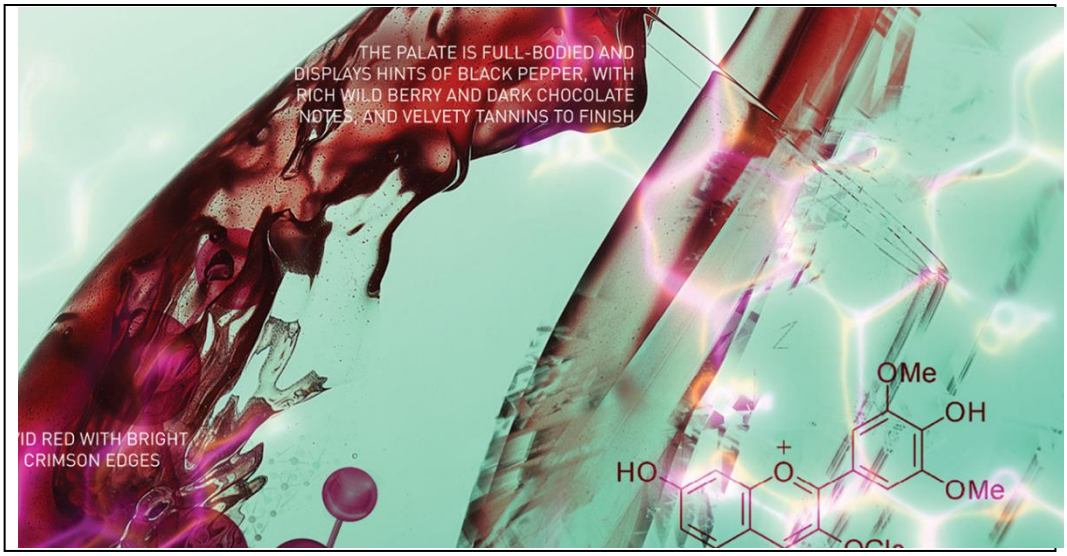
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THE UNIVERSITY of ADELAIDE

TRANSLATION OF "WHOLE-OF-PRODUCTION-CHAIN" WINE SCIENCE RESEARCH TO INDUSTRY OUTCOMES



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

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UA 1304: TRANSLATION OF "WHOLE-OF-PRODUCTION-CHAIN" WINE SCIENCE RESEARCH TO INDUSTRY OUTCOMES

The ARC TC-IWP Integrated Strategy for Flavour and Alcohol Modulations

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1. ABSTRACT

The Australian Research Council (ARC) Training Centre for Innovative Wine Production (TC-IWP) was established at The University of Adelaide with support from industry partners and Wine Australia. It linked scientific and industrial expertise, contributions and facilities of two universities, four scientific organisations and six industry partners. The research of the ARC TC-IWP focused on strategies to reduce the alcohol content of wine through an integrated, whole-of-production-chain approach that starts in the vineyard, continues through fermentation and post-fermentation and finishes with wine consumers. The projects involved in the TC-IWP investigated: early harvest and blending regimes, the use of *Saccharomyces* and non-*Saccharomyces* yeast strains, the individual or combined addition of commercially available winemaking additives and the effects of reverse osmosis/evaporative perstraction (RO/EP) treatment on wine composition and sensory properties. Research into the alcohol 'sweet spot' phenomenon and consumers' acceptability for lower alcohol wines is also being conducted. The outcomes have been compiled in the integrated strategy for alcohol and flavour modulations which aimed to deliver new tools or to optimise existing viticultural and winemaking practices to enhance the quality of grapes and wines and define market and consumer preferences for wines with a lower alcohol level.

1. EXECUTIVE SUMMARY

In last three decades the average alcohol level for Australian red wines has risen from 12.4% up to 14.4% v/v, which has negative financial implications for winemakers due to higher taxes and increased retail prices. The observed trend in an increasing level of alcohol is partially due to a hotter climate and improvements in viticultural and winemaking practices. Hotter and shorter vintages also put a lot of strain on wineries, which may struggle to process larger amounts of fruit in a very short period. Higher alcohol wines are also driven by winemaker and consumer fondness for aromatic, full-bodied wines. However, there is a growing interest among wine consumers for wines with lower alcohol content driven by health concerns and social issues associated with greater consumption of alcohol (feeling out of control, hangover, not able to drive after drinking) and wine and food pairing. As a result many winemakers are seeking methods to decrease the alcohol content of their wines, without significant effects on wine quality.

The ARC TC-IWP has taken an integrated, 'whole-of-production-chain' approach to modulate alcohol levels in wines by developing new and/or evaluating existing viticultural and winemaking methods and techniques either before, during or after fermentation. The following is a summary of the key project areas:

Grape berry cell death

Grape berry cell death is accelerated by impacts of warmer temperatures and elevated evaporative conditions during ripening period, so it is important to understand the mechanisms underlying cell death in order to devise mitigation strategies. The investigations included the physiological aspects (oxygen deficiency and loss of cell vitality) and molecular events (reactive oxygen species (ROS) and ubiquitination in programmed berry cell death) under control, drought and heat stress conditions. During the ripening period the beginning of cell death in berries corresponded to a decreased internal oxygen concentration [O_2] and increased levels of ROS. Elevated temperature and water stress accelerated cell death in Shiraz berries and under water deficit berry respiration rate also decreased. A list of genes (e.g. *VvBAG1* and *VvLOXA*) that could be linked to ROS signalling and programmed cell death were identified and VvPub13 was observed to reduce H_2O_2-induced cell death by inducing genes involved in anti-oxidant responses. The results suggested that bunch shading and irrigation to manipulate plant hydration status may reduce berry cell death and rates of weight loss in Shiraz berries.

The sugar-potassium nexus within the grapevine

For the first time the sugar-K^+ relationship was illustrated in individual grape berries and in the grape berry tissues. Potassium and sugar accumulation were closely correlated in the berry pulp, skin and seeds, especially from véraison onwards. However, the sugar content increased more rapidly than K^+ with a ten-fold difference observed at harvest. There was also extensive plasticity from berry to berry in the ratio between the two components. A strong ternary relationship between berry K^+, sugar and water content was also evident. Rootstock selection and mild water stress through deficit irrigation strategies can be considered for reducing potassium uptake, but this approach is complex due to variety differences, climatic variations and soil variability and warrants further research.

Optimisation of an early harvest and blending regime

An early harvest regime and a blending approach were investigated by i) using either "green harvest wine" (GHW) or water to substitute for some juice prior to fermentation and ii) 'double harvest' or blending early and late harvest wines. Large decreases in final wine alcohol concentration (3% v/v) could be readily achieved purely through a pre-fermentation approach. Adjusting the alcohol level via juice substitution led to mostly marginal changes in wine colour density, anthocyanin and phenolic composition as well as wine sensory properties in Shiraz and Cabernet Sauvignon wines. Even in a compressed vintage with a high percentage of Cabernet Sauvignon berry shrivel (2015 vintage) the blending treatments showed promise to produce wines with moderate alcohol concentrations without significantly alternating its quality parameters. In comparison to GHW, the implementation of water was found to be the better way to decrease wine alcohol content due to its ubiquitous availability, low cost and minimal impact on wine composition.

The 'double harvest' method of blending early and late harvest final wines may also reduce alcohol level without significant implications on wine flavour profile. Verdelho and Petit Verdot blends maintained sensory profiles similar to those of the wines made from more mature fruit despite being prepared from less ripe grapes. However, dealcoholisation of late harvest wines of Petit Verdot, Verdelho and Shiraz by using a combined reverse osmosis-evaporative perstraction (RO-EP) process to the same alcohol levels as an early harvest (up to -6% v/v) had a significant effect on some volatile compounds such as esters and high alcohols, but not on others (e.g. monoterpenes and C13-norisoprenoids). Changes in the volatile composition of wines reflected in sensory properties of all wines, but it appears that Verdelho wines were the most affected. Interestingly, 13.5% v/v Shiraz wines produced by selecting a specific harvest date, blending or dealcoholisation did not differ for any of the sensory attributes examined. Although similar trends were observed for different varieties, prediction of the dealcoholisation effects on the sensory profile of wines is very difficult due to the complex matrix of the initial wine and the dealcoholisation operating conditions. On another side, the use of a blending practice on wines produced from different harvest dates is an easy-to-adopt, flexible and cost-effective alternative to dealing with increasing levels of alcohol.

Yeast strains in alcohol management and flavour enhancement

The use of yeasts strains capable of yielding lower ethanol in the fermentation is of high interest, as it is does not require additional labour, equipment or handling. Research in this project focused on selection, characterisation and improvement of non-*Saccharomyces* yeasts for lowering ethanol in wines, whilst enhancing the sensory properties. Firstly, all (to date) commercially available non-*Saccharomyces* starter cultures were evaluated for their performance in earlier and later harvested Shiraz fruit. The purpose was to test whether the commercial (thus readily-implementable for the industry) non-*Saccharomyces* treatments have the potential to boost quality of sub-optimally ripe grapes, and/or could they lead to ethanol decrease in later harvest. In comparison to the *S. cerevisiae* control, enhanced sensory attributes were noted in earlier harvest wines fermented with non-*Saccharomyces*. However, these treatments were also related to an increased risk of arrested fermentation in higher ripeness conditions. In parallel, characterisation of isolates from un-inoculated

grape fermentations in South Australia highlighted a *Metschnikowia pulcherrima* isolate that, in conjunction with *S. cerevisiae*, was capable of significantly lowering wine ethanol content (1.2% v/v in white grape juice) compared to the *S. cerevisiae* monoculture. The ethanol decrease was achieved across a number of conditions without any apparent off-flavour production. Finally, a genetic diversity study of *Lachancea thermotolerans* was conducted revealing a grouping of isolates based on their geographic origin and isolation habitat. A subset of isolates (94) was further evaluated for their oenological potential in Chardonnay fermentations. They showed great potential in terms of lower ethanol yield and biological acidification due to lactic acid production.

To produce wines with distinctive characteristic winemakers may utilise spontaneous fermentations using 'wild' yeasts (non-*Saccharomyces*) in order to impart some desirable characteristics via a specific intermediates of their metabolic pathways and/or end products. However, the final product is hard to predict, so the aim of this project was to determine the factors influencing the success of spontaneous fermentation in a high sugar environment. Twenty yeast strains were isolated from an in-house collection and tested for physiological responses to osmotic and ethanol stress. Yeast reaction to osmotic stress was clear and similar for all the evaluated strains, while ethanol stress appeared to be more challenging and the responses were more differentiated and genera/strain specific. *Torulaspora delbrueckii* showed high potential as an alternative to an *S. cerevisiae* monoculture during wine fermentation, but its performance in a mixed population in a high sugar environment needs to be investigated further in order to allow winemakers to use this yeast species in an informed manner for modulating sensory profile of wines.

Winemaking techniques for alcohol management and flavour enhancement

Additives, such as oenological tannin and mannoproteins, can be used to improve mouthfeel and consequently quality of lower alcohol wines. However, a greater understanding of the compositional consequences of tannin and mannoprotein (MP) additions, and their interactions in the wine matrix, are needed. Trials involving the addition of commercial additives to 'early' and 'late' harvest Shiraz wines were undertaken, but the outcomes were inconclusive, likely due to the large compositional variation amongst the commercial additives. As a consequence, the composition of 14 grape-based oenotannins and 8 MPs were profiled. Analysis showed that some products exhibited compositions in agreement with the labelled origin of material (i.e. grape seed and/or skin), while others did not. Furthermore, some products were marketed under different names for different oenological purposes, but their compositions were actually quite similar; with the same products marketed by different manufacturers (under different labels) showing significant compositional differences. Based on those results, a subset of tannins and mannoproteins was selected and introduced into wine in different combinations and at different concentrations. However, no significant effect on wine body or astringency was perceivable by sensory analysis. It remains unclear if the difference in tannin levels between treatments was too subtle for the sensory panel to detect, or if the panel needed more training to achieve higher sensitivity. However, significantly different interactions between two selected mannoprotein products and tannin were observed, suggesting that addition of polysaccharide fractions could modify wine polyphenolic composition. Preliminary trials may therefore be required during winemaking to determine the outcomes of their addition in particular wine matrices.

Trials were conducted to investigate the impact of alcohol removal by reverse osmosis–evaporative perstraction (RO-EP). Although the applied technique showed some impacts on wine chemical and sensory properties, they were not detrimental. Some volatile compounds were lost through membrane filtration, which may be considered as the major drawback of this technique. However, only small effects on wine aroma and flavour were observed, which was consistent with the small changes observed in basic wine composition and wine volatile profiles. The perception of hotness was most affected in all wines, but in some wines, body, acidity and bitterness were reduced, while astringency increased, suggesting that the impact of RO-EP depends on the initial alcohol level and wine composition. The

'sweetspot' phenomena was investigated, but for now remains undefined due to the time-consuming nature of the exercise, which required involvement of a large number of winemakers with experience in alcohol 'sweetspotting'.

Novel techniques for flavour enhancement

A novel method of using magnetic molecularly imprinted polymers (MMIPs) to specifically remove methoxypyrazines (MPs) from wines is being developed. A range of molecularly imprinted polymers (MIPs) were synthesised for comparison with their magnetic counterparts and non-imprinted polymers (NIPs), and trialled in Cabernet Sauvignon grape must spiked with 3-Isobutyl-2-methoxypyrazine (IBMP). Chemical and sensory evaluation of wines arising from MMIPs and MNIPs treatments showed the polymers could effectively decrease green sensory characters without largely compromising overall aroma intensity of the wines, especially when added pre-fermentation. However, this novel and promising technique needs further improvement of the efficiency and specificity of MMIPs before they can be used in a commercial context.

The use of cyclodextrins for enhancement of wine sensory profiles has been trialled for the first time, despite their wide use in the food industry for removal/delivery of flavour compounds and modification of mouth-feel and taste. α-, β-, and γ-cyclodextrins were added to model, white and red wines spiked with volatile phenols associated with smoke taint and *Brettanomyces* spoilage: guaiacol, 4-methylguaiacol, 4-ethylphenol, 4-ethylguaiacol, ortho-cresol, meta-cresol, para-cresol and eugenol. β-cyclodextrin, with the strongest hydrogen bonding capability, gave the best results in terms of binding volatile phenols. Sensory analysis confirmed a significant reduction in off-flavours after cyclodextrin addition, although some loss of other wine aroma compounds, particularly long chain acids, was also observed. Further work is underway.

Biochemical response of grapevines to smoke exposure

Smoke taint research has largely focused on the chemical and sensory consequences of vineyard exposure to smoke while molecular and biochemical events underlying smoke uptake in berries has received less attention. RNA sequencing of potted Shiraz and Chardonnay grapevines exposed to smoke indicated higher expression of heat shock proteins and glucosyltransferases in smoke affected berries, compared to control berries. Four glucosyltransferases (GT) that yielded higher expression in both varieties and one additional GT that previously showed preferential activity towards smoke derived volatile phenols were selected for further investigation. This study also showed that the volatile phenol glycoconjugate profiles of smoke-affected grapes was variety dependent, with Merlot showing higher levels of glycoconjugates compared to Sauvignon Blanc and Chardonnay. The application of agrichemicals (i.e. kaolin, a particulate clay and Envy, a polymer-based anti-transpirant), prior to smoke exposure did not significantly affect the volatile phenol glycoconjugate profiles in Sauvignon Blanc, Chardonnay and Cabernet Sauvignon, but kaolin provided some protection for Merlot grapes after foliar treatment.

Consumer acceptance of lower alcohol wines

Wine is a very traditional product with high symbolic value, but recent trends in wine consumers' behaviour support modifying (reducing) alcohol levels, either by partial or complete dealcoholisation. Existing lower/low alcohol wines have not been very successful due to people experiencing these wines as less traditional, less complex and without varietal character. This study examined whether the intrinsic innovation of a product will elicit a stronger influence on perceived authenticity when the product is traditional rather than not traditional. The preliminary exploratory approach towards low alcohol wines, involving twelve focus groups and wine tastings, was conducted in Indonesia, where wine is not a traditional product, Australia, where wine consumption is part of the culture and in France where wine is considered as a very traditional product. Overall results indicated that Indonesian participants are more open to consuming low/no alcohol wine and still consider the product to be wine

in contrast to Australian and French participants, who reacted more negatively to the product innovation and did not consider the product to be wine. Quantitative results indicated that traditionality perceptions influence perceptions of authenticity, which in turn significantly influences purchase intention.

2. BACKGROUND

Alcohol levels in wines have been increasing in recent decades largely due to climate change and improvements in viticultural practices and winemaking techniques (Pickering 2000). In the last three decades there was a rise of more than 9% in the alcohol content of Californian wines (Alston et al. 2011). In Australia alone, the average alcohol level in red wines has risen steadily from 12.4% to 14.4% (Godden and Muhlack 2010, Varela et al. 2015). This trend was also driven by winemakers' and consumers' fondness for riper grapes, which make more aromatic and full-bodied wines (Wilkinson and Jiranek 2013). However, this has negative financial implications for winemakers due to higher taxes and increased retail prices. Concurrent to this trend, however, is a growing market interest in reduced alcohol beverages (Bruwer et al. 2014). These products include and can be classified as de-alcoholised or alcohol-free (< 0.5% v/v), low alcohol (0.5% - 1.2% v/v), reduced alcohol (1.2 % - 5.5/6.5 % v/v) and lower alcohol wine (5.5% - 10.5% v/v), although these categories vary between countries based on legislation (Pickering 2000, Saliba et al. 2013a). There is also a wine consumer demand for lower alcohol wines, driven by health concerns and social issues associated with greater consumption of alcohol (feeling out of control, hangover, not able to drive after drinking) and wine and food pairing (Meillon et al. 2010b, Saliba et al. 2013a, Bruwer et al. 2014).

High alcohol levels can affect must fermentation and wine sensory perception. High alcohol levels are positively associated with bitterness (Fischer and Noble 1994, Vidal et al. 2004a) and hotness (Gawel et al. 2007), may affect viscosity (Nurgel and Pickering 2005, Runnebaum et al. 2011) and enhance sugar sweetness in wine (Nurgel and Pickering 2005, Zamora et al. 2006). Higher alcohol may also reduce astringency elicited by grape seed tannin (Vidal et al. 2002, Fontoin et al. 2008) and affect aroma intensity by altering distribution coefficients between the aqueous solution and the headspace of volatile compounds (Escudero et al. 2007, Goldner et al. 2009). Higher alcohol wines are usually characterised with intense 'hotness' and 'overripe fruit' characters with modified district varietal attributes (de Orduna 2010).

Many winemakers are seeking methods to decrease the alcohol content of their wines, without significant effects on the concentrations of other compounds associated with wine quality. Different approaches have been explored to produce wines with lower ethanol content (as reviewed in (Varela et al. 2008, Schmidtke et al. 2012, Palliotti et al. 2014) and they can be grouped into four main strategies:

1. Viticultural practices, such as the reduction of the sugar content of the grape in the vineyard either by manipulating the ratio leaf area to fruit weight (LA/FW) (Palliotti et al. 2013, Poni et al. 2013, Parker et al. 2015a, 2015b) and/or early and sequential harvests, in some cases including juice dilution, freezing concentration and fractionation, and blending regimes of juices from these (Pickering 2000, Kalua and Boss 2009, Kontoudakis et al. 2011, Bindon et al. 2013, Fedrizzi et al. 2014);
2. Pre-fermentation methods include treatments of grape juice with glucose oxidase enzyme (to lower glucose concentrations and consequently alcohol levels in the finished wines) (Pickering et al. 1999a, 1999b, 1999c) and applications of meso-porous membranes, evaporation under-vacuum or nanofiltration (Arriagada-Carrazana et al. 2005, Vincze et al. 2006, Kozak et al. 2008, Massot et al. 2008);
3. During-fermentation methods include early arrest of fermentation (Pickering 2000), methods for extraction of ethanol (Aguera et al. 2010) and the use of modified yeast strains (Palacios et al. 2007, Tilloy et al. 2015);

4. Post-fermentation methods include applying technological protocols to decrease the ethanol level of the final wine using non-membrane extraction such as ion exchange and a spinning cone column (Sykes et al. 1992, Makarytchev et al. 2005, Schmidtke et al. 2012) and membrane protocols including reverse osmosis procedures (RO) (Bui et al. 1986, Pilipovik and Riverol 2005), evaporation perstraction (EP) (Diban et al. 2008, Varavuth et al. 2009), pervaporation (Vatai et al. 2007), RO with EP (Wollan 2005) or RO with nanofiltration (Labanda et al. 2009, Catarino and Mendes 2011).

The ARC Training Centre for Innovative Wine Production (TC-IWP) was awarded $3 million from the Australian Research Council (ARC) and industry support to tackle some of the major problems facing the wine industry including rising wine alcohol content and changing wine consumers' preferences. The research of the ARC TC-IWP focused on reduction of alcohol levels in wines through an integrated whole-of-production-chain approach that started in the vineyard, integrated vinification and post-vinification, and finished with wine consumers. The TC-IWP projects investigated berry cell death, blending regimes, the use of *Saccharomyces* and non-*Saccharomyces* yeast stains, individual or combined additions of commercially available additives (e.g. pre-fermentation maceration enzymes, mannoproteins and tannins) and the effects of RO/EP treatment on reduced alcohol wine composition and sensory properties. The research into the alcohol 'sweet spot' and wine consumers' acceptance and liking of wines with lower alcohol content has also been conducted.

The Centre aimed to deliver new tools and develop new techniques for mitigation of undesirable compounds, while enhancing wine chemical and sensory profiles. Excess amounts of methoxypyrazines (MPs), compounds known to be responsible for green characters such as vegetative, herbaceous and capsicum-like flavour in grapes and wines, could be detrimental for quality of Sauvignon Cabernet (King et al. 2011), thus a method for their management and/or removal has been widely sought from winemakers from all over the world. Bushfires can also cause considerable loss as vineyard exposure to bush fires and smoke result in smoke taint in wines which makes wines faulty and not suitable for sale.

The main aim of the UA 1304 project was to compile the outcomes of viticultural and winemaking methods and techniques from a range of TC-IWP projects into an integrated strategy for flavour and alcohol modulation. The TC-IWP projects were grouped under the following themes:

a. Berry shrivel and grape berry cell death
b. The sugar:potassium nexus within the grapevine
c. Optimisation of harvest blending regimes
d. Yeast strains in ethanol management and flavour enhancement
e. Techniques for ethanol management and flavour enhancement
f. Novel techniques for flavour enhancement
g. Biochemical response of grapevine to smoke
h. Wine innovation and the importance of authenticity

The integrated strategy for flavour and alcohol modulation will help the wine industry to tackle challenges currently created by environmental and social changes.

3. PROJECT AIMS AND PERFORMANCE TARGETS

Project UA1304 was directly involved in the conduct of research activities that utilised expertise and facilities of two universities (University of Adelaide and Charles Sturt University) and thirteen industry partners by coordinating and facilitating collaboration between 16 TC-IWP projects and driving the translation of TC-IWP research outputs into industry-ready applications.

Building on collaborative research the project specific objectives were:

1. To determine the cumulative magnitude of change that can be achieved via a series of incremental modulations across a range of viticultural and winemaking techniques including:

a. Viticultural practices that may affect sugar accumulation in berries, reduce berry cell death, and minimise intake of taint compounds in the grape. A series of harvest blending regimes across several varieties and vineyards were compared for key compositional features.
b. Fermentation techniques to remove sugar prior to fermentation, divert sugar away from alcohol, improve the reliability and reduce the duration of high sugar fermentations using pure and mixed culture of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast strains
c. Post fermentation techniques for alcohol reduction (reverse osmosis/evaporative perstraction and/or enchaining wine quality
d. Consumer studies to define consumer perceptions and preferences for lowered-alcohol wine and, in new markets, for Australian wines generally, and use this knowledge to inform the production process

2. To collate, interpret and summarise research outcomes from all TC-IWP projects in an integrated strategy for flavour and alcohol modulation.
3. To provide support to HRDs, PDFs, PIs and CIs to maximise benefits of shared resources and build a strong and compact team around wine and grape research at the TC-IWP.
4. To disseminate outcomes through various extension mechanisms such as articles in peer-reviewed or industry journals, industry reports, industry seminars, workshops, and domestic and international symposia.

4. METHOD

A wide range of methods have been used in various TC-IWP projects. In some cases, small scale winemaking was conducted, and grapes and wines were analysed for chemical composition, flavour and sensory profiles, while other projects required gene expression studies, therefore material and methods were briefly described for each project below.

5. RESULTS AND DISCUSSION

Many outcomes from the TC-IWP projects project have been published in scientific, industry journals or conference proceedings, hence, they are under copyright restriction. In this report, wherever applicable, reference is made to the relevant publications to prevent breaking copyright.

6.1. Berry shrivel and grape berry cell death

6.1.1. Cell death in the berry and berry weight loss

Introduction

Mesocarp cell death is important to the production of lower alcohol wines because vital grape berry cells are thought to be necessary for maintaining a high water content in the fruit. Vital berry cells promote water inflow from the parent plant, compensating for water lost to the atmosphere through the berry surface. Vital cells are also thought to retain water in the grape berry that would otherwise be drawn back to the parent plant to help satisfy the water requirements of the canopy. In the absence of vascular inflows, a lack of vital cells will decrease the fruit water content and increase the concentration of berry solutes. The concentrated sugars resulting from these processes are anticipated to increase the alcohol content of wine.

Previous research suggests that programmed cell death (PCD) takes place at a specific developmental stage, but its onset and severity can also be influenced by biotic and abiotic factors. Warmer temperatures and elevated evaporative conditions during ripening may hasten the onset of cell death and berry water loss and the aim of this project was to further characterize the role of heat accumulation on cell vitality. During this project two experiments were completed: (1) a cuvette-based method was developed to assess rates of water vapour efflux from berries, and (2) an experiment on berry exposure was used to further characterize the effect of temperature on loss in cell vitality.

Materials and methods

Experiment 1: An LI-6400XT portable photosynthesis system manufactured by LI-COR (Lincoln, Nebraska) equipped with an opaque cuvette (LI-6400-22) and light source (LI-6400-18) designed to measure gas exchange from conifer leaves was modified to house grape berries. The system was pre-tested to ensure that it was capable of accurately estimating the water vapour efflux from simple samples such as a vial of deionised water. Grape berries were cut from the bunch and the pedicel was covered with heat shrink tubing to prevent transpiration from this area. The temporal responses of fruit transpiration and surface temperature to changes in the block temperature were assessed as was the response of fruit transpiration to temperature-induced increases in the vapour pressure deficit. Comparisons of measured versus modelled decreases in sample mass during the course of experimental runs were also made.

Experiment 2: Potted Shiraz vines grown in an outdoor bird-proof enclosure were pruned to three shoots, each carrying a bunch of approximately 100 berries. Exposed and shaded sampling sites on each bunch were identified and thermocouples within the bunch were used to monitor temperature at these sites. Light-sensitive acetylcellulose film was used to assess radiation input and GDD (growing degree days) at the berry centre was modelled. Berries from the different exposure sites were sampled during the ripening period and assessed for fresh weight, TSS, skin albedo, transpiration rates and cell vitality using fluorescein diacetate staining.

Results and discussion

The first set of observations in 2014/2015 did not demonstrate berry dehydration in the lead up to harvest. This result was most likely a response to repeated rain events during the ripening period as the rainfall provided the fruit with an exogenous water source and enabled berries to continue accumulating dry matter several weeks after the typical harvest point. The rainfall enabled the berry water budget to be decoupled from the normal developmental trends in, and interactions between, berry and whole plant physiology. Grape bunches in this set of observations and subsequent experiments also exhibited a surprising and unwanted degree of variability in cell vitality, particularly once mean cell vitality had progressed below 90 %.

In the 2015/2016 season a field-portable system designed for conifer leaves was successfully modified to a lab-based method to monitor transpiration rates of fruits. Low transpiration made the system sensitive to noise, but this was reduced to some degree by signal averaging, increasing the transpiring surface area and sourcing reference air with a stable (or slowly changing) water vapour concentration. Leakage of water vapour from the cuvette was minimised by bagging the sensor head. Improvements in precision and accuracy were obtained by utilising fruit pre-conditioned to the initial operating temperature of the system, reducing the size of stepped increases in system temperature, and eliminating the use of artificial light sources to minimise thermal gradients across fruit surfaces.

Counter to expectations and previous research (Bonada et al. 2013a, 2013b, Caravia et al. 2016), light interception did not result in differences in mesocarp cell vitality of berries until several weeks after berries were deemed suitable for harvest. Therefore, in this study, temperature of the grape berry over the ripening period was not a strong predictor of the extent of cell vitality at harvest. This may be because (a) the temperature differences between exposed and shaded parts of the bunch were not stark enough to result in differences in cell vitality, or (2) the previous studies used to manipulate cell vitality were conducted at the whole-plant scale rather than within the bunch and differences in plant

hydration may reflect on the extent of cell vitality. Moreover, it was found that rainfall had a detectable effect on neutralising the rate of cell senescence and therefore thermal time alone could not predict the phenology of berry cell death-associated symptoms.

Conclusion

Mesocarp cell death is potentially important to the production of lower alcohol wines because vital grape berry cells are thought to be necessary for maintaining a high water content in the fruit. Methods to assess water vapour efflux from plant organs with low transpiration rates are notoriously difficult, but the method proposed here entailing minor modifications to a commercially available cuvette was capable of overcoming limitations frequently encountered with small fruit and thus allowed better understanding of the underlying processes driving grape berry dehydration and losses in cell vitality. Our data suggest that bunch shading and rainfall may counteract loss in cell vitality and rates of berry weight loss in Shiraz, but not in all instances.

The results of this study have been compiled in the following publications:

1. Clarke SJ. 2018. Challenges of measuring the surface water vapour permeance of detached fruits. *In review.*
2. Clarke SJ, Rogiers SY. 2018. The role of grape berry temperature in the late season decline of mesocarp cell vitality. *In review.*

6.1.2. Investigation of the physiological cause of grape berry cell death

Introduction

Cell death in the mesocarp of berries occurs late in the ripening process and may influence berry sensory attributes and water retention. There are cultivar-dependent correlations between mesocarp cell death and berry shrivel. Cell death is likely to be associated with yield losses of up to 30% for Shiraz due to berry shrivel, which concentrates sugars and leads to high alcohol content in wine. The object of this study was to investigate whether oxygen deficiency was the cause of grape berry cell death.

Materials and methods

Experimental trials were conducted in the 2014/2015 and 2015/2016 seasons in the Shiraz vineyard situated in Nuriootpa, the Barossa Valley and in the Coombe vineyard, Waite Campus, South Australia. The internal oxygen concentration ([O2]) across the mesocarp was measured in berries from Chardonnay and Shiraz, both seeded, and Ruby Seedless, from the Waite vineyards, using an oxygen micro-sensor. Berry and seed respiration was monitored in Chardonnay berries, while the lenticel density of berry pedicels (stem and receptacle) was assessed in Chardonnay and Shiraz berries, followed by an assessment of the long-term effect of blocking pedicel lenticels on berry internal [O_2] profiles and cell death. Air spaces within the Chardonnay berries at different development stages were visualized using x-ray micro-CT.

A factorial trial of two irrigation regimes was applied in season 2015 and a factorial trial of two irrigation regimes and two temperatures was applied in season 2016, in Nuriootpa. Midday stem water potential, stomatal conductivity and photosynthetic rate were measured to examine the efficiency of drought and canopy heating treatments. The oxygen micro-sensor was used in measuring oxygen concentration in grapes and respiration rate of the grapes. The effects of overhead shading (2015), rootstocks and kaolin application on the vines (2017), on Shiraz berry cell death and berry shrivel were examined.

Results and discussion

In Chardonnay, Shiraz and Ruby Seedless grapes, steep [O_2] gradients were observed across the skin and [O_2] decreased toward the middle of the mesocarp. As ripening progressed the minimum [O_2] approached zero in the seeded cultivars and correlated to the profile of cell death (CD) across the mesocarp (**Figure 1**).

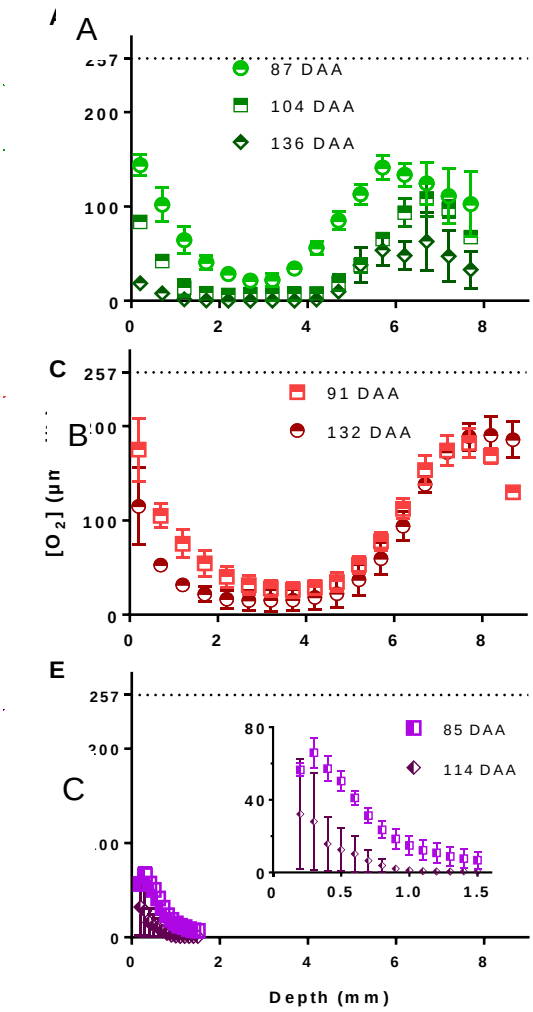


Figure 1. [O_2] profiles of Chardonnay (A), Ruby Seedless (B) and Shiraz berries (C) at various ripening stages. Derived from Xiao et al. (2017).

Seed respiration declined during ripening, from a large proportion of total berry respiration early to negligible at later stages. [O_2] increased towards the central axis corresponding to the presence of air spaces visualised using x-ray micro-CT (**Figure 2**). These air spaces connect to the pedicel where lenticels are located that are critical for berry O_2 uptake as a function of temperature, and when blocked caused hypoxia in Chardonnay berries, ethanol accumulation and CD.

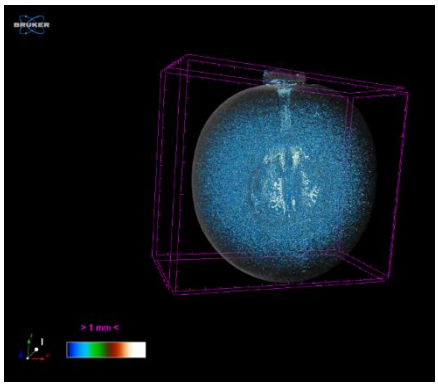


Figure 2. Structural arrangement of air space inside a grape. Dark/blue indicate thinner volume and white thicker volume.

In the factorial field experiment comprising two thermal regimes (control and heated) and two irrigation regimes (irrigated and non-irrigated), conducted in Nuriootpa in 2015 showed that non-irrigation increased the rate of cell death relative to control. In the second season, despite temperature treatment, non-irrigation advanced the onset of cell death relative to the irrigated treatments. Non-irrigation treatments in the second season also decreased [O_2] within the berry mesocarp relative to the irrigated treatments. An association was established between mesocarp [O_2] and berry cell death. Berry respiration and total berry porosity were also found to decrease during berry ripening.

The progression of mesocarp cell vitality was monitored in berries from Shiraz scions on three rootstocks, with variable drought resistance properties, including Schwarzmann, Ruggeri 140 and 420 A. There was no difference in the water relations of rootstocks, while rootstocks showed different effects on both cell death and berry shrinkage. The effect of applying water to the grapevine canopy (control), and kaolin in the bunch zone (bunch) and the whole canopy (canopy) on berry cell death and weigh loss was also examined. Both kaolin treatments resulted in an increase in berry weight compared with control berries. There was a decrease in photosynthesis (light saturation) at a given stomatal conductance in the canopy treatment. However, cell vitality was not affected by the treatments. Overhead shading reduced canopy temperature and berry internal oxygen concentration, which could have resulted from reduced respiration with lower oxygen demand.

Conclusion

Grape internal [O_2] declines during fruit development and is correlated with the profile of mesocarp cell death. Lenticels on the pedicel provide a pathway for O_2 diffusion into the berry and when covered to restrict O_2 diffusion into the berry cause a large reduction in [O_2] in the centre of the berry, an increase in ethanol concentration and cell death. Differences in internal O_2 availability of berries between cultivars could be associated with seed development and differences in lenticel surface area. A higher rate of mesocarp cell death rate linked with non-irrigation was also associated with hypoxia within grape berries.

Rootstocks with different drought resistance properties can affect Shiraz berry weight loss and cell death. Further research is needed on impacts of other commonly used rootstocks on berry cell death and berry weight loss during ripening. Kaolin can effectively reduce Shiraz berry weight loss after the peak berry weight was reached. Kaolin can also reduce photosynthesis at a given stomatal conductance.

The data generated in this study provide the basis for further research into the role of berry gas exchange on berry quality and cultivar selection for adapting viticulture to a warming climate. Understanding the association between berry internal oxygen statuses and berry shrivel and cell death, as well as the effect of strategies to mitigate berry shrivel, will provide researchers and growers new insights into berry ripening and the basis for future research into berry flavour development and yield optimization.

The results of this study have been compiled in the following publication:

Xiao, Z., S. Rogiers, V. Sadras, and S. Tyerman (2017) Hypoxia in the grape berry linked to mesocarp cell death: the role of seed respiration and lenticels on the berry pedicel. bioRxiv. doi: 10.1101/209890 (under review).

6.1.3. Molecular events underlying cell death in the grape berry

Introduction

Mesocarp cell death in some varieties of *Vitis vinifera* L. is characterised by a breakdown of cell membrane integrity, which is believed to have an effect on flavour and aroma development, extractability of the juice and ultimate wine quality. In order to improve wine industry profitability, it is important to gain a better understanding of the mechanisms underlying cell death in grape berry. Reactive oxygen species (ROS) are versatile signalling molecules that play an essential part in regulating apoptosis-like plant cell death (AL-PCD), of which loss of cell membrane competence is a hallmark. In this project the primary objective was to assess whether the mesocarp cell death in the grape berry is programmed as an apoptosis-like cell death. This primary objective was combined with (1) information on the temporal and spatial coordination of ROS and associated factors involved in the induction of mesocarp cell death, and (2) the expression of ROS and AL-PCD related genes during critical stages of berry ripening. The significance of this project in terms of the wine industry are: 1) to gain profitability outcomes through better balancing yield and wine quality; 2) to lower alcohol content in wine (link to berry shrinkage which is increased with cell death); 3) to improve regulation of grape berry development with different treatments and varieties; 4) cell death is accelerated by impacts of climate warming, like water stress and heat waves, so it is important to understand the process in order to devise mitigation strategies.

Materials and methods

The experimental trials were conducted in the 2015 and 2016 seasons in the Shiraz vineyard situated in Nuriootpa, the Barossa Valley and in the Coombe vineyard, Waite Campus, South Australia. A factorial trial of two irrigation regimes was applied in season 2015 and a factorial trial of two irrigation regimes and two temperatures was applied in season 2016. Conducted trails were the same as described in 6.1.2 project.

Results and discussion

Observations from season 2014/2015 suggested that the large reduction in berry weight (berry shrinkage) from 91 days after flowering (DAF) to 119 DAF contributed to increased sugar accumulation. The commencement of berry weight loss at 91 DAF corresponded to the peak production of ROS in both control and drought vines in Shiraz. The impedance (membrane leakage) results of the same sets of berry samples indicated that the decrease of living tissue started from 91 DAF suggesting the beginning of mesocarp cell death. Thereafter, ROS signal started to decline and at 112 DAF, the impedance results of control vines were significantly higher (more vital tissue) than the drought and the drought plus heat treatments (**Figure 3**).

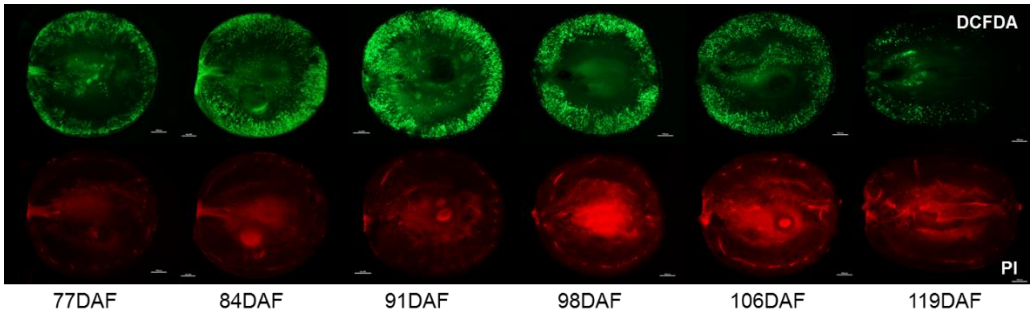


Figure 3. Image of grape berry signal of DCFDA (ROS sensor; green) and PI (cell death; red).

Based on those results, three key time points during berry development were selected for the transcriptome analysis: i) 77 DAF (early berry development), ii) 91 DAF (the beginning of berry weight reduction and the possible onset of mesocarp cell death) and iii) 106 DAF (late ripening stage of berry development).

In the first instance approximately eighteen thousand genes came out from RNA sequencing, but only 260 genes showed differential expression in the comparisons of drought and control treatment in at least one of the developmental stages. Among them, 32 genes underwent changes above 3-fold with 67% of these changes observed at 106 DAF. The increasing number of genes that expressed differentially along three development stages suggested there was a large transcriptome switch happening after 91 DAF in both treatments. This progressed more aggressively in the drought than the control treatment and the drought had almost twice the number of genes that showed differential expression than the control vines (**Figure 4**).

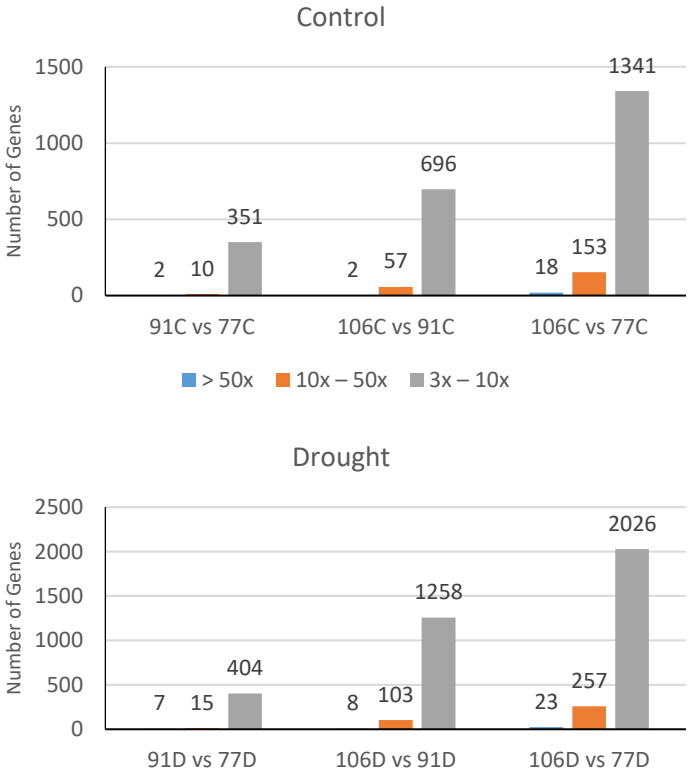


Figure 4. Number of differentially expressed genes with fold-change of FPKM value equal or greater than 3 in each of the comparisons. Time point comparisons were made between any two of the three sampling dates (77 DAF, 91 DAF and 106 DAF) from control (up) and drought (down) treatment respectively. ≥3-fold to <10-fold change (3x-10x; ■), ≥10-fold to <50-fold change (10x-50x; ■) and ≥50-fold change (>50x; ■) as illustrated. FPKM, fragments per kilobase of transcript per million mapped reads.

Candidate genes, such as *VvBAG1 (BCL-2-associated athanogene 1)* and *VvLOXA (LOX2; lipoxygenase 2/A)*, showing over 3-fold change of gene expression in the comparison between the control and water stressed vines, were picked out for further analysis (**Figure 5**). Gene *BAG1* is involved in the induction of cell death and encodes the protein BAG1, which can bind to BCL-2 and enhances its anti-apoptosis effect through blocking a pathway leading to apoptosis. *VvBAG1* expression reduced from 98 DAF to 106 DAF in both treatments suggesting an apoptotic-like cell death might be programmed towards berry ripening in Shiraz. LOXA is a member of the lipoxygenase family, which is not only involved in lipid based aroma and flavour compound formation, but also plays an important role in promoting oxidative injury during senescence. LOXA is able to catalyse membrane galactolipid peroxidation when overexpressed in tobacco. During berry development, the reduction of *VvLOXA* expression in both treatments was expected and was more pronounced in the drought treatment. Gene expression of *VvBAG1* and *VvLOXA* in berries from two distinct maturity stages was also analysed during season 2017. Results indicated both *VvBAG1* and *VvLOXA* expressed down-regulation as the corresponding impedance value decreasing in berry samples from two different maturity stages.

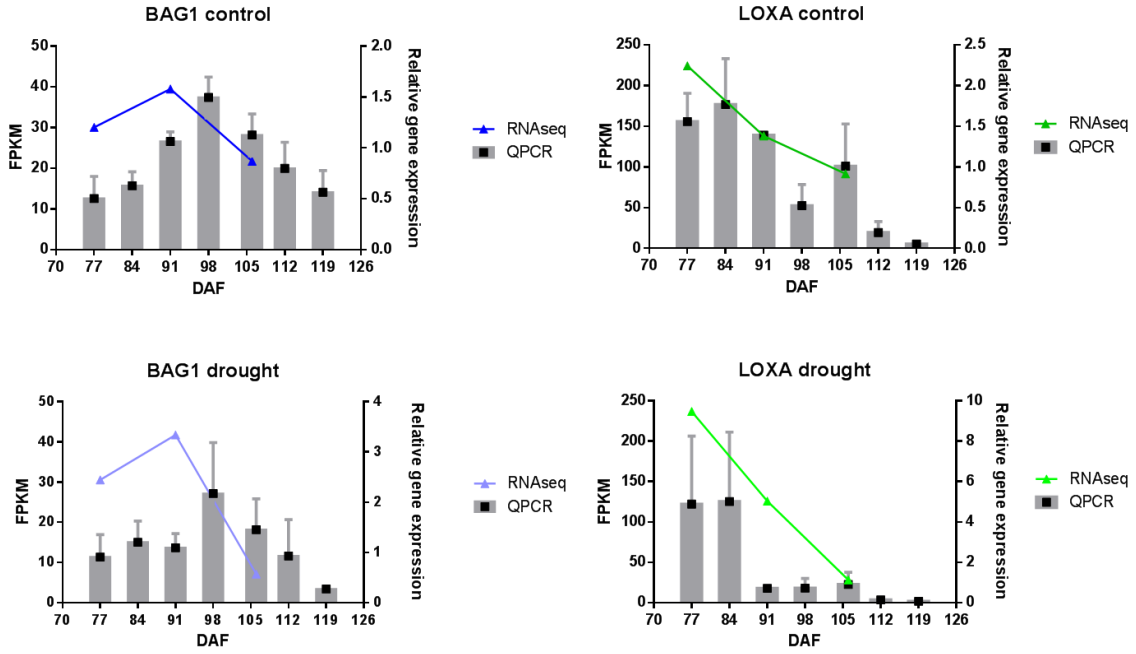


Figure 5. Gene expression of *VvBAG1* and *VvLOXA* in season 2014/2015. QPCR results were normalized with two reference genes. DAF, days after flowering. FPKM, fragments per kilobase of transcript per million mapped reads.

By using a ROS sensor, DCFDA (2', 7'-dichlorofluorescein diacetate), the accumulation of ROS could be detected during berry development. In both seasons, the imaging of ROS signal showed a similar trend along ripening in Shiraz berries. Moreover, to investigate the varietal differences in the distribution of ROS during berry development, Chardonnay was studied in season 2016. Interestingly, Chardonnay berries had a relatively intact and clear signal of ROS accumulated in the skin, whereas the ROS signal was largely lost in the skin of Shiraz.

Conclusion

The increased levels of ROS were associated with the beginning of mesocarp cell death in both control and water stressed Shiraz berries. Elevated temperature and water stress further accelerated cell death in Shiraz berries. Shiraz and Chardonnay demonstarted distinct patterns of ROS accumulation during berry development.

RNA-seq analysis identified lists of candidate genes that showed differential expression in at least one of the comparisons between either three critical development stages or different treatments. Some candidate genes (e.g. *VvBAG1* and *VvLOXA*) might be related more tightly with cell vitality rather than maturity stages, but further studies are necessary.

6.1.4. Ubiquitination in programmed cell death in grapevine berries

Introduction

Programmed cell death (PCD) is an organized process by which organisms selectively remove cells according to developmental needs or in response to biotic or abiotic stress. In plants, PCD plays a fundamental role in plant development, senescence, and pathogen infection. Mesocarp cell death in some varieties of *Vitis vinifera* L. is characterised by a breakdown of cell membrane integrity, which is believed to have an effect on flavour and aroma development, juice extractability and wine quality. A better understanding of the cell death mechanism in grape berry is therefore anticipated to be of significance to the grape and wine industry.

Ubiquitin is a stable, highly conserved, and universally expressed protein that mediates growth and development of all eukaryotic species. This is achieved by ubiquitination – the attachment of ubiquitin to select proteins to regulate their stability and activity. Ubiquitination has also been implicated in a growing number of plant signalling pathways, including those mediating responses to hormones, light, sucrose, developmental cues, pathogens and cell death.

The attachment of ubiquitin to proteins designated for degradation is catalysed sequentially by three enzymes (known as E1, E2 and a ligase, E3). It is the last step, whereby the E3 ligase catalyses the attachment of ubiquitin to specific substrates, that is of particular interest in this research. E3 ligases are classified into different groups based on the presence of specific HECT, RING, or U-box domains. From previous research, it appears several E3 ligases are involved in PCD activation. However, the mechanisms by which these ligases mediate plant PCD are poorly characterized, especially in grapevines.

Materials and methods

RNA from grape berries, tobacco and *Arabidopsis* leaves was isolated using the Spectrum Plant Total RNA kit (Sigma) following the manufacturer's procedures. RNA was treated with DNase I using Turbo DNA-free (Ambion) for 1 hour at 37 ^{o}C, then ethanol precipitated and resuspended in water. An RNA quality threshold was set for 260/280 and 260/230 absorbance ratios at > 1.8. Gene expression was determined by quantitative PCR.

Transient expression in tobacco: genes to be expressed were cloned into binary vectors and transformed into *Agrobacterium tumefaciens* strain *GV3101*. The *Pub13* and *Pub13* (*V274I*) genes were expressed with the CaMV 35S promoter in the binary p1301-eGFP vector. Agrobacterial cells containing *Pub13*, *Pub13* (*V274I*) and the empty vector were each resuspended in the infiltration buffer (10 mM $MgCl_2$, 10 mM MES, and 150 mM Acetosyringone) at 0.6 OD_{600}. 1 M of H_2O_2 was sprayed onto *Nicotiana benthamiana* leaves 15 h after infiltration.

Electrolyte leakage: Leaves of *N. benthamiana* plants were harvested after infiltration with *A. tumefaciens*. Leaf discs (0.5 cm in diameter) were removed with a cork borer and washed in 10 mL of sterile double-distilled water for 30 min with gentle agitation. Washed leaf discs were transferred to 20 mL of sterile double-distilled water and incubated for 2 h at room temperature with gentle agitation. The conductivity of the leaf samples was measured using a conductivity meter.

Transformation in Arabidopsis: *VvPub13* was recombined into the constitutive expression vector p1301. *A. tumefaciens* strain Agl-1 was transformed with 35S:*VvPub13* by the freeze thaw method and then used to transform *Arabidopsis* by floral dipping. Transformed (T1) seed were selected on hygromycin plates (15 µg.m/L) following the rapid method reported previously.

Results and discussion

VvPub13 encoding E3 ligase is of particular interest in this research. *VvPUB13* is related to a gene reported to negatively regulate cell death and H_2O_2 accumulation. The expression of this was quantified during grape berry development under drought stress. The expression of *VvPub13* decreased during fruit development. *VvPub13* was strongly inhibited by drought on 106 DAF.

To investigate the potential relationship between *VvPUB13* and cell death regulation, agrobacterium-mediated transient expression of this gene on tobacco *Nicotiana benthamiana* leaves was performed. Leaves were transfected by *35S*-driven GFP, *VvPub13*-GFP and *VvPub13*(*V274I*)-GFP for transient expression of the proteins. 40 µM MG132 as a proteasome inhibitor was injected into tobacco leaves with *VvPub13*-GFP. A total of 1 M of H_2O_2 was sprayed onto the leaves 15 h after infiltration. After 2 days of H_2O_2 treatment, the tobacco leaves with the control vector (*35S*:GFP) showed strong cell death, however, agrobacterium-mediated transient expression of *VvPub13* (*35S*:*VvPub13*) alleviated the cell death response. The U-box domain mutant and *VvPub13* together with the proteasome inhibitor *MG132* exhibited distinctly increased cell death phenotypes compared with the wild-type *35S*:*VvPub13*. In agroinfiltrated tobacco leaves, *35S*:*VvPub13*-inhibited cell death led to a significant decrease in electrolyte leakage compared with empty vector controls. In contrast, the *VvPub13* mutant or *VvPub13* combined with *MG132* could not generate low levels of electrolyte leakage.

Hsr203J has been discovered to be a PCD-related gene in plant. To investigate the nature of the death events regulated by the indicated genes, the expression of *hsr203J* was analysed. After 2 days of H_2O_2 treatment, the tobacco leaves with the control vector (*35S*:GFP) showed strong *hsr203J* expression, however, agrobacterium-mediated transient expression of *VvPub13* (*35S*:*VvPub13*) inhibited its expression. In contrast, the *VvPub13* mutant or *VvPub13* combined with *MG132* could not inhibit the transcripts of *HSR203J*. Taken together, these results indicate that expression and ubiquitination of *VvPub13* are required to inhibit cell death effectively in tobacco leaves.

Among 10 T2 lines of *VvPub13*-over expressing (OX) plants, lines 2, 3 and 8 displayed strong *VvPub13* expression levels. These lines were selected for further study after confirmation of *VvPub13* transcript levels using RT-PCR. No apparent phenotypic differences were observed between the wild type and *VvPub13*-OX lines. To test the effect of H_2O_2 on cell death in transgenic plants, the leaves from 5-week old plants were infiltrated using a needless syringe with 50 mM H_2O_2. Treatment of wild type leaves with H_2O_2 triggered cell death after 5 days, but transgenic leaves did not show such death, which was confirmed by the electrolyte leakage measurement. In *Arabidopsis*, H_2O_2 content is often associated with the expression of several anti-oxidative genes. RT-PCR was used to examine the expression of *AtMSD1*, *AtCAT1*, *AtAPX1* and *AtGPX1*. H_2O_2 infiltration increased expression of these four genes in transgenic plants compared to the wild type on day one. In conclusion, overexpression of *VvPub13* in *Arabidopsis* alleviates cell death symptoms when challenged with H_2O_2. This new knowledge might be used in breeding or selecting grapevine clones that are more resistant to berry shrivel.

Conclusion

The outcomes of this research highlighted the role of *VvPub13* transiently expressed in tobacco leaves that inhibits H_2O_2-induced cell death. *VvPub13* in grape berries can also inhibit cell death. Furthermore, overexpression of *VvPub13* in *Arabidopsis* reduces H_2O_2-induced cell death by inducing genes involved in anti-oxidant responses.

6.2. The sugar-potassium nexus within the grapevine

Introduction

Several authors have speculated on the relationship between potassium (K^+) and sugar transport through the phloem of plants and the role of K^+ in sugar accumulation into the grape berry (Lang 1983, Deeken et al. 2000, Davies et al. 2006, Rogiers et al. 2006). This is mainly due to similar patterns in the accumulation of these two compounds in the grape berry during ripening. It has been hypothesised that sugar accumulation may be dependent on K^+ import through the important role that K^+ plays in sugar loading into the phloem at carbon sources and unloading from the phloem into the grape berry (Ache et al. 2001, Davies et al. 2006). Potassium assists in turgor regulation by creating a low-viscous pressure gradient within the phloem and therefore contributing to the transport of photoassimilates and phloem unloading (Lang 1983, Very and Sentenac 2002, Davies et al. 2006, Kumar et al. 2006, Lebaudy et al. 2007). According to other research, K^+ may have a role in activating membrane channels and even plant signalling through the transmission of electrical potentials in sieve tubes (Deeken et al. 2000, 2002, Ache et al. 2001, Davies et al. 2006).

The functionality of K^+ and the co-transport of sugar and K^+ into the grape berry is, however, not well understood or defined as yet. A detailed review of the knowledge on the transport and functionality of K^+ in the grape berry was summarised in Rogiers et al. (2017). The aim of this project was therefore to better understand the mechanism of the proposed link (i.e. to determine if it is incidental or causal) and to determine if the sugar content in the grape berry can be manipulated through altering K^+ supply to the grape berry. The final aim was to characterise the expression patterns of several sugar and K^+ transporter proteins of the grape berry pericarp during ripening.

In order to assess the aforementioned aims, the following main research questions were postulated:

1. Is there a relationship between sugar and K^+ accumulation in the grape berry and is this correlation evident throughout ripening?
2. Is this correlation evident in the different tissues of the berry?
3. Does the manipulation of either the sugar or K^+ component within the grape berry, alter the accumulation rate, pattern and content of the other component?
4. Are there similarities in the up- and down regulation of sugar and K^+ transporter proteins during ripening and are these patterns related to the accumulation of sugar and K^+ in the grape berry pericarp?

Materials and methods

Experiment 1: Sauvignon Blanc (clone F4V6) grape berries were collected weekly (n = 48) from pre-véraison to when berries were considered to be harvest ripe (n = 7 sampling dates). On each sampling date the berries were classified into four berry volume classes according to the diameter (Šuklje et al. 2012) and the individual berries were then separated into the skin, pulp and seeds. Each single tissue was analysed for the glucose and fructose content with enzymatic analyses, and the K^+ content with flame atomic absorption spectroscopy (FAAS) after wet digestion. The methodology for experiment 1 is illustrated in **Figure 6.**

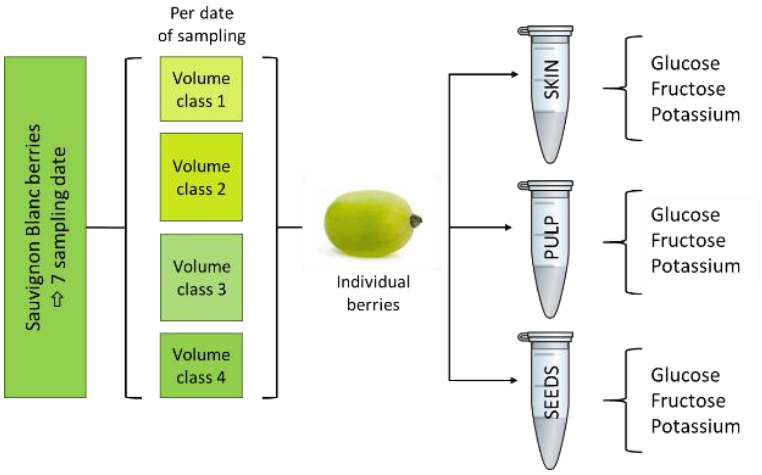


Figure 6: Methodology for Experiment 1.

Experiment 2: Shiraz (clone SA1654) grapevines (n = 48) were initially grown in the bird-proof cages located at the National Grape and Wine Industry Centre in Wagga Wagga. Prior to the onset of véraison, the vines were standardised to one shoot with 21 leaves and bearing one representative bunch. The vines were randomly allocated to four environmentally controlled chambers (n = 12 per chamber) set at a 14-hour photoperiod and at similar climatic conditions. In order to reduce the photoassimilation rate, and potentially the quantity of sugar translocated to the grape berries from the leaves, the atmospheric CO_2 concentration was reduced by 34 % in two of the chambers, while two chambers were kept at ambient CO_2 conditions (≈ 350 µmol.mol^{-1}). To theoretically increase the K^+ content in the grape berries, half the vines per chamber (n = 6) were soil fertilised with a modified Hoagland's solution (Baby et al. 2014) and the remainder of the vines with the same solution in which the K^+ concentration was increased by 60%.

The abiotic conditions (temperature, relative humidity, photosynthetically active radiation and atmospheric CO_2 concentration) were monitored continuously. Photosynthesis, soil water content and the SPAD units, potentially indicating the chlorophyll concentration of the leaves, were measured fortnightly.

To assess the kinetic changes in the accumulation of K^+ and sugar within the grape berries during ripening, two berries per vine were collected weekly from pre-véraison until harvest (n = 7 sampling dates), flash frozen and stored at -80 °C until further analyses. One berry per vine per sampling date was allocated for chemical analyses and the other for later molecular analyses. The pericarp of the individual berries assigned for chemical analyses (n = 336) were homogenised with a handheld homogeniser and the sugar and organic acids content determined by HPLC (Eyéghé-Bickong et al. 2012) and the K^+ content by FAAS after wet digestion.

At the end of the experimentation period, each vine was partitioned into the main organs (n = 9), oven dried (with the exception of the berries and leaves) and enzymatically analysed for the carbohydrate (starch and soluble sugars) and the nutrient content by inductively coupled plasma optical emission spectroscopy (ICP-OES). After determining their surface area, leaves were individually flash frozen and kept at -80 °C until further analyses. Leaves from a subset of vines were individually ground under liquid nitrogen (N) with a mortar and pestle and the chlorophyll (Moran and Porath 1980, Moran 1982) and K^+ content (by FAAS after wet digestion) per leaf was determined. The leaves of the remainder of the vines were combined, ground with an analytical mill and analysed for the chlorophyll, soluble sugars (enzymatically) and nutrient content (ICP-OES) per vine.

The remaining berries on the bunch were ground under liquid N, after removal of the seeds, with an analytical mill and the powdered pericarp was used to analyse the sugar and organic acid content by HPLC (Eyéghé-Bickong et al. 2012) and the nutrient content by ICP-OES.

Experiment 3: The second set of kinetic berry samples, allocated for molecular analyses, was divided into three biological repeats. Each group consisted of four berries, with the berries allocated per biological repeat according to the date of véraison. The berries were ground with a mortar and pestle under liquid nitrogen after removal of the seeds.

Ten genes of interest (GOI), associated with the transport or sugar, K^+ and water in the grape berry during ripening, were identified from literature and presented in **Figure 7**.

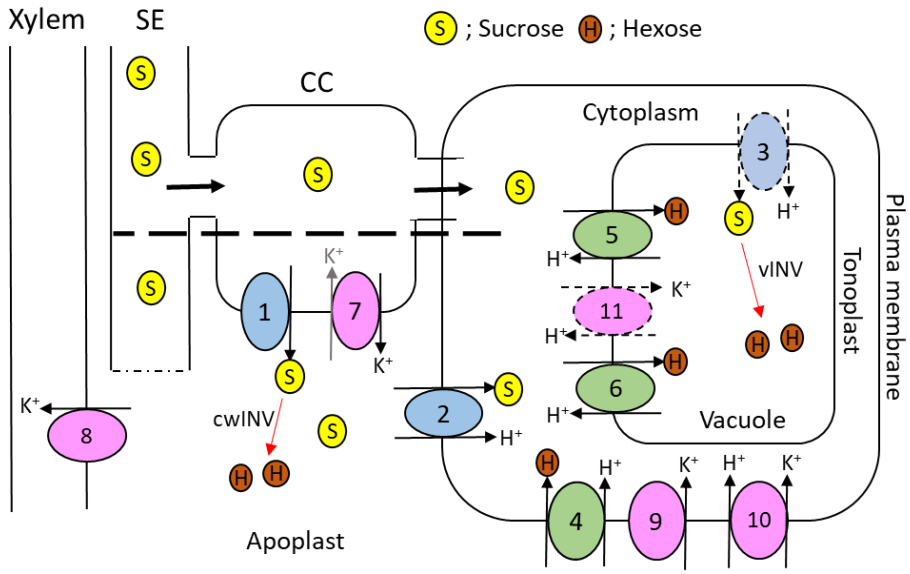


Figure 7: Diagram indicating the putative location and functionality of the sugar and potassium transporter proteins, associated with the accumulation of these metabolites in the grape berry. Investigated sucrose transporter proteins (blue) included *VvSWEET15* (Chong et al. 2014) and *VvSUC12* (Afoufa-Bastien et al. 2010). The hexose transporters (green) explored were *VvHT3* (Hayes et al. 2007), *VvTMT1* (Afoufa-Bastien et al. 2010) and *VvTMT2* (Çakir and Giachino 2012). Four transporter proteins associated with K^+ transport (pink) were studied: *VvKT2* (Deeken et al. 2002), *VvSKOR* (Pilot et al. 2003), *VvK1.2* (Cuellar et al. 2013) and *VvKUP2* (Davies et al. 2006). Transporter proteins identified from the literature, but not investigated in this study, were *VvSUC11* (Manning et al. 2001) and VvNHX1 (Hanana et al. 2007).

RNA was isolated from ≈ 100 mg of the ground pericarp tissue with commercially available kits and cDNA synthesised through reverse transcription from 1 µg of total RNA. Gene specific primers were designed and optimised and their relative expression determined through comparative real-time qPCR normalised to three housekeeping genes (*VvActin7*, *VvEF1γ* and *VvGAPDH*). A full description of methods and methods used in this study can be found in Coetzee (2017).

Results and discussion

*Experiment 1***.** Sugar and K^+ had similar accumulation patterns within the grape, however, sugar accumulated to ten-fold greater concentrations. When comparing individual berries on any particular date, high variability in the ratio of sugar to K^+ was apparent, suggesting plasticity in the accumulation of these metabolites (**Figure 8**). Furthermore, sugar and K^+ content increased as the berry volume increased, potentially indicating a ternary relationship between sugar, K^+ and water accumulation in the berry. Davies et al. (2006) suggested that the influx of K^+ coupled with the accumulation of sugars, may help in turgor-driven expansion of grape berries. More results from this experiment can be viewed in Coetzee (2017a).

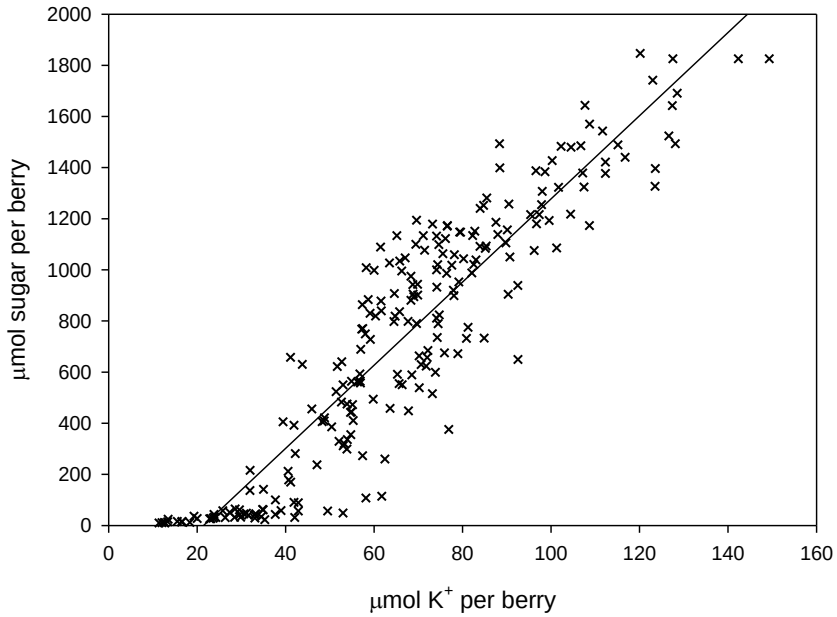


Figure 8: Relationship between the sugar and total potassium content (µmoles) per berry (sum of the skin, pulp and seeds) from one week prior to the onset of véraison to when the berries were considered to be harvest ripe (n = 226 berries) where y = -348.832 + 16.268x, r2 = 0.85 and r = 0.92.

Experiment 2. By decreasing the atmospheric CO_2 concentration by 34 % the photosynthesis rate was decreased by 35 %, but the K^+ treatment did not affect the assimilation rate which suggested that many of the observed physiological responses were mainly driven by the atmospheric treatments. The reduction in CO_2 postponed the date of véraison by four days, however, at harvest the berries of both atmospheric treatments contained the same amount of sugar, despite a delay in the onset of ripening and a significant reduction in the CO_2 assimilation rate (**Table 1**).

Table 1: Treatment effect on the pericarp attributes per berry at harvest adapted from Coetzee et al. (2017b). Values are the treatment means ± SE (n = 12) of the berry pericarp attributes.

	Low CO_2, standard K^+	**Low CO_2, increased K^+**	**Ambient CO_2, standard K^+**	**Ambient CO_2, increased K^+**	**Interaction [1]**
Water content (%)	74.5 ± 0.3	74.4 ± 0.8	73.2 ± 0.4	72.5 ± 0.4	CO_2**
Sugar content (mg)	287 ± 9	303 ± 18	312 ± 13	295 ± 12	n.s.
K^+ content (mg)	4.6 ± 0.2	5.2 ± 0.3	4.6 ± 0.2	4.2 ± 0.1	CO_2*, CO_2 x K^+ *

[1] Treatment interactions followed by * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively, as determined by two-way analyses of variance. Adapted from Coetzee et al. (2017b).

In accordance with Experiment 1, sugar and K^+ followed similar increasing accumulation patterns during berry ripening. Potassium accumulation within the berries followed very similar accumulation patterns in both atmospheric treatments up to the fifth week of sampling. The K^+ content in the ambient treatment remained stable until the date of harvest, whereas the K^+ content per berry in the low CO_2 treatment increased. Therefore, at harvest, berries from the low CO_2 atmosphere had higher K^+, despite a similar sugar content (**Table 1**). This data thus indicate that sugar and K^+ accumulation do not necessarily accumulate in a consistent relationship. A tight correlation between K^+ and water again points towards the possibility of a ternary relationship.

At harvest, the vines in the low CO_2 treatment were both smaller (less fresh mass) and had less biomass than the vines of the ambient treatment. This could be attributed to a decrease in starch levels within the woody components of the vine as a result of the decreased availability of photoassimilates, the mobilisation of carbohydrates towards the reproductive organs (berries), or a combination of both. The root system underwent the largest decrease in starch content and was the only organ (except for the rachis) that showed a decrease in the K^+ content. More results from this experiment are compiled in Coetzee et al. (2017b).

Experiment 3. The expression patterns of several GOI followed similar patters to that of the accumulation of sugar, K^+ and water in the pericarp. Hierarchical clustering analysis indicated that the expression of the genes putatively attributed to the transport of hexose sugars across the tonoplast was highly related to the expression of an aquaporin irrespective of the treatment, indicating that sugar is likely the main osmoregulatory solute in the grape berry cell as expected. A manuscript describing the findings of this experiment is in preparation.

Conclusion

There is a clear similarity in the accumulation patterns of sugar and K^+ into the grape berry, but their interdependence is still under discussion. It was possible to successfully manipulate the accumulation of both sugar and K^+ into the grape berry in a tightly controlled setting, but this is not applicable on an industrial scale and needs further investigation as the grapevine is a complex system able to compensate and regulate internal processes in spite of environmental alterations. New theories about the functional role of K^+ in the grapevine have however emerged from this study and these are currently being investigated further.

According to the theory, lowering the K^+ transport into the grape berry may decrease sugar accumulation in the grape berry. Australian soils, however, have a high K^+ content resulting in a high uptake rate of K^+ by the grapevine. It is not feasible to decrease the K^+ in the soil, but intensive research has previously been conducted on the selection of rootstocks to decrease K^+ uptake. Whether this decline in K^+ uptake by the vine would result in lowered sugar accumulation into the berry remains to be tested, along with other carry-over effects on vine physiology, berry growth and flavour development. Other solutions may include the selection of varieties and rootstock or clonal selections of widely used varieties that may uptake and accumulate less sugar in the berries.

The results from this study have been compiled and published as a PhD thesis: Coetzee, Z.A., 2017. The sugar-potassium nexus within the grape berry. Doctor of Philosophy, Charles Sturt University, 145 p. and the following publications:

1. Coetzee, Z.A., R.R. Walker, A. Deloire, S.J. Clarke, C. Barril, and S.Y. Rogiers. 2017a. Spatiotemporal changes in the accumulation of sugar and potassium within individual 'Sauvignon Blanc' (*Vitis vinifera* L.) berries. Vitis 56: 189-195.
2. Coetzee, Z.A., R.R. Walker, A.J. Deloire, C. Barril, S.J. Clarke, and S.Y. Rogiers. 2017b. Impact of reduced atmospheric CO_2 and varied potassium supply on carbohydrate and potassium distribution in grapevine and grape berries (*Vitis vinifera* L.). Plant Physiol. Biochem. 120: 252-260.

6.3. Optimisation of an early harvest regime

6.3.1. Optimisation of an early harvest regime – impact on grape and wine composition and quality

Introduction

To control the sugar accumulation in berries (and thus potential alcohol in the wines), several viticultural practices can be used to manipulate the leaf area to fruit weight ratio, including shoot trimming, and modified irrigation and pruning regimes (Keller 2010, Martínez De Toda and Balda 2013, Martínez De Toda et al. 2013, 2014, Palliotti et al. 2014). An early harvest approach as a mean to decrease wine alcohol content through lower grape sugar concentrations has increasingly become of interest for the wine industry, but picking fruit earlier may result in wine composition tending towards green and unripe sensory attributes due to higher levels of methoxypyrazines and/or of C_6 alcohol or aldehyde volatiles ('green apple', 'grass') from the grapes (Kalua and Boss 2009, 2010). Indeed, insufficient aroma/flavour and phenolic maturity of early-harvest fruit usually produces wines low in flavour intensity and higher in bitter and herbaceous characters (Pineau et al. 2011, Bindon et al. 2013). Potentially overcoming this issue, unripe grapes could be used to produce a low alcohol, highly acidic blending material that is subsequently incorporated into must from the more mature fruit prior to fermentation, in order to produce wines with moderate alcohol levels and better sensory properties (Kontoudakis et al. 2011). Furthermore, regulations in Australia and several other countries, including USA, have changed in favour of legal water additions into must/wine during winemaking to decrease initial sugar levels. This approach offers another way to moderate wine alcohol content but it may also impact sensory properties through dilution of components.

Materials and methods

In 2015 and 2016, Cabernet Sauvignon grapes were sourced from a commercial vineyard in McLaren Vale, South Australia. Green harvest wine (GHW), made from bunches harvested during véraison (≈50% coloured berries), was fined with charcoal and bentonite, and stored at 0°C until required for blending. Consecutive harvest wines were made from grapes picked and vinified at four maturity levels (H1-H4), producing wines with different alcohol levels (further referred to as % alcohol by volume V/V). The commercial harvest (H4) was used as a control treatment and as a base for pre-fermentation blending treatments with GHW or water (**Table 2**).

The substitution volume was determined with the following equation:
Substitution volume (L) = Y × (G2-G1)/(G2-GH)
Y = grape juice yield in L (based on 50% yield/kg of fruit)
G2 = potential alcohol of grape juice
G1 = desired wine alcohol content
GH = alcohol content of green harvest wine

Consecutive harvest and blending treatment wines were prepared in triplicate. Basic chemical composition of grapes and wines was determined, and wines were profiled for their chemical attributes (more details of this work can be found in Schelezki et al. (2018)). In addition, descriptive analyses of the wines were conducted according to the consensus-based approach (Lawless and Heymann 2010).

Table 2. Proportions of juice substituted with either GHW (B1-B3) or water (Bw1-Bw3) and the resulting wine alcohol concentrations from vintages 2015 and 2016

Vintage	Parameter	B1	B2	B3	Bw1	Bw2	Bw3	Control
2015[1]	Substitution [% v/v]	43.7	27.3	13.6	32.0	19.9	10.1	-
	Alcohol (% v/v)	14.4^{d}	15.8^{b}	17.0^{c}	14.7^{d}	16.0^{c}	17.4^{b}	18.2^{a}
2016	Substitution [% v/v]	39.5	25.7	16.3	25.3	16.4	14.4	-
	Alcohol (% v/v)	12.6^{e}	13.8c	14.5^{b}	12.9^{d}	13.8^{c}	14.6^{b}	15.5^{a}

Alcohol (% v/v) values are means of 3 replicates ± standard error. Values followed by different letters within rows are significantly different ($p \leq 0.05$ one way ANOVA). [1]Data retrieved from Schelezki et al (2018).

Results and discussion

Wine colour and phenolic composition

Weather conditions of both vintages were distinct in such that grapes were subjected to heatwaves coupled with low water availability in 2015. This resulted in the occurrence of berry shrivel from H3 to H4 (commercial harvest date/control (Schelezki and Jeffery 2017) and a TSS increase from 27.4^{o} to 30.4^{o} Brix within only four days (translating to 15.1% V/V and 18.2% V/V, respectively), in line with a significant yield loss. In contrast, in 2016 grapes reached commercial maturity (15.5% V/V) in the absence of berry shrivel with a similar phenological development, giving two exemplary vintage situations for the pre-fermentative alcohol management. As observed in both vintages, juice substitution with GHW or water had minimal effect on colour density of the Cabernet Sauvignon wines, including the wines resulting from the highest substitution levels of 43.7% (2015)/39.5% (2016) for GHW and 32.0% (2015)/25.3% (2016) for water in B1 and Bw1, respectively (**Figure 9, Table 2**). Although no differences were noted in 2015, intermediate and low levels of juice substitution enhanced wine colour density in B3, Bw2 and Bw3 treatments in 2016 compared to the control (H4). Concurrently, the observed trend of a decrease in stable SO_2 resistant pigments relative to the control (H4) values with higher substitution rates appeared to be non significant in 2016 whereas in 2015 the highest additions of water or GHW (B1/Bw1) resulted in significantly inferior levels of stable pigments. This contrasted with the lack of impact of the treatments on anthocyanin concentrations in both vintages, and on wine tannins in 2015 (two factors usually associated with increasing colour density and stability), regardless of the blending component or substitution rate employed (compared to the respective controls). However, the concentration of wine tannins was significantly lowered in 2016 with the highest addition of GHW (B1); apparently, GHW treatments caused a drop in wine tannin concentration, whereas substitution with water tended to yield an increase with respect to the control. This resulted in significantly higher tannin levels in wines Bw1-Bw3 compared to their respective counterparts B1-B3, hence, in accord with the steady SO_2 resistant pigments, indicating a greater ageing potential when alcohol levels are managed via water additions. In general, these results suggest that the effects of substitution rate and blending component were more pronounced in vintage 2016 than in 2015. Further, while the lower alcohol wines produced with the blending treatment resembled those of the respective controls in terms of the presented quality parameters, wines of similar alcohol levels made from earlier harvested grapes showed significantly inferior values (data not shown). More details can be found in Ristic et al. (2018) and a full account of vintage 2015 in Schelezki at al. (2018).

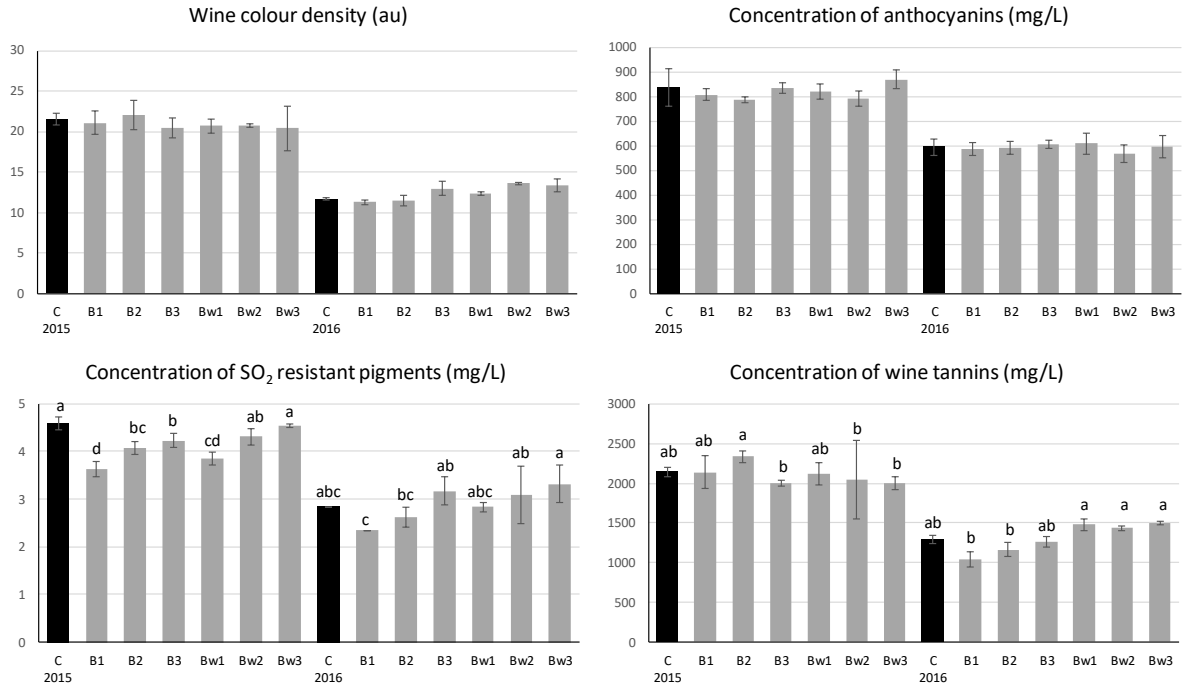


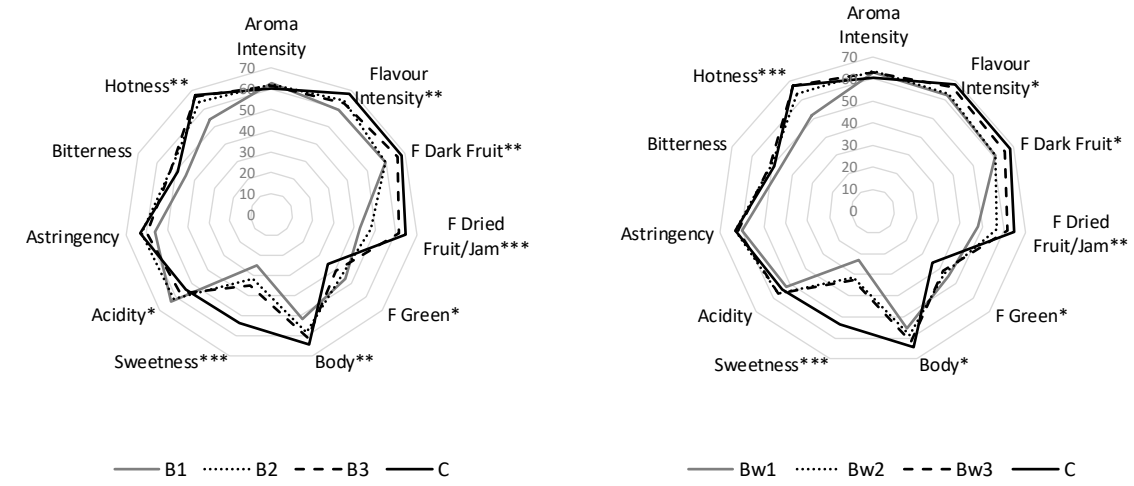
Figure 9. Wine colour density, the concentration of total anthocyanins, SO_2-resistant pigments and wine tannins of Cabernet Sauvignon wines made via blending treatments in 2015 and 2016 vintage. B1-B3 and Bw1-Bw3 refer to GHW and water substitution treatments, respectively. Column values are means of 3 wine replicates and error bars represent ± standard error. Different letters are significantly different ($p \leq 0.05$, one way ANOVA).

Wine sensory characteristics

No significant differences in overall aroma intensity were observed across the blending treatments or vintages compared to the respective controls (**Figure 10**), rather, it appeared that the flavour attributes changed with the treatments. In 2015, 'flavour intensity', 'dark fruit', 'dried fruit/jam', and 'green' decreased with higher substitution rates for both blending components, however statistical significance was mostly evident with the highest substitution rates. Thus, an important change of the pre-fermentative TSS concentration was seemingly required (via substitution of juice with water or GHW) before a perceptible sensory effect was observed. Further, the perception of 'sweetness' and 'body' declined with lower alcohol levels, particularly with a first steep drop from the control (18.2% v/v) to B3/Bw3 (17.0/17.4% v/v). Accordingly with the similar tannin concentrations presented above, wine 'astringency' and 'bitterness' were not perceived differently despite the significant change in alcohol levels. Interestingly, a significant decline in 'hotness' was only achieved in B1/Bw1, with an alcohol level of slightly above 14% v/v (down from >18% v/v in the control), which may indicate a prevailing effect of grape over-maturity even upon pre-fermentatively adjusting alcohol levels.

With the absence of berry shrivel and a lower initial alcohol level of the control wine (15.5% v/v) in 2016, obvious differences emerged with the type of blending component used. Juice substitution with GHW lowered the ratings for the flavour attributes 'flavour intensity', 'dark fruit' and 'dried fruit', similarly to that observed in 2015, whereas substituting juice for water apparently preserved these attributes, regardless of the alcohol level of the respective wines (**Figure 10**). On the other hand, the flavour perception of 'green' characters or 'sweetness' remained unchanged for all treatments. Interestingly, use of water did not induce changes in the 'astringency' perception of the wines, in contrast to the lower 'astringency' ratings of the GHW blended wines that were in line with the different tannin concentrations of those treatments (**Figure 10**).

2015



2016

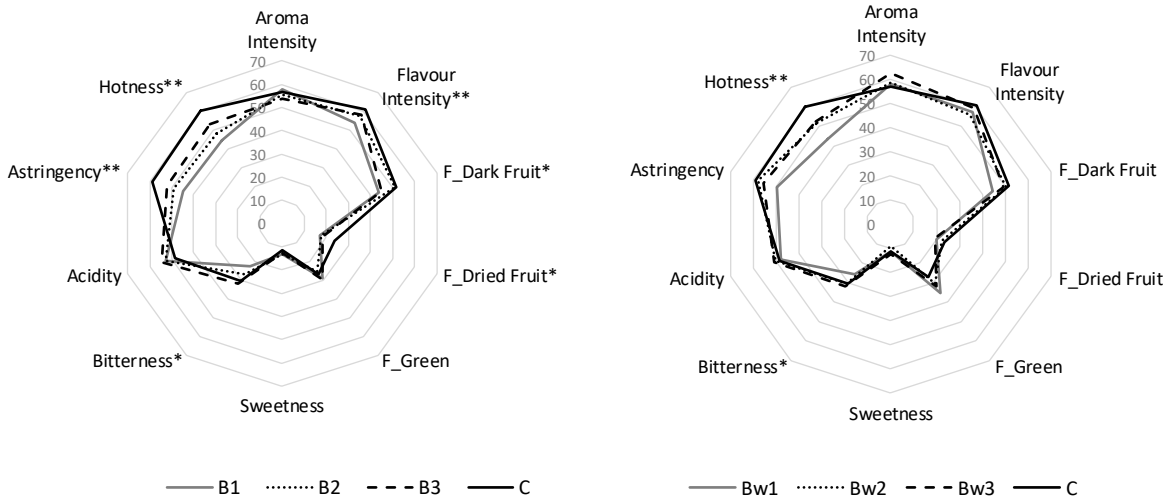


Figure 10. Sensory profiles of Cabernet Sauvignon wines Cabernet Sauvignon wines made via blending treatments in 2015 and 2016 vintage. B1-B3 and Bw1-Bw3 refer to GHW and water substitution treatments, respectively. C = control; F = flavour attribute. *,**,*** denote significant differences at $p<0.5$, $p<0.01$ and $p<0.01$. Derived from Ristic et al. (2018)

Conclusion

For both vintages, moderating the alcohol level by up to 4% v/v via pre-fermentative juice substitution led to mostly marginal changes as opposed to an early harvest regime, where lower values for colour densities, total phenolics and stable pigments indicated that grape ripening was still in progress, even though ethanol concentrations (and the preceding grape sugars levels) were already moderate to high. In the context of berry shrivel (as a result of compressed ripening dynamics), which was the situation during the 2015 vintage of this project, the blending treatment could have been a promising tool (as envisaged with the changes in regulation regarding water addition) to lower the ethanol content of wines with initially high must sugar concentrations (> 30°Brix in this study) to produce wines with moderate alcohol concentrations without significantly impacting quality parameters. However, an earlier harvest in 2015 (i.e. H3, assuming this was logistically possible) would have avoided a significant yield loss and led to a more desirable aroma profile (especially less hotness). In 2016, the grapes achieved commercial ripeness without being affected by berry shrivel. The presented data suggest that, in order to produce alcohol-adjusted wines under milder vintage conditions, the substitution of juice exclusively with water could have been recommended over an earlier harvest due

to better expression of more mature fruit characteristics in the wines. Further, the inferior colour and tannin parameters and decreasing intensity of desirable flavour attributes resulting from the GHW blending treatments in 2016, the absence of advantages of using GHW over water (with one exception being that less tartaric acid is needed to adjust wine pH using GHW) in 2015, and the costs involved with producing GHW in the first place, challenge its suitability for a commercial application, leaving water as the preferred pre-fermentative blending component and more feasible alternative to adjust alcohol concentrations in wines.

The results from this study have been compiled in the following publication:

1. Schelezki, O.J., P.A. Smith, A. Hranilovic, K.A. Bindon, and D.W. Jeffery. 2018. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition. Food Chem. 244: 50-59.

6.3.2. Effects of harvest timing and technological approaches on volatile compounds and sensory profiles of lower alcohol wines

Introduction

Although several methods have been implemented to reduce wine alcohol content, removal of alcohol from wine at a post-fermentation stage (dealcoholisation) is the most accepted at the industrial scale (Schmidtke et al. 2012). Among the dealcoholisation processes, membrane filtration, such as reverse osmosis and evaporative perstraction (membrane contactor), is one of the most widely employed. However, these processes also cause significant losses of important volatile compounds such as esters, which are known to confer fruity aromas to wine (Longo et al. 2017). Aside from these chemical and sensory alterations, dealcoholisation requires a high capital outlay and has poor eco-sustainability because of high energy inputs and water requirements (Margallo et al. 2015b). A more attractive and environmentally friendly approach would be to harvest grapes at an early stage of ripening, when they naturally contain lower concentrations of fermentable sugars. Unfortunately, insufficiently ripened fruit may not have adequate phenolic and aromatic profiles to produce commercially acceptable wines. Early harvested grapes tend to produce wines with high levels of acidity, and lower levels of yeast-contributed and grape-derived aroma compounds (Bindon et al. 2013). This combination typically results in wines with undeveloped, green sensory flavours (Bindon et al. 2014).

A better understanding of how changes in aromatic compounds, particularly fermentative by-products and grape derived compounds, arise during dealcoholisation or blending processes and how these changes impact the sensory profile of reduced alcohol wines is lacking. Research on the reduction of alcohol content has mainly focused on small decreases in alcohol content (1-2% v/v), with minimal emphasis on understanding how chemical changes in wine affect sensory characteristics at greater alcohol reductions. Analysis of the volatile composition of wines before and after each treatment has allowed the identification through multivariate analyses such as partial least square regression (PLS2) and the Common Dimension (ComDim) approach of the analytes responsible for the perceived sensory differences.

To summarise, the main aims of this body of research were to:

1. Evaluate wine blending as tool to produce wines with more balanced, riper flavours and lower levels of alcohol;
2. Assess the effect of dealcoholisation on the volatile composition and sensory profile of wines produced from mature fruit;
3. Identify potential differences in the volatile and sensory composition of wines produced from early harvest regimes and dealcoholisation strategies;

4. Understand of how changes in chemical composition during alcohol reduction impacts on the sensory profile of wine.

Materials and methods

In 2015, Verdelho and Petit Verdot grapes were harvested from two adjacent commercial vineyards sited in Rylstone (Mudgee Region, NSW) when fruit reached a total soluble solids (TSS) of 14.6 and 19.1 °Brix for Verdelho, and 19.3 and 22.7 °Brix for Petit Verdot. Same grape varieties (from the same vineyard site) were harvested in 2016 at a TSS of 17.2 and 23.3 °Brix for Verdelho, and at 21.4 and 23.6 °Brix for Petit Verdot. In the second trial carried out in 2016, Shiraz grapes were sequentially harvested from the Gundagai Region (NSW) at 19.3, 24 and 29.3°Brix representing an early, middle and commercial harvest date. A laboratory scale bench-top Micro AA MEM-074 (Memstar®, Oakleigh, Australia) apparatus consisting of reverse osmosis followed by a membrane contactor was used to dealcoholise.

Basic wine parameters including ethanol, TA, pH, sugars (glucose, fructose), free/total SO_2, malic and acetic acid were quantified per established methods. A total of 46 volatile compounds (esters, higher alcohols, terpenes) were identified and quantified in the wine headspace by SPME/GC-MS (Antalick et al. 2015, Šuklje et al. 2016). A sensory descriptive analysis was performed one week after dealcoholization, approximately three months after bottling, according to a previously outlined method (Blackman and Saliba 2009). Wines were evaluated by a 12-member panel. A detailed description of materials and methods used in this project is given in Rocco Longo's PhD thesis "*Effects of harvest timing and technological approaches on volatile compounds and sensory profiles of lower alcohol wines*", CSU, Wagga Wagga and Longo et al. 2018 (in press).

Results and discussion

Evaluation of blending as a tool to produce wines with more balanced, riper flavours and lower levels of alcohol

In vintage 2015 the blend of Verdelho wines produced from less ripe grapes (14.6 °Brix) and riper fruits (19.5 °Brix) resulted in a wine without significant differences from the ripe fruit wine for any of the sensory descriptors, except for some mouthfeel attributes. While the perceptions of 'acidity' and 'bitterness' were scored significantly higher in the blended wine compared to the ripe treatment, the perception of 'alcohol' was significantly lower. Even more positive outcomes were achieved with the blend of a Petit Verdot wine made from less (19.6 °Brix) and more (22.1 °Brix) ripe grapes which resulted in a wine with the same sensory profile as the ripe treatment (**Figure 11**). The same blending trials were repeated in vintage 2016 with addition of Shiraz wines, which were produced from grapes picked at three ripeness stages that were grown in a single vineyard located in a warm-climate area (Gundagai, NSW). The Shiraz blend (19.3 °Brix) did not differ from the later harvest treatment (commercial ripeness at 29.3 °Brix) for most of the sensory attributes, except 'raisin/prune' and 'alcohol' attributes which were scored significantly lower in the blended treatment.

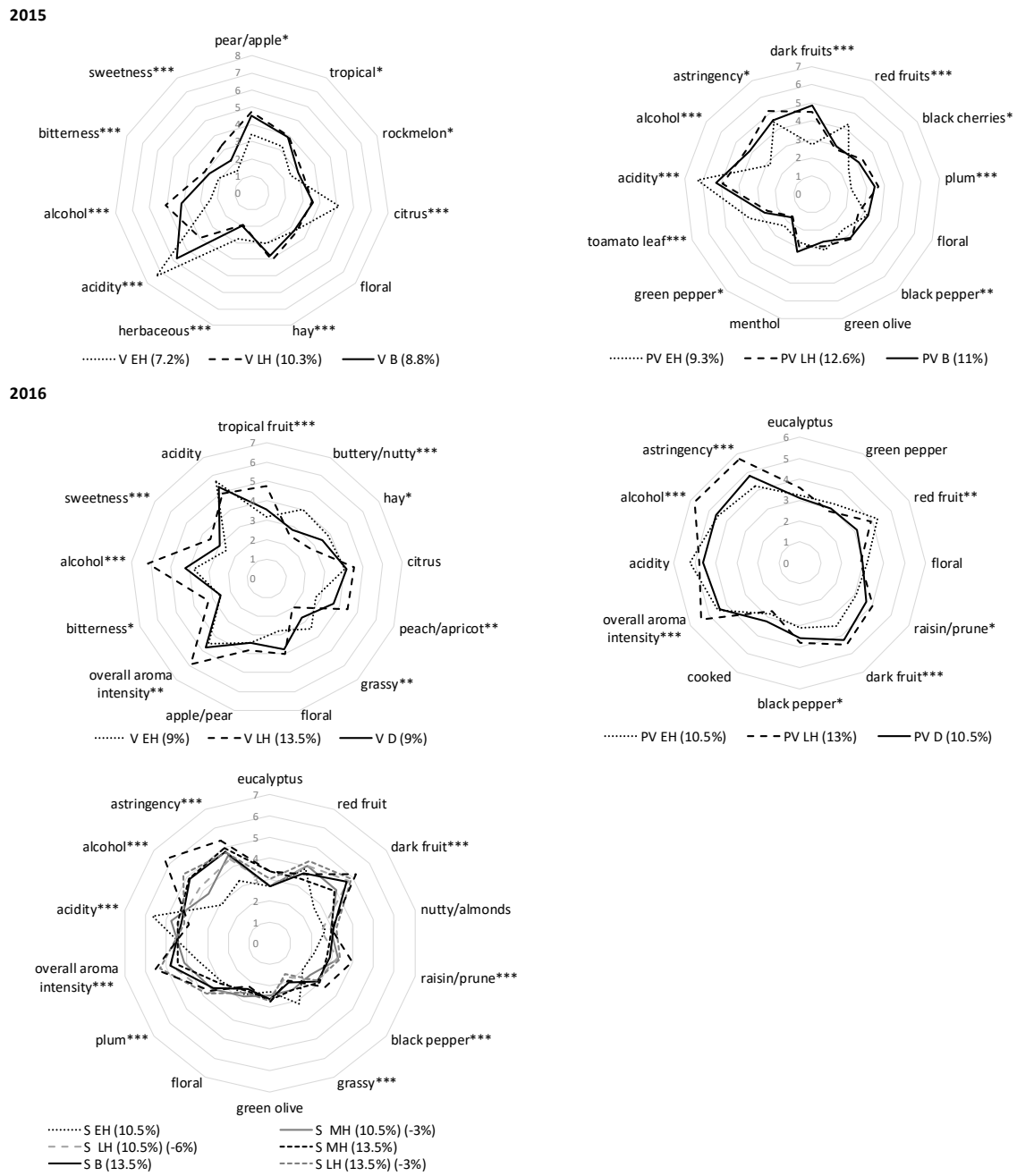


Figure 11. The sensory profile of Petit Verdot (PV) and Verdelho (V) wines made in 2015 and 2016, and Shiraz (S) in 2016 from an early (EH) and late harvest (LH) and their blends (B) (n=3)

The blending procedure carried out in two vintages on different grape varieties allowed the production of wines with reduced alcohol content, but with similar sensory properties of the riper fruit wines. The proposed procedure is easy to apply, does not require specific equipment and offers a means of addressing the problem of overly alcoholic wines because of overripe grapes. However, wines produced from early harvest grapes had higher levels of organic acids, particularly malic acid, thus deacidifying could be recommended prior to blending. A full account of vintage 2014/15 can be found in Longo (2018) and Ristic et al. (2016).

Assessing the effect of dealcoholisation on volatile composition and sensory profile of wines produced from mature fruit

Verdelho and Petit Verdot wines containing 13.5% and 13% v/v alcohol were dealcoholised to 9% and 10.5% v/v respectively, using a combined reverse osmosis-evaporative perstraction process. This process significantly affected concentrations of highly hydrophobic compounds such as ethyl esters, particularly ethyl hexanoate, ethyl decanoate and ethyl dodecanoate, which decreased by up to 80% from their original concentration; with decrease more evident in white than red wines. However, some volatile compounds were not affected by dealcoholisation. For example, the concentrations of many monoterpenes and C_{13}-norisoprenoids did not significantly decrease in Petit Verdot dealcoholised wines, which could be attributed to the ability of non-volatile components in red wines (e.g. phenolic substances) to retain monoterpenes and C_{13}-norisoprenoids (Rodriguez-Bencomo et al. 2011). Changes in the volatile composition of wines reflected in sensory properties of wines, such that the perceptions of 'tropical', 'overall aroma', 'bitterness' and 'alcohol' attributes were significantly lower in dealcoholised Verdelho wines compared to the original samples. The perception of 'overall aroma' attribute also decreased in Petit Verdot dealcoholised wines, together with 'alcohol' and 'astringency' mouthfeel attributes.

In 2016 vintage Shiraz wines with 13.6% and 16.3% v/v were dealcoholised to 10.3% and 10.5% v/v respectively, which mostly affected esters and alcohols (C_6-alcohols, higher alcohols), while most of the monoterpenes and C_{13}-norisoprenoids remained unchanged. Interestingly, the concentration of β-damascenone remained unaffected by harvest date (13.6% and 16.3% v/v) or the dealcoholisation extent (-3% and -6% v/v). These results, consistent with the previous findings for Petit Verdot, confirmed that the matrix composition of initial wine could actually facilitate the retention of terpenoids in red wines (Rodriguez-Bencomo et al. 2011). Importantly, it appears that dealcoholized Shiraz wines were able to retain the majority of the sensory attributes of original wines even at 6% of dealcoholisation level, while the perception of 'alcohol' and 'astringency' significantly decreased. Although similar trends were observed for dealcoholized Petit Verdot and Shiraz wines, prediction of the dealcoholisation effects on the sensory profile of wines is very difficult due to complex matrix of the initial wine and the dealcoholisation operating conditions. However, this study has highlighted the key role of non-volatile compounds such as polyphenols exerting a retention effect towards volatile compounds and their possible interaction with monoterpenes and C_{13}-norisoprenoids which could explain the unaltered perception of some ripe fruit descriptors, i.e. the dark fruit aroma, following dealcoholisation of Petit Verdot and Shiraz wines (Escudero et al. 2007, Pons et al. 2008). More details about this experiment can be found in Longo (2018) and Ristic et al. (2016).

Identifying potential differences in the volatile composition and sensory profile of wines produced from early harvest and dealcoholisation

In vintage 2016, Verdelho and Petit Verdot wines containing 9% and 10.5% v/v alcohol respectively, were produced using two methodologies: fermentation of early harvest grapes and dealcoholisation of wines from late harvest. GC-MS analyses on wine samples indicated that the total concentration of ethyl esters was significantly higher in both Verdelho and Petit Verdot early harvest wines compared to the dealcoholised treatments which demonstrated significant removal of ethyl esters together with alcohol. However, the total concentration of C_{13}-norisoprenoids in Petit Verdot dealcoholised treatments was higher than the early harvest wines and only small differences were found in the sensory profiles between the dealcoholised and early harvest Verdelho wine; the dealcoholised treatment was highly rated in 'buttery/nutty' aroma and early harvest wine in 'red fruit' aroma (**Figure 11**).

Similar experiment was conducted in vintage 2016 with Shiraz wines produced by harvest timing and dealcoholisation to achieve 10.5% and 13.5% v/v alcohol levels. Among the wines containing 10.5% v/v alcohol, early harvest treatments had significantly higher concentrations of C_6-alcohols (particularly *cis*-3-hexenol), higher alcohols and ethyl esters. From a sensory perspective, most differences were found between early harvest and the dealcoholised wines which was produced by removing 6% v/v

alcohol from the later harvest wine (16.3% v/v). The perception of unripe characters such as 'grassy' and 'acidity' were significantly higher in early harvest wines compared to the dealcoholised treatments, while that of 'dark fruit', 'raisin/prune', 'black pepper', 'overall aroma', 'alcohol' and 'astringency' were significantly higher. In the same experiment, a moderate alcohol level of 13.5% v/v was targeted, which is considerably a lower concentration than the commercial wine (up to 17.0% v/v) produced from this vineyard site. Descriptive sensory analysis of 13.5% v/v Shiraz wines produced by dealcoholisation and blending showed that the wine produced from grapes picked to achieve 13.5% v/v alcohol was not sensorially different from the dealcoholised treatment for any of the sensory attributes assessed. Nevertheless, the dealcoholised wine was the most similar to the wine produced from grapes picked at commercial ripeness (29.3 °Brix), differing only for a lower 'alcohol' perception (**Figure 11**).

Both experiments carried out in vintage 2016 showed that dealcoholised and early harvest wines can have different sensory profiles, as observed for the 10.5% v/v Shiraz wines, inferring that other components, different from alcohol, such as yeast-contributed and grape derived aroma compounds, can have a larger impact on some of the aroma attributes. Finally, anticipating the harvest date in order to control or moderate the levels of alcohol could be one solution in particular for smaller wineries that struggle to benefit from dealcoholisation technologies because of the initial and running costs of the equipment (Margallo et al. 2015a). More details about this experiment can be found in Longo et al. (2018).

Conclusion

Correlations between sets of sensory and analytical data as derived with the aid of multivariate statistical procedures were used to improve our current understanding of alcohol reduction effects. Partial Least Square Regression (PLS2) and the Common Dimension (ComDim) approach were selected for the multivariate data analysis. In the 2015 experiment, PLS2 regression exposed a clear separation of each treatment for Verdelho and Petit Verdot respectively, with earlier harvest wines clearly parting from the ripe fruit treatments. Accordingly, the blended samples of each grape variety were located around the center of the plots between the two harvest treatments. PLS2 outcomes infer that blending results in an averaging effect of their analytical and sensory measures. PLS2 models also revealed that ethyl esters and higher alcohol acetates contributed differently to Verdelho and Petit Verdot sensory profiles. Whereas esters were associated with 'tropical fruit', 'rockmelon' and 'pear/apple' aromas of the ripe Verdelho wines, they were linked to 'red fruit', 'tomato leaf' and 'green pepper' perceptions in early harvest Petit Verdot, together with *cis*-3-hexenol and 1-hexanol. Another noteworthy finding was the strong association between ethyl-2-methyl butyrate, ethyl isovalerate and γ-nonalactone with perceived 'dark fruit', 'black cherry' and 'plum' aromas in ripe Petit Verdot wines, confirming previous findings for other red wines (Pineau et al. 2009, Lytra et al. 2012).

In both 2016 experiments, the analytical attributes that were responsible for the perceived sensory differences in the wines were identified through the ComDim approach, which enabled several blocks of chemical data to be explicitly linked to sensory properties (Bouveresse et al. 2011). Whereas the PLS2 models used for the 2015 experiments were developed using only the analytical and sensory attributes that differed significantly across the treatments, the ComDim plots included all quantified variables to ensure that no important associations were overlooked. Similar to the previous findings obtained from the 2015 growing season, the esters of Verdelho and Petit Verdot wines from the 2016 trial were projected opposite each other along the horizontal axis of the ComDim plots. While this trend likely reflects the origin of esters, either directly from the grape or as a product from yeast metabolism (Antalick et al. 2014), similar sensory relationships to those observed in the previous growing season were revealed. An important finding, however, was the association of the C_{13}-norisoprenoids β-ionone, α-ionone and β-damascenone to perceived 'raisin/prune', 'dark fruit' and 'overall aroma intensity' in the ripe Petit Verdot treatment. Contributions of β-ionone and β-damascenone in red wines has previously been reported (Escudero et al. 2007, Pons et al. 2008). Since the concentration of β-damascenone and the perception of 'dark fruit' aroma did not significantly change with

dealcoholisation, it was also hypothesised that this C_{13}-norisoprenoid may have contributed to the perception of 'dark fruit' in the dealcoholised Petit Verdot.

In 2016 it was possible to observe by means of the ComDim approach that the chemistry data block, which included acetic acid, pH and glycerol for example, contributed to the higher proportion of variation, thus to the separation of the treatments in the respective 10.5% and 13.5% v/v ComDim plots. Among the 10.5% v/v Shiraz wines the early harvest replicates were clearly separated from the -6% v/v dealcoholised samples. Similarly, 13.5% v/v harvest timing wines were clearly separated from the -3% v/v dealcoholised replicates. The extent of dealcoholisation and the original wine composition were greater contributors to the sensory differences than the final alcohol content.

In summary, the ComDim approach appears to be a suitable multi-block analysis method to describe several data blocks observed for the same sample set. However, aroma reconstitution and omission studies are warranted to confirm our results (Longo 2018).

The results from this study have been compiled in the following publications:

1. Longo, R., J.W. Blackman, G. Antalick, P.J. Torley, S.Y. Rogiers, and L.M. Schmidtke. 2018. Harvesting and blending options for lower alcohol wines: a sensory and chemical investigation. J. Sci. Food Agric. 98: 33-42
2. Longo, R., J.W. Blackman, G. Antalick, P.J. Torley, S.Y. Rogiers, and L.M. Schmidtke. 2018. A comparative study of partial dealcoholisation versus early harvest: Effects on wine volatile and sensory profiles. Food Chem. (in press)
3. Longo, R., J.W. Blackman, P.J. Torley, S.Y. Rogiers, and L.M. Schmidtke. 2017. Changes in volatile composition and sensory attributes of wines during alcohol content reduction. J. Sci. Food Agric. 97: 8-16
4. *Longo, R. Effects of harvest timing and technological approaches on volatile compounds and sensory profiles of lower alcohol wines, PhD thesis, Charles Sturt University (under examination)*

6.4. Yeast strains in alcohol management and flavour enhancement

6.4.1. Non-*Saccharomyces* in alcohol management and flavour enhancement

Introduction

In oenology, the term 'non-*Saccharomyces* yeasts' refers to about 50 yeast species, excluding *S. cerevisiae,* that are native to the wine-related environment (Jolly et al. 2014). Unlike *S. cerevisiae,* these yeasts are generally unable to deplete all sugars from the grape juice, i.e. 'complete' the fermentation. Their prevalence in the fermentation medium is generally limited, as they are sensitive to a range of biotic and abiotic stressors. Moreover, due to isolation from incomplete or protracted fermentations and/or analytically anomalous wines, these yeasts were originally regarded as spoilage organisms. Winemakers therefore generally seek to unselectively inhibit their growth. This is achieved in most cases by the addition of commercially acquired *S. cerevisiae* as a high density inoculum, commonly coupled with SO_2 concentrations toxic for most other microorganisms. Inoculating a fermentation in such a manner has become a common practice in oenology, as it ensures a reliable and timely process with a consistent outcome. Nowadays, however, the large inter- and intra-species diversity amongst non-*Saccharomyces* has become more apparent; while some strains cause wine spoilage, others can improve its overall quality. Moreover, co-existence and progression of multiple species results in a more diverse metabolic matrix compared to a *S. cerevisiae* monoculture, leading in turn to increased aroma/flavour

complexity and palate structure. Promoting the proliferation of native microflora by omitting a *S. cerevisiae* starter culture can therefore be beneficial for the wine quality. However, the lack of predictability and reproducibility hinders wider industrial applicability of the 'spontaneous'/'un-inoculated' fermentation modality.

In an attempt to address the sensory uniformity and decreased complexity of inoculated wines, while avoiding risks of un-inoculated fermentation, mixed culture inoculation has been proposed as an innovative fermentation management modality in winemaking. It implies simultaneous or sequential inoculation of selected non-*Saccharomyces* and *Saccharomyces* strains, where non-*Saccharomyces* proliferate in the early stages of the fermentation, contributing to the chemical and sensory properties of the wine, while the later stages are dominated by more competitive *Saccharomyces* yeasts, ensuring fermentation completion.

This concept is, however, still at its infancy; as of now only around a dozen non-*Saccharomyces* inocula representing four yeast species (i.e. *Torulaspora delbrueckii*, *Lachancea thermotolerans, Metschnikowia pulcherrima* and *Pichia kluyveri*) are available on a market saturated with hundreds of *Saccharomyces* yeasts. The use of non-*Saccharomyces* co-starters can lead to dramatic modulations of wine chemical and sensory profile, and as such may even offer solutions for the challenges the global wine industry is facing.

A number of microbiology laboratories are therefore on a quest for a yeast capable of lowering wine ethanol content while enhancing, rather than lessening, its overall quality. Besides GM and non-GM techniques employed to expand low diversity in ethanol yields among *S. cerevisiae* wine strains (Tilloy et al. 2015), these efforts encompass exploring the diversity of non-*Saccharomyces* yeasts (Ciani et al. 2016). Indeed, several selection programmes focused on identifying non-*Saccharomyces* strains that are less efficient in converting grape hexoses to ethanol. These candidate yeasts were further trialled in conjunction with *Saccharomyces* yeasts, resulting in up to 1.6% v/v less ethanol than the *S. cerevisiae* control (Contreras et al. 2014, 2015, Ciani et al. 2016). However, the potential of non-*Saccharomyces* yeasts is thus far still under-explored, and low-ethanol yielding strains are yet to be commercialised for the industry.

This project has therefore addressed the following:

1. Potential and limitations of existing commercial non-*Saccharomyces* co-inocula in Shiraz fermentations at two maturity levels
2. Selection and characterisation of lower-ethanol non-*Saccharomyces* strain(s) for sequential fermentations with *S. cerevisiae*
3. Genotypic and phenotypic diversity of *Lachancea thermotolerans*, a species with remarkable oenological potential due to lower ethanol yield and acidifying character.

Materials and methods

Experiment 1. The performance of eight yeast treatments was evaluated in 12 kg Shiraz fermentations at two grape maturity levels, i.e. earlier harvest (24 °Brix) and later, commercial harvest (29 °Brix). Yeast treatments included five non-*Saccharomyces* co-starters (three *Torulaspora delbrueckii* strains, one *Lachancea thermotolerans* and one *Metschnikowia pulcherrima* strain) with sequentially inoculated *S. cerevisiae*, a commercial blend of non-*Saccharomyces* and *S. cerevisiae* strains, and a *S. cerevisiae* inoculum. Identical fermentation management was applied to all treatments. The wines were subjected to comprehensive chemical analysis, including basic chemistry, volatile composition and phenolic measurements, and sensory profiling (descriptive analysis).

Experiment 2. A number of non-*Saccharomyces* isolates were tested in pure culture fermentation trials in a synthetic grape juice-like medium, allowing for the selection of yeasts capable of yielding lower ethanol content than *S. cerevisiae* control, without production of apparent analytical or sensory anomalies. Selected candidates were then trialled in co-cultures with sequentially inoculated *S. cerevisiae*. *Metschnikowia pulcherrima* MP2 isolate resulting in the most prominent ethanol reduction

in wines finished off with *S. cerevisiae*, was further tested in both synthetic grape juice with increase sugar concentration (i.e. ~250 g/L), as well as a white grape juice. Six consecutive sequential inoculation treatments (**Table 3**) were conducted in triplicate in 100 mL fermentations (**Figure 12**), alongside a *S. cerevisiae* single culture control. Samples were taken regularly to monitor microbial growth) and sugar consumption. Residual sugars, gross metabolites and organic acids were determined prior to the sequential inoculation, and at the fermentation completion by HPLC. Treatments resulting in statistically different ethanol levels were analysed for their volatile profile by a SPME-GC-MS method.

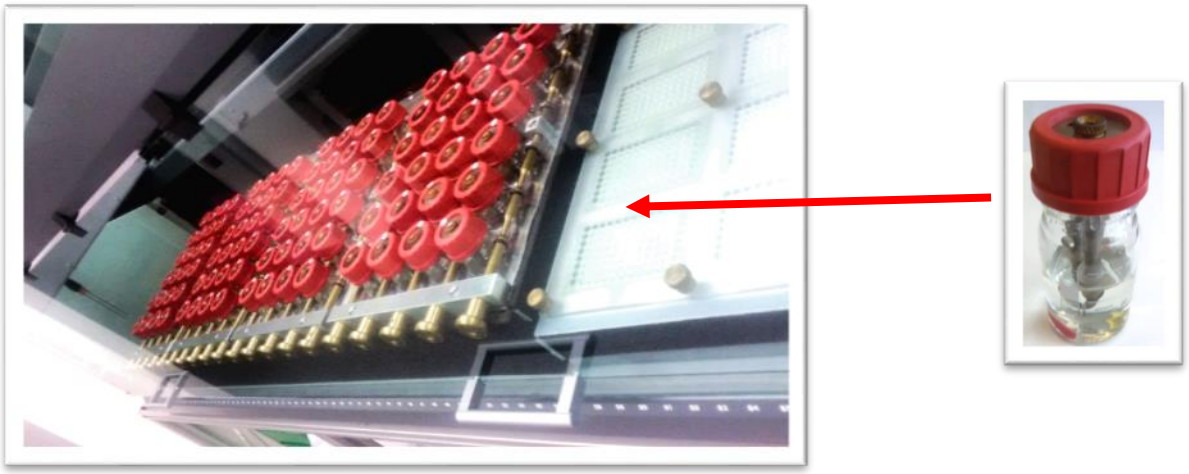


Figure 12. Set-up of 100 mL fermentations in the automatic handling platform 'Tee-bot'. This platform allows for the set-up of up to 96 simultaneous fermentations. The samples, collected at user-defined intervals, are aliquoted in the 96-well plates, thus being compatible for a number of downstream analyses.

Table 3. Inoculation regimes of fermentation treatments conducted in both sterile Chemically Defined Grape Juice Medium and grape juice inoculated with 5 x 10^6 viable cells/mL.

Treatment	***M. pulcherrima* inoculation**	***S. cerevisiae* inoculation**						
	t = 0	t = 0	3 days	4 days	5 days	6 days	7 days	50% sugar consumed
SC	-	$5x10^6$						
MP2 x SC3	$5x10^6$		$5x10^6$					
MP2 x SC4	$5x10^6$			$5x10^6$				
MP2 x SC5	$5x10^6$				$5x10^6$			
MP2 x SC6	$5x10^6$					$5x10^6$		
MP2 x SC7	$5x10^6$						$5x10^6$	
MP2 x SC50%	$5x10^6$							$5x10^6$

Experiment 3. To study genetic diversity and population structure in *L. thermotolerans*, 172 isolates were sourced from different isolation substrates and continents worldwide. These were analysed using a newly developed set of 14 microsatellite markers. In addition, plate-based growth assays using different carbon sources and physicochemical conditions were conducted to compare the phenotypic diversity of genotyped isolates, followed by an in-depth study of oenological performance of 94 strains in Chardonnay fermentations.

Results and discussion

Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-Saccharomyces co-starters

Both harvest date and yeast treatments significantly affected a range of compositional parameters of the wines. Of particular interest was the increased sensory appeal of earlier harvest wines compared to the *S. cerevisiae* control. However, some treatments were related to an increased risk of stuck fermentation in higher ripeness conditions (**Figure 13**). The findings of this study are outlined in detail in a resultant research publication (Hranilovic et al. 2017b).

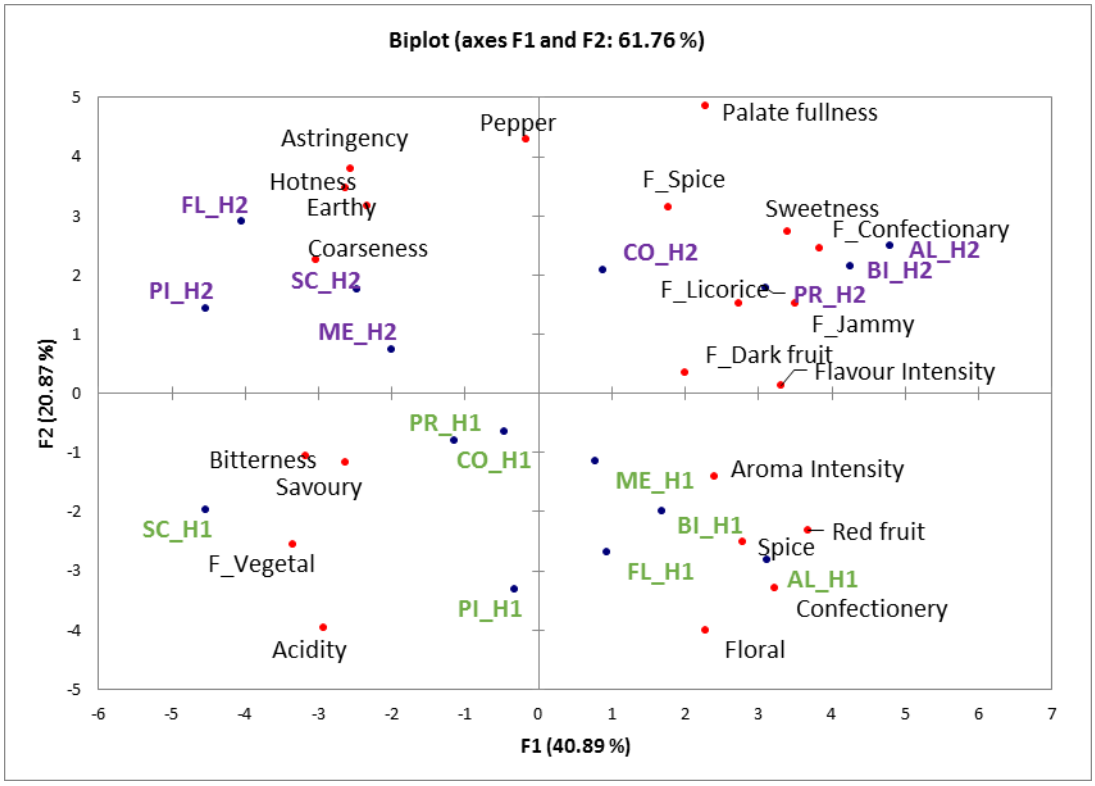


Figure 13. PCA biplot of sensory data for Shiraz wines produced with eight yeast treatments using earlier (H1) and later (H2) harvested fruit. AL, BI, PR *Torulaspora delbrueckii* strains; CO, *Lachancea thermotolerans*; FL, *Metschnikowia pulcherrima*; PI, an initially uninoculated treatment; ME, a commercial blend of *Saccharomyces cerevisiae*, *T. delbrueckii* and *L. thermotolerans*; and SC, a *S. cerevisiae* strain. Derived from Hranilovic et al. (2017b).

Descriptive sensory analysis of wines comprised of training sessions to generate and gain familiarity with wine attributes, and formal assessments to rate these attributes. Wines were discriminated based on the harvest date, separated on the y axis of the PCA plot (**Figure 13**). Interestingly, the wines made from earlier harvested fruit with non-*Saccharomyces* treatments, as well as the initially un-inoculated treatment, were in general characterised by more appealing sensory attributes, such as flavour and aroma intensity, palate fullness, fruit sweetness, 'red fruit', 'floral', 'confectionary' and 'spice' aromas. On the other hand, the earlier harvested PDM control tended towards descriptors such as acidic and green. Wines made from the fruit harvested at commercial ripeness without residual sugar were characterised by hotness, increased astringency and surface smoothness, and 'peppery' and 'earthy' aromas, whereas the remainder of the later harvested wines containing variable concentrations of residual sugars were perceived as sweeter, fuller on the palate, with 'dark fruit', 'confectionary', 'jammy', 'liquorice' and 'spicy' aromas (**Figure 13**).

Selection and characterisation of lower-ethanol Metschnikowia pulcherrima in sequential fermentations with Saccharomyces cerevisiae

Sequential inoculation trials with pre-selected non-*Saccharom*yces candidate strains and *S. cerevisiae* revealed a number of strains capable of decreasing final wine ethanol content in dry wines. The most prominent reduction in ethanol in synthetic grape-juice like medium (230 g/L sugar, 300 mg/L N) was observed with *M. pulcherrima* strain MP2. To validate this finding, a series of fermentations were conducted in both synthetic and real grape juice (~250 g/L sugars, 350 mg/L N), comprising six different sequential inoculation modalities alongside a *S. cerevisiae* control (**Table 3**). This experimental set-up was chosen to allow for fermentation management optimisation, in terms of timely fermentation progression, with concomitant wine compositional modulation. As expected, a delay in *S. cerevisiae* sequential inoculation resulted in a greater ethanol decrease in sequentially fermented wines (**Table 4**). For example, the delay of three days led to 0.6% v/v lower wine ethanol content, whereas *S. cerevisiae* addition upon 50% sugar depletion decreased ethanol by 1.2% in white grape juice. The decrease in ethanol was accompanied with an increase in glycerol, and a decrease in acetic acid.

Table 4. Concentrations of metabolites at different fermentation stage produced with six *Metschnikowia pulcherrima* (MP2) sequential inoculation treatments (**Table 3**) and a *S. cerevisiae* (SC) control in a white grape juice and a synthetic grape juice.

Media	Parameter	Yeast treatment						
		MP2 x SC3	MP2 x SC4	MP2 x SC5	MP2 x SC6	MP2 x SC7	MP2 x SC50%	SC
White grape juice	Glucose (g/L)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Fructose (g/L)	0.3d	0.2^{bc}	0.2^{c}	0.3^{b}	0.2^{bc}	0.3^{bc}	0.4^{a}
	Ethanol (% v/v)	14.5^{b}	14.4^{c}	14.4^{c}	14.2^{d}	14.2^{d}	13.9^{e}	15.1^{a}
	Glycerol (g/L)	10.9^{b}	11.0^{ab}	11.1^{ab}	11.4^{a}	11.2^{ab}	11.3^{ab}	7.8^{c}
	Acetic acid (mg/g)	0.46^{a}	0.38^{b}	0.35^{bc}	0.33^{c}	0.32^{c}	0.26^{d}	0.45^{a}
Synthetic grape juice	Glucose (g/L)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Fructose (g/L)	0.3	0.2	0.2	0.1	0.0	0.0	0.4
	Ethanol (% v/v)	14.5^{b}	14.4^{bc}	14.4^{c}	14.3^{c}	14.2^{d}	14.0^{e}	15.5^{a}
	Glycerol (g/L)	11.0^{a}	10.9^{ab}	10.9^{ab}	10.6^{b}	10.7^{b}	10.7^{b}	6.0^{c}
	Acetic acid (mg/g)	0.17^{bc}	0.22^{b}	0.14^{c}	0.16^{c}	0.13^{c}	0.14^{c}	0.67^{a}

Values are means of triplicates ± standard deviation. Values followed by different letters within rows are significantly different ($p \leq 0.05$, one way ANOVA).

A total of 31 volatiles was analysed in a sub-set of samples with different ethanol content. A number of yeast-derived volatiles showed significant differences (data not show), and the apparent off-flavour compounds could not be perceived/measured in the obtained wines. Further studies are therefore focusing on evaluation of this promising non-*Saccharomyces* strain.

Lachancea thermotolerans diversity study

Lachancea thermotolerans (ex *Kluyveromyces thermotolerans*) is a ubiquitous yeast of underexplored biotechnological potential. In the winemaking context, *L. thermotolerans* is of particular interest, as it produces substantial amounts of lactic acid during alcoholic fermentation (Jolly et al. 2014). The maximum reported lactate concentrations achieved during *L. thermotolerans* fermentations are 16.6 g/L (Banilas et al. 2016). In comparison, *S. cerevisiae* strains in the same conditions only produced up to about 0.4 g/L lactate. The produced lactic acid leads to pH decrease/total acidity increase, ameliorating the wine, thus potentially alleviating requirements for external inputs (e.g. tartaric acid). Moreover, in co-culture with *S. cerevisiae*, *L. thermotolerans* is reported to lead to a final ethanol decrease of about 1% v/v; (Gobbi et al. 2013). Other compounds positively affecting wine quality

(e.g. 2-phenyethanol, glycerol, etc.) are also reported to be increased in co-cultures (Jolly et al. 2014). Nonetheless, information on the ecology, evolution and diversity in *L. thermotolerans* was still scarce. We therefore sourced a large number (172) of *L. thermotolerans* isolates from different isolation substrates and continents worldwide and tested them at a genetic level (microsatellite markers). The resultant clustering revealed that the evolution of *L. thermotolerans* has been shaped by the geographical localisation, human influence and flux between different ecosystems. Genetic proximity of isolates originating from anthropic environments, in particular grapes and wine, is suggestive of domestication events within the species. Further support for the genetic clustering was provided via plate-based assays testing growth on several substrates and physicochemical conditions. These findings were published in PLOS ONE (Hranilovic et al. 2017a). Furthermore, 94 *L. thermotolerans* strains were tested for their fermentation performance (the rate and extent of growth and sugar consumption) and outcome (gross metabolite production, acidification and volatile composition) in Chardonnay grape juice. This exercise highlighted their large phenotypic diversity (**Table 5**).

Table 5. Analytical profile range of *L. thermotolerans* and *S. cerevisiae* final fermentations in Chardonnay juice (230 g/L sugar, pH 3.5).

Parameter	***S. cerevisiae***	***L. thermotolerans***	
		Minimum	**Maximum**
Residual sugar (g/L)	0.1	12.6	76.6
Ethanol (% v/v)	13.5	7.3	10.6
Ethanol yield	0.45	0.34	0.39
pH	3.37	3.16	3.81
Lactic acid (g/L)	0.1	1.8	12.0
Acetic acid (g/L)	0.6	0.1	0.3

Conclusion

Observed differences in wine composition and sensory profiles in this study suggest the potential applicability of non-*Saccharomyces* yeasts to enhance the quality of earlier harvested grapes and thereby become a complimentary, rather than alternative, approach in microbiological wine ethanol management. Selection and characterisation of *M. pulcherrima* strain MP2 in sequential inoculations with *S. cerevisiae* revealed significant ethanol decreases compared to *S. cerevisiae* monoculture. In lower-alcohol wines, compositional alterations strongly depended on sequential inoculation timing, and no apparent off-flavours were seen in the wines. Further studies are therefore aiming to establish the inoculation effectiveness in conditions better mimicking real winemaking scenarios. The *Lachancea thermotolerans* study revealed remarkable intra-specific diversity at a genetic and phenotypic level. In general, the non-*Saccharomyces* yeasts have a large potential to modulate wine chemical and sensory profile, and even mitigate negative trends in winemaking, related to excess ethanol and insufficient acidity.

The results from this study have been compiled in the following publications:

1. Hranilovic, A., M. Bely, I. Masneuf-Pomarede, V. Jiranek, and W. Albertin. 2017a. The evolution of *Lachancea thermotolerans* is driven by geographical determination, anthropisation and flux between different ecosystems. PLOS One 12.
2. Hranilovic, A., S. Li, P. Boss, K. Bindon, R. Ristic, P. Grbin, T. Van der Westhuizen, and V. Jiranek. 2017b. Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-*Saccharomyces* inocula. Australian Journal of Grape and Wine Research (in press).

6.4.2. Impact of high sugar content on the efficiency and sensory outcomes of un-inoculated fermentations

Introduction

Yeast species are widely distributed around the globe and occur saprophytically on substances rich in sugar, such as the grape surface (Renouf et al. 2005). Consequently several yeast species are present at the beginning of fermentation, although the dominance of *S. cerevisiae* is expected and desired. Indeed, the oenological practice of culturing selected inocula of *S. cerevisiae* has dominated winemaking for decades, especially in difficult situation such as fermentation of high sugar juices. However, even though 'spontaneous' fermentations where the micro-flora present on grapes or in a winery initiate the alcoholic fermentation but may present a higher risk of spoilage, the improved complexity, mouth-feel (texture) and integration of flavours are qualities winemakers seek (Jolly et al. 2014). One of these species making up the micro-flora of the grape surface is *Torulaspora delbrueckii*. Recently its impact on fermentation has been described as beneficial (Bely et al. 2008) thanks to desired oenological traits such as low acetic acid yield. As a consequence some *T. delbrueckii* strains have been commercialised and their employment, hand in hand with other non-*Saccharomyces* starters, is increasing in cellars (Curiel et al. 2017). However its oenological traits have not been investigate at a molecular level. The aim of the project is to study the genome and transcriptome of *T. delbrueckii* during fermentation to highlight the physiological differences between it and *S. cerevisiae.*

Materials and methods

Yeast selection

An in-house wild yeast collection was created from samples previously taken at different stages during spontaneous fermentations of Shiraz and Viognier grapes at Yalumba winery. The genetic identification of non-*Saccharomyces* and *Saccharomyces* species was made following DNA extraction and analysis of ITS regions (Pramateftaki et al. 2000). The fitness of these yeast species was measured individually in environments mimicking wine fermentations of increased stresses (osmotic and ethanol), to analyse the differences in growth rate and fermentative metabolism.

RNA extraction and transcriptome analysis

Selected indigenous yeast species, *T. delbruckii* and *S. cerevisae*, were grown in CDGJM with 250 g/L of sugar. RNA was harvested from fermentation samples during the exponential growth and stationary phases, and sequenced using Illumina technology. Assembly and downstream analysis were perform following the Trinity protocol (Grabherr et al. 2011, Haas et al. 2013).

Quantum dot labelling

Quantum dots from different suppliers were conjugated with glutathione through EDC coupling (Gustafsson et al. 2014). Different yeast species were fed with QDs at a concentration of 0.1 µM overnight in YNB w/o amino acids and sugar. Confocal microscopy and flow cytometry were used to monitor yeast uptake and the intracellular fluorescence, respectively, during fermentation.

Results and discussion

Characterization of wine yeast isolates through fermentation kinetics and fitness advantage

The physiological responses of several indigenous yeasts to high sugar fermentation in terms of (i) cell viability, (ii) cell growth and (iii) sugar metabolism were monitored. Through individual quantification of the effects of sugar and ethanol, we assessed the impact on the yeast population and the gain or loss of fitness advantage through mathematical models. The results showed that stress, such as osmotic and ethanol, reduce the competiveness of the yeast species. Imposition of *Saccharomyces cerevisiae* over the other species was made possible by ethanol formation. Meanwhile osmotic stress didn't represent a selective advantage for this species.

Assembly and characterization of indigenous Torulaspora delbrueckii and Saccharomyces cerevisiae strain transcriptomes under winemaking conditions

Differences in the production of secondary metabolites, such as acetic acid, glycerol, acetates, esters and higher alcohols, highlighted large differences between the metabolism of the two species. Transcriptome analysis proved to be a powerful tool in understanding the genetic basis for differences between a well-annotated species (*Saccharomyces*) and a non-reference species with a sparely annotated genome (*Torulaspora*).

Defining mixed fermentation dynamics in high sugar musts using quantum dots

CdSe/ZnS core/shell quantum dots with poly (ethylene glycol)-appended dihydrolipoic acid (DHLA-PEG) coating were successfully conjugated with glutathione. Commercial yeast RC212 showed uptake of the QDs. Optimisation of the protocol for QD uptake by indigenous yeasts (*T. delbrueckii* and *S. cerevisiae*) is under investigation, as well as the monitoring of the intracellular QD fluorescence with flow cytometry during fermentation (**Figure 14**).

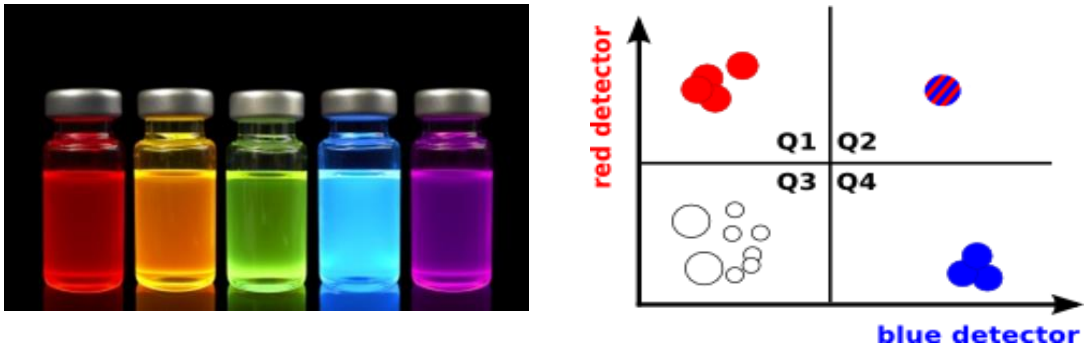


Figure 14. Quantum dot fluorescence and the theoretical spatial distribution of labelled cells using flow cytometry

Conclusion

Yeast cells must coordinate gene expression to rapidly respond to external changes and to maintain competitive fitness and cell survival (Causton et al. 2001). Although, several studies have analysed the yeast response to specific stress conditions, there is a need to expand our knowledge about spontaneous, multicultural fermentations where multiple stresses are present. In this study, twenty isolated yeast strains have been tested for physiological responses to osmotic and ethanol stress. Yeast reaction to osmotic stress was clear and similar for all the evaluated strains, while ethanol stress appeared to be more challenging and the responses were more differentiated and genera/strain specific. Based on these results three wild yeast strains have been selected for transcriptome analyses to explore how different yeasts change their gene expression in response to stress conditions.

Torulaspora delbrueckii shows high potential as an alternative to an *S. cerevisiae* monoculture during wine fermentation. The results confirmed earlier work of Belly el al (2008) and Tataridis et al (2013) that *T. delbrueckii* produced less volatile acidity and different aromatic compounds during fermentation. Furthermore, transcriptome analyses revealed the relations between genotype and phenotype. Further characterisation of its oenological traits will allow winemakers to use this yeast species in an informed manner as another tool to improve wine sensory profile. The interaction with *Saccharomyces* and consequently the performance of a mixed population in high sugar mixed fermentation needs to be investigated further.

6.5. Winemaking techniques for alcohol management and flavour enhancement

6.5.1. Selective and deliberative use of winemaking supplements to modulate sensory properties in wine

Introduction

Harvesting grapes earlier (i.e. at lower sugar levels) can be an effective method to reduce alcohol levels in wine. However, fruit maturity has a significant influence on wine composition. Wines made from early harvested fruit can be deficient in the desirable organoleptic characters usually associated with full-bodied wines made from mature fruit; aroma and flavour intensity in particular, as well as mouthfeel attributes. Tannins and mannoproteins (MP) are two key macromolecules present in wines that are responsible for important mouthfeel attributes, such as astringency and viscosity. However, these macromolecules are lacking in wines made from early harvested fruit. This project therefore aimed to improve the mouthfeel of Shiraz wines made from early harvested fruit, through selective application of commercial winemaking supplements to modulate tannin and mannoprotein levels.

Pre-fermentation maceration enzymes and oenotannins were used to modify wine tannin composition. Maceration enzymes can degrade pectin in the grape cell walls thereby facilitating the extraction of phenolic compounds into wine. Oenotannin is widely used in the Australian wine industry for colour stabilisation, creating specific wine styles, masking faults and for general risk management (Hill and Kaine 2007). However previous research concerning the effects of tannin addition on wine composition and sensory properties shows diverse outcomes, probably due to a range of factors, such as additive origin (grape, oak and other material), dose rates, grape variety and timing of addition (Versari et al. 2013).

The effects of mannoprotein are far less studied, despite mannoprotein being one of the main polysaccharides in red wine. MP accounted for 35% of the total wine polysaccharide in a Carignan wine (Vidal et al. 2003). Evidence suggests that mannoprotein can contribute to palate fullness and reduce astringency (Vidal et al. 2004b, Quijada-Morín et al. 2014). Although mannoproteins are not as widely used in wine production as oenotannins, there are still several winemaking supply companies that produce and market this product.

Materials and methods

A winemaking trial conducted in 2015 involved harvesting Shiraz grapes at two levels of maturity, with a maceration enzyme, an oenotannin and a mannoprotein being introduced into early harvest wines, either individually or in combination. Compositional and sensory analyses were subsequently performed on finished wines. A subsequent trial characterised the composition of 14 oenotannin and 8 mannoprotein products available on the Australian market. Based on these results, 2 oenotannins and 1 mannoprotein were selected for addition to two Shiraz wines (one with lower alcohol content, the other with typical alcohol content) in different combinations and at different concentration, prior to chemical and sensory analyses.

Experiment 1. Winemaking Trial in 2015

Shiraz grapes were sourced from a commercial vineyard in the McLaren Vale region of South Australia when the total soluble solids (TSS) reached 24 °Brix (i.e. 'early' harvest) and again, from the same plot, at 28 °Brix (i.e. 'late' harvest). The early harvest fruit was randomly assigned to one of the following seven treatments (in triplicate):

1. Control (early harvest). Wines were made following the small-lot winemaking procedures at the University of Adelaide.

2. Cold soak. Grape must was stored at 10°C for 61 hours before being warmed to room temperature and fermented as per the control.
3. Enzyme. Grape must underwent cold soak as in Treatment 2 but with the addition of a commercial maceration enzyme at crushing.
4. Enzyme and Mannoprotein (Enzyme_manno). Grapes were vinified as in Treatment 3 with a commercial mannoprotein product added after racking of gross lees.
5. Tannin. A mixture of two commercial powdered tannin products were blended thoroughly and added to the must at first plunging, as per manufacturer's suggestion.
6. Tannin and Mannoprotein (tannin_manno). Conditions were the same as for Treatment 5, but mannoprotein was also added after racking of gross lees.
7. Mannoprotein (manno). Mannoprotein was added to wines after racking of gross lees.

Experiment 2: Evaluation of Commercial Oenotannin and Mannoprotein Products

Fourteen grape-based tannin products were sourced from five different manufacturers, twelve of which were in powdered form; the other two were in liquid form. Eight mannoprotein products were then sourced from five different manufacturers (not necessarily the same as for the tannin products), five of which were in powdered form; the other three were in liquid form. The liquid products were freeze-dried and powdered for ease of comparison. In order to obscure product names, as per manufacturers' request, tannin products were labelled as 'skin', 'seed', or 'skin+seed', according to the origin of material reported by the manufacturers. Products were analysed for the tannin concentration and composition. More detail can be found in Li et al. (2017a).

Experiment 3: The Impact of Selected Oenotannin and Mannoprotein products on Shiraz Wines Made from Two Distinctive Harvest

Three commercial products, two oenotannins (one derived from grape seed and one from skins) and one mannoprotein, were selected for further trials, based on results obtained from Experiment 2; i.e. because they showed compositional characters that typically define their counterparts when isolated from grapes and wine. These products were added to two Shiraz wines to study their impact on wine composition and sensory properties, in particular, mouthfeel characters.

In contrast to Experiment 1 where only the wines made from early harvested grapes were supplemented, Experiment 3 involved the same supplementation regimes for wines made from both unripe and mature grapes. In this way, a series of wines comprising different ethanol, tannin and polysaccharide concentrations and/or compositions were created, which enabled any interactions attributable to these three wine components to be evaluated using sensory analysis techniques.

Results and discussion

Experiment 1. Wines made from riper grapes were naturally higher in tannin and mannoprotein than wines made from grapes harvested earlier. Maceration enzyme had a marked effect on the breakdown of grape cell walls, which led to significantly higher concentrations and average molecular mass of wine tannins; i.e. levels were comparable with those of wines made from mature grapes. The enzyme treated wines were rated highest for astringency and palate coarseness, as expected based on their chemical composition. In contrast, MP addition achieved the lowest tannin concentration and corresponding wines were rated lowest for palate coarseness. However, the increase in MP concentration in the treated wines was considerably lower than expected. Analyses of the MP product revealed that it contained only 10% mannan, and instead contained around 25% arabinogalactan (AG). Oenotannin addition did not influence wine tannin composition, colour parameters or mouthfeel properties. However, it increased red fruit and confectionary aromas. When enzyme or oenotannin were applied in combination with MP, the effects were less apparent. The enzyme + MP treatment was similar to when the enzyme was used alone, whereas the tannin + MP treatment had a significant impact on aroma and flavour, but not on mouthfeel, compared to when the tannin was used alone. Principal component analysis (**Figure 15**) revealed that later harvest wines were separated from earlier harvest

wines based on their more intense aroma and flavour, sweetness, palate fullness and hotness. Furthermore, of all supplement regimes, the tannin + MP wine most closely resembled the wines made from mature grapes (based on the proximity of the tannin_manno and late harvest treatments in **Figure 15**).

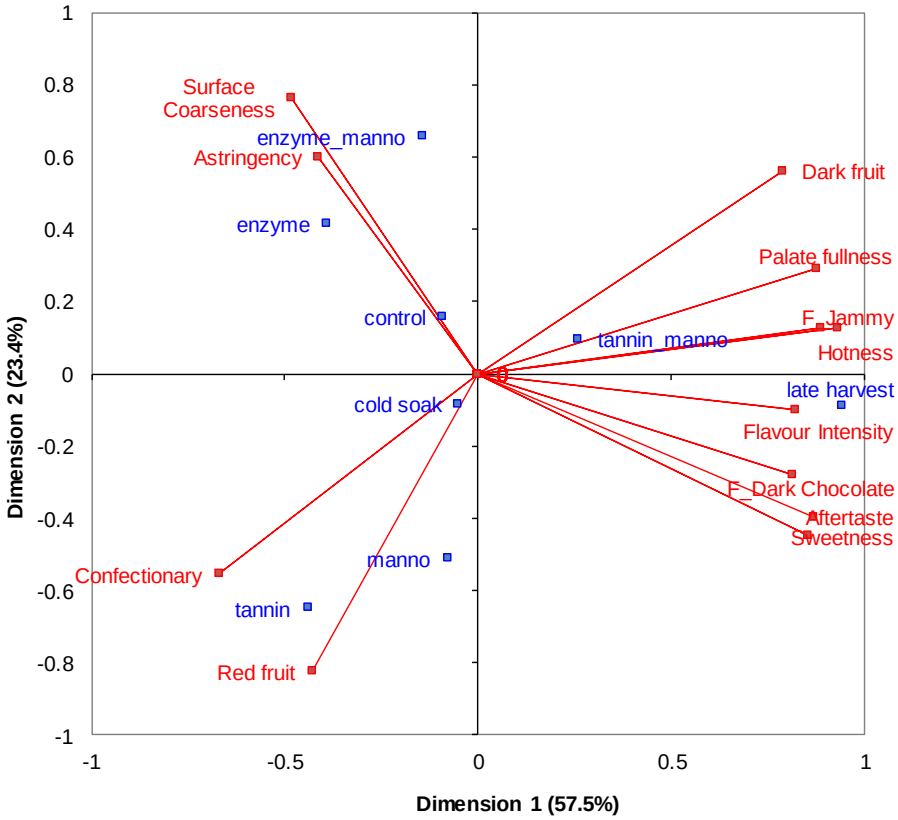


Figure 15. Principal component analysis of sensory profiles of Shiraz wines. F= flavour attributes. Derived from Li et al. (2017b).

This study confirmed the hypothesis that altering tannin concentration, composition and size could affect the perception of astringent mouthfeel. However, although the parameters of wine tannin measured in this study were similar between the enzyme treatment and the late harvest Shiraz, the former was perceived to be astringent while the latter was not. This observation indicated that mouthfeel is likely to be affected by other wine sensory components, such as the intensity of fruit characters and/or sweetness. Modifying one factor alone may result in the mouthfeel becoming unbalanced with other wine components. Furthermore, the unexpected composition of MP product indicated that there may be large compositional variation amongst commercial products, even within the same types of supplement. Thus the results reported above might be only applied to the three products used in the current study. Also, the current study demonstrated a great loss of added oenotannin, and inconsistent recovery of mannoprotein. This may be due to both the composition of the particular products used and the processes of precipitation and subsequent racking during vinification. Lastly, the vintage conditions of 2015 were warmer than expected, and so both wines contained more ethanol than intended. This did not meet the initial aim of the study, to use wines containing only 2% - 3% lower alcohol levels than for the average red wine produced in Australia (i.e. 14.5%). Thus, future studies were designed to (i) use lower alcohol wines, (ii) evaluate a broader selection of winemaking supplements and (iii) investigate the sensory impact of additives in finished wines.

Details of this study can be found in Li et al. (2017b).

Experiment 2. Two of the products used in the previous study were selected for further examination, i.e. grape-derived oenotannin and mannoprotein (MP). The maceration enzyme was not included in the subsequent studies because it alters both tannin and polysaccharide compositions and it can not be applied to a finished wine. The composition of 14 grape-based oenotannin products and 8 MP products were determined. For oenotannins, methylcellulose precipitable tannin (MCPT) was measured and calculated as a percentage of the dry weight to represent product purity (**Figure 16**). The MCPT values among products were highly variable, and the contents of major monomeric phenolic compounds were found to be relatively low across all products. Principal component analysis based on tannin composition and size (**Figure 17**) revealed that some products exhibited chemical compositions that strongly agreed with the labelled origin of material (i.e. seed and skin), while others did not. Furthermore, for certain manufacturers, although products were marketed under different names for different oenological purposes, their compositions were actually quite similar, while other manufacturers' products (sold under different labels) showed significant compositional differences.

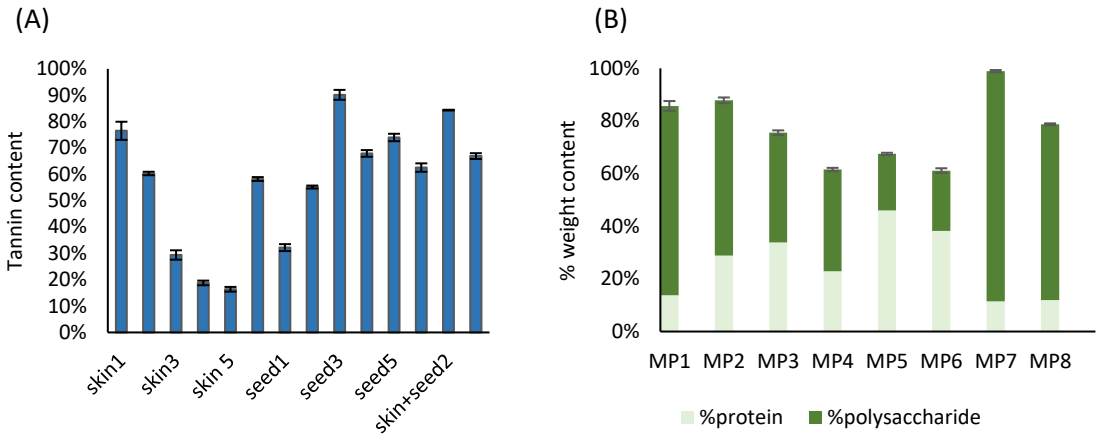


Figure 16. (A) Weight percentage of measurable tannin content (methylcellulose precipitable tannin) in oenotannin products 'skin' (1 - 6), 'seed' (1-5) and 'skin+seed' (1-3) and (B) total polysaccharides and protein contents (%) in mannoprotein products (right)

The monosaccharide and protein analyses accounted for 60 to almost 100% of the dry weight of MP products. The composition of the polysaccharide fraction of products were also highly variable. All products contained different amounts of mannose and glucose residues. However, some products also contained a considerable amount of arabinose and galactose residues, which indicated presence of arabinogalactans, a polysaccharide not derived from yeast. The protein content of products ranged between 10% and 50%. This is likely to have significant impact on the products' effect on wine, as yeast derived proteins have higher interactions with wine polyphenolics than the polysaccharide fraction. Furthermore, molecular distribution of the products spanned 5 to 800 kD, with products containing arabinogalactan, leaning towards higher size averages than products contained only MP.

The impact on mouthfeel of tannin and MP has previously been attributed to concentration, composition (subunit composition for tannin and protein content for MP) and molecular size. It is therefore reasonable to assume that the choice of products will affect the potential sensory impact on the treated wines. It is therefore crucial for researchers to report product characterisation involved in the study and for winemakers to conduct bench trials using the wines to be treated with different products, in order to make informed decisions regarding the use of supplements.

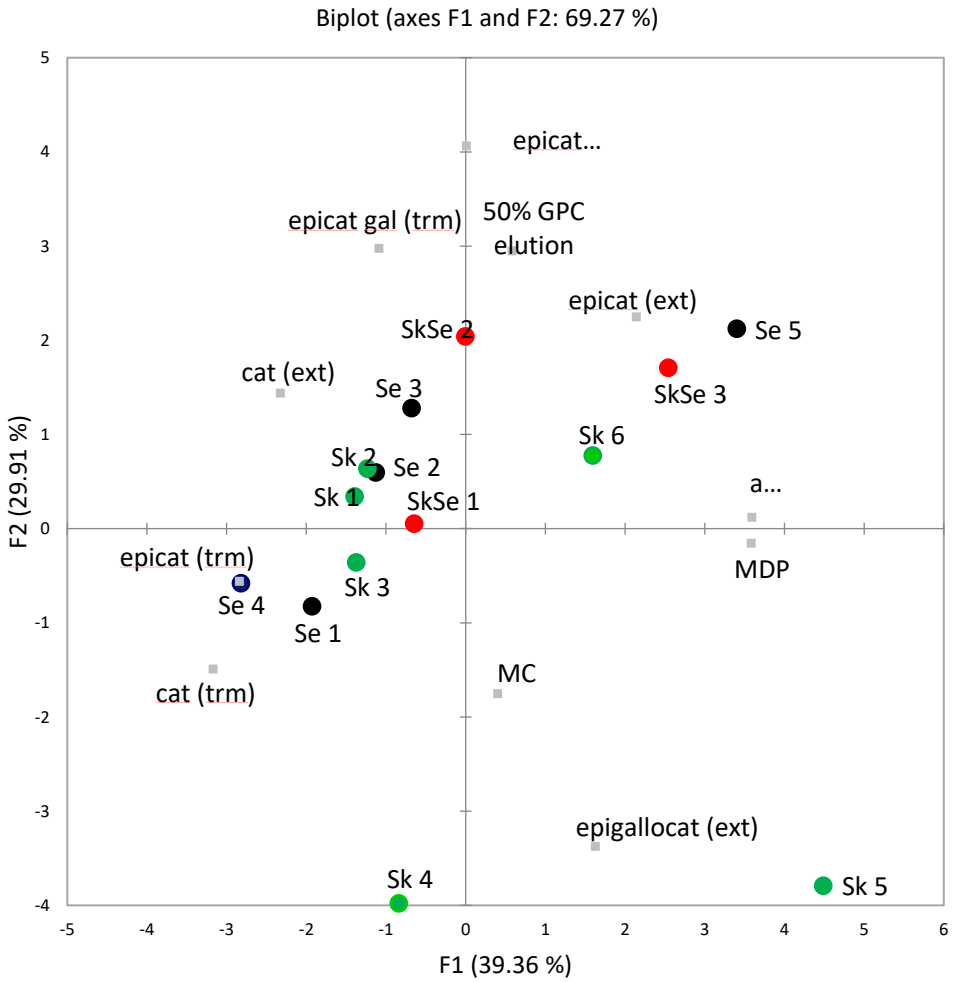


Figure 17. PCA plot of tannin products based chemical composition. % = percentage of terminal (trm) and extension (ext) units of catechin (cat), epicatechin, (epicat) epicatechin gallate (epicat gal) and epigallocatechin. Sk= skin; Se = seed, SkSe = skin + seed; MDP = mean degree of polymerisation; aMM = average molecular mass; MM = % mass conversion. Dervived from Li et al. (2017a).

Experiment 3. Two oenotannins (Se4 and Sk5) and one MP (MP 7) were selected for further study, based on results from experiment 2. Two Shiraz wines, made from sequential harvests of grapes from the same vineyard, containing 11.5% and 14.5% alcohol, respectively were made. Supplements were introduced to these wines in different combinations and at different concentrations. The supplementation regimes created a series of wines with tannin concentrations from 326 to 1067 mg/L, and mannoprotein concentrations of 68 to 452 mg/L. DA revealed panelists perceived wines of 14.5% alcohol as having more 'sweetness', 'body', 'hotness' and 'flavour intensity', than wines with 11.5% alcohol. However, no significant differences were found for 'astringency' or 'body' across treatments. A further experiment was performed where judges rated: (i) 'astringency' for a series of wines (containing 11.5% alcohol), spiked seed tannin (at 300, 600, 1000 and 1500 mg/L); and (ii) 'body' for a series of wines spiked with mannoprotein (at 400, 1000, 3000 and 6000 mg/L). The sensory panel could not distinguish astringency levels when the differences in tannin concentration between spiked samples were 300 or 600 mg/L, but they could differentiate between samples with 1000 mg/L or more differences in tannin concentration. The judges were also unable to perceive an increase in body in wines with higher MP concentrations, even at 6 g/L. It was unclear if the difference in tannin levels between treatments was too subtle to be examined by DA or the judges were not sufficiently sensitive and in need of more training. It is likely that the increased 'body' perception between wines of the two harvests was due to

complex interactions of flavour, viscosity and other compounding factors. However, increasing mannoprotein concentrations alone could not achieve similar effects.

Conclusion

This project provides valuable insight into the changes in mouthfeel characters that can be induced by modifying wine tannin and mannoprotein composition, especially in the context of improving the mouthfeel properties of wine made from early harvest grapes. Increasing tannin molecular mass as well as concentration, such as seen in the application of maceration enzymes, produced a wine that was more coarse on the palate. Conversely, the application of oenotannin to finished wine increased tannin concentration without modifying tannin composition and size, but did not result in any change in astringency perception. Similarly, addition of a protein-rich mannoprotein at 400 mg/L during the vinification process could achieve a softer mouthfeel, likely due to fining of wine tannin. However, supplementing a finished red wine with a polysaccharide-rich mannoprotein did not modify either astringency or wine body, even at 1000 mg/L addition rate. It was also demonstrated that there is considerable variation amongst commercial oenotannin and mannoprotein products, which are therefore likely to achieve different effects on wine composition and, by extension, mouthfeel characters. In summary, this project furthered the current level of understanding of commercial wine supplements and showed that their selective and deliberate application can be used to modify the composition and sensory properties of red wine.

Results from this study have been compiled in the following publications:

1. Li, S., Bindon, K., Bastian, S.E.P., Jiranek, V., Wilkinson, K.L. (2017). Use of winemaking supplements to modify the composition and sensory properties of Shiraz wine. J. Ag. Food Chem. 65 (7), pp 1353–1364. DOI: 10.1021/acs.jafc.6b04505.
2. Li, S., Wilkinson, K.L. and Bindon, K.A. (2017). Compositional variability in commercial tannin and mannoprotein products, Am. J. Enol. Vitic. (AJEV-D-17-00057). DOI: 10.5344/ajev.2017.17057
3. Bindon, K., Li, S., Kassara, S., Smith, P. (2016). Retention of proanthocyanidin in wine-like solution is conferred by a dynamic Interaction between soluble and insoluble grape cell wall components, J. Ag. Food Chem. 64:8406-8419.
4. *Li, S. Selective use of winemaking supplements to modulate the chemical composition and sensory properties of Shiraz wine. PhD thesis, The University of Adelaide (under examination).*

6.5.2. Getting alcohol content right: The compositional and sensory basis for an alcohol 'sweetspot'

Introduction

Demand for reduced alcohol wines (RAW), as well as the range of treatments employed by the wine industry to reduce wine ethanol levels, has been increasing. However, there is a lack of research on the chemical composition and sensory profiles of RAW. The impact of dealcoholisation on the composition of RAW is complex, particularly the impact on volatile and non-volatile compounds. Up until now, a loss or reduction of volatiles during dealcoholisation has been considered a major drawback of this approach to achieving lower alcohol levels in wine. Since ethanol is essentially a solvent in wine, when ethanol levels are reduced, the concentration of most non-volatile and some volatile compounds changes, irrespective of whether or not the volume of these compounds change. Ethanol is also directly involved in the acid-ethyl ester equilibrium, so when ethanol decreases, the concentration of ethyl esters (i.e. volatiles that positively influence wine aroma) may also decrease. The complexity of ester

interactions within the wine matrix, particularly taking into consideration that these compounds are typically present at around detection threshold concentrations, means that modest changes in ethanol concentrations might have dramatic effects on wine aroma and flavour, and consequently, wine quality. Moreover, the optimal ethanol level for RAW remains unclear, as well as any scientific basis for the "sweet spot" phenomenon. This project therefore aims to provide insight into the effects of alcohol reduction on the wine equilibrium.

Materials and methods

A series of trials was conducted to investigate the impact of ethanol removal by reverse osmosis–evaporative perstraction (RO-EP). Red wines were partially dealcoholised by RO-EP, such that alcohol content was reduced by 1.0–2.4 % v/v. Wine composition was analysed: (i) pre-treatment, (ii) post-treatment and (iii) post-treatment following alcohol adjustment (i.e. the addition of ethanol to achieve the original alcohol content). Wine colour was analysed by spectrophotometric methods, while other compositional changes were determined by WineScan, HPLC and GC-MS. The sensory profile of wines (before and after partial dealcoholisation) was evaluated by descriptive analysis. Sensory trials were undertaken (with a panel comprising winemakers) to investigate the basis for sweetspotting (i.e. optimising the ethanol content of wine from a bracket of 9 wine samples with 0.2 % v/v increment) and the influence of sample presentation on panel performance.

Results and discussion

Experiment 1. Analyses of wines A, B and C with reduced alcohol levels showed that RAWs were slightly more concentrated than pre-treatment wines, resulting in RAWs having darker colour, higher astringency and increased acidity (**Table 6**). The RAWs also displayed lower concentrations of small molecular weight volatile compounds, particularly ethyl esters, which could be due to increased ester hydrolysis (data not shown). They also appeared to be of lower viscosity, which in turn, may contribute to the lower wine body.

Table 6. Chemical composition of wines A, B and C treated by RO-EP.

	Wine A			Wine B			Wine C		
	pre	post	adj	pre	post	adj	pre	post	adj
Ethanol (% v/v)	14.1	12.4	14.1	17	14.6	17	15.1	14.2	15.1
Methanol (g/L)	4.26	4.50	4.49	4.48	4.49	4.38	7.50	7.60	7.11
Glucose (g/L)	0.91	0.11	0.07	0.05	0.06	0.05	0.02	0.03	0.02
Fructose (g/L)	2.02	0.23	0.48	0.42	0.43	0.41	0.38	0.38	0.37
Free SO_2 (mg/L)	3.8	0.0	0.0	6.4	0.0	0.0	7.9	2.1	2.3
pH	3.69	3.60	3.68	3.59	3.60	3.59	3.67	3.65	3.67
TA (g/L)	5.9	6.1	5.9	7.2	7.1	7.2	6.4	6.5	6.4
VA (g/L)	0.40	0.30	0.36	0.53	0.75	0.48	0.63	0.62	0.61
Citric acid (g/L)	1.17	1.17	1.14	0.75	0.73	0.76	0.87	0.89	0.85
Succinic acid (g/L)	6.22	6.22	6.07	8.19	8.33	8.29	6.47	6.51	6.39
Lactic acid (g/L)	3.68	3.81	3.71	2.81	2.83	2.75	3.83	3.79	3.68
Acetic acid (g/L)	0.28	0.22	0.25	0.63	0.62	0.57	0.57	0.56	0.48
Colour density (au)	10.8	12.6	12.2	19.6	21.0	18.8	17.2	17.6	15.7
Hue/Tint (au)	0.68	0.65	0.68	0.60	0.58	0.59	0.55	0.58	0.63
Polymeric pigments (mg/L)	2.79	4.91	3.77	4.08	4.24	3.95	3.39	3.64	3.40
Flavonols (mg/L)	1158	1108	1145	1346	1377	1314	1284	1302	1290
Total phenolic (mg/L)	1701	1638	1695	2685	2755	2640	1987	1988	1998
Gelatin index (%)	38.4	52.5	47.1	50.7	57.5	48.5	43.5	43.7	40.8
Density (g/mL)	0.9949	0.9951	0.9932	0.9927	0.9957	0.9925	0.9929	0.9938	0.9928
Viscosity (Pa.s)	1.837	1.708	1.793	2.005	1.834	1.979	1.859	1.75	1.831

TA = titratable acidity; VA = volatile acidity; au = absorbance units

Wines WA, WB and WC were 2014 Barossa Valley Shiraz Cabernet Sauvignon, 2015 McLaren Vale Cabernet Sauvignon and 2015 Adelaide Hills Shiraz, respectively.

Experiment 2. Triangle tests were conducted to determine ethanol detection thresholds in RAWs. Wine samples were blended to achieve 0.2, 0.5 and 1.0 % v/v alcohol increments, by mixing pre- (high alcohol) and post-treatment (low alcohol) wines. There were no significant differences between the lower alcohol content sample set (i.e. wines comprising 14–15% ethanol v/v), where 0.2, 0.5 and 1.0% alcohol differences yielded only 5, 6 and 5/18 correct responses, respectively. However, in the higher alcohol content sample set (i.e. wines comprising 15.3–16.3 % ethanol v/v), the number of correct responses increased (to 5, 7 and 9/18) with increasing alcohol content (i.e. 0.2, 0.5 and 1.0% difference, respectively). This is in agreement with previous studies that found differences in ethanol concentration were detectable between 0.5 and 2.0% v/v, depending on the wine style and the assessor, i.e. wine experts vs. consumers (Yu and Pickering 2008, Schmitt et al. 2013).

Experiment 3. The fourth study involved sweetspotting trials for two red wines, by an expert panel of 14 winemakers. Shiraz and Cabernet Sauvignon wines with 16.1% v/v alcohol were dealcoholised to 14.4 % v/v and thereafter blended at 0.2 % v/v alcohol increments, giving 9 wine samples in total for each grape variety. Wine brackets comprising all 9 wines were presented to panellists in duplicate, in four presentation orders: row and circle, and ordered (i.e. low to high) or randomised. Panelists were asked to taste the wines and identify the wine(s) they considered to represent the 'sweetspot'; i.e. the wine(s) that exhibited the best aroma and palate, in terms of overall sensory perception. Interestingly, the sweetspotted wine was found to be the 15.6 % v/v wine in the circle presentation and the 15.4 % v/v wine in the row presentation, and the 15.4 % v/v wine considering the overall results. Statistical analysis found that while there was no statistically significant wine for the entire range of wine samples, there was a significant difference for the highest preference value, which indicated a 'sweetspotted' wine.

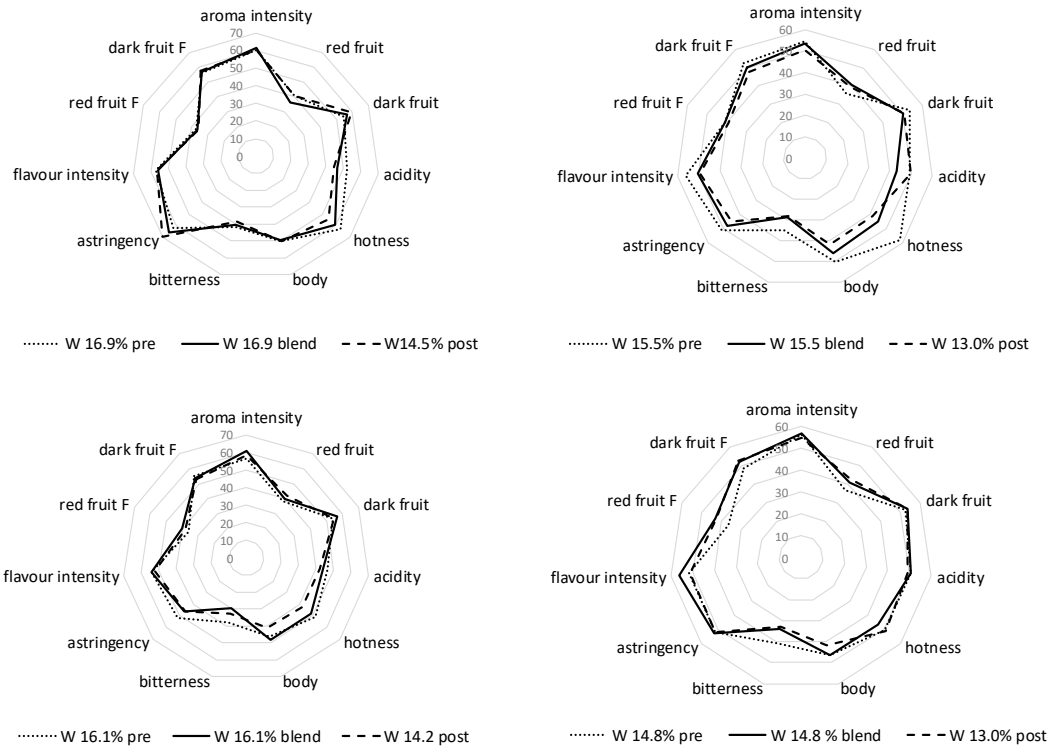


Figure 18. The sensory profiles of pre, post and blended dealcoholised Cabernet Sauvignon wines sourced from the South Australian wine regions, vintage 2015. Derived from Ristic et al. (2016).

Experiment 4. Descriptive analysis of RAW Cabernet Sauvignon wines indicated that dealcoholisation affected the perception of wine sensory attributes, in particular hotness, astringency and acidity (**Figure 18**).

The DA panel also attempted to identify the alcohol 'sweetspot' using two approaches: a 'circle-order' presentation style and a 'knockout' or elimination process (in which only two samples were evaluated at a time). The 'knockout' method showed the most promising results and will be used in future trials, with wine samples presented randomly and in order.

Conclusion

The use of RO-EP technologies to manage wine alcohol levels by industry will allow customers to enjoy lower alcohol wines with improved aroma, flavour, taste and mouthfeel properties. In general, chemical analysis showed that removal of ethanol by RO-EP had some impacts on wine compositional properties, depending on the wine and the initial level of ethanol. Some volatile compounds were lost through membrane filtration and while there was no significant change in pH, TA and VA, small changes in density and viscosity were observed. Sensory analysis showed RO-EP treatment had only small effects on wine aroma and flavour, which was consistent with the small changes observed in basic wine composition and volatile profiles. However, the perception of hotness was most affected while sweetness and saltiness were less affected. In some wines, body, acidity and bitterness were reduced, but astringency increased.

6.6. Novel techniques for flavour enhancement

6.6.1. Controlling unripe characters using magnetic molecularly imprinted polymers to eliminate excessive methoxypyrazines from wines

Introduction

Methoxypyrazines (MPs) are known to be responsible for green sensory characters in grapes and wines, contributing vegetative, herbaceous and capsicum-like flavours (King et al. 2011). These are potent odorants with extremely low olfactory detection thresholds ranging from 0.3 to 16 ng/L in wine (3-isopropyl-2-methoxypyrazine (IPMP) and 3-isobutyl-2-methoxypyrazine (IBMP)) and water (3-secbutyl-2-methoxypyrazine (SBMP)) (Kotseridis et al. 1998, Sala et al. 2004, Pickering et al. 2007). Although important character impact compounds for varietals such as Cabernet Sauvignon, suprathreshold concentrations of MPs can be deemed overpowering and undesirable (Hein et al. 2009). Early harvested grapes used to produce lower alcohol wine, or grapes from cool climate regions, may contain higher concentrations of MPs, leading to wines with 'unripe' sensory characters. MPs present in grapes at harvest are largely unaffected by winemaking procedures, so methods for removing excessive MPs post-harvest are warranted.

Materials and methods

This project involved the synthesis of magnetic molecularly imprinted polymers (MMIPs) to specifically remove MPs from wines. The target molecules or structurally similar compounds were used as templates during polymer synthesis, whereupon removal of the template liberates cavities that selectively recognise and bind target molecules (Haupt et al. 2012). The synthesis of MMIPs is a multi-step process. Firstly, Fe_3O_4@SiO_2-MPS nanoparticles as magnetic substrates were prepared (Chen et al. 2013), and then they were added into toluene mixture with methyl methacrylate as a functional monomer and ethylene glycol dimethacrylate as a cross-linker to complete polymerisation (Belbruno and Kelm 2014). Molecularly imprinted polymers (MIPs) were also synthesised for comparison with their magnetic counterparts and non-imprinted polymers (NIPs), made the same way but without the template molecule, acted as controls. Microwave synthesis (MW) was adopted to compare with

conventional thermal synthesis. IBMP was chosen as a target molecule and physical characterisations and adsorption tests were carried out to evaluate the polymers (**Figure 19**). Furthermore, MMIPs were tested in winemaking trials as pre- and post-fermentation treatments using Cabernet Sauvignon grape must spiked with IBMP.

Results and discussion

Magnetic polymers were designed so that their separation from a liquid medium could be assisted by adding an external magnetic field (vs decanting/using in a packed column for other polymers). Magnetic polymers with dosage of 10 mg/mL were found to have removed 40-60% of IBMP (initial concentration around 30 ng/L) from model wine and white wine within ten minutes, according to GC-MS analysis. The polymer could be regenerated by washing in diethyl ether. The addition of magnetic nanoparticles and microwave-induced polymerisation did not affect adsorption isotherms compared to regular imprinted polymers (**Figure 20**). The amount of IBMP binding to the polymers increased with increasing initial concentrations. Adsorption isotherm models were applied to the adsorption data, showing that the 'Freundlich' isotherm was the best fit for both MMIPs and MNIPs. The Freundlich isotherm implies multisite adsorption of the polymers with heterogeneous surfaces, meaning there is stacking of the adsorbed molecules rather than covering the polymer surface with a monolayer molecules. However, imprinted polymers were found to have no specific binding towards IBMP compared with non-imprinted controls, so different template, monomer and reaction solvent combinations have been trialled to improve polymer specificity for IBMP.

Chemical and sensory evaluation of wines arising from MMIPs and MNIPs treatments of grape must with elevated levels of IBMP showed the MMIPs and MNIPs could effectively decrease green sensory characters without largely compromising overall aroma intensity of the wines, especially when added pre-fermentation. The concentration of IBMP in wines decreased from 20.55 ng/L to 11.62 ng/L (just around the detection threshold of IBMP in red wines (10 ng/L) (Roujou de Boubée et al. 2000)) by pre-fermentation treatment with MMIPs. This is a useful (and sensible) outcome, given that IBMP is present as a free volatile compound in grape juice/must but many other varietal compounds are bound as non-volatile precusors, and yet others are formed by yeast. As such, most wine aroma compounds are produced (or released) during fermentation, making pre-fermentation a more appropriate time for treatment to remove compounds like IBMP. Furthermore, red wine colour components are initially extracted from the skins during fermentation, and pre-fermentation treatments had less effect on wine colour properties than the post-fermentation treatments. For instance, the concentrations of total phenolics were decreased by 14.75% through pre-fermentation treatment and by 33.07% through post-fermentation treatment with MMIPs.

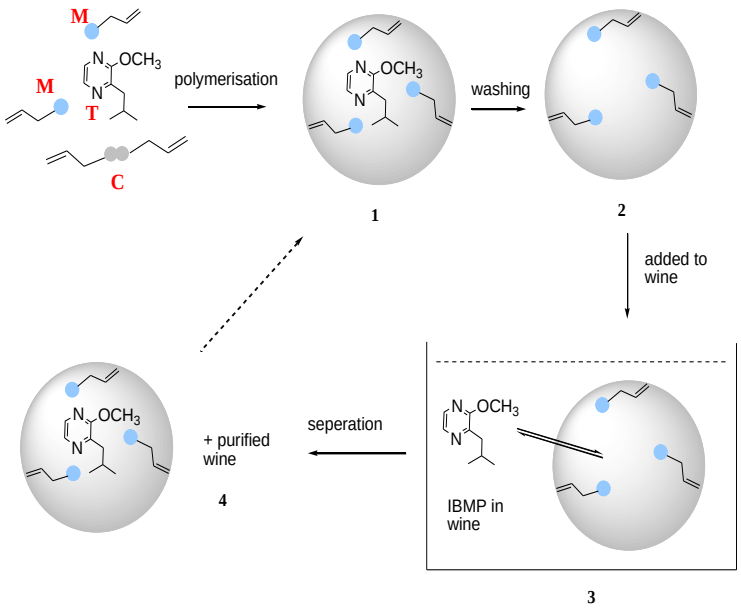


Figure 19. Molecular template (T) of IBMP; functional monomers (M); cross-linker (C). Self-assembly and polymerisation of the system produces a rigid polymer bearing imprinted sites (1). Removal of the template through washing liberates cavities that can specifically recognize and bind the target molecule (IBMP) (2). Imprinted polymer added to wine which contains IBMP (3). Separation of the polymer results in purified wine (4). Polymer (1) may be recycled.

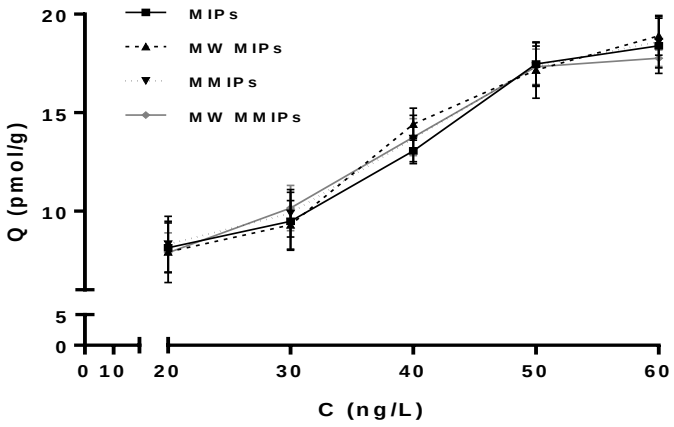


Figure 20. Adsorption isotherms of IBMP on different imprinted polymers.
Q: The equilibrium adsorption amounts of IBMP (pmol/g).
C: Concentration of IBMP (ng/L) in model wine (12% v/v ethanol, pH 3.4).

Conclusion

Winemaking options for controlling “green” characters due to MPs are generally lacking and the selective removal of IBMP from wine was investigated. A range of polymers were trialled, including magnetic and molecularly-imprinted variants. MMIPs were found to offer moderate adsorption ability towards IBMP, were regenerable, and could be removed by applying a magnetic field, which shows that they might be an effective option to remediate wines with elevated MP concentrations. Furthermore, it was revealed that treatment of grape must with MMIPs to remove spiked IBMP was better than treatment of the finished wine, in terms of the impact on an array of other aroma volatiles and colour.

However, further improvement of the efficiency and specificity of MMIPs will need to be a focus of future work if they are to become a better option than their non-imprinted analogues.

6.6.2. The use of cyclodextrins to manipulate off-flavours in wines

Introduction

Cyclodextrins, as a group of natural oligosaccharides, have been widely used in the food industry for removal/delivery of flavour compounds and modification of mouth-feel and taste (Szente and Szejtli 2004). Their application is based on the ability of the cyclodextrin ring structure to encapsulate hydrophobic molecules in the cavity. The physico-chemical changes brought upon the guest molecules, including decreased volatility, increased solubility and decreased perception of flavour (Hedges et al. 1995). The hydrophobicity and size of the guest molecule were the most influential factors in this effect (Marques 2010).

Volatile phenol compounds are hydrophobic molecules mainly responsible for some off-odours resulted from undesirable conditions in viticultural and wine making processes, such as barnyard and medicinal characters due to *Brettanomyces bruxellensis* spoilage (Suárez et al. 2007) and smoke taint characters from bush fire (Kennison et al. 2007). A number of techniques have been developed in the wine industry to deal with such problems on a molecular level, and the number of publications in related topics has been increasing rapidly in recent years. For example, Carrasco-Sánchez et al. (2015) reported the use of polyaniline-based compounds to remove 4-ethylphenol and 4-ethylguaiacol molecules that caused the *Brettanomyces* off-odour in red wine; Fudge at al. (2011, 2012) reported varied adsorption results on several commercial fining agents and adsorption resin to remove "smoke taint" related volatile phenols; Larcher et al. (2012) reported the capability of esterified cellulose to remove 4-ethylpheol and 4-ethylguaiacol. It is worth noticing that most of these off-odour removal techniques are based on hydrophobic interactions between off-odour phenol compounds and the reagents.

A few studies have looked into the hydrophobic inclusion effect of cyclodextrins, and the possibility of utilizing cyclodextrins as additives or processing-aids during wine making to supress or remove hydrophobic molecules. De and Vinho (2011), despite a relatively simplistic methodology, reported that β-cyclodextrin addition effectively suppressed the perception of "*Brett*" characters caused by elevated concentrations of 4-ethylphenol and 4-ethylguaiacol. It wasn't clear if this removal technique was accompanied by loss of desired aromas or other detrimental effects on wine composition. According to the study by Ratnasooriya and Rupasinghe (2012) cyclodextrins could be used to extract phenolic compounds from grape and pomace. While these studies exploited the encapsulation effect between cyclodextrin cavities and phenolic compounds in wine, other studies have investigated the encapsulation effect with aroma/flavour compounds, albeit not in wine (Reineccius et al. 2002, Kant et al. 2004, Suratman et al. 2004). Cyclodextrin polymers, sometimes molecularly imprinted, were also used for dye adsorption (Kyzas et al. 2013), removal of 2,4-dichlorophenol from water (Yamasaki et al. 2008, Surikumaran et al. 2014), and retention of aroma compounds in essential oil (Ciobanu et al. 2013). Based on those studies, it can be hypothesised that cyclodextrins and their polymers can be used in off-odour removal and mouth-feel/taste modification during wine making.

Materials and methods

α-, β-, and γ-cyclodextrins were added to model wine, white wine and red wine samples spiked with off-odour compounds, including guaiacol, 4-methylguaiacol, 4-ethylphenol, 4-ethylguaicol, ortho-cresol, meta-cresol, para-cresol and eugenol. The headspace residual of the volatile phenols was analysed using GC-MS with solid-phase microextraction (SPME). A new 4-phase headspace SPME sampling method that allows isolation of internal standards from the reacting matrix while the headspace is sampled was developed to overcome the complexation between cyclodextrins and internal

standards. Sensory analysis was carried out to determine the olfactory effect of the retention effect using triangular sniff tests.

The complexation structure and binding constants between cyclodextrins and off-odour compounds was characterised by ^{1}H 2D Rotating-frame overhauser spectroscopy (ROESY) nuclear magnetic resonance (NMR) and UV-Vis spectroscopy.

To evaluate performance of cyclodextrin polymers in removing off-odours, hexamethylene diisocyanate (HDI) cross-linked cyclodextrin polymer, molecularly imprinted and non-molecularly imprinted methacrylic acid (MAA) cross-linked cyclodextrin polymer are being produced. They will be used as a fining resin on the permeate portion of an initial reverse osmosis filtration in order to minimize the overall impact on the wine. Headspace GCMS with SPME sampling and sensory analysis will be carried out to analyse the removal effect.

Results and discussion

The new 4-phase headspace SPME sampling method provided good consistency and reproducibility. It was found that cyclodextrins could retain the off-odour volatile phenols in wine to different levels (see **Figure 21**), but this effect was accompanied by a loss in the volatility of other wine aroma compounds, particularly long chain acids. α-, β-, and γ-cyclodextrins showed different specificity across the board, with β-cyclodextrin being the most active. This is consistent with previous findings that β-cyclodextrin provides the strongest hydrogen bonding capability among the three evaluated cyclodextrins (Del Valle 2004). It appeared that hydrophobicity of the guest molecule was a selective parameter as the compounds with more hydrophobic C-H bonds were more effectively retained than others. However, this effect was coupled with the selection of size of the guest molecule, because it was observed that compounds with branches were not encapsulated as well as those without branches in the structure. This differentiates cyclodextrins from conventional fining agents, which are mainly based on hydrophobic binding. Sensory analysis confirmed the reduction effect of the volatile phenols by cyclodextrin additions. Out of 38 panellists, 24 and 20 panellists, respectively, reported difference in the smoke taint bracket and *Brettanomyces* bracket in the triangular test.

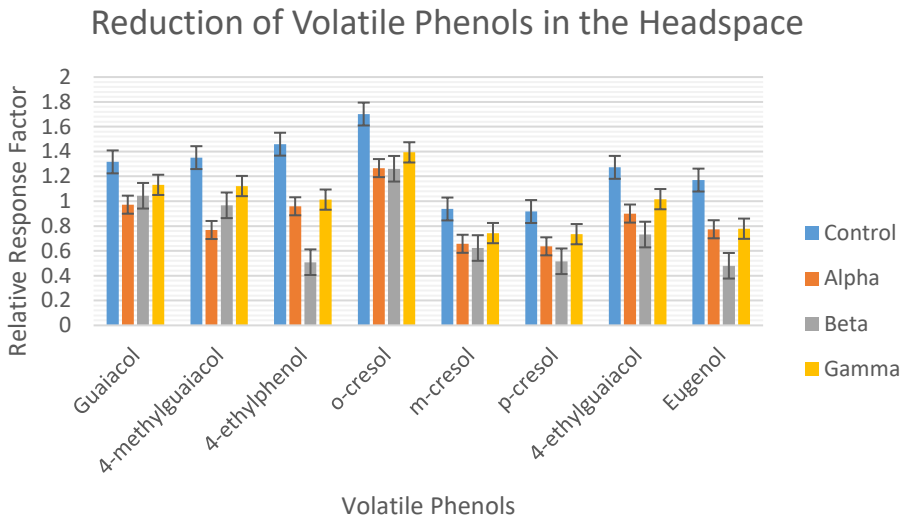


Figure 21. Decrease of off-odour compounds in wine with cyclodextrin addition (10 g/L).

The structure of the complexation between β-cyclodextrin and 4-ethylphenol is illustrated in **Figure 22**. The cross-peaks arising from the nuclear overhauser effect (NOE) interactions between the annular H3, H5 and H6 protons of β-cyclodextrin and the aromatic and methyl protons of 4-ethylphenol shows closer positions of these protons in the spatial distribution of the complex.

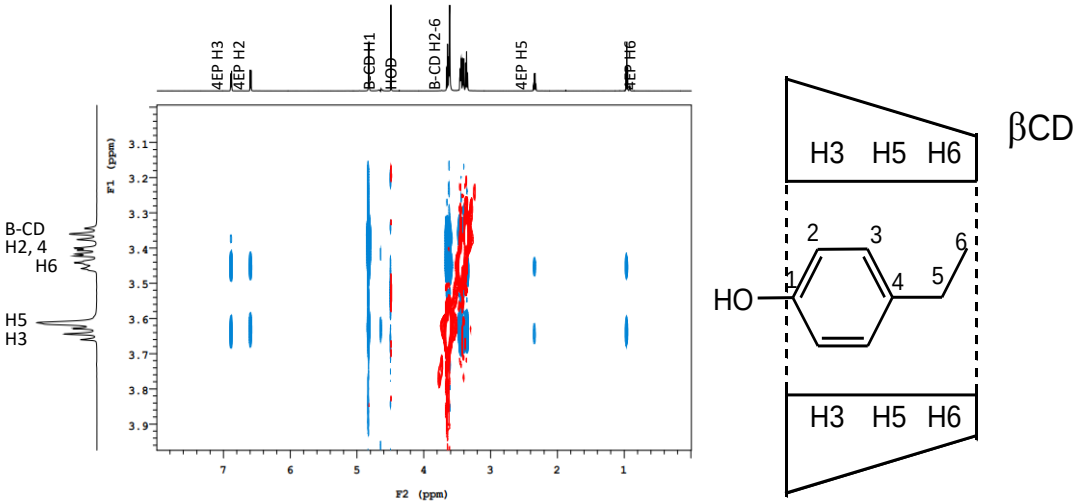


Figure 22. Cross peaks between hydrogens on β-cyclodextrin and 4-ethylphenol shown by NMR (left panel) and illustration of the insertion (right panel).

Conclusion

Obtained results indicate that natural cyclodextrins, being listed as novel foods in many countries, have a potential to be used as additives in wine making in order to remove off-odours. Cyclodextrin polymers are expected to provide promising results, but their effect on the overall profile of the wine is yet to be determined.

6.7. Biochemical response of grapevines to smoke exposure

Introduction

For the past decade, the impact of smoke taint on the wine industry has been the focus of considerable research (Jiranek 2011, Krstic et al. 2015). The occurrence of smoke exposure by vineyards during fruit development can result in grapes taking up smoke-derived volatile compounds, which negatively affect wine as a taint characterised by smoky and ashy aromas and flavours, and an extremely drying, ashy mouthfeel (Fudge et al. 2011). Most research into the subject has focussed on the compositional and sensory consequences of grapevine exposure to smoke, as well as the potential for ameliorating smoke-affected wines (Ristic et al. 2011). Some work has been done towards understanding the uptake of smoke-derived compounds by grapes and leaves, with an emphasis on the possibility of translocation through the vine (Hayasaka et al. 2010). The research presented here has mostly been focussed on questions relating to the prevention of smoke taint, as well as the role of enzymatic conjugation of smoke-derived volatile phenols as glycosides, to further understanding of the biochemical response of grapevines to smoke. Glycoconjugation is a common process in various types of vegetation, and in grapes is thought of as a way to establish flavour reserves, as well as a means of detoxification (Høj et al. 2003, Bowles and Lim 2010, Hjelmeland and Ebeler 2015). Especially in the case of the uptake of exogenous volatiles (e.g. from smoke), the latter might be an important consideration for smoke affected grapes.

Material and methods

Initial work investigated the uptake of smoke derived compounds by grapes and other types of fruit at key phenological time points, in order to further our understanding of the influence of berry morphology on the uptake of smoke-derived volatile compounds, and their subsequent

glycoconjugation. Field trials were performed by exposing grapevines and apple trees to smoke for an hour, whereas strawberry and tomato plants were exposed to smoke for 30 minutes. Samples were subsequently collected at several time points to investigate changes in fruit composition, up until maturity. Samples were analysed by GC-MS and HPLC-MSMS to determine volatile phenol and glycoconjugate concentrations, respectively, using previously published analytical methods (Kennison et al. 2009, Ristic et al. 2011). However, instrument issues rendered the data obtained for these sample sets unsuitable for publication. However, results identified a new research direction based on the use of kaolin (a particulate clay) in apple orchards in the Adelaide Hills, which may have provided protection for apples exposed to smoke from the Sampson Flat bushfires. Consequently, in the following year, the field trial involving apple trees was repeated, with samples being analysed by HPLC-MSMS following smoke exposure of an hour.

In order to investigate smoke taint prevention by means of a physical barrier, three different varieties of grapevine (Sauvignon Blanc, Chardonnay and Merlot) were exposed to smoke, with and without foliar application of kaolin. Furthermore, during this trial, spectral measurements were taken to investigate the potential for a handheld spectrometer to identify environmental disturbances of berries due to smoke exposure (Fudge et al. 2012). A Jaz miniature spectrometer (Ocean Optics Inc., Dunedin, FL) was used to measure spectral reflectance relative to a white Spectralon standard (Kronstadt et al. 2013). Measurements were taken for all treatments included in this field trial (i.e. fruit from: (i) control (unsmoked) grapevines; (ii) grapevines exposed to smoke; and (iii) grapevines treated with kaolin and subsequently exposed to smoke). As in previous trials, grapevines were exposed to smoke for an hour, followed by GC-MS and HPLC-MSMS analysis, to measure volatile phenols and their glycoconjugates, respectively.

In the following year, an additional field trial was established to confirm the reflectance spectrometry results obtained; this time four grape varieties (Sauvignon Blanc, Chardonnay, Merlot and Cabernet Sauvignon) were studied, to better determine the varietal responses identified. This field trial also included the use of a second agrichemical, being a polymer-based anti-transpirant (sold under the trade name Envy).

In the final year of this project, two complementary trials investigating the molecular response of grapevines to smoke exposure were completed; trials involved RNA sequencing of samples harvested from smoke exposed grapevines, based on the *Vitis vinifera* genome (Jaillon et al. 2007). Two grape varieties, one white (Chardonnay) and one red (Shiraz), were grown in a controlled environment. Close to maturity, grapes were exposed to smoke for an hour, after which samples were taken for further analysis. Glycosyltransferase activity can vary, depending on many biotic and abiotic influences, such as variety, phenology and other factors that might promote or inhibit fruit development (Tikunov et al. 2010, 2013, Hjelmeland and Ebeler 2015). Preliminary analysis of RNA Seq data showed upregulation of a range of genes following smoke exposure, including a set of glycosyltransferase (GT) genes, mostly from GT family 1 and 8.

To further investigate the RNA Seq data obtained from the potted grapevines, a field trial was conducted, in which four grape varieties (Sauvignon Blanc, Chardonnay, Merlot and Cabernet Sauvignon) were exposed to smoke; samples were subsequently analysed by Q-PCR to determine the expression of previously identified glycosyltransferases (i.e. from family 1 and 8). Sample sets were divided into skin and pulp fractions, to identify differences in expression in for these tissue samples.

Results and discussion

Investigations identified changes in the accumulation of volatile phenol glycoconjugates in smoke-affected grapes, over time. Smoke exposure of *Vitis vinifera* cultivars Sauvignon Blanc, Chardonnay and Merlot at approximately 10 days post-veraison showed varietal differences in the glycoconjugate profiles of smoke-affected grapes. Merlot grapes showed the highest levels of volatile phenol glycoconjugates, with the most abundant precursors being pentose-glucosides of guaiacol and cresol. For Sauvignon Blanc however, rutinosides of cresol and phenol were most abundant, whereas for Chardonnay, pentose-glucosides of guaiacol, cresol and phenol, as well as syringol gentiobioside,

were observed at high levels. Furthermore, changes in volatile phenol glycoconjugate profiles were observed over time, i.e. for fruit sampled 1 day after smoke exposure compared with fruit sampled at maturity.

The application of agrichemicals (i.e. kaolin and Envy), prior to smoke exposure did not significantly affect the volatile phenol glycoconjugate profiles of Sauvignon Blanc, Chardonnay and Cabernet Sauvignon fruit; indeed, some precursor levels were actually higher in grapes following the application of Envy. However, significantly lower levels of glycoconjugate precursors were identified in Merlot grapes after treatment with kaolin, suggesting kaolin may have afforded some protection from smoke exposure. Protection is likely dependent on the level of coverage, and differences in fruit composition may have reflected the efficiency of kaolin applications to grapevine fruit and foliage. Differences were also seen in the spectral measurements of control and smoke-affected grapes, albeit differences varied by cultivar, and were no longer significant for measurements taken 7 days after smoke exposure. This research could potentially impact the way smoke-affected fruit is analysed in the future. Currently, grape and wine producers tend to rely on commercial laboratories to conduct volatile phenol and/or glycoconjugate analyses. During vintage, when both time and money are precious, the ability to detect smoke-affected grapes in the vineyard would be highly valuable. As such, this study was repeated in 2017, using four grape varieties (Sauvignon Blanc, Chardonnay, Merlot and Cabernet Sauvignon).

Grapevines grown in both a controlled growth room environment and the field were exposed to smoke under experimental conditions, and their transcriptional response determined. RNA sequencing of control and smoke-affected grapes from potted Shiraz and Chardonnay indicated higher expression of heat shock proteins and glucosyltransferases following smoke exposure. Six glucosyltransferases yielded higher expression in both Chardonnay and Shiraz, and four of these were selected as candidates for further investigation in subsequent field trials. One additional GT was included in this investigation as it had previously been reported to show preferential activity towards smoke derived volatile phenols, and has a high overall abundance in grapevines (Härtl et al. 2017). Real time quantitative PCR of Chardonnay, Sauvignon Blanc, Cabernet Sauvignon and Merlot fruit indicated a putative hydroquinone glucosyltransferase, crocetin glucosyltransferase and 7-deoxyloganetic acid glucosyltransferase were more highly expressed in smoke-affected grapes at specific time points; with differences observed in relative expression for skin and pulp fractions also.

An additional trial involving the application of smoke to apple trees was performed to determine the potential for smoke taint to occur in a crop other than wine grapes. Low levels of volatile phenol glycoconjugates were observed in apples harvested from trees exposed to smoke for an hour, but smoke exposure did not affect the development or maturation of apples.

Conclusion

The results from this work will contribute to our knowledge of the biochemical response of grapevines to smoke exposure, in particular (i) the accumulation of glycoconjugate precursors in grapes, and (ii) expression of glucosyltransferase enzymes following grapevine exposure to smoke. Understanding glycosyltransferase activity will inform future work into not only the volatile compounds contributing to the aroma and flavour associated with smoke affected wine, but grape and wine aroma and flavour profiles more broadly (Tikunov et al. 2010, 2013). The effects of the application of agrichemicals (i.e. kaolin and Envy, a polymerbased anti-transpirant) prior to smoke exposure were inconclusive as kaolin provided some protection for Merlot berries, but not for other investigated varieties.

The results from this study have been compiled as a PhD thesis: L. van der Hulst (2018) *The analysis of grapevine response to smoke exposure, Doctor of Philosophy, The University of Adelaide.*

6.8. Wine Innovation and the importance of authenticity

Introduction

Meeting consumer expectations can be challenging; it is hard for companies to introduce innovated products and even harder to innovate traditional ones (Katz 2003). Launching innovated products comes with high risks and the success rate is usually under 50% (Taylor and Bearden 2003) due to feelings of inherent uncertainty experienced by consumers when purchasing these products. Typically, they avoid risk and/or use supportive information to reduce uncertainty levels (Martínez et al. 2009). Important factors influencing the success of a product innovation relate to consumer perceptions of authenticity (Gilmore and Pine 2007), better value/quality and a good understanding of customer needs (Kenneth 2013); hence the acceptance of a new (or innovated) product is critically connected to belief in superior product attributes as well as consumer characteristics (frequency of consumption and product class involvement) (d'Hauteville 1994). Whilst consumers may well expect and welcome product innovation in categories such as computers, software, cars and numerous other categories, the examination of intrinsic innovation of more traditional products, like wine, is limited resulting in a substantial gap in our current knowledge. As a result, important attributes of any innovated products may include: their perceived 'traditionality' (how traditional a product is perceived to be), the perceived enhanced value of the innovation and the level of innovativeness offered by the new product over the original.

Wine is a very traditional product with high symbolic value (Meillon et al. 2010a). The intrinsic innovation of modifying (reducing) alcohol levels (by partial or complete dealcoholization) is gaining support in society as the alcohol level in wines has continued to increase through the years, leading to an increassed per capita consumption (Chikritzh et al. 2010). This has led the World Health Organization (WHO) to launch a global strategy specifically aimed at lowering alcohol consumption. This global strategy, together with consumers' increasing health consciousness, has increased the need to develop new wines and other forms of alcoholic drinks to enhance consumer choice for lower alcohol alternatives. However, lower/low alcohol wines already exist but have not been very successful due to people experiencing these wines as less traditional, less complex and without varietal character (Masson et al. 2008, Meillon et al. 2010a, Saliba et al. 2013b). This is particularly true for red wines, which are perceived to be more complex and are not expected to withstand the dealcoholization process without losing quality (Meillon et al. 2010a). Moreover, red wine is considered more traditional with a somewhat 'sacred' status (Meillon et al. 2010a). In summary, the process of dealcoholization is perceived by consumers to prevent the traditional winemaking process from being performed properly, and thus hinders their acceptance of such products. Hence, determining an acceptable level of innovation in wine products, one that would still allow the product to be deemed 'authentic' (in terms of style, varietal, level of alcohol, color), is gaining importance. The purpose of this study, therefore, was to test consumers' response in three diverse wine markets, one where wine is a traditionally consumed product, one considered an emerging wine market and one where wine is deeply rooted into the country's history. Wine products tested were of varying alcohol levels, varietals and styles. The purpose of the qualitative research was to inform the conceptual framework to be empirically investigated.

Materials and methods

Focus groups in Indonesia, Australia & France

Exploratory research in the form of focus group interviews was employed to explore consumer reactions to a variety of alcohol-reduced wines in depth (Morgan 1997). This technique allows probing for participants' perceptions (Albrecht et al. 1993) , providing an amicable environment and has been successfully used in previous studies about wine and authenticity (Beverland 2005).

Table 7. Demographic profile of focus group participants conducted in Adelaide, Jakarta & Dijon

	Demographic	Adelaide	Jakarta	Dijon
Focus Group 1-3	Age	18-35	18-35	18-35
	Gender	6 males	6 males	7 males
	Nationality	Australian	Indonesian	French
Focus Group 4-6	Age	36-65	36-65	36-65
	Gender	6 males	8 males	8 males
	Nationality	Australian	Indonesian	French
Focus Group 7-9	Age	18-35	18-35	18-35
	Gender	6 females	6 females	9 females
	Nationality	Australian	Indonesian	French
Focus Group 10-12	Age	36-65	36-65	36-65
	Gender	5 females	6 females	6 females
	Nationality	Australian	Indonesian	French

Convenience sampling was employed to examine the relationship between the perception of product traditionality and authenticity. All participants were recruited through a marketing company in Jakarta (Indonesia) and a university network in Australia and France. These countries were chosen as they exhibit different levels of historical involvement with, and consumption of, wine and thus it is expected that wine will not be considered equally 'traditional' in the three locations. Focus groups demographic data are given in Table 7 (Qesja 2017).

Interview protocol and data analysis

A semi-structured interview guide was developed based on the gaps in the literature with a view to encourage discussion, provide flexibility, and ensure consistency across several focus groups (Stewart and Shamdasani 2014). Key questions were directed to period of drinking wine ('How long have you been drinking wine?'), situation ('When do you like consuming wine?'), criteria of wine selection ('What do you look for when buying a bottle of wine?'), frequency of drinking, perception of low alcohol wines, benefits perceived from consuming low/no alcohol wines, authenticity of wine, whether they viewed wine as a traditional product, etc. Participants were given eight different wines to taste. The wines varied in attributes and alcohol level (three reds: 0.5 %, 7.5%, 15.5%; two rose: 0% and 13.5 %; three white wines: 0.5%, 5.5% and 13.5 % alcohol). No information was given about the wines and the labels were covered. After the tasting, participants were told that three wines had no alcohol and were asked whether their perception of those wines changed after finding out the alcohol level and whether they still considered the beverages to be wine. Participants were also asked to rate the wine overall from 0 (really disliked it) to 10 (liked it very much). Moreover, they were asked to guess the alcohol level and to give any comments on the wine. Identical methodology was repeated in Adelaide and Dijon (Qesja et al. 2016a, 2016b, 2017).

Quantitative data collection

Following the same logic as for the focus groups, surveys were launched in three different countries (Australia, France and Singapore) resulting in a total sample of 1517 participants (503, 508 and 506, respectively). A professional research company in the USA, Qualtrics, was contracted to recruit all respondents in each country. Samples were comprised of members of the general public and all respondents had to be over 18 yo and citizens of their respective countries. In the wine survey, the unit of analysis was defined as individual consumers, female or male (with a ratio of 1:1), that consume more than 5 glasses of wine in an average month. Data collection was finalised in June 2016, and the analysis was perfomed in Amos via the use of structural equation modelling (SEM). The influence of the situation of consumption on perceived gain and sacrifice from the innovation was analysed via a multiple paired-samples t-test in order to evaluate the size and significance of the impact of situation on perceived gain and sacrifice (Qesja 2017).

Results and discussion

Qualitative results

Qualitative results in Indonesia indicated that as expected, the no alcohol wines were considered to have the lowest quality overall, irrespective of varietal and style. However, the small range (4.4-6.5) is a positive sign that the difference in preference is not as vast. The participants had trouble associating the character of the wine with the alcohol level, and overestimated the level of alcohol in the wine. The no alcohol red wine was ranked last, following the prediction made from the literature review that 'red wine does not handle the dealcoholisation process the same way as rose and white wines'. The rose wine was met with the most positive reaction, being characterized as 'refreshing' and 'easy to drink'. Men were the most against the dealcoholized wines, however the history of drinking and frequency (years of drinking wine) played a moderating role. Men that had not been drinking wine for long were more open to accepting lower alcohol wines. The same acceptance of these wines was indicated by females. However, overall, they were more open to the idea of consuming these wines on occasion, when wanting to relax after a long day of work, when not eating and when not wanting to get drunk. The majority still considered the no alcohol products to be 'wine' and their perceptions did not change after finding out the information. As predicted, wine was not considered a traditional product by most participants. These participants reacted more positively to the innovation and saw the benefits as overcoming the downfalls in particular situations. The innovated product was still perceived as authentic (Qesja et al. 2016a, Qesja 2017).

On the other hand, the participants that were raised with the culture of drinking wine reacted more negatively to the innovation, seeing the new product as not authentic, and the perceived sacrifice as high. Qualitative results in Australia indicated that wine was considered a traditional product and perceived to be authentic at standard or more 'normal' alcohol levels. Authenticity was associated more with methods of production, location, and producer. Similarly to the data collected in Jakarta, the no alcohol wines were ranked lowest in preference; however there was a discrepancy between the rating of the white and rose wines and the red. The white and rose wines were considered light and refreshing, while the red was deemed to be 'undrinkable' and 'like fruit juice'. Respondents reacted more negatively upon finding out that the wines contained no alcohol with one participant stating that *'now that I found out that it contained no alcohol, I would never buy it'* regardless of whether they had liked the taste. Judgement of the wine was also related to a quality benchmark created through years of drinking wine, particularly when they started as young adults, linking their judgement to the tradition of drinking wine with their parents. Upon finding out the alcohol content, the majority did not see the product as authentic and did not agree with the idea of calling it wine. As predicted, wine was considered a traditional product by most participants. These participants reacted more negatively to the innovation and saw the downfalls as overcoming the benefits (Qesja 2017).

However, the participants that were not raised with the culture of drinking wine reacted more positively to the innovation, and the perceived sacrifice was lower. Frequency of drinking, situation and history of drinking were found to play a moderating role similar to the results in Jakarta. French focus group results indicate that wine was considered a traditional product and perceived to be authentic by all participants. Similarly to the data collected in Jakarta and Adelaide, a low/no alcohol red wine was met with a more negative reaction as compared to white and rose wines. The majority of the participants were raised drinking diluted red wine since childhood and mentioned that one of the reasons for drinking wine was that it is part of the culture. As a result, they had a strong consideration about how red wine should be. When asked about the role of the alcohol level in their decision making, participants stated that it did not play a role and overestimated the level of alcohol in the wines tasted; however, when asked if they would drink lower alcohol wines the reaction was negative. Upon finding out that the wines contained no alcohol, in contrast to the Indonesian and Australian participants, French respondents reacted more negatively. The majority did not see the product as authentic and did not agree with the idea of calling it wine. Situation and history of drinking were found to play a moderating role similar to the results with the Indonesian and Australian participants (Qesja et al. 2017).

Quantitative results

Results indicated that, as hypothesized, product traditionality has a positive and significant impact on perceived authenticity. Perceived congruence of the innovation partially mediates the impact of product traditionality, degree of complexity of the innovation and degree of innovation on perceived authenticity of the innovation. Perceived authenticity positively and significantly influences perceived advantages from the innovation which in turn significantly and positively influences perceived gain and sacrifice and purchase intention. Perceived authenticity had a positive and significant direct effect on purchase intention. The effect was stronger than the indirect effect via perceived gain and sacrifice rendering the indirect effect insignificant. Perceived gain and sacrifice has a positive and significant impact on purchase intention (Qesja 2017).

Moreover, results indicate that all situations presented had a statistically significant impact on perceived gain. The degree of perceived gain decreased moderately when participants envisioned themselves drinking alone with a meal, and during a business lunch. The effect size of the decrease was large when participants envisioned drinking when pregnant, and small to moderate when drinking alone to relax after work. The degree of perceived gain increased when participants envisioned themselves drinking with friends at a restaurant, and when drinking with company on a special occasion. In regard to the impact of consumption situation on perceived sacrifice from the innovation, results indicate that all situations presented had a statistically significant impact. The degree of perceived sacrifice decreased when participants envisioned themselves drinking alone with a meal, during a business lunch and when drinking alone to relax after work. The decrease in perceived sacrifice was large when participants envisioned themselves drinking when pregnant. The degree of perceived sacrifice increased when participants envisioned themselves drinking with friends at a restaurant, and when drinking with company on a special occasion. These results are consistent with the focus group results where participants envisioned themselves consuming a lower alcoholic wine only under specific situations. An observation of the impact of the situation on perceived gain and sacrifice from the innovation on a country level, indicates that the country the participants were from plays an important role in influencing feelings of gain and sacrifice (Qesja 2017).

Conclusion

Focus groups conducted in Australia and France indicated that wine was considered to be part of the culture and perceived to be authentic at some standard alcohol levels. These respondents, in contrast to Indonesian participants (that were not raised in a culture of wine drinking), reacted more negatively upon finding out that the evaluated beverages contained no alcohol, regardless whether they had liked the taste. This indicates that the negative attitudes towards low alcohol wines were not simply a result of the alteration of sensory properties of wines, but rather an outcome of traditionality perceptions, history of drinking, and authenticity perceptions among others.

This study offers important managerial implications as it explicates how consumers react to innovations of traditional products, as well as theoretical contributions about authenticity in the context of product innovations. Moreover, it will also be a contribution to the alcohol industry by providing an insight as to how consumers perceive the innovation as well as what is the 'optimum' innovated product in terms of style, varietal and alcohol level. This may provide the foundation for lowering alcohol consumption per capital.

The results from this study have been compiled as a PhD thesis and the following publications:

1. *Qesja, B. (2017) Innovating traditional products: Product authenticity vs perceived sacrifice from the innovation, Doctor of Philosophy, The University of Adelaide.*
2. Qesja, B., R. Crouch, and P. Quester (2016a) Innovating Traditional Products:"Self-Sacrifice vs. Product Authenticity". *In* Proceedings of the AWBR conference proceedings (in press).
3. Qesja, B., R. Crouch, and P.G. Quester (2016b) Reduced alcohol wine: Product innovation and authenticity: The case of wine. Wine & Viticulture Journal 31: 67.

7. CONCLUSION

The UA 1304 project complied a comprehensive research from sixteen ARC TC-IWP projects into an integrated strategy, which delivers improved knowledge and tools to assist winemakers to objectively manipulate flavours and alcohol content in wines. The integrated strategy was achieved through an integrated, 'whole-of-production-chain' approach that started in the vineyard, explored fermentation and post-fermentation and finished with wine consumers. It included: i) viticultural practices that may affect sugar accumulation in berries, reduce berry cell death, and minimise intake of taint compounds in the grape. A series of harvest blending regimes across several varieties and vineyards were compared for key compositional features; ii) fermentation techniques to remove sugar prior to fermentation, divert sugar away from alcohol, improve their reliability and reduce the duration of high sugar fermentations using pure and mixed culture of *Saccharomyces* cerevisiae and non-*Saccharomyces* yeast strains; iii) post fermentation techniques for alcohol reduction (reverse osmosis/evaporative perstraction and/or enchaining wine quality; iv) novel techniques to remove off-flavours in wines and v) consumer studies to define consumers' perceptions and preferences for lowered-alcohol wine which could be used for marketing strategies in Australian export (Indonesia and France) and domestic market.

Additionally, the project achieved the following objectives:

1. The PDF built a strong and compact team around wine and grape research at the TC-IWP. It linked several projects that sourced grape material from the same vineyard and maximised benefits of shared resources. Strong collaborations have been established between NWG 1301 (CSU), several TC-IWP projects (UA), Memstar and dealcoholisation facilities in TWE.
2. The PDF provided training and support to HRDs for wine chemical analysis and sensory studies, and interpretation of collated data.
3. Based on the recommendation of the TC-IWP Advisory Committee, the PDF conducted research into development of smoke taint in bottles during wine ageing. The outcomes have been published in the peer-review paper (see attachment)
4. The PDF disseminated outcomes through various extension mechanisms: peer-reviewed articles (1 senior author and 3 co-author), industry articles (4), industry reports (4), industry seminars (4), workshops (1), domestic (2) and international symposia (2).

8. OUTCOMES AND RECOMMENDATIONS

The integrated strategy highlighted the following outcomes:

Grape berry cell death

There are several factors, under varying degrees of viticultural control, with the potential to influence grape berry mesocarp cell vitality at harvest: temperature, irrigation and grape variety.

1. Minimise vineyard temperature – within-canopy records of cumulative growing season temperature correlate well with the decline in berry mesocarp cell vitality. If this is a causal relationship, then efforts to cool the microclimate of grape bunches will reduce the extent of cell vitality decline observed at harvest. Such efforts include site selection based on aspect and exposure, and providing sufficient irrigation water for transpiration to cool the canopy via sensible heat exchange.
2. Maintain plant hydration status – long-term deficit irrigation has been shown to accelerate the rate at which cell vitality declines late in the ripening period. The corollary is that persistent maintenance of plant hydration status will reduce the extent to which cell vitality has declined at harvest. The precise mechanism by which this system operates is unclear. For example, it is not

known if late season cell vitality is particularly sensitive to a decrease in hydration status at a particular time point nor whether the dehydration response occurs at the berry level or perhaps a larger scale (e.g. canopy). However, the results suggested that bunch shading and rainfall may counteract loss in cell vitality and rates of berry weight loss in Shiraz, but not in all instances.

3. Grape variety – there is a genetic component to the propensity for cell vitality to decrease late in ripening. Importantly, it also appears there is scope for other important determinants of berry hydration status under genetic control to interact with cell vitality. The best example is hydraulic conductivity of the pedicel, which varies between cultivars. Relatively high conductivity increases berry susceptibility to dehydration via negative hydrostatic pressure generated by the parent vine.
4. Rootstocks with drought resistance characteristics may affect grapevine hydration status and reduce berry cell death.

Of these factors, plant hydration status is most readily influenced by viticultural practices via irrigation management. Treatments can also be put in place to reduce vineyard temperature by shading, but these would involve greater time and resource inputs. Grape variety and rootstock selection is an impractical factor for viticulturists to manipulate because this decision must respond to consumer demands as interpreted by winemakers and brand owners. However, knowledge of cultivar susceptibility to berry dehydration and cell vitality loss may help identify where in a viticultural enterprise other efforts to reduce these effects will be most productive.

1. Objectively measuring cell death – this research has sought to commercialise an objective method of measuring cell vitality based on the electrical impedance of the fruit. This research has repeatedly demonstrated that a rapid and objective measure is needed if industry and the scientific community are to relate the extent of berry cell vitality to grape and wine quality.
2. Temperature and water stress – this research has further developed our understanding of how high air temperature and low soil water stress results in a decline in grape berry cell vitality. For example, the experiment conducted with normal irrigated and water deficit treated vines in the last season showed water deficit berries had more significant reduction in cell vitality during ripening, which is closely related to the lower oxygen content inside those berries. Internal oxygen concentration ($[O_2]$) decreased in correlation with berry cell death during berry development. However, berry respiration rate decreased during late ripening only under water deficit. During late development stages, naturally occurring fermentation produced ethanol in the berries, resulting in an increased membrane permeability. Information on berry physiological responses to abiotic stresses and, importantly, to treatments designed to ameliorate them (such as irrigation and shading), will underpin decisions in industry on whether to promote or avoid fruit grown under these various conditions.
3. The beginning of mesocarp cell death in both control and water stressed Shiraz berries corresponded to the increased level of ROS. RNA-seq analysis identified lists of candidate genes that showed differential expression in at least one of the comparisons between either three critical development stages or different treatments. Genes such as e.g. *VvBAG1* and *VvLOXA* could be linked to ROS signalling and programmed cell death. Furthermore, *VvPub13*, transiently expressed in tobacco leaves, was observed to reduce H_2O_2-induced cell death by inducing genes involved in anti-oxidant responses. Consequently, more research has been conducted to document that *VvPub13* in grape berries can inhibit cell death.
4. Understanding the process of cell death – detailed information directed at understanding key molecules and genes associated with the loss of grape berry cell vitality must underpin practical efforts to influence the process. This research is collecting such information across genetically diverse grape cultivars that differ in their susceptibility to berry cell vitality loss and dehydration, across a wide range of berry developmental stages, and across vineyards that differ in their abiotic conditions.

At the conclusion of this research the method most readily available to vineyard managers, and potentially most effective, for manipulating grape berry cell death at harvest is likely to be the application of irrigation water to manipulate plant hydration status.

The sugar-potassium nexus within the grapevine

The accumulation of sugar and potassium (K^+) are integral processes occurring during fruit ripening, and as such, may influence grape and wine composition, but their interdependence is still unclear. Given that K^+ is integral to normal berry development and because of its close links with water and sugar accumulation during ripening it is clear that K+ deficiency should be avoided. While many Australian soils are naturally high in K^+, deficiency can occur through leaching or crop removal. The availability of K^+ can also be limited in sandy soils, heavy clays and acid soils. Potassium can be applied as potassium chloride (in low saline soils), potassium nitrate or potassium sulphate. Mulches and composts may provide an additional source of K^+ to the vineyard. Care is needed to satisfy the K+ requirements of grapevines without excess, given that excess can lead to high pH in grape juice and wine and negative effects on wine quality. Soil and petiole testing will give a good indication of additional requirements to be rendered through fertilisation.

From a viticultural perspective, information is required on the optimal potassium concentrations in the berry at various stages of growth and development. Given the ability of potassium to translocate from roots to leaves and then back down to roots again, the grapevine is an extremely responsive and adaptable system, capable of maintaining internal homeostasis and driving nutrient flow to areas of greatest demand. Considering predicted increases in heat and drought periods, as well as a predicted increase in grapevine water requirements within warm viticultural regions due to climate change, together with increased competition for available water supplies, sustainable cost-effective management options are required. Grape variety and rootstock selection has also been proposed as a possible way of reducing potassium uptake and consequently lowering sugar accumulation, which require further investigation into different grape varieties, clones and/or rootstocks. The effects of vine vigour, canopy shading, crop load and foliar potassium application on berry potassium accumulation are inconclusive and require further research (Coetzee and Rogiers 2017a, 2017b).

Optimisation of an early harvest and blending regime

A sequential harvest regime (i.e., harvesting grapes at earlier time points) or juice substitution with a green harvest (i.e., unripe) “wine” or water prior to fermentation can be used as a method for the production of wine with a lower alcohol content (up to 3% alcohol by volume) compared to commercially harvested fruit. Particularly interesting was the apparent absence of dilution effects when using water, which enabled easy moderation of alcohol levels with no or small changes in wine phenolic composition and sensory profile. Due to its ubiquitous availability and minimal impact on wine composition, the implementation of water was found to be the most convenient way to decrease wine alcohol content in this study on Cabernet Sauvignon. However, because this approach tends to retain the compositional attributes determined by grape maturity at the time of harvest time, it could be regarded as a useful last resort to limit the negative implications of a highly mature crop, rather than being broadly implemented after deliberately prolonging the maturation of grapes on the vine (Schelezki and Jeffery 2017). This pioneering work initiated further research into optimisations of the blending techniques, which are particularly interesting for winemakers in light of recent changes in the Australian and New Zealand Food Standard regulations that now permit the pre-fermentative addition of water under certain conditions.

The ‘double harvest’ method also showed promising results in reducing the alcohol level in wines without significant implications for wine sensory profile. The potential use of a blending practice on wines produced from different harvest dates as an easy-to-adopt, flexible and cost-effective alternative to deal with increasing levels of alcohol and aspects of phenolic based analytes deserve particular attention. The results of this research could be specific to unoaked and young wines. However, given

that many highly alcoholic wines are often treated with oak, it would be interesting to expand these studies to a range of wine styles and wine age. A more comprehensive investigation comprising significantly more samples derived from a number of harvest dates and grape varieties is warranted. Dealcoholisation experiments could be targeted to a broader range in grape ripeness degrees to further examine the importance of harvest date on the specific dealcoholisation process. This study showed that 13.5% v/v Shiraz wines produced by selecting a specific harvest date, blending or dealcoholisation did not differ for any of the sensory attributes examined. It would be worthwhile to investigate consumer liking responses to these wines to provide further insight at the commercial scale.

Further studies of other varieties with a different range of alcohol levels and blending ratios would be valuable in order to further investigate the efficacy of this procedure for the production of lower alcohol wines.

Yeast strains in alcohol management and flavour enhancement

1. The commercially available (thus readily-implementable) non-*Saccharomyces* yeast treatments were able to increase the intensity of descriptors generally regarded as more appealing in earlier harvest wines. As such, non-*Saccharomyces* yeasts appear to be a useful tool for optimising the quality of wines made from earlier harvests.
2. An indigenous *Metschnikowia pulcherrima* isolate was selected based on its ability to lower wine ethanol content in sequential fermentations with *Saccharomyces cerevisiae,* and characterised across a range of conditions. Depending on the inoculation regime, alcohol decrease in white wines ranged between 0.6 and 1.2% (v/v).
3. Remarkable diversity of *Lachancea thermotolerans* isolates was observe at a genetic and a phenotypic level, as well as the potential of certain isolates to decrease ethanol and pH in wines.
4. *Torulaspora delbrueckii* showed high potential as an alternative to an *S. cerevisiae* monoculture in high sugar environment, but its performance and contributions in mono and mixed cultures need to be defined for different varieties and conditions.

Winemaking techniques for alcohol management and flavour enhancement

Wine mouthfeel properties can be modulated by the addition of winemaking supplements that modify wine tannin and mannoprotein composition, especially in the context of improving the quality of wines made from early harvest grapes. However, the final outcome depends on the composition of additives; MPs of different protein content and size distribution (e.g. with low or high protein content) could behave differently in the presence of tannins. Findings suggest certain polysaccharides will aggregate with tannin particles which may have an effect on wine molecular assembly and colloidal stability. Furthermore, a 3% alcohol difference can significantly influence aggregation in some instances. Nanoparticle Tracking Analysis (NTA) was successfully used to characterise interactions between tannins and polysaccharide, and provides a novel technique which could be further developed for studying wine macromolecules.

Defining the alcohol ‘sweetspot’ remains difficult and time-consuming, and despite the progress achieved to date, the current methods have still not been adequately validated. It is therefore important that a combination of sensory and chemical analyses be used to validate the alcohol ‘sweetspot’ phenomenon, to provide clear guidelines for winemakers to manage the optimal alcohol content of wines. There were some limitations of this study that could be improved in the future. Firstly, the red wines used for the study were young and high in both alcohol and astringency. This undoubtedly made it more difficult to investigate the alcohol ‘sweetspot’. Sweetspotting of white wines or wines with less hotness and/or astringency might be easier for panellists, and therefore yield better results. Further research is also needed to understand the influence of wine presentation. Secondly, although the p-value outcomes would be more reliable if the number of panellists was increased (e.g. to 50 or more), it was already difficult to recruit winemakers with experience in alcohol sweetspotting. Given that many high alcohol wines in need of some alcohol correction are red wines, and that winemakers currently

spend a significant amount of time optimising the wine alcohol level, the results of this study are likely to be relevant to many winemakers working to achieve lower alcohol wines.

Novel techniques for flavour enhancement

Recent research has developed novel approaches to manipulate undesirable flavours in wines. Using molecularly imprinted polymers (MIPs) to reduce unripe characters within wine, such as methoxypyrazines, caused by using early harvested grapes, can contribute to beneficial development of lower alcohol wines. This approach is based on MIPs structural matrix that possesses complementary cavities for target molecules (IBMP), but it appeared more efficient in grape must than in final wine, thus future work should focus on improvement of their efficiency and specificity. The use of magnetic molecularly imprinted polymers may be an innovation and add convenience to practical industrial operations, since MIPs can be removed from a wine by simply applying an external magnetic field.

Cyclodextrins, being listed as novel foods in most countries and frequently used in the food and pharmaceutical industry as additives to remove odours and modify tastes, can potentially be used during the winemaking process, given positive legislation. Some suppression of off-odours (e.g. guaiacol, 4-ethylphenol, 4-ethylguaiacol) under cyclodextrin treatments were achieved, with β-cyclodextrin being the most effective. However, this effect was coupled with minor loss of wine aromas and changes of the taste and mouth-feel of wine due to cyclodextrin's mild sweetening and emulsifying characters. While promising outcomes have been noted, more investigations into cyclodextrin polymers impact on wine is needed.

Biochemical response of grapevines to smoke exposure

Despite an extensive body of knowledge having been accumulated on the topic of smoke taint in recent years, there is still scope for further research; particularly given improved methods for preventing and/or ameliorating smoke taint are still required.

1. Identification of volatile phenol glycoconjugate profiles for a broader range of grape cultivars. The work described in this project included the determination of volatile phenol glycoconjugate profiles for Sauvignon Blanc, Chardonnay, Merlot and Cabernet Sauvignon. However, bushfires occur in regions in which other grape cultivars are grown. For example, in 2017, grape-growing areas of Chile were affected by significant bushfires, where the most prominent grape varieties include Pais, Merlot and Malbec. The provision of benchmarking data to establish the glycoconjugate profiles of a broader range of grape varieties, both naturally occurring (i.e. the glycoconjugate levels present in control fruit) and smoke derived (i.e. the distribution and levels present in smoke-affected fruit), would enable industry to determine levels of smoke taint in fruit following vineyard smoke exposure. Furthermore, the occurrence of more highly conjugated precursors, e.g. trisaccharides, could be investigated, as to date, only glucosides and disaccharides have been identified.
2. Identification of the pathway for uptake of smoke derived volatile phenols. The mechanism by which smoke derived volatile phenols are taken up by grapevine leaves and fruit has not been adequately investigated. In the current study, the application of kaolin to Merlot grapevines mitigated the impact of subsequent smoke exposure, giving fruit with lower levels of volatile phenol glycoconjugates (compared with smoke-affected fruit from grapevines that were not treated with kaolin). Identification of pathways by which the constituents of smoke are taken up by grapevines would help to establish more effective preventative measures, and therefore warrant further research.
3. Identification of genes that respond to smoke exposure. The transcriptomic analysis of smoke-affected grapevine tissue identified upregulation of heat shock proteins associated with abiotic stress. Further investigation into the functionality of these genes is warranted. Given gene transcription is often influenced by multiple factors, the contribution of other abiotic factors towards the development of smoke taint could also be studied.

Consumer acceptance of lower alcohol wines

Results from this study support several recommendations to the wine industy related to launching partially/completely dealcoholised wines. When launching wines with a lower alcohol level, gender should be considered as results indicated that females are more open to consuming low alcohol wines. However, history of consumption and level of involvement in the product category influenced both males and females. Consumers with a shorter history of drinking and lower level of involvement are more likely to accept the innovation. Perceptions of authenticity of the innovated product also played an important role in influencing perceptions of gain from the innovation, thus wine makers and marketers should focus on preserving perceptions of authenticity. Moreover, situation plays an important role in influencing feelings of perceived sacrifice and gain from the innovation, thus it could be an important selling point when marketing the product. However, marketers should also consider the impact of culture on situational influence, as differences existed between countries. The type of wine also played a role in impacting consumer perceptions. Generally, participants were more open to the dealcoholisation (partial or full) of rosé, sparkling or white wines. Having identified that authenticity of the innovation stands to create value in the minds of the consumers, enough to influence purchase intention, an interesting next step would be examining whether a higher degree of change in the authenticity of a product, will have a negative impact on value generation and purchase intention.

The ARC TC-IWP research has taken a multidisciplinary approach to investigate existing and develop new viticultural and winemaking techniques to modulate alcohol and flavour in wines. Working closely with industry partners a few new processes and products have arisen from the Centre research:

1. Strategic irrigation and shading can reduce berry shrivel and yield loss
2. Early harvest dates and blending regimes can achieve wines with lower alcohol while maintaining desirable flavours. Blending mature fruit with extremely early-harvest low alcohol wine yielded good results, but was not deemed to be particularly practical, whereas blending with water affords a very promising way to manage wine alcohol concentrations
3. Harvest blending regime in collaboration with the industry partner 'Lowe Wines' produced two commercially available wines (TINJA – 2015 Mudgee Verdelho low alcohol and TINJA – 2016 Mudgee Petit Verdot low alcohol)
4. Certain commercial non-*Saccharomyces* co-inocula were found to increase the quality and appeal of wine made from the earlier harvested fruit. Indigenous *Metchnikowia sp*. isolates capable of lowering wine ethanol content for up to ~1.5% v/v were isolated and characterised
5. Sensory method for defining alcohol 'sweetspot' is being developed and validated
6. Using molecularly imprinted polymers (MIPs) to reduce unripe characters within wine, such as methoxypyrazines, showed promising results, but more work is required to improve their efficiency and specificity
7. Cyclodextrins can be applied to wines faulted by 'Brett' and 'smoke taint', but these techniques need to be approved by the legislative authority to be formally used in the wine industry. However, if being used as a resin fining agent, polymerized cyclodextrins will encounter much less legislative difficulty
8. Greater insight into wine consumers' perceived authenticity and congruence of low/partially dealcoholised wines was obtained. These findings may contribute to marketing strategies of lower alcohol wines and consequently lowering the alcohol consumption per capita.

All researchers involved in the ARC TC-IWP, with immense support from the industry partners, have contributed to the creation of the integrated strategy for alcohol and flavour modulation, which offers real practical management solutions for current challenges facing grapegrowers and winemakers. The project UA 1304 was directly involved in the conduct of TC-IWP research activities by coordinating and facilitating collaboration between 16 TC-IWP projects, which resulted in the strategy that offers translation of research outputs into industry-ready applications.

9. APPENDIX 1: COMMUNICATIONS

9.1. Peer-reviewed publications

1. Bindon, K.A., S. Li, S. Kassara, and P.A. Smith (2016). Retention of proanthocyanidin in wine-like solution is conferred by a dynamic interaction between soluble and insoluble grape cell wall components. Journal of Agricultural and Food Chemistry 64, 8406-8419. doi: 10.1021/acs.jafc.6b02900.

Abstract
For better understanding of the factors that impact proanthocyanidin (PA) adsorption by insoluble cell walls or interaction with soluble cell wall-derived components, application of a commercial polygalacturonase enzyme preparation was investigated to modify grape cell wall structure. Soluble and insoluble cell wall material was isolated from the skin and mesocarp components of Vitis vinifera Shiraz grapes. It was observed that significant depolymerization of the insoluble grape cell wall occurred following enzyme application to both grape cell wall fractions, with increased solubilization of rhamnogalacturonan-enriched, low molecular weight polysaccharides. However, in the case of grape mesocarp, the solubilization of protein from cellwalls (in buffer) was significant and increased only slightly by the enzyme treatment. Enzyme treatment significantly reduced the adsorption of PA by insoluble cell walls, but this effect was observed only when material solubilized from grape cell walls had been removed. The loss of PA through interaction with the soluble cell wall fraction was observed to be greater for mesocarp than skin cell walls. Subsequent experiments on the soluble mesocarp cell wall fraction confirmed a role for protein in the precipitation of PA. This identified a potential mechanism by which extracted grape PA may be lost from wine during vinification, as a precipitate with solubilized grape mesocarp proteins. Although protein was a minor component in terms of total concentration, losses of PA via precipitation with proteins were in the order of 50% of available PA. PA-induced precipitation could proceed until all protein was removed from solution and may account for the very low levels of residual protein observed in red wines. The results point to a dynamic interaction of grape insoluble and soluble components in modulating PA retention in wine.

2. Coetzee, Z., Walker, R., Deloire, A., Clarke, S., Barril, C. Rogiers, S. (2017) Spatiotemporal changes in the accumulation of sugar and potassium within single Sauvignon Blanc (*Vitis vinifera* L.) berries. Vitis 56, 189-195, doi: Vitis: 10.5073/vitis.2017.56.193-195.

Abstract
It has been speculated that there may be a link between the transport of sugar and potassium into grape berries during ripening as they exhibit similar accumulation patterns. It is unclear if this proposed link is apparent in individual grape berries and in the grape berry compartments. Single grape berries were therefore analysed for sugar and potassium content and concentration within the skin, seeds and the pulp from pre-véraison until harvest. Sugar and potassium had similar accumulation patterns and positive relationships were confirmed between the sugar and potassium content within individual berries and compartments. The sugar content in the grape berry, however, increased 5-fold during ripening whereas the potassium content only doubled. Both sugar and potassium increased with berry size, suggesting a ternary relationship with berry water. The high variability in sugar and potassium contents between berries however affirms plasticity in their accumulation within individual berries.

3. Coetzee, Z., Walker, R., Deloire, A., Clarke, S., Barril, C. Rogiers, S. Impact of reduced atmospheric CO_2 and varied potassium supply on carbohydrate and potassium distribution in grapevine and grape berries (*Vitis vinifera* L.). Plant Physiology and Biochemistry. doi.org/10.1016/j.plaphy.2017.10.008.

Abstract
To assess the robustness of the apparent sugar-potassium relationship during ripening of grape berries, a controlled-environment study was conducted on Shiraz vines involving ambient and reduced (by 34%) atmospheric CO_2 concentrations, and standard and increased (by 67%) soil potassium applications from prior to the onset of ripening. The leaf net photoassimilation rate was decreased by 35% in the reduced CO_2 treatment. The reduction in CO_2 delayed the onset of ripening, but at harvest the sugar content of the berry pericarp was similar to that of plants grown in ambient conditions. The potassium content of the berry pericarp in the reduced CO_2 treatment was however higher than for the ambient CO_2. Berry potassium, sugar and water content were strongly correlated, regardless of treatments, alluding to a ternary link during ripening. Root starch content was lower under reduced CO_2 conditions, and therefore likely acted as a source of carbohydrates during berry ripening. Root carbohydrate reserve replenishment could also have been moderated under reduced CO_2 at the expense of berry ripening. Given that root potassium concentration was less in the vines grown in the low CO_2 atmosphere, these results point toward whole-plant fine-tuning of carbohydrate and potassium partitioning aimed at optimising fruit ripening.

4. Hranilovic, A., Bely, M., Masneuf-Pomarede, I., Jiranek, V., Albertin, W. (2017) The evolution of *Lachancea thermotolerans* is driven by geographical determination, anthropisation and flux between different ecosystems PLOS ONE. doi.org/10.1371/journal.pone.0184652.

Abstract
The yeast *Lachancea thermotolerans* (formerly *Kluyveromyces thermotolerans*) is a species with remarkable, yet underexplored, biotechnological potential. This ubiquist occupies a range of natural into *L. thermotolerans* population diversity and structure, 172 isolates sourced from diverse habitats worldwide were analysed using a set of 14 microsatellite markers. The resultant clustering revealed that the evolution of *L. thermotolerans* has been driven by the geography and ecological niche of the isolation sources. Isolates originating from anthropic environments, in particular grapes and wine, were genetically close, thus suggesting domestication events within the species. The observed clustering was further validated by several means including, population structure analysis, F-statistics, Mantel's test and the analysis of molecular variance (AMOVA). Phenotypic performance of isolates was tested using several growth substrates and physicochemical conditions, providing added support for the clustering. Altogether, this study sheds light on the genotypic and phenotypic diversity of *L. thermotolerans*, contributing to a better understanding of the population structure, ecology and evolution of this non-*Saccharomyces* yeast.

5. Hranilovic, A, Li, S, Boss, PK, Bindon, K, Ristic, R, Grbin, PR, Van der Westhuizen, T and Jiranek, V. (2017) Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-*Saccharomyces* inocula. Australian Journal of Grape and Wine Research (in press). doi: 10.1111/ajgw.12320.

Abstract
The choice of yeast strain(s) to conduct the fermentation can greatly affect wine chemical and sensory profile. Even though the use of non-*Saccharomyces* co-inocula to build complexity and diversify styles is increasingly in vogue, a limited number of such products are available to date, and more research is required to guide their use in the wine industry. This study evaluates the potential of commercial yeast inocula to modulate the quality of Shiraz wines at two maturity levels. Vinification outcomes of eight yeast treatments were compared in earlier (24°Brix) and later (29°Brix) harvested Shiraz fruit. Yeast treatments included five non-*Saccharomyces* products with sequentially inoculated *Saccharomyces cerevisiae*, a commercial blend of non-*Saccharomyces* and *S. cerevisiae* strains, and a *S. cerevisiae* inoculum. Fermentation monitoring, and comprehensive analytical profiling in terms of basic chemistry, volatile composition, phenolic measurements and descriptive sensory analysis allowed for the

comparison of the resulting wines. Both harvest date and yeast inoculation treatments had a significant impact on a range of compositional and, in turn, sensory parameters of the wines. Certain non-*Saccharomyces* sequential inoculation treatments led to increased appeal of earlier harvest wines compared to the *S. cerevisiae* control. These treatments, however, were related to an increased risk of arrested fermentation in higher ripeness conditions. This study contributes to a better understanding of yeast inoculum-derived modulation of Shiraz wine quality parameters at different maturity levels.

6. Li, S., Bindon, K., Bastian, S.E.P., Jiranek, V., Wilkinson, K. L. (2017). Use of winemaking supplements to modify the composition and sensory properties of Shiraz wine. Journal of Agricultural and Food Chemistry 65 (7); 1353–1364. doi: 10.1021/acs.jafc.6b04505.

Abstract
Wine quality can be significantly affected by tannin and polysaccharide composition, which can in turn be influenced by grape maturity and winemaking practices. This study explored the impact of three commercial wine additives, a maceration enzyme, an enotannin, and a mannoprotein, on the composition and sensory properties of red wine, in particular, in mimicking the mouthfeel associated with wines made from riper grapes. Shiraz grapes were harvested at 24 and 28 °Brix and the former vinified with commercial additives introduced either individually or in combination. Compositional analyses of finished wines included tannin and polysaccharide concentration, composition and size distribution by high-performance liquid chromatography, whereas the sensory profiles of wines were assessed by descriptive analysis. As expected, wines made from riper grapes were naturally higher in tannin and mannoprotein than wines made from grapes harvested earlier. Enzyme addition resulted in a significantly higher concentration and average molecular mass of wine tannin, which increased wine astringency. Conversely, mannoprotein addition reduced tannin concentration and astringency. Addition of enotannin did not meaningfully influence wine composition or sensory properties.

7. Li, S., Wilkinson, K.L. and Bindon, K.A. (2017) Compositional variability in commercial tannin and mannoprotein products, American Journal of Enology and Viticulture (in press). doi: 10.5344/ajev.2017.17057.

Abstract
Enotannin and mannoprotein additives are applied in order to achieve protein, cold or color 14 stability in wine, or alternatively to modify wine sensory properties. In most cases, only basic 15 compositional information and a proposed effect in wine are provided by the manufacturer. In this study, 16 14 grape-based enotannins and 8 mannoproteins were sourced from the Australian market and their 17 composition and molecular size distribution were determined. Diverse product composition was 18 observed for both categories, suggesting a range of effects could potentially be achieved by applying 19 different products. Moreover, some products showed good agreement between product composition and 20 their designated material of origin, while others showed significant differences.

8. Longo, R., J.W. Blackman, P.J. Torley, S.Y. Rogiers, and L.M. Schmidtke (2017). Changes in volatile composition and sensory attributes of wines during alcohol content reduction. Journal of the Science of Food and Agriculture 97, 8-16. doi: 10.1002/jsfa.7757

Abstract
A desirable sensory profile is a major consumer driver for wine acceptability and should be considered during the production of reduced-alcohol wines. Although various viticultural practices and microbiological approaches show promising results, separation technologies such as membrane filtration, in particular reverse osmosis and evaporative perstraction, in addition to vacuum distillation, represent the most common commercial methods used to produce reduced-alcohol wine. However, ethanol removal from wine can result in a significant loss of volatile compounds such as esters (ethyl

octanoate, ethyl acetate, isoamyl acetate) that contribute positively to the overall perceived aroma. These losses can potentially reduce the acceptability of the wine to consumers and decrease their willingness to purchase wines that have had their alcohol level reduced. The change in aroma as a result of the ethanol removal processes is influenced by a number of factors: the type of alcohol reduction process; the chemical-physical properties (volatility, hydrophobicity, steric hindrance) of the aroma compounds; the retention properties of the wine non-volatile matrix; and the ethanol level. This review identifies and summarises possible deleterious influences of the dealcoholisation process and describes best practice strategies to maintain the original wine composition.

9. Longo R, Blackman J, Antalick G, Torley P, Rogiers S, Schmidtke L, (2017) Harvesting and blending options for lower alcohol wines: A chemical and sensory investigation. Journal of the Science of Food and Agriculture 98, 33-42. doi: 10.1002/jsfa.8434.

Abstract

Lower alcohol wines often have a poor reputation among consumers, in part due to their unsatisfactory flavours such as reducedoverall aromaintensity or herbaceous characters. The aim of this study,performedonVerdelho andPetitVerdot, was to quantify the effectiveness of a monovarietal blend in which wines made from less ripe grapes were blended with an equivalent volume of a wine vinified from riper fruit to produce wines with a lower alcohol content and desirable ripe fruit flavours.
Elevenand13 attributes, forVerdelho andPetitVerdot, respectively,were selected during sensory descriptive analysis. Intensities of perceived 'acidity', 'sweetness' and 'alcohol' attributes were significantly different ($P \leq 0.05$) between the blend (8.8±0.1% v/v) and mature Verdelho (10.3±0.1% v/v) wines, while no significant differences were found between the Petit Verdot blend (11.0±0.1% v/v) and mature (12.6±0.2% v/v) treatments. Volatile composition of wines was assessed using HS-SPME-GC-MS. Partial least square regression suggested relationships between sensory descriptors and chemical attributes in the wines, as well as themodifications of sensory and compositional profiles following blending. The blending practice described allowed the production of wines with lower alcohol content while retaining similar sensory profiles of the later harvested, riper fruit wines.

10. Ristic, R., L. van der Hulst, D.L. Capone, and K.L. Wilkinson (2017). Impact of bottle aging on smoke-tainted wines from different grape cultivars. Journal of Agricultural and Food Chemistry 65, 4146-4152. doi: 10.1111/j.1755-0238.2011.00146.x.

Abstract

Smoke taint is the term given to the objectionable smoky, medicinal, and ashy characters that can be exhibited in wines following vineyard exposure to bushfire smoke. This study sought to investigate the stability of smoke taint by determining changes in the composition and sensory properties of wines following 5 to 6 years of bottle aging. Small increases in guaiacol and 4-methylguaiacol (of up to 6 µg/L) were observed after bottle aging of smoke-affected red and white wines, while syringol
increased by as much as 29 µg/L. However, increased volatile phenol levels were also observed in control red wines, which indicated that changes in the composition of smoke-affected wines were due to acid hydrolysis of conjugate forms of both naturally occurring and smoke-derived volatile phenols. Acid hydrolysis of smoke-affected wines (post-bottle aging) released additional quantities of volatile phenols, which demonstrated the relative stability of glycoconjugate precursors to the mildly acidic conditions of wine. Bottle aging affected the sensory profiles of smoke-affected wines in different ways. Diminished fruit aroma and flavor led to the intensification of smoke taint in some wines, but smoke-related sensory attributes became less apparent in smoke-affected Shiraz wines, post-bottle aging.

11. Rogiers SY, Coetzee ZA, Deloire A, Walker RR, Tyerman SD. (2017). Potassium in the grape berry: transport and function. Frontiers in Plant Science 8: 1629. doi: 10.3389/fpls.2017.01629.

Abstract
K^+ is the most abundant cation in the grape berry. Here we focus on the most recent information in the long distance transport and partitioning of K^+ within the grapevine and postulate on the potential role of K^+ in berry sugar accumulation, berry water relations, cellular growth, disease resistance, abiotic stress tolerance and mitigating senescence. By integrating information from several different plant systems we have been able to generate new hypotheses on the integral functions of this predominant cation and to improve our understanding of how these functions contribute to grape berry growth and ripening. Valuable contributions to the study of K^+ in membrane stabilization, turgor maintenance and phloem transport have allowed us to propose a mechanistic model for the role of this cation in grape berry development.

12. Schelezki, O.J., Smith, P.A., Hranilovic, A., Bindon, K.A, Jeffery, D.W. (2018) Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition. Food Chemistry 244; 50-59. doi.org/10.1016/j.foodchem.2017.10.024.

Abstract
A changing climate has led to winegrapes being harvested with increased sugar levels and at greater risk of berry shrivel. A suggested easy-to-adopt strategy to manage the associated rising wine alcohol levels is the pre-fermentative substitution of juice with either “green harvest wine” or water. Our study investigates the effects of this approach on Vitis vinifera L. cv. Cabernet Sauvignon wine quality attributes. Wines were also made from fruit collected at consecutive earlier harvest time points to produce wines comparable in alcohol to the substituted wines. Tannin concentrations and colour did not change significantly in the wines with modified alcohol content even at higher juice substitution rates. Differences in polysaccharide and tannin composition indicated variability in extraction dynamics according to substitution rate and type of blending component. In scenarios where berry shrivel is inevitable, the incorporation of water in particular offers much promise as part of a strategy to manage wine alcohol content.

13. Wollan, D., D.-T. Pham, and K.L. Wilkinson (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. doi: 10.1021/acs.jafc.6b02691.

Abstract
The relative proportion of water and ethanol present in alcoholic beverages can significantly influence the perception of wine sensory attributes. This study therefore investigated changes in wine ethanol concentration due to evaporation from wine glasses. The ethanol content of commercial wines exposed to ambient conditions while in wine glasses was monitored over time. No change in wine ethanol content was observed where glasses were covered with plastic lids, but where glasses were not covered, evaporation had a significant impact on wine ethanol content, with losses from 0.9 to 1.9% alcohol by volume observed for wines that received direct exposure to airflow for 2 h. Evaporation also resulted in decreases in the concentration of some fermentation volatiles (determined by gas chromatography–mass spectrometry) and a perceptible change in wine aroma. The rate of ethanol loss was strongly influenced by exposure to airflow (i.e., from the laboratory air-conditioning unit), together with certain glass shape and wine parameters; glass headspace in particular. This is the first study to demonstrate the significant potential for ethanol evaporation from wine in wine glasses. Research findings have important implications for the technical evaluation of wine sensory properties; in particular, informal sensory trials and wine show judging, where the use of covers on wine glasses is not standard practice.

9.2. Industry articles

1. Ristic, R. 2015. TC-IWP: New training centre projects to deliver new tools and optimise existing practices for industry. Wine & Viticulture Journal 30: 20-24.
2. Ristic, R., A. Hranilovic, S. Li, R. Longo, D.-T. Pham, B. Qesja, O.J. Schelezki, and V. Jiranek. 2016. Integrated strategies to moderate the alcohol content of wines. Wine & Viticulture Journal 31: 33-38.
3. Qesja, B., R. Crouch, and P.G. Quester. 2016. Reduced alcohol wine: Product innovation and authenticity: The case of wine. Wine & Viticulture Journal 31: 67-69.
4. Ristic, R., Schelezki, O. and Jeffery, D. (2018) Water into wine: pre-fermentation strategies for producing lower alcohol wines. Wine & Viticulture Journal (in press).
5. Ristic, R., van der Hulst, L. and Wilkinson, K. (2018) Development of smoke taint in wine during bottle aging. Wine & Viticulture Journal (in press).

9.3. Conference publications

1. Qesja. B., Crouch. R., Quester. P. (2016) Innovating Traditional Products "Self-Sacrifice vs. Product Authenticity", Proc 9th AWBR Conference (Academic Conference, 9th Academy of Wine Business Research Conference), 17-19 Feb 2016, Adelaide
2. Ristic R. and Jiranek V. (2016) A national initiative aimed at developing a multi-faceted approach to managing alcohol and flavour, Proc 62nd German Winegrowers' Cong., 27–30 Nov 2016, Stuttgart, Germany. (in press).
3. Olaf Schelezki. Maturity variability. Australian Society for Viticulture & Oenology Seminar: Earlier, shorter, hotter; Managing compressed vintages. Adelaide, Nov 2015. (in press).
4. Ana Hranilovic. Yeast for high (and low alcohol). Australian Society for Viticulture & Oenology Seminar: Earlier, shorter, hotter; Managing compressed vintages. Adelaide, Nov 2015. (in press).
5. Bora Qesja. Public perception of high alcohol wines. Australian Society for Viticulture & Oenology Seminar: Earlier, shorter, hotter; Managing compressed vintages. Adelaide, Nov 2015. (in press).
6. Wilkinson, K and V Jiranek (2013) Consumer demand vs. industry willingness and ability to deliver. Proceedings of the 1st International Symposium on Alcohol Reduction in Wine. pp 98-104.

9.4. Other publications

1. The ARC TC-IWP Annual report 2014: https://www.adelaide.edu.au/tc-iwp/docs/annual-report/annual-report2014.pdf
2. The ARC TC-IWP Annual report 2015: https://www.adelaide.edu.au/tc-iwp/docs/annual-report/annual-report2015.pdf
3. The ARC TC-IWP Annual report 2016: https://www.adelaide.edu.au/tc-iwp/publications/annual-report/arc-tc-iwp-annualreport-2016.pdf
4. Technical note 1: Smoke taint development in grapes. https://www.adelaide.edu.au/tc-iwp/docs/publications/tn1-smoke-taint.pdf
5. Technical note 2: Changes in wine ethanol content due to evaporation from wine glasses. https://www.adelaide.edu.au/tc-iwp/docs/publications/tn2-ethanol-evaporation.pdf
6. Technical note 3: Use of winemaking supplements to modify the composition and sensory properties of Shiraz wine. https://www.adelaide.edu.au/tc-iwp/docs/publications/tn3-winemaking-supplements.pdf
7. Technical note 4: Innovating Traditional Products: "Self-Sacrifice vs. Product Authenticity". The Case of Wine. https://www.adelaide.edu.au/tc-iwp/docs/publications/tn4-low-alcohol-wines.pdf
8. Technical note 5: Harvesting and blending options for lower alcohol wine. https://www.adelaide.edu.au/tc-iwp/publications/technical/TN_5_harvestingandblending.pdf

9. Technical note 6: Changes in wine during alcohol content reduction. https://www.adelaide.edu.au/tc-iwp/publications/technical/TN_6_Loweralcoholwines.pdf
10. Technical note 7: Water into wine: Pre-fermentation strategies for producing lower alcohol wines. https://www.adelaide.edu.au/tc-iwp/publications/technical/TN7-Water-into-wine.pdf
11. Technical note 8: Implications of potassium nutrition on grapes and wine. https://www.adelaide.edu.au/tc-iwp/publications/technical/TN8-role-of-k-in-grapevines.pdf
12. Technical note 9: The sugar-potassium nexus within the grape berry. https://www.adelaide.edu.au/tc-iwp/publications/technical/TN9-k-sugar-nexus.pdf

9.5. Industry seminars

1. 'How to modulate flavour and alcohol levels in times of climate and market change?' Industry Seminar, Launceston, Tasmania, 11/5/2015
2. 'How can we modulate flavour and alcohol levels in times of climate and market change?', Industry Seminar, Wagga Wagga, NSW, 19/5/2016
3. 'How can we modulate flavour and alcohol levels in times of climate and market change?' Industry Seminar, Coonawarra, SA, 12/10/2016
4. 'Alcohol corrected wines: from production to consumption' workshop at the 16th Australian Wine Industry Technical Conference, 24/7/2016
   During industry seminars TC-IWP researchers presented their projects in a 5-10 minute 'pitch' that highlighted their key research objectives and outcomes, followed by a discussion session during which they received valuable input and feedbacks from grape growers, winemakers and fellow researchers. The success of the industry seminars were covered in the local TV and newspapers.
5. 'Grapes for style: the impact of berry ripening on wine quality' workshop at the 16th Australian Wine Industry Technical Conference, 24/7/2016
6. 'Smoke taint in grapes and wines', Industry Seminar, Chile
   Following bushfires in several wine regions throughout Chile in February 2017, Vinos de Chile invited Dr Renata Ristic and TC-IWP HDR Lieke van der Hulst to share knowledge on the effects of grapevine exposure to smoke on vine health, and grape and wine quality. They presented a seminar in Talca for 150+ attendees. A short television report was produced by Chilean Central TVN Red Maule and more than 30 articles in various newspapers, magazines and newsletters covered this event. The visit to Chile included meetings with the Ministry of Agriculture, directors of Vinos of Chile and numerous representatives from Santiago and Talca universities.

9.6. Conference presentations

1. 'Alcohol corrected wines: from production to consumption' W03 **workshop conveyor** at the 16th AWITC, 24/7/2016
2. Ristic, R., Deloire, A. and Jiranek, V. 'The ARC TC-IWP integrated approach to alcohol corrected wines: from production to consumption', **poster presentation** at the 16th AWITC, 21-24/7/2016
3. Ristic R. and Jiranek V. 'A national initiative aimed at developing a multi-faceted approach to managing alcohol and flavour', **oral presentation** at the 62nd German Winegrowers' Congress, Stuttgart, Germany, 27–30/11/2016
4. Ristic, R., J. 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', **oral presentation** at the 254th American Chemical Society National Meeting & Exposition, Washington, DC, 20-24/8/2017
5. Ristic, R., J. 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', **oral**

presentation at the Crush 2017, the grape and wine science symposium, Adelaide, SA, 13-14/11/2017

10. APPENDIX 2: REFERENCES

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11. APPENDIX 3: PROJECTS AND RESEARCHERS

Projects	**Researchers**
Berry shrivel and grape berry cell death	
Cell death in the berry and berry weight loss	Dr Simon Clarke
Investigation of the physiological cause of grape berry cell death	Zeyu Xiao
Molecular events underlying death in the grape berry	Siyang Liao
Programmed cell death in grape berries	Dr Shifeng Cao
The sugar: potassium nexus within the grapevine	
The sugar-potassium nexus within the grape berry	Zelmari Coetzee
Optimisation of harvest blending regimes	
Optimisation of an early harvest regime – impact on grape and wine composition and quality	Olaf Schelezki
Application of reverse osmosis/perstraction to wines made from grapes with different levels of maturity: chemical and sensory evaluation	Rocco Longo
Yeast strains in ethanol management and flavour enhancement	
Managing ethanol and sensory compounds by non-*Saccharomyces* yeasts	Ana Hranilovic
Impact of high sugar content on the efficiency and sensory outcomes of un-inoculated fermentations	Federico Tondini
Winemaking techniques for ethanol management and flavour enhancement	
Selective and deliberative use of winemaking supplements to modulate sensory properties of wines	Sijing Li
Getting alcohol content right: The compositional and sensory basic for an alcohol sweet spot	Dr Duc-Truc Pham
Novel techniques for flavour enhancement	
Controlling unripe characters using molecularly imprinted polymers or specific microbials to eliminate methoxypyrazines from wine	Chen Liang
The use of cyclodextrins to manipulate off-flavours in wine	Chao Dang
Biochemical response of grapevine to smoke	
The biochemical response of grapevines to smoke exposure	Lieke van der Hulst
Wine innovation and the importance of authenticity	
Self-sacrifice vs. product authenticity (The case of wine)	Bora Qesja
Translation of 'whole of production chain' wine science research to industry outcomes	Dr Renata Ristic

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