically significant preference for the diluted to 13.5 °Bé mod ACE treatment group when compared to the diluted to 13.5 °Bé (no ACE), 15.5 °Bé (non-diluted no ACE) and the diluted to 13.5 °Bé low ACE treatment groups. These results are not statistically significantly different from the diluted to 13.5 °Bé high ACE treatment group; however, both the diluted to 13.5 °Bé mod ACE and diluted to 13.5 °Bé high ACE treatment groups show statistically significantly increased preference for purchase likelihood when

compared to diluted to 13.5 °Bé (no ACE) and 15.5 °Bé (non-diluted no ACE) treatment groups (Figure 6.5).

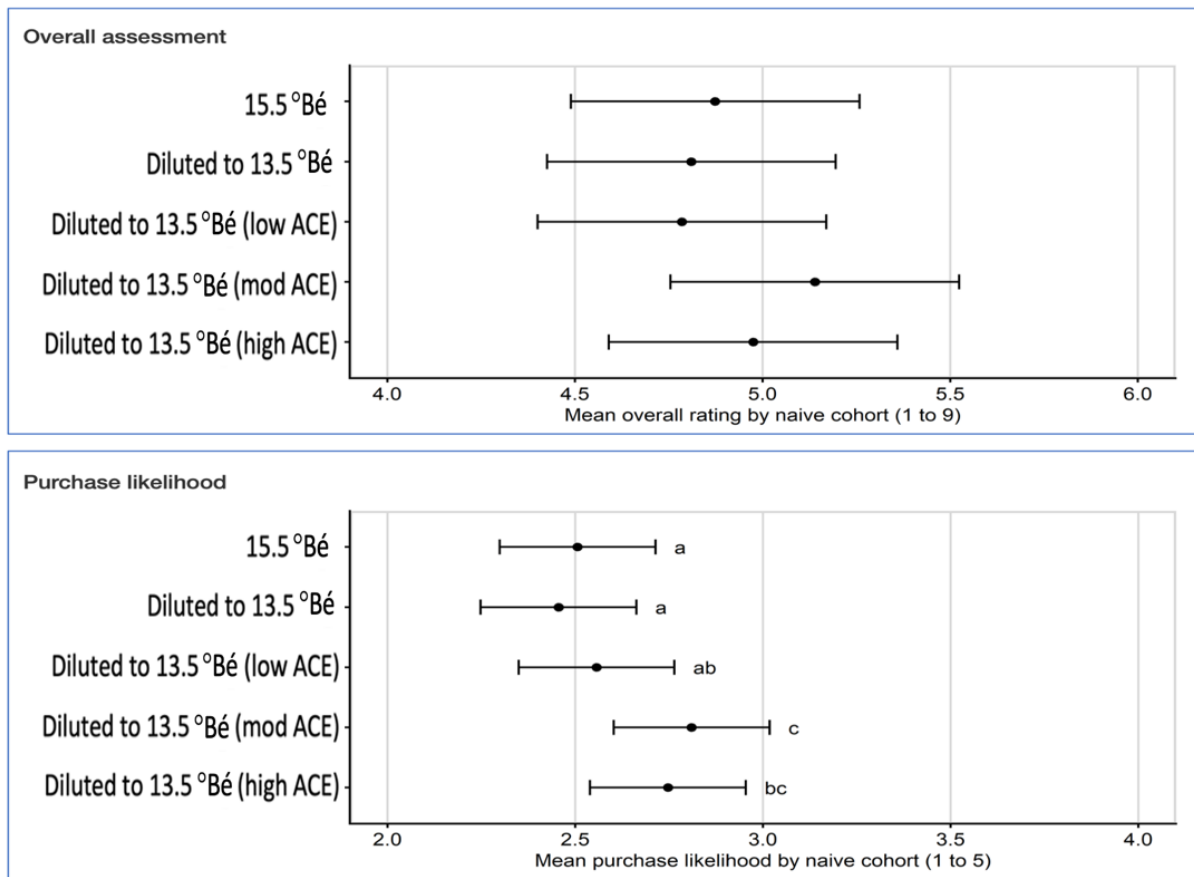


Figure 6.5. Overall assessment and purchase likelihood for the naïve wine consumer panellists

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

Penalty analysis results demonstrate that ‘too little’ body and ‘too little’ red fruit taste are the only two attributes in the critical corner. ‘Too little’ of these two attributes are observed to have the most negative impact on overall liking when observed in the JAR scale. The diluted to 13.5 °Bé low ACE treatment group has the lowest in observed body. The diluted to 13.5 °Bé (no ACE) treatment group has the lowest observed red fruit taste. Consumer perception is that these two treatment groups have the lowest observed concentration of the attributes; however, this is shown to be not statistically significantly different from any other treatment group in each attribute category (Figure 6.6).

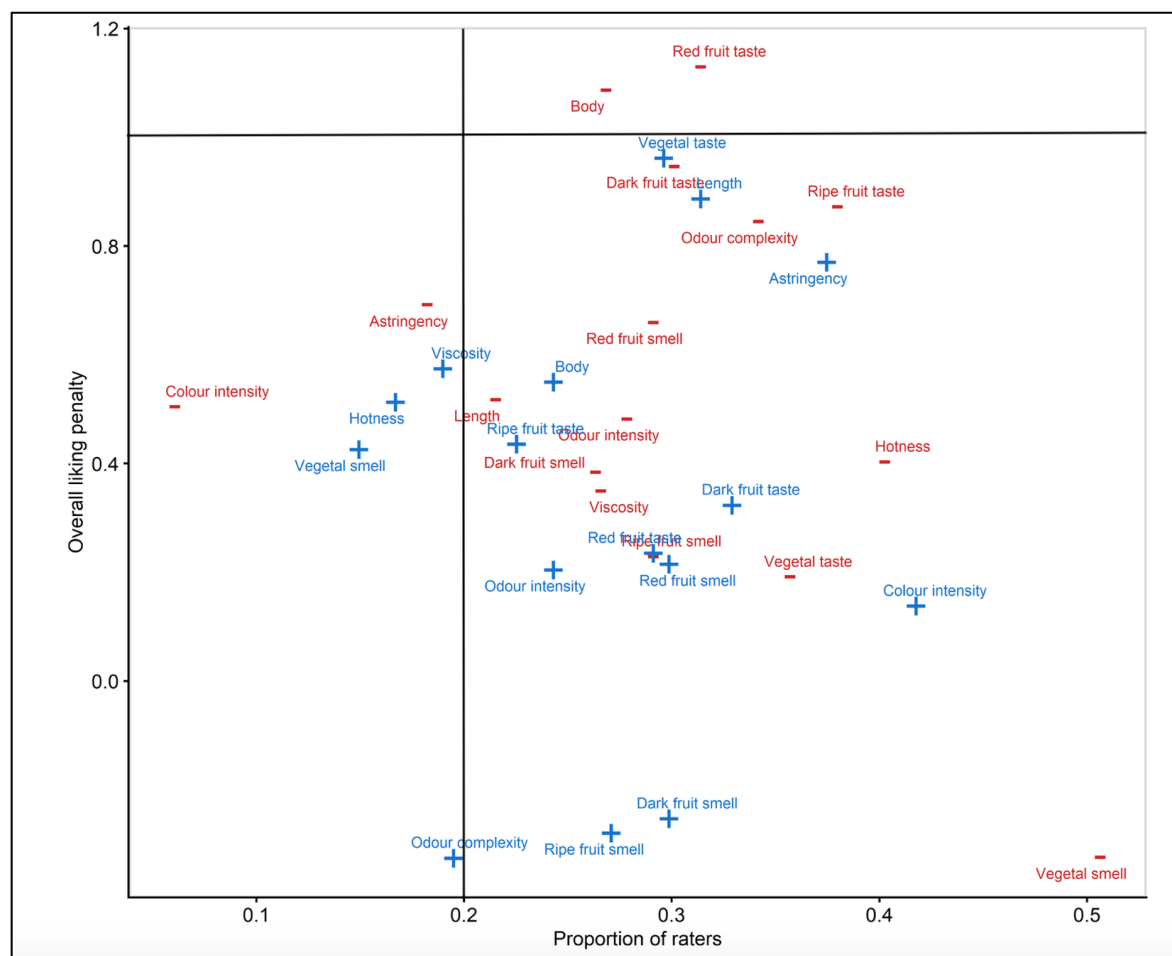


Figure 6.6. Overall liking penalty and proportion of raters for sensory attributes

‘+’ denotes too much of an attribute, and ‘-’ denotes too little of an attribute.

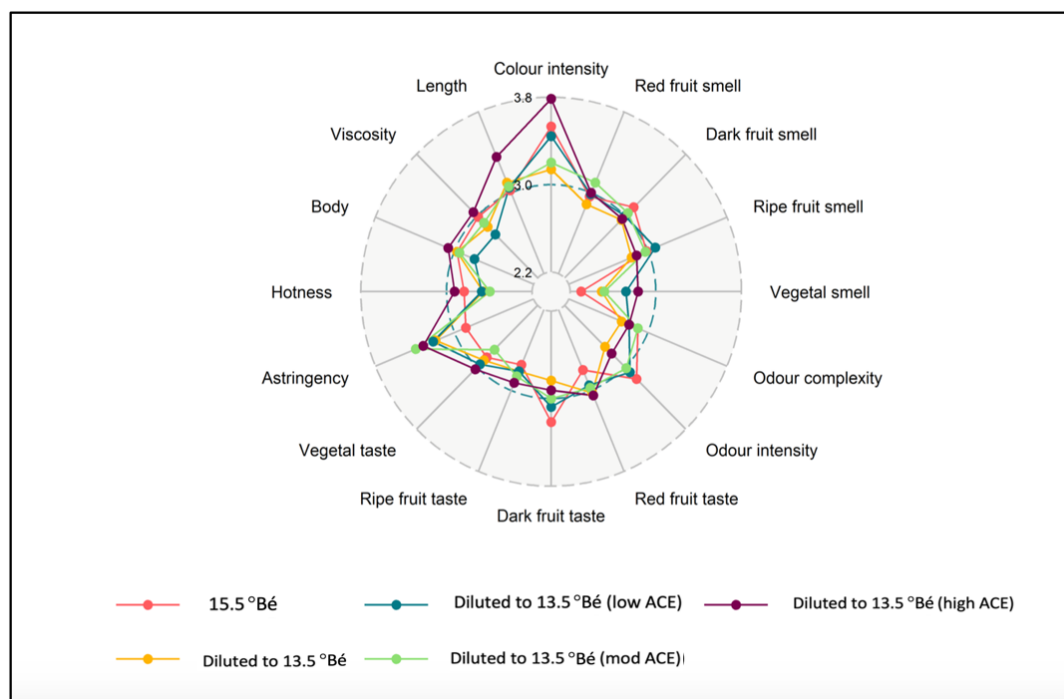


Figure 6.7A. Descriptive sensory analysis of the wines made with ACE-treated must

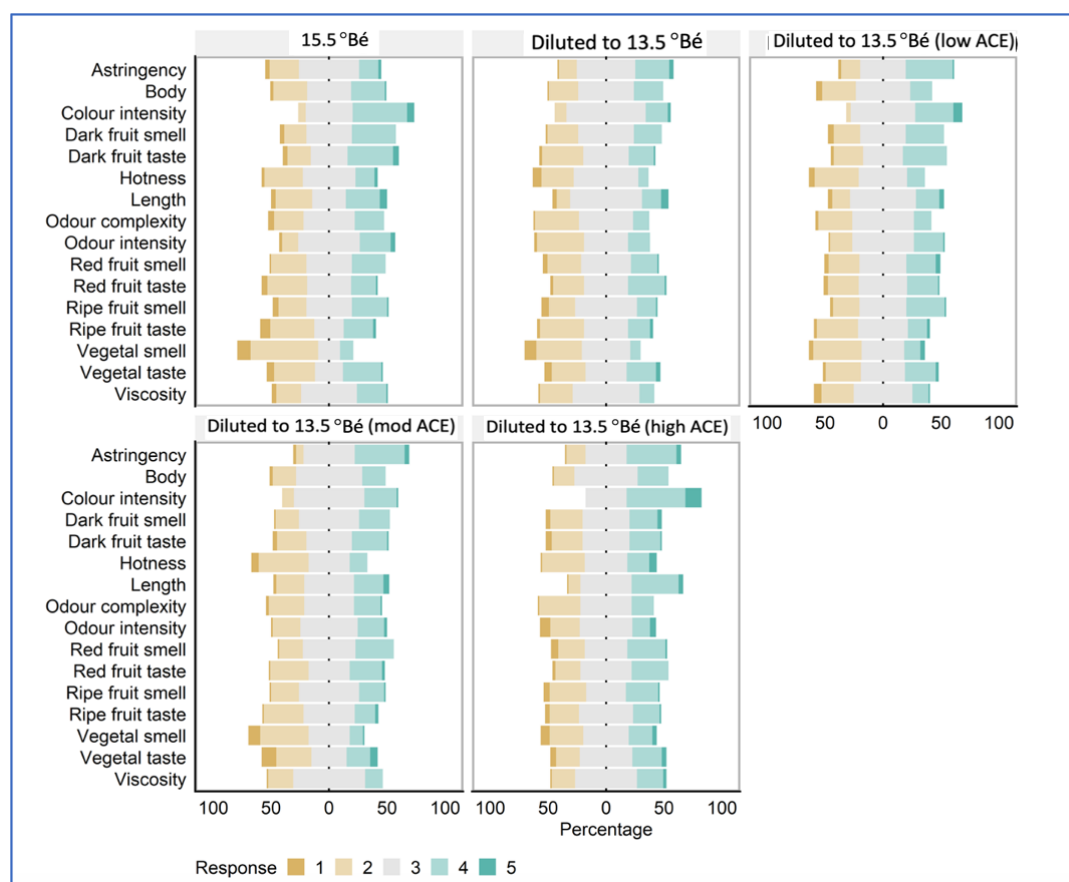


Figure 6.7B. Likert plot – visual representation of the sensory just about right (JAR) naïve sensory panellist JAR datasets

The larger the grey mid-section, the higher preference a particular attribute has.

4. Discussion

Concerns regarding wine quality (Jackson, 2008) and consumer aversion to the production of high-alcohol wines (Burro et al., 2023; Gawel et al., 2013; Saliba et al., 2013) have led to a number of fermentation strategies being adopted, both in the laboratory and commercially, to address the reduction of ethanol yields during fermentation of musts, while also considering the impact on other wine organoleptic qualities. One approach is water addition or water substitution into pre-fermentation high-sugar musts (Australian Government, 2017; Bindon et al., 2019; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020). However, recent studies show that this winemaking approach can lead to a significant decrease in phenolic concentrations and can also negatively impact sensory parameters when compared to a late harvest non-treated wine (Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020). Previous studies have also indicated that because ethanol will contribute to final wine aroma, tannins and flavour (Carpena et al., 2021), there is also a need to balance desired reduction in ethanol production with other desirable wine flavonoids (Remize et al., 1999). It is these concerns that are addressed by this study where the implementation of the accentuated cut edges (ACE) winemaking technique to maintain final wine quality is examined.

4.1 Diluted 13.5 °Bé ACE treatment can restore total phenolics (a.u.), but none of the ACE treatments can restore free anthocyanin (mg/L) levels for a water-treated must young Shiraz wine

The extraction of phenolic compounds has an important role in the determination of final wine quality attributes (Kassara & Kennedy, 2011; Rousserie et al., 2019). Total phenolics in wines which have been subjected to water treatment of their musts pre fermentation have been shown to be negatively impacted (Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020). We find that the diluted to 13.5 °Bé high ACE treatment can restore total phenolics (53.00 a.u.) to similar levels to that of 15.5 °Bé (non-diluted no ACE) (54.33 a.u.) treatment, where all other treatments are observed to have statistically significantly lower results for total phenolics. These outcomes agree with other previous work implementing ACE winemaking approaches (Cheng et al., 2022; Kang et al., 2020; Sparrow, Smart, et al., 2016; Wang et al., 2022).

Anthocyanins are water-soluble flavonoid pigments that, depending on pH, and in some cases complexing agents, can contribute diverse colours in red wine from slight red to

dark purple (Gil-Muñoz et al., 2021). Water dilution treatment of high-sugar musts has been shown to lead to decreases in wine anthocyanins or colour density (Teng et al., 2020). Our findings also find that water addition to the 15.5 °Bé must results in a statistically significant decrease in free anthocyanins in the diluted 13.5 °Bé (no ACE) treatment (538.00 mg/L; 459.00 mg/L). We show that the application of ACE at all levels into water-diluted musts do not significantly address this impact. These findings can be related to all ACE treatment wine samples being tested early, only one and half years post bottling. In young wines, wine anthocyanins have not yet had time to react with other acids and compounds in the wine, such as tannins, pyruvic acid and acetaldehyde, which will change the colour of the wine, causing it to develop more ‘brick red’ hues (Teng et al., 2020). Our outcomes indicate that for this wine quality parameter, ACE implemented at all levels in a water-treated must young Shiraz wine will have minimal influence.

4.2 Diluted to 13.5 °Bé high ACE treatment can increase total tannin (%), but has no effect for chemical age 1 or 2 for a water-treated must young Shiraz wine

Tannins are important flavonoids in wine production. Tannins will come from the grape skins, pips (seeds) and stems, and the wood barrels used during aging. Tannins provide texture and mouthfeel to wine as well as a sense of weight, structure and aging potential (Schelezki, Antalick, et al., 2020). The extraction and retention of tannins from the skins and seeds of red grapes in must are significantly influenced by the conditions throughout the wine fermentation process, including temperature, ethanol concentration and pH levels (Beaver et al., 2019; Watrelot & Norton, 2020). Grape skin tannins are readily extractable in the must due to grape skin breakage and the presence of ethanol, which serves as a solvent during fermentation and maceration, allowing for early release of tannins and anthocyanins into the wine (Kennedy, 2008). Extraction of tannins from grape seeds takes longer and is dependent on ethanol produced by yeasts during alcoholic fermentation of grape sugars present in the must (González-Manzano et al., 2004).

In our study, temperatures of all ferments were maintained so as to address any associated possible impacts on chemical compounds. However, we find that water dilution treatments affect chemical compound concentrations to varying degrees. Previous research has shown that adding water to the fermentation process inevitably dilutes the

must, affecting the overall sugar concentration, which then impacts the fermentation process's ability to produce ethanol. In turn, changes in ethanol content caused by adding water to a must have a significant adverse impact on the tannin extraction kinetics since ethanol plays a major role in the extraction of tannins (Harbertson et al., 2009; Schelezi, Antalick, et al., 2020; Teng et al., 2020). Our results are in line with these findings, where we show that the diluted to 13.5 °Bé (no ACE) treatment wine (with a corresponding decrease in final alcohol levels [13.5% alc/vol]) has a statistically significant decrease in total tannin (0.89%) when compared to the 15.5 °Bé (non-diluted no ACE) treatment wine (1.19%). By implementing ACE treatment, we show that total tannin (%) in the diluted high ACE treatment group (1.20%) is restored to a similar level as the 15.5 °Bé (non-diluted no ACE) treatment group (1.19%) (Figure 6.3; Supplementary materials: Supp. Table S6.2). Our results are consistent with Kang et al. (2020), who also show that ACE can increase the concentration of tannin and total phenolics in wine which has had its must subject to pre-fermentation water treatment (Kang et al., 2020). It could be that the high ACE treatment in our study is able to enhance tannin extraction, where the usual binding of tannin molecules by polysaccharide structures of cell wall fibres reached saturation point before the seed tannins are fully extracted (Bindon et al., 2010), which allows for a significant proportion of extracted tannins to remain in the wine matrix rather than being discarded with the grape marc at pressing. In early maceration and fermentation, anthocyanins can bind with proteins in the grapes and drop out as sediment. Keeping tannins in the must in this way can allow for some tannins to bind with the proteins instead. Tannins will also bind with anthocyanins, where this can stabilise dissolved pigments, which will also assist in preserving colour (Li et al., 2020). The restoration of tannins by high ACE treatment in water-treated musts is thus an important positive outcome that winemakers can consider.

Notably, while chemical age 1 level is highest for the diluted to 13.5 °Bé high ACE treatment group, and chemical age 2 level is highest for the diluted to 13.5 °Bé low ACE treatment group, these levels are not statistically significantly different from any other treatment groups. Chemical age 1 and chemical age 2 have been defined by the extent to which monomeric anthocyanins are displaced by polymeric pigment forms and can indicate wine aging potential (Somers & Evans, 1977). Previous findings that have found that chemical age 1 and 2 indices are significantly correlated with maturation time regardless of other influences (Basalekou et al., 2017). Our results agree with this, where

they may be attributed to the Shiraz treatment wines in this study all being young (1 year after bottling), where no statistically significant changes occurred for these chemical attributes regardless of treatments implemented.

4.3 ACE treatments can restore some sensory parameters in water-treated must young Shiraz wine

4.3.1 Diluted to 13.5 °Bé high ACE treatment can restore colour intensity

In this study, all wine samples were first tested visually. This is because colour and appearance will generally provide a first impression and can be associated with wine quality. If the colour is not what is expected for a wine in question, then this may impact on all other sensory parameters being tested (Ailer et al., 2020). Water-diluted must treated wines have been shown to display a negative impact on wine colour intensity (Teng et al., 2020). By combining ACE with water treatment of musts, we show that for colour intensity, diluted to 13.5 °Bé high ACE treatment wine has the greatest overall colour intensity score. This finding is supported by Sparrow, Holt, et al. (2016) study which showed that for ACE-treated Pinot noir wine, this approach produced the highest proportion of red colour, when compared to non-ACE-treated wine samples (Sparrow, Holt, et al., 2016). However, in our study, sensory panellists find it ‘too high’, with the lowest preference of all treatment groups. Interestingly, 15.5 °Bé (non-diluted no ACE) treatment wine and diluted to 13.5 °Bé moderate ACE treatment wine show greatest preference for colour intensity. For this sensory parameter alone, it can be deduced that while ACE can restore colour intensity in wines which have had their pre-fermentation musts subject to water treatment, untreated wines can still be preferred.

4.3.2 Diluted to 13.5 °Bé high ACE treatment can restore mouthfeel and texture

The mouthfeel and texture properties of wine are a complex mixture of taste and mouthfeel sensations, mostly astringency, and flavour (Canon et al., 2022). These non-taste sensations are a consequence of oral–tactile stimulations and are considered just as important as wine appearance, aroma and taste sensory parameters of a wine (Canon et al., 2022). Studies have shown that water addition treatments of musts implemented to reduce final alcohol levels in wines can have negative impacts on mouthfeel and texture sensory parameters (Tang et al., 2020). Petrie et al. (2019) showed that late harvest 15.5 °Bé non-treated wines displayed higher hotness, viscosity, astringency and opacity

than wines produced from water-treated musts (Petrie et al., 2019). In this study, we assess the mouthfeel and texture sensory parameters of astringency, hotness, body, viscosity and length for all diluted ACE treatment wines in order to determine if ACE can address the impacts on these sensory parameters from water treatment of musts.

Addressing the sensory parameter of hotness in particular can be important, especially for water-diluted treated wines where ethanol levels are decreased. This is because the sensation of hotness is associated with ethanol, which can increase the complexity perceived in a wine (Meillon et al., 2009). In this study, our naïve sensory study panellists score the diluted to 13.5 °Bé high ACE treatment wine with the greatest overall preference for hotness along with the 15.5 °Bé (non-diluted no ACE) treatment wine, where all other treatment wines are observed as having hotness as ‘too low’. This indicates that the diluted to 13.5 °Bé high ACE treatment can replicate or offset the loss in hotness, when compared to other treatments with dilution, and match that of the 15.5 °Bé (non-diluted no ACE) treatment wine effectively. This could be as a result of an increase in other structural chemical compounds in the wine, such as total phenolics and total tannin, as noted above (Li et al., 2020). A high ACE winemaking implementation in a water diluted wine grape must may therefore address the negative impact of the loss of ethanol on sensory parameters in a water-treated wine alone. Related to this is the sensory parameter of wine astringency (Piombino et al., 2020; Vidal et al., 2015). Total tannins and total phenolic chemical measures will also contribute to the perception of astringency. As noted above, the diluted to 13.5 °Bé high ACE diluted treatment will have a positive impact on these chemical parameters. We also find that all ACE treatments will modify the astringency reduction caused by water dilution alone (diluted to 13.5 °Bé [no ACE]). This may be associated with an increased perception of graininess intensities (Bajec & Pickering, 2008). This result is consistent with previous studies undertaken for the Pinot noir variety (Sparrow, Holt, et al., 2016). In our study, however, sensory panellists found that astringency levels were ‘too high’ for all ACE treatment wines, with no preference for any of the treatments indicated by panellists.

The sensory parameter of length is also important for a wine, as it can also indicate its quality. The longer the taste of the wine lingers, the higher the quality it is perceived to have (Canon et al., 2022). This study’s sensory outcomes show that the diluted to 13.5 °Bé high ACE treatment wine has the greatest overall observed length. However, it

is observed as ‘too high’ and has the lowest preference of all treatment wines. No difference in length is observed between other treatments. It may be deduced that the diluted to 13.5 °Bé high ACE treatment increases length too much. However, in lower implementation, diluted to 13.5 °Bé low ACE and diluted to 13.5 °Bé mod ACE treatments are able to maintain a preference for length comparable to that of other treatments without ACE (15.5 °Bé [non-diluted no ACE], diluted to 13.5 °Bé [no ACE]).

4.3.3 Diluted to 13.5 °Bé high ACE treatment can produce preferred vegetal smell, and diluted to 13.5 °Bé moderate ACE treatment can produce preferred odour intensity

Wine aromas will also play an important role in acceptability of a wine and will influence consumer preferences (André et al., 2023; Sáenz-Navajas et al., 2016). For this study, treatment wines without ACE (15.5 °Bé [non-diluted no ACE]; diluted to 13.5 °Bé [no ACE]) have the lowest preference relating to vegetal smell. We find that ACE is able to increase preference for vegetal smell, where sensory panellists indicate highest preference for the diluted to 13.5 °Bé high ACE treatment wine. For odour intensity, 15.5 °Bé (non-diluted no ACE) treatment wine exhibits the highest score; however, this is rated as ‘too high’ by the sensory panellists and has the lowest preference of all treatment wines. This may be attributed to corresponding high ethanol levels of 15.5% alc/vol compared to other diluted treatments at 13.5% alc/vol. Lowest odour intensity is shown in the diluted to 13.5 °Bé (no ACE) treatment wine, where all ACE treatment groups are effectively able to increase odour intensity. This is in line with previous studies that found that ACE can positively address decreases in odour intensity for wines that have been subject to water treatment of must pre fermentation (Kang et al., 2020). Interestingly, our study finds that the diluted to 13.5 °Bé mod ACE treatment wine has the highest preference for odour intensity, indicating that ACE at this level is an effective strategy to increase odour intensity concentrations and preference in water-diluted wines, where it does not become ‘too high’.

4.3.4 All ACE treatments can restore dark fruit taste sensory parameter, with diluted to 13.5 °Bé moderate ACE treatment most preferred

Taste is also an essential element that will indicate wine quality and can demonstrate varying levels of consumer liking (Canon et al., 2022). Taste covers four main receptor-mediated, gustatory sensations: sweet, bitter, sour and salty. Flavour is the combination

of the taste buds and the olfactory receptors. Non-volatile compounds contribute to taste and volatile compounds contribute to aroma (Canon et al., 2022). Previous studies have found that for wines which have had their musts subject to water treatment pre fermentation, negative impacts on wine sensory parameters of taste and flavour will result (Petrie et al., 2019; Piccardo et al., 2019; Schelezi, Smith, et al., 2018; Schelezi, Šuklje, et al., 2018). This can include ‘cooked vegetable’, drain, savoury characteristics and acidic characters (Petrie et al., 2019; Piccardo et al., 2019). By adding ACE to water-treated must, we find that ACE can restore some of the favourable taste/flavour sensory parameters in a final wine. 15.5 °Bé (non-diluted no ACE) treatment wine has the highest observed dark fruit taste; however, this is noted as ‘too high’ with the lowest preference of all treatments. Diluted to 13.5 °Bé (no ACE) treatment wine shows the lowest observed dark fruit taste. The diluted to 13.5 °Bé mod ACE treatment wine has the highest preference, which indicates this ACE treatment can effectively offset the dilution of dark flavours. However, there is no statistically significant difference between diluted to 13.5 °Bé (no ACE) treatment and all other ACE treatments despite their increased preference. We also show that with respect to red fruits taste and flavour, ripe fruit taste and flavour, and vegetal taste and flavour, there are no statistically significant differences observed between any of the treatments. These results may be related to Shiraz wines, which are generally associated with attributes of dark fruits (Pearson et al., 2018), where ACE may have less impact for this variety on these sensory parameters.

4.4 Consumer preference for ACE- and water-treated must young Shiraz wines

By adding ACE to water-treated musts, we find that diluted to 13.5 °Bé high ACE and diluted to 13.5 °Bé mod ACE treatment wines are highest rated overall compared to other treatment wines. However, there is no statistically significant difference observed in consumer overall liking of the wines between treatments. Regarding purchase likelihood, the diluted to 13.5 °Bé mod ACE treatment wine shows a statistically significant preference compared to the 15.5 °Bé (non-diluted no ACE), diluted to 13.5 °Bé low ACE, and diluted to 13.5 °Bé (no ACE) treatment wines, but these are not statistically significantly different from the diluted to 13.5 °Bé high ACE treatment wine. Both the diluted to 13.5 °Bé high ACE and diluted to 13.5 °Bé mod ACE treatment wines demonstrate a statistically significant increase in purchase likelihood when compared to 15.5 °Bé (non-diluted no ACE) and diluted to 13.5 °Bé (no ACE) treatment wines. The

overall increase in purchase likelihood is consistent with statistically significant differences observed in individual attribute preferences. Overall liking analysis is undertaken by a penalty analysis approach. This penalty analysis demonstrates that ‘too little’ body and ‘too little’ red fruit taste are the only two attributes in the critical corner. ‘Too little’ of these two attributes is shown to have the most negative impact on overall liking when observed in the JAR scale. Diluted to 13.5 °Bé low ACE treatment wine is shown as having the lowest score for body. Diluted to 13.5 °Bé (no ACE) treatment wine shows the lowest score for red fruit taste. It could be noted that although consumer perception is that these two treatments have the lowest observed concentration of these attributes, this is not statistically significantly different from any other treatment in each attribute category, so definite conclusions cannot be adequately drawn from these results.

5. Conclusion

In this study, the must extraction winemaking technique accentuated cut edges (ACE) was applied at three different levels (low ACE, moderate ACE and high ACE), where the ACE-treated musts were then subjected with water dilution treatment pre fermentation. The diluted to 13.5 °Bé high ACE treatment was effectively able to offset the outcome of reduced total tannin (%) and total phenolics (a.u.) due to water dilution alone, but not for free anthocyanins (mg/L). Sensory results demonstrated that the diluted to 13.5 °Bé moderate ACE treatment wine had the greatest preference for colour intensity, odour intensity and dark fruit taste for the Shiraz cultivar. In addition, the diluted to 13.5 °Bé high ACE treatment wine showed greatest preference in hotness and vegetal smell, and an increase in preference for dark fruit taste. Wine producers can implement ACE must extraction techniques, in those circumstances where they are required to address final wine alcohol levels while still addressing impacts on flavour and taste, by using pre-fermentation water treatments of wine grape must winemaking. While water treatments of pre-fermentation musts are not yet approved winemaking approaches in many countries, we suggest that as global warming and its impacts on grape growing and wine production continue to be of increasing concern, international winemakers can be kept abreast of developments in Australia. This study demonstrates ACE as a valid winemaking process for Shiraz, which has the potential to positively address any resultant negative impacts on final wine phenolic and sensory aspects related to water dilution fermentation approaches.

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Chapter 6 Supplementary materials

Supp. Table S6.1. ACE skin fragmentation parameters including: number of particles, total surface area, average surface area per particle, average perimeter per particle and perimeter/surface area ratio

	Diluted to 13.5 °Bé (no ACE)	Diluted to 13.5 °Bé (low ACE)	Diluted to 13.5 °Bé (mod ACE)	Diluted to 13.5 °Bé (high ACE)
Number of particles	5.67	22.67	41.00	68.67
Total surface area (cm ²)	7.22	7.65	8.72	10.07
Total perimeter (cm)	31.26	50.46	81.29	110.04
Average surface area per particle (cm ²)	1.34	0.34	0.22	0.15
Average perimeter per particle (cm)	5.68	2.22	2.00	1.62
Perimeter/surface area ratio	4.24	6.45	9.24	10.87

Mean results for triplicate samples are presented.

Supp. Table S6.2. ACE treatment effects on the measures of chemical compounds, for alternative process/treatment groups

Phenolic compound	15.5 °Bé (non-diluted no ACE)			Diluted to 13.5 °Bé (no ACE)			Diluted to 13.5 °Bé low ACE			Diluted to 13.5 °Bé mod ACE			Diluted to 13.5 °Bé high ACE		
	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI	Mean	Standard error (SE)	95% CI
Pigmented tannin (%)	9.83	1.47	6.55, 13.11	9.37	1.47	6.55, 13.11	12.66	1.47	9.38, 15.94	12.29	1.47	9.01, 15.57	12.21	1.47	8.93, 15.49
Chemical age 1	0.36	0.03	0.30, 0.41	0.35	0.03	0.29, 0.41	0.38	0.03	0.33, 0.44	0.38	0.03	0.32, 0.43	0.39	0.03	0.33, 0.44
Chemical age 2	0.10	0.02	0.07, 0.13	0.09	0.02	0.06, 0.13	0.13	0.02	0.09, 0.16	0.12	0.02	0.09, 0.16	0.12	0.02	0.09, 0.15
Colour density (a.u.)	14.73	0.66	13.26, 16.21	11.90	0.66	10.42, 13.38	13.57	0.66	12.09, 15.04	12.97	0.66	11.49, 14.44	14.10	0.66	12.62, 15.58
Free anthocyanins (mg/L)	538.00	21.83	489.35, 586.65 b	459.00	21.83	410.35, 507.65 a	410.33	21.83	352.69, 449.98 a	398.33	21.83	349.69, 446.98 a	449.00	21.83	400.35, 497.65 a
Hue	0.65	0.01	0.63, 0.66	0.64	0.01	0.63, 0.66	0.64	0.01	0.62, 0.65	0.62	0.01	0.60, 0.64	0.62	0.01	0.61, 0.64
Pigmented tannin (a.u.)	3.18	0.36	2.38, 3.97	2.54	0.36	1.75, 3.34	3.20	0.36	2.41, 3.99	3.06	0.36	2.27, 3.85	3.40	0.36	2.61, 4.19
Total phenolics (a.u.)	54.33	0.88	52.37, 56.30 b	46.00	0.88	44.03, 47.97 a	46.00	0.88	44.03, 47.97 a	46.33	0.88	44.37, 48.30 a	53.00	0.88	51.03, 54.97 b
Total pigment (a.u.)	32.20	0.75	30.53, 33.87 c	27.18	0.75	25.51, 28.85 ab	25.40	0.75	23.73, 27.07 a	25.02	0.75	23.35, 26.69 a	28.11	0.75	26.44, 29.78 b
Total tannin (%)	1.19	0.03	1.12, 1.25 b	0.89	0.03	0.82, 0.95 a	0.93	0.03	0.87, 1.00 a	0.93	0.03	0.87, 1.00 a	1.20	0.03	1.14, 1.27 b

Treatment groups that do not have statistically significant differences ($p < 0.05$) will share the same letter (95% CI); and those that are statistically significantly different will have different letters. For the cells where no letters are present, this indicates no overall difference between any treatment groups for that attribute. Mean results for triplicate samples are presented.

Supp. Table S6.3. The linear mixed effects model

Attribute	Model term	df1	df2	F.ratio	p.value
Colour intensity	Process	4	312	18.292	<0.001
Red fruit smell	Process	4	312	0.801	0.525
Dark fruit smell	Process	4	312	0.663	0.618
Ripe fruit smell	Process	4	312	1.323	0.261
Vegetal smell	Process	4	312	6.733	<0.001
Odour complexity	Process	4	312	0.808	0.521
Odour intensity	Process	4	312	4.278	0.002
Red fruit taste	Process	4	312	1.339	0.255
Dark fruit taste	Process	4	312	2.674	0.032
Ripe fruit taste	Process	4	312	0.655	0.624
Vegetal taste	Process	4	312	1.107	0.353
Astringency	Process	4	312	6.249	<0.001
Hotness	Process	4	312	2.956	0.020
Body	Process	4	312	1.832	0.122
Viscosity	Process	4	312	2.273	0.061
Length	Process	4	312	2.544	0.040
Overall	Process	4	312	0.902	0.463
Purchase likelihood	Process	4	312	3.408	0.010

The linear mixed effects models were fitted to the ratings of each attribute. A separate model was used for each attribute: 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, length, and overall liking and purchase likelihood for all ACE experiments. Process (treatment) was included as a fixed effect and panellist taster was included as random effect. Where the p-value is equal to or less than significance level (≤ 0.05), it is assumed that process (treatment) significantly affects the response received. Mean results for triplicate samples are presented.

Chapter 7 – ‘Water into pre-fermentation wine grape must’ and thesis conclusions

1. Summary of the thesis, overall findings and significance

Climate change presents substantial issues for wine grape producers and winemakers. Wine grape growing regions around the world, including Australia, are particularly affected because premium wine grape production happens within restricted temperate ranges, where even small shifts in climate can have an impact on productivity and wine quality (Gladstones, 2011; Mozell & Thach, 2014; Santisi, 2011). The literature review in **Chapter 2** of this thesis presents previous studies that demonstrate a correlation between later harvests and resultant increased levels of ethanol in wine must, together with adverse effects on fermentation, causing unfavourable impacts on volatile aromas, chemical compounds and sensory attributes in the final wine product (Bindon et al., 2019; Teng et al., 2020). The mini literature review in **Chapter 3** of the thesis reveals that there is a need to produce wines with lower alcohol due to concerns about adverse effects of high alcohol consumption on human health (Saliba et al., 2013), as well as associated changing demands of modern wine consumers. Wine producers who now need to address increasing alcohol levels in wine due to a warming climate face the challenge of finding adaptive and alternative winemaking methods that will produce wines with lower alcohol levels, while maintaining organoleptic elements, and ultimately addressing wine consumer demands.

While many vineyard management approaches can address these challenges, winemaking approaches ‘in winery’ can effectively achieve lower alcohol levels in a final wine. The literature review in **Chapter 2** highlights ‘water treatment of pre-fermentation wine grape must’ as one easy-to-implement winemaking approach that winemakers can use to address problematic ‘fermentation issues’ associated with later harvest high-sugar musts. This approach can also produce wines with lower, more-favourable final alcohol levels, and is now approved for use in Australia. The literature review in **Chapter 2** found a significant gap in the research for this winemaking technique. In particular, the impacts from this technique on wine volatile aromas, chemical compounds and correlated sensory attributes of the final treated wines produced is lacking for the most commonly grown grape variety, *V. vinifera* cv. Shiraz (Wine Australia, 2023b). Further, commercial applications based on a number of earlier laboratory findings for this technique appear to be limited as previous studies have primarily concentrated on a single vintage, one or two harvest timings, small wine samples, and limited numbers of panellists for sensory

analysis (Piccardo et al., 2019; Schelezki, Antalick, et al., 2020; Schelezki, Deloire, & Jeffrey, 2020; Teng et al., 2020). Addressing these gaps is at the core of this thesis.

To address the **first research question** of the thesis – “Do expert or naïve wine consumers accept lower-alcohol wines, produced by the addition/substitution of water to pre-fermentation wine grape must winemaking approach, but only where final wine quality including taste and flavour is unaffected?” – I formulated and distributed two wine consumer (both naïve and expert) preference surveys. **Chapter 3** presents the results from these surveys. The self-administered surveys confirmed the hypothesis that expert wine consumers are less concerned about water adjustments during fermentation than naïve wine consumers. I discovered that expert wine consumers prioritised quality over other factors, where 80% of expert wine consumer participants expressed a willingness to purchase wines that had had their alcohol reduced using water dilution. Further, results show that while alcohol content and health considerations did play a role in expert wine consumer preferences, they did not hold the utmost importance for naïve wine consumers. Naïve wine consumers prioritised attributes of personal taste and value for money when making wine purchasing decisions. For both naïve and expert wine consumers, the perception of different alcohol reduction techniques indicated a preference for natural early harvest winemaking approaches. The results also indicate that transparency in winemaking practices and clear labelling were integral for all naïve wine consumers, reflecting their desire for informed and empowered choices.

My findings add to the existing body of knowledge that relates to wine consumer preferences (**Chapter 3**). Outcomes from both surveys identified, amongst other things, what role alcohol content, health considerations, personal taste preference and value for money played in shaping consumer choices. Further, I was able to establish the extent to which both naïve and expert wine consumers expressed their perceptions for different alcohol reduction techniques that can be used by winemakers. I highlighted the differences between expert and naïve wine consumers’ willingness to purchase lower-alcohol wines produced from alternative winemaking approaches, including the ‘water treatment of wine grape must’ technique. Knowing what wine consumers expect from ‘water-treated’ wines with respect to final wine attributes, including alcohol levels, can provide winemakers with the justifications required to adjust their winemaking approaches. In addition, the results give winemakers an appreciation of the preferences

of different types of wine consumers, and determines what information is important to have made available to the broader Australian and global wine industry.

The findings and analysis from the **Chapter 3** consumer preference surveys also set the scene for the scientific experimental approaches taken, as presented in **Chapters 4, 5 and 6** of the thesis. The experimental studies relied on wines produced from *V. vinifera* cv. Shiraz cultivar as it is recognised as the most-planted red grape variety in Australia and globally, where it can grow in both warm and cool climates (Onetto et al., 2023; Schelezki, Deloire, & Jeffrey, 2020; Wine Australia, 2023b). Therefore, any winemaking approach taken to reduce final alcohol levels in Shiraz wine was noted to have a potentially high economic impact for wine producers, wine distributors and wine consumers (Onetto et al., 2023).

To address the **second research question** of the thesis – “Will the addition/substitution of water to later harvest wine grape must pre-fermentation winemaking approach affect fermentation-derived volatile aroma compounds?” – gas chromatography–mass spectrometry (GC–MS) was used to isolate and analyse free volatile compounds for each treatment group wine. Results are presented in **Chapter 4** of the thesis. By clustering 43 common volatile aromas (across the 2020 [Year 1] and 2021 [Year 2] vintages) into 11 compound groups, both harvest timing and alternative water treatments of later harvest pre-fermentation wine grape musts were shown to affect wine alcohol concentrations and influence the volatile aroma compositions of wine to various degrees. The **fourth research question** of the thesis – “Will the addition/substitution of water to late harvest wine grape must pre-fermentation winemaking approach lead to unfavourable sensory parameters including colour, olfactory (aroma), olfactory (taste/flavour), taste, mouthfeel and palate sensation, persistence of flavours on the palate, and overall perception, when compared to non-treated wines? – was initially addressed by collecting sensory data from expert wine consumer panellists; these results are also presented in **Chapter 4**. Expert wine consumer panellists were first relied on because it was recognised that they could add an extra dimension to the results, especially with regard to their perceived influence on the reputation and commerciality of a wine, based on their recognised prior expertise (Hendrickx et al., 2016).

The outcomes of two sensory expert wine consumer sensory surveys conducted as part of the **Chapter 4** study – just about right (JAR) and quantitative descriptive analysis

(QDA) – were then compared to the chemical volatile aromas data obtained through GC–MS. Results demonstrate that wine aroma fingerprinting results were dependent on harvest timings and alternative water treatments, with significant correlations to JAR and QDA sensory attribute outcomes. Most significantly, volatile aroma profiling indicated lowest observed concentrations of isoprenoids, C6 compounds and ‘higher alcohols’ in the early 13.5 °Bé wine. It was noted that C6 compounds in particular could be seen to contribute to grassy or green characteristics of a wine (Bindon, Kassara, et al., 2014). In addition, JAR results for early 13.5 °Bé wine (as well as middle diluted 13.5 °Bé wine) indicated the least preference for dark fruit taste, sweet spice smell, earthy smell, ripe fruit taste, chocolate flavour and earthy flavour, which were all observed as being ‘too low’ by expert wine consumer panellists. Similar results were observed for bitterness, astringency, hotness, body and length, where the early 13.5 °Bé wine had the lowest JAR preference scores, all also observed as being ‘too low’. Correlated QDA results supported these JAR outcomes. It was concluded that harvesting early was to be avoided if winemakers were to avoid negative impacts on volatile aromas and sensory attributes correlated to the early harvest resultant wines. These outcomes were then compared with the wine produced from the late 15.5 °Bé harvest. Here too, there were negative impacts observed for wine aroma profiles, together with many associated negative impacts on sensory parameters. The late 15.5 °Bé wine displayed a decrease in an array of fermentation volatiles and was the least preferred wine for most JAR sensory attributes; these observations were correlated with QDA outcomes. In particular, the late 15.5 °Bé wine showed the lowest observed concentration of acids (hexanoic acid and octanoic acid). While octanoic acid was recognised as an important volatile that could contribute to fruity odour in a wine, it was recognised that it could also have negative rancid and harsh effects (Siebert et al., 2005), which may indicate why many other JAR sensory attributes of the late 15.5 °Bé wine were least preferred. In particular, the late 15.5 °Bé wine was observed to be least preferred for flavour intensity, which was considered ‘too high’ by the expert wine consumer sensory panellists. This observation was closely correlated with its higher QDA mean rating.

Importantly, JAR and QDA expert wine consumer sensory findings (as presented in **Chapter 4**) demonstrated that the late diluted 14.5 °Bé wine was frequently preferred across most sensory attributes, with correlated QDA mean results. This wine was most preferred for colour intensity, which aligned with higher colour intensity mean scores in

QDA quantitative data. Other sensory attributes for this wine, such as odour intensity, red fruit taste, dark fruit taste, liquorice and chocolate flavours, flavour intensity, bitterness, astringency, viscosity, body and length, also demonstrated higher preferences under JAR, where all were correlated closely to their QDA mean value outcomes. It was noted that most of these positive sensory attributes may have been correlated to the observed increase in β -damascenone concentrations in this wine, compared to the late 15.5 °Bé wine, given that β -damascenone has the ability to enhance ‘fruity’ attributes (Pineau et al., 2007). It was concluded that treatment of late harvest pre-fermentation wine grape must with lower levels of water addition to produce a late diluted 14.5 °Bé wine is an alternative winemaking approach that winemakers could adopt to effectively minimise many of the negative impacts on wine volatile compounds due to higher water addition levels, while still maintaining some of the positive flavonoid parameters associated with ethanol, and with less effect on sensory attributes.

To address the **third research question** of the thesis – “Can the addition/substitution of water to late harvest wine grape must pre-fermentation winemaking approach produce negative outcomes with respect to wine chemistry compounds, when compared to non-treated wines?” – **Chapter 5** presents a further and extended analysis of the identical wine samples produced for the **Chapter 4** study. The focus was to test a number of more common chemical compounds in the wine samples, together with correlated sensory parameters as judged by naïve wine consumers. Naïve wine consumers were used in this additional study because they too could add an extra dimension to the thesis results. This is because it was recognised that naïve wine consumers could provide winemakers with an informed perspective as to what typical wine consumers expected from a wine. The objective of this approach was to allow for winemakers to adjust their winemaking and distribution approaches accordingly, based on economic, social and health considerations.

Importantly, results in **Chapter 5** indicate that chemical attributes were at their lowest levels for early 13.5 °Bé wine, except for wine hue (which was consistent across all different harvest timing wines). Principal component analysis (PCA) results confirmed these observations. In addition, for the early 13.5 °Bé wine, the lowest JAR sensory mean ratings (when compared to all other treatment wines) were observed for red fruit smell, vegetal smell, vegetal taste, astringency, viscosity, length and body. The sensory

parameter of ‘colour intensity’ was the only sensory parameter that was most preferred by naïve wine consumer panellists for this wine. It was noted that this result could have been attributed to Shiraz being a full-bodied red wine, which can have high colour intensity even in early harvest wines. These findings correlated to those results observed in **Chapter 4** for this wine, where once again it was determined that an early harvest approach to producing wines with lower alcohol levels is one that winemakers should avoid, especially for full-bodied grape cultivars such as Shiraz. This position was based on the notion that the production of more-favourable chemical compounds in a final wine, which are generally associated with a later harvest, could not be achieved by early harvesting. This technique also had associated negative impacts on a higher number of final wine sensory characteristics, as indicated by naïve wine consumer panellists.

Results for chemical compounds and sensory parameters obtained from naïve wine consumer panellists pertaining to late 15.5 °Bé wine, presented in **Chapter 5**, followed the **Chapter 4** scientific experimental results. The results from the **Chapter 5** study show that the later harvest wine had a final higher alcohol level and higher levels of most chemical compounds when compared to all other harvest timing wines and all water treatment wines. PCA results showed that the late 15.5 °Bé wine and the middle 14.5 °Bé wine were located in the space defined by negative Dim 1 and negative Dim 2, where there were increased colour density (a.u.), total tannin (%), total pigment (a.u.), total phenolics (a.u.) and pigmented tannin (a.u.) levels compared to other treatments. The late 15.5 °Bé wine also had lower preference for many sensory attributes, except for vegetal taste and red fruit taste. While it was noted that these two sensory attributes could be agreeable ones, given the negative impact on all other sensory parameters, together with the production of higher levels of less-favourable chemical compounds, winemakers are advised against harvesting late if their objective is to address the production of undesirable final high alcohol levels in a wine and also avoid other unfavourable wine quality attributes associated with late harvest wines.

Notably, **Chapter 5** results also show that while the late diluted 13.5 °Bé wine displayed some unfavourable impacts for a number of chemical compounds, this wine nevertheless had preferred ripe fruit taste, red fruit smell and hotness sensory attributes. For instance, the lowest level for total phenolics (a.u.) was observed in the late diluted 13.5 °Bé wine, and while this level was not statistically significantly different from all other water

treatment wines, it was still recognised as a relevant consideration for winemakers. This is because total phenolics (a.u.) can have a role in the determination of quality attributes of a final wine, where they can impart important characteristics such as colour, aroma and flavour (Aleixandre-Tudó et al., 2016). Interestingly, naïve wine consumer JAR sensory results indicated that late diluted 13.5 °Bé wine had the highest preference for ripe fruit taste and red fruit smell, which was supported with previous findings that ‘port-like’ sensory attributes generally associated with wines from a late harvest can continue to be present in wines whose late harvest high-sugar musts have been subject to water treatments (Schelezk, Smith, et al., 2018; Schelezk, Šuklje, et al., 2018). This indicated that these quality parameters may have been a preferable aspect of a full-bodied wine such as Shiraz, which could be maintained even with water treatment implemented. In addition, JAR sensory results together with penalty analysis outcomes showed the highest preference for hotness for the late diluted 13.5 °Bé wine, which had a final lower alcohol level of 13.5% ABV. Interestingly, penalty analysis showed that late diluted 13.5 °Bé wine brought the preference rating for hotness closer to the ideal mean value of 3.0; however, the result shifted from ‘too high’ to ‘too low’, indicating that the reduction in hotness by this water treatment into a late 15.5 °Bé harvest was too significant. Nevertheless, this was recognised as an important outcome because hotness can increase the complexity perceived in a wine (Meillon et al., 2009). The thesis findings suggested that water treatment in a late harvest could effectively decrease the hotness to more desirable and preferred levels compared to the untreated late 15.5 °Bé wine.

The most interesting outcomes related to wines produced from a middle 14.5 °Bé harvest and a middle diluted 13.5 °Bé treatment, where each were shown to be more effective at preserving many chemical compounds and favourable sensory attributes in a final wine. This could have been related to the middle 14.5 °Bé wine having a lower starting Bé than a late harvest, but higher Bé than an early harvest, and also to lower water addition levels. There are no previous studies known to date that have tested these particular winemaking approaches and then compared the outcomes for chemical and sensory parameters to those from wines produced from water-treated late 15.5 °Bé musts. For free anthocyanin (mg/L), levels for the middle diluted 13.5 °Bé wine were observed to be less adversely affected when compared to those observed in late diluted 13.5 °Bé wine. Further, the middle diluted 13.5 °Bé wine had a statistically significant decrease in colour density, which was 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’ was noted for this treatment; however, naïve wine consumer panellists indicated a lower preference for this attribute when compared to early 13.5 °Bé wine. It was noted that this outcome may be an important one because wine colour and associated sensory wine quality parameters can be generally attributed to the chemical parameter of colour density (Schelezi, Antalick, et al., 2020). It was concluded that water dilution into a middle 14.5 °Bé harvest was therefore one winemaking approach that winemakers could find more preferable if they seek to modulate colour density, where it brings colour intensity closer to the preferred levels of an early 13.5 °Bé harvest wine. I also found that the middle diluted 13.5 °Bé wine had the highest preference ratings by naïve wine consumers.

To address the **fifth research question** of the thesis – “Can accentuated cut edges (ACE) together with the water addition/substitution into a high-sugar grape must pre-fermentation winemaking approach positively change the chemical composition and sensory attributes of treated wines, when compared to a diluted to 13.5 °Bé (no ACE) wine?” – **Chapter 6** presents scientific experimental results for wines produced from a 2022 late harvest, which were subject to the ACE winemaking process. ACE in this study involved grape skins being mechanically cut into smaller fragments, to assist the extraction of phenolic components from the grape skin of the floating pomace cap in the early stages of fermentation (Sparrow et al., 2016). The literature review in **Chapters 2** revealed that ACE could address some of negative impacts related to the ‘water treatment of pre-fermentation wine grape musts’ winemaking approach. There are very few studies that cover this winemaking technique, especially for the grape cultivar Shiraz, with none testing different levels of ACE fragmentation approaches into pre-fermentation wine must, which are then subject to ‘water treatment’ to produce wines with lower final alcohol levels.

Chapter 6 presents an extended study related to wine produced from the 2022 harvest. The study investigated the ACE winemaking technique to expand on the existing scientific knowledge for ACE. It did so by implementing the ACE winemaking technique at three grape skin fragmentation levels – 10-second ACE treatment (low), 20-second ACE treatment (moderate) and 40-second ACE treatment (high) – into pre-fermentation 15.5 °Bé Shiraz musts, which were then diluted with water to 13.5 °Bé. It was found that reduced total phenolics (a.u.) in diluted to 13.5 °Bé (no ACE) treatment wine (53.00 a.u.)

could be effectively addressed by a diluted to 13.5 °Bé high ACE treatment, restoring levels similar to 15.5 °Bé (non-diluted no ACE) wine (54.33 a.u.). It was also found that total tannins (%) were restored to a similar level as 15.5 °Bé (non-diluted no ACE) wine (1.19%) by a diluted to 13.5 °Bé high ACE treatment (1.20%). Complementing these chemical outcomes, sensory analysis for naïve wine consumers further showed that the diluted to 13.5 °Bé moderate ACE treatment produced wine with the greatest preference for colour intensity, and increased preference for odour intensity and dark fruit taste. Further, the diluted 13.5 °Bé high ACE treatment produced a wine with greatest preference for hotness and vegetal smell, and an increase in preference for dark fruit taste. Both diluted to 13.5 °Bé high ACE and diluted to 13.5 °Bé moderate ACE treatment wines also demonstrated a statistically significant increase in purchase likelihood when compared to the 15.5 °Bé (non-diluted no ACE) wine and the diluted to 13.5 °Bé (no ACE) wine. It was noted that if and when water treatment of pre-fermentation musts winemaking approaches become more widely accepted globally, ACE can be an important winemaking technique for winemakers as it provides them with an additional winemaking strategy that may effectively address the negative impacts on chemical compounds and sensory attributes associated with water treatment winemaking alone.

2. Impact, value and significance for winemakers, wine consumers and the Australian wine industry

Climate change and global warming can affect grape-ripening periods from year to year; however, a longer-term strategy is required, as climate science demonstrates that global warming is real and is not abating (Holland & Smit, 2010). The outcomes and datasets collected in this thesis for the winemaking techniques tested in Chapters 4, 5 and 6 provide evidence-based backing for wine provenance and measures of wine quality, while maintaining Australian wines' unique terroirs. Applying the outcomes of the novel wine consumer preference surveys in this thesis (**Chapter 3**), together with the scientific experimental findings (**Chapters 4, 5 and 6**), can further protect the reputation of Australian wines. In addition, the final experimental data results provided can inform winemakers in their winemaking approaches, especially those taken for commercial wine production. Ultimately, the underpinning goals of providing the thesis results to winemakers are to improve resource management and sustainability, while supporting informed business decision-making. Further, the aim is to effectively and efficiently address challenges for winemaking in both short-term climate cycles, and also as

winemaking approaches that can be taken to address challenges that long-term climate change will present. These goals also go beyond Australian shores, as they are also important for the global wine industry.

The outcomes from the two consumer preference surveys (**Chapter 3**) are beneficial for winemakers as they provide them with preliminary information about what wine consumers expect from water-treated wines with respect to final wine attributes, including alcohol levels, while also providing winemakers with the justifications required to adjust their winemaking approaches. In addition, not only do the research outcomes give winemakers an appreciation of consumer preferences, they also determine what information is important to have made available to the broader wine industry. The survey outcomes also set the scene for the scientific experimental approaches taken, as presented in **Chapters 4, 5** and **6** of the thesis. Harvest timings and water treatments affect wine aroma volatile parameters. The analysis of volatile aromas for each of the various treatment groups provides an efficient prediction of Shiraz wine quality as it relates to volatile concentrations (**Chapter 4**). Combining sensory data obtained from expert wine consumer panellists with aroma volatile data shows that wine consumer perception and preferences provide for a new approach to understanding the correlation of aroma fingerprinting of a wine with its overall liking and preference. The findings in **Chapter 4** provide practical suggestions for winemakers, which have been discussed in detail in Section 1 above. Harvest timings and water treatments also affect wine chemical parameters (**Chapter 5**). These outcomes when taken together with JAR sensory data collected from a larger than typical sample size of naïve wine consumer panellists demonstrates that, while the water treatment of a pre-fermentation wine grape must winemaking approach can have a number of negative impacts on wine chemical compounds, the impacts for sensory attributes are less severe. Overall, naïve wine consumer sensory panellists indicated that they prefer a middle diluted 13.5 °Bé wine. Overall, the data collected has higher than general statistical power due to the large number of panellists involved (n=159) (**Chapter 5**). While it is acknowledged that reliance on naïve wine consumer outcomes can skew results, given that these wine consumers may rate a wine not by reference to its ‘quality’ but on their ‘likings’, the outcomes in **Chapter 5** nevertheless provide winemakers with additional knowledge so that they can make informed decisions about what type of wines will be favoured by general wine consumers, where economic decision-making is also relevant in this regard.

As presented in **Chapter 6**, the diluted to 13.5 °Bé high ACE treatment effectively offset many of the negative chemical impacts on wine due to water dilution alone, where this wine also had the greatest preference among naïve wine consumers for many of the sensory attributes. While water treatments of pre-fermentation wine grape musts are not yet approved winemaking approaches in many countries, as global warming and its impacts on grape growing and wine production continue to be of increasing concern, international winemakers can be kept abreast of developments in Australia. Importantly, wine producers can implement ACE must extraction techniques at differing levels, in those circumstances where they seek to reduce final wine alcohol levels. ACE can also positively address impacts on chemical attributes and correlated wine flavour and taste in those circumstances where winemakers use water treatment of pre-fermentation wine grape must winemaking approaches to produce wines with more-favourable lower alcohol levels. The outcomes as noted above demonstrate that ACE is a valid winemaking process for Shiraz that has the potential to positively address many resultant negative impacts on final wine chemical compounds and sensory parameter aspects related to ‘water treatment of pre-fermentation wine grape must’ winemaking approaches.

3. Considerations for future directions

This thesis research investigated the potential for water treatment of pre-fermentation wine grape musts as an accepted, readily implemented winemaking approach, both for the Australian wine industry, and as an approach that can be adopted globally. However, further research can be conducted to improve the results collected, and the assessment of the testing conducted.

For example, testing could be applied to other grape cultivars. The experimental approach in this thesis tested wines produced from only one wine grape variety, Shiraz. While Shiraz is one of the most important cultivars in Australia, and the most planted red wine grape variety grown (26% of all red wine grape cultivars grown in Australia in 2023), Australia produces wine from many other grape varieties, including white wine varieties such as Chardonnay (19% of all white wine grape cultivars grown in Australia in 2023) (Wine Australia, 2023b). In this thesis study, reducing final alcohol levels by water treatment of wine grape musts, and the impact on wine aroma, chemical composition and sensory attributes, were investigated for treated Shiraz wines. However, the literature review in **Chapter 2** demonstrates that experimental outcomes for this newly permissible

winemaking technique are especially lacking for other key wine grape varieties. Thus further research is required for other wine grape cultivars, as differences in berry compositions and variety-related growing conditions may lead to outcomes that differ from those of the thesis study.

Further studies could also be done using trained wine assessors. Sensory approaches in this thesis study in **Chapters 4, 5 and 6** relied on expert and naïve wine consumer panellists for the testing. While no one type of wine assessor group is better than another, given that each assessor group performs different tasks (Ares & Varela, 2017; Schelezk, Antalick, et al., 2020), it is recognised that the result outcomes can be improved upon by extended studies that use trained wine consumer assessors, who can bring a more nuanced approach to assessing wines for their olfactory, mouthfeel and visual sensory attributes. While it is recognised that using trained assessors can be expensive and time-consuming because of the extensive prior training they need (Sáenz-Navajas et al., 2016), they nevertheless serve as the ‘standard’ for establishing sensory properties of a given food item (Ares & Varela, 2017), having undergone significant prior training to equip them for the sensory tasks ahead (Savelle-Huovinen et al., 2018). This approach can especially address any limitations associated with using naïve wine panellists, who may rate a wine based on their preferences regardless of the quality of the given wine (Ares & Varela, 2017; Schelezk, Antalick, et al., 2020), which means that even a poor-quality wine could have been assessed as a preferred wine, depending on consumer ‘likings’.

4. Conclusion

Wine has a long history for humankind. It is connected to culture, to terroir and to food. Wine continues to be connected to social relations for trade and commerce (Inglis & Almila, 2019). Indeed, in modern times, wine continues to be produced and distributed in a global context across many cultural, social and economic borders (Huber, 2011).

The impetus to undertake this thesis study was the concern that global warming now presents the global wine industry with alarming associated consequences for both wine production and for meeting today’s wine consumer preferences. Climate change affects wine grape growing seasons, where delayed harvests are producing overripened fruit with high sugar levels. In turn, the threat is the production of excessively alcoholic wines or unbalanced wines with unfavourable cooked flavours. In addition, the consumption of

wine has seen a decline in more recent times worldwide. Health and wellness considerations have driven many previous wine consumers to abstain from drinking wine altogether, while others are drinking less but paying more, while some are seeking out no-alcohol and low-alcohol wine options (Wine Australia, 2023a). It is here that winemakers and the wine industry overall need to be more adept at addressing the changing requirements of all wine consumers.

The processes involved in the production of wine have developed over many centuries. One recent consideration for producing wine that can meet the modern wine production challenges in a warming climate relates to an old winemaking approach, but with a new-world application, that is, the ‘water treatment of pre fermentation wine grape must’. Indeed, this technique was an accepted practice in winemaking processes in ancient Greece (Henderson, 2014). However, there are concerns that this winemaking approach can negatively impact final wine flavour, taste and other quality parameters (Petrie et al., 2019). These concerns necessitate that today’s winemakers are provided with evidence-based outcomes and robust datasets to further support wine provenance and measures of wine quality. Linking the outcomes of the two consumer preference surveys (**Chapter 3**) with outcomes for the impacts on volatile aromas, chemical compounds and sensory attributes (as tested by expert and naïve wine consumers) (**Chapters 4 and 5**) demonstrates that the addition or substitution of water to pre-fermentation high-sugar wine grape musts together with ACE winemaking, to address both ethanol production and final wine quality parameters (**Chapter 6**), can be considered as progressive, effective and commercially viable long-term approaches in the future of winemaking.

The results from this thesis support alternative winemaking techniques that can be implemented in a warming climate. This is especially important for wine grape growers, winemakers and wine distributors, where it can provide for the ongoing evolution of wine production and supply, while meeting current wine consumer demands in a global wine economy. Maintaining wine provenance in a warming climate and in response to the shifts in current wine consumer demands have now become the modern challenges of utmost importance for today’s winemakers. Considering the future effects of long-term climate change, the growth of the wine industry in Australia and worldwide demands that modernism and innovation are adopted.

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Appendix 1

Chapter 3: The survey questionnaires

Naïve wine consumer survey

Background	
1	Select your age
	a. under 18 years1 b. 18 to 29 years2 c. 30 to 39 years.....3 d. 40 to 49 years4 e. 50 to 59 years5 f. 60 to 69 years6 g. 70+7 <i>Terminate if (a.) was selected</i>
2	Select gender
	a. Female.....1 b. Male2 c. Other/ Prefer not to disclose3
3	How often do you drink wine?
	Approximately which range best represents the average amount of glasses of wine consumed per week? (Circle only one) a. Daily1 b. 2 days or more per week2 c. Once per week3 d. Once per month.....4 e. Never.....5
4	How much would you spend on average on a bottle of wine?
	Which range best represents a typical price paid for a bottle of wine? (Circle only one) a. \$0.00-\$9.991 b. \$10.00-\$19.992 c. \$20.00-\$34.993 d. \$35.00-\$49.994 e. \$50.00+5
5	What style of wine do you drink usually? (Circle only one)
	a. Red1 b. White2 c. Other3
6	Is there a region in Australia that you like to drink wine from? (multiple selections available)

	a. Barossa Valley1 b. Hunter Valley2 c. Margaret River3 d. Coonawarra4 e. Yarra Valley5 f. Clare Valley6 g. Rutherglen7 h. McLaren Vale8 i. Tasmania9 j. Other10
Specific Questions	
7	Does the percentage alcohol of the wine you are looking to purchase effect your purchasing decisions?
	Yes No
8	Do you think that wines made in Australia have alcohol concentrations that are too high?
	Yes No
9	If your answer to Question 8 is Yes, would you like to see this alcohol level reduced? Explain your reasoning.
	Open ended answer
10	Would you like to see more lower alcohol wines available for purchase?
	Yes No
11	Based on your answer to question 10 explain your reasons
	Open ended answer
12	Are you aware that water is a permitted addition into grape juice during the winemaking process since 2017 as per the change in the AU/NZ food standards act?
	Yes No

13	Would you purchase a wine that has have its alcohol reduced in the winemaking process using water dilution?
	Yes No
14	Based on your answer to question 13 explain your reasons
	Open ended answer
15	Would you purchase a wine that has have its alcohol reduced using other chemical techniques such as reverse osmosis or spinning cone technologies?
	Yes No Indifferent
16	Based on your answer to question 15 explain your reasons
	Open ended answer
17	Would you purchase a wine that has had its alcohol reduced by earlier harvest of wine grapes at lower sugar levels?
	Yes No Indifferent
18	Based on your answer to question 17 explain your reasons
	Open ended answer
19	If you were to purchase a wine which had undergone alcohol reduction which would be your preference (number 1 to 3 with 1 being your first preference and 3 being your last)
	- Early harvest (less ripe grapes produce wines with lower alcohol) - Normal harvest then add water to grape juice before ferment (diluting the sugar concentration in grape juice before fermentation reduces the final alcohol of resultant wines) - Normal harvest then remove alcohol by chemical techniques such as centrifugation or reverse osmosis
20	The following is a list of attributes that people may look for when purchasing wine. Please indicate the desirability of each by rating them using the 1-4 desirability scale described below.
	LOW HIGH 1. Health benefits 1 2 3 4 5 2. Health risks 1 2 3 4 5 3. Alcohol content 1 2 3 4 5 4. Calorie intake 1 2 3 4 5

21	Water can be added during the process of making wine in Australia without any indication on the labelling. How important do you feel it is that there should be regulation in food packaging regarding the mandatory disclosure of water additions into wine?																				
	Extremely important.....1 Very important.....2 Somewhat important.....3 Slightly important4 Not at all important5																				
22	The following is a list of features of wine people may look for wine making a wine purchasing decision. Please indicate the desirability of each feature to you when you purchase wine by indicating a number from 1-5. 5. Extremely desirable 4. Very desirable 3. Desirable 2. Slightly desirable 1. Not at all desirable																				
	<table border="0"> <tr> <td>Brand I have tasted before</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Good value for money</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Varietal I like</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Alcohol Level</td> <td>1 2 3 4 5</td> </tr> <tr> <td>A complement to food</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Attractive label</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Region of origin</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Produced sustainably</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Organically grown</td> <td>1 2 3 4 5</td> </tr> <tr> <td>Additives</td> <td>1 2 3 4 5</td> </tr> </table>	Brand I have tasted before	1 2 3 4 5	Good value for money	1 2 3 4 5	Varietal I like	1 2 3 4 5	Alcohol Level	1 2 3 4 5	A complement to food	1 2 3 4 5	Attractive label	1 2 3 4 5	Region of origin	1 2 3 4 5	Produced sustainably	1 2 3 4 5	Organically grown	1 2 3 4 5	Additives	1 2 3 4 5
Brand I have tasted before	1 2 3 4 5																				
Good value for money	1 2 3 4 5																				
Varietal I like	1 2 3 4 5																				
Alcohol Level	1 2 3 4 5																				
A complement to food	1 2 3 4 5																				
Attractive label	1 2 3 4 5																				
Region of origin	1 2 3 4 5																				
Produced sustainably	1 2 3 4 5																				
Organically grown	1 2 3 4 5																				
Additives	1 2 3 4 5																				

Expert wine consumer/industry survey

Background	
1	Select your age
	a. under 18 years..... 1 b. 18 to 29 years..... 2 c. 30 to 39 years 3 d. 40 to 49 years..... 4 e. 50 to 59 years..... 5 f. 60 to 69 years..... 6 g. 70+ 7 <i>Terminate if (a.) was selected</i>
2	Select your gender
	a. Female 1 b. Male 2 c. Other/ Prefer not to disclose..... 3
3.	What is your frequency of consumption of wine
	a. Daily b. Two or more times a week c. Once a week d. Once a month e. Never
4	What is your occupation in the wine industry (select all that apply)
	a. Winemaker b. Wine sales representative c. Wine retailer d. Wine taster/ judge e. Other – please specify _____
5	How many years have you been in your primary role?
	a. 2-4 years b. 5-9 years c. 9-15 years d. 15 + years

6	How many years have you been in the wine industry
	a. 2-4 years b. 5-9 years c. 9-15 years d. 15 + years
Specific Questions	
7	Are you aware of the changes in 2017 to the AU/NZ Food Standards Act that allow the addition of water to grape must pre-fermentation?
	Yes No
8	If yes, what is your understanding of the change?
	Open ended question
9	Do you think that the addition of water to grape must can have a positive contribution to final wine products?
	Yes No Unsure
10	Based on your answer to question 9 explain your reasons
	Open ended answer
11	Have you performed or been made aware of any water additions into grape must?
	Yes No
12	Have you added water to grape must before?
	Yes No
13	Would you purchase a wine that has have its alcohol reduced during the winemaking process using water dilution?
	Yes No

14	What proportion of wines produced in Australia approx. would you estimate you have water added to them?
	a. 0% b. 1-10% c. 11 – 25% d. 26 % - 50% e. Over 50%
15	From your perspective, do you think the addition of water to grape must is a widespread practice in the Australian wine industry?
	Yes No
16	Are you aware of any producers (including yourself) adding water to grape must before 2017?
	Yes No
17	Water can be added during the process of making wine in Australia without any indication on the labeling. How important do you feel it is that there should be regulation in food packaging regarding the mandatory disclosure of water additions into wine?
	Extremely important 1 Very important..... 2 Somewhat important 3 Slightly important..... 4 Not at all important..... 5

Appendix 2

Chapter 4: Just about right (JAR) and quantitative
descriptive analysis (QDA) expert consumer survey
questionnaires

Faculty of Veterinary and Agricultural Sciences

Consent form for persons participating in a research project

PROJECT TITLE: Assessment of wine and wine labels using sensory analysis and biometric techniques

Name of participant:

Name of investigator(s): **Dr Pangzhen Zhang, Ms Sonja Needs, A/Prof Kate Howell, Mr Chris Barnes, Mr Billy Xynas**

1. I consent to participate in this project, the details of which have been explained to me, and I have been provided with a written plain language statement to keep.
2. I understand that after I sign and return this consent form it will be retained by the researcher.
3. I understand that my participation will involve answering a sensory form, and I agree that the researcher may use the results as described in the plain language statement.
4. I acknowledge that:
 - (a) The possible effects of participating in the sensory panel have been explained to my satisfaction;
 - (b) I have been informed that I am free to withdraw from the project at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
 - (c) The project is for the purpose of research;
 - (d) I have been informed that the confidentiality of the information I provide will be safeguarded subject to any legal requirements;
 - (e) I have been informed that with my consent the sensory panel sessions will be recorded in sensory forms, and I understand that sensory forms will be stored at University of Melbourne and will be destroyed after five years;
 - (f) My name will be referred to by a pseudonym in any publications arising from the research;
 - (g) I will not take part of the study if I declare I have any food allergy related to the samples involved in this study. (Please specify any food allergy: _____)

Participant signature: _____

Date: _____

Please circle your answer

Age: 18-24 25-30 31-40 41-55 56 and above

Gender: Female Male Other

Occupation: Student non-wine related work wine related work retired not work

If you circled 'wine related work' please circle which of the following applies:

Wine Educator Winemaker Wine Trader Wine Show Judge Viticulturist Wine Lover

Wine Retail Other: _____.

How long have you been doing wine sensory evaluation 0-1 year 2-5 years 6-10 years 11-20 years 21-30 years > 30 years

Have you done any professional training in wine sensory evaluation: (if yes please indicate)

Background	Australian Born and live in Australia for more than 1.5 years	Chinese Born live in Australia for less than 1.5 years	India Born live in Australia for less than 1.5 years	Europe or US born, live in Australia over 10 years	Asian (other than China) Born live in Australia over 10 years	Other
------------	---	--	--	--	---	-------

Do/have you drunk red wine before? Yes No

If Yes, do you like *red* wine? Don't like at all much Like it little Neither like nor dislike Like it a lot Like it very

If Yes, how long have you drunk *red* wine for? < 2 years 2-5 years 6-10 years 11-20 years > 20 years

If Yes, how often do you drink *red* wine? ≥ once a day ≥ 3 times a week ≥ once a week ≥ once a month ≥ once a year < once a year

If Yes, you drink *red* wine: (circle all that apply) With meals By itself Socially I don't drink it

Have you undertaken wine sensory analysis before? Yes No

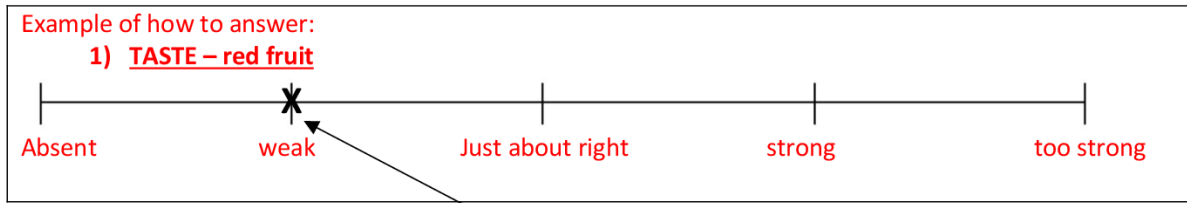
(if yes how many times) once <10 times <50 times >50 times

How much would you spend on a bottle of *red* wine for yourself? (AUD) ≤ \$10 \$11-20 \$21-30 \$31-40 \$41-50 \$51-100 >\$100

How much would you spend on a bottle of *red* wine for a gift? (AUD) ≤ \$10 \$11-20 \$21-30 \$31-40 \$41-50 \$51-100 >\$100

How to answer:

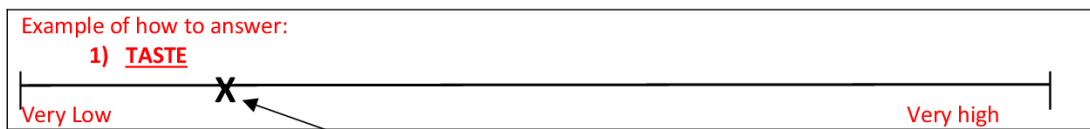
Just About Right Method:



Mark X on the line at the indicted points on the line, where you feel the appropriate level is perceived.

Do not mark in the middle between two levels.

Quantitative Descriptive Analysis Mehtod:

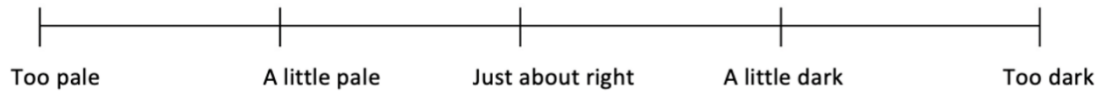
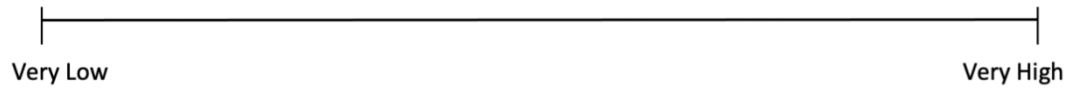


Mark X on the line anywhere you feel the appropriate level is perceived

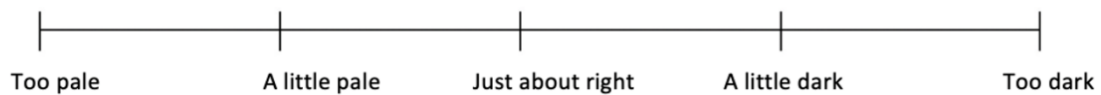
WINE

A) VISUAL (View the glass on a 45° angle on white paper)

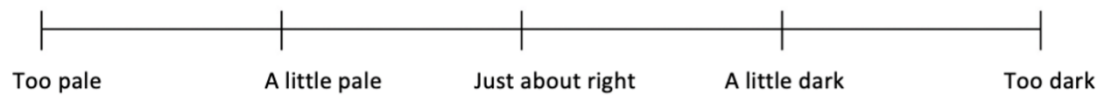
Opacity (transparency)



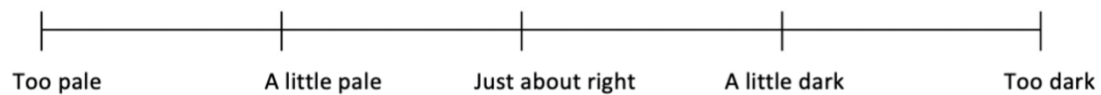
Purple Colour



Red Colour

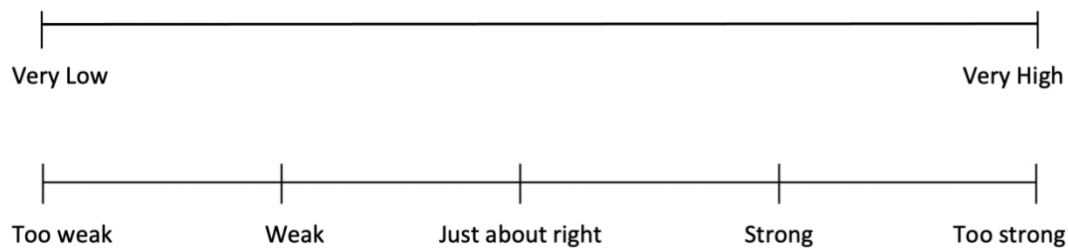


Colour Intensity

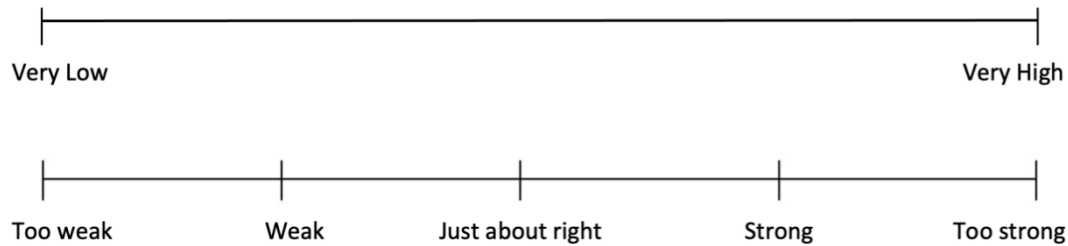


B) OLFACTORY (AROMA) (DO NOT drink wine yet - Swirl the wine a few times, then smell for better aroma release)

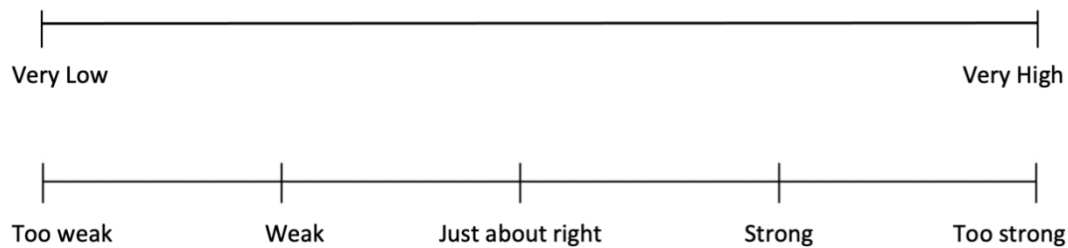
Red Fruit smell (includes smell of cherries, raspberries and strawberries)



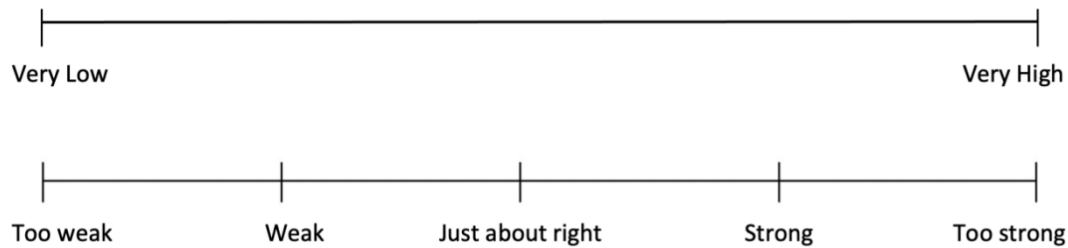
Dark Fruit smell (includes the smell of cassis, black berries)



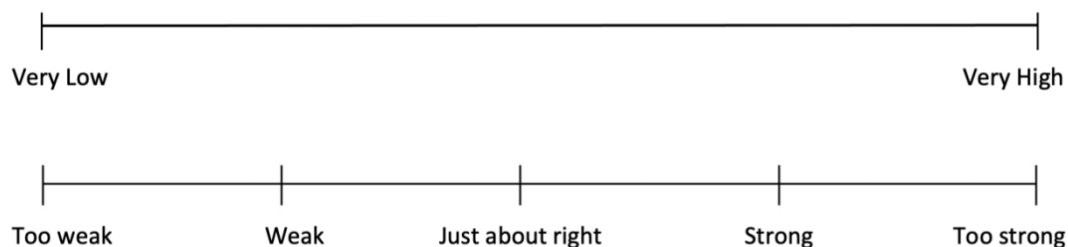
Ripe fruit smell (includes smells of ripe fruits, candied fruits)



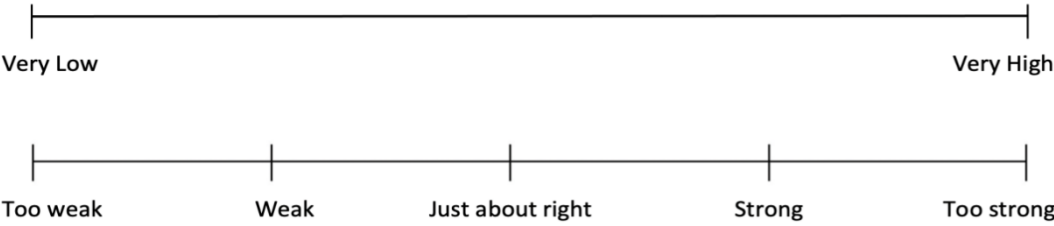
Vegetal smell (includes smells of vegetal, herbaceous, sweet pepper)



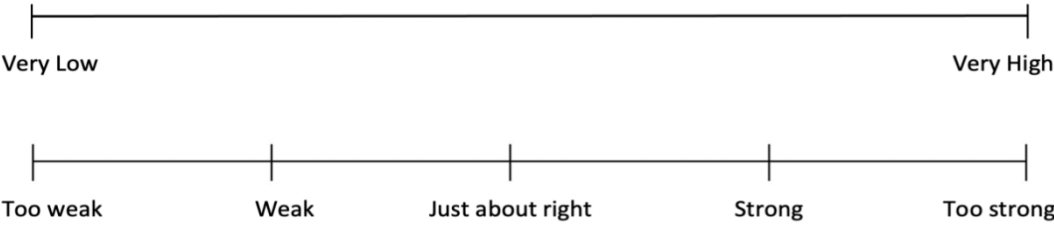
Sweet Spice smell (includes the smell of cloves, cinnamon, cardamon, mixed oriental spice)



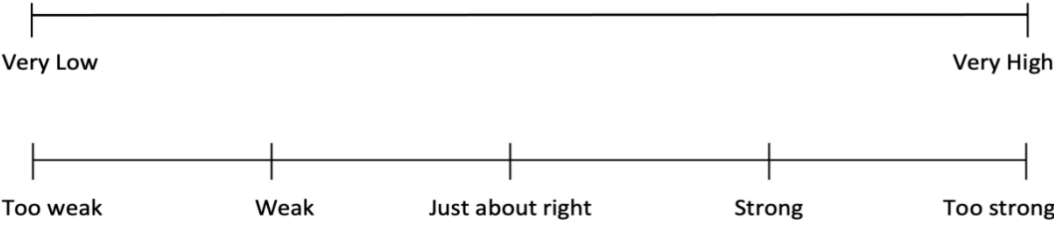
Liquorice smell (includes the smell of liquorice)



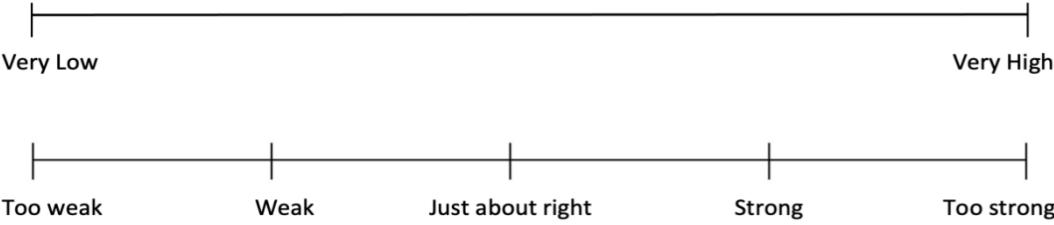
Chocolate smell (includes the smell of dark chocolate)



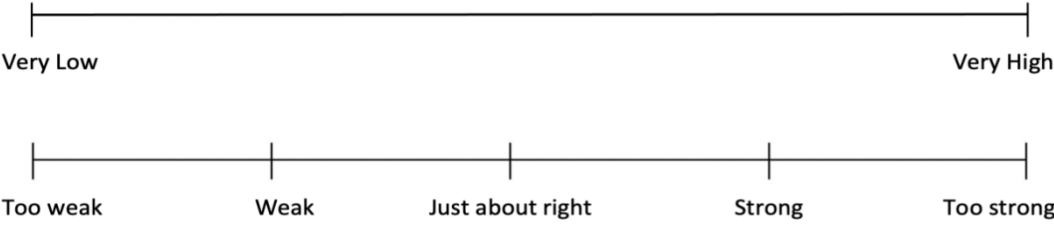
Confectionary smell (includes the smell of lollies)



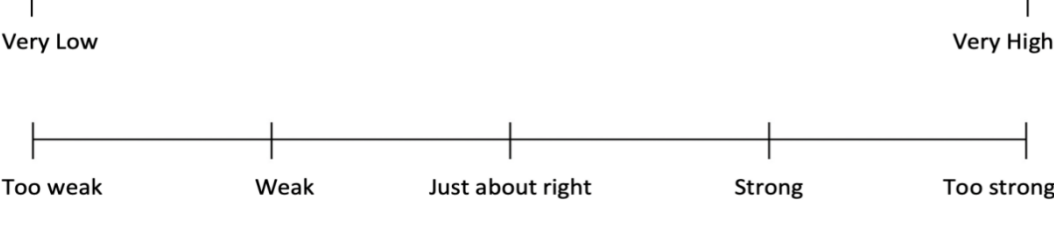
Earthy smell (includes the smell of soil, dirt and dust)



Aroma Intensity (includes the smell of the sum of all aroma attributes perceived in the wine)

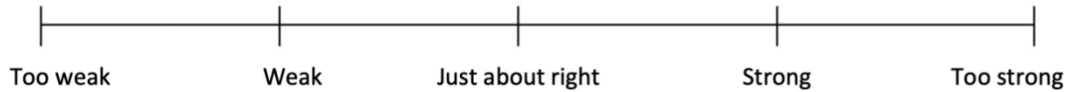
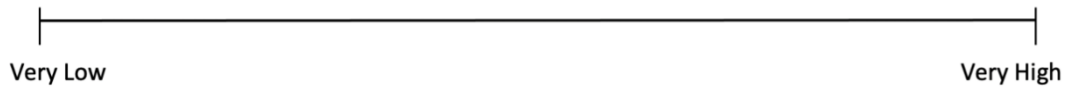


Odour complexity (Overall smell, Complexity, richness)

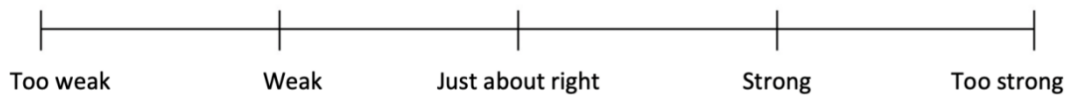
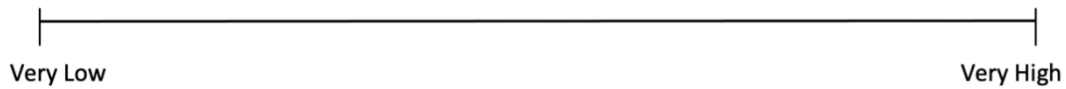


C. OLFACTORY (TASTE/FLAVOUR) *(Take a sip of wine, coat all of mouth and tongue, exhale through nose)*

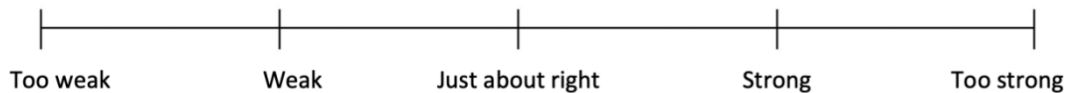
Red Fruit flavour *(includes the flavour of cherry, raspberry and strawberry)*



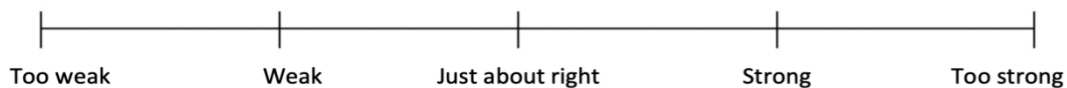
Dark fruit flavour *(includes the flavour of cassis, black berries)*



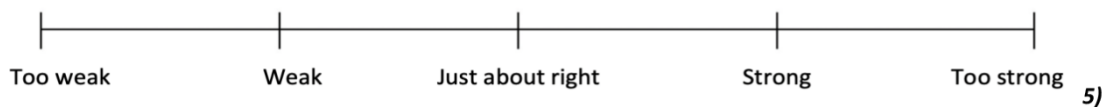
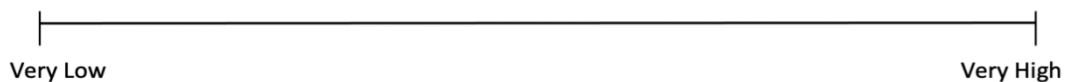
Vegetal taste and flavour *(includes the flavour of vegetal, herbaceous, sweet pepper)*



Ripe fruits taste/flavour *(includes the flavour of ripe fruits, candied fruits)*

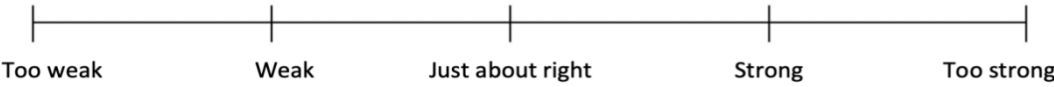
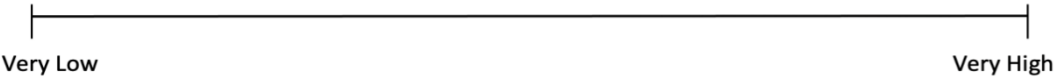


Sweet Spice flavour *(includes the flavour of cloves, cinnamon, cardamon, mixed oriental spice)*



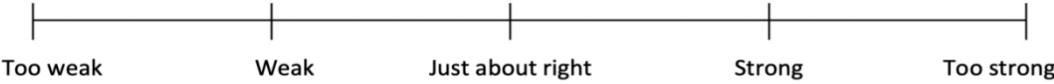
5)

Liquorice flavour (includes the flavour of liquorice)

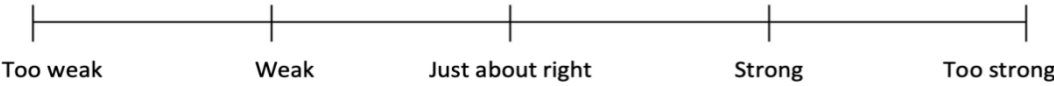
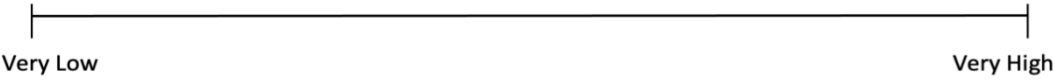


6)

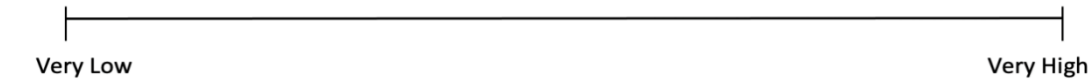
Chocolate flavour (includes the flavour of dark chocolate)



Earthy flavour (includes the flavour of soil, dirt or dust)

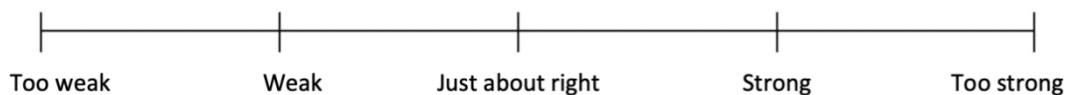


Flavour Intensity (includes the flavour overall perceived in the wine)

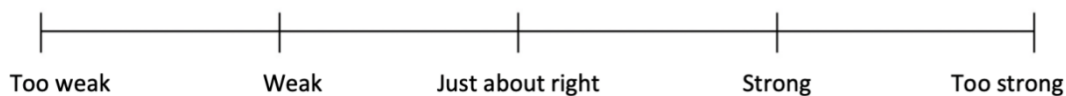


D. TASTE/ MOUTHFEEL/ PALATE SENSATION

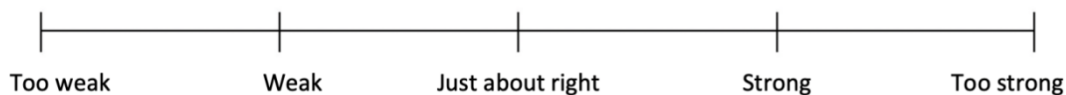
Acidity (the perceived acidity on your tongue)



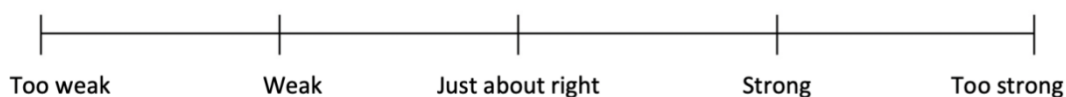
Sweetness (the perceived sweetness on your tongue)



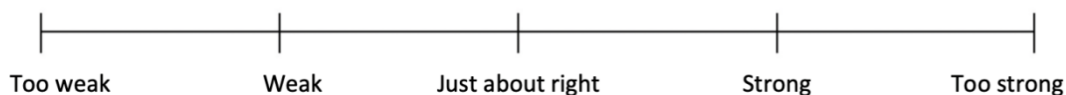
Bitterness (the perceived bitterness on your tongue)



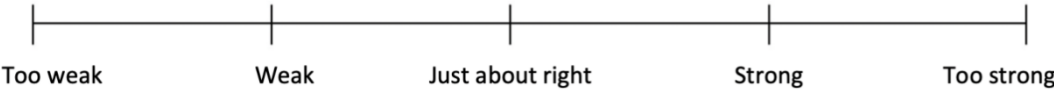
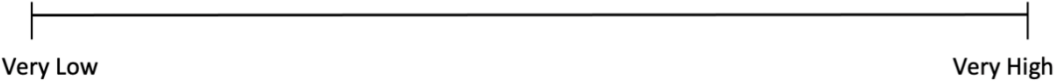
Astringency (the astringency, perceived as puckering and drying sensation on the oral mucosa)



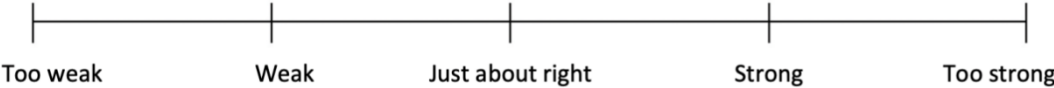
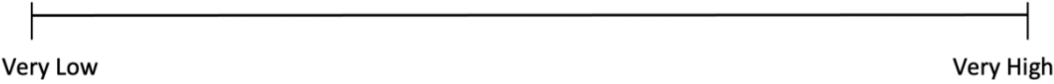
Hotness (the perceived hotness that is caused by alcohol)



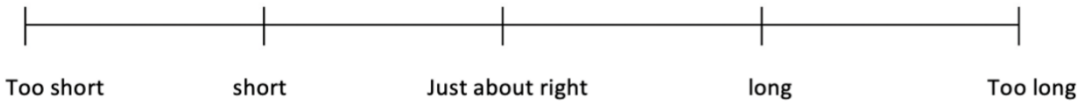
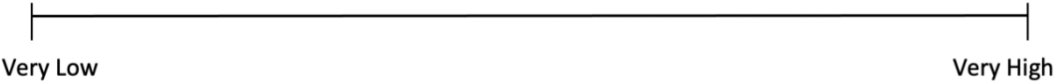
Viscosity (*thickness of the wine in the mouth*)



Body (*The perception of the body, richness, fullness*)

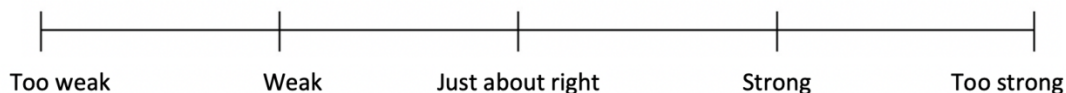
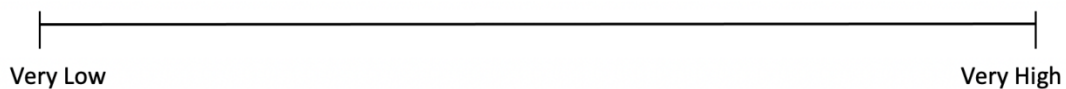


Length (*The time that aftertaste persists in the mouth*)

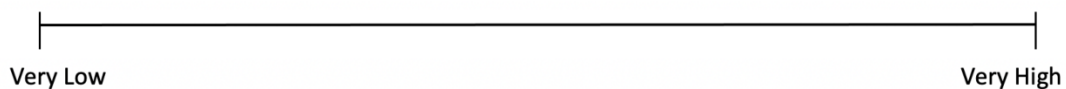


E) PERSISTENCE OF FLAVOURS ON THE PALATE

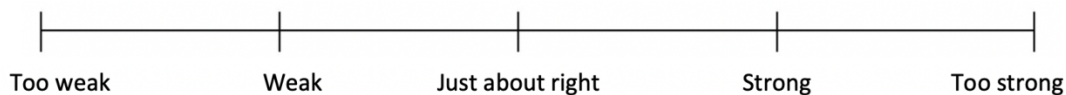
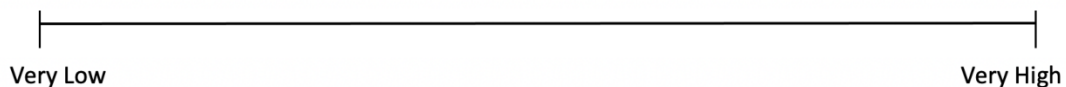
Fruit aftertaste (The time that fruit aftertaste persists in the mouth)



Sour aftertaste (The time that aftertaste persists in the mouth)

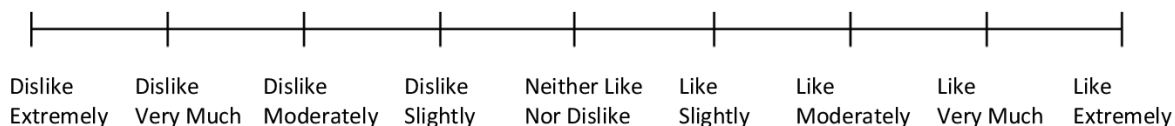
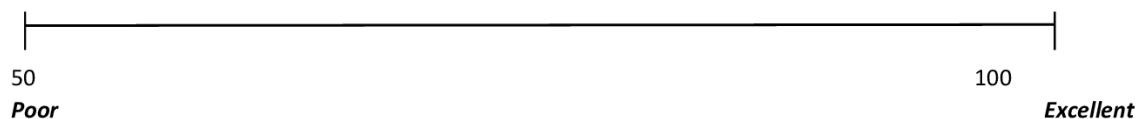


Balance (a balance of aromas, flavour, structure and mouthfeel)

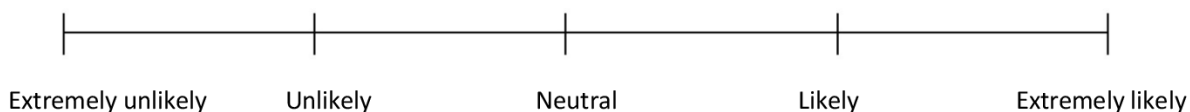


F) OVERALL PERCEPTION

Overall liking of the wine (taking into account all aspects)



How likely are you to purchase this wine?



Appendix 3

Chapter 5: Just about right (JAR) naïve wine consumer questionnaire



Faculty of Veterinary and Agricultural Sciences

Consent form for persons participating in a research project

PROJECT TITLE: Assessment of wine and wine labels using sensory analysis and biometric techniques

Name of participant:

Name of investigator(s): Dr Pangzhen Zhang, Ms Sonja Needs, A/Prof Kate Howell, Mr Chris Barnes, Mr Billy Xynas

I consent to participate in this project, the details of which have been explained to me, and I have been provided with a written plain language statement to keep.

I understand that after I sign and return this consent form it will be retained by the researcher.

I understand that my participation will involve answering a sensory form, and I agree that the researcher may use the results as described in the plain language statement.

I acknowledge that:

- The possible effects of participating in the sensory panel have been explained to my satisfaction;
- I have been informed that I am free to withdraw from the project at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
- The project is for the purpose of research;
- I have been informed that the confidentiality of the information I provide will be safeguarded subject to any legal requirements;
- I have been informed that with my consent the sensory panel sessions will be recorded in sensory forms, and I understand that sensory forms will be stored at University of Melbourne and will be destroyed after five years;
- My name will be referred to by a pseudonym in any publications arising from the research;
- I will not take part of the study if I declare I have any food allergy related to the samples involved in this study. (Please specify any food allergy: _____)

Participant signature:

Date:

Please circle your answer

Age: 18-24 25-30 31-40 41-55 56 and above

Gender: Female Male Other

Occupation: Student non-wine related work wine related work retired not work

If you circled 'wine related work' please circle which of the following applies:

Wine Educator Winemaker Wine Trader Wine Show Judge Viticulturist Wine Lover

Wine Retail Other: _____.

How long have you been doing wine sensory evaluation 0-1 year 2-5 years 6-10 years 11-20 years 21-30 years > 30 years

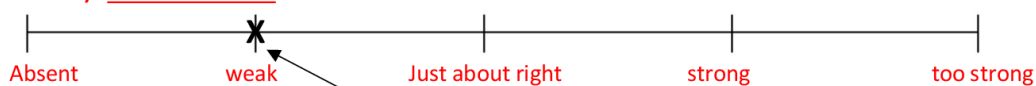
Have you done any professional training in wine sensory evaluation: (if yes please indicate)

Background	Australian Born and live in Australia for more than 1.5 years	Chinese Born live in Australia for less than 1.5 years	India Born live in Australia for less than 1.5 years	Europe or US born, live in Australia over 10 years	Asian (other than China) Born live in Australia over 10 years	Other	
Do/have you drunk red wine before?	Yes					No	
If Yes, do you like <i>red</i> wine?	Don't like at all much	Like it little	Neither like nor dislike	Like it a lot	Like it very		
If Yes, how long have you drunk <i>red</i> wine for?	< 2 years	2-5 years	6-10 years	11-20 years	> 20 years		
If Yes, how often do you drink <i>red</i> wine?	≥ once a day	≥ 3 times a week	≥ once a week	≥ once a month	≥ once a year	< once a year	
If Yes, you drink <i>red</i> wine: (circle all that apply)	With meals		By itself		Socially	I don't drink it	
Have you undertaken wine sensory analysis before?	Yes		No				
(if yes how many times)	once		<10 times		<50 times	>50 times	
How much would you spend on a bottle of <i>red</i> wine for yourself? (AUD)	≤ \$10	\$11-20	\$21-30	\$31-40	\$41-50	\$51-100	>\$100
How much would you spend on a bottle of <i>red</i> wine for a gift? (AUD)	≤ \$10	\$11-20	\$21-30	\$31-40	\$41-50	\$51-100	>\$100

The Survey

Example of how to answer:

1) TASTE – red fruit

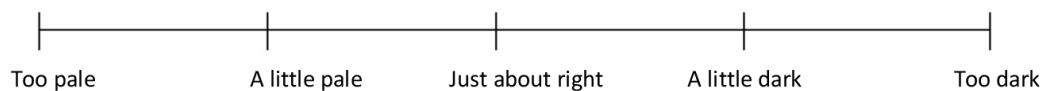


Mark X on the line at the indicted points on the line, where you feel the appropriate level is perceived. Do not mark in the middle between two levels.



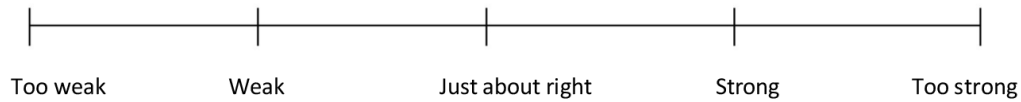
A) VISUAL (View the glass on a 45° angle on white paper)

1) *Colour Intensity*

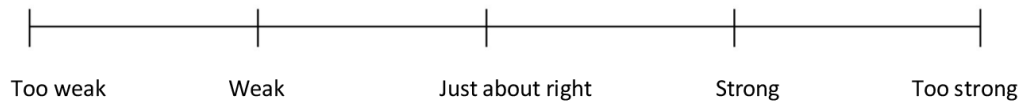


B) OLFACTORY (AROMA) *(DO NOT drink wine yet - Swirl the wine a few times, then smell for better aroma release)*

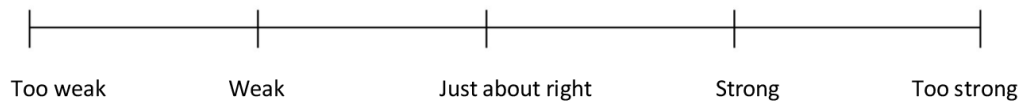
1) Red fruit smell *(includes smell of cherries, raspberries and strawberries)*



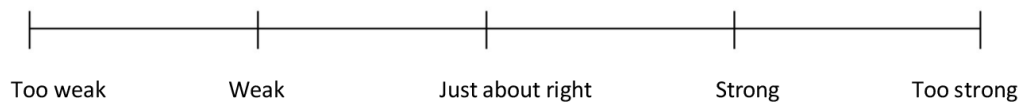
2) Dark fruit smell *(includes the smell of cassis, black berries)*



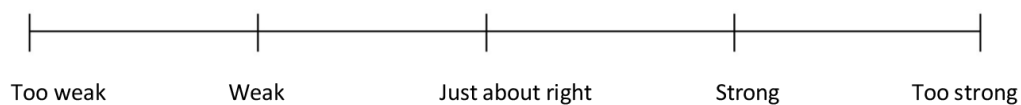
3) Ripe fruit smell *(includes smells of ripe fruits, candied fruits)*



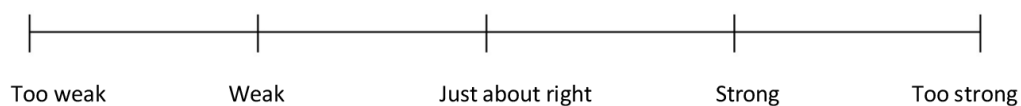
4) Vegetal smell *(includes smells of vegetal, herbaceous, sweet pepper)*



5) Odour complexity *(Overall smell, Complexity, richness)*

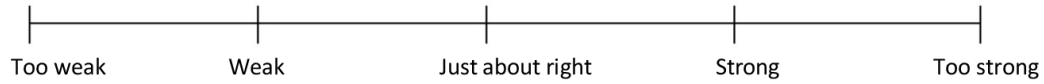


6) Odour intensity *(Intensity of odours overall)*

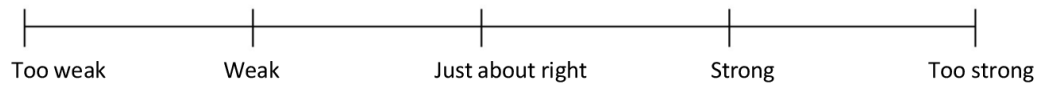


C. OLFACTORY (TASTE/FLAVOUR) *(Take a sip of wine, coat all of mouth and tongue, exhale through nose)*

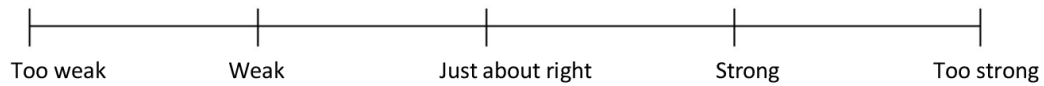
1) Red Fruits taste/ flavour *(includes the flavour of cherries, raspberries and strawberries)*



2) Dark Fruits taste/flavour *(includes the flavour of cassis, black berries)*



3) Ripe fruits taste/flavour *(includes the flavour of ripe fruits, candied fruits)*

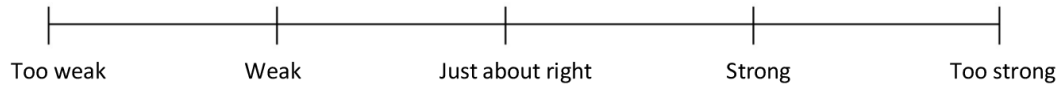


4) Vegetal taste and flavour *(includes the flavour of vegetal, herbaceous, sweet pepper)*

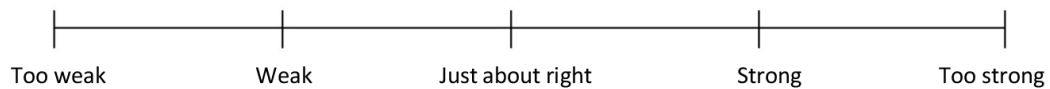


D) MOUTHFEEL/ TEXTURE

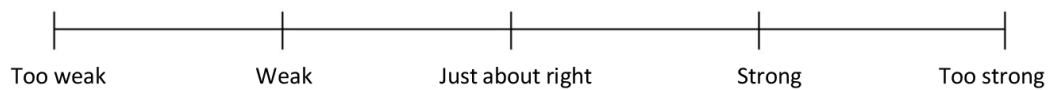
1) Astringency *(the astringency, perceived as puckering and drying sensation on the oral mucosa)*



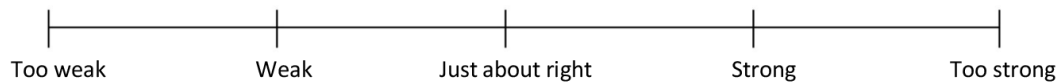
2) Hotness *(the perceived hotness that is caused by alcohol)*



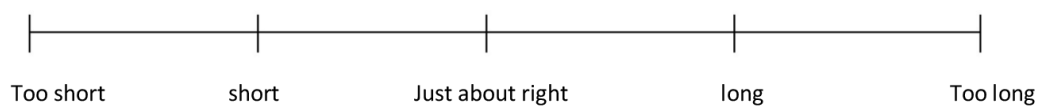
3) Body *(The perception of the body, richness, fullness)*



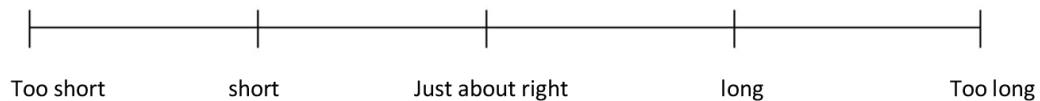
4) Viscosity *(thickness of the wine in the mouth)*



5) Length *(The time that aftertaste persists in the mouth)*

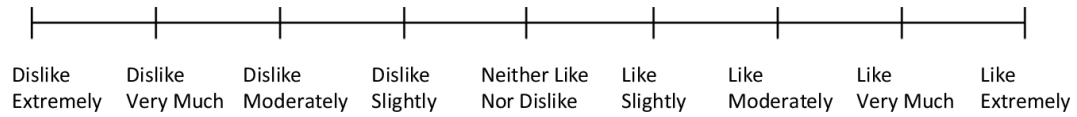


5) Balance *(Balance between aromas, flavour, structure and mouthfeel)*

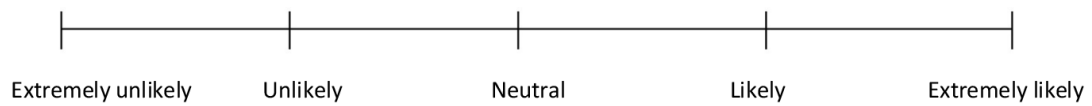


F) OVERALL PERCEPTION

1) Overall liking of the wine (taking into account all aspects)



2) How likely are you to purchase this wine?



Appendix 4

Chapter 6: Just about right (JAR) naïve wine consumer questionnaire



Faculty of Veterinary and Agricultural Sciences

Consent form for persons participating in a research project

PROJECT TITLE: Assessment of wine and wine labels using sensory analysis and biometric techniques

Name of participant:

Name of investigator(s): Dr Pangzhen Zhang, Ms Sonja Needs, A/Prof Kate Howell, Mr Chris Barnes, Mr Billy Xynas

I consent to participate in this project, the details of which have been explained to me, and I have been provided with a written plain language statement to keep.

I understand that after I sign and return this consent form it will be retained by the researcher.

I understand that my participation will involve answering a sensory form, and I agree that the researcher may use the results as described in the plain language statement.

I acknowledge that:

- The possible effects of participating in the sensory panel have been explained to my satisfaction;
- I have been informed that I am free to withdraw from the project at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
- The project is for the purpose of research;
- I have been informed that the confidentiality of the information I provide will be safeguarded subject to any legal requirements;
- I have been informed that with my consent the sensory panel sessions will be recorded in sensory forms, and I understand that sensory forms will be stored at University of Melbourne and will be destroyed after five years;
- My name will be referred to by a pseudonym in any publications arising from the research;
- I will not take part of the study if I declare I have any food allergy related to the samples involved in this study. (Please specify any food allergy: _____)

Participant signature: _____

Date: _____

Please circle your answer

Age: 18-24 25-30 31-40 41-55 56 and above

Gender: Female Male Other

Occupation: Student non-wine related work wine related work retired not work

If you circled 'wine related work' please circle which of the following applies:

Wine Educator Winemaker Wine Trader Wine Show Judge Viticulturist Wine Lover
Wine Retail Other: _____.

How long have you been doing wine sensory evaluation 0-1 year 2-5 years 6-10 years 11-20 years 21-30 years > 30 years

Have you done any professional training in wine sensory evaluation: (if yes please indicate)

Background	Australian Born and live in Australia for more than 1.5 years	Chinese Born live in Australia for less than 1.5 years	India Born live in Australia for less than 1.5 years	Europe or US born, live in Australia over 10 years	Asian (other than China) Born live in Australia over 10 years	Other
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Do/have you drunk red wine before? Yes No

If Yes, do you like *red* wine? Don't like at all much Like it little Neither like nor dislike Like it a lot Like it very

If Yes, how long have you drunk *red* wine for? < 2 years 2-5 years 6-10 years 11-20 years > 20 years

If Yes, how often do you drink *red* wine? ≥ once a day ≥ 3 times a week ≥ once a week ≥ once a month ≥ once a year < once a year

If Yes, you drink *red* wine: (circle all that apply) With meals By itself Socially I don't drink it

Have you undertaken wine sensory analysis before? Yes No

(if yes how many times) once <10 times <50 times >50 times

How much would you spend on a bottle of *red* wine for yourself? (AUD) ≤ \$10 \$11-20 \$21-30 \$31-40 \$41-50 \$51-100 >\$100

How much would you spend on a bottle of *red* wine for a gift? (AUD) ≤ \$10 \$11-20 \$21-30 \$31-40 \$41-50 \$51-100 >\$100

The Survey

Example of how to answer:

1) **TASTE – red fruit**



Mark X on the line at the indicted points on the line, where you feel the appropriate level is perceived. Do not mark in the middle between two levels.

A) **VISUAL** (View the glass on a 45° angle on white paper)

1) **Colour Intensity**

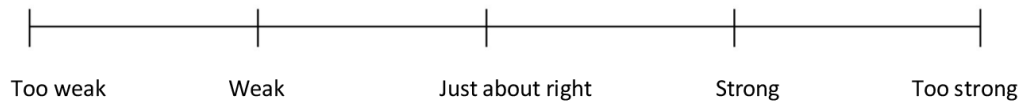


B) OLFACTORY (AROMA) *(DO NOT drink wine yet - Swirl the wine a few times, then smell for better aroma release)*

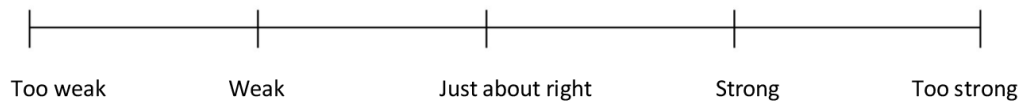
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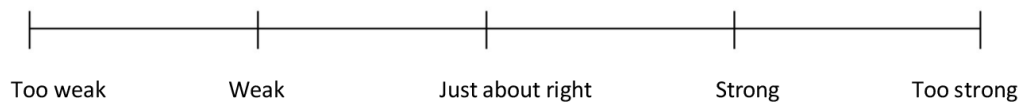
2) Dark fruit smell *(includes the smell of cassis, black berries)*



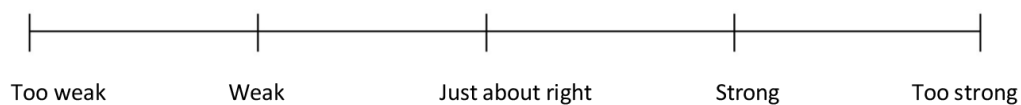
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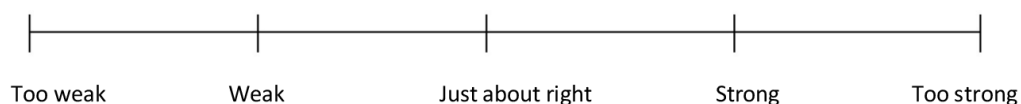
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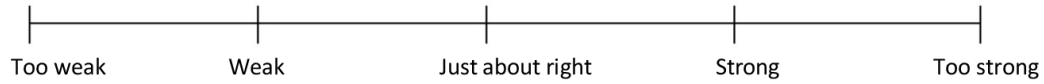


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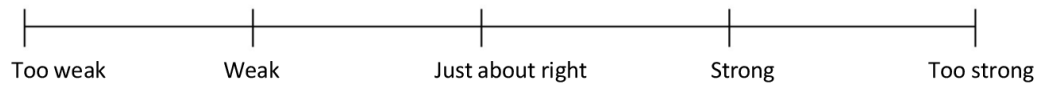


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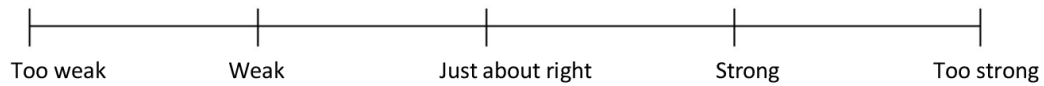
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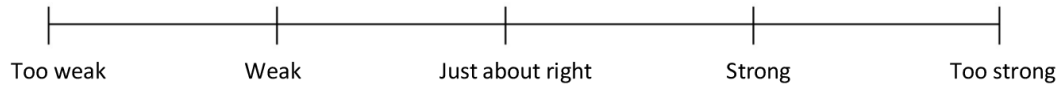


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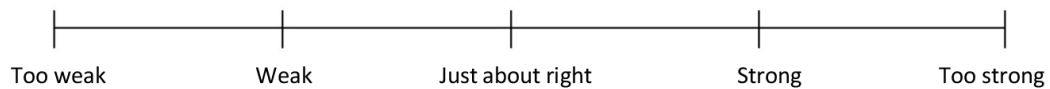


D) MOUTHFEEL/ TEXTURE

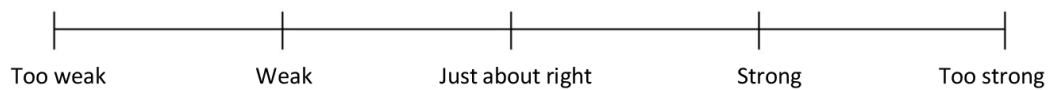
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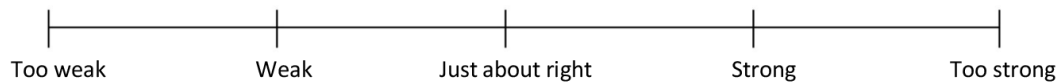
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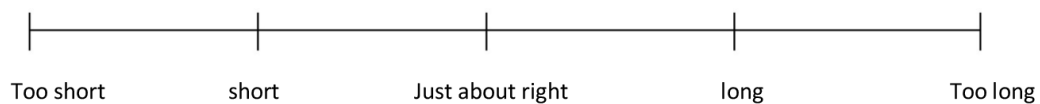
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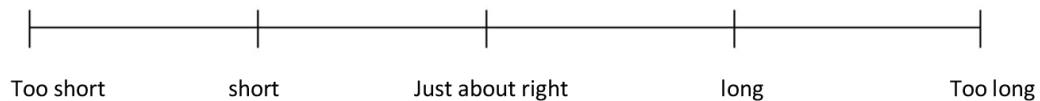
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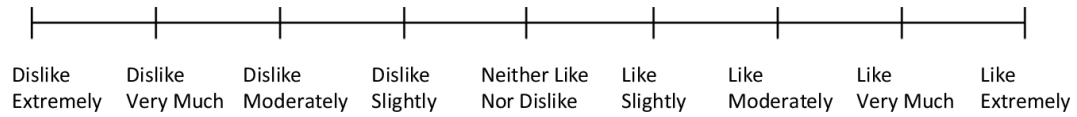


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F) OVERALL PERCEPTION

1) Overall liking of the wine (taking into account all aspects)



2) How likely are you to purchase this wine?

