



Getting Alcohol Content Right: The Compositional and Sensory Basis for the Alcohol Sweetspot

Duc-Truc Pham

Thesis submitted for the degree of
Doctor of Philosophy
in
School of Agriculture, Food and Wine
The University of Adelaide

June, 2020

CONTENTS

ABSTRACT	ii
DECLARATION.....	iv
ACKNOWLEDGEMENTS.....	v
PUBLICATIONS AND PRESENTATIONS	vi
PANEL OF SUPERVISORS.....	viii
ABBREVIATIONS	viii
Chapter 1. Introduction	1
1. RAW regulations	2
2. Approaches for alcohol reduction in wines	2
3. Impact of ethanol removal on wine composition.....	11
4. Impact of ethanol removal on wine sensory properties	14
5. The sweetspot phenomenon in reduced alcohol wine	16
6. Research aims	20
References.....	21
 Chapter 2. Compositional consequences of partial dealcoholization of red wine by reverse osmosis–evaporative perstraction	 31
1. Introduction.....	32
2. Results and Discussion	33
3. Materials and Methods.....	38
4. Conclusions.....	44
References.....	45
Supplementary Materials	48
 Chapter 3. Investigating alcohol sweetspot phenomena in reduced alcohol red wines	 49
1. Introduction.....	50
2. Materials and Methods.....	52
3. Results and Discussion	54
4. Conclusions.....	59
References.....	60
Supplementary Materials	62
 Chapter 4. Influence of partial dealcoholization on the composition and sensory properties of Cabernet Sauvignon wines	 68
1. Introduction.....	69
2. Materials and Methods.....	70
3. Results and Discussion	71
4. Conclusions.....	75
References.....	76
Supplementary Materials	77
 Chapter 5. Summary and Conclusion.....	 82

ABSTRACT

The alcohol content of wine has been steadily increasing due to both climate change and improvements to viticultural management practices and winemaking techniques. Wines containing high alcohol levels tend to exhibit increased hotness, a characteristic considered to negatively impact wine quality. Winemakers are therefore managing their wine alcohol levels, using various methods. The objectives of this research included: (i) determination of the compositional and sensory changes resulting from partial dealcoholisation of wine using reverse osmosis–evaporative perstraction (RO-EP); and (ii) optimisation of the ethanol content for reduced alcohol wine (RAW), i.e. a study into the alcohol ‘sweetspot’ phenomenon. The thesis comprises an introduction, 3 manuscripts describing experimental work completed, and a conclusion, including limitations and future work.

Chapter 1 comprises a literature review outlining: the different approaches available for ethanol reduction of wine, together with their relative advantages and disadvantages; the chemical and sensory consequences of removing ethanol from wine; and previous investigations into the wine alcohol ‘sweetspot’ phenomenon.

Chapter 2, published in *Molecules*, is titled: Compositional consequences of partial dealcoholization of red wine by reverse osmosis–evaporative perstraction. This study investigated compositional changes in red wines resulting from ethanol removal by reverse osmosis–evaporative perstraction (RO-EP), and provides insight into the physical and chemical changes to RAW. Trial 1 involved RO-EP treatment of three wines analysed pre-treatment, post-treatment, and post-treatment with alcohol adjustment (i.e. addition of ethanol to the original alcohol content). Trial 2 involved partial dealcoholisation of two wines and analysis of samples collected before, during and after RO-EP treatment. RAWs were slightly more concentrated than pre-treatment wines, which resulted in greater colour

intensity, and increased phenolics and organic acids. However, partial dealcoholisation resulted in lower concentrations of some fermentation volatiles, particularly ethyl esters, which may reflect ester hydrolysis following ethanol removal.

Chapter 3, submitted to *Foods*, is titled: Investigating alcohol sweetspot phenomena in reduced alcohol red wines. In this study, different methods of presenting wine samples (ordered vs. randomised, and linear vs. circular) were employed to determine to what extent presentation order influences the outcome of alcohol sweetspotting trials. Two different statistical analyses, i.e. chi-square goodness of fit vs. one proportion tests, were also evaluated. Statistical analyses confirmed alcohol sweetspots were apparent in some evaluations, but outcomes were not reproducible (by panel or by individual panellist). One proportion tests improved the likelihood of identifying statistically significant differences between RAWs, but variation in individuals' sensitivity prevented validation of the alcohol sweetspot phenomenon.

Chapter 4, prepared for submission to *Foods*, is titled: Influence of partial dealcoholization on the composition and sensory properties of Cabernet Sauvignon wines. This study investigated changes in the chemical and sensory profiles of five Cabernet Sauvignon wines following partial dealcoholisation by RO-EP. Descriptive analysis of wine indicated dealcoholisation did not strongly affect aroma and flavour, consistent with the small changes observed in wine composition. However, significant difference were observed for several palate attributes, with dealcoholised wines found to exhibit lower acidity, sweetness, bitterness, saltiness and/or body. A novel approach to alcohol sweetspotting trials was also investigated, but evidence is still needed to validate any alcohol sweetspot phenomenon.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Duc Truc Pham

26/ 06 / 2020

ACKNOWLEDGEMENTS

There are a number of people without whom I may not have completed my PhD thesis. I would like to sincerely thank these people and acknowledge them here:

My supervisors Professor Kerry Wilkinson, A/Professor David Jeffery and Dr Vanessa Stockdale for their scientific advice and encouragement during my PhD. They have always been a dedicated supervisor panel during the course of my PhD.

Dr Renata Ristic, my external advisor, for incredible assistance given to me in data processing and plenty of encouragement over the years. Dr Jonathan Tuke for incredible support in statistical analysis.

The industry partners, Treasury Wine Estates and Memstar, for their collaboration and for the provision of technical support and wine samples; and AWRI for support in chemical analysis.

Everyone in the Wilkinson Group (Julie, Lieke and Sijing) for their friendship and support throughout the years.

Members of the ARC Training Centre for Innovative Wine Production, at the University of Adelaide, for the invaluable assistance given to me.

Scholarship funding from ARC Training Centre for Innovative Wine Production, conference travel funding from Wine Australia and project funding from industry partners.

Last but not least, I wish to thank my friends, my family and relatives, who have encouraged me throughout my studies with endless love and support.

Duc-Truc Pham

PUBLICATIONS AND PRESENTATIONS

SCIENTIFIC JOURNAL ARTICLES:

Pham, D-T; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L. Compositional consequences of partial dealcoholization of red wine by reverse osmosis-evaporative perstraction. *Molecules* 2019, 24, 1404; doi:10.3390/molecules24071404

Wollan, D.; Pham, D-T; Wilkinson, K.L. Changes in wine ethanol content due to evaporation from wine glasses and implications for sensory analysis. *J. Agric. Food Chem.*, 2016, 64 (40), pp 7569–7575

Pham, D-T; Stockdale, V.J.; Jeffery, D.W.; Tuke, J.; Wilkinson, K.L. Investigating Alcohol Sweetspot Phenomena in Reduced Alcohol Red Wines. *Foods* 2019, 8, 491; doi:10.3390/foods8100491

Pham, D-T; Ristic, R.; Stockdale, V.J.; Jeffery, D.W.; Tuke, J.; Wilkinson, K.L. Influence of Partial Dealcoholization on the Composition and Sensory Properties of Cabernet Sauvignon Wines. *Food Chemistry*, 2020, 325, 126839. DOI: 10.1016/j.foodchem.2020.126869

INDUSTRY JOURNAL ARTICLES:

Wollan, D.; Pham, D-T; Wilkinson, K.L. Changes in wine ethanol content due to evaporation from wine glasses. *Wine & Viticulture Journal*, 2016, 31 (6), 32.

Ristic, R.; Hranilovic, A., Li, S.; Longo, R.; Pham, D-T; Qesja, B.; Schelezki, O.J.; Jiranek, V. Alcohol: Integrated strategies to moderate the alcohol content of wines. *Wine & Viticulture Journal*. 2016, 31 (5), 33.

CONFERENCE PRESENTATIONS:

Ristic, R., Schelezki, O.J., Hranilovic, A., Li, S., Pham, D-T., Wollan, D., Bindon, K.A., Jeffery, D.W., Jiranek, V., Wilkinson, K.L. An integrated approach to achieving lower alcohol levels in wine. 254th ACS National Meeting & Exposition, August 2017, Washington DC, USA.

Dang, C; Pham, D-T; Taylor, D. Cyclodextrins and polymers as novel additives and processing aids to remove off-odours in wine. 10th In Vino Analytica Scientia Symposium, July 2017, University of Salamanca, Spain.

D.-T. Pham, Wilkinson, K.L. Walking the line: Getting the alcohol content right. Treasury Wine Estates Technical Conference, Nov 2016. Magill, South Australia.

Pham, D-T; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L. Changes in red wine composition following partial dealcoholisation by reverse osmosis-evaporative perstraction. The 16th Australian Wine Industry Technical Conference. July 2016, Adelaide, Australia. (poster presentation).

Dang, C; Pham, D-T; Taylor, D. Cyclodextrins as novel additives to remove off-odours in wine. The 16th Australian Wine Industry Technical Conference, 2016. Adelaide, Australia. (poster presentation).

Wollan, D.; Pham, D-T; Wilkinson, K.L. Changes in wine ethanol content due to evaporation from wine glasses. The 16th Australian Wine Industry Technical Conference. July 2016, Adelaide, Australia. (poster presentation, Winner of the People's Choice for Best Poster).

Pham, D-T; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L. Partial dealcoholisation of red wine by reverse osmosis-evaporative perstraction: impact on wine composition. Macrowine- Macromolecules and Secondary Metabolites of Grapevine and Wine, 2016. Changins-Nyon, Switzerland. (poster presentation).

Pham, D-T; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L. Compositional consequences of partly dealcoholising red wine. The Wartburg Symposium on Flavor Chemistry & Biologyn, 2016. Eisenach, Germany. (poster presentation).

Pham, D-T; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L. Impact of ethanol reduction by membrane techniques on wine composition. Crush 2015 – The Grape and Wine Science Symposium. Adelaide, Australia. (oral presentation).

PANEL OF SUPERVISORS

Main supervisor: Professor Kerry L. Wilkinson

Co supervisor: A/Professor David W. Jeffery

External supervisor: Dr Vanessa J. Stockdale

Independent Advisor: Dr Renata Ristic

ABBREVIATIONS

RAW	reduced alcohol wine
SCC	spinning cone column
RO	reverse osmosis
EP	evaporative perstraction
MWCO	molecular weight cut off
TA	titratable acidity
VA	volatile acidity
HPLC	high performance liquid chromatography
GC-MS	gas chromatography-mass spectroscopy
SIDA	stable isotope dilution analysis
SPME	solid phase micro-extraction
SIM	selective ion monitoring
ANOVA	analysis of variance
LSD	least significant difference
DA	descriptive analysis

Chapter 1.

Introduction



In recent decades, the alcohol content of wine has been increasing worldwide, largely due to climate change and improvements in viticultural practices and winemaking techniques.¹ For example, over the past 20 years, Australian red wines have shown a steady increase in alcohol levels, such that the mean for all samples analysed by the Australian Wine Research Institute (AWRI) in 2008 was 14.4% (v/v), compared with 12.4% v/v in 1984,² with harvest times changing from mid-March to mid-February in South Australia.³ These increases have negative financial implications for winemakers, because of higher tax rates; whereas for wine consumers, there are health and social issues associated with increased alcohol consumption.¹ Moreover, excessive ethanol levels may have negative effects on must fermentation and wine sensory perception.⁴⁻⁸ For these reasons, there is worldwide demand for the production of reduced alcohol wine (RAW).⁹

1. RAW regulations

Legal definitions of wine and wine products specify minimum ethanol concentrations, which vary for different countries. In the United States, the Federal Alcohol Administration Act states that wine must contain between 7 and 24% v/v ethanol.^{10,11} In Australia, until recently, wine was required to contain greater than 8% v/v ethanol,¹² but amendments made in 2011 revised the minimum alcohol requirement to 4.5% v/v.¹³ Recent European regulations (EC Reg. 606/2009) allow for the reduction of wine ethanol levels to 2% v/v, prompting new interest in RAW.¹⁴

2. Approaches for alcohol reduction in wines

Many winemakers and researchers have been seeking methods to decrease the alcohol level of wine, without significantly affecting the concentrations of other compounds associated with wine quality, especially aroma compounds.¹⁵ Methods for producing RAW have been available since the early 1900s.¹ Approaches can be grouped into three main

categories (**Table 1**): (i) pre-fermentation - the reduction of grape sugar levels in the vineyard;¹⁶⁻¹⁹ (ii) during-fermentation - limiting the amount of ethanol produced during the fermentation process;²⁰⁻²³ and (iii) post-fermentation - applying technological protocols that decrease the ethanol level of finished wine.^{1,10,14,24,25}

Table 1. Key methods for reducing the ethanol content of wine

Stages	Methods	References
Pre-fermentation	• Adjusting vine leaf area	16,17
	• Early fruit harvest	10
	• Glucose oxidase enzyme	26-29
	• Dilution	14
	• Membrane	30
During fermentation	• Early arrest of fermentation	1
	• Extraction of ethanol	15
	• Modified yeast strains	10
Post-fermentation	• Spinning cone column (SCC)	1,10,31
	• Reverse osmosis (RO)	32
	• Evaporative Perstraction (EP)	33
	• Pervaporation	34
	• RO with distillation	35
	• RO with EP	36

Pre-fermentation. This approach, which has been widely used in the past, involves choosing particular viticultural practices that reduce the ripeness of berries produced by the grapevine. *Adjusting the vine leaf area-to-crop* ratio can control the fermentable carbohydrate/sugar level of wine grapes.¹⁶ This approach may help to address the imbalance between the accumulation carbohydrates and other grape constituents. However, significant research is still required to determine the optimum leaf area-to-crop ratios, the influence of timing and location of leaf removal from vines relative to fruit location, and any long-term impact upon vine physiology.^{16,17}

Harvesting fruit at an early stage of development and subsequent vinification will reduce the sugar content of fruit and thus the alcohol content of wine. However, harvesting grapes earlier may result in wines that are organoleptically immature due to decreased

flavour precursor development prior to harvest, excessively high acidity levels, and the likelihood of unripe flavour attributes.¹⁰

Treatment of grape juice with glucose oxidase can lower glucose concentrations and consequently the ethanol level of finished wines.²⁶ Studies have shown that glucose can be converted to gluconic acid, which is not metabolised by yeast. As the glucose fraction in grape juice represents approximately 50% of total fermentable sugars, the theoretical maximum reduction in ethanol production is 50%. In practice, however, the efficiency of glucose oxidation is dependent upon enzyme concentration, juice pH, dissolved oxygen concentration, processing time and temperature, thus the ethanol reductions reported for wines produced via this method range from 4-40% v/v.²⁷⁻²⁹

Other methods for reducing the sugar content of grape juice have significant limitations when applied at an industrial scale. Must *dilution* with water¹⁴ or blending with water or 4.5% v/v wine made from unripe grapes following proportional juice removal³⁷ have legal consequences in wine-producing countries which limit or prohibit water addition. The application of *membrane-based* techniques that remove sugar from juice tend to remove only small amounts of sugar due to problems associated with suspended solids, and/or the high operating pressure required involves significant energy consumption so the process is not economically viable.^{30,37}

During fermentation. *Early arrest of fermentation* is a relatively simple approach to ethanol reduction. However, as the resulting wine contains high levels of residual sugar, it may need to be stabilised through the addition of higher levels of sulfur dioxide. Furthermore, the residual sugar will impart sweetness to the wine, which may not be desirable for certain wine styles.¹ *Extraction of ethanol during fermentation* has been performed, allowing reductions in ethanol content of ~ 2% v/v, either through distillation under vacuum or by stripping with CO₂.¹⁵ These methods offer some advantages such as

increased fermentation rates (likely due to the concurrent extraction of inhibitory compounds), while any loss of desirable aroma compounds can be partly compensated for with additional fermentation. Sensory analyses found no significant difference between wines produced with or without extraction treatment, albeit a slight negative effect was associated with stripping. However, while these methods have been successful at a *pilot scale* (i.e. at 100 L), routine application of this method is difficult at a commercial scale, because it requires specialised equipment and must be performed at a highly specific time during fermentation. Furthermore, in the case of stripping, additional treatments are required to recover the “strip water”. Yeast can also respond negatively to the environmental stresses caused by the use of distillation and stripping, so fermentation performance needs to be monitored carefully.

Microbiological methods that decrease alcohol during fermentation have been achieved mainly by employing novel yeast strains developed either by genetic modification or adaptive evolution.³⁸ Sequential fermentations utilising the yeast strain AWRI1149 produced wines with alcohol levels of 0.9% and 1.6% v/v lower.²³ This has led to the development of ‘low-alcohol’ yeast strains which work by redirecting their carbon metabolism away from ethanol production to other metabolites, such as glycerol.³⁹ Although *Saccharomyces cerevisiae* displays a wide range of phenotypic diversity, the difference in percentage ethanol production between wild strains does not typically exceed 0.5% v/v.⁴⁰ A strategy involving the use of non-*Saccharomyces* yeast to initially inoculate grape must, followed by the use of *S. cerevisiae* to finish fermentation, may lower wine ethanol levels by up to 1.6% v/v.^{21,41} However, this approach carries the risk of compromising wine quality in some grape musts, with ethyl acetate being present at much higher concentrations in wines produced using sequential fermentation.⁴²

Genetically engineering strains of *S. cerevisiae* that divert grape-derived sugars from ethanol production to the formation of other metabolites (e.g. glycerol or lactate) is a relatively new strategy being trialled to achieve lower wine ethanol concentrations.¹⁰ However, the accumulation of undesirable fermentation by-products (e.g., acetate- and acetaldehyde-derived volatiles) may negatively affect the sensory properties of wine.¹⁵ Research into genetically modified organisms may overcome these side effects; however, the underlying issue associated with public acceptance of genetically-modified-organism-based foods and food products prohibits their use in many winemaking countries.⁴³

Post-fermentation. Currently, most of the ethanol reduction strategies employed in winemaking are carried out post-fermentation. Dealcoholisation has been achieved using a range of techniques, including thermal distillation, adsorption, extraction and membrane-based methods. However, the most common techniques are spinning cone column (SCC) distillation, reverse osmosis (RO), pervaporation and evaporative perstraction (EP).^{1,10} These techniques offer greater advantages when scaled-up to the industrial level, as they do not produce waste materials. Their by-products (e.g. ethanol) can be utilised according to demand, and the processes meet the criteria for ‘clean’ technology.³⁴

SCC is a modern, multi-stage strip column developed in the USA in the 1930s,¹ but improved more recently in Australia.¹⁰ The SCC involves gravity pulling wine over the upper surfaces of stationary cones then down into the centre of the column. The wine is then pushed up over the edges of the rotating cones by centrifugal force. These flow processes between stationary and rotating cones produce a thin film of wine and the vapour flows up the column together with an inert gas that is introduced from the bottom through the thin film (**Figure 1**). SCC is used as a two-stage process for wine dealcoholisation. In the first stage, volatile components are stripped at a low temperature (i.e. ~28°C). Then in the second stage, ethanol is removed (under vacuum) at a higher temperature (i.e. ~38 °C). The final

dealcoholised wine is produced by blending the aroma fraction collected during the first stage of distillation with the wine produced in the second stage of distillation.¹⁶

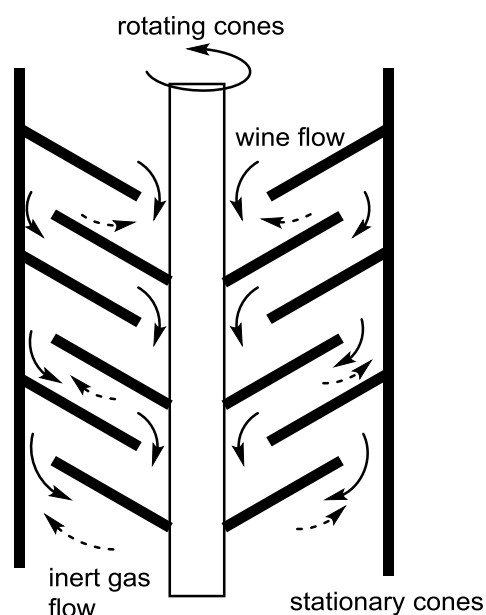


Figure 1. Structure of spinning cone column.

Advantages of the SCC system include high efficiency, low liquid residence times, low entrainment, minimal thermal damage and the ability to handle highly viscous liquid.¹ It is possible for ethanol concentrations to be decreased from 15% to less than 1% v/v, using SCC distillation.¹⁰ A *disadvantage* of SCC distillation is that some heating of the wine is required, which may negatively impact the wine organoleptically. The capital costs associated with SCC distillation equipment are relatively high, and given it is not easily moved, wine must be transported for processing.^{10,31}

Reverse osmosis (RO) is probably the most widely used technique involving membrane filtration. The first patent for the application of RO to an alcoholic beverage was obtained by the West German brewing company, Lowenbrau, in 1975, where it was used for the ethanol reduction of beer and wine.³⁷ The technique involves pressure filtration of lower turbidity wine (less than 20 NTU) across a porous membrane, such that the feed is separated into two streams: retentate (concentrate) and permeate (filtrate). A membrane is selected

with a low molecular weight cut off (MWCO) - for 'tight' RO membranes, the MWCO is 90 Da, whereas for 'loose' RO membranes, the MWCO is 180 Da³ - so water and ethanol, being small molecules, predominantly pass through the membrane into the permeate stream. Compounds with a molecular weight higher than the MWCO of the membrane will be retained in the stream passing across the membrane surface. *Advantages* of RO include the low-energy input requirement, the operation at ambient temperatures, the ability to achieve reproducible control over multiple separations, without disposable filtration media, and its easy automation and continuous operation. It should be noted, however, that since water is removed along with ethanol, treated wines contain ethanol at levels higher than the original wines; with the addition of water prohibited in many countries. The combination of two RO processes could allow the conversion of wine into two products, of high and low alcohol content, but its application is still limited within the industry.³² Modifications to the RO technique that either increase the pore size of the membrane or decrease the applied pressure give rise to a gradient of membrane filtration processes with increasing solute permeability, i.e. reverse osmosis, nanofiltration, ultrafiltration, and microfiltration.¹⁰ For example, reverse osmosis was used to achieve partial dealcoholisation of two wines, a Cabernet Sauvignon (14.8 % v/v) and a Grenache Carignan blend (16.2 % v/v) by 1 and 2 % (v/v), respectively.⁴⁴ Chemical analysis showed there were no significant changes in pH, colour intensity or total phenolic index, while sensory analysis found statistically significant differences were only apparent in some of the comparisons made by triangular tests. However, it should be noted that wines were only subjected to chemical and sensory analysis following aging in oak barrels for 9 months, and the compositional changes associated with oak maturation may have masked differences arising from partial dealcoholisation.

Other emerging membrane technologies such as *evaporative perstraction (EP)* (also referred to as osmotic distillation or isothermal membrane distillation)³³ and *pervaporation* share similar processes, in that they involve selective removal of ethanol, arising from the vapour pressure differential that occurs across a membrane. In this technology, wine is circulated through a hydrophobic, hollow fibre membrane contactor, while a degassed stripping water phase flows along the downstream side of the membrane.³³

EP membranes have hydrophobic properties, which are essential as this critical factor determines the flux of ethanol from the retentate to the permeate. Aroma and flavour compounds have lower vapour pressures (in ethanolic solutions) than ethanol itself; thus, the flux ratios of these compounds are considerably lower and so they are mostly retained within the wine. However, to increase the rate of ethanol removal from the feed stream, the processing conditions (e.g. feed velocity, stripping solution velocity and operating temperature) can be varied; albeit some aroma losses can occur with increased operating times and/or temperatures.^{33,45}

Pervaporation membranes are nonporous and can be hydrophilic or organophilic.³⁴ Pervaporation can produce a more ethanol-rich permeate, thus the wine becomes less dense in comparison with other membrane-based techniques.³⁴ However, as *higher temperatures* are required to achieve effective ethanol permeation there is also increased flux of aroma compounds into the permeate.³⁴

Combined technologies can be used to minimise the disadvantages associated with individual methods, and to increase the quality and performance of ethanol reduction. There have been several approaches, but the most effective combinations are RO/distillation and RO/EP (**Figure 2**).

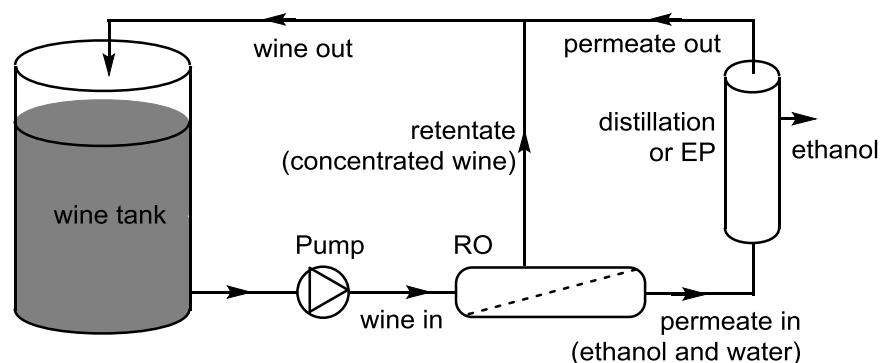


Figure 2. Simplified diagram of the dealcoholisation process using combined RO and EP technologies.^{35,36}

The combination of reverse osmosis and distillation, proposed in the 1996 US Patent by Smith,³⁵ involves wine being processed through a reverse osmosis system to generate retentate (i.e. concentrated wine) and permeate (largely comprising ethanol and water). The retentate is recycled back to the feed wine, while permeate is distilled in a high-energy distillation column and the distillate, which consists substantially of high strength ethanol, is removed as a by-product. The residual material, being dealcoholised permeate, is then returned to the wine tank, thus diluting its overall ethanol content (**Figure 2**). This process is effective, but because of the relative volatility of ethanol and water, distillation is difficult and costly, in terms of both energy and infrastructure costs.³⁵

The combination of RO with EP was proposed in the 2008 US Patent by Wollan.³⁶ In this system, wine is first subjected to RO (in a similar manner to that of the RO/distillation process, albeit pressure and temperature conditions may differ) to produce retentate and permeate streams. Again, the retentate is returned to the feed wine, but the permeate is degassed, heated to between 45-55 °C, and circulated through a hydrophobic hollow fibre membrane contactor. Meanwhile, pure degassed water flows across the downstream surface of the membrane (i.e. as stripping liquid). Ethanol is transported by vapour diffusion through the membrane pores and condenses in the stripping water on the other side of the membrane. In this way, the ethanol content of the permeate is decreased and the treated permeate is

returned to the wine tank to produce RAW. The wine returning to the feed tank typically has an ethanol content 0.5-1.5% v/v lower than the initial wine. Chile and Australia first began to use RO/EP systems for alcohol adjustment of wine in 2008.⁴⁶

Finally, production methods may incorporate a *combination* of pre-, during- and/or post-fermentation techniques to achieve the low alcohol product desired. However, if winemakers use multiple techniques, the cost of the final wine may increase beyond an acceptable (i.e. profitable) level.

Despite many wineries employing both RO and EP technologies for alcohol adjustment of wine, very little research has been undertaken to compare the chemical and sensory profiles of wine before and after treatment. This research is essential to give winemakers a better understanding of the consequences of partial dealcoholisation by RO-EP, and to enable optimisation of alcohol adjustment strategies.

3. Impact of ethanol removal on wine composition

There have been many studies and/or reviews concerning the chemical composition, sensory profiles and quality of wine. However, there is a general lack of research focussed on the composition of RAW, despite ethanol reduction processes influencing wine composition in complex ways.

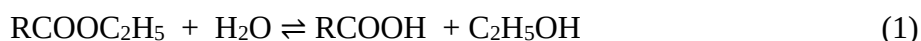
Firstly, dealcoholisation can cause the loss or reduction of volatiles during processing. The extent to which volatile compounds are lost depends on the nature of the dealcoholisation process and the volatility of particular aroma compounds. For example, the product obtained after processing wine by distillation⁴⁷ or evaporative perstraction³³ typically lacks many of the volatile compounds found pre-treatment. Other volatiles are detected at much lower concentrations than in the original wine.^{10,34,37} However, with improvements to membrane technology and the combination of dealcoholisation techniques (e.g., RO/distillation and RO/EP), the loss of volatiles has been substantially decreased.^{35,36}

Secondly, since ethanol essentially acts as a solvent in wine, removal of ethanol can increase the concentration of non-volatile and volatile compounds, even if the relative amounts of these compounds do not change. For every litre of pure ethanol removed, only about 900 mL of total volume is lost, as a consequence of the different densities of ethanol and ethanol/water solutions.⁴⁸ For example, a recent study using SCC distillation to produce RAW wine found that the concentration of resveratrol and other phenolic compounds in treated wine were either unchanged or elevated.⁴⁹

Thirdly, the colour of RAW may increase, even though the concentration of anthocyanins may not change significantly. Previous studies have shown large decreases in colour density during the production of red wines due to increasing ethanol levels during fermentation.⁵⁰ Conversely, in a study by Gambuti and colleagues, involving the partial dealcoholisation of red wines performed using a polypropylene hollow fibre membrane contactor apparatus, wine colour density increased by 11.0%, 7.2% and 8.1% after removal of 2% ethanol from Aglianico, Merlot and Piediroso wines, respectively.⁹ Similarly, Gonçalves and coworkers showed 2% ethanol removal achieved in red wine by nanofiltration or RO, increased colour density by 5.5% and 11%, respectively.⁵¹ Since copigmentation based on hydrophobic interactions and hydrogen bonds are destroyed by the presence of ethanol,⁵² in RAW, the more ethanol removed, the more copigmentation occurs, which increases colour density.

Finally, the level of ethyl esters in RAW may change significantly due to ethanol removal. Ethanol plays a part in the acid/ethyl ester equilibrium, so when ethanol is removed, the concentration of ethyl esters may decrease due to an equilibrium shift to the right (Equation 1), due to hydrolysis of esters. This process is entirely reversible, so factors affecting the reaction rate in one direction will also affect the reverse reaction.⁵³ The rates of both processes (i.e. forward: hydrolysis, and reverse: esterification) are accelerated at higher

temperatures.⁵⁴ Conversions will reach maximum or minimum rates at certain points for each type of ester.^{55,56}



The ester equilibrium has been studied in wine during ageing and in model wine, however, there is limited literature concerning the ester equilibrium in RAW. Previous studies found that the concentrations of *straight chain acid ethyl esters* (e.g. ethyl hexanoate, ethyl octanoate and ethyl decanoate) decreased slightly during wine ageing.⁵⁷ This slight decrease may occur because these esters are introduced enzymatically into wine at levels in excess of their equilibrium concentrations; i.e. they gradually hydrolyse until they approach equilibrium with their respective acids and alcohols. In contrast, concentrations of *branched acid ethyl esters* (e.g. ethyl isobutanoate and ethyl isopentanoate), which usually occur at low levels in young wines, increase with ageing due to chemical esterification of ethanol. In these cases, the branched fatty acids might be produced during non-oxidative ageing, so that these ethyl esters are formed by a shifting of the acid-ester equilibrium.⁵⁸

Another study on the esterification of tartaric acid with ethanol in model wine systems highlighted the importance of ethanol concentration.⁵⁹ An increase in the rate and quantity of ester formation occurred with increasing ethanol concentrations, i.e. 8, 12 and 18% ethanol v/v. In a recent study, two red wines (with initial ethanol levels of 15.4 and 13.3% v/v), were partially dealcoholised to decrease the alcohol content by 2, 3 and 5% v/v) using a polypropylene hollow fibre membrane contactor apparatus.⁶⁰ Following dealcoholisation, ethyl esters levels decreased by between 11 and 100%. Another study decreased ethanol levels from 13% to either 8 or 5%, using a benchtop RO/EP system.³ Ethyl ester levels in the resultant wines decreased by 20 to 70%.⁶¹ Interestingly, in these studies, some acid concentrations increased, e.g. hexanoic acid increased by 24%,⁶⁰ and propanoic acid increased by 173%.⁶¹

Esters with higher molecular weights (and thus higher boiling points) have been found to decrease in concentration more than their lighter counterparts, despite the fact that RO membranes allow smaller esters to pass through and/or evaporate.³² However, this is consistent with literature that suggests the more complicated the acid (i.e. the higher its *molecular weight*), the more *slowly* it will be esterified (i.e. the faster it will undergo hydrolysis).⁵³ Esterification rates were found to decrease for ethyl decanoate more than for ethyl octanoate, which in turn decreased more than for ethyl hexanoate.⁵⁴ These results support the hypothesis that the ester hydrolysis reaction shifts to the right to counter the lower ethanol concentrations.

In general, the fact that many esters are present at concentrations *around* their aroma detection threshold values (and that their contributions to aroma can be additive), suggests that modest concentration changes might have dramatic effects on wine aroma and flavour.⁶² These changes to ethyl esters may affect wine styles. Therefore, the loss of ethyl esters due to ethanol removal based on chemical equilibrium may not be easily remedied and may require more attention for RAWs.

Also, if 10% alcohol is removed, the concentration of the compounds measured should increase by about 10% (assuming no volume change); however, this difference may not be observed if the error associated with analytical methods is greater than 10%.

4. Impact of ethanol removal on wine sensory properties

Wines are complex substrates, meaning that sensory profiles are not always in agreement with compositional data, in addition to being difficult to predict.¹⁴ Sensory studies are therefore of critical importance when determining treatment effects on wine aroma, flavour, taste and mouthfeel properties.⁶³

Firstly, wine is a complex matrix of compounds that are present at varied concentrations, and small changes in ethanol levels due to processing can significantly

influence the perception of wine, in ways that are difficult to predict. There are several types of binding that may occur between wine constituents, e.g., chemical binding of covalent bonds, physical binding through π - π stacking, hydrophobic interactions, hydrogen bonding, as well as the formation of inclusion complexes.⁶³ The extent to which binding occurs depends on the physico-chemical properties of each aroma compound (e.g. molecular size, functional groups and volatility). The strength and nature of binding can alter the partitioning of aroma compounds between the gas (headspace) and liquid (wine) phases at equilibrium.⁶⁴ Changing the concentration of free volatile compounds thus leads to changes in aroma or flavour perception. Consequently, the concentration of aroma compounds found in the headspace above the wine may either be increased (due to volatile release or as a result of ‘salting-out’ effects) or decreased (due to retention effects), when the concentrations of other wine components changes.⁶³

For example, the addition of ethanol, tannin and fructose to wine resulted in 57–75% reductions in the headspace concentrations of several odorants.⁶⁴ The removal of ethanol from wine may increase the binding of aroma compounds to proteinaceous materials due to the less hydrophobic environment, which can subsequently lead to diminished headspace concentrations of volatiles and, consequently, decreased aroma perception.⁶⁵ A recent study used isothermal titration calorimetry to measure the binding strength of the model salivary protein, poly(L-proline), and a range of tannin fractions derived from Cabernet Sauvignon wine.⁶⁶ The study found that with increasing ethanol concentrations, the strength of wine tannin-poly(L-proline) binding decreased, in agreement with perceived decreases in wine astringency, determined by sensory analysis.⁶⁷ Several studies have reported the occurrence of significant two-way,⁶⁸ three-way or higher-order⁶⁹ wine matrix interactions in model wine systems. However, it is still difficult to predict or optimise the release and/or perception of aroma volatiles in grapes and wine.⁷⁰

Secondly, ethanol strongly influences wine quality due to its effects on acidity, astringency and the volatility of aroma compounds, and therefore the organoleptic properties.³³ Increased ethanol concentrations typically enhance the perception of bitterness, body and hotness, and to a lesser extent sweetness.⁴ Conversely, lower ethanol levels can decrease the perception of acidity and astringency. The magnitude of sensory changes associated with RAW depends upon the quantity of ethanol remaining in the final wine.^{7,10,14} For example, a study found that the sensory perception of aroma at low ethanol concentrations (i.e. 10.0–12.0%) was ‘fruity’, whereas higher ethanol levels (i.e. 14.5–17.2%) were considered ‘herbaceous’.⁶

Aqueous solutions of ethanol have been shown to have an approximately linear relationship to physical viscosity and density.⁵ When the ethanol concentration increases, viscosity increases and density decreases. However, the perceived fullness response to ethanol concentration in white wine is more complex. Within the ethanol range of 0–14% v/v, the intensity maxima for perceived viscosity and density occurs at 10 and 12% v/v.⁵

Most of studies outlined above involve partial dealcoholisation of wine with alcohol levels less than 15% v/v. However, there are instances where industry needs to manage the alcohol content of wines containing higher ethanol levels (i.e. > 15% v/v). For example, the rate of duties tax applied to wine is often tied to alcohol content, so there are financial incentives for adjusting the alcohol content of wines produced for export. Further research is therefore warranted to better understand the sensory impact of partial dealcoholisation of wines with alcohol levels greater than 15% v/v.

5. The sweetspot phenomenon in reduced alcohol wine

The term ‘alcohol sweetspot’ has been used by several winemakers in relation to wine alcohol reduction and refers to a tasting process employed to identify the optimal alcohol content of dealcoholised wine.^{71,72} Sweetspots have been described as the one or two specific

ethanol levels at which wine seems to ‘stand out’ from a bracket comprising a series of wine of different ethanol levels, each varying by 0.1–0.2% v/v; i.e. the sweetspot purportedly displays significantly enhanced aroma and flavour intensity, and superior overall balance.^{73,74}

There is some evidence to support the sweetspot phenomenon. Ethanol plays a contradictory role in wine sensation, depending on its concentration. At certain levels, ethanol can affect taste and mouthfeel by enhancing or suppressing astringency, bitterness, sweetness, saltiness, hotness and sourness.⁴ On the other hand, it can make wine more attractive by masking undesirable sensory attributes and harmonising imbalances;⁷⁵ however, it can also mask desirable flavours.^{69,76,77} At lower levels (i.e. 5–12% v/v), ethanol enhances the release of aroma compounds under dynamic conditions and helps to maintain the headspace concentration of volatile compounds.⁷⁸ Conversely, higher ethanol levels (i.e. 10–18% v/v) are linearly and negatively correlated with volatile headspace concentrations, which may be attributable to the increased solubility of volatiles in the ethanolic solution.⁶⁸

Discrimination/difference tests are sensory methods used to establish whether or not any perceptible differences exist between two or more products.⁷⁹ Previous studies suggest the difference in ethanol concentration at which wines can be distinguished varies from 0.5 to 2.0% v/v, depending on the wine style and the assessor (i.e. wine expert vs. consumer).^{75,80} Therefore, variation in ethanol levels of as little as 0.1% v/v may or may not influence the perception of a ‘sweetspot’. Some Australian winemakers are nevertheless adamant that the sweetspot phenomenon exists, and agree that when the ethanol levels of a given wine are varied by as little as ± 0.1 –0.2% v/v, significant changes in flavour intensity and balance occur.⁷¹

Informal sweetspotting trials involve presentation of samples derived from the same wine, comprising different ethanol concentrations, and in most cases, a panel of tasters

evaluate the samples and determine the alcohol content that exhibits superior sensory qualities.⁷¹ Anecdotally, it is also accepted that varying the ethanol content can profoundly affect the perceived style of a given wine. Depending on the ethanol content, a given wine may have more than one appealing sweetspot, corresponding to quite different wine styles. For example, a Chardonnay with 14.2% alcohol may display rich, “New World” qualities, but at 13.6% alcohol, the flavours present in the same wine may be more subtle, representative of Burgundian Chardonnay, whereas at 12.8% alcohol, the wine may be Chablis-like. The issue of wine style therefore confounds identification of the sweetspot phenomenon for a given wine.

In an attempt to validate the sweetspot phenomenon, a recent study⁸ evaluated 11 wines, divided into six subsets, each comprising six wines spanning an ethanol range of approximately 1% v/v. In this study, groups of consumers (n=17-18) were randomly allocated to a subset of samples and asked to rate their liking of each, using a nine-point hedonic categorical scale, anchored from 1 = “extremely dislike” to 9 = “extremely like”. Only one consumer group provided results that supported a sweetspot ethanol level for the wines (i.e. a positive quadratic curve for a subset), being approximately 13.8 to 14.0% v/v. Data from other consumer groups was inconsistent, suggesting that consumer-based panels may not be suitable for sweetspotting trials.

Another sweetspotting study involved three white wines and one red wine, with ranking tests performed by a panel comprising experts.⁸⁰ The ethanol level of samples ranged by 3% v/v, at 0.5% intervals (i.e. 12.1, 12.6, ... 15.1% v/v). Panellists ranked samples according to their individual preferences, i.e. from 1, for their most preferred wine, to 7, their least preferred wine. Each wine was tasted in three separate ranking tests, using different presentation orders, i.e. increasing, decreasing or randomised ethanol concentration. Results from this study showed no clear preference for any particular wine ethanol level, but wines

with ethanol concentrations above 15% v/v were ranked significantly lower than other samples. The authors concluded the interval between ethanol levels may have been too great to allow for sweetspot identification.⁸⁰

An important consideration is the potential for the ethanol level of wines to influence wine perception. Presentation order is critical when sweetspotting is conducted by winemakers and researchers. Winemakers tend to evaluate brackets of wine from highest to lowest ethanol content (i.e. High-Low), then back again, to determine their preferred wine(s),⁸¹ whereas researchers would generally present samples in random order.^{8,80} A recent study confirmed the impact of tasting order on wine perception.⁷ The sensory profiles of commercial Cabernet Sauvignon wines ranging from 12–16% v/v (at 0.5% v/v intervals) were evaluated using three tasting orders: randomised, High-Low and the reverse (i.e. Low-High), using both descriptive sensory analysis and wine judging conditions.⁷ The presentation order employed during wine assessment significantly influenced sensory perception, particularly for assessment of palate attributes. In some instances, the two specified tasting orders showed opposing effects of ethanol on wine sensory profiles, with the randomised group giving intermediary responses. The largest differences occurred when low alcohol wines were tasted first, with substantial changes observed in the sensory profiles of higher alcohol content wines.⁷

Most of these studies present wine samples to sensory panellists in rows of ascending or descending alcohol content, which may cause bias if panellists avoid samples at the ends of rows, in favour of samples in the middle of the row. Additionally, statistical analyses tend to establish whether or not there is significance amongst the ratings given to all samples, rather than to consider whether individual wines are different from one another.

6. Research aims

The research described in this thesis aimed to determine the compositional and sensory changes resulting from partial dealcoholisation of wine by RO/EP, and to investigate the sweetspot phenomenon through chemical, sensory and statistical analyses. The research undertaken investigated changes in wine composition immediately after treatment by RO-EP to determine the impact of partial dealcoholisation, with minimal re-balancing of wine equilibria. Samples were also collected during the dealcoholisation process to characterise membrane performance and the consequences of membrane fractionation on volatile and non-volatile wine compounds. Sweetspotting trials were also performed, following the approach used by some wine producers to optimise their use of dealcoholisation, but with modifications that attempted to correct bias associated with sample presentation. Two different statistical analyses were also evaluated for testing the robustness of data from sweetspotting trials. Research aims were achieved via the following studies:

Study 1: RO/EP was used to partially dealcoholise red wine, with samples collected from each stage of the dealcoholisation process. The composition of wine was then determined by HPLC (for non-volatile compounds), by GC-MS (for volatiles compounds) and other basic analyses. The extent to which partial dealcoholisation of wine impacts the hydrolysis of ethyl esters associated with fruit aroma and flavor was also investigated.

Study 2: Difference tests were conducted to determine the alcohol perception threshold (i.e. 0.1, 0.2, 0.5 % v/v) that winemakers use when conducting sweetspot experiments. A series of alcohol sweetspotting trials involving expert panels were also undertaken, using four methods of sample presentation: (i) ordered vs. randomised; and (ii) linear (i.e. samples presented in a row) vs. circular (i.e. samples presented in a circle). Results from sweetspotting trials were then analysed using two different statistical tests. Chemical analyses of partially dealcoholised wine samples were also performed.

Study 3: The chemical and sensory consequences of partial dealcoholization of five Cabernet Sauvignon wines were determined. Further sweetspotting trials were conducted using a trained panel in an attempt to validate the alcohol sweetspot phenomenon.

References

1. Pickering, G. J., Low- and reduced-alcohol wine: A review. *J. Wine Res.* **2000**, *11*, 129-144.
2. Godden, P.; Muhlack, R., Trends in the composition of Australian wine. *Aust. N.Z. Grapegrow. Winemak.* **2010**, 47.
3. Memstar, Pty Ltd, 712 Research Road, Nuriootpa SA 5355, Australia.
4. Fischer, U.; Noble, A. C., The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. *Am. J. Enol. Vitic.* **1994**, *45*, 6-10.
5. Pickering, G. J.; Heatherbell, D.; Vanhanen, L.; Barnes, M., The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. *Am. J. Enol. Vitic.* **1998**, *49*, 306-318.
6. Goldner, M. C.; Zamora, M. C.; Di Leo Lira, P.; Gianninoto, H.; Bandoni, A., Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *J. Sens. Stud.* **2009**, *24*, 243-257.
7. King, E. S.; Dunn, R. L.; Heymann, H., The influence of alcohol on the sensory perception of red wines. *Food Qual. Pref.* **2013**, *28*, 235-243.
8. King, E. S.; Heymann, H., The effect of reduced alcohol on the sensory profiles and consumer preferences of white wine. *J. Sens. Stud.* **2014**, *29*, 33-42.
9. Gambuti, A.; Rinaldi, A.; Lisanti, M. T.; Pessina, R.; Moio, L., Partial dealcoholisation of red wines by membrane contactor technique: influence on colour, phenolic compounds and saliva precipitation index. *Eur. Food Res. Technol.* **2011**, *233*, 647-655.

10. Schmidtke, L. M.; Blackman, J. W.; Agboola, S. O., Production technologies for reduced alcoholic wines. *J. Food Sci.* **2012**, 77, R25-41.
11. Anon, CPG Sec. 510.400 dealcoholized wine and malt beverages—labeling. Washington D.C.: Food and Drug Administration. **2005**.
12. ANZFA, Standard 2.7.1 labelling of alcoholic beverages and food containing alcohol. Canberra: Commonwealth of Australia. 6 p. **2002**.
13. Wilkinson, K.; Jiranek, V., Wine of reduced alcohol content: Consumer and society demand vs industry willingness and ability to deliver. In *1st International symposium of alcohol level reduction in wine*, Bordeaux, 2013; Vol. 15, p 98.
14. Fedrizzi, B.; Nicolis, E.; Camin, F.; Bocca, E.; Carbognin, C.; Scholz, M.; Barbieri, P.; Finato, F.; Ferrarini, R., Stable isotope ratios and aroma profile changes induced due to innovative wine dealcoholisation approaches. *Food Bio. Technol.* **2014**, 7, 62-70.
15. Aguera, E.; Bes, M.; Roy, A.; Camarasa, C.; Sablayrolles, J.-M., Partial removal of ethanol during fermentation to obtain reduced-alcohol wines. *Am. J. Enol. Vitic.* **2010**, 61, 53-60.
16. Stoll, M.; Lafontaine, M.; Schultz, H. R., Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis vinifera* L. Riesling. *Le Progrès Agricole et Viticole* **2010**, 127, 68-71.
17. Poni, S.; Gatti, M.; Bernizzoni, F.; Civardi, S.; Bobeica, N.; Magnanini, E.; Palliotti, A., Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance. *Aust. J. Grape Wine Res.* **2013**, 19, 378-387.
18. Olego, M. Á.; Álvarez-Pérez, J. M.; Quiroga, M. J.; Cobos, R.; Sánchez-García, M.; Medina, J. E.; González-García, S.; Coque, J. J. R.; Garzón-Jimeno, J. E., Viticultural and Biotechnological Strategies to Reduce Alcohol Content in Red Wines. *Grape and Wine Biotechnology* **2016**, 361-382.

19. Valencia, P.; Espinoza, K.; Ramirez, C.; Franco, W.; Urtubia, A., Technical Feasibility of Glucose Oxidase as a Prefermentation Treatment for Lowering the Alcoholic Degree of Red Wine. *Am. J. Enol. Vitic.* **2017**, *68*, 386-389.
20. Rossouw, D.; Bauer, F. F., Exploring the phenotypic space of non-Saccharomyces wine yeast biodiversity. *Food Microbiol.* **2016**, *55*, 32-46.
21. Canonico, L.; Comitini, F.; Oro, L.; Ciani, M., Sequential Fermentation with Selected Immobilized Non-Saccharomyces Yeast for Reduction of Ethanol Content in Wine. *Front Microbiol* **2016**, *7*, 278.
22. Varela, C.; Barker, A.; Tran, T.; Borneman, A.; Curtin, C., Sensory profile and volatile aroma composition of reduced alcohol Merlot wines fermented with *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. *Int. J. Food Microbiol.* **2017**, *252*, 1-9.
23. Varela, C.; Sengler, F.; Solomon, M.; Curtin, C., Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. *Food Chem.* **2016**, *209*, 57-64.
24. Dequin, S.; Escudier, J.-L.; Bely, M.; Noble, J.; Albertin, W.; Masneuf-Pomarède, I.; Marullo, P.; Salmon, J.-M.; Sablayrolles, J. M., How to adapt winemaking practices to modified grape composition under climate change conditions. *OENO One* **2017**, *51*, 205.
25. Longo, R.; Blackman, J. W.; Torley, P. J.; Rogiers, S. Y.; Schmidtke, L. M., Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *J. Sci. Food Agric.* **2016**.
26. Pickering, G. J.; Heatherbell, D. A.; Barnes, M. F., Optimising glucose conversion in the production of reduced alcohol wine using glucose oxidase. *Food Res. Int.* **1998**, *31*, 685-692.

27. Pickering, G. J.; Heatherbell, D. A.; Barnes, M. F., The production of reduced-alcohol wine using glucose oxidase treated juice. Part I. Composition. *Am. J. Enol. Vitic.* **1999**, 50, 291-298.
28. Pickering, G. J.; Heatherbell, D. A.; Barnes, M. F., The production of reduced-alcohol wine using glucose oxidase-treated juice. Part II. Stability and SO₂-binding. *Am. J. Enol. Vitic.* **1999**, 50, 299-306.
29. Pickering, G. J.; Heatherbell, D. A.; Barnes, M. F., The production of reduced-alcohol wine using glucose oxidase-treated juice. Part III. Sensory. *Am. J. Enol. Vitic.* **1999**, 50, 307-316.
30. Vincze, I.; Stefanovits-Bányai, É.; Vatai, G., Using nanofiltration and reverse osmosis for the concentration of seabuckthorn (*Hippophae rhamnoides* L.) juice. *Desalination* **2006**, 200, 528-530.
31. Saha, B.; Torley, P.; Blackmann, J. W.; Schmidtke, L. M., Review of processing technology to reduce alcohol levels in wines. In *1st International symposium of alcohol level reduction in wine*, Bordeaux, 2013; p 78.
32. Bui, K.; Dick, R.; Moulin, G.; Galzy, P., A reverse osmosis for the production of low ethanol content wine. *Am. J. Enol. Vitic.* **1986**, 37, 297-300.
33. Diban, N.; Athes, V.; Bes, M.; Souchon, I., Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. *J. Membr. Sci.* **2008**, 311, 136-146.
34. Takács, L.; Vatai, G.; Korány, K., Production of alcohol free wine by pervaporation. *J. Food Eng.* **2007**, 78, 118-125.
35. Smith, C. R., Apparatus and method for removing compounds from a solution. US Patent: 5,480,665. **1996**.

36. Wollan, D. Alcohol reduction in beverages. Patent Number: US 2008/0272041 A1, **2008**
37. Rayess, Y. E.; Mietton-Peuchot, M., Membrane technologies in wine industry: An overview of applications and recent developments. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 2005-2020.
38. Bovo, B.; Nadai, C.; Vendramini, C.; Fernandes Lemos Junior, W. J.; Carlot, M.; Skelin, A.; Giacomini, A.; Corich, V., Aptitude of *Saccharomyces* yeasts to ferment unripe grapes harvested during cluster thinning for reducing alcohol content of wine. *Int. J. Food Microbiol.* **2016**, *236*, 56-64.
39. Goold, H. D.; Kroukamp, H.; Williams, T. C.; Paulsen, I. T.; Varela, C.; Pretorius, I. S., Yeast's balancing act between ethanol and glycerol production in low-alcohol wines. *Microb Biotechnol* **2017**, *10*, 264-278.
40. Dequin, S., Managing excess alcohol in wine: A new challenge for wine yeast. *Glo. Warm.: N. Oenol. Chall.* **2007**, *1*, 5-1.
41. Liu, P.-T.; Lu, L.; Duan, C.-Q.; Yan, G.-L., The contribution of indigenous non-*Saccharomyces* wine yeast to improved aromatic quality of Cabernet Sauvignon wines by spontaneous fermentation. *LWT - Food Science and Technology* **2016**, *71*, 356-363.
42. Contreras, A.; Hidalgo, C.; Henschke, P.; Chambers, P.; Curtin, C.; Varela, C., Evaluation of non-*Saccharomyces* yeasts for the reduction of alcohol content in wine. *Appl. Environ. Microbiol.* **2014**, *80*, 1670-1678.
43. Kutyna, D. R.; Varela, C.; Henschke, P. A.; Chambers, P. J.; Stanley, G. A., Microbiological approaches to lowering ethanol concentration in wine. *Trends Food Sci. Technol.* **2010**, *21*, 293-302.

44. Gil, M.; Estévez, S.; Kontoudakis, N.; Fort, F.; Canals, J. M.; Zamora, F., Influence of partial dealcoholization by reverse osmosis on red wine composition and sensory characteristics. *Eur. Food Res. Technol.* **2013**, 237, 481-488.
45. Banvolgyi, S.; Savas Bahceci, K.; Vatai, G.; Bekassy, S.; Bekassy-Molnar, E., Partial dealcoholization of red wine by nanofiltration and its effect on anthocyanin and resveratrol levels. *Food Sci. Technol. Int.* **2016**, 22, 677-687.
46. Massot, A.; Mietton-Peuchot, M.; Peuchot, C.; Milisic, V., Nanofiltration and reverse osmosis in winemaking. *Desalination* **2008**, 231, 283-289.
47. Gómez-Plaza, E.; López-Nicolás, J.; López-Roca, J.; Martinez-Cutillas, A., Dealcoholization of wine. Behaviour of the aroma components during the process. *LWT-Food Sci. Technol.* **1999**, 32, 384-386.
48. Wollan, D., VA Filtration, PO Box 794, Nuriootpa, SA 5355. Private communication.
49. Belisario-Sánchez, Y. Y.; Taboada-Rodríguez, A.; Marín-Iniesta, F.; López-Gómez, A., Dealcoholized wines by spinning cone column distillation: phenolic compounds and antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl method. *J. Agric. Food Chem.* **2009**, 57, 6770-6778.
50. Chris Somers, T.; Evans, M. E., Grape pigment phenomena: Interpretation of major colour losses during vinification. *J. Sci. Food Agric.* **1979**, 30, 623-633.
51. Gonçalves, F.; Ribeiro, R.; Neves, L.; Lemperle, T.; Lança, M.; Ricardo da Silva, J.; Laureano, O., Alcohol reduction in wine by nanofiltration. Some comparisons with reverse osmosis technique. In *1st International symposium of alcohol level reduction in wine*, Bordeaux, 6 Sep 2013; pp 64-67.
52. Boulton, R., The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Am. J. Enol. Vitic.* **2001**, 52, 67-87.

53. Ramey, D. D.; Ough, C. S., Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agric. Food Chem.* **1980**, 28, 928-934.
54. Makhotkina, O.; Kilmartin, P. A., Hydrolysis and formation of volatile esters in New Zealand Sauvignon blanc wine. *Food Chem.* **2012**, 135, 486-493.
55. Euranto, E. K., Esterification and ester hydrolysis. In *Carboxylic Acids and Esters* (1969), John Wiley & Sons, Ltd.: 2010; pp 505-588.
56. Fan, Y.; Xie, Z.; Zhang, H.; Qian, J., Kinetic resolution of both 1-phenylethanol enantiomers produced by hydrolysis of 1-phenylethyl acetate with *Candida antarctica* lipase B in different solvent systems. *Kinet. Catal.* **2011**, 52, 686-690.
57. Marais, J., The effect of pH on esters and quality of Colombar wine during maturation. *Vitis* **1978**, 17, 396-403.
58. Diaz-Maroto, M. C.; Schneider, R.; Baumes, R., Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. *J. Agric. Food Chem.* **2005**, 53, 3503-3509.
59. Edwards, T. L.; Singleton, V. L.; Boulton, R., Formation of ethyl esters of tartaric acid during wine aging: chemical and sensory effects. *Am. J. Enol. Vitic.* **1985**, 36, 118-124.
60. Lisanti, M. T.; Gambuti, A.; Moio, L., Partial dealcoholization of red wines, sensory and composition quality. In *1st International symposium of alcohol level reduction in wine*, Bordeaux, 6 Sep 2013; pp 89-97.
61. Saha, B. GC-MS head space and consumer preference evaluation of wines with reduced ethanol content. Master of Philosophy Thesis, Charles Sturt University, Australia, **2013**.
62. Wollan, D., Alcohol sweet spot seminars in McLaren Vale. *Aust. N. Z. Grapegrower Winemaker* **2005**, 70-71.

63. Sumby, K. M.; Grbin, P. R.; Jiranek, V., Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chem.* **2010**, *121*, 1-16.
64. Villamor, R. R.; Evans, M. A.; Mattinson, D. S.; Ross, C. F., Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Res. Int.* **2013**, *50*, 38-45.
65. Voilley, A.; Lubbers, S., *Flavor-Matrix interactions in wine*. In: Waterhouse AL, Ebeler SE, editors. *Chemistry of wine flavour*. American Chemical Society: Washington, D.C., 1998.
66. McRae, J. M.; Ziora, Z. M.; Kassara, S.; Cooper, M. A.; Smith, P. A., Ethanol Concentration Influences the Mechanisms of Wine Tannin Interactions with Poly(l-proline) in Model Wine. *J. Agric. Food Chem.* **2015**, *63*, 4345-52.
67. Demiglio, P.; Pickering, G. J., The influence of ethanol and pH on the taste and mouthfeel sensations elicited by red wine. *J. Food Agric. Environ* **2008**, *6*, 143-150.
68. Robinson, A. L.; Ebeler, S. E.; Heymann, H.; Boss, P. K.; Solomon, P. S.; Trengove, R. D., Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *J. Agric. Food Chem.* **2009**, *57*, 10313-22.
69. Jones, P.; Gawel, R.; Francis, I.; Waters, E., The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Qual. Pref.* **2008**, *19*, 596-607.
70. Polášková, P.; Herszage, J.; Ebeler, S. E., Wine flavor: chemistry in a glass. *Chem. Soc. Rev.* **2008**, *37*, 2478-2489.
71. MEMSTAR, Alcohol “sweet spot” seminar held in McLaren Vale. Memstar, Australia.

<http://memstar.webmad.com.au/themes/default/images/alcoholadjust/seminarReport.pdf>

(accessed July 1, 2014). **2005**.

72. Baker, A. K.; Ross, C. F., Sensory evaluation of impact of wine matrix on red wine finish: A preliminary study. *J. Sens. Stud.* **2014**, 29, 139-148.
73. Wollan, D., Controlling excess alcohol in wine. *Aust. N.Z. Wine Ind. J.* **2005**, 20, 48-50.
74. Wollan, D., The right tools for the job: dealing with cool climate wine styles. In *Proceedings of the sixth international symposium for cool climate viticulture and oenology. Auckland: New Zealand Society for Viticulture and Oenology*, 2006; pp 159-168.
75. Yu, P.; Pickering, G. J., Ethanol difference thresholds in wine and the influence of mode of evaluation and wine style. *Am. J. Enol. Vitic.* **2008**, 59, 146-152.
76. Mattes, R. D.; DiMeglio, D., Ethanol perception and ingestion. *Physiol. Behav.* **2001**, 72, 217-229.
77. Fontoin, H.; Saucier, C.; Teissedre, P.-L.; Glories, Y., Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Qual. Pref.* **2008**, 19, 286-291.
78. Tsachaki, M.; Linfoth, R. S. T.; Taylor, A. J., Dynamic headspace analysis of the release of volatile organic compounds from ethanolic systems by direct APCI-MS. *J. Agric. Food Chem.* **2005**, 53, 8328-8333.
79. Meilgaard, M. C.; Carr, B. T.; Civille, G. V., *Sensory evaluation techniques*. CRC press: 2006.
80. Schmitt, M.; Christmann, M.; Jung, R.; Schübler, C., Enological/technical approaches to reduce elevated alcohol levels in wine (alcohol management)–sensory results. *Bulletin de l'OIV* **2013**, 86, 485-492.

81. TWE, Treasury Wine Estates, 97 Sturt Highway, Nuriootpa, South Australia 5352.

Statement of Authorship

Title of Paper	Compositional Consequences of Partial Dealcoholization of Red Wine by Reverse Osmosis-Evaporative Perstraction		
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style		
Publication Details	Duc-Truc Pham, Vanessa J. Stockdale, David Wollan, David W. Jeffery and Kerry L. Wilkinson. Molecules 2019, 24, 1404; doi:10.3390/molecules24071404.		

Principal Author

Name of Principal Author (Candidate)	Duc-Truc Pham		
Contribution to the Paper	Designed and performed experiments; conducted chemical analysis of wines; performed statistical analysis of data (using ANOVA); interpreted the data; and drafted and revised the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	26-6-2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Vanessa J. Stockdale		
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design and data interpretation; and edited the manuscript.		
Signature		Date	26-6-2020

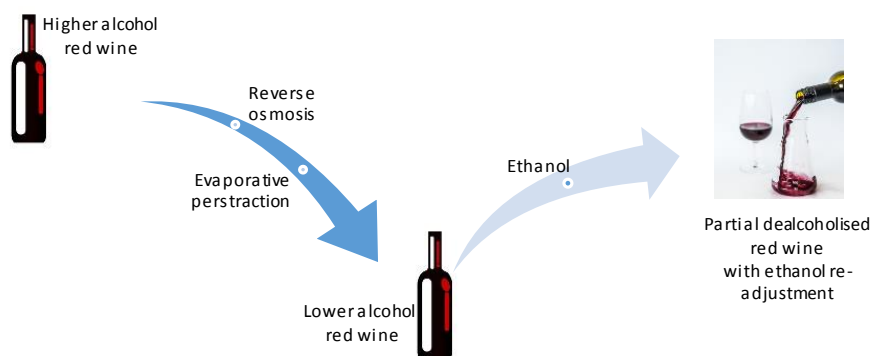
Name of Co-Author	David Wollan		
Contribution to the Paper	Contributed to the research idea, experimental design, partial dealcoholisation of wines and data interpretation; and edited the manuscript.		
Signature		Date	26-6-2020

Name of Co-Author	David W. Jeffery		
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design and data interpretation; and edited the manuscript.		
Signature		Date	26-6-2020

Name of Co-Author	Kerry L. Wilkinson		
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design, and data analysis and interpretation; edited and revised the manuscript, and acted as the corresponding author.		
Signature		Date	26-6-2020

Chapter 2.

Compositional Consequences of Partial Dealcoholization of Red Wine by Reverse Osmosis–Evaporative Perstraction



Pham, D-T; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L.

Molecules 2019, 24, 1404; doi:10.3390/molecules24071404

Article

Compositional Consequences of Partial Dealcoholization of Red Wine by Reverse Osmosis-Evaporative Perstraction

Duc-Truc Pham ^{1,2}, Vanessa J. Stockdale ^{2,3}, David Wollan ^{2,4} , David W. Jeffery ^{1,2} 
and Kerry L. Wilkinson ^{1,2,*} 

¹ School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia; duc-truc.pham@adelaide.edu.au (D.-T.P.); david.jeffery@adelaide.edu.au (D.W.J.)

² The Australian Research Council Training Centre for Innovative Wine Production, PMB 1, Glen Osmond, SA 5064, Australia

³ Treasury Wine Estates, 97 Sturt Highway, Nuriootpa, SA 5352, Australia; Vanessa.Stockdale@tweglobal.com

⁴ VA Filtration, PO Box 794, Nuriootpa, SA 5355, Australia; DWollan@memstar.com.au

* Correspondence: kerry.wilkinson@adelaide.edu.au; Tel.: +61-8-8313-7360

Received: 20 February 2019; Accepted: 1 April 2019; Published: 10 April 2019



Abstract: This study investigated compositional changes in red wines resulting from wine alcohol removal by reverse osmosis-evaporative perstraction (RO-EP) and provides insight into the physical and chemical changes in reduced alcohol wine (RAW). Trial 1 involved RO-EP treatment of three wines that were analyzed pre-treatment, post-treatment, and post-treatment with alcohol adjustment (i.e., addition of ethanol to achieve the original alcohol content). Trial 2 involved partial dealcoholization of two wines and analysis of samples collected during RO-EP treatment, i.e., wine in, wine out, retentate, permeate (pre- and post-EP treatment) and strip water. Wine color was analyzed by spectrophotometric methods, while other compositional changes were determined by WineScan, high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) analyses. In general, RAWs were slightly more concentrated than pre-treatment wines, which resulted in greater color intensity and increased phenolics and organic acids. However, partial dealcoholization resulted in lower concentrations of some fermentation volatiles, particularly ethyl esters, which may reflect ester hydrolysis following ethanol removal.

Keywords: alcohol; evaporative perstraction; dealcoholization; reduced alcohol wine; reverse osmosis

1. Introduction

Globally, the ethanol content of wine has progressively increased over time, which has been attributed to warmer growing conditions resulting from climate change, together with improvements to viticultural management practices and winemaking techniques [1–3]. Concurrently, market research suggests consumer preferences are tending towards lighter wine styles (i.e., light-bodied white, rosé and sparkling wines), comprising lower levels of alcohol [4]. Winemakers are therefore employing a range of strategies to achieve wines of lower ethanol content, applied either: (i) pre-fermentation, e.g., harvesting grapes ‘early’, when sugar levels are lower [5–7]; (ii) during fermentation, e.g., by arresting fermentation before sugars are fully converted to ethanol [1], by fermenting with low-alcohol-producing yeast [8], or by diluting the concentration of sugar and/or alcohol through the addition of water to juice or wine [7]; or (iii) post-fermentation, e.g., by removing alcohol from wine through distillation [9,10]. For now, alcohol removal post-fermentation remains the most widely practiced approach [3].

Dealcoholization of wine can be achieved via means such as thermal distillation, adsorption, extraction and fractionation methods [1]. However, the two most common techniques are spinning

cone column (SCC) distillation [11] and reverse osmosis combined with evaporative perstraction (RO-EP) [12]. Besides water and ethanol, these techniques do not produce waste materials, and both have been implemented at industrial scale. Furthermore, the recovery and subsequent use of ethanol generated during dealcoholization ensures these processes meet the criteria for 'clean' technology [9]. SCC involves heating wine, which may negatively impact wine sensory properties, and therefore quality; the capital costs associated with SCC distillation equipment are also relatively high [5,13].

The first patent for the application of RO for dealcoholization of beer and wine was obtained by the German brewing company Lowenbrau in 1975 [3]. Since RO membranes remove smaller molecules (e.g., water) more readily than larger molecules (e.g., ethanol), RO-treated wine can actually have higher ethanol levels than untreated wine; i.e., the addition of water is necessary to achieve dealcoholization. This has limited the application of RO, because traditionally the addition of water to wine has been prohibited (or limited) in many wine-producing countries [14].

Evaporative perstraction (EP) membranes have been used to remove ethanol from the permeate fraction obtained following RO treatment of wine. EP membranes have hydrophobic properties that ensure retention of a bulk liquid, whilst permitting the flux of ethanol vapor from either wine or RO permeate to a water 'stripping' solution. The rate at which ethanol is removed during EP depends on processing conditions such as the membrane surface area, feed flow rate, the stripping solution flow rate, and temperature, thus aroma loss can occur with prolonged treatment times and/or at elevated temperatures [15]. Nevertheless, the combined RO-EP treatment process has achieved commercial success [12] and has overcome many of the issues associated with dealcoholization methods that make use of a single membrane [16]. However, the impact on wine chemistry of the RO-EP treatment has not been well studied, particularly on an industrial scale.

This study aimed to investigate compositional changes in red wines as a consequence of partial dealcoholization (i.e., decreases of 0.5 to 5.0% alcohol by volume, (abv)), achieved via RO-EP treatment. Wines were analyzed pre-treatment, post-treatment, and post-treatment following alcohol re-adjustment (i.e., the addition of ethanol to achieve the original wine alcohol content) to determine compositional changes associated with alcohol removal. In a subsequent trial, samples were collected during RO-EP treatment to enable the composition of permeate, retentate and strip water fractions to be studied. Given the contribution of ethyl esters to the fruity aromas and flavors of red wine, this study also sought to determine to what extent dealcoholization of wine might impact ester concentrations via changes to ester-acid equilibria.

2. Results and Discussion

The intended outcome of RO-EP treatment is the partial dealcoholization of wine but the process may also impact other wine constituents. For example, the concentrations of non-volatile compounds such as tannins and anthocyanins, which typically have molecular weights that far exceed the molecular weight cut-off (MWCO) of RO membranes, can increase because the removal of alcohol effectively concentrates the wine by 0.5 to 5.0% [3]. In contrast, lower molecular weight compounds including wine volatiles may decrease in concentration, as compounds pass through both the reverse osmosis and perstractive membranes [3]. Chemical analysis of red wines was therefore performed before, during and after RO-EP treatment to determine compositional changes due to partial dealcoholization.

2.1. Effect of RO-EP Treatment and Alcohol Re-Adjustment on Basic Wine Composition

Basic wine parameters, including alcohol, density, pH, titratable acidity (TA), volatile acidity (VA), the gelatin index (a chemical measure of astringency), and wine color measurements for Trial 1 wines are shown in Table 1. Partial dealcoholization by RO-EP achieved a significant decrease in the alcohol content of wines, being 1.6, 2.6 and 0.7% abv for wines A, B and C, respectively. Analysis confirmed the addition of ethanol to wines following RO-EP (i.e., alcohol re-adjustment) restored the alcohol content to the same levels as that of the initial wines. Differences in alcohol content did not significantly influence wine density, but significant differences in viscosity were observed amongst

wine samples. In each case, RO-EP treatment of wine significantly decreased viscosity (by between 6 and 9%) which was likely driven by changes to ethanol levels, in agreement with a previous study involving partial dealcoholization of model wines [17]. The subsequent addition of alcohol increased viscosity, but not to the same levels observed prior to dealcoholization (Table 1). This might reflect the loss of some wine components through EP, such that alcohol-adjusted wines were not physically the same as pre-treatment wines.

There were no significant changes in the pH, TA, VA, or organic acid concentrations of wines as a consequence of either RO-EP treatment or the subsequent alcohol re-adjustment. However, dealcoholization significantly affected free sulfur dioxide levels. In the case of wines A and B, there was no detectable sulfur dioxide remaining after RO-EP treatment, whereas 1.9 mg/L remained in Wine C after dealcoholization. Although the initial levels of free sulfur dioxide were low, given its role in preventing oxidation and microbial spoilage of wine, this was an important finding and highlights the need for sulfur dioxide levels to be checked following RO-EP dealcoholization. As expected, there was no change in free sulfur dioxide levels following alcohol re-adjustment.

Changes in alcohol content are thought to affect interactions between salivary proteins and wine tannins [18], influencing the perception of mouthfeel properties, including astringency. The gelatin index of wines was therefore determined as a chemical measure of astringency, with RO-EP treatment found to affect wines differently (Table 1). A significant increase was observed for wine A (from 38 to 55%, $P < 0.001$), suggesting dealcoholization would likely increase the perceived astringency, in agreement with previous studies [18,19]; whereas the 5% increase observed for wine B was not significant and there was no change for wine C. Alcohol re-adjustment significantly decreased the gelatin index values obtained for wines A and C (by 9 and 3%, respectively), but the 6% decrease observed for wine B was not statistically significant. The varied effects of (partial) dealcoholization on salivary protein interactions by wine type (i.e., variety) has previously been reported and was attributed to compositional differences besides alcohol content (i.e., wine pH, and tannin and organic acid concentrations) [18]. Similar compositional variation amongst Trial 1 wines might therefore explain why RO-EP treatment impacted the gelatin index measurements differently.

For wines A and B, RO-EP treatment resulted in significant intensification of wine color, whereas the hue of all wines increased with dealcoholization, albeit by relatively small amounts in the case of wines B and C (Table 1). Differences in wine color following dealcoholization were further characterized by CIELab measurements, with significant differences observed for each parameter, i.e., lightness and hue intensities. However, RO-EP treatment affected the color of individual wines differently. For wines A and B, the lightness (L^*) decreased by 7 and 4%, and hue intensities (a^* and b^*) increased by 6 and 4%, respectively; whereas for wine C, lightness stayed the same, a^* decreased slightly and b^* increased substantially. The subsequent addition of ethanol to dealcoholized wines did not restore the color properties of wines to those observed prior to RO-EP treatment, with alcohol re-adjustment affecting the color of individual wines in different ways. Wine C was not significantly different in color but for wines A and B, ethanol re-adjustment decreased the intensity of wine color compared to that of wine post RO-EP, presumably as a consequence of dilution. In the case of wine B, wine color following ethanol addition was even significantly lower than that observed prior to RO-EP treatment (18.8 vs. 19.6, Table 1), which was not the case for Wine A (12.2 vs. 10.8). The hue of dealcoholized wines decreased following alcohol re-adjustment compared to that of wine pre RO-EP, but to higher, lower and comparable levels for wines A, B and C, respectively. Differences in color are expected to be detectable to the human eye where $\Delta E^* \geq 3.0$ [20], thus, partial dealcoholization visibly affected the wine color for wines A and C ($\Delta E^* = 5.3$ and 3.0, respectively, Table 1), with a perceivable color difference remaining in wine A even after alcohol re-adjustment ($\Delta E^* = 4.0$).

Table 1. Density, viscosity and basic composition of Trial 1 wines before and after RO-EP treatment, and following RO-EP treatment and alcohol re-adjustment.

	Wine A				Wine B				Wine C			
	Pre-RO-EP	Post-RO-EP	Post-EtOH	P	Pre-RO-EP	Post-RO-EP	Post-EtOH	P	Pre-RO-EP	Post-RO-EP	Post-EtOH	P
alcohol (% abv)	14.1a	12.5b	14.1a	<0.001	17.1a	14.5b	17.1a	<0.001	14.9a	14.2b	14.9a	<0.001
free SO ₂ (mg/L)	3.8	nd	nd	–	6.4	nd	nd	–	7.7	1.9	1.9	–
density (g/mL)	0.995	0.995	0.993	–	0.993	0.996	0.993	–	0.993	0.994	0.993	–
viscosity (mPa s)	1836a	1711c	1796b	<0.001	2009a	1829c	1982b	<0.001	1858a	1751c	1836b	<0.001
glycerol (g/L)	10.2a	10.2a	10.0b	<0.001	10.9b	11.0a	10.7c	<0.001	11.9a	11.8a	11.6b	0.039
gelatin index (%)	38c	55a	46 b	<0.001	49	54	48	–	44a	44a	41b	0.040
pH	3.7	3.6	3.7	–	3.6	3.6	3.6	–	3.7	3.7	3.7	–
TA (g/L)	5.9	6.1	5.9	–	7.2	7.1	7.2	–	6.4	6.5	6.4	–
VA (g/L)	0.4	0.3	0.4	–	0.5	0.8	0.5	–	0.6	0.6	0.6	–
citric acid (g/L)	1.2	1.2	1.1	–	0.7	0.7	0.7	–	0.9	0.9	0.8	–
succinic acid (g/L)	6.2	6.2	6.1	–	8.2	8.3	8.3	–	6.5	6.5	6.4	–
lactic acid (g/L)	3.7	3.8	3.7	–	2.8	2.8	2.8	–	3.8	3.8	3.7	–
wine color (au)	10.8c	12.6a	12.2b	<0.001	19.6b	21.0a	18.8c	<0.001	17.2	17.3	17.1	–
wine hue	2.8c	4.9a	3.8b	<0.001	4.1b	4.2a	3.9c	<0.001	3.4b	3.6a	3.4b	<0.001
L*	67.6a	62.7c	64.0b	<0.001	49.7a	47.5b	49.8a	<0.001	52.9b	53.1a	52.7c	0.003
a*	34.7c	36.8a	36.1b	<0.001	50.8b	52.6a	50.9b	<0.001	49.5a	48.5b	49.6a	<0.001
b*	5.0c	5.6b	5.9a	<0.001	5.3a	5.3a	3.3b	<0.001	0.6b	3.4a	0.5b	<0.001
ΔE^*^1	–	5.3	4.0	–	–	2.7	2.0	–	–	3.0	0.3	–

Values are means of duplicate measurements (n = 2). Standard errors were $\leq 10\%$; nd = not detected. Values followed by different letters within rows (for each wine) are statistically significantly different. ¹ Total color differences (ΔE^*) were calculated relative to the color of each wine prior to RO-EP treatment.

As with gelatin index measurements, the variation in wine color properties observed amongst treatments likely reflected more than just the concentration or dilution of anthocyanins as a consequence of RO-EP or ethanol re-adjustment, respectively. A study involving the removal of 2% ethanol from wine by nanofiltration and reverse osmosis found the intensity of wine color increased by 6 and 11%, respectively [21], whereas the dilution of wine by the addition of ethanol decreased wine color as a result of lower co-pigmentation [22] and vice versa [23]. However, dealcoholization may also have affected the extent of co-pigmentation and/or formation of derived pigments as a consequence of oxygen uptake, the loss of sulfur dioxide and/or adsorption on the membrane surface, as suggested by Gambuti and colleagues [18]; decreased color following the addition of ethanol might be explained by the disruption of copigmentation stacks [23].

2.2. Effect of RO-EP Treatment and Alcohol Re-adjustment on Wine Fermentation Volatiles

Among the various volatile compounds formed as a result of fermentation, esters contribute many of the important fruity aromas and flavors typically found in wine [24]. Ethanol plays an important role in the acid-ethyl ester equilibrium (Equation (1)). Thus, the removal of ethanol through dealcoholization of wine could be expected to impact the concentrations of ethyl esters and their corresponding acids, due to the resulting equilibrium shift towards ester hydrolysis. Esterification and hydrolysis are reversible reactions [25], with the rate of each reaction being influenced by factors including temperature and activation energy [26], and different points of equilibrium being established for individual esters [27,28].



To determine the impact of dealcoholization on esters, the concentrations of a range of volatile acids, esters and alcohols were measured before and after RO-EP treatment, and following alcohol re-adjustment (Table 2). RO-EP treatment of wine resulted in a decrease in the concentration of acids by as much as 50%, with higher proportions of acids with lower molecular weights, e.g., acetic, propionic, and butanoic acids, being diminished as a result of dealcoholization than acids of higher molecular weight such as hexanoic, octanoic, and decanoic acids. Indeed, decanoic acid concentrations appeared to increase with RO-EP treatment. The concentrations of branched acids similarly decreased with dealcoholization, but not significantly, and only by $\leq 10\%$. Theoretically, the concentration of acids should increase slightly if ester hydrolysis occurred. However, the loss of acids through EP meant that most acid concentrations decreased; for wine A, the concentrations of butanoic, hexanoic, octanoic and decanoic acids decreased by 45, 7, 1 and 25%, respectively.

Decreases in ethyl ester concentrations were also observed, with ethyl esters of higher molecular weight, e.g., ethyl octanoate and ethyl decanoate, decreasing the most despite smaller, more volatile esters being expected to transition across the RO membrane and be removed via EP more readily. Nevertheless, the loss of ethyl esters may not only be wholly attributable to membrane filtration. The removal of ethanol might also have affected the ester equilibrium (Equation (1)), such that esters were hydrolyzed to release ethanol and their corresponding acids.

Previous research found esters with higher molecular weights (or longer carbon chains) undergo higher rates of hydrolysis [25]. During wine aging, the concentrations of straight chain ethyl esters (i.e., ethyl hexanoate, octanoate and decanoate) were found to decrease, which was attributed to their enzymatic formation during fermentation being at levels exceeding their equilibrium concentrations [29]. A subsequent study on esterification of tartaric acid with ethanol in model wine demonstrated the importance of ethanol concentration; with an increase in both the rate and quantity of ester formation observed at higher ethanol concentrations [30]. Furthermore, esterification rates were found to decrease as molecular weight increased, i.e., ethyl decanoate > ethyl octanoate > ethyl hexanoate [26]. When two red wines with different initial ethanol concentrations (being 15.4 and 13.3% v/v) were partially dealcoholized (to remove 2, 3 and 5% v/v ethanol) using a polypropylene

hollow fiber membrane contactor apparatus, ethyl ester levels decreased by between 11 and 100% [31]. Similar results were observed in a study involving dealcoholization of wine using a benchtop RO-EP system; ethyl ester concentrations decreased by 20 to 80% as ethanol concentrations decreased from 13.7% (for Shiraz wine) and 12.2% (for Chardonnay wine), to 8% (or even to 5%) [32]. Interestingly, in these studies, the concentrations of some acids increased, e.g., hexanoic and propanoic acid levels increased by 24% [31] and by 11 to 173% [32], respectively, which may reflect ester hydrolysis.

The addition of ethanol to dealcoholized wines again had variable consequences on wine composition. The concentrations of fermentation volatiles were expected to decrease due to dilution following the addition of ethanol, and in some instances, this was observed; e.g., the concentrations of octanoic and decanoic acids decreased significantly ($P \leq 0.021$). However, the concentrations of many volatiles did not change substantially and the levels of ethyl decanoate were found to increase significantly ($P = 0.019$). This could reflect shifts in the acid-ester equilibrium, i.e., in favor of esterification to produce ethyl decanoate. Importantly, there were very few instances in which the fermentation volatile concentrations of ethanol re-adjusted wines were comparable to those of wines prior to RO-EP treatment, which demonstrates the impact on wine aroma chemistry of the dealcoholization process. The compositional consequences of RO-EP treatment of red wines was investigated further in Trial 2, with different samples (i.e., wine, retentate, permeate and strip water) being collected during the dealcoholization process.

2.3. Basic Composition of Samples Collected during RO-EP Treatment of Wine

RO-EP treatment of wines D and E achieved decreases in alcohol content of 2.6 and 2.4%, respectively (Table 3). Retentate fractions generated by RO yielded a higher ethanol concentration than the initial wine in both cases, for reasons given above. That is, although both ethanol and water permeate the RO membrane, water permeates at a higher rate due to its considerably lower molecular weight. The removal of alcohol due to EP can be clearly seen by the significant decrease in ethanol content for permeate fractions before and after EP (i.e., >10% abv difference), with the ethanol subsequently being transferred to the strip water (which contained ~8–9% abv). Small but significant differences in density were observed, in particular for permeate and strip water fractions. Glycerol concentrations increased in retentate due to RO fractionation, with the lower levels observed in permeate being substantially affected by EP, despite glycerol not being detected in strip water. The levels of glycerol in wine out samples were slightly higher than in untreated wine, which may impact the perception of viscosity [33].

The pH of wine, retentate and permeate remained relatively consistent, despite the increased TA of retentate and decreased TA of permeate following RO as a consequence of the retention of organic acids, including succinic and lactic acids (Table 3). Notably, EP had no effect on TA which may be due to the levels of organic acids pre-EP being quite low. VA was not affected by RO-EP treatment.

The intensity of wine color increased significantly due to dealcoholization (Table 3), but with minimal impact on wine hue, similar to that of wines A, B and C in Trial 1 (Table 1). CIELab measurements suggested the color increase was reflective of wines becoming darker, with significantly lower L^* and higher a^* values obtained. The color properties of retentate fractions were augmented (relative to wine samples before or after treatment) due to the concentration of anthocyanins and derived pigments during RO. This was further reflected by the complete absence of color in permeate and strip water fractions. For wine D, the ΔE^* following RO-EP treatment was only 2.0, which is not expected to be evident to the naked eye, but for wine E, ΔE^* was 3.7, and so the change in color would likely be perceptible. The impact of RO treatment on wine color was more obvious due to the intensification of color in retentate from both wines; with ΔE^* being >10 (Table 3), this color change would also be expected to be readily observed.

2.4. Concentration of Fermentation Volatiles in Samples Collected during RO-EP Treatment of Wine

The concentrations of volatile acids, esters and alcohols present in wine, retentate, permeate and strip water samples are shown in Table 4. It should be noted that these concentrations do not represent absolute quantities of volatiles since the volume of wine, permeate and strip water are not the same. Therefore, mass flow values were calculated (Table 5) to better demonstrate changes in fermentation volatiles.

It is evident from Table 4 that all of the fermentation volatiles measured were capable of permeating the RO membrane. Different proportions of each volatile were distributed in the retentate and permeate fractions but fermentation volatiles were typically present at higher concentrations in retentate compared with permeate. Following EP treatment of permeate, the concentrations of most volatiles decreased, suggesting they were being removed. This notion was supported by their detection in strip water. As a consequence, volatile concentrations in dealcoholized wines were generally lower than the levels observed in wine prior to RO-EP treatment. As outlined above, this outcome may also reflect shifts in the equilibrium between ester formation and hydrolysis due to the removal of ethanol. This was consistent with an earlier study that reported decreases in ester concentrations of up to 60% in red wines following alcohol removal using a polypropylene membrane [34]. A separate study also observed a significant loss of esters (ethyl octanoate, ethyl acetate and isoamyl acetate) following RO-EP treatment of wine to remove alcohol [35].

3. Materials and Methods

3.1. Chemicals

Chemicals and solvents (analytical grade) were purchased from Sigma Aldrich (Castle Hill, NSW, Australia) and Merck (Darmstadt, Germany), respectively. Deuterated internal standards (d_5 -ethyl propanoate, d_5 -ethyl 2-methylpropanoate, d_9 -2-methylpropyl acetate, d_5 -ethyl butanoate, d_5 -ethyl 2-methylbutanoate, d_5 -ethyl 3-methylbutanoate, d_5 -3-methylbutyl acetate, d_5 -2-methylbutyl acetate, d_{13} -1-hexanol, d_5 -ethyl hexanoate, d_{13} -hexyl acetate, d_3 -2-phenylethanol, d_5 -ethyl octanoate, d_3 -2-phenylethyl acetate and d_5 -ethyl decanoate) were synthesized as previously reported [36].

3.2. Wine Samples

Wines were sourced from several industry partners who made use of industrial scale RO-EP units (Model Midi 10, VA Filtration, Nuriootpa, Australia) to achieve partial dealcoholization (i.e., decreases in alcohol content of between 1.0 and 2.5% abv) in accordance with the manufacturer's operating instructions. Briefly, wines were pumped from a feed tank (industrial scale 20–30 kL) across a series of 10 spiral wound 4040 reverse osmosis membranes (nominal MWCO of 220–270 atomic mass units; filtering area 75 m²), under approximately 3,000 kPa of pressure, to generate retentate and permeate fractions. The permeate was degassed, heated to between 45 and 55 °C, and passed across one side of a microporous, hydrophobic hollow fiber perstractive membrane (filtering area 130 m²). Filtered, degassed water was passed across the other side of the membrane, as the stripping liquid. In this way, ethanol was vaporized from the permeate, diffused across the perstractive membrane and condensed in the strip water. The EP-treated permeate was subsequently cooled, recombined with the retentate and returned to the feed tank. Wine was circulated through the RO-EP unit in this way, until the desired alcohol level was achieved, as shown in Figure 1. The volume of wine in tanks was about 20–30 kL, and at this scale, industry partners found that for every 1000 L of pure ethanol removed, the wine volume decreased by 900 L, due to the mixing factor of ethanol/water. Some compounds may have been bound to the membranes, however, this would be saturated quickly at a typical flow rate of 4000 L/h. To avoid this impact, samples were collected a few hours after the commencement of processing.

Table 2. Concentrations of fermentation volatiles present in Trial 1 wines before and after RO-EP treatment, and following RO-EP treatment and alcohol re-adjustment.

		Wine A			Wine B			Wine C			P
		Pre-RO-EP	Post-RO-EP	Post-EtOH	Pre-RO-EP	Post-RO-EP	Post-EtOH	Pre-RO-EP	Post-RO-EP	Post-EtOH	
straight chain acids	acetic acid ¹	488	428	459	855	796	824	871	646	722	0.107
	propanoic acid	2009	1873	1763	1954	1846	1741	3057	2060	1838	0.190
	butanoic acid	2100	1156	1562	3399	1614	1799	2577	1514	1804	0.014
	hexanoic acid	2277	2111	2172	1757	1656	1624	1828	1778	1641	0.055
	octanoic acid	3384	3344	3075	2986	2940	2622	3016	2962	2719	<0.001
	decanoic acid	2129	2652	1870	2121	2246	1604	2066	2491	2070	0.021
straight chain ethyl esters	ethyl acetate ¹	60	54	55	108	89	88	53	51	50	0.158
	ethyl propanoate	322	286	317	229	191	195	167	152	156	0.059
	ethyl butanoate	214	207	210	209	166	163	137	126	123	0.163
	ethyl hexanoate	411	385	402	375	301	300	325	294	302	0.084
	ethyl octanoate	41.8	33.8	33.7	74.0	50.5	51.8	31.5	28.0	30.5	0.145
	ethyl decanoate	114	75.7	89.0	167	93.6	109	133	96.3	116	0.019
branched acids	2-methylpropanoic acid	2011	1959	1818	4824	4578	4378	2300	2108	2135	0.051
	2-methylbutanoic acid	1219	1179	1206	1808	1642	1596	781	770	761	0.289
	3-methylbutanoic acid	1912	1720	1665	2337	2194	2140	1631	1625	1594	0.068
branched ethyl esters	ethyl 2-methylpropanoate	120	115	114	312	258	258	69.6	62.5	67.3	0.290
	ethyl 2-methylbutanoate	27.1	26.8	26.6	28.5	24.2	24.3	10.3	9.5	9.7	0.241
	ethyl 3-methylbutanoate	39.3	38.4	38.4	50.9	42.7	43.2	17.4	15.6	15.9	0.201
acetates	2-methylpropyl acetate	39.5	38.6	36.3	126	105	100	27.5	27.3	26.7	0.340
	2-methylbutyl acetate	356	397	338	401	334	314	179	166	162	0.335
	3-methylbutyl acetate	1140	1379	1128	1334	1111	1038	597	564	541	0.469
	hexyl acetate	24.4	31.0	13.0	12.3	9.86	9.50	8.07	6.49	6.70	0.392
	2-phenylethyl acetate	158	157	140	76.4	68.8	63.3	67.6	67.3	67.4	0.164
alcohols	2-methylpropanol ¹	46	43	42	114	102	99	47	46	46	0.201
	butanol	2047	1693	1752	1232	1030	1057	1487	1340	1290	0.011
	2/3-methylbutanol ¹	176	161	160	198	182	171	148	146	143	0.067
	1-hexanol	2695	2290	2296	2271	1977	1876	1876	1774	1744	0.027
	2-phenylethanol ¹	78	74	73	73	70	68	55	53	52	0.003

¹ Concentrations are µg/L, except for acetic acid, ethyl acetate, 2-methylpropanol, 2-methylbutanol and 2-phenylethyl ethanol, which are mg/L.

Table 3. Flow rate, density and basic composition of samples collected during RO-EP treatment of Trial 2 wines.

	Wine D							Wine E						
	Wine in	Wine out	Retentate	Permeate Pre-EP	Permeate Post-EP	Strip Water	P	Wine in	Wine out	Retentate	Permeate Pre-EP	Permeate Post-EP	Strip Water	P
flow rate (L/h)	3144	3059	2198	946	861	1138	–	3750	3664	2820	930	844	1555	–
alcohol (% abv)	15.2b	12.6d	15.9a	14.2c	3.7f	8.8e	<0.001	14.7b	12.3d	15.2a	13.3c	1.7f	7.6e	<0.001
density (g/mL)	0.993d	0.996c	0.997b	0.985f	0.998a	0.988e	<0.001	0.994d	0.997b	0.997b	0.995c	0.999a	0.990e	<0.001
glycerol (g/L)	10.0c	10.3b	12.3a	4.9e	5.4d	nd	<0.001	10.9c	11.3b	13.3a	4.1e	4.6d	nd	<0.001
pH	3.5c	3.5c	3.5c	3.6b	3.4d	4.0a	<0.001	3.6c	3.6c	3.7b	3.5d	3.4e	4.7a	<0.001
TA (g/L)	6.3c	6.6b	7.8a	2.3d	2.4d	0.1e	<0.001	6.4c	6.6b	7.7a	2.1d	2.1d	0.2e	<0.001
VA (g/L)	0.5	0.4	0.5	0.5	0.4	0.1	–	0.6	0.5	0.7	0.4	0.4	0.1	–
succinic acid (g/L)	5.3b	5.5b	7.2a	1.0c	1.0c	nd	<0.001	1.2b	1.2b	1.4a	0.6c	0.7c	nd	<0.001
lactic acid (g/L)	3.7b	3.9b	4.4a	2.5c	2.1c	nd	<0.001	1.8b	2.2a	2.3a	1.4c	1.3c	nd	<0.001
wine color (au)	14.8c	16.3b	20.9a	nd	nd	nd	<0.001	12.9c	14.2b	17.4a	nd	nd	nd	<0.001
wine hue	0.73a	0.71c	0.72b	nd	nd	nd	0.005	0.67	0.66	0.67	nd	nd	nd	ns
L*	57.2a	55.7b	45.9c	nd	nd	nd	<0.001	61.4a	58.9b	53.1c	nd	nd	nd	<0.001
a*	41.2c	42.6b	49.8a	nd	nd	nd	<0.001	38.2c	40.9b	44.9a	nd	nd	nd	<0.001
b*	9.6c	9.5b	13.9a	nd	nd	nd	<0.001	1.0c	1.3b	2.9a	nd	nd	nd	<0.001
$\Delta E^* a$	–	2.0	14.9	–	–	–	–	–	3.7	10.8	–	–	–	–

With the exception of flow rate, values are means of two replicate measurements (n = 2). Standard errors were $\leq 10\%$; nd = not detected. Values followed by different letters within rows (for each wine) are statistically significantly different; ns = not significant. ¹ Total color differences (ΔE^*) were calculated relative to the color of each wine prior to RO-EP treatment.

Table 4. Concentrations of fermentation volatiles present in samples collected during RO-EP treatment of Trial 2 wines.

		Wine D						Wine E						P
		Wine in	Wine out	Retentate	Permeate Pre-EP	Permeate Post-EP	Strip Water	Wine in	Wine out	Retentate	Permeate Pre-EP	Permeate Post-EP	Strip Water	
straight chain acids	acetic acid ¹	682	653	672	600	614	301	461	445	468	425	364	100	<0.001
	propanoic acid	2207	2264	2107	3632	3126	1423	1287	1286	1465	1010	778	401	0.417
	butanoic acid	1152	714	1289	677	<250	<250	756	736	868	589	<250	<250	0.009
	hexanoic acid	2116	2080	2488	1574	1022	728	1275	1237	1477	759	<250	<250	<0.001
	octanoic acid	2470	2283	2518	1023	529	466	1070	988	1242	587	<250	362	0.041
straight chain ethyl esters	decanoic acid	514	518	565	254	<100	162	493	461	542	317	207	286	0.003
	ethyl acetate ¹	123	98	137	99	21	47	61	51	67	47	4	27	0.013
	ethyl propanoate	473	353	548	345	59	164	207	174	238	146	<25	80	0.032
	ethyl butanoate	307	239	359	183	35	92	171	145	202	85	5	47	0.004
	ethyl hexanoate	452	346	552	226	46	120	27	23	33	13	2	8	0.400
branched acids	ethyl octanoate	298	221	359	132	32	63	28	23	34	17	2	11	0.355
	ethyl decanoate	161	105	178	42	7	14	4	3	6	1	<1	<1	0.455
	2-methylpropanoic acid	1696	1583	1890	1084	738	506	1578	1535	1946	722	321	265	<0.001
	2-methylbutanoic acid	14586	12746	18916	5819	2474	1705	1202	1169	1506	335	177	187	0.361
	3-methylbutanoic acid	1651	1558	2141	787	447	280	1345	1311	1619	364	173	175	<0.001
branched ethyl esters	ethyl 2-methylpropanoate	339	307	466	121	31	52	124	102	157	41	<5	21	0.070
	ethyl 2-methylbutanoate	51	44	68	13	2	4	25	22	34	6	<1	3	0.017
	ethyl 3-methylbutanoate	82	68	111	21	3	7	37	33	48	10	<1	5	0.031
	2-methylpropyl acetate	51	44	67	20	<5	11	45	42	58	15	<5	<5	<0.001
	2-methylbutyl acetate	191	158	244	64	11	51	106	97	140	24	<10	14	0.004
acetates	3-methylbutyl acetate	593	474	747	202	42	181	1052	956	1345	269	23	165	0.017
	hexyl acetate	8	4	9	4	<2	5	20	18	23	10	2	6	0.142
	2-phenylethyl acetate	297	240	358	170	35	111	855	806	1040	321	39	192	0.112
	2-methylpropanol ¹	68	63	85	30	7	19	66	63	81	22	2	14	<0.001
	butanol	1787	1587	1917	1458	649	989	1556	1460	1800	992	304	605	<0.001
alcohols	2/3-methylbutanol ¹	152	143	189	59	15	38	330	339	445	158	12	80	0.037
	1-hexanol	2229	1952	2649	1262	252	761	3654	3322	4220	1900	<50	1099	0.007
	2-phenylethanol ¹	287	280	328	171	124	43	414	409	473	238	159	67	0.001

¹ Concentrations are µg/L, except for acetic acid, ethyl acetate, 2-methylpropanol, 2-methylbutanol and 2-phenylethyl ethanol, which are mg/L; < denotes below limit of detection.

Table 5. Mass flow of fermentation volatiles present in samples collected during RO-EP treatment of Trial 2 wines.

		Wine D						Wine E						P
		Wine in	Wine out	Retentate	Permeate Pre-EP	Permeate Post-EP	Strip Water	Wine in	Wine out	Retentate	Permeate Pre-EP	Permeate Post-EP	Strip Water	
straight chain acids	acetic acid ¹	2.15	2.00	1.48	0.57	0.53	0.34	1.73	1.63	1.32	0.40	0.31	0.16	<0.001
	propanoic acid	6.94	6.93	4.63	3.44	2.69	1.62	4.82	4.71	4.13	0.94	0.66	0.62	0.001
	butanoic acid	3.62	2.18	2.83	0.64	<0.22	<0.28	2.83	2.69	2.45	0.55	<0.21	<0.39	<0.001
	hexanoic acid	6.65	6.36	5.47	1.49	0.88	0.83	4.78	4.53	4.17	0.71	<0.21	<0.39	<0.001
	octanoic acid	7.77	6.98	5.54	0.97	0.46	0.53	4.01	3.62	3.50	0.55	<0.21	0.56	0.013
straight chain ethyl esters	decanoic acid	1.62	1.58	1.24	0.24	<0.09	0.18	1.85	1.69	1.53	0.29	0.17	0.44	<0.001
	ethyl acetate ¹	0.39	0.30	0.30	0.09	0.02	0.05	0.23	0.19	0.19	0.04	<0.01	0.04	0.004
	ethyl propanoate	1.49	1.08	1.20	0.33	0.05	0.19	0.78	0.64	0.67	0.14	<0.02	0.12	0.008
	ethyl butanoate	0.97	0.73	0.79	0.17	0.03	0.10	0.64	0.53	0.57	0.08	<0.01	0.07	<0.001
	ethyl hexanoate	1.42	1.06	1.21	0.21	0.04	0.14	0.10	0.09	0.09	0.01	<0.01	0.01	0.377
branched acids	ethyl octanoate	0.94	0.68	0.79	0.13	0.03	0.07	0.11	0.08	0.10	0.02	<0.01	0.02	0.319
	ethyl decanoate	0.51	0.32	0.39	0.04	0.01	0.02	0.02	0.01	0.02	<0.01	<0.01	<0.01	0.467
	2-methylpropanoic acid	5.33	4.84	4.15	1.03	0.64	0.58	5.92	5.63	5.49	0.67	0.27	0.41	<0.001
	2-methylbutanoic acid	45.86	38.99	41.58	5.50	2.13	1.94	4.51	4.28	4.25	0.31	0.15	0.29	0.329
	3-methylbutanoic acid	5.19	4.77	4.71	0.74	0.39	0.32	5.05	4.80	4.57	0.34	0.15	0.27	<0.001
branched ethyl esters	ethyl 2-methylpropanoate	1.06	0.94	1.02	0.11	0.03	0.06	0.47	0.37	0.44	0.04	<0.01	0.03	0.033
	ethyl 2-methylbutanoate	0.16	0.13	0.15	0.01	<0.01	<0.01	0.09	0.08	0.10	0.01	<0.01	0.01	0.006
	ethyl 3-methylbutanoate	0.26	0.21	0.24	0.02	<0.01	0.01	0.14	0.12	0.14	0.01	<0.01	0.01	0.009
	2-methylpropyl acetate	0.16	0.13	0.15	0.02	<0.01	0.01	0.17	0.15	0.16	0.01	<0.01	<0.01	<0.001
	2-methylbutyl acetate	0.60	0.48	0.54	0.06	0.01	0.06	0.40	0.36	0.39	0.02	<0.01	0.02	<0.001
acetates	3-methylbutyl acetate	1.86	1.45	1.64	0.19	0.04	0.21	3.94	3.50	3.79	0.25	0.02	0.26	0.034
	hexyl acetate	0.03	0.01	0.02	0.00	<0.01	0.01	0.08	0.06	0.06	0.01	<0.01	0.01	0.141
	2-phenylethyl acetate	0.93	0.73	0.79	0.16	0.03	0.13	3.21	2.95	2.93	0.30	0.03	0.30	0.140
	2-methylpropanol ¹	0.21	0.19	0.19	0.03	0.01	0.02	0.25	0.23	0.23	0.02	<0.01	0.02	<0.001
	butanol	5.62	4.85	4.21	1.38	0.56	1.13	5.83	5.35	5.08	0.92	0.26	0.94	<0.001
alcohols	2/3-methylbutanol ¹	0.48	0.44	0.42	0.06	0.01	0.04	1.24	1.24	1.25	0.15	0.01	0.12	0.064
	1-hexanol	7.01	5.97	5.82	1.19	0.22	0.87	13.70	12.17	11.90	1.77	<0.04	1.71	0.018
	2-phenylethanol ¹	0.90	0.86	0.72	0.16	0.11	0.05	1.55	1.50	1.33	0.22	0.13	0.10	0.008

¹ Concentrations are g/h, except for acetic acid, ethyl acetate, 2-methylpropanol, 2-methylbutanol and 2-phenylethyl ethanol, which are kg/h < denotes below limit of detection.

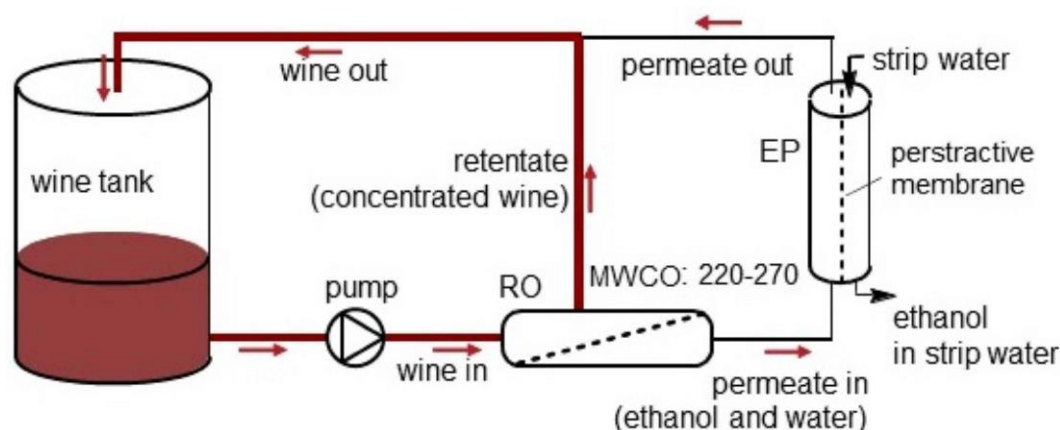


Figure 1. Schematic diagram of the reverse osmosis-evaporative perstraction process.

Two trials involving partial dealcoholization of red wines were undertaken. Trial 1 involved RO-EP treatment of three wines: A 2014 Barossa Valley Shiraz Cabernet Sauvignon (Wine A), a 2015 McLaren Vale Cabernet Sauvignon (Wine B) and a 2015 Adelaide Hills Shiraz (Wine C). Samples were collected before and after dealcoholization and also following ethanol re-adjustment, i.e., the addition of ethanol to dealcoholized wine, to restore the initial wine alcohol content. Trial 2 involved RO-EP treatment of two wines: A 2013 Barossa Valley Shiraz (Wine D) and a 2015 McLaren Vale Shiraz (Wine E). Samples (i.e., wine in, wine out, retentate, permeate (pre- and post-EP treatment) and strip water) were collected 1.5 h after RO-EP treatment commenced. Samples were bottled in 750 mL glass bottles under screw cap closures and cellared at 15 °C prior to chemical analysis, which was performed within 2 to 3 days of RO-EP treatment.

Since RO-EP was performed on industrial volumes, it was not practical to replicate treatments. Instead Trial 1 involved RO-EP treatment of three wines and Trial 2 involved RO-EP treatment of two wines.

3.3. Basic Wine Analysis

The alcohol content, density, pH, TA (as g/L tartaric acid) and VA (as g/L acetic acid) were measured (in duplicate) by the Australian Wine Research Institute's Commercial Services Laboratory, using a FOSS FTIR WineScan (Mulgrave, Victoria, Australia). Glycerol and organic acid concentrations were determined by high performance liquid chromatography (HPLC) using methods described previously [37]. Analyses were performed with an Agilent 1100 series HPLC (Agilent Technologies, Forest Hill, Victoria, Australia) equipped with an Aminex HPX-87H cation exchange column (Bio-Rad Laboratories, Gladesville, NSW, Australia), diode array, and refractive index detectors. The mobile phase was 2.5 mM sulfuric acid. Wine color, hue and CIELab were determined via spectral measurements made with a Cintra 4040 spectrometer (GBC Scientific Equipment, Melbourne, Vic., Australia) operating between 380 and 780 nm (at 2 nm intervals). CIELab measurements comprised: lightness (L^*); the intensity of red and green hues (a^*); and the intensity of yellow and blue hues (b^*). The total color difference (ΔE^*) was calculated according to the equation $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Viscosity was measured using an Ostwald-type viscometer (Sigma-Aldrich, 0.5 mm capillary diameter), as previously described [17], and the gelatin index of wines was measured using methodology developed by Glories, as described by Goldner and Zamora [38].

3.4. Gas Chromatography-Mass Spectrometry Analysis

The concentrations of the predominant fermentation volatiles (acids, alcohols and esters) were determined by Metabolomics Australia (the Australian Wine Research Institute, Adelaide, Australia) using gas chromatography-mass spectroscopy (GC-MS), according to stable isotope dilution analysis (SIDA) methods reported elsewhere [36]. Wine samples (1 mL, diluted with 9 mL of pH 3.7

buffer) were saturated with sodium chloride (2 g), prior to the addition of an internal standard mixture (Supplementary Table S1). Samples were extracted with a DVB/CAR/PDMS solid phase micro-extraction (SPME) fiber (Sigma Aldrich) for 10 min at 35 °C, prior to desorption (splitless mode), at an injector temperature of 200 °C, onto an Agilent 7890A gas chromatograph equipped with a Gerstel MPS2 multi-purpose sampler, and coupled to an Agilent 5975C mass selective detector. Separation was achieved with a Phenomenex wax column (60 m × 0.25 mm i.d. × 0.25 µm film thickness), with helium (Ultra High Purity) as the carrier gas (in constant flow mode). The initial oven temperature was 35 °C (held for 3 min) and then increased to 220 °C (at 5 °C/min, held for 3 min). The mass spectrometer quadrupole temperature was 150 °C, the source was set at 230 °C and the transfer line was held at 250 °C. Positive electron ionization spectra (at 70 eV) were recorded in selected ion monitoring (SIM) mode with a solvent delay of 5 min. Raw data from Agilent ChemStation software (v E.02.02.1431, Agilent Technologies, Forest Hill, Victoria, Australia) were converted into MassHunter data files and processed using MassHunter Workstation Software (Agilent Technologies) for Quantitative Analysis (v B.04.00). Fermentation volatiles were identified by comparing mass spectral data with the NIST mass spectral database and were subsequently quantified against their corresponding isotopically-labelled internal standard.

3.5. Statistical Analysis

Basic compositional data were analyzed by one-way analysis of variance (ANOVA) using GenStat (15th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by least significant difference (LSD) multiple comparison test at $P < 0.05$. Volatile data were analyzed via an ANOVA F-test using the lmerTest package in R statistical software [39]. For each dataset (i.e., Trials 1 and 2), mixed effect linear models were fitted individually for each volatile, with the response variable being the concentration at each treatment level. A fixed effect predictor was included for treatments, together with a random intercept for wine to account for the repeated measures on each wine. The fitting was performed using the lme4 package in R [40].

4. Conclusions

A number of options are available to winemakers to manage the final alcohol content of their wines. In this study, RO-EP was shown to effectively remove ethanol to controllable extents, but also impacted wine composition depending on both the wine in question and the amount of ethanol being removed. In general, the dealcoholization process had a concentrating effect on wines. While free SO₂ was lost through RO-EP, some acids, and therefore TA, increased slightly but there were no significant changes to pH or VA. However, due to the contribution of ethanol to the physical properties of wine, there were significant effects of partial dealcoholization on wine color properties and the gelatin index (a chemical measure of astringency), which were attributable to changes in the concentration of anthocyanins and phenolics. Changes in ethanol concentration also affected the concentrations of ethyl esters, which may reflect both the loss of esters into strip water during EP and a shift in the chemical equilibrium responsible for ester formation and hydrolysis, following ethanol removal. From a practical consideration, winemakers need to manage the risks associated with the introduction of oxygen and loss of sulfur dioxide during RO-EP treatment, and be aware of the potential for dealcoholization to enhance wine astringency.

Supplementary Materials: The following are available online, Table S1: Deuterated internal standards used for determination of fermentation volatiles by GC–MS.

Author Contributions: All authors were involved in conceptualization; methodology, D.-T.P.; formal analysis, D.-T.P. and K.L.W.; investigation, D.-T.P.; resources, V.J.S. and D.W.; writing—original draft preparation, D.-T.P.; writing—review and editing, V.J.S., D.W., D.W.J. and K.L.W.; supervision, V.J.S., D.W.J. and K.L.W.; funding acquisition, V.J.S., D.W., D.W.J. and K.L.W.

Funding: This research was funded by the Australian Research Council Training Centre for Innovative Wine Production (www.adelaide.edu.au/tc-iwp), which is funded as part of the ARC's Industrial Transformation Research Program (Project No. ICI30100005), with support from Wine Australia and industry partners.

Acknowledgments: The authors gratefully acknowledge: the industry partners who provided wine; the staff from VA Filtration who provided technical assistance with RO-EP dealcoholization of wines; Natoiya Lloyd and Esther Kristianto from Metabolomics Australia (AWRI) for GC-MS analysis of fermentation volatiles; and Jonathan Tuke from the University of Adelaide for assistance with statistical analysis.

Conflicts of Interest: D.W. declares a financial conflict of interest as both the inventor of RO-EP and an employee of VA Filtration. The funders had no role in the design of the study, i.e., in the collection, analysis or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

References

- Pickering, G.J. Low- and reduced-alcohol wine: A review. *J. Wine Res.* **2000**, *11*, 129–144. [[CrossRef](#)]
- Godden, P.; Muhlack, R. Trends in the composition of Australian wine. *Aust. N. Z. Grapegr. Winemaker* **2010**, *558*, 47–61.
- Rayess, Y.E.; Mietton-Peuchot, M. Membrane technologies in wine industry: An overview. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 2005–2020. [[CrossRef](#)]
- Zamora, F. Dealcoholised wines and low-alcohol wines. In *Wine Safety, Consumer Preference, and Human Health*; Moreno-Arribas, M., Bartolomé Suáldea, B., Eds.; Springer: Cham, Switzerland, 2016; pp. 163–182.
- Schmidtke, L.M.; Blackman, J.W.; Agboola, S.O. Production technologies for reduced alcoholic wines. *J. Food Sci.* **2012**, *77*, R25–R41. [[CrossRef](#)] [[PubMed](#)]
- Longo, R.; Blackman, J.W.; Antalick, G.; Torley, P.J.; Rogiers, S.Y.; Schmidtke, L.M. Harvesting and blending options for lower alcohol wines: A sensory and chemical investigation. *J. Sci. Food Agric.* **2017**, *98*, 33–42. [[CrossRef](#)]
- Schelezi, O.J.; Šuklje, K.; Boss, P.K.; Jeffery, D.W. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. *Food Chem.* **2018**, *259*, 196–206. [[CrossRef](#)]
- Hranilovic, A.; Li, S.; Boss, P.K.; Bindon, K.; Ristic, R.; Grbin, P.R.; Van der Westhuizen, T.; Jiranek, V. Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-*Saccharomyces* inocula. *Aust. J. Grape Wine Res.* **2018**, *24*, 166–180. [[CrossRef](#)]
- Takács, L.; Vatai, G.; Korány, K. Production of alcohol free wine by pervaporation. *J. Food Eng.* **2007**, *78*, 118–125. [[CrossRef](#)]
- Smith, C.R. Apparatus and Method for Removing Compounds from a Solution. U.S. Patent 5480665, 2 January 1996.
- Belisario-Sánchez, Y.Y.; Taboada-Rodríguez, A.; Marín-Iniesta, F.; López-Gómez, A. Dealcoholized wines by spinning cone column distillation: Phenolic compounds and antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl method. *J. Agric. Food Chem.* **2009**, *57*, 6770–6778. [[CrossRef](#)] [[PubMed](#)]
- Wollan, D. Alcohol Reduction in Beverages. U.S. Patent 20080272041, 6 November 2008.
- Saha, B.; Torley, P.; Blackmann, J.W.; Schmidtke, L.M. Review of processing technology to reduce alcohol levels in wines. In Proceedings of the 1st Oenoviti International Symposium—Alcohol Level Reduction in Wine, Bordeaux, France, 6 September 2013; VIGNE et vin Publications Internationales: Bordeaux, France, 2013; pp. 78–86.
- Bui, K.; Dick, R.; Moulin, G.; Galzy, P. A reverse osmosis for the production of low ethanol content wine. *Am. J. Enol. Vitic.* **1986**, *37*, 297–300.
- Diban, N.; Athes, V.; Bes, M.; Souchon, I. Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. *J. Memb. Sci.* **2008**, *311*, 136–146. [[CrossRef](#)]
- Massot, A.; Mietton-Peuchot, M.; Peuchot, C.; Milisic, V. Nanofiltration and reverse osmosis in winemaking. *Desalination* **2008**, *231*, 283–289. [[CrossRef](#)]
- Pickering, G.J.; Heatherbell, D.; Vanhanen, L.; Barnes, M. The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. *Am. J. Enol. Vitic.* **1998**, *49*, 306–318.
- Gambuti, A.; Rinaldi, A.; Lisanti, M.T.; Pessina, R.; Moio, L. Partial dealcoholisation of red wines by membrane contactor technique: Influence on colour, phenolic compounds and saline precipitation index. *Eur. Food Res. Technol.* **2011**, *233*, 647–655. [[CrossRef](#)]

19. Meillon, S.; Urbano, C.; Schlich, P. Contribution of the temporal dominance of sensations (TDS) method to the sensory description of subtle differences in partially dealcoholized red wines. *Food Qual. Pref.* **2009**, *20*, 490–499. [\[CrossRef\]](#)
20. Gonzalez-Manzano, S.; Dueñas, M.; Rivasgonzalo, J.; Escribano-Bailón, M.; Santos-Buelga, C. Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. *Food Chem.* **2009**, *114*, 649–656. [\[CrossRef\]](#)
21. Gonçalves, F.; Ribeiro, R.; Neves, L.; Lemperle, T.; Lança, M.; Ricardo da Silva, J.; Laureano, O. Alcohol reduction in wine by nanofiltration. Some comparisons with reverse osmosis technique. In Proceedings of the 1st Oenoviti International Symposium—Alcohol Level Reduction in Wine, Bordeaux, France, 6 September 2013; VIGNE et vin Publications Internationales: Bordeaux, France, 2013; pp. 64–67.
22. Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.* **2001**, *52*, 67–87.
23. Somers, T.C.; Evans, M.E. Grape pigment phenomena: Interpretation of major colour losses during vinification. *J. Sci. Food Agric.* **1979**, *30*, 623–633. [\[CrossRef\]](#)
24. Villamor, R.R.; Ross, C.F. Wine matrix compounds affect perception of wine aromas. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 1–20. [\[CrossRef\]](#)
25. Ramey, D.D.; Ough, C.S. Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agric. Food Chem.* **1980**, *28*, 928–934. [\[CrossRef\]](#)
26. Makhotkina, O.; Kilmartin, P.A. Hydrolysis and formation of volatile esters in New Zealand Sauvignon blanc wine. *Food Chem.* **2012**, *135*, 486–493. [\[CrossRef\]](#)
27. Euranto, E.K. Esterification and ester hydrolysis. In *Carboxylic Acids and Esters (1969)*; John Wiley & Sons Ltd.: Chichester, UK, 1969; pp. 505–588.
28. Fan, Y.; Xie, Z.; Zhang, H.; Qian, J. Kinetic resolution of both 1-phenylethanol enantiomers produced by hydrolysis of 1-phenylethyl acetate with *Candida antarctica* lipase B in different solvent systems. *Kinet. Catal.* **2011**, *52*, 686–690. [\[CrossRef\]](#)
29. Marais, J. The effect of pH on esters and quality of Colombard wine during maturation. *Vitis* **1978**, *17*, 396–403.
30. Edwards, T.L.; Singleton, V.L.; Boulton, R. Formation of ethyl esters of tartaric acid during wine aging: Chemical and sensory effects. *Am. J. Enol. Vitic.* **1985**, *36*, 118–124.
31. Lisanti, M.T.; Gambuti, A.; Genovese, A.; Piombino, P.; Moio, L. Partial dealcoholization of red wines by membrane contactor technique: Effect on sensory characteristics and volatile composition. *Food Bioprocess Technol.* **2013**, *6*, 2289–2305. [\[CrossRef\]](#)
32. Saha, B.; Longo, R.; Torley, P.; Saliba, A.; Schmidtke, L. SPME method optimized by Box-Behnken design for impact odorants in reduced alcohol wines. *Foods* **2018**, *7*, 127. [\[CrossRef\]](#)
33. Gawel, R.; Sluyter, S.V.; Waters, E.J. The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. *Aust. J. Grape Wine Res.* **2007**, *13*, 38–45. [\[CrossRef\]](#)
34. Rolle, L.; Englezos, V.; Torchio, F.; Cravero, F.; Río Segade, S.; Rantsiou, K.; Giacosa, S.; Gambuti, A.; Gerbi, V.; Cocolin, L. Alcohol reduction in red wines by technological and microbiological approaches: A comparative study. *Aust. J. Grape Wine Res.* **2017**, *24*, 62–74. [\[CrossRef\]](#)
35. Longo, R.; Blackman, J.W.; Torley, P.J.; Rogiers, S.Y.; Schmidtke, L.M. Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *J. Sci. Food Agric.* **2016**, *97*, 8–16. [\[CrossRef\]](#)
36. Siebert, T.E.; Smyth, H.E.; Capone, D.L.; Neuwohner, C.; Pardon, K.H.; Skouroumounis, G.K.; Herderich, M.J.; Sefton, M.A.; Pollnitz, A.P. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Anal. Bioanal. Chem.* **2005**, *381*, 937–947. [\[CrossRef\]](#)
37. Frayne, R.F. Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. *Am. J. Enol. Vitic.* **1986**, *37*, 281–287.
38. Goldner, M.C.; Zamora, M.C. Effect of polyphenol concentrations on astringency perception and its correlation with gelatin index of red wine. *J. Sens. Stud.* **2010**, *25*, 761–777. [\[CrossRef\]](#)
39. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* **2017**, *82*, 1–26. [\[CrossRef\]](#)

40. Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [[CrossRef](#)]

Sample Availability: Samples of the compounds are not available from the authors.



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

**Table S1.** Deuterated internal standards used for determination of fermentation volatiles by GC-MS.

Analyte	RT (min)	Ions ¹ (<i>m/z</i>)	Internal standard
ethyl acetate	6.783	61 , 70, 88	d ₈ -ethyl acetate
ethyl propanoate	8.492	75, 80, 102	d ₅ -ethyl propanoate
ethyl 2-methylpropanoate	8.712	88, 101, 116	d ₅ -ethyl 2-methyl propanoate
2-methylpropyl acetate	10.107	56 , 73, 86	d ₉ -2-methylpropyl acetate
ethyl butanoate	10.870	71, 88, 101	d ₅ -ethyl butanoate
ethyl 2-methylbutanoate	11.375	85, 102 , 115	d ₅ -ethyl 2-methyl butanoate
ethyl 3-methylbutanoate	11.872	85, 88 , 115	d ₅ -ethyl 2-methyl butanoate
2-methyl-1-propanol	12.781	41, 43 , 74	d ₉ -2-methyl-1-propanol
2-methylbutyl acetate	13.681	57, 72, 74	d ₉ -3-methylbutyl acetate
3-methylbutyl acetate	13.718	55, 87, 88	d ₉ -3-methylbutyl acetate
1-butanol	14.473	41, 44, 56	d ₁₀ -1-butanol
2-methyl-1-butanol	16.522	41, 56, 70	d ₉ -3-methyl-1-butanol
3-methyl-1-butanol	16.522	55, 60, 70	d ₉ -3-methyl-1-butanol
ethyl hexanoate	17.419	88 , 99, 115	d ₅ -ethyl hexanoate
hexyl acetate	18.692	69, 73, 84	d ₁₃ -hexyl acetate
hexanol	21.018	55, 56 , 69	d ₁₃ -hexanol
ethyl octanoate	23.441	88, 101 , 127	d ₅ -ethyl octanoate
acetic acid	24.058	43, 60	d ₃ -acetic acid
propanoic acid	26.283	57, 73, 74	d ₅ -propanoic acid
2-methylpropanoic acid	26.979	43, 73 , 88	d ₇ -2-methylpropanoic acid
butanoic acid	28.553	45, 60 , 73	d ₇ -butanoic acid
ethyl decanoate	28.811	101, 157, 200	d ₅ -ethyl decanoate
3-methylbutanoic acid	29.686	60 , 61	d ₇ -3-methylbutanoic acid
2-methylbutanoic acid	29.704	57, 74	d ₇ -3-methylbutanoic acid
2-phenylethyl acetate	33.010	65, 91, 104	d ₃ -2-phenylethyl acetate
hexanoic acid	33.435	60 , 73, 87	d ₁₁ -hexanoic acid
2-phenylethanol	34.667	91 , 92, 122	d ₃ -2-phenylethanol
octanoic acid	36.641	60, 85, 115	d ₁₅ -octanoic acid
decanoic acid	39.304	73, 129 , 172	d ₁₉ -decanoic acid

¹ The bolded ion was used for quantification.

© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Statement of Authorship

Title of Paper	Investigating Alcohol Sweetspot Phenomena in Reduced Alcohol Red Wines
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Duc-Truc Pham, Vanessa J. Stockdale, David W. Jeffery, Jonathan Tuke and Kerry L. Wilkinson. Foods, 2019, 8, 491; doi:10.3390/foods8100491.

Principal Author

Name of Principal Author (Candidate)	Duc-Truc Pham		
Contribution to the Paper	Designed and performed experiments; conducted chemical and sensory analysis of wines; performed statistical analyses of data (using ANOVA, chi-squared goodness of fit and one proportion tests); interpreted the data; and drafted and revised the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this		
Signature		Date	26-6-2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Vanessa J. Stockdale		
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design and data interpretation; and edited the manuscript.		
Signature		Date	26-6-2020

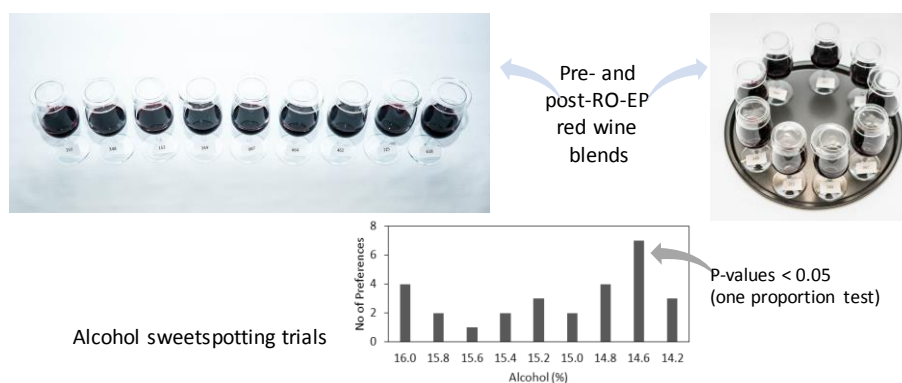
Name of Co-Author	David W. Jeffery		
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design and data interpretation; <u>and edited the manuscript.</u>		
Signature		Date	26-6-2020

Name of Co-Author	Jonathan Tuke		
Contribution to the Paper	Contributed to statistical analysis and interpretation of data; and edited the manuscript.		
Signature		Date	26-6-2020

Name of Co-Author	Kerry L. Wilkinson		
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design, and data analysis and interpretation; edited and revised the manuscript, and acted as the corresponding author.		
Signature		Date	26-6-2020

Chapter 3.

Investigating Alcohol Sweetspot Phenomena in Reduced Alcohol Red Wines



Duc-Truc Pham, Vanessa J. Stockdale, David W. Jeffery, Jonathan Tuke and Kerry L. Wilkinson. *Foods* 2019, 8, 491; doi:10.3390/foods8100491

Article

Investigating Alcohol Sweetspot Phenomena in Reduced Alcohol Red Wines

Duc-Truc Pham ^{1,2} , Vanessa J. Stockdale ^{2,3}, David W. Jeffery ^{1,2} , Jonathan Tuke ⁴ and Kerry L. Wilkinson ^{1,2,*} 

¹ School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond 5064, Australia; duc-truc.pham@adelaide.edu.au (D.-T.P.); david.jeffery@adelaide.edu.au (D.W.J.)

² The Australian Research Council Training Centre for Innovative Wine Production, PMB 1, Glen Osmond 5064, Australia; Vanessa.Stockdale@tweglobal.com

³ Treasury Wine Estates, 97 Sturt Highway, Nuriootpa 5352, Australia

⁴ School of Mathematical Sciences, The University of Adelaide, Adelaide 5000, Australia; simon.tuke@adelaide.edu.au

* Correspondence: kerry.wilkinson@adelaide.edu.au; Tel.: +61-8-8313-7360

Received: 11 September 2019; Accepted: 11 October 2019; Published: 14 October 2019



Abstract: Warmer growing seasons, variations to grape ripening dynamics, and stylistic changes have contributed to increased wine alcohol levels, which can negatively impact sensory properties. As a consequence, winemakers have sought technological innovations to produce reduced alcohol wine (RAW). The sensory methodology used by industry to optimize the ethanol content of RAW is known as ‘alcohol sweetspotting’. However, to date, there is no scientific evidence to support the alcohol sweetspot phenomenon, and the sensory methodology used for alcohol sweetspotting has not been validated. In this study, different methods of presenting wine samples (i.e., ordered vs. randomized, and linear vs. circular) were employed to determine to what extent presentation order influences the outcome of alcohol sweetspotting trials. Two different approaches to statistical analysis of sensory data, i.e., chi-square goodness of fit vs. one proportion tests, were also evaluated. Statistical analyses confirmed alcohol sweetspots were apparent in some sweetspot determination trials, but outcomes were not reproducible in replicate determinations (either by panel or by individual panelists). Analysis of data using the one proportion test improved the likelihood of identifying statistically significant differences between RAWs, but variation in individuals’ sensitivity to differences in sensory properties following ethanol removal prevented validation of the alcohol sweetspot phenomenon based on the wines studied.

Keywords: alcohol adjustment; evaporative perstraction; one proportion test; partial dealcoholization; reverse osmosis; sensory analysis

1. Introduction

The alcohol content of wine has steadily increased in recent years [1], in part due to the warmer temperatures experienced during the growing season, as a consequence of climate change. This has financial implications for winemakers, not only because wines with higher alcohol levels attract higher import duties and taxes, but because wine producers and consumers alike, are increasingly mindful of the health and social issues associated with excessive alcohol consumption [2]. The wine industry is therefore adopting various strategies for correcting the alcohol content of wine, including the partial dealcoholization of wine using reverse osmosis-evaporative perstraction (RO-EP) [3]. This process employs membrane technologies to fractionate wine and remove ethanol from the resulting permeate,

before the (partially) dealcoholized permeate is recombined with the retentate, to give reduced alcohol wine (RAW). Partial dealcoholization of wine has also been achieved using pervaporation and spinning cone column distillation technologies [4,5]. It is still unclear, however, how to determine optimal ethanol levels for RAW from a sensory perspective.

Some wine producers employ a process referred to as ‘alcohol sweetspotting’ to optimize wine alcohol levels [6]. This involves partial dealcoholization of wine (by RO-EP) and subsequent blending of the base wine and RAW to generate a series of wines (typically 8–10) comprising alcohol levels that differ by small, incremental amounts (e.g., 0.2% alcohol by volume (abv)). The blends are then evaluated (typically in ascending or descending order of ethanol concentration) by three or four winemakers, who identify the wine (or wines) they considered to exhibit superior organoleptic properties (i.e., optimal aroma, flavor, taste, mouthfeel and balance). To date, however, the existence of an alcohol sweetspot phenomenon has not been scientifically validated.

The impacts of ethanol on wine sensory properties tend to support the concept of an alcohol sweetspot. Depending on its concentration, ethanol can affect the perception of taste and mouthfeel properties by enhancing or suppressing sweetness, sourness, bitterness, saltiness, astringency and hotness [7–10]. Moreover, ethanol can enhance wine aroma by masking undesirable attributes and/or harmonising imbalances, but the perception of desirable aromas and flavors can also be diminished by ethanol [11,12]. At lower levels, i.e., 5–12% abv, ethanol facilitates the release of volatile compounds under dynamic conditions and maintains their headspace concentrations to enhance wine aroma [13]. Conversely, higher ethanol levels, i.e., 10–18% abv, are negatively correlated with volatile headspace concentrations, reflecting the increased solubility of volatiles in ethanol, relative to water [14].

Several previous studies have attempted to validate the alcohol sweetspot phenomenon. A 2013 study investigated alcohol sweetspots in three white wines and a red wine, using an expert panel [15]. The ethanol content of the wines evaluated via ranking and triangle tests ranged by 3% abv, spaced at 0.5% intervals, e.g., 12.1, 12.6, 13.1, 13.6, 14.1, 14.6 and 15.1% abv for a bracket of Riesling wines. Panelists ranked wines according to their individual preferences, with the most preferred wine being ranked 1 and the least preferred wine ranked 7. Each bracket was evaluated in triplicate, using three different orders of presentation: increasing ethanol content; decreasing ethanol content; and a randomized presentation order. Although there were no clear preferences for wines based on alcohol content, wines with ethanol levels above 15% abv were consistently ranked lower than other samples. It is not clear to what extent the spacing between wine ethanol levels (i.e., 0.5%) may have influenced the identification of any alcohol sweetspots.

In a subsequent study, consumers evaluated subsets of Chardonnay wine comprising samples for which the ethanol content had been adjusted to span a concentration range of approximately 1% abv (at 0.2% increments), using a wine that was partially dealcoholized by spinning cone column distillation [16]. Consumers were randomly allocated to wine subsets and asked to rate their liking of samples using a 9-point hedonic category scale, but only one consumer group yielded results (i.e., a positive quadratic curve) that suggested the existence of an alcohol sweetspot (being 13.8 to 14.0% abv) for their subset of wines. Consistent liking scores were not obtained for other wine subsets and therefore did not enable identification of a sweetspot for that wine.

The alcohol sweetspotting process is likely affected by a combination of factors, including the order in which samples are presented, the incremental difference in alcohol levels between samples, and the composition of the sensory panel (i.e., consumers vs. winemakers/experts). Standardized sensory methodology typically requires randomized presentation of samples, whereas industry based sweetspotting trials usually present samples in sequential order (i.e., increasing or decreasing ethanol concentration [5]). Randomization of samples may confound the perception of subtle differences between samples of similar alcohol content, whereas sequential presentation of samples may introduce bias (i.e., away from the samples of highest and/or lowest alcohol content).

This study aimed to investigate to what extent different methods of presenting samples (i.e., ordered vs. randomized, and linear vs. circular) might influence the outcome of alcohol sweetspotting trials.

These approaches were intended to evaluate the ability of panelists to choose the same sample in replicate brackets, as well as to investigate whether the order in which samples were assessed influenced the sensory perceptions of wines. Different approaches to statistical analysis of data from sweetspotting trials were also evaluated, i.e., chi-square tests for ‘goodness of fit’ vs. ‘one proportion’, in an attempt to further provide scientific evidence of an alcohol sweetspot.

2. Materials and Methods

2.1. Wine Samples

Three 2015 Barossa Valley red wines, a Shiraz Cabernet Sauvignon blend (60:40) and two Shiraz wines, hereafter wines A, B and C, respectively, were sourced from a commercial winery who had deemed the wines to be in need of alcohol adjustment. The wines were partially dealcoholized using an industrial scale RO-EP unit (VA Filtration, Nuriootpa, Australia), in accordance with manufacturer operating instructions [3]; their alcohol concentrations before and after RO-EP treatment were: 16.0 and 14.4% abv for wine and RAW A; 16.0 and 14.2% abv for wine and RAW B; and 16.3 and 14.0% abv for wine and RAW C.

Wines A and B were subsequently blended with different proportions of their corresponding RAW to generate a series of samples with ethanol concentrations that differed by 0.2% abv, for use in alcohol sweetspotting trials, according to the practices typically employed by the winery. Trial 1 comprised nine blends of wine A and RAW A, with alcohol percentage levels: 16.0 (wine A), 15.8, 15.6, 15.4, 15.2, 15.0, 14.8, 14.6, and 14.4 (RAW A); while Trial 2 comprised nine blends of wine B and RAW B, with alcohol percentage levels: 16.0 (wine B), 15.8, 15.6, 15.4, 15.2, 15.0, 14.8, 14.6, and 14.2 (RAW B). Wine C was blended with its corresponding RAW to achieve samples which differed in ethanol concentration by 0.2, 0.5 and 1.0% abv from both wine C (i.e., 16.1, 15.8 and 15.3% abv) and RAW C (i.e., 14.2, 14.5 and 15.0% abv), for use in difference tests.

Wines, RAWs and blends thereof were subsequently bottled (under screw cap) in 750 mL glass bottles and cellared (in darkness at 15 °C) prior to chemical and sensory analyses (performed approximately 1 month after RO-EP treatment, blending and bottling).

2.2. Sensory Analysis of Wines

For each of the sensory analyses performed, i.e., alcohol sweetspotting trials and difference tests: samples (30 mL) were served at ambient temperature (i.e., 22–24 °C), in covered, three-digit coded XL5 wine glasses (International Organization for Standardization, ISO 3591:1977), under LED white lighting. Alcohol sweetspotting trials were held in an open-plan sensory laboratory (with each panelist on a separate bench) at a commercial winery in the Barossa Valley wine region. Difference tests were held in a sensory laboratory at the University of Adelaide’s Waite Campus. Sensory trials were approved by the Human Research Ethics Committee of the University of Adelaide (H-2015-094).

2.2.1. Alcohol Sweetspotting Trials

Expert panels, each comprising 14 winemakers, were assembled for alcohol sweetspotting trials. Prior to evaluation, each panelist completed a short survey comprising demographic questions. For each trial, a higher proportion of panelists were male (64–79%) and aged between 31 and 50 years (72–85%), but the majority had more than 10 years industry experience, all had wine judging experience, and 79% had previously undertaken alcohol sweetspotting trials (Table S1).

Panelists were presented with brackets of nine samples at a time (comprising wine, RAW and blends thereof), using four different orders of presentation: (i) samples presented in a linear format, in random order, hereafter ‘row, randomized’; (ii) samples presented in a linear format, with alcohol content decreasing from left to right, hereafter referred to as ‘row, ordered’; (iii) samples presented in a circular format (i.e., evenly spaced around a circular tray), in random order, hereafter ‘circular,

randomized'; and (iv) samples presented in a circular format with alcohol content increasing in a clockwise direction, hereafter referred to as 'circular, ordered' (Figure 1).



Figure 1. (a) Linear and (b) circular presentation formats used in alcohol sweetspotting trials.

Panelists were instructed to evaluate samples presented in rows from left to right, whereas for samples presented in a circle, panelists were instructed to evaluate samples in a clockwise direction, starting with any sample. For each trial, panelists evaluated eight brackets of samples in total, i.e., four presentation orders in duplicate, with brackets also being presented in a randomized order. Short breaks (2 min) were enforced between brackets, with longer breaks (30–60 min) enforced after the first four brackets. In Trial 1, panelists were asked to identify one or more samples from each bracket that they considered exhibited superior overall sensory properties; but in Trial 2, the panel was asked to identify only one sample. Trials 1 and 2 were held on different days.

2.2.2. Difference Tests

A series of triangle tests [17] were conducted with a panel of 18 assessors comprising wine science staff and students from the University of Adelaide (nine males and nine females, aged between 18 and 55 years) to determine the change in ethanol concentration that resulted in perceptible differences in sensory properties for wine C and RAW C (using blends thereof, prepared as outlined above). Samples were presented in a balanced, randomized presentation order comprising all possible configurations (i.e., XXY , XYX , YXX , YYX , YXY and XYX , where X denotes wine C or RAW C, and Y denotes blends that differed in ethanol concentration by 0.2, 0.5 and 1.0% abv), an equal number of times. Panelists were asked to smell and taste the samples presented in each bracket, and to identify the sample that was different. Sensory data were collected and processed using RedJade online software (RedJade, Redwood Shores, CA, USA).

2.3. Chemical Analysis of Wines

The alcohol content, density, pH, titratable acidity (TA, as g/L of tartaric acid equivalents, to an endpoint of pH 8.2) and volatile acidity (VA, as g/L of acetic acid equivalents) were determined (in duplicate) by the Australian Wine Research Institute's (AWRI) Commercial Services laboratory (Adelaide, Australia), using a Foss WineScan analyzer (Mulgrave, Australia). Glucose, fructose, glycerol and organic acids were determined (in duplicate) by high-performance liquid chromatography (HPLC), as described previously [18]. Briefly, an Agilent 1100 series HPLC (Agilent Technologies, Forest Hill, Australia), fitted with diode array and refractive index detectors was used, with separation achieved using an Aminex HPX-87H cation exchange column (Bio-Rad Laboratories, Gladesville, NSW, Australia) and 2.5 mM sulfuric acid as the mobile phase. Calibration curves relating concentrations to optical density or refractive index were fitted using ChemStation software (Agilent Technologies). Wine color measurements, including CIELab, were performed (in duplicate) using a Cintra 4040 spectrophotometer (GBC Scientific Equipment, Melbourne, Australia). Samples were filtered through 0.45 μm filters (Acrodisc, Sigma-Aldrich, Castle Hill, Australia) after which absorbance was recorded at 420, 520 and 800 nm. Wine color density and hue were calculated as: color density (au) = $(A_{520} - A_{800}) + (A_{420} - A_{800})$ and hue = $(A_{420} - A_{800}) / (A_{520} - A_{800})$ [19]. CIELab measurements determined L^* ,

a^* and b^* , being coordinate values corresponding to the degree of lightness, and the intensity of red (when $a^* > 0$), green (when $a^* < 0$), yellow (when $b^* > 0$) and blue (when $b^* < 0$) hues [19].

The concentrations of several fermentation volatiles (acids, alcohols and esters) were determined (without replication, i.e., $n = 1$) by Metabolomics Australia (AWRI) using an Agilent 7890A gas chromatograph, equipped with a Gerstel MPS2 multipurpose autosampler and coupled to an Agilent 5875C mass selective detector, and previously reported stable isotope dilution analysis (SIDA) methods [20]. Headspace solid phase micro-extraction (HS-SPME) sampling of diluted wine (1 in 10 dilution in water) was performed in a 20 mL vial containing 2 g of sodium chloride, with the SPME fiber being exposed to the headspace for 10 min prior to desorption. Separation was achieved using a Phenomenex ZB-Wax column (60 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness) and helium as the carrier gas (2.0 mL/min in constant flow mode). Preparation of isotopically labelled internal standards, method validation and instrument operating conditions are described extensively in the aforementioned publication [20].

2.4. Statistical Analysis

Basic compositional data were analyzed by one-way analysis of variance (ANOVA) using GenStat (15th Edition, VSN International Limited, Herts, England, UK). Mean comparisons were performed by a least significant difference (LSD) multiple comparison test at $\alpha < 0.05$. Volatile data were analyzed via an ANOVA F -test using the lmerTest package in R statistical software (www.R-project.org/) [21]. Mixed effect linear models were fitted individually for each volatile, with the response variable being the concentration at each treatment level. A fixed effect predictor was included for treatment, together with a random intercept for wine, to account for the repeated measures on each wine. The fitting was performed using the lme4 package in R [22]. Two statistical analyses were employed using XLSTAT (version 2015.4.1, Addinsoft, NY, USA): (i) a chi-square goodness of fit test, to determine statistical significance of the observed distribution of sample preferences vs. random choice; and (ii) a one sample proportion test, to determine statistical significance between the preferred sample, i.e., the alcohol sweetspot, vs. random choice.

3. Results and Discussion

3.1. Influence of Partial Dealcoholization by Ro-Ep on Wine Composition

Basic compositional parameters, i.e., alcohol, residual sugar, density, glycerol, pH, TA, VA, organic acids and color, for wines A and B (and their corresponding RAWs) are reported in Table 1. Decreases in alcohol content of 1.6 and 1.8% abv were achieved by RO-EP treatment of wines A and B, respectively. Statistically significant increases in residual sugar levels were observed, but these were not considered meaningful given both wines were dry, i.e., contained < 1 g/L of sugar. Similarly, slight increases in density and glycerol were not considered to be meaningful. In the case of glycerol, concentration increases might be explained by its molecular weight (180 atomic mass units, amu), being close to the molecular weight cut-off of the membrane (180–220 amu), such that glycerol was retained during dealcoholization and concentrated with ethanol removal [3]. Anecdotal evidence suggests that for commercial scale dealcoholization (i.e., at the 20–30 kL scale employed in the current study), for every 1000 L of ethanol removed, wine volume decreases by 900 L due to the mixing phenomena of ethanol and water. There were no significant changes in pH, TA or VA following dealcoholization, and with the exception of lactic acid, which increased in concentration by 26% due to RO-EP treatment of wine B, there were no significant changes in organic acid levels, in agreement with previous studies that suggest wine is a well-buffered system [23,24].

Table 1. Composition of wines A and B before and after partial dealcoholization.

Parameter	Wine A	RAW A	<i>p</i>	Wine B	RAW B	<i>p</i>
alcohol (% abv)	16.0 a	14.4 b	<0.01	16.0 a	14.2 b	<0.01
residual sugar (g/L)	0.56 a	0.68 b	<0.01	0.5 a	1.1 b	<0.01
density (g/mL)	0.993	0.995	ns	0.993 a	0.996 a	<0.01
glycerol (g/L)	10.7	11.1	ns	10.9 b	11.6 a	0.01
pH	3.7	3.7	–	3.6	3.6	–
TA (g/L)	6.8	6.8	–	6.8	6.9	–
VA (g/L)	0.7	0.8	–	0.7	0.7	–
succinic acid (g/L)	1.4	1.5	ns	1.5	1.5	ns
lactic acid (g/L)	1.9	2.0	ns	1.9 b	2.4 a	0.01
malic acid (g/L)	0.20	0.23	ns	0.15	0.10	ns
tartaric acid (g/L)	2.6	2.8	ns	2.8	2.7	ns
wine color density (au)	12.4 a	12.6 b	<0.01	15.4	15.4	ns
wine hue	0.7	0.7	–	0.7	0.7	–
L*	62.3 a	61.8 b	<0.01	55.7	55.7	ns
a*	35.5 a	35.9 b	<0.01	41.4	41.4	ns
b*	2.77 a	2.89 b	0.05	4.6	4.5	ns

Values are means of duplicate measurements ($n = 2$). Standard errors were $\leq 10\%$. Values followed by different letters within rows (for each wine) are statistically significant ($p = 0.05$, one-way ANOVA); ns = not significant. RAW = reduced alcohol wine; au = absorbance units.

Wine color intensity increased in RAW A compared to wine A, while L* (lightness) decreased, indicating color became darker, and a* and b* increased, indicating intensification of red and yellow hues. Significant differences in color were not observed between wine B and its corresponding RAW. According to the literature, changes in color (ΔE^*ab) ≥ 3.0 can be readily distinguished [25]. In the current study, ΔE^*ab values were 0.7 and 0.2 for wine/RAW A and B respectively, meaning color changes would not be visually perceptible. Nevertheless, color changes could be explained by a combination of increased anthocyanin concentrations and the formation of copigmented/polymeric anthocyanin complexes, as a consequence of wine concentration with ethanol removal. Previous research has shown that 2% ethanol removal from wine by nanofiltration or reverse osmosis resulted in increases in color intensity of 5.5 and 11%, respectively [26].

In terms of aroma and flavor, partial dealcoholization of wine by RO-EP can affect the concentration of volatile compounds in three ways: i) volatiles can be lost during processing as a result of their passage into the waste stream; ii) the concentration of volatiles can increase in the headspace of wine as ethanol removal affects their solubility and volatility (i.e., ethanol acts essentially as a solvent); and iii) changes in ethanol levels may positively or negatively influence the concentration of volatiles that exist in equilibrium with ethanol [3,27,28], e.g., alcohols, acids and esters. In the current study, ethyl and acetate ester concentrations typically decreased with dealcoholization, in some cases by as much as 50–80% (Table 2); albeit 2-phenylethyl acetate levels increased substantially with dealcoholization of wine A. In contrast, 2-phenylethanol concentrations increased slightly, whereas 1-hexanol levels increased following RO-EP treatment of wine A, but decreased in wine B. Whereas RO-EP treatment was only found to have significantly impacted the concentrations of ethyl hexanoate, ethyl 2-methylpropanoate and ethyl 3-methylbutanoate, this likely reflects the limited sample size (i.e., $n = 2$) available for statistical analysis. Nevertheless, where dealcoholization results in sub-threshold concentrations of volatiles (derived from either grapes or fermentation), this may diminish the perception of wine aroma and flavor.

Table 2. Volatile composition of wines A and B before and after partial dealcoholization.

Volatile Compound	Descriptors	Threshold	Wine A	RAW A	Wine B	RAW B	<i>p</i>	SD
ethyl propanoate	fruity	1840	170	75	251	88	ns	3
ethyl butanoate	acid fruit	20	94	21	136	32	ns	1
ethyl hexanoate	green apple	5	11	3	13	5	<0.01	6
ethyl octanoate	sweet, soap	2	7	3	8	6	ns	3
ethyl decanoate	soap	200	2	1	2	2	ns	5
1-hexanol	green, grass	4000	1950	2265	2132	2096	ns	10
2-phenylethanol	roses	10	381	399	376	399	ns	0.7
ethyl 2-methylpropanoate	fruity	15	115	14	95	40	<0.05	4
ethyl 2-methylbutanoate	sweet fruit	1	13	2	14	5	ns	1
ethyl 3-methylbutanoate	berry	3	20	4	22	8	<0.05	0.3
2-methylpropyl acetate	banana, fruity	1600	27	5	38	11	ns	2
3-methylbutyl acetate	banana	30	255	110	723	136	ns	20
2-methylbutyl acetate	banana, fruity	1600	93	35	231	46	ns	5
2-phenylethyl acetate	floral	250	247	739	698	439	ns	1
hexyl acetate	sweet, perfume	670	1	1	7	1	ns	0.1

Concentrations are µg/L, except for 2-phenylethanol which was mg/L. Aroma descriptors and thresholds obtained from the literature [29]. Standard deviation (SD) based on calibration as analyses were not replicated (i.e., $n = 1$). *p* values were calculated from an ANOVA of the likelihood ratio test of a treatment effect ($p = 0.05$).

3.2. Influence of Presentation Order on the Outcomes of Alcohol Sweetspotting Trials

In sensory analysis, it is usual for samples to be presented in a randomized order, so as to minimize bias that might favor certain outcomes over others [17]. It has been suggested that the methodological approach used by industry during alcohol sweetspotting trials, i.e., the evaluation of samples in ascending or descending order of ethanol concentration, might influence panelists' decision-making [6]. For example, even where the alcohol content of samples is not disclosed, panelists who are familiar with the evaluation of samples comprising incremental changes in ethanol content may tend to avoid selection of sample extremities, i.e., the 'end' samples with the lowest and highest ethanol levels, potentially biasing their alcohol sweetspot determination. A key aim of this study was therefore to evaluate to what extent the way in which samples are presented (i.e., in linear (row) vs. circular and randomized vs. ordered formats) might influence the outcome of alcohol sweetspotting trials. The ease with which individual panelists, as well as the panel as a whole, could reproduce sweetspot determinations (using the four presentation formats) was also evaluated. Samples presented in a linear format were tasted from left to right (being from highest to lowest alcohol content), and back again, because the panel (predominantly comprising winemakers with alcohol sweetspotting experience) indicated they felt this approach facilitated the perception of changes within brackets of samples, particularly for palate attributes such as acidity, hotness and astringency. Samples presented in a circular format were tasted in a clockwise direction (starting with a randomly selected sample), and back again.

In Trial 1, panelists were permitted to identify one or more samples they considered to exhibit superior overall sensory qualities. The 15.4% blend of wine/RAW A from the row, randomized format was the only apparent sweetspot (Figure 2), with both chi-square goodness of fit and one proportion tests confirming sample preference within this bracket was statistically significant (Table 3). However, this sample was not consistently identified as a sweetspot. Wine A and its 15.6% blend were chosen more frequently than other samples when a circular, randomized format was used for evaluation (Figure 2c), whereas for samples evaluated according to alcohol content (i.e., an ordered format), panelist preferences were distributed across each bracket (i.e., with 4 to 7 preferences given to 7 of 9 samples, Figure 2b,d). Statistical analyses confirmed there were no significant differences amongst these brackets (Table 3). When the outcomes of sweetspotting trials were compared as individual brackets (Figure S1), it can be seen that the 15.4% blend of wine/RAW A was only identified as a sweetspot in one bracket replicate (Figure S1a). Furthermore, when the performance of individual panelists was considered (Table S2), only 16 of the 56 trial 1 sweetspot determinations yielded the same preferences. This suggests panelists found it difficult to identify samples that clearly exhibited

superior overall sensory properties, either individually or collectively, and irrespective of presentation format. Disagreement within a panel (i.e., between panelists) might be explained by differences in winemakers' perceptions of what constitutes 'superior sensory properties' (i.e., stylistic preferences), but disagreement in individual panelist's determinations suggests differences in wine sensory properties as a consequence of compositional changes achieved by partial dealcoholization (Tables 1 and 2), but ethanol removal in particular, could not be reproducibly discriminated via alcohol sweetspotting.

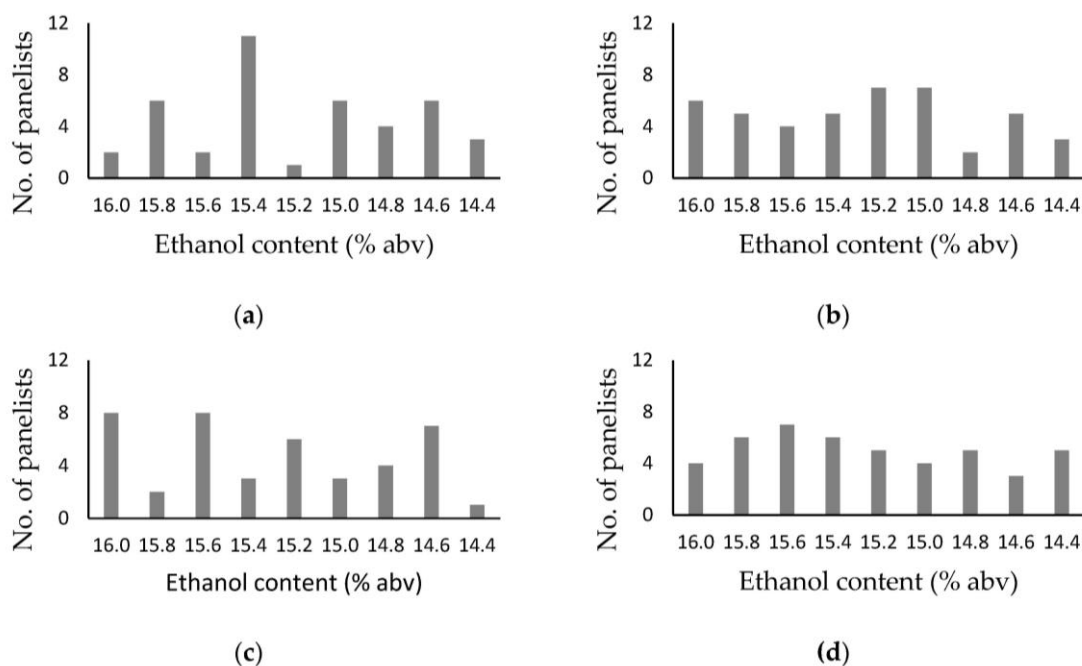


Figure 2. Histograms showing sample preferences from alcohol sweetspotting trials (performed in duplicate, with an expert panel of 14 winemakers) with samples comprising wine A, RAW A and blends thereof, spanning ethanol levels from 14.4 to 16.0% abv, using: (a) row, randomized, (b) row, ordered, (c) circular, randomized and (d) circular ordered, presentation formats.

Table 3. *p* values calculated for chi-square goodness of fit and one proportion tests of preference data from alcohol sweetspotting trials; the proportion of preferred samples are shown in brackets.

Presentation Order	Goodness of Fit Test		One Proportion Test	
	Trial 1	Trial 2	Trial 1	Trial 2
row, randomized	0.033	0.227	0.004 (11/41)	0.083 (6/28)
row, ordered	0.791	0.270	0.212 (7/44)	0.030 (7/28)
circular, randomized	0.966	0.434	0.089 (8/42)	0.194 (5/28)
circular, ordered	0.151	0.639	0.229 (7/45)	0.030 (7/28)

In Trial 2, panelists were only permitted to identify one sample, i.e., the sample considered to exhibit superior overall sensory qualities. Chi-square goodness of fit tests indicated that irrespective of presentation format, the distribution of sample preferences observed for wine B, RAW B, and blends thereof (Figure 3) were not statistically significant (Table 3). However, the 15.2 and 14.6% blends of wine/RAW B from the row, ordered and circular, ordered formats respectively (Figure 3b,d), each received 7/28 preferences, which one proportion tests deemed statistically significant ($p = 0.030$, Table 3). Again, the outcomes of sweetspotting trials were not consistent across individual brackets (Figure S2); only RAW B and the 14.6% blend of wine/RAW B received statistically significant numbers of preference (being 5/14, $p = 0.014$), in one circular, randomized bracket and one circular, ordered bracket, respectively (Figure S2f,h). When the performance of individual panelists was considered

(Table S3), only 7 of the 56 trial 2 sweetspot determinations yielded the same preferences; all of which were from ordered presentation formats.

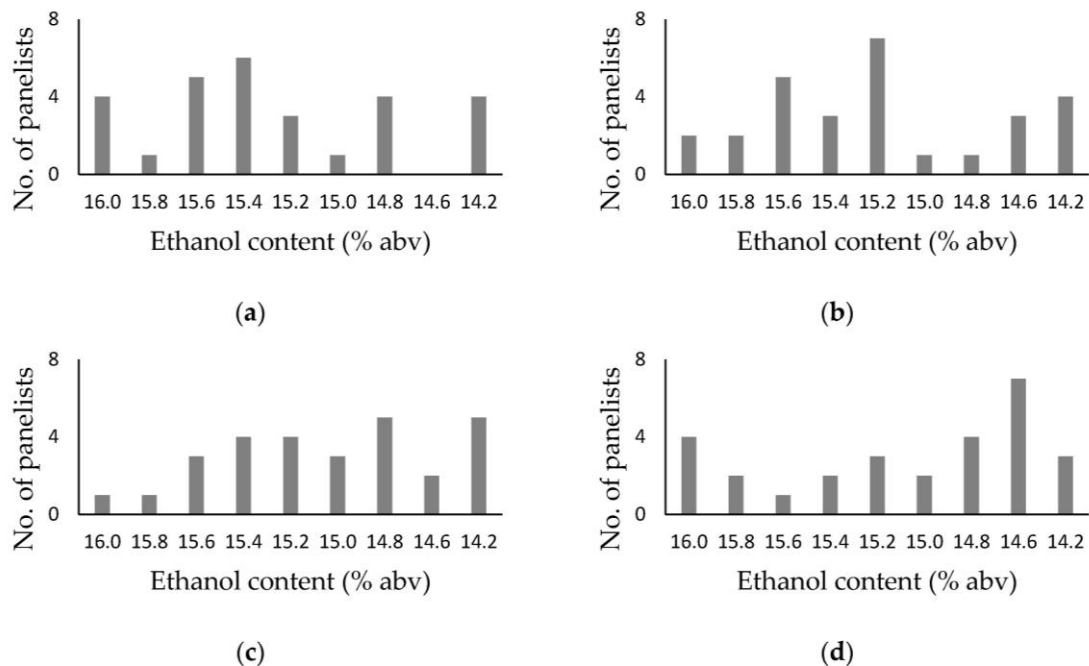


Figure 3. Histograms showing sample preferences from alcohol sweetspotting trials (performed in duplicate, with an expert panel of 14 winemakers) with samples comprising wine B, RAW B and blends thereof, spanning ethanol levels from 14.2 to 16.0% abv, using: (a) row, randomized, (b) row, ordered, (c) circular, randomized and (d) circular ordered, presentation formats.

A study investigating the influence of tasting order on the perception of red wines with varying levels of alcohol (from 12 to 16% abv) found alcohol concentration affected wine perception, with panelists who assessed wines in order of decreasing alcohol less able to discern differences amongst wines than panelists who assessed wines according to ascending alcohol levels [30]. The authors suggested sensory differences might be more strongly contrasted between lower and higher alcohol wines (rather than between higher and lower alcohol wines). In the current study, it is unclear if the moderate differences in alcohol concentration (i.e., 0.2% abv) were simply too small to perceptively impact wine sensory properties, or if the order of sample presentation, either randomized, or from highest to lowest alcohol content (and back again), did not sufficiently contrast any sensory differences amongst samples of different ethanol concentrations. Sensory differences may have been more apparent if samples had instead been presented in order of lowest to highest alcohol content and/or if the incremental difference in alcohol content between samples had been larger, albeit this might also have increased the potential for alcohol sweetspots to be missed (i.e., where a sweetspot exists at an alcohol concentration that falls between the incremental change in ethanol content).

3.3. Influence of Ethanol Content on the Outcomes of Difference Tests

Triangle tests were performed with wine C and RAW C blends to determine the change in ethanol concentration required to give perceptible differences in wine sensory properties. Although the number of correct responses increased as the difference in alcohol content between wine C and its blends increased (Table 4), the number of correct responses required to establish differences at acceptable levels of confidence (i.e., 10, 12 or 13 for 95, 99 and 99.9% confidence, respectively [17]) were not achieved. Only 5 or 6 correct responses were obtained for brackets comprising RAW C and its blends, suggesting the differences in alcohol content for these samples did not appreciably influence their sensory properties. Informal feedback from panelists indicated these samples were quite astringent,

which may have confounded their detection of sensory differences attributable to variation in ethanol content. The panel's inability to discriminate samples which differed in ethanol concentration by as much as 1.0% was consistent with a previous study that reported ethanol difference thresholds for red wine of 1.08 to 1.32% abv [12]. Sensitivity to ethanol differences have also been shown to depend on wine style, evaluation mode (i.e., orthonasal vs. retronasal) and the assessor (i.e., wine experts vs. consumers) [12,15]. This may further explain the difficulty in ascertaining clear alcohol sweetspots from the sweetspotting trials described above, despite the use of an expert panel, as well as the lack of scientific evidence in support of the alcohol sweetspot phenomenon, more broadly.

Table 4. Results from difference tests for blends of wine C and RAW C.

	Ethanol Concentration (% Abv) of Samples Evaluated in Difference Tests					
	16.3 v 16.1 (0.2%)	16.3 v 15.5 (0.5%)	16.3 v 15.3 (1.0%)	15.0 v 14.0 (1.0%)	14.5 v 14.0 (0.5%)	14.2 v 14.0 (0.2%)
correct responses	5/18	7/18	9/18	5/18	6/18	5/18

4. Conclusions

Partial dealcoholization of wines A and B decreased alcohol levels by 1.6 and 1.8%, respectively, with no significant changes to key compositional parameters such as pH, TA, VA or color, but some loss of ethyl esters which could affect wine aroma. Chi-square goodness of fit and one proportion tests indicated preference data were statistically significant for some sweetspot determinations, with the one proportion test discriminating preferences for individual samples relative to random choice, thereby improving the likelihood of statistically significant preferences being identified. However, the outcomes of sweetspotting trials could not be replicated (either by panel or by individual panelists), suggesting identification of samples comprising superior sensory properties was challenging for this set of samples, irrespective of presentation order. It is unclear to what extent this can be attributed to the absence of perceptible differences in sensory properties due to moderate differences in (incremental) ethanol concentrations and/or the order of sample presentation, vs. the non-existence of alcohol sweetspot phenomena. Regardless, the global wine industry employs various strategies to adjust the alcohol content of wine in response to environmental, financial and market challenges, and it is likely that winemakers will continue to use alcohol sweetspotting practices to inform decisions regarding dealcoholization. The use of statistical analyses such as one proportion tests are therefore recommended, to validate any significance of outcomes from alcohol sweetspotting. Future research involving paired comparisons of samples could also be undertaken as an alternate approach to discrimination of samples that exhibit optimal overall sensory properties (i.e., to facilitate identification of alcohol sweetspots, if the alcohol sweetspot phenomena does exist).

Supplementary Materials: The following are available online at <http://www.mdpi.com/2304-8158/8/10/491/s1>, Figure S1: Histograms comparing sample preferences from replicated of alcohol sweetspotting trials comprising wine A, RAW A and blends thereof, spanning alcohol levels from 14.4 to 16.0% abv, using: (a,b) row, randomized, (c,d) row, ordered, (e,f) circular, randomized, and (g,h) circular, ordered presentation formats; Figure S2: Histograms comparing sample preferences from replicated of alcohol sweetspotting trials comprising wine B, RAW B and blends thereof, spanning alcohol levels from 14.2 to 16.0% abv, using: (a,b) row, randomized, (c,d) row, ordered, (e,f) circular, randomized, and (g,h) circular, ordered presentation formats; Table S1: Demographics and experience of expert panelists; Table S2: Panelist sample preferences from alcohol sweetspotting trials comprising wine A, RAW A and blends thereof, spanning alcohol levels from 14.4 to 16.0% abv, for each presentation format; Table S3: Panelist sample preferences from alcohol sweetspotting trials comprising wine B, RAW B and blends thereof, spanning alcohol levels from 14.2 to 16.0% abv, for each presentation format.

Author Contributions: All authors were involved in conceptualization; methodology, D.-T.P.; formal analysis, D.-T.P., J.T. and K.L.W.; resources, V.J.S.; writing—original draft preparation, D.-T.P.; writing—review and editing, V.J.S., J.T., D.W.J. and K.L.W.; supervision, V.J.S., D.W.J. and K.L.W.; funding, V.J.S., D.W.J. and K.L.W.

Funding: This research was conducted by the Australian Research Council Training Centre for Innovative Wine Production (www.adelaide.edu.au/tc-iwp), which is funded as part of the ARC's Industrial Transformation Research Program (Project No. ICI30100005), with support from Wine Australia and industry partners.

Acknowledgments: The authors gratefully acknowledge: VA Filtration for technical assistance with RO-EP dealcoholization of wines; the winemakers, staff and students who participated in sensory analysis; and Metabolomics South Australia which is funded through Bioplatforms Australia Pty Ltd. (BPA), a National Collaborative Research Infrastructure Strategy (NCRIS), and investment from the South Australian State Government and The Australian Wine Research Institute, for GC-MS analysis of fermentation volatiles.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, i.e., in the collection, analysis or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

References

- Godden, P.; Muhlack, R. Trends in the composition of Australian wine. *Aust. N. Z. Grapegrow. Winemak.* **2010**, *558*, 47–61.
- Zamora, F. Dealcoholised wines. In *Wine Safety, Consumer Preference, and Human Health*; Moreno-Arribas, M., Bartolomé Suáldea, B., Eds.; Springer: Cham, Switzerland, 2016; pp. 163–182. [\[CrossRef\]](#)
- Pham, D.-T.; Stockdale, V.J.; Wollan, D.; Jeffery, D.W.; Wilkinson, K.L. Compositional consequences of partial dealcoholization of red wine by reverse osmosis-evaporative perstraction. *Molecules* **2019**, *24*, 1404. [\[CrossRef\]](#) [\[PubMed\]](#)
- Takács, L.; Vatai, G.; Korány, K. Production of alcohol free wine by pervaporation. *J. Food Eng.* **2007**, *78*, 136–146. [\[CrossRef\]](#)
- Schmidtke, L.M.; Blackman, J.W.; Agboola, S.O. Production technologies for reduced alcoholic wines. *J. Food Sci.* **2012**, *77*, R25–R41. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wollan, D. Alcohol sweet spot seminars in McLaren Vale. *Aust. N. Z. Grapegrow. Winemak.* **2005**, *502*, 70–71.
- Martin, S.; Pangborn, R.M. Taste interaction of ethyl alcohol with sweet, salty, sour and bitter compounds. *J. Sci. Food Agric.* **1970**, *21*, 653–655. [\[CrossRef\]](#)
- Fischer, U.; Noble, A.C. The effect of ethanol, catechin, and pH on sourness and bitterness of wine. *Am. J. Enol. Vitic.* **1994**, *45*, 6–10.
- Mattes, R.D.; DiMeglio, D. Ethanol perception and ingestion. *Physiol. Behav.* **2001**, *72*, 217–229. [\[CrossRef\]](#)
- Fontoin, H.; Saucier, C.; Teissedre, P.-L.; Glories, Y. Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Qual. Pref* **2008**, *19*, 286–291. [\[CrossRef\]](#)
- Jones, P.R.; Gawel, R.; Francis, I.L.; Waters, E. The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Qual. Pref* **2008**, *19*, 596–607. [\[CrossRef\]](#)
- Yu, P.; Pickering, G.J. Ethanol difference thresholds in wine and the influence of mode of evaluation and wine style. *Am. J. Enol. Vitic.* **2008**, *59*, 146–152.
- Tsachaki, M.; Linforth, R.S.T.; Taylor, A.J. Dynamic headspace analysis of the release of volatile organic compounds from ethanolic systems by direct APCI-MS. *J. Agric. Food Chem.* **2005**, *53*, 8328–8333. [\[CrossRef\]](#) [\[PubMed\]](#)
- Robinson, A.L.; Ebeler, S.E.; Heymann, H.; Boss, P.K.; Solomon, P.S.; Trengove, R.D. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compounds headspace partitioning. *J. Agric. Food Chem.* **2009**, *57*, 10313–10322. [\[CrossRef\]](#) [\[PubMed\]](#)
- Schmitt, M.; Christmann, M.; Jung, R.; Schübler, C. Enological/technical approaches to reduce elevated alcohol levels in wine (alcohol management) – Sensory results. *Bull. de l'OIV* **2013**, *86*, 485–492.
- King, E.S.; Heymann, H. The effect of reduced alcohol on the sensory profiles and consumer preferences of white wine. *J. Sens. Stud.* **2014**, *29*, 33–42. [\[CrossRef\]](#)
- Meilgaard, M.C.; Carr, B.T.; Civille, G.V. *Sensory Evaluation Techniques*, 4th ed.; CRC Press: Boca Raton, FL, USA, 2007.
- Frayne, R.F. Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. *Am. J. Enol. Vitic.* **1986**, *37*, 281–287.
- Cliff, M.A.; King, M.C.; Schlosser, J. Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. *Food Res. Int.* **2007**, *40*, 92–100. [\[CrossRef\]](#)

20. Siebert, T.E.; Smyth, H.E.; Capone, D.L.; Neuwohner, C.; Pardon, K.H.; Skouroumounis, G.K.; Herderich, M.J.; Sefton, M.A.; Pollnitz, A.P. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Anal. Bioanal. Chem.* **2005**, *381*, 937–947. [\[CrossRef\]](#)
21. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* **2017**, *82*, 1–26. [\[CrossRef\]](#)
22. Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [\[CrossRef\]](#)
23. Dartiguenave, C.; Jeandet, P.; Maujean, A. Study of the contribution of the major organic acids of wine to the buffering capacity of wine in model solutions. *Am. J. Enol. Vitic.* **2000**, *51*, 352–356.
24. Torija, M.J.; Beltran, G.; Novo, M.; Poblet, M.; Rozes, N.; Mas, A.; Guillamon, J.M. Effect of organic acids and nitrogen source on alcohol fermentation: Study of their buffering capacity. *J. Agric. Food Chem.* **2003**, *51*, 916–922. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Gonzalez-Manzano, S.; Dueñas, M.; Rivasgonzalo, J.; Escribano-Bailón, M.; Santos-Buelga, C. Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. *Food Chem.* **2009**, *114*, 649–656. [\[CrossRef\]](#)
26. Gonçalves, F.; Ribeiro, R.; Neves, L.; Lemperle, T.; Lança, M.; Ricardo da Silva, J.; Laureano, O. Alcohol reduction in wine by nanofiltration. Some comparisons with reverse osmosis technique. In Proceedings of the 1st Oenoviti International Symposium—Alcohol Level Reduction in Wine, Bordeaux, France, 6 September 2013; VIGNE et vin Publications Internationales: Bordeaux, France, 2013; pp. 64–67.
27. Longo, R.; Blackman, J.W.; Torley, P.J.; Rogiers, S.Y.; Schmidtke, L.M. Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *J. Sci. Food Agric.* **2016**, *97*, 8–16. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Rayess, Y.E.; Mietton-Peuchot, M. Membrane technologies in wine industry: An overview. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 2005–2020. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Francis, I.L.; Newton, J. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **2005**, *11*, 114–126. [\[CrossRef\]](#)
30. King, E.S.; Dunn, R.L.; Heymann, H. The influence of alcohol on the sensory perception of red wines. *Food Qual Pref.* **2013**, *28*, 235–243. [\[CrossRef\]](#)



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Supplementary Materials

Investigating Alcohol Sweetspot Phenomena in Reduced Alcohol Red Wines

Duc-Truc Pham ^{1,2}, Vanessa J. Stockdale ^{2,3}, David W. Jeffery ^{1,2}, Jonathan Tuke ⁴ and Kerry L. Wilkinson ^{1,2,*}

¹ School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, 5064 Glen Osmond, Australia; duc-truc.pham@adelaide.edu.au (D.-T.P.); david.jeffery@adelaide.edu.au (D.W.J.)

² The Australian Research Council Training Centre for Innovative Wine Production, PMB 1, 5064 Glen Osmond, Australia; Vanessa.Stockdale@tweglobal.com

³ Treasury Wine Estates, 97 Sturt Highway, 5352 Nuriootpa Australia

⁴ School of Mathematical Sciences, The University of Adelaide, 5000 Adelaide, Australia; simon.tuke@adelaide.edu.au

* Correspondence: kerry.wilkinson@adelaide.edu.au; Tel.: +61-8-8313-7360

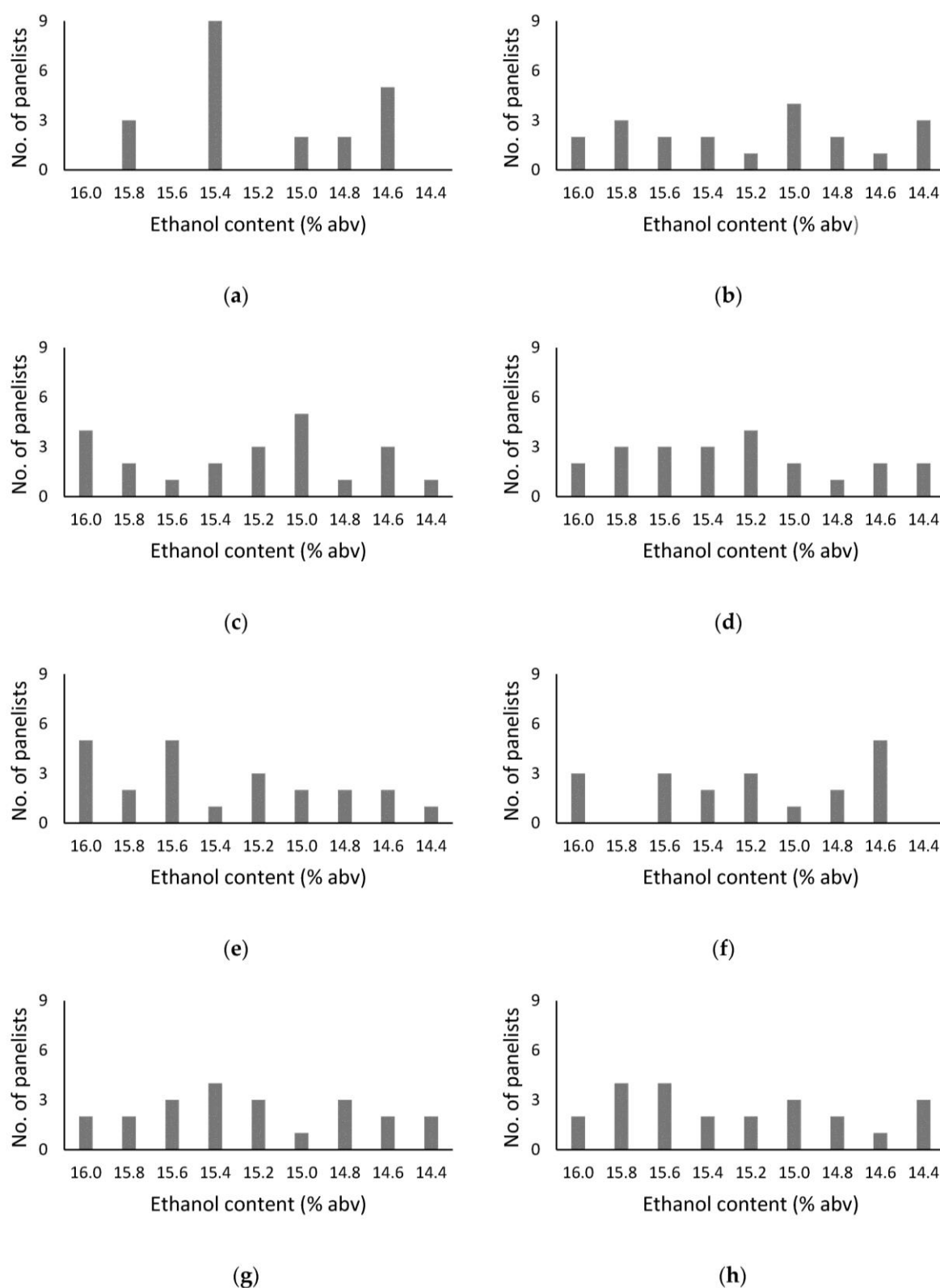


Figure S1. Histograms comparing sample preferences from individual replicates of alcohol sweetspotting trials comprising wine A, RAW A and blends thereof, spanning alcohol levels from 14.4 to 16.0% abv, using: (a,b) row, randomized, (c,d) row, ordered, (e,f) circular, randomized, and (g,h) circular, ordered presentation formats.

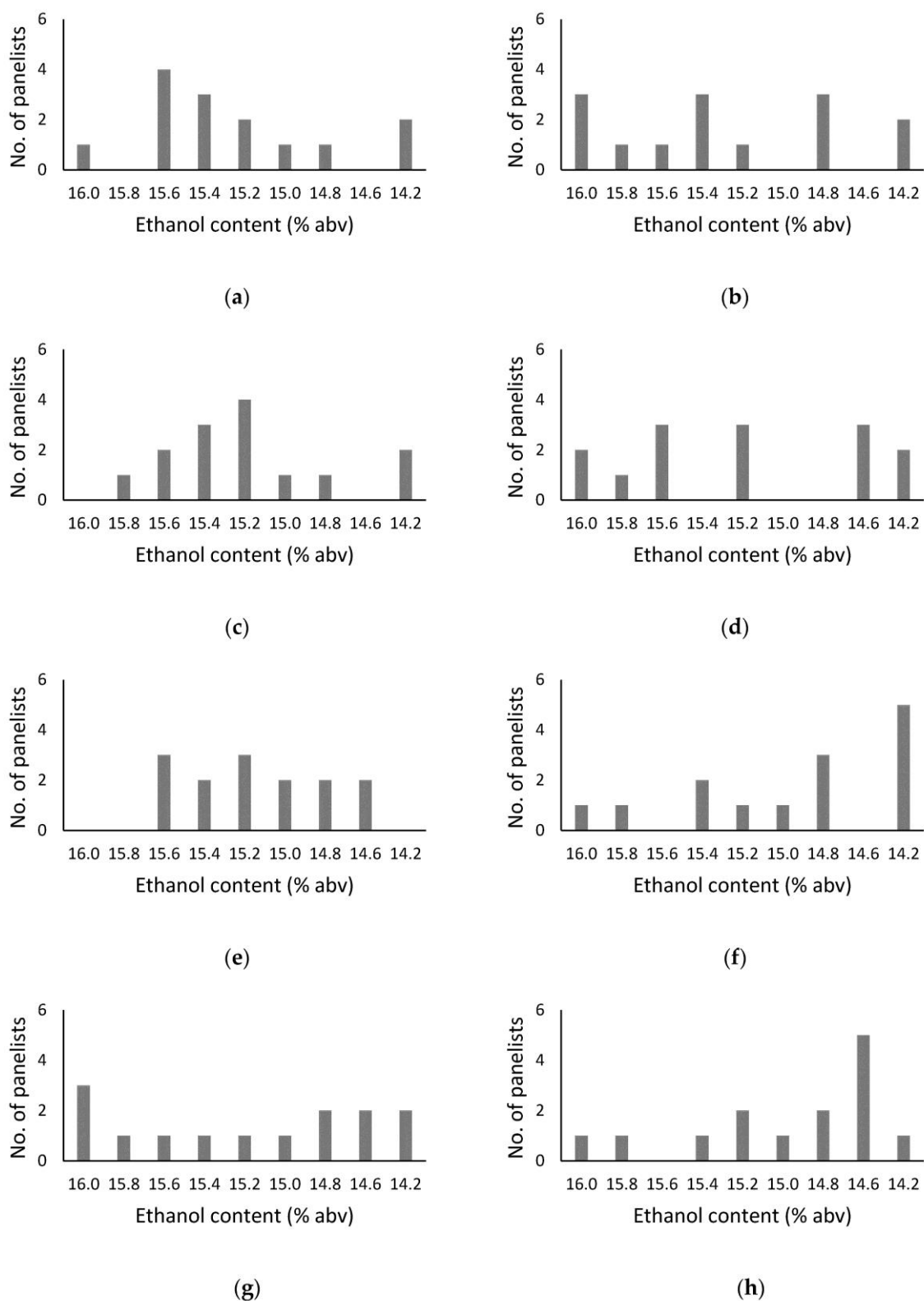


Figure S2. Histograms comparing sample preferences from individual replicates of alcohol sweetspotting trials comprising wine B, RAW B and blends thereof, spanning alcohol levels from 14.2 to 16.0% abv, using: (a,b) row, randomized, (c,d) row, ordered, (e,f) circular, randomized, and (g,h) circular, ordered presentation formats.

Table S1. Demographics and experience of expert panelists.

	Trial 1 (n=14)	Trial 2 (n=14)
<i>Sex</i>		
female	21	36
male	79	64
<i>Age (years)</i>		
≤ 30	7	7
31–40	43	64
41–50	29	21
≥ 51	21	7
<i>Wine industry experience (years)</i>		
≤ 10	7	28
11–20	58	58
≥ 21	35	14
<i>Wine judging experience?</i>		
Yes	100	100
No	0	0
<i>Alcohol sweetspot experience?</i>		
Yes	79	79
No	21	21

Data are presented as percentages.

Table S2. Panelist sample preferences from alcohol sweetspotting trials comprising wine A, RAW A and blends thereof, spanning alcohol levels from 16.0 to 14.4% abv, for each presentation format.

Presentation format	Panelist													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
row, randomized 1	8	8,2	2,4	4, 7	4,2	4	4	4	8,4	6	4,8	8,6	4	7
row, randomized 2	9	1,3	1,9	7	2	4	2	6	4,8	2	6	9,6	7,3,5	6
row, ordered 1	5	3,6	2,8	4	1,6	1,5	1	5,6	8,7	6	4	6,8	1,9	2
row, ordered 2	3	5,4,6	2,8	5	2	3,9	9	7	1,5	4	5,8	6,3	1,2	4
circular, randomized 1	1	7,3	7	2,6,4	5,1	1,9	2	5	1,3	3	1,3	6,8,5	8	3
circular, randomized 2	5	3	1,5	4,8	3	8	8	4	7,8	1	7	6,3	1,8	5
circular, ordered 1	7	7	3,9	1,6	2,4	9	4	4,5	1,4	8	8,7	3,5	3,2	5
circular, ordered 2	6	4	6,9	7,2,5	3	2	2	7	9,5	1	2,3	1,3,8	4,9,3	6

Sample preference numbers (i.e., 1 to 9) correspond to decreasing ethanol concentrations (i.e. 16.0 to 14.4%).

Table S3. Panelist sample preferences from alcohol sweetspotting trials comprising wine B, RAW B and blends thereof, spanning alcohol levels from 16.0 to 14.2% abv, for each presentation format.

Presentation format	Panelist													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
row, randomized 1	3	5	3	1	4	5	4	3	6	9	3	4	9	7
row, randomized 2	4	3	1	4	9	7	9	1	7	7	5	1	2	4
row, ordered 1	4	7	3	3	5	5	9	4	5	4	6	2	5	9
row, ordered 2	3	9	5	2	3	8	8	3	5	8	9	1	5	1
circular, randomized 1	7	6	4	8	3	4	3	5	5	3	8	5	7	6
circular, randomized 2	9	4	1	9	9	9	9	2	7	5	7	7	6	4
circular, ordered 1	6	9	7	7	8	9	4	3	5	2	8	1	1	1
circular, ordered 2	8	8	7	6	2	9	4	8	5	7	5	8	8	1

Sample preference numbers (i.e., 1 to 9) correspond to decreasing ethanol concentrations (i.e. 16.0 to 14.2%).

Statement of Authorship

Title of Paper	Influence of Partial Dealcoholization on the Composition and Sensory Properties of Cabernet Sauvignon Wines
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Duc-Truc Pham, Renata Ristic, Vanessa J. Stockdale, David W. Jeffery, Jonathan Tuke and Kerry L. Wilkinson. Food Chemistry, 2020, 325, 126839. DOI: 10.1016/j.foodchem.2020.126869.

Principal Author

Name of Principal Author (Candidate)	Duc-Truc Pham
Contribution to the Paper	Designed and performed experiments; conducted chemical and sensory analysis of wines; performed statistical analyses of data (using ANOVA, chi-squared goodness of fit and one proportion tests); interpreted the data; and drafted and revised the manuscript.
Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 26-06-2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Renata Ristic
Contribution to the Paper	Contributed to the research idea, sensory analysis, experimental design and data interpretation; and edited the manuscript.
Signature	Date 26-06-2020

Name of Co-Author	Vanessa J. Stockdale
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design and data interpretation; and edited the manuscript.
Signature	Date 26-06-2020

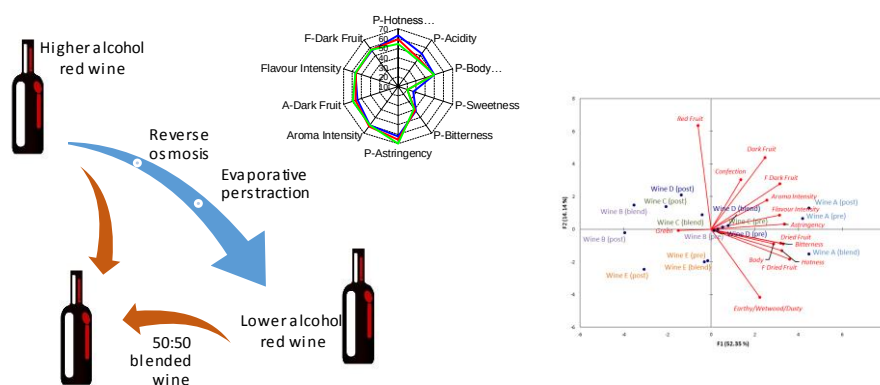
Name of Co-Author	David W. Jeffery
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design and data interpretation; and edited the manuscript.
Signature	Date 26-06-2020

Name of Co-Author	Jonathan Tuke
Contribution to the Paper	Contributed to statistical analysis and interpretation of data; and edited the manuscript.
Signature	Date 26-06-2020

Name of Co-Author	Kerry L. Wilkinson
Contribution to the Paper	Supervised the work; contributed to the research idea, experimental design, and data analysis and interpretation; edited and revised the manuscript, and acted as the corresponding author.
Signature	Date 26-06-2020

Chapter 4.

Influence of Partial Dealcoholization on the Composition and Sensory Properties of Cabernet Sauvignon Wines



Duc-Truc Pham, Renata Ristic, Vanessa J. Stockdale, David W. Jeffery, Jonathan Tuke and Kerry L. Wilkinson. Food Chemistry, 2020, 325, 126839. DOI: 10.1016/j.foodchem.2020.126869



Influence of partial dealcoholization on the composition and sensory properties of Cabernet Sauvignon wines

Duc-Truc Pham^{a,b}, Renata Ristic^{a,b}, Vanessa J. Stockdale^{b,c}, David W. Jeffery^{a,b}, Jonathan Tuke^d, Kerry Wilkinson^{a,b,*}

^a The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia

^b The Australian Research Council Training Centre for Innovative Wine Production, PMB 1, Glen Osmond, SA 5064, Australia

^c Treasury Wine Estate, 97 Sturt Highway, PMB 1, Nuriootpa, SA 5352, Australia

^d The University of Adelaide, School of Mathematical Sciences, Adelaide, SA 5000, Australia

ARTICLE INFO

Keywords:

Alcohol sweetspot
Descriptive analysis
Evaporative perstraction
Reverse osmosis
Reduced alcohol wine

ABSTRACT

Worldwide, winemakers are increasingly adopting alcohol management strategies to counter the higher wine ethanol concentrations observed over the past ~30 years. Wines with high ethanol levels exhibit increased 'hotness' on the palate, which is generally considered to negatively impact wine quality. This study investigated changes in the chemical and sensory profiles of five Cabernet Sauvignon wines following their partial dealcoholization by reverse osmosis-evaporative perstraction (RO-EP). Descriptive analysis (DA) of wine before and after RO-EP treatment indicated dealcoholization did not strongly affect wine aroma and flavor, consistent with the small changes observed in wine chemical composition. However, significant differences in the perception of wine palate attributes were observed, with dealcoholized wines found to exhibit lower acidity, sweetness, bitterness, saltiness and/or body. Astringency was enhanced following partial dealcoholization of one wine, but diminished in another. Sensory trials were also undertaken to determine the optimal ethanol concentration of wines, or the 'alcohol sweetspot'. Two approaches to alcohol sweetspotting of dealcoholized wines were evaluated, together with two methods of statistical analysis, chi-squared goodness of fit and one proportion tests.

1. Introduction

Winemakers can enhance the fruit intensity of their wines by harvesting riper grapes, but this invariably leads to fruit with higher sugar levels, and thus, wines of higher alcohol content. High levels of alcohol can be problematic where they result in wines being perceived as 'warm', or even 'hot' (Baker & Ross, 2014; King, Dunn, & Heymann, 2013), since 'hotness' is increasingly being considered detrimental to wine quality, by winemakers and consumers alike. Ethanol influences the perception of a range of wine sensory properties, not only taste and mouthfeel sensations, but aroma and flavor (Diban, Athes, Bes, & Souchon, 2008). Elevated ethanol concentrations typically enhance the perception of 'bitterness', 'body' and 'hotness', whereas lower ethanol levels can diminish the perception of 'acidity', 'astringency', 'aroma', and 'flavor' (Fischer & Noble, 1994). The sensory impact of partial dealcoholization of wine therefore depends on the quantity of ethanol remaining in the final reduced alcohol wine (RAW) (Fedrizzi et al., 2014; King et al., 2013; Schmidtke, Blackman, & Agboola, 2012). For

example, wines that were considered 'herbaceous' at alcohol levels of 14.5–17.2% alcohol by volume (abv) were described as 'fruity' at lower ethanol concentrations, i.e., at 10.0–12.0% abv (Goldner, Zamora, Di Leo Lira, Gianninoto, & Bandoni, 2009).

Internationally, winemakers are increasingly adopting strategies to manage and/or adjust wine ethanol levels. In the vineyard, viticultural management practices aim to decrease grape sugar levels (Olego et al., 2016); whereas in the winery, strategies seek to limit the amount of ethanol produced during fermentation (Dequin et al., 2017; Zamora, 2016). Ethanol can also be removed from wine after fermentation, using technologies such as reverse osmosis-evaporative perstraction (RO-EP) and spinning cone column distillation (Belisario-Sánchez, Taboada-Rodríguez, Marín-Iniesta, & López-Gómez, 2009; Pham, Stockdale, Wollan, Jeffery, & Wilkinson, 2019; Rayess & Mietton-Peuchot, 2016; Wollan, 2005). Cabernet Sauvignon is a cultivar of commercial importance, not only in Australia, but worldwide (Ciatti Global Wine and Grape Brokers, 2017). It typically produces full-bodied (higher alcohol) wines, and so it is a varietal for which ethanol

* Corresponding author at: The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia.

E-mail addresses: duc-truc.pham@adelaide.edu.au (D.-T. Pham), renata.ristic@adelaide.edu.au (R. Ristic), vanessastockdale@bigpond.com (V.J. Stockdale), david.jeffery@adelaide.edu.au (D.W. Jeffery), simon.tuke@adelaide.edu.au (J. Tuke), kerry.wilkinson@adelaide.edu.au (K. Wilkinson).

<https://doi.org/10.1016/j.foodchem.2020.126869>

Received 6 December 2019; Received in revised form 18 April 2020; Accepted 18 April 2020

Available online 20 April 2020

0308-8146/ © 2020 Elsevier Ltd. All rights reserved.

management might be beneficial. Whereas lower levels of alcohol can be achieved in wines made from grapes that are harvested 'early', i.e., at moderate grape sugar levels (Schelezi, Šuklje, Boss, & Jeffery, 2018), early-harvested Cabernet Sauvignon grapes tend to exhibit unripe 'green' and 'vegetative' aromas and flavors (Brodison & Bell, 2014), and higher acidity (Schmidtke et al., 2012). The addition of water to must can also achieve lower wine alcohol levels (Harbertson, Mireles, Weller, Harwood, & Ross, 2009; Schelezi et al., 2018). However, this practice is not permitted in all wine producing countries.

Adding to this challenge, wine is highly complex, and as such, compositional analyses cannot reliably predict wine sensory profiles (Fedrizzi et al., 2014). Sensory studies therefore continue to provide valuable information when determining the outcome of treatments on wine aroma, flavor, taste and mouthfeel properties (Gawel & Godden, 2008; Mattes & DiMeglio, 2001). Amongst the various sensory methods that are available, descriptive analysis (DA) is frequently used to characterize and/or contrast wine sensory profiles (Lawless and Heymann, 2010). However, few studies to date have employed descriptive analysis to profile the sensory properties of reduced alcohol wines (RAW). A key aim of this study was therefore to identify changes in the chemical and sensory profiles of five Cabernet Sauvignon wines, following partial dealcoholization by RO-EP treatment.

In addition, winemakers who employ RO-EP treatment of wine sometimes use a sensory-based practice referred to as 'alcohol sweet-spotting' to determine the alcohol content at which wine displays enhanced aroma and flavor intensity, and superior overall balance, compared to the same wine comprising different ethanol concentrations (Baker & Ross, 2014; Wollan, 2005). 'Alcohol sweetspots' have been defined as the one or two specific ethanol levels at which wine seems to 'stand out' from incremental blends of wine and its corresponding RAW (Wollan, 2005; Wollan, 2006). However, to date, there is no scientific evidence to support the existence of an alcohol sweetspot. Sweet-spotting trials typically involve samples being evaluated in order of lowest to highest alcohol concentration, or vice versa (King & Heymann, 2014; Schmitt, Christmann, Jung, & Schübler, 2013), but this presentation format might create bias, if panelists avoid end samples. This might be overcome by using a randomized presentation order, except, this approach might prevent discrimination of subtle differences between samples of similar alcohol content. A secondary aim of this project was therefore to evaluate alternate approaches to alcohol sweet-spotting, both sample presentation and statistical analysis, in order to standardize the optimization of dealcoholization processes.

2. Materials and methods

2.1. Wine samples

Five 2015 Cabernet Sauvignon wines (hereafter referred to as wines A, B, C, D and E) were sourced from South Australian wine producers who used industrial scale RO-EP (VA Filtration, Nuriootpa, SA, Australia) to achieve partial dealcoholization; i.e., ethanol was removed to lower the alcohol content by between 1.8 and 2.5% alcohol by volume, (abv). Given that RO-EP was performed on industrial volumes (i.e., on 20–30 kL of wine) during real winemaking operations, it was not possible to replicate treatments. Instead, RO-EP was performed on five different wines, in accordance with manufacturer operating instructions, as previously described (Pham, Stockdale, Wollan et al., 2019). Control (i.e., untreated) and partially dealcoholized wines (12 bottles per treatment) were bottled in 750 mL glass bottles under screw cap closures and cellared (in darkness at 15 °C) prior to chemical and sensory analysis approximately 3 months after RO-EP treatment. Blends of control and dealcoholized wines (50:50) were also prepared for sensory analysis.

2.2. Chemical analysis of wines

The alcohol content, density, pH, titratable acidity (TA, as g/L of tartaric acid equivalents, to an endpoint of pH 8.2) and volatile acidity (VA, as g/L of acetic acid equivalents) were determined (in duplicate) by the Australian Wine Research Institute's (AWRI) Commercial Services laboratory (Adelaide, SA, Australia), using a Foss WineScan analyzer (Mulgrave, Vic., Australia). Glucose, fructose, glycerol and organic acids were determined (in duplicate) by high-performance liquid chromatography (HPLC), as described previously (Frayne, 1986), using an Agilent 1100 series HPLC (Agilent Technologies, Forest Hill, Vic., Australia), fitted with diode array and refractive index detectors. The concentrations of several fermentation volatiles (acids, alcohols and esters) were determined by Metabolomics Australia (AWRI) using gas chromatography-mass spectrometry (GC-MS), according to previously reported stable isotope dilution analysis (SIDA) methods (Siebert et al., 2005).

2.3. Sensory analysis of wines

Descriptive analysis and alcohol sweet-spotting trials were performed in a purpose-built sensory laboratory at the University of Adelaide's Waite Campus. For each of the sensory analyses performed: samples (30 mL) were served at ambient temperature (i.e., 22–24 °C), in four digit coded XL5 (ISO standard) 215 mL wine glasses, under LED white lighting; with wine glasses covered to avoid ethanol evaporation (Wollan, Pham, & Wilkinson, 2016). Sensory trials were approved by the Human Research Ethics Committee of the University of Adelaide (H-2015–094).

2.3.1. Descriptive analysis

The sensory profiles of control and dealcoholized wines (and 50:50 blends thereof), were determined by descriptive analysis (DA) (Lawless and Heymann, 2010), with a trained panel comprising nine University of Adelaide staff and students (five women and four men, aged between 18 and 55 years). Panelists were recruited based on their availability and prior sensory experience, and underwent 10 hr of training (5 × 2 hr sessions), during which they generated appropriate descriptive terms, definitions and reference standards (Table 1), and then gained familiarity with the use of intensity scales, and recognition and scoring of sensory attributes, using published protocols (Bastian et al., 2010). The panel generated a total of 27 attributes, comprising 10 aroma, 10 flavor and 7 palate descriptors (Table 1). Reference standards were prepared as shown in Table 1 and panelists could refer to standards prior to and during the formal evaluation sessions. Training sessions also included two practice evaluation sessions, which were held in individual sensory booths under the conditions used during formal assessment. Data from training sessions were analyzed using PanelCheck software (www.panelcheck.com) to evaluate panelist performance (reproducibility and repeatability) and determined when formal assessment could commence. Formal evaluation sessions were held weekly over 4 weeks, such that all wines were assessed in triplicate. Panelists were presented with brackets of no more than four samples at a time (presented in random order) and rated the intensity of each sensory attribute using 15-cm unstructured line scales with anchor points of 'low' and 'high' placed at 10 and 90% on the scale, respectively. Breaks were enforced between samples (1 min) and brackets (5 min) to prevent sensory fatigue. Panelists rinsed thoroughly with pectin solution (1 g/L) after each sample, with distilled water and plain crackers also provided as palate cleansers. Data were acquired with RedJade software (Redwood Shores, CA, USA).

2.3.2. Alcohol sweet-spotting trials

Three wines, A, D and E, were selected for alcohol sweet-spotting trials based on their chemical and sensory profiles. Each control wine was blended with its corresponding dealcoholized wine to generate a

Table 1
Aroma/flavor and palate attributes used in descriptive analysis of wines.

Attribute	Definition	Standard (mixed in 30 mL of Cabernet Sauvignon bag-in-box wine)
Aroma/Flavor	<i>perception of...</i>	
overall intensity	overall aroma or flavor intensity	
red fruit	fresh raspberry, strawberry, sour cherry and red plum	half a strawberry and 2 raspberries
dark fruit	fresh/stewed blackberry, blueberry and plum aroma	2 blackberries and 2 blueberries
dried fruit	cooked fruit and dried fruit	half a teaspoon of dried minced fruit and 5 dried cranberries
jam	jam and prune	1 teaspoon fruit jam (Cottee's)
green	cut grass, leaf and fresh herb	half a eucalyptus leaf and 1 blade of grass (shredded)
confection	strawberry cream and bubble gum	1 strawberry cream lolly (Allen's) and 1 red gummy lolly (Allen's)
sweet spice	spicy, clove	1 pinch ground all spice and 1 pinch mixed spice
chocolate	dark chocolate	1 square of dark chocolate (chopped, Lindt)
earthy	dusty, damp soil and mushroom	1 pinch damp soil
Palate	<i>intensity of...</i>	
acidity	acid taste perceived in the mouth or after expectorating	
sweetness	sweet taste perceived in the mouth or after expectorating	
bitterness	bitter taste perceived in the mouth or after expectorating	
saltiness	salty taste perceived in the mouth or after expectorating	
hotness	warmth or heat in the mouth or after expectorating	
body	viscosity and density in the mouth or after expectorating	
astringency	astringency perceived in the mouth or after expectorating	

series of samples with ethanol concentrations that differed by 0.2–0.4% abv. Samples were subsequently evaluated (in duplicate) by the DA panel. For trials involving wines D and E, samples (comprising control and dealcoholized wines, and blends thereof) were presented to panelists ($n = 8$) in a circular format (i.e., evenly spaced around a circular tray), with the alcohol content increasing in a clockwise direction (hereafter a 'circular, ordered' presentation format). Panelists were instructed to evaluate samples in a clockwise direction, then back again, starting with any sample, and to identify the sample that they considered exhibited superior overall sensory properties. For the trial involving wine A, samples were presented to panelists ($n = 9$) according to a 'knockout' style format (Fig. S1), i.e., panelists were instructed to evaluate randomly selected pairs of samples, and to identify the sample from each pair that they considered exhibited superior overall sensory properties. These samples were retained for a second round of evaluation; with samples evaluated in this way until only a single sample remained, i.e., the sample deemed to exhibit the best overall sensory properties. To avoid sensory fatigue, breaks were enforced between pairs of samples (1 min) and after brackets of eight samples (5 min). During each alcohol sweetspotting trial, panelists rinsed thoroughly with pectin solution (1 g/L) after each sample. Distilled water and plain crackers were also provided as palate cleansers.

2.4. Statistical analysis

Basic compositional data were analyzed by one-way analysis of variance (ANOVA) using GenStat (15th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by a least significant difference (LSD) multiple comparison test at $\alpha < 0.05$. Volatile data were analyzed via an ANOVA F-test using the lmerTest package in R statistical software (Kuznetsova, Brockhoff, & Christensen, 2017). Mixed effect linear models were fitted individually for each volatile, with the response variable being the concentration at each treatment level. A fixed effect predictor was included for treatment, together with a random intercept for wine, to account for the repeated measures on each wine. The fitting was performed using the lme4 package in R (Bates, Maechler, Bolker, & Walker, 2015). Sensory data were analyzed using SenPaq (version 6.03, Qi Statistics, Reading UK) and a mixed model ANOVA performed to determine the effects of treatment, replicate and panelist). The method for discrimination of means was Fisher's least significant difference at $\alpha < 0.05$. Principal component analysis (PCA) was performed on mean ratings of statistically significant sensory attributes for all wines, using XLSTAT (version 2015.4.1, Addinsoft, Melbourne, Vic., Australia). Two statistical

analyses were employed to analyze alcohol sweetspotting data: (i) a chi-square goodness of fit test, to determine statistical significance of the observed distribution of sample preferences vs. random choice; and (ii) a one sample proportion test, to determine statistical significance between the preferred sample, i.e., the alcohol sweetspot, vs. random choice.

3. Results and discussion

Winemakers employ various strategies to manage the ethanol content of their wine, including removal of ethanol post-fermentation. In this study, chemical and sensory analyses were employed before and after RO-EP treatment of Cabernet Sauvignon wines, to determine the effects of partial dealcoholization. An alternate approach to alcohol sweetspotting was also evaluated.

3.1. Influence of partial dealcoholization on the composition of wine

The alcohol content, density, pH, TA, VA, glycerol and acid profiles of control and dealcoholized wines are reported in Table 2. RO-EP treatment of wines achieved decreases in ethanol concentration of between 1.3 and 2.5% abv, as intended, which resulted in small, but statistically significant increases in density. Whereas partial dealcoholization did not meaningfully impact pH, TA or VA, some small increases in glycerol, succinic acid and lactic acid concentrations were observed; in the case of Wine E, a small decrease in lactic acid occurred. However, changes were small ($< 8\%$). The unusually high succinic acid concentrations for Wine A (i.e., 8.2 and 8.3 g/L in comparison to usual levels of < 3.0 g/L) could not be explained, but the change in concentration due to RO-EP treatment was negligible (0.1 g/L).

RO-EP treatment generally led to small concentration losses for most of the fermentation volatiles measured (Table 3), but compositional changes varied amongst the wines studied, such that there was no statistical significance attributable to treatment. Partial dealcoholization of Wine A decreased the concentration of all volatiles, typically by between 5 and 20%, but by approximately 30 and 50% for ethyl octanoate and butanoic acid, respectively. Decreased volatile concentrations were similarly observed for Wine D, particularly for ethyl and acetate esters. In the case of wine B, C and E, some volatile concentrations decreased, whereas others increased, notably: ethyl and acetate esters for Wine B; acetate esters and some acids for Wine C; and a combination of acids, esters and alcohols for Wine E. These results were consistent with previous research concerning the influence of RO-EP treatment on the volatile composition of wine (Longo et al., 2018;

Table 2

Basic composition of Cabernet Sauvignon wines before and after partial dealcoholization by RO-EP.

	Wine A		Wine B		Wine C		Wine D		Wine E		P
	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	
alcohol (% abv)	17.0a	14.5b	15.5a	13.3b	14.9a	13.3b	14.5a	13.2b	16.0a	14.2b	< 0.001
density (g/mL)	0.993b	0.996a	0.994b	0.997a	0.994b	0.997a	0.995b	0.997b	0.992b	0.994b	< 0.001
pH	3.6	3.6	3.8	3.8	3.7	3.7	3.6	3.6	3.5	3.5	–
TA (g/L)	7.2	7.1	6.6	6.8	6.8	7.1	6.9	7.1	6.5	6.5	–
VA (g/L)	0.5	0.8	0.6	0.5	0.5	0.4	0.6	0.6	0.6	0.6	–
glycerol (g/L)	10.9	11.0	11.7	12.0	10.3b	10.6a	10.6b	10.7a	10.2b	10.4b	< 0.001
succinic acid (g/L)	8.2b	8.3a	1.7	1.7	1.3b	1.4 a	1.3b	1.4 a	1.2	1.2	≤ 0.002
lactic acid (g/L)	2.8	2.8	2.5b	2.6a	1.7	1.7	2.0	2.0	1.8 a	1.7b	≤ 0.008

Values are means of duplicate measurements (n = 2). Standard errors were ≤ 5%.

Values followed by different letters within rows (for each wine) are statistically significant ($P = 0.05$, one-way ANOVA).

Pham, Stockdale, Wollan et al., 2019; Pham, Stockdale, Jeffery, Tuke, & Wilkinson, 2019), and reflect the potential for volatiles to be: removed via evaporative perstraction; concentrated as a consequence of ethanol being removed; and/or affected by changes in equilibrium between acids, alcohols and esters.

The degree of dealcoholization (by RO-EP) has been shown to affect the loss of esters from wine, with 6% ethanol removal resulting in a 50% decrease in the overall ester concentration of Shiraz wine (Longo et al., 2018). In the current study, where alcohol levels were adjusted by ≤ 2.5% abv, the majority of volatile compounds were present in wines (both before and after RO-EP treatment) at concentrations above their odor detection threshold levels; the exceptions being 2-methylpropanoic acid (cheese, rancid), ethyl hexanoate (apple peel), ethyl octanoate (fruit, fat), ethyl 3-methylbutanoate (berry) and 2-phenylethyl acetate (rose, honey, tobacco) (Francis & Newton, 2005). As such, in the current study, partial dealcoholization of the Cabernet Sauvignon wines in this study was not expected to significantly affect the intensity of their aromas and flavors.

3.2. Influence of partial dealcoholization on the sensory properties of wine

The sensory profiles of wines were determined by DA before and after RO-EP treatment, and after blending (50:50) of control and dealcoholized wines (Fig. 1, Table S1). The RO-EP treatment had

limited effects on wine aroma and flavor. Significant differences were observed amongst the intensity ratings of only five aroma attributes (i.e., 'red fruit', 'dark fruit', 'green', 'confectionery' and 'chocolate'), but there were no significant differences in the overall intensity of wine aroma (Table S1). 'Dark fruit' aroma increased and 'green' aroma decreased following treatment of Wine A, whereas 'confection' and 'chocolate' aromas were diminished in Wine B and Wine C, respectively. 'Red fruit' aroma increased in Wine D; no significant changes in aroma were perceived in Wine E. Partial dealcoholization resulted in small, but significant decreases in the overall flavor intensity of Wine B, largely attributed to the diminished intensity of 'dark fruit', 'sweet spice' and 'chocolate' flavors. The overall flavor intensity of Wine E also decreased, but the DA panel did not identify significant changes to the intensity of any specific flavors. Small decreases in 'dried fruit' and 'chocolate' flavors were perceived in Wines A and C, respectively (Fig. 1, Table S1).

The concentrations of volatile compounds in the headspace above dealcoholized wine have been found to both increase (due to enhanced volatility) and decrease (due to retention), in response to changes in concentration of other wine constituents (Polášková, Herszage, & Ebeler, 2008; Voilley, Beghim, Charpentier, & Peyron, 1991). Removing ethanol from wine can enhance interactions between aroma compounds and proteinaceous materials present in wine, i.e., due to the less hydrophobic environment, which can lead to diminished headspace

Table 3

Concentrations (μg/L) of volatile acids, esters and alcohols in Cabernet Sauvignon wines before and after partial dealcoholization by RO-EP.

	Odor thresholds ^a (µg/L)	Wine A		Wine B		Wine C		Wine D		Wine E		SD
		pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	pre-RO-EP	post-RO-EP	
Acids												
butanoic acid	173	3399	1614	533	466	528	886	466	482	546	997	50
hexanoic acid	420	1757	1656	1672	1495	1416	1732	1534	1476	1760	1659	10
octanoic acid	500	2986	2940	1737	1694	1471	1403	1648	1416	1609	1623	90
2-methylpropanoic acid	2300	4824	4578	1175	1040	2676	7509	1950	1929	1595	2163	40
2-methylbutanoic acid	33	1808	1642	4808	4035	2036	2362	1939	1881	2135	1975	50
3-methylbutanoic acid	33	2337	2194	1244	1137	1319	3190	982	970	840	878	20
Ethyl esters												
ethyl hexanoate	14	375	301	3	30	30	1.7	36	3.1	5	12	6.0
ethyl octanoate	5	74	50	10	29	30	2.7	34	7.4	15	27	2.5
ethyl 3-methylbutanoate	3	51	43	1	16	26	3.4	24	1.2	1	5	0.3
Acetates												
2-methylbutyl acetate	160	401	334	26	474	467	3347	399	20	20	111	45
3-methylbutyl acetate	30	1334	1111	95	1592	1555	8866	1346	73	76	400	20
2-phenyl ethyl acetate	250	76	69	1444	1349	600	11,125	539	454	607	618	1
Alcohols												
2-methylpropanol	40,000	113,945	101,922	34,885	34,381	75,821	49,829	67,344	55,880	49,363	56,078	700
2-methylbutanol	65,000	198,369	181,776	145,852	133,718	156,608	102,370	127,806	108,521	100,802	101,855	400
2-phenylethanol	10,000	73,198	70,123	490,195	477,378	346,053	331,255	244,820	244,362	208,659	215,812	700

Standard deviation (SD) based on calibration. ^aThreshold data from Francis & Newton, 2005.

A one-way ANOVA was fitted to test for a treatment effect (with a random intercept included to account for repeated measures), but no statistically significant effect of RO-EP treatment was found at $P = 0.05$.

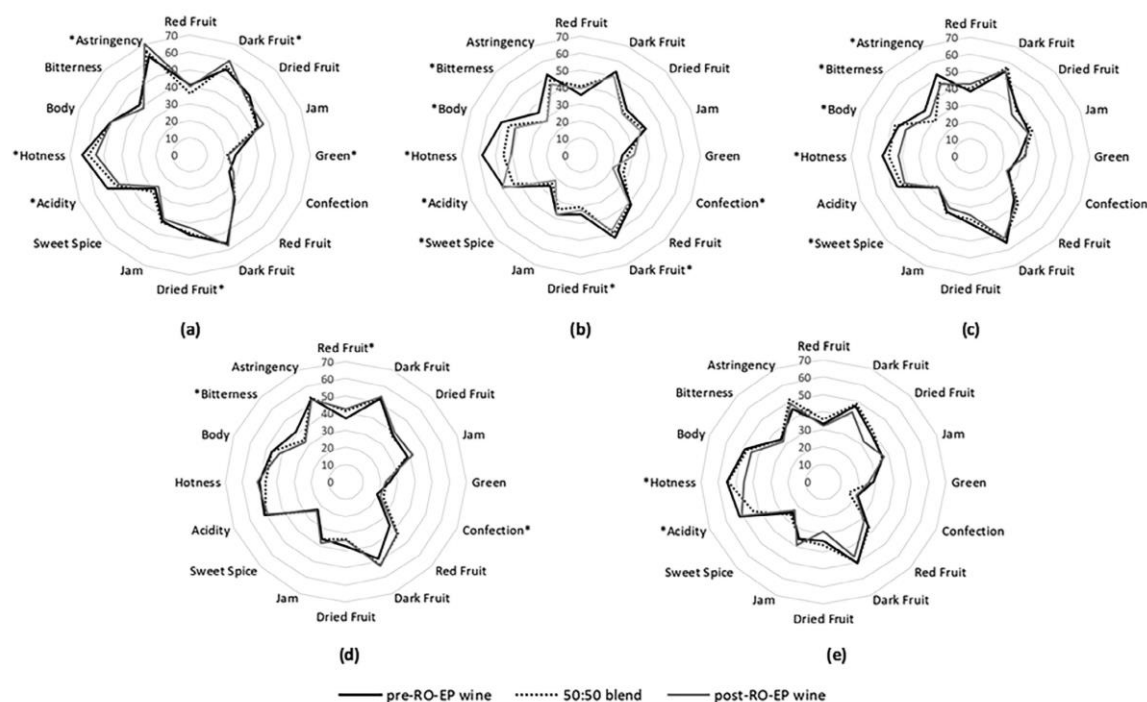


Fig. 1. Sensory profiles of Cabernet Sauvignon wines before and after partial dealcoholization by RO-EP, and 50:50 blends thereof; (a) to (e) = Wines A to E; * denotes statistical significance ($P = 0.05$, one-way ANOVA).

concentrations of volatiles and, consequently, decreased aroma perception (Voilley & Lubbers, 1998). On the other hand, the removal of ethanol effectively concentrates wine, enhancing wine color, as reported previously (Pham, Stockdale, Wollan, et al., 2019; Pham, Stockdale, Jeffery, et al., 2019), as well as other constituents, which may explain the increase in flavor intensity observed in the current study for some attributes.

Significant differences were detected in the intensity ratings of all seven palate attributes (Table S1). 'Hotness' decreased in four of five wines (i.e., Wines A, B, C and E) following RO-EP treatment (Fig. 1). This was attributable to lower ethanol levels, in agreement with previous research (Fischer & Noble, 1994), and in the case of Wine B, it resulted in a perceivable decrease in 'body'. Small differences in 'acidity' (for Wine A) and 'bitterness' (for Wines B and D) were also perceived after RO-EP treatment. In the case of acidity, this was surprising, since ethanol typically masks acidity, but the finding was not unexpected for bitterness, since the perception of bitterness has been shown to be enhanced by higher ethanol levels (Fedrizzi et al., 2014; King et al., 2013; Schmidtke et al., 2012). Small but significant decreases in sweetness and saltiness were perceived for Wines A and E respectively, but the intensity of these attributes were low compared with other attributes (i.e., they were not prominent sensory attributes). In contrast, all wines exhibited varying degrees of astringency, and RO-EP treatment enhanced the perception of this attribute in Wine A, but slightly diminished it in Wine C. A previous study found that lower ethanol concentrations (in model wine) increased binding between the model salivary protein poly(L-proline) and a range of wine tannin fractions isolated from Cabernet Sauvignon (McRae, Ziora, Kassara, Cooper, & Smith, 2015), which might explain the increase in astringency observed in Wine A, being the wine from the current study from which the most ethanol was removed. DA of 50:50 blends of control and dealcoholized wines was performed to further characterize the sensory consequences of partial dealcoholization by RO-EP

treatment. However, there were few significant differences between the sensory profiles of blended wines and control and/or partially dealcoholized wines.

Principal component analysis (PCA) was performed on the sensory data obtained from all wines (Fig. 2). The first two principal components (PC) accounted for 66.5% abv of the total variation observed with wines being separated along the first PC according to positive associations with higher ratings for most sensory attributes, except for 'green' and 'red fruit' characters. Importantly, the position of control (pre-RO-EP), dealcoholized (post-RO-EP) and blended samples derived from each wine were quite close within the PCA biplot, suggesting the sensory differences between the five Cabernet Sauvignon wines, A to E, were larger than differences arising from dealcoholization and/or blending. 'Hotness' was positively correlated with 'body', 'bitterness' and 'astringency' ($R^2 = 0.63$, 0.72 and 0.69 , respectively), but also with 'dried fruit' aroma ($R^2 = 0.842$) and flavor intensity ($R^2 = 0.805$), specifically 'dark fruit' and 'dried fruit' flavors ($R^2 = 0.675$ and 0.683 , respectively). Following dealcoholization, Wines C and D were characterized by enhanced 'red fruit' characters and diminished 'green' character, which remained prominent in Wine B.

Alternate strategies for achieving wines with lower alcohol levels include blending wines made from sequentially harvested fruit (Longo et al., 2018; Schelezki et al., 2018), substituting juice with either water or wine made from early harvested fruit (Schelezki et al., 2018; Schelezki, Antalick, Šuklje, & Jeffery, 2020), the addition of water to must (Harbertson, Mireles, Harwood, Weller, & Ross, 2009) and co-fermentation with non-*Saccharomyces* yeast, as lower ethanol-yielding yeast strains (Hranilovic et al., 2018; Rolle et al., 2018). Alcohol management strategies involving early harvested fruit have achieved substantial decreases in alcohol content (as much as 4–6% abv), but the resulting wines can exhibit green or herbaceous characters and there are negative implications for color that may be variety-dependent (Longo et al., 2018; Schelezki et al., 2018; Schelezki et al., 2020).

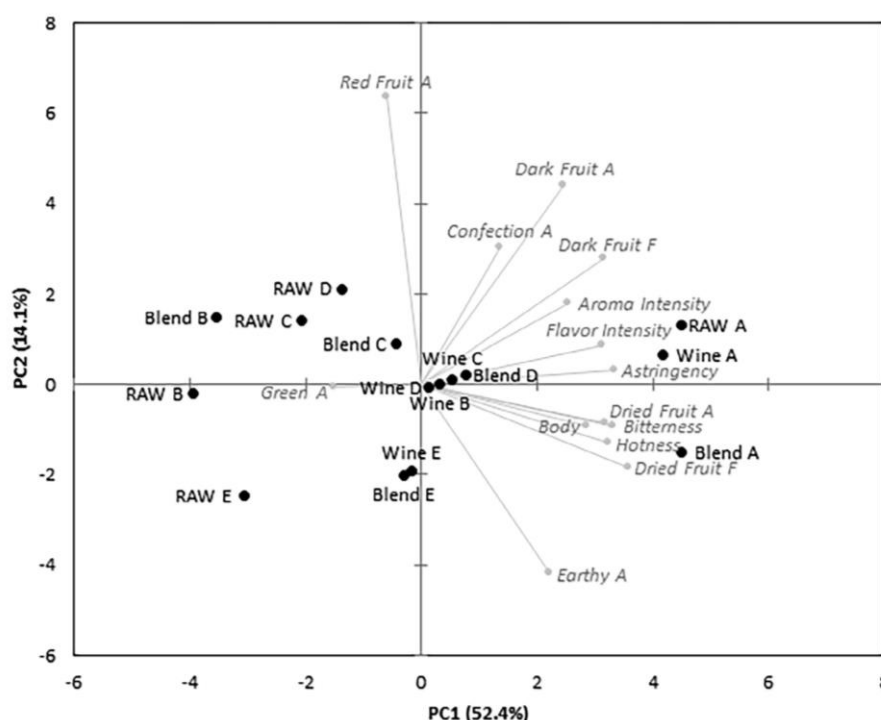


Fig. 2. PCA biplot of mean sensory attribute ratings of Cabernet Sauvignon wines before and after partial dealcoholization by RO-EP (Wine and RAW, respectively), and 50:50 blends thereof (Blend).

Substitution of juice with water (where regulations permit) could overcome the issue of unripe fruit characters, and changes in wine composition and sensory properties were less pronounced than with 'wine' made from early harvested fruit (Schelezi et al., 2018), although variable differences in sensory profiles have also been found to depend on the cultivar (Schelezi et al., 2020). Importantly, water substitution could mitigate the perception of 'hotness' (Schelezi et al., 2020), but not always, especially when considering wines derived from overripe fruit in a hot growing season (Schelezi et al., 2018). In contrast, microbiological approaches to alcohol adjustment of red wine can give higher concentrations of wine volatiles associated with desirable aromas and flavors, but typically achieve only small decreases in alcohol content (i.e. < 1% abv) (Hranilovic et al., 2018; Rolle et al., 2018). Overall, it can be seen that the perceived sensory outcomes of alcohol management clearly depend on the method and the degree of dealcoholization. Results from the present study suggested that RO-EP treatment can achieve decreases in wine alcohol content that mitigate the perception of 'hotness', without comprising sensory quality.

3.3. Influence of sample presentation on the outcome of alcohol sweetspotting trials

Alcohol sweetspotting trials were undertaken by DA panelists in an attempt to identify the ethanol content at which the wines studied exhibited optimal sensory properties. Two statistical tests were applied to results from sweetspotting trials: (i) chi-square goodness-of-fit tests were used to determine if there were significant differences in the preference of judges across each bracket of samples; whereas (ii) one sample proportion tests (www.measuringu.com/onep/) determined if the preferred sample was significantly different from random choice.

The first two trials comprised evaluations of Wines D and E, which had initial ethanol concentrations of 14.5 and 16.0% abv respectively,

and ethanol concentrations after RO-EP treatment of 13.2 and 14.2% abv respectively. Control and dealcoholized wines were blended to achieve brackets of eight samples with incremental changes in ethanol content spanning their different alcohol levels. The DA panelists tasted each bracket (in duplicate), with samples presented in a circular ordered presentation format (as described above in section 2.3.2.), based on findings from a previous study (Pham, Stockdale, Jeffery, et al., 2019). This method presents samples in order of alcohol content (rather than in a randomized order), in accordance with current industry practice, but the circular format avoids obvious identification of samples with the highest and lowest alcohol levels (i.e., as occurs in a linear format). Nevertheless, for these two trials, the most preferred samples were only selected by 3 or 4 panelists respectively; i.e., different samples were preferred by different panelists (Fig. 3a and 3b), such that no apparent sweetspots were identified. Statistical analysis confirmed this outcome, with *P* values ranging from 0.130 to 0.429 (Table S2). Panelists clearly did not agree on what constituted 'superior sensory properties', which was perhaps not surprising, given expert panelists could not reproducibly discriminate wines in this way (Pham, Stockdale, Jeffery, et al., 2019).

The potential for sensory fatigue to occur during evaluation of samples using the circular, ordered presentation format was considered, and as such, a novel approach to alcohol sweetspotting was employed, i.e., a 'knockout' format, in which panelists were presented randomly selected pairs of samples and asked to identify the sample from each pair that exhibited superior overall sensory properties. These samples were retained in a second round of evaluation, with samples evaluated in this way until only a single sample remained, i.e., the sample deemed to exhibit the best overall sensory properties. Wine A was blended with its corresponding dealcoholized wine to generate a bracket of samples comprising 17.0, 16.6, 16.3, 15.9, 15.6, 15.2, 14.9 and 14.5% abv. Nine panelists evaluated the samples (in duplicate) using the knockout

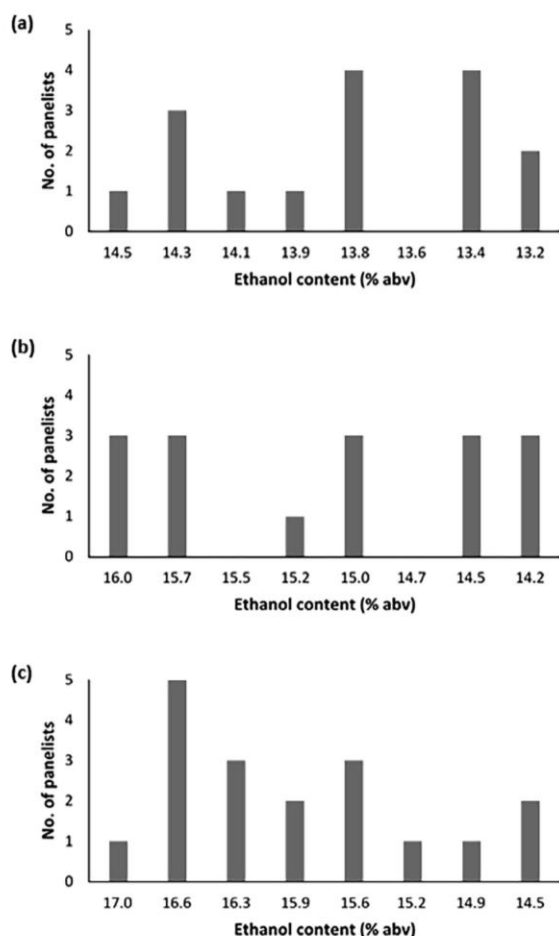


Fig. 3. Histograms showing sample preferences from alcohol sweetspotting trials for: (a) control and dealcoholized wine D, and blends thereof, spanning ethanol levels from 13.2 to 14.5% abv, presented in a circular ordered format; (b) control and dealcoholized wine E, and blends thereof, spanning ethanol levels from 14.2 to 16.0% abv, and (c) control and dealcoholized wine A, and blends thereof, spanning ethanol levels from 14.5 to 17.0%, presented in a 'knockout' format.

format, and on five occasions, the sample comprising 16.6% abv was found to be the most preferred wine (Fig. 3c). Chi-square goodness-of-fit and one proportion tests were again used to determine the statistical significance of the results (Table S2). However, despite the one proportion test returning a much lower *P* value of 0.065, for the 16.6% abv sample, the outcome was still not statistically significant.

Informal feedback from panelists indicated they preferred the knockout format over the circular, ordered format, finding it easier to perform. Ideally, additional alcohol sweetspotting trials using this format would have been undertaken with the other wines and/or with expert panelists (i.e., winemakers), but insufficient wine was available. Nevertheless, for winemakers who employ alcohol sweetspotting practices to optimize the ethanol content of wine, the knockout format and one proportion test may offer more rigorous approaches to alcohol adjustment.

4. Conclusion

This was the first study to consider the chemical and sensory

consequences of industrial scale RO-EP treatment of multiple wines (i.e., five commercial Cabernet Sauvignon wines). The RO-EP treatment did not meaningfully affect wine aroma and flavor, which was consistent with the relatively small compositional changes observed between wines before and after partial dealcoholization (with the exception of ethanol content). The perception of 'hotness' on the palate was significantly lower in most of the dealcoholized wines, as expected due to the removal of ethanol. In some instances, other palate attributes including 'body', 'acidity', 'bitterness' and 'astringency' were also modified, with the greatest sensory impact observed following partial dealcoholization of Wine A, where a 2.5% abv adjustment was made. The process used to optimize the ethanol concentration of selected wines with alcohol sweetspotting practices, i.e., to identify an ethanol concentration for which the wine exhibited superior overall sensory properties, was evaluated with the two different approaches. The methodology employed circular, ordered and knockout sample presentation, in an attempt to avoid the inherent bias associated with the presentation format typically employed during sweetspotting trials (i.e., presentation of samples in ascending order of ethanol content). The knockout format showed greater promise, and could be instituted by winemaker panels conducting sweetspotting trials. Nevertheless, further research should be undertaken with samples comprising smaller incremental differences in alcohol content, expert panelists (i.e., winemakers) and/or a broader range of wines to validate these methods and the existence of an alcohol sweetspot phenomenon.

CRediT authorship contribution statement

Duc-Truc Pham: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Renata Ristic:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Vanessa J. Stockdale:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition. **David W. Jeffery:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Jonathan Tuke:** Methodology, Formal analysis, Writing - review & editing. **Kerry Wilkinson:** Conceptualization, Methodology, Visualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was conducted by the Australian Research Council's Training Centre for Innovative Wine Production (ARC's TC-IWP, www.adelaide.edu.au/tc-iwp/), which is funded as part of the ARC's Industrial Transformation Research program (Project No IC130100005) with support from Wine Australia and industry partners. The authors gratefully acknowledge: VA Filtration for technical assistance with RO-EP dealcoholization of wines; the winemakers, staff and students who participated in sensory analysis; and Metabolomics Australia which is funded through Bioplatforms Australia Pty Ltd (BPA), a National Collaborative Research Infrastructure Strategy (NCRIS), and investment from the South Australian State Government and The Australian Wine Research Institute, for GC-MS analysis of fermentation volatiles.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2020.126869>.

References

- Baker, A. K., & Ross, C. F. (2014). Sensory evaluation of impact of wine matrix on red wine finish: A preliminary study. *Journal of Sensory Studies*, 29, 139–148. <https://doi.org/10.1111/joss.12089>.
- Bastian, S. E. P., Collins, C., & Johnson, T. E. (2010). Understanding consumer preferences for Shiraz wine and Cheddar cheese pairings. *Food Quality and Preference*, 21, 668–678. <https://doi.org/10.1016/j.foodqual.2010.02.002>.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Belisario-Sánchez, Y. Y., Taboada-Rodríguez, A., Marín-Iniesta, F., & López-Gómez, A. (2009). Dealcoholized wines by spinning cone column distillation: Phenolic compounds and antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl method. *Journal of Agricultural and Food Chemistry*, 57, 6770–6778. <https://doi.org/10.1021/jf900387g>.
- Brodison, K., & Bell, C. (2014). Cabernet fruit maturity and hang time. *Australian and New Zealand Grapegrower and Winemaker*, 610, 28–29.
- Ciatti Global Wine and Grape Brokers: Global Market Report. (2017) 8, pp. 1–21: www.ciatti.com/ciatti-reports/ Accessed September 19, 2019.
- Dequin, S., Escuyer, J.-L., Bely, M., Noble, J., Albertin, W., Masneuf-Pomarède, I., et al. (2017). How to adapt winemaking practices to modified grape composition under climate change conditions. *OENO One*, 51, 205–214. <https://doi.org/10.20870/oeno-one.2016.0.0.1584>.
- Diban, N., Athes, V., Bes, M., & Souchon, I. (2008). Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. *Journal of Membrane Science*, 311, 136–146. <https://doi.org/10.1016/j.memsci.2007.12.004>.
- Fedrizzi, B., Nicolis, E., Camin, F., Bocca, E., Carbognin, C., Scholz, M., et al. (2014). Stable isotope ratios and aroma profile changes induced due to innovative wine dealcoholisation approaches. *Food Bioprocess Technology*, 7, 62–70. <https://doi.org/10.1007/s11947-013-1068-x>.
- Fischer, U., & Noble, A. C. (1994). The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. *American Journal of Enology and Viticulture*, 45, 6–10.
- Francis, I. L., & Newton, J. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research*, 11, 114–126. <https://doi.org/10.1111/j.1755-0238.2005.tb00283.x>.
- Frayne, R. F. (1986). Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. *American Journal of Enology and Viticulture*, 37, 281–287.
- Gawel, R., & Godden, P. W. (2008). Evaluation of the consistency of wine quality assessments from expert wine tasters. *Australian Journal of Grape and Wine Research*, 14, 1–8. <https://doi.org/10.1111/j.1755-0238.2008.00001.x>.
- Goldner, M. C., Zamora, M. C., Di Leo Lira, P., Gianninoto, H., & Bandoni, A. (2009). Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *Journal of Sensory Studies*, 24, 243–257. <https://doi.org/10.1111/j.1745-459X.2009.00208.x>.
- Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K. M., & Ross, C. F. (2009). Chemical and sensory effects of saignée, water addition, and extended maceration on high Brix must. *American Journal of Enology and Viticulture*, 60, 450–460.
- Hranilovic, A., Li, S., Boss, P. K., Bindon, K., Ristic, R., Grbin, P. R., et al. (2018). Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-Saccharomyces inocula. *Australian Journal of Grape and Wine Research*, 24, 160–180. <https://doi.org/10.1111/ajgw.12320>.
- Lawless, H. T., & Heymann, H. (2010). Descriptive analysis. *Sensory evaluation of food: Principles and practices* (pp. 227–253). New York: Springer. <https://doi.org/10.1007/978-1-4419-6488-5>.
- Longo, R., Blackman, J. W., Antalick, G., Torley, P. J., Rogiers, S. Y., & Schmidtke, L. M. (2018). Volatile and sensory profiling of Shiraz wine in response to alcohol management: Comparison of harvest timing versus technological approaches. *Food Research International*, 109, 561–571. <https://doi.org/10.1016/j.foodres.2008.04.057>.
- King, E. S., Dunn, R. L., & Heymann, H. (2013). The influence of alcohol on the sensory perception of red wines. *Food Quality and Preference*, 28, 235–243. <https://doi.org/10.1016/j.foodqual.2012.08.013>.
- King, E. S., & Heymann, H. (2014). The effect of reduced alcohol on the sensory profiles and consumer preferences of white wine. *Journal of Sensory Studies*, 29, 33–42. <https://doi.org/10.1111/joss.12079>.
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82, 1–26. <https://doi.org/10.18637/jss.v082.i13>.
- Mattes, R. D., & DiMeglio, D. (2001). Ethanol perception and ingestion. *Physiology and Behavior*, 72, 217–229. [https://doi.org/10.1016/S0031-9384\(00\)00397-8](https://doi.org/10.1016/S0031-9384(00)00397-8).
- McRae, J. M., Ziora, Z. M., Kassara, S., Cooper, M. A., & Smith, P. A. (2015). Ethanol concentration influences the mechanisms of wine tannin interactions with poly(1-proline) in model wine. *Journal of Agricultural and Food Chemistry*, 63, 4345–4352. <https://doi.org/10.1021/acs.jafc.5b00758>.
- Olego, M. Á., Álvarez-Pérez, J. M., Quiroga, M. J., Cobos, R., Sánchez-García, M., Medina, J. E., et al. (2016). Viticultural and biotechnological strategies to reduce alcohol content in red wines. *Grape and wine biotechnology* (pp. 361–382). London: InTech Open. <https://doi.org/10.5772/64982>.
- Pham, D.-T., Stockdale, V. J., Wollan, D., Jeffery, D. W., & Wilkinson, K. L. (2019). Compositional consequences of partial dealcoholization of red wine by reverse osmosis-evaporative perstraction. *Molecules*, 24, 1404. <https://doi.org/10.3390/molecules24071404>.
- Pham, D.-T., Stockdale, V. J., Jeffery, D. W., Tuke, J., & Wilkinson, K. L. (2019). Investigating alcohol sweetspot phenomena in reduced alcohol red wines. *Foods*, 8, 491. <https://doi.org/10.1390/foods8100491>.
- Polášková, P., Herszage, J., & Ebeler, S. E. (2008). Wine flavor: Chemistry in a glass. *Chemical Society Reviews*, 37, 2478–2489. <https://doi.org/10.1039/B714455P>.
- Rayess, Y. E., & Miettton-Peuchot, M. (2016). Membrane technologies in wine industry: An overview. *Critical Reviews of Food Science and Nutrition*, 56, 2005–2020. <https://doi.org/10.1080/10408398.2013.809566>.
- Rolle, L., Englezos, V., Torchio, F., Cravero, F., Río Segade, S., Rantsiou, K., et al. (2018). Alcohol reduction in red wines by technological and microbiological approaches: A comparative study. *Australian Journal of Grape and Wine Research*, 24, 62–74. <https://doi.org/10.1111/ajgw.12301>.
- Schelezi, O. J., Šuklje, K., Boss, P. K., & Jeffery, D. W. (2018). Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. *Food Chemistry*, 259, 196–206. <https://doi.org/10.1016/j.foodchem.2018.03.118>.
- Schelezi, O. J., Antalick, G., Šuklje, K., & Jeffery, D. W. (2020). Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis. *Food Chemistry*, 309, 125698. <https://doi.org/10.1016/j.foodchem.2019.125698>.
- Schmidtke, L. M., Blackman, J. W., & Agboola, S. O. (2012). Production technologies for reduced alcoholic wines. *Journal of Food Science*, 77, R25–R41. <https://doi.org/10.1111/j.1750-3841.2011.02448.x>.
- Schmitt, M., Christmann, M., Jung, R., & Schübler, C. (2013). Enological/technical approaches to reduce elevated alcohol levels in wine (alcohol management) – Sensory results. *Bulletin de l'IVV*, 86, 485–492.
- Siebert, T. E., Smyth, H. E., Capone, D. L., Neuwohner, C., Pardon, K. H., Skouroumounis, G. K., et al. (2005). Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Analytical Bioanalytical Chemistry*, 381, 937–947. <https://doi.org/10.1007/s00216-004-2992-4>.
- Voilley, A., Beghim, V., Charpentier, C., & Peyron, D. (1991). Interactions between aroma substances and macromolecules in a model wine. *LWT Food Science and Technology*, 24, 469–472.
- Voilley, A., & Lubbers, S. (1998). Flavor-matrix interactions in wine. *Chemistry of wine flavor* (pp. 217–229). American Chemical Society. <https://doi.org/10.1021/bk-1998-0714.ch016>.
- Wollan, D. (2005). Controlling excess alcohol in wine. *Australian and New Zealand Grapegrower and Winemaker*, 20, 48–50.
- Wollan, D. (2006) The right tools for the job: Dealing with cool climate wine styles. In Proceedings of the 6th Cool Climate Symposium for Viticulture and Oenology, Auckland, NZ, February 2006, pp. 159–168.
- Wollan, D., Pham, D.-T., & Wilkinson, K. L. (2016). Changes in wine ethanol content due to evaporation from wine glasses and implications for sensory analysis. *Journal of Agricultural and Food Chemistry*, 64, 7569–7575. <https://doi.org/10.1021/acs.jafc.6b02691>.
- Zamora, F. (2016). Dealcoholised wines and low-alcohol wines. *Wine Safety, consumer preference, and human health* (pp. 163–182). Cham: Springer. https://doi.org/10.1007/978-3-319-24514-0_8.

Supplementary Data for

**Influence of Partial Dealcoholization on the Composition and Sensory Properties of
Cabernet Sauvignon Wines**

Duc-Truc Pham ^{1,2}, Renata Ristic ^{1,2}, Vanessa J. Stockdale ^{2,3}, David W. Jeffery ^{1,2},
Jonathan Tuke ⁴ and Kerry L. Wilkinson ^{1,2,*}

¹ The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond,
SA 5064, Australia

² The Australian Research Council Training Centre for Innovative Wine Production, PMB 1,
Glen Osmond, SA 5064, Australia

³ Treasury Wine Estate, 97 Sturt Highway, PMB 1, Nuriootpa, SA 5352, Australia

⁴ The University of Adelaide, School of Mathematical Sciences, Adelaide, SA 5000,
Australia

* Corresponding Author: Professor Kerry Wilkinson, telephone: + 61 8 8313 7360, facsimile:
+ 61 8 8313 7716, email: kerry.wilkinson@adelaide.edu.au

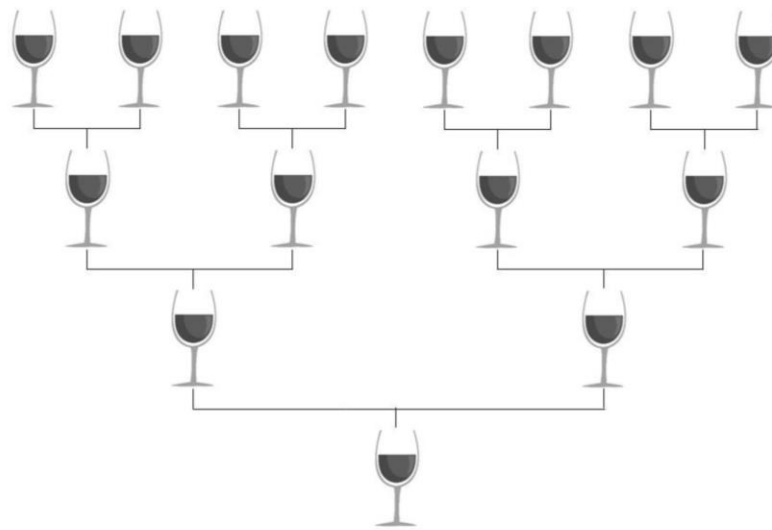


Figure S1. Knockout presentation format used in alcohol sweetspotting trial.

Table S1 Mean intensity ratings for sensory attributes of Cabernet Sauvignon wines before and after partial dealcoholization by RO-EP, and 50:50 blends thereof.

	Wine A			Wine B			Wine C			Wine D			Wine E		
	pre-RO-EP	blend	post-RO-EP	pre-RO-EP	blend	post-RO-EP	pre-RO-EP	blend	post-RO-EP	pre-RO-EP	blend	post-RO-EP	pre-RO-EP	blend	post-RO-EP
Aroma															
intensity	60	62	60	55	54	51	56	61	58	56	57	55	57	57	52
red fruit	40	36	40	36	41	40	38	40	42	37 b	41 ab	42 a	34	35	32
dark fruit	54 b	56 ab	59 b	53	50	51	54	57	54	52	53	54	47	49	43
dried fruit	49	47	46	38	36	35	39	38	34	39	37	40	39	41	32
jam	43	42	47	41	40	38	37	39	37	39	39	42	37	36	38
green	27 a	22 b	22 b	25	28	31	29	32	33	25	23	23	29	29	26
confection	25	27	27	24 ab	27 a	20 b	25	24	23	19 b	23 a	22 ab	21	16	20
sweet spice	32	29	28	23	23	21	28	31	29	24	25	26	24	26	25
chocolate	18	19	16	16 a	11 b	15 ab	17 a	11 b	9 b	16	14	12	13	15	11
earthy	16	22	18	16	16	14	15	17	16	16	16	14	17	18	16
Flavor															
intensity	57	56	57	56 a	51 b	49 b	54	54	53	52 b	56 a	51 b	55 a	55 a	47 b
red fruit	37	37	36	41	41	39	36	40	38	36	43	42	36	36	33
dark fruit	56	57	58	52 a	50 ab	48 b	55	53	53	49	52	53	50	51	46
dried fruit	47 a	46 ab	42 b	35	31	32	40	37	35	37	33	34	34	36	28
jam	42	43	40	38	35	38	36	37	33	36	36	38	36	36	39
green	30	26	26	25	28	27	26	28	30	25	22	25	27	25	28
confection	19	21	20	22 b	26 a	24 ab	18	21	21	18	19	21	18	16	15
sweet spice	28	30	26	26 a	24 ab	21 b	27	26	26	23	24	24	25	26	23
chocolate	14	14	14	12 a	10 ab	9 b	12 a	9 ab	7 b	13	11	12	11	10	10

earthy	17	21	16	13	14	14	14	14	14	19	15	15	18	17	16
Palate															
acidity	52 a	47 ab	45 b	49 a	43 b	50 a	47	44	42	51	50	49	51 a	43 b	50 ab
sweetness	27 a	21 b	20 b	26	23	25	25	28	24	22	22	25	25	26	22
bitterness	41	41	39	35 a	29 b	28 b	38 a	29 b	33 ab	41 a	34 ab	33 b	34	34	33
saltiness	23	20	20	21	19	16	20	19	19	20	20	20	28 a	21 b	21 b
hotness	63 a	59 ab	55 b	58 a	45 b	41 b	51 a	48 ab	42 b	50	47	51	55 a	54 ab	45 b
body	50	49	50	50 a	46 b	42 c	46 ab	48 a	41 b	47	46	42	48	47	45
astringency	62 b	66 ab	70 a	51	48	45	52 a	47 ab	46 b	52	53	52	45	51	48

Values followed by different letters within rows (for each wine) are statistically significant ($P=0.05$, one-way ANOVA).

Table S2 *P* values calculated for chi-square goodness of fit and one proportion tests of preference data from alcohol sweetspotting trials; the proportion of preferred samples are shown in brackets.

Sample	Presentation	Goodness of fit test	One proportion test
Wine A	knockout	<i>0.540</i>	<i>0.065</i> (5/18)
Wine D	circular, ordered	<i>0.333</i>	<i>0.130</i> (4/16)
Wine E	circular, ordered	<i>0.429</i>	<i>0.323</i> (3/16)

Chapter 5

Summary and Conclusion



In summary, this research investigated compositional and sensory perception changes in red wines resulting from ethanol removal by RO-EP. In Chapter 2, a series of trials conducted to investigate the impact of ethanol removal by reverse osmosis–evaporative perstraction (RO-EP), are reported. Red wines were partially dealcoholised by RO-EP, such that their alcohol contents were reduced by 1.0–2.4 % abv. Wine composition was analysed: (i) pre-RO-EP, (ii) post-RO-EP and (iii) post-RO-EP following alcohol re-adjustment. In Chapter 3, sensory trials were undertaken (with a panel comprising winemakers who were familiar with the practice of alcohol sweetspotting) to investigate the basis for sweetspotting and the influence of sample presentation on the panel’s performance. Different methods of presenting wine samples (ordered vs. randomised, and linear vs. circular) were employed to determine to what extent presentation order influenced the outcome of alcohol sweetspotting trials. Two different statistical analyses, i.e. chi-square goodness of fit vs. one proportion tests, were also evaluated. In Chapter 4, descriptive analysis trials were undertaken to investigate changes in sensory profiles of five Cabernet Sauvignon wines following their partial dealcoholisation by RO-EP. Wine composition was also determined.

Key Findings

The research presented in this thesis demonstrates that removal of ethanol from wine by RO-EP influences both wine composition and sensory properties, but that changes depend on the initial style and profile of the wine being treated, including the initial level of ethanol. In general, the removal of ethanol by RO-EP resulted in the concentration of wines; whilst some volatile compounds were lost through membrane filtration, there were no significant changes in pH, TA, or VA, albeit small changes in wine density and viscosity were observed. Due to the influence of ethanol on the physical properties of wine, some significant effects on wine colour and astringency were observed, probably as a consequence of increased

concentrations of phenolic compounds, and the interaction of these compounds with ethanol. At lower levels of ethanol (i.e. following partial dealcoholisation), the chemical equilibrium of wine was also affected, such that wines with higher levels of residual sugars required the addition of SO₂ to prevent further fermentation. Hydrolysis of ethyl esters may also have occurred due to shifts in the chemical equilibrium between acids, alcohols (especially ethanol) and esters, following partial dealcoholisation of wine.

RO-EP treatment of wine had only small effects on the perceived intensity of aroma and flavour attributes, which was consistent with the small changes observed in both basic wine composition and the concentrations of volatile compounds. However, the perception of hotness on the palate was notably affected. In some wines, the perception of body, acidity and bitterness was diminished, while the perception of astringency increased. These impacts on wine composition and sensory properties suggest that it is necessary for winemakers to optimise not only the final levels of alcohol in RAW, but to ensure the finished product achieves the best overall expression of sensory characteristics.

Several approaches to alcohol sweetspotting were evaluated in this study and were intended to determine the importance of sample presentation and panel composition to the outcome of sweetspotting trials. Although there was limited evidence of the existence of an alcohol sweetspot, findings suggest the most reliable alcohol sweetspotting methods involve either: (i) the circular-ordered format: i.e. the presentation of samples in order of increasing alcohol content, in which the samples containing the lowest and highest alcohol levels are not visually identifiable (as occurs in a linear or row format); or (ii) the 'knockout' format: in which panellists evaluate a series of paired samples, with only the sample perceived to exhibit the 'best' combination of sensory properties being retained for further evaluation, until only a single sample, being the 'alcohol sweetspot' remains. The most appropriate method of statistical analysis for sweetspotting data is then the one proportion test.

Research Limitations

There were some limitations to this study that should be acknowledged and taken into account for any future research. Firstly, the red wines used in the study were young, high in alcohol and quite astringent, which almost certainly made it more difficult for panellists to identify the alcohol sweetspot. Performing sweetspotting trials on either white wines, or wines with less apparent hotness and/or astringency, may have made it easier for the panellists; i.e. may have yielded more reproducible results. However, young, hot and astringent red wines are, by their very nature, most in need of alcohol management. Secondly, recruitment of winemakers with experience in alcohol sweetspotting was an ongoing challenge. Ideally, sweetspotting trials would have been conducted with a panel comprising 50 or more winemakers, but logistically this wasn't possible. Nevertheless, given the incidence of higher alcohol wines that require some degree of alcohol correction, full-bodied red wines in particular, and the significant amount of time required to optimise alcohol levels using RO-EP, the results from this study are expected to be of particular interest and relevance to winemakers intent on achieving reduced alcohol wines.

In conclusion, this research has demonstrated the potential for the wine industry to employ membrane technologies to achieve reduced alcohol wines with improved aroma, flavour, taste and mouthfeel attributes, so as to appease consumer demand for low alcohol wines. Importantly, this approach overcomes issues associated with the production of low alcohol wines from early-harvested fruit, i.e. the tendency for these wines to exhibit unripe fruit characters, high levels of acidity and harsh tannins. Winemakers can instead harvest fruit at optimal flavour ripeness, and mitigate any apparent hotness arising from excessive levels of alcohol by ethanol removal, whilst retaining aroma and flavour intensity.

Future Directions

There is still additional research that could be undertaken to further understand the impact of ethanol removal on the composition, quality and acceptance of RAW, as well as sensory and chemical analyses that could be used to validate the alcohol sweetspot phenomenon. The use of different membranes for the fractionation of wine and/or the recovery of volatile compounds from the strip water following evaporative perstraction could be investigated, in addition to the application of RO-EP treatment to other wine styles (including white wines).

Whereas the current research focused on changes in the concentrations of volatile esters, acids and alcohols, more detailed volatile analysis could be explored, to investigate the effect of partial dealcoholisation on a broader range of grape-derived volatile compounds, such as terpene alcohols and C13-norisoprenoids, as well as polythiols. Analysis of these compounds might explain the changes in wine sensory properties, i.e. in the intensity of 'red fruit', 'dark fruit', 'green', 'confectionery' and 'chocolate' attributes, observed by descriptive analysis.

In terms of alcohol sweetspotting trials, the knockout format showed greater promise and could be readily implemented by winemakers who routinely employ alcohol adjustment. Further trials need to be undertaken to validate the alcohol sweetspot phenomenon; for example trials involving: samples comprising smaller incremental differences in alcohol content; panels comprising experts (i.e. winemakers) or larger numbers of consumers, assessing a broader range of wines; and/or assessment of wines of even lower alcohol content (i.e., samples from which greater quantities of ethanol have been removed). It is also entirely possible that the alcohol sweetspot phenomenon does not exist, in which case industry needs to consider other approaches to optimising the alcohol content of their wines. Consumer acceptance trials could also be considered to better define the RAW styles that meet the specific needs and expectations of those segments of the consumer market who are

specifically seeking wines of lower alcohol content. The findings from future studies will continue to inform winemaking decisions concerning the management of wine alcohol content.
