INVESTIGATION OF AN EARLY HARVEST REGIME AND PRE-FERMENTATIVE BLENDING TREATMENTS TO PRODUCE LOWER ALCOHOL WINES: IMPACT ON GRAPE AND WINE COMPOSITION AND QUALITY

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Thesis Summary

Warmer and shorter grape ripening periods, as well as concomitant extreme weather events like heatwaves, have been posing considerable challenges for wine producers, particularly when winemakers seek to extend fruit hang-times to meet consumer demands of fuller flavoured wines. Consequently, grapes may not be harvested at desired qualities and may be exposed to over-ripeness or berry shrivel, which likely translate into excessive wine alcohol concentrations. As the nature of these weather events is rather unpredictable and succumbs to annual fluctuations, winemakers rely on flexible and economic strategies (e.g. alternative to current physical dealcoholisation techniques) to ameliorate situations of excessive grape ripeness.

Among the various methods to manage wine alcohol levels, one recently proposed strategy is the pre-fermentative dilution of sugar in juice with either a very low alcohol wine (~5 % alcohol by volume) or water. However, the effect of such manipulations on wine compositional and sensory qualities was not entirely understood. Further, it has been unclear how the resulting wines would compare to those of similar alcohol levels made from earlier harvested grapes that are picked to avoid grape over-maturity. For this purpose, studies were undertaken to evaluate these approaches to alcohol management for the production of Cabernet Sauvignon and Shiraz wines under a variety of vintage conditions. The studies have been drafted as manuscripts that have been prepared for publication or have already been submitted or published in peer-reviewed journals. The manuscripts are presented in chapters as outlined below after an introductory chapter.

The first study reports on the initial vintage (2015), in which extremely warm and dry conditions caused an exemplary over-maturity of the Cabernet Sauvignon grapes. Experimental wines of various lower alcohol concentrations were produced via pre-fermentative substitution of juice with a very low alcohol green harvest wine (GHW) or

water at various rates. The consequences for non-volatile wine composition (colour parameters, tannin and polysaccharide composition) of these pre-fermentative approaches were reported relative to the high alcohol control wine. The characteristics of the substituted wines were discussed in the context of wines of similar alcohol content produced from earlier harvested grapes, thereby providing an evaluation of the role of harvest decision in this extreme vintage scenario. It was shown that colour and tannin parameters were not significantly affected even by the highest substitution rates (with GHW and water) compared to the control and in fact retained values superior to those in wines resulting from earlier harvests. A manuscript detailing this work has been published in Food Chemistry, 244 (2018) 50-59.

Further building on the 2015 experimental winemaking, a second manuscript presents the consequences of the alcohol management treatments on wine volatile composition (qualitative and quantitative data obtained with GC-MS analysis) and wine sensory profiles (determined with descriptive sensory analysis). Analysing the same wines as before, the substitution treatments were contrasted with wines arising from earlier harvests to outline potential merits and disadvantages of each approach, this time in terms of volatile and sensory profiles. According to the GC-MS data, the implementation of water had the least effect on the volatile composition, causing rather minor concentration changes of a small fraction of the analysed volatiles. This was further mirrored in the sensory profiles of the lower alcohol wines, which were found to be strongly reminiscent of the overripe control wine, hence there not only positive results (aroma intensity, red fruit, dark fruit) but also negative attributes (hotness) sustained when adjusting the wine alcohol levels via the proposed pre-fermentative treatments. This indicated that wine styles are more affected by harvest date than the substitution treatments. This study has been published in Food Chemistry 259 (2018) 196-206.

The two studies reporting on the 2015 winemaking trial provided evidence that pre-fermentative additions of GHW or water are suitable for the production of lower alcohol wines from highly mature Cabernet Sauvignon grapes without greatly affecting the wine quality. The objective of the subsequent study in 2016 was to confirm these findings and to further evaluate an early harvest regime and pre-fermentative substitution treatments as means to produce lower alcohol wines under milder vintage conditions and with relevance to changes in regulation allowing water addition under certain circumstances. Cabernet Sauvignon and Shiraz were investigated and the resulting wines were examined for colour, tannin parameters, volatile composition and sensory properties. The benign nature of the substitution treatments on wine quality parameters, for instance stable levels of anthocyanin and tannin concentrations, was confirmed for Cabernet Sauvignon, but less so in case of Shiraz, where more pronounced differences emerged according to the blending component used. In this case, water substitution was identified as the more suitable treatment to manage wine alcohol levels under mild vintage conditions while preserving the wine quality as defined by harvest date. Wine volatile profiles were generally more affected by the blending treatments in the 2016 vintage context and as a function of the variety. Different responses for Cabernet Sauvignon and Shiraz were associated with the different blending components, however without largely influencing the volatile profiles in comparison to the controls. This study was prepared as a manuscript for submission to a peer-reviewed journal.

Following a recent change in regulation that allows the addition of water into the winemaking process (and consequently lowering the wine alcohol concentrations), there has been a particular interest by the wine industry to understand the consequences on wine composition, wine style and quality to facilitate decision making around this newly available winemaking technique. In this context, the final study of experimental Shiraz wines from 2017 extended upon the conclusions drawn in the preceding studies and addressed additional gaps in knowledge about adding water during winemaking. The experiments focused on

evaluating two options for pre-fermentative water addition during the winemaking process – that is, substitution versus addition without juice removal (i.e., dilution). In addition, the importance of grape maturity on producing high quality, lower alcohol wines with pre-fermentative water addition was assessed using grapes harvested at two distinct maturity levels, targeting "fresh" and "mature" stages of fruit development.

Based on a lower grape maturity (i.e., Fresh Fruit), low juice substitution with water did not change colour properties, whereas the analogous dilution treatment with water elicited declines in colour intensity and stability in line with decreases in total phenolics and tannin concentrations. The juice dilution further resulted in declines of important sensory attributes, such as 'flavour intensity' and 'body', diverging from the more benign substitution treatments. When applied at a greater grape maturity level (i.e., Mature Fruit), substitution or dilution with water appeared to have a greater effect on colour properties with only small implementation volumes, but high dilution rates in particular resulted in dramatically decreased tannin concentrations. In terms of wine sensory profiles, the differences between substitution and dilution treatments appeared to be less pronounced for Mature Fruit Shiraz wines compared to the Fresh Fruit counterparts, but high implementation rates well beyond the legal limit of must dilution (minimum of 13.5 °Bé) led a noticeable decline in an array of sensory attributes. Analysis of the volatile data is underway and the study will be reported in form of a manuscript for submission to peer-reviewed journal.

In conclusion, this work has provided knowledge on the consequences for wine quality associated with pre-fermentative alcohol management approaches involving the implementation of water or a very low alcohol wine into the must. The managed wines were hereby around 1% - 6% lower in alcohol by volume, so generally exceeding the capabilities of other viticultural or microbiologic strategies, but lying within the possibilities of post-fermentative physical dealcoholisation technologies. Although observed implications on final wine sensory attributes were marginal particularly at low to moderate levels of alcohol

adjustment, this study has illustrated that higher grape maturities with subsequent alcohol management provides only limited merits to the wine quality, so that an earlier harvest could be a more appropriate solution. Given that this project included three distinct vintage situations and two important red wine varieties, the results can help support winemakers to make informed decisions regarding wine alcohol management according to harvest situation and preferred wine style.

Declaration

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

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18/10/2018

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Olaf Schelezki   Date

Publications

This thesis contains two manuscripts that were published in Food Chemistry during candidature. Food Chemistry had an impact factor of 4.529 according to Thomson Reuters Journal Citation Reports (2016). Two additional manuscripts are prepared for subsequent submission to scientific journals.

The publications included in this thesis are:

Chapter 2 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 (2018) 50-59.

Chapter 3 Schelezki, O.J., Suklje, K., Boss, P., Jeffery, D.W., 2018. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. Food Chemistry 259 (2018) 196-206.

The following chapters contain the manuscripts prepared for submission to scientific journals are:

Chapter 4 Schelezki, O.J., Antalick, G., Suklje, K., Jeffery, D.W., 2018. Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis.

Chapter 5 Schelezki, O.J., Deloire, A., Jeffery, D.W., 2018. Substitution or dilution? Assessing pre-fermentative water implementation to produce lower alcohol Shiraz wines.

Conferences and presentations

Australian Society of Viticulture and Oenology (ASVO) Seminar 'Earlier, shorter, hotter – Managing compressed vintages', 19 November 2015, Adelaide, Australia

Presentation given titled 'Berry heterogeneity and wine quality'. A proceedings manuscript was accepted by the ASVO titled 'Berry heterogeneity and wine quality' and can be found in the appendix.

The 16th Australian Wine Industry Technical Conference, 24 - 28 July 2016, Adelaide, Australia.

Presentation and workshop given titled 'Optimisation of an early harvest regime – Impact on grape & wine composition & quality.'

ARC Training Centre Industry Workshop, 12 October 2016, Coonawarra, Australia.

Presentation given titled 'Optimisation of an early harvest regime – Impact on grape & wine composition & quality.

Treasury Wine Estates (TWE) Technical Conference, 16 November 2016, Adelaide, Australia

Presentation given titled 'Does size matter? Berry, berry much.'

Margaret River Cabernet Hang-Time Forum, 24 January 2017, Margaret River, Australia.

Presentation given titled 'Berry variability and harvest data – Impact on wine quality.'

In Vino Analytica Scientia 2017 10th Symposium, 17 - 20 July 2017, Salamanca, Spain.

Presentation given titled 'Managing red wine alcohol content using pre-fermentative juice substitution or sequential grape harvesting: Impact on wine composition and quality.'

Crush 2017 – The Grape and Wine Science Symposium, 13 – 14 November 2017, Adelaide, Australia.

Presentation given titled 'Pre-fermentative addition of water into must to manage alcohol levels of red wine.'

Further presentations were given at Australian Research Council Training Centre for Innovative Wine Production annual meetings in 2015 (Launceston, Tasmania), 2016 (Wagga Wagga, New South Wales) and 2017 (Adelaide, South Australia) presenting highlights of the PhD project.

Panel of supervisors

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Chapter 1

LITERATURE REVIEW

This literature review was prepared within the first six months of candidature and comprises

the literature up to April 2015. Any relevant additional literature has been included in the

introduction sections of the publications addressed under Chapters 2 to 5.

1.1   Introductory background

1.1.1 Grape and wine composition

The final chemical composition of wines, which defines quality indicators such as clarity, colour, aroma and flavour, results from evolutionary changes throughout the winemaking chain from grapevine to glass. During formation and ripening of the grapes, a series of volatile and non-volatile compounds accumulate in the berry and define to a large extent the final quality of the wine in the glass. For example it is assumed that the maximum concentration of methoxypyrazines is already determined around two weeks before veraison (Ryona et al. 2008[1], Kalua and Boss 2010[2], Harris et al. 2012[3]), enough time for the winemaker to plan eventual interventions in the vineyard. Current research by CSIRO pursues this assessment investigating the potential to evaluate fruit and wine aroma profiles early in the season, which eventually could provide tools for early assessments of the desired fruit quality and respective adaptation of vineyard operations, beyond just methoxypyrazines. For instance, (Bindon et al. 2013[4]) observed steady concentrations of C_{13}-norisoprenoids such as β-damascenone and linalool already five weeks before harvest. Further knowledge could give valuable indications of potential varietal aroma levels early in the season aiding viticultural and enologic planning.

1.1.2 Volatile sensory contributors

The grape compositional profile affecting wine aroma, as defined by external factors surrounding the vine, namely the genome of the cultivar, the site (soil and climate) and viticultural practices is generally divided into three groups (Ebeler 2001[5]). The first group includes volatiles derived from the grapes, either directly or indirectly as a result of precursors and is generally known to define the varietal character of a wine. Some volatile

compounds, for instance 3-isobutyl-2-methoxypyrazine (IBMP) and rotundone (Wood et al. 2008[6], Siebert et al. 2008[7]), that may be characteristic for Cabernet Sauvignon and Shiraz wines, respectively, are present in the grapes and can be extracted into the wines without further modification (de Boubee et al. 2000[8], Robinson et al. 2011[9]). Table 1 gives an overview on pyrazines frequently associated with wine aroma. Pyrazines are found within a very low concentration range of, in case if IBMP, 0.5-50 ng/l (Clarke and Bakker 2004[10]) but the perception threshold of 2 ng/l (in water) turns it into a powerful odorant.

Table 1 Descriptions and olfactory perception thresholds of the main methoxypyrazines. From Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., *Handbook of Enology Vol. 2*, 2nd Edition, Copyright (2006) by John Wiley Sons, Inc. Reprinted with permission from John Wiley & Sons, Inc.

Pyrazine	Olfactory perception threshold in water (ng/l)	Description
2-Methoxy-3-isobutyl	2	Green pepper
2-Methoxy-3-isopropyl	2	Green pepper, earthy
2-Methoxy-3-*sec*-butyl	1	Green pepper
2-Methoxy-3-ethyl	400	Green pepper, earthy

Other aroma compounds in the grapes on the other hand become active during the winemaking procedures. Monoterpenoids, such as linalool and geraniol, are mostly derived as glycosides from the grape cell vacuoles that are transferred to the must through crushing or pressing, where they become sensorial detectable odorants with the β-glucosidase activity of yeasts during fermentation. Same observations are made for C_{13}-norisoprenoids such as β-damascenone ('rose', 'honey') and β-ionone ('violets') (Fischer 2007[12]). Exogenous addition of pectinase, or yeasts with an enhanced β-glucosidase activity are used in the wine industry to increase the varietal odour impact in wines. Examples are given in Table 2, which shows the importance of terpene glycosides on the potential aroma composition in Gewurztraminer or Riesling wines.

Table 2 Overview of free and glycosidic bond terpenes and C_{13}-norisoprenoid glycosides concentrations found in various *Vitis vinivera* cultivars. From Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., *Handbook of Enology Vol. 2*, 2nd Edition, Copyright (2006) by John Wiley Sons, Inc. Reprinted with permission from John Wiley & Sons, Inc.

Grape variety	Free terpenols (μg/l)	Terpene glycosides (μg/l)	C_{13}-norisoprenoid glycosides[a] (μg/l)	Reference
Muscats:				
Alexandria	1513	4040	ND[b]	Günata (1984)
Frontignan	1640	1398	ND	Günata (1984)
Hamburg	594	1047	ND	Günata (1984)
Ottonel	1679	2873	ND	Günata (1984)
Gewürztraminer	282	4325	ND	Günata (1984)
Riesling	73	262	182	Razungles *et al.* (1993)
Sauvignon Blanc	5	107	104	Razungles *et al.* (1993)
Sémillon	17	91	265	Razungles *et al.* (1993)
Syrah	13	65	84	Razungles *et al.* (1993)
Chardonnay	41	12	140	Razungles *et al.* (1993)
Cabernet Sauvignon	0	13	100	Razungles *et al.* (1993)

[a]C_{13}-norisoprenoids analyzed: hydroxy-3-β-D-damascone, oxo-3-α-ionol, oxo-4-β-ionol, hydroxy-3-β-ionol and hydroxy-3-dihydro-7,8-β-ionol.
[b]ND = not detected.

Another group in the aroma composition of wines are sulphur compounds, which, besides being associated with off-flavours such as the smell of 'rotten eggs' (H_2S), discriminate grape vine cultivars such as Sauvignon blanc. The thiol 4-mercapto-4-methyl-pentan-2-one for instance is responsible for the 'black currant' or 'boxwood' perception in Sauvignon blanc wines. Others like 3-mercapto-hexan-1-ol ('tropical') or 4-mercapto-3-methylpentan-2-ol ('citrus') can be found, among others, in Gewurztraminer or Riesling (Fischer 2007[12]). Table 3 shows thiols particularly important for Sauvignon blanc wines and indicates their strong odor impact potentials by comparing the perception thresholds with observed content ranges in wines. In grapes, thiols are odourless due to their presence as cysteine or glutathione conjugates and first become active after exhibition to the yeast derived β-lyase. This mechanism has also opened the opportunity for the wine industry to actually influence the odorous impact by yeast selection upon different β-lyase activities.

Table 3 Thiol compounds, their discriptors, perception thresholds and content range as found in wines, after Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., *Handbook of Enology Vol. 2*, 2nd Edition, Copyright (2006) by John Wiley Sons, Inc. Reprinted with permission from John Wiley & Sons, Inc.

Compound identified	Description	Perception threshold* ng/l	Content (ng/l)
4-mercapto-4-methyl-pentan-2-one	Boxwood, broom	0.8	0–120
3-mercaptohexyl acetate	Boxwood, passion fruit	4	0–500
3-mercaptohexanol	Passion fruit, grapefruit zest	60	150–3500
4-mercapto-4-methyl-pentan-2-ol	Citrus zest	55	15–150
3-mercapto-3-methyl-butan-1-ol	Cooked leeks	1500	20–150
benzenemethanethiol	Gunflint, smoke	0.3	5–20

*in model dilute alcohol solution.

A second group of volatile compounds is formed during alcoholic fermentation by the secondary metabolism of the yeasts (Ebeler et al. 2001[5]), where amino acids and lipids extracted from grapes or biosynthesised by yeasts are transformed into higher alcohols and esters (Swiegers et al. 2005[13]). The precise nature of the higher alcohols or esters finally produced and their relative quantities mainly depend on species and strain of the yeast and their various activities (e.g. conversion efficiencies reliant on diffusion rates based on precursor type, and different acetyltransferase (ATT) activity rates for different substrates (Dennis et al. 2012[14]) between *Saccharomyces* (Malcorps et al. 1992[15]) and non-*Saccharomyces* yeasts (Rojas et al. 2001[16]). Further, the presence of C6 compounds from the grapes, as precursors that are modified by yeasts, plays a major role for cultivar specific ester profiles (Kalua et al. 2012[14]). Wondra and Beroviv (2001) used different Saccharomyces cerevisiae strains isolated from different vineyard sites for fermentations and could show the significance of yeast strain impact on the higher alcohol and ester profiles (Table 4).

Table 4 Concentrations of Higher alcohols (HA), lower fatty acids (LFA) and Esters as produced by various *Saccharomyces cerevisiae* strains isolated from different vineyards. From Faculty of Food Technology and Biotechnology, Vol 39 (2), Wondra, M., Beroviv, M., Analysis of aroma components of Chardonnay wine fermented by different yeast strains, 141-148, Copyright (2001), reprinted with permission.

γ (aromatic compound) mg L^{-1}	*Saccharomyces cerevisiae* strain										Control
	1	2	3	4	5	6	7	8	9	10	
HA											
3-methyl-1-butanol	323.00	353.00	384.80	359.30	262.50	261.00	399.20	299.30	536.80	284.80	13.00
2-methyl-1-butanol	76.00	88.80	91.50	92.80	51.50	78.00	113.30	77.00	115.50	93.00	7.60
2-methyl-1-pentanol	6.30	6.80	5.30	5.80	5.00	6.00	5.50	5.50	6.80	6.30	0.00
2-phenyl ethanol	50.50	62.80	58.80	43.80	54.80	34.30	85.80	42.80	79.30	50.80	4.60
Σ HA	455.80	511.40	540.40	501.70	373.80	379.30	603.80	424.60	738.40	434.90	25.20
LFA											
pyruvic acid	20.00	20.00	13.50	10.50	10.50	10.80	18.20	11.00	14.00	19.80	5.00
hexanoic acid	32.00	28.50	8.30	5.00	4.00	7.00	16.30	4.00	9.50	11.30	0.00
octanoic acid	158.00	167.30	148.80	142.30	141.80	142.80	192.00	120.30	150.30	157.00	4.80
decanoic acid	136.00	155.80	116.30	125.00	140.50	128.00	173.00	113.30	139.30	152.00	6.60
Σ LFA	326.00	351.60	273.40	272.30	286.30	277.80	381.30	237.60	299.10	320.30	16.40
ESTERS											
ethyl acetate	29.00	36.80	41.00	41.30	21.00	34.50	32.20	12.30	33.30	28.80	6.20
ethyl isobutirate	5.00	5.50	6.00	8.00	7.80	7.30	7.30	5.50	8.30	6.30	0.00
isobutyl acetate	12.80	11.50	8.80	16.50	11.00	13.80	19.30	7.50	9.00	12.50	0.00
ethyl lactate	15.30	15.80	12.30	18.80	14.80	15.80	18.80	11.00	16.30	16.50	0.00
ethyl isovalerate	1.30	1.00	1.00	1.00	1.00	1.00	1.50	1.00	1.30	1.00	0.00
3-methyl-butyl acetate	227.00	228.30	213.30	392.30	199.30	204.80	338.00	194.00	265.00	202.50	5.40

Besides the positive contribution of microorganisms on wine aroma, a number of compounds responsible for so called off flavours can be produced by yeast and bacteria (Table 5). Due to a lack of yeast available nitrogen yeasts may metabolise sulfur containing amino acids, such as cysteine, and compounds like ethyl mercaptan or hydrogen sulphide may be formed causing the perception of 'rotten-eggs', 'garlic' or 'onion' (Ebeler 2001[5]). Volatile compounds derived from maturation in oak barrels or which are formed chemically during wine ageing form the third compositional group that determines the overall aroma perception of wine. The evolution of wine aroma over time is rather complex and wine characteristics such as pH, the SO_2 concentration, oxygen intake, temperature and wood contact influence the alterations of the wine aroma composition over time.

Table 5 List of sulfur containing compounds found in wine with negative impact on quality, respective perception thresholds, descriptors and concentrations found in wines. From Ribéreau-Gayon, P., Glories, Y.; Maujean, A.; Dubourdieu, D., *Handbook of Enology Vol. 2*, 2nd Edition, Copyright 2006 by John Wiley Sons, Inc. Reprinted by permission from John Wiley & Sons, Inc.

Substances	Perception thresholds (μg/l)	Description	'Clean' wine (concentrations in μg/l)	Wine with 'reduction' odors (concentrations in μg/l)	Boiling point (°C)
Carbonyl sulfide[a]		Ether	0.7	0.4	−50
Hydrogen sulfide	0.8	Rotten eggs	0.3	16.3	−61
Methanethiol	0.3	Stagnant water	0.7	5.1	6
Ethanethiol	0.1	Onion	0	10.8	35
Dimethyl sulfide	5	Quince, truffle	1.4	2	35
Carbon disulfide		Rubber	1.7	2.4	46

[a]measured by the ratio of the peak surface to that of the internal standard.

Acetate- and ethyl esters formed during fermentation happen to be chemically hydrolysed through acid catalysation and reconstituted into different esters, or new esters are formed with the presence of organic acids and alcohols, both changing the aroma profile over the time the wine ages in the bottle (Perez-Prieto et al. 2003[18]). Even a major part of varietal aroma compounds like terpenes are still present in their glycolised form after fermentation an may be liberated through hydrolisation in wine (Ugliano et al. 2006[19]). Loscos et al. (2010[20]) showed that many terpenes in a model wine increased in concentration in the early stages of ageing and decreased again during later stages below the initial values due to acid-catalysed rearrangements, for example to respective oxides such as nerol- or linalool oxide. Where the bouquet of Riesling or Chenin blanc may benefit from an 'ageing aroma' (with moderate increments of 1,1,6-Trimethyl-1,2-dihydronaphthalene for instance), terpene rich varieties such as Gewürztraminer or Scheurebe rather lose their desired varietal characters and hence their expected qualities during bottle ageing (Perez-Coello et al. 2009[21]).

Barrel ageing of wines on the other hand is associated with an increase product value as the sensory impact on the wine, most of all the release of oak wood aromas, is

generally appreciated by the customer (Perez-Coello et al. 2009[21]). Typical oak derived compounds associated with barrel ageing are the lactone isomers cis- and trans-β-methyl-γ-octalactone (mainly associated for the perception of "oak" and "coconut"), the phenolic aldehyde vanillin ('vanilla') and volatile phenols such as guaiacol ('Spicy', 'toasty', 'smoky') and eugenol ('spicy', 'clove'). The choice of oak species, wood treatments, number of uses and extraction time influences the final composition extracted to the wine and is an important tool for the winemaker to influence the final product quality. Table 6 illustrates the impact of the mentioned factors on the final compound concentrations in wine.

Table 6 Mean concentrations (µg/l) of volatile compounds in wine aged in barrels with different number of uses (new barrels, barrels with three uses, barrels with five uses). Reprinted from Trends in Food Science & Technology, Vol 17, Garde-Cerdán, T., Ancín-Azpilicueta, C., Review of quality factors on wine ageing in oak barrels, 438-447, Copyright (2006), with permission from Elsevier.

Compounds	New barrels[a]		Barrels with 3 uses[b]		Barrels with 5 uses[c]	
	American oak	French oak	American oak	French oak	American oak	French oak
Furfural	∼4500	∼4700	n.s.	n.s.	90	148
Vanillin	n.s.	n.s.	∼90	∼50	15	30
Eugenol	∼22	∼22	n.s.	n.s.	20	18
Guaiacol	∼53	∼49	∼10	∼10	6	5
4-Methylguaiacol	∼15	∼15	∼8	∼8	0.05	0.03
cis-Oak lactone	∼225	∼120	∼120	∼80	129	66
trans-Oak lactone	∼40	∼90	∼10	∼25	26	41

n.s., not study.
[a] *Chardonnay* wine aged in barrels for 7 months (From Towey & Waterhouse, 1996).
[b] *Monastrell* wine aged in barrels for 6 months (From Pérez-Prieto et al., 2002).
[c] Blend wine (*Tempranillo* 41% and *Cabernet Sauvignon* 59%) aged in barrels for 12 months (From Garde-Cerdán, Rodríguez-Mozaz et al., 2002).

Barrel ageing however can also have negative impact on wine aroma, mainly associated with the spoilage of *Brettanomyces* yeast. This yeasts ability to convert p-coumaric acid, present in red wines, to 4-vinylphenol and further to 4-ethylphenol provokes the unpleasant 'medical' or 'band-aids' odour and even 'horse stable' in higher concentrations (Chatonnet et al. 1992[23]). The number of origins different volatile groups can

be assigned to and their complex changes throughout winemaking and ageing imply the difficulty to predict the final compositional matrix when the bottle is opened.

1.1.3 Analysis of volatiles to aid understanding

Technologic progress has enabled the detection of more than 800 volatile compounds in wine so far. With concentrations between 10^{-4} to 10^{-9} g/l, most of those volatiles are present below the limit of human detection (Rapp et al. 1990[24], Guth et al. 1997[25], Ferreira et al. 2000[26]) but still may have significant contribution to the final wine bouquet, as explained later on in this text. Improvements in analytical capability and detection of volatiles have already helped to advance winemaking practices. For instance, a characteristic (varietal) trait for Cabernet Sauvignon is the aroma of 'green capsicum' created by the compound IBMP mentioned above, which is produced in primary leaves and mainly transported into the exocarp tissue of the grape berry (Maggu et al. 2007[27], Ryona et al. 2008[1]). IBMP concentration in grapes are reported to increase with higher vine vigour, resulting in more growth of the primary and secondary shoots and leaf area and consequently the shading of the bunch zone. Water and nitrogen mainly determine vine vigour, but through leaf removal, hence viticultural practice, bunch exposure to sunlight can be increased and IBMP can be managed (Ryona et al. 2008[1], Suklje et al. 2012[28] and 2014[29]) to reach the desired product quality or different wine styles. The frequent application of this knowledge by the wine industry today is the result of great research efforts dealing with one compound, but there are others to be studied to be able to control other sensory aspects of the wine.

Recent GC-O studies by Mayr et al. (2014[30]) showed 44 volatile compounds as being directly responsible for the aroma perception of Shiraz wines. None-the-less, a model wine reconstituting those 44 volatiles could not mimic the original one in its quality and all real wines were higher scored in 'overall fruit'. Synergistic, additive and antagonistic effects among the volatiles existing below their aroma detection thresholds, and their effect on the volatiles present above their detection thresholds, are presumably the causes. This example

shows the limit of GC-O analysis regarding the extrapolation to wine aroma profiles without respecting the volatile (Atanasova et al. 2005[31], Escudero et al. 2007[32], Pineau et al. 2009[33]) and non-volatile context (Pineau et al. 2007[34], Robinson et al. 2009[35], Saenz-Navajas et al. 2010[36]), which affects overall perceived flavour intensity and quality. In fact, the reconstitution studies conducted by Mayr et al. (2014[30]) showed that the omission of non-volatiles has a bigger effect on aroma perception than the absence of other odorants. The perception of 'chocolate' and 'dark fruit' flavour in Shiraz wine, for instance, increases with the absence of non-volatiles whereas the perception of 'green' and 'pepper' decreases. However, it is not clear which compounds of the non-volatile matrix can be held responsible for this particular effect. The study of the effects of the non-volatile matrix on the aroma perception of wine by Saenz-Navajas et al. (2010[36]) also showed a strong interaction effect. The aromatic perception of a defined set of volatiles changed dramatically while being introduced to different non-volatile matrices, to an extent that white wines can be mistakenly perceived as red wines (Saenz-Navajas et al. 2010[36]).

1.1.4 Non-volatile sensory contributors

Beside the influence on wine aroma, non-volatiles play a direct role in colour and mouthfeel perception of wines. In particular, polyphenols such as anthocyanins and tannins (i.e. polymeric flavan-3-ols, so called proanthocyanidins) essentially contribute to astringency, flavour and colour of wine (Dallas et al. 1995[37], De Freitas et al. 2000[38], Cheynier et al. 2005[39]). Starting with veraison, anthocyanins accumulate in the exocarp tissue of the berry, following a curve similar to the accumulation of sugar, such that during berry ripening, anthocyanin levels, and hence wine colour intensities, increase (Cadot et al. 2012[40]). It was shown that higher anthocyanin levels in grapes correlate strongly with final wine colour (Ristic et al. 2010[41], Mercurio et al. 2010[42], Gil et al. 2015[43]) and ageing capacity (through higher total phenolic concentration and more anthocyanin-tannin complexes) and are therefore associated with high wine quality scores (Somers and Evans 1974[44]). For those

reasons winemakers as well as consumers may assess the colour of wines for quality evaluation.

The correlation between grape phenolic composition and the final concentrations in wine or their impact on sensory is not yet fully understood. Higher anthocyanin contents in berries do not necessarily produce wines with deeper colour (Holt et al. 2008), which also depends on the presence of other compound group like flavonols (which enhance colour through favouring copigmentation) (Perez-Magarino and Gonzales-San Jose 2006[45]). The extractability of those compounds varies among winemaking procedures (Castillo-Sanchez et al. 2006[46]), which adds significant complexity to the causality-impact assessment. Another work by Romero-Cascales et al. (2005[47]) showed a poor relationship between increasing concentration in grape and final concentration in wines of not only anthocyanins, but also of skin tannins (which is illustrated in Table 7).

Table 7 Correlation among grape and wine variables (ApH1: anthocyanins extracted at pH1; ApH3.6: anthocyanins extracted at pH 3.6; EI: extractability index; TP (pH3.6): total phenol content of the solution at pH 3.6; SMI: seed maturity index) (Romero-Cascales et al. 2005[47])

	Wine color intensity	A^{acet}	A^{20}	A^{SO2}	TP_{wine}	Wine tannins
Grape anthocyanins[a] (mg/kg)	0.62*	0.03	-0.08	-0.25	-0.39	-0.48
ApH1[b]	0.64*	0.25	0.14	-0.01	-0.24	-0.60*
ApH3.6[b]	0.69**	0.86**	0.87**	0.85**	0.68**	0.10
EI[b]	-0.22	-0.67**	-0.77**	-0.88**	-0.92**	-0.63*
SMI[b]	-0.64**	-0.53*	-0.41	-0.23	0.11	0.69**
Total phenols (pH 3.6)[b]	0.04	0.34	0.47	0.63*	0.81**	0.77**
Skin tannins (mg/kg)	0.21	-0.25	-0.34	-0.45	-0.44	-0.11
Seed tannins (mg/kg)	0.05	0.15	0.28	0.43	0.71**	0.90**

[a]Sum of grape anthocyanins identified and quantified by HPLC.
[b]Parameters calculated according to Saint-Criq et al. (1998).
*, ** indicate significant correlation at the 95 and 99% confidence level, respectively.

Additionally, no clear trend among wines made from different berry sizes could be observed for individual anthocyanins or total flavonols and total proanthocyanidins just

differed significantly for small berries, not for large and medium sized ones (Gil et al. 2015[43]) (Table 8). This contradicts at least partially the anecdotal claim that wines made of smaller berries are richer in polyphenols though increased skin surface to volume ratio, and suggested reasons are differences in extractability as well as polymerisation of degradation reactions (Guidoni and Hunter 2012[48]). Similarly to the above point regarding interactions among volatiles, anthocyanins also experience complex interplays with other substances present in wine such as tannins, resulting in compositional changes leading to deeper and more stable colour (Perez-Magarino et al. 2006[45]).

Table 8 Effect of berry size on the composition of phenolic substances of the wines made from small (Ø<11.5 ±0.5 mm), medium (11.5<Ø<14.5 ± 0.5 mm) and large (Ø>14.5 ± 0.5 mm) and from control Cabernet Sauvignon berries. Reprinted from The Australian Journal of Grape and Wine Resarch, Vol 21, Gil, M., Pascual, O., Gómez-Alonso, S.; García-Romero, E., Hermosín-Gutiérrez, I., Zamora, F., Canals, J. M., *Influence of berry size on red wine colour and composition*, 200-211, Copyright (2015) by John Wiley Sons, Inc., with permission from John Wiley & Sons, Inc.

Wine		Large	Medium	Small	Control
Monomeric flavan-3-ols and proanthocyanidins					
Monomeric flavan-3-ols[a]	Racked	110.5 ± 0.7 a	100.6 ± 9.1 a	134.6 ± 13.0 b	131.4 ± 8.3 b
	Press	100.4 ± 14.8	113.9 ± 10.8	126.5 ± 13.6	88.05 ± 24.1
	Average	*107.1 ± 12.5 a*	*105.0 ± 2.6 a*	*131.9 ± 11.9 b*	*116.9 ± 2.6 a*
Total PA[b]	Racked	1414 ± 183 ab	1336 ± 55 a	1702 ± 157 b	1534 ± 128 ab
	Press	1192 ± 65	1348 ± 102	1460 ± 206	1337 ± 53
	Average	*1336 ± 103 a*	*1342 ± 50 a*	*1615 ± 142 b*	*1465 ± 87 ab*
mDP[c]	Racked	6.06 ± 0.18 b*	5.45 ± 0.07 a	6.08 ± 0.07 b	6.07 ± 0.16 b*
	Press	5.39 ± 0.02	5.42 ± 0.50	5.70 ± 0.27	5.67 ± 0.14
	Average	*5.82 ± 0.13 b*	*5.45 ± 0.21 a*	*5.94 ± 0.14 b*	*5.93 ± 0.09 b*
PD (%)[d]	Racked	20.10 ± 0.69 a	21.14 ± 0.38 ab	22.81 ± 0.26 b	22.45 ± 1.41 b
	Press	19.82 ± 0.35 a	21.30 ± 0.87 b	22.32 ± 1.02 b	21.83 ± 0.08 b
	Average	*20.00 ± 0.54 a*	*21.22 ± 0.32 b*	*22.64 ± 0.38 c*	*22.22 ± 0.96 bc*
Gal (%)[e]	Racked	8.15 ± 0.45 b	8.19 ± 0.30 b	6.40 ± 0.15 a	6.63 ± 0.25 a
	Press	8.91 ± 0.29 c	8.37 ± 0.25 b	6.86 ± 0.37 a	7.31 ± 0.12 a*
	Average	*8.42 ± 0.24 b*	*8.25 ± 0.26 b*	*6.56 ± 0.12 a*	*6.87 ± 0.18 a*

Tannins are extracted from skins and seeds during fermentation as a result of maceration, and are mainly associated with astringency and bitterness in wines (Bindon et al. 2014[49]). In a study on Cabernet Franc wines, both attributes were shown to increase with

later harvest dates (Cadot et al. 2012[40]). However, the correlation between perceived astringency and bitterness of wines and their tannin concentration was not evident, which implies the consideration of the tannin structure from both, skins and seeds. The overall tannin composition of final wines was found to be influenced by the harvest date (Cadot et al. 2012[40]) and higher tannin concentrations were found in wines made from mature grapes. But the total phenolic concentration in grapes seems to decrease with higher maturity (Bindon et al. 2013[4]), which suggests the influence of changing extractability with grape maturity. It was found that during ripening, tannin concentration in seeds declines while the concentration in skins increases (Bindon et al. 2013[4]). As seeds contain more tannins than skins, the total tannin concentration in berries declines despite the relative increase of skin tannin concentration, but the increasing extractability of those skin tannins means, a higher proanthocyanidin concentration can be observed in wines made from riper grapes (Cadot et al. 2012[40]). This is an important factor for winemakers regarding harvest decision because higher concentrations of total phenolics are strongly associated with an increase in quality perception and wine scores (Kassara et al. 2011[50]).

The increasing concentrations of proanthocyanidins in final wines made of riper fruit lead to higher perception of astringency (Kennedy et al. 2006). This is also due to structural changes of those proanthocyanidins like elevated mean degree of polymerisation (mDP) and polymerisation with the involvement of gallic acid (de Freitas and Mateus 2001[51], Chira et al. 2011[52]). This sets importance on the structures within the spectrum of phenolic composition.

1.1.5 Impact of ethanol levels on aroma and mouthfeel

Besides water, ethanol is a main constituent of wines and the main volatile compound, which content can vary between, for instance, 7% ABV to even 16% ABV or higher, dependent on the wine style. Hence it plays a significant role in the wine matrix influencing the aroma and flavour of wines due to its direct impact on the volatility of aroma

compounds (Goldner et al. 2009[53]). In fact, certain aroma perceptions disappear with increasing ethanol concentrations and constant volatile and non-volatile matrices. Escudero et al. (2007) showed, with a model mixture of esters, an intensity decrease of 'fruity' attribute (due to suppression of the esters) at increasing ethanol levels (0%, 10%, 12% and 14,5%). This effect could also be seen with real wines, in a study by Goldner et al. (2009[53]) with Malbec wines, where 'fruitness' perceived at low ABV levels shifted to 'herbaceous'. Ethanol shows as well significant alterations in wine flavour and mouthfeel, as for example 'sourness' perception weakens (Williams 1972[54]) and perceived 'sweetness' enhances (Nurgel and Pickering 2006[55]) with rising ABV. An important descriptor frequently associated with higher alcohol content is 'hotness'. Those are relationships winemakers have been concerned about since recent years since increasing ethanol levels have changed wine profiles, however there is no published direct evidence that consumers actually dislike the resulting wines. Several studies have rather observed that consumers are not able to significantly detect differences within small increases within 1 and 3 % ABV (King and Heymann 2014[56], Meillon 2010[57]). If ethanol reduction would not be necessary to increase wine qualities for customers, as they don't have any preference, it still would be feasible for the sake of tax savings and health benefits, as mentioned further in this text. This means that ethanol reducing technologies need to full fill the criteria to not decrease product quality and that respective research and development needs to be conducted.

1.1.6 Why are ethanol levels increasing

The past three decades have seen a trend towards increased alcohol content in wines as a result of winemakers seeking riper fruit flavours desired by consumers, which is particularly obvious in red wines (Godden and Muhlack 2010[58]). In addition to that, improvements in modern fungicides and application techniques has reduced the pressure of *Botrytis cinerea* giving more flexibility in harvest time and hence maturity levels (Edder et al. 2009[59]). The general increase in alcohol can also be ascribed to climate change, where

hotter weather leads to compressed vintages (Schultz and Jones 2010[60]) and winemaking logistics so that harvest delays are inevitable. Together with restrictions on irrigation, especially for varieties that are susceptible to berry shrivel such as Shiraz (as seen recently during the vintage 2015 in Shiraz and Cabernet Sauvignon vineyards in McLaren Vale, with estimated yield losses of up to 30% and high Brix levels (as resulting from the first winemaking of this project), these factors lead to increased sugar accumulation and subsequently to wines with higher alcohol. The increase of value, which winemakers expect with later harvests, should be critically tested against the volume loss due to berry shrivelling. Further, such wines are subjected to higher taxes in the export market and can be viewed negatively in terms of social and health concerns related to alcohol consumption (Pickering et al. 2000[61]).

1.1.7 Contribution of grape and berry heterogeneity to wine composition and ethanol levels

Vine development and physiology are predominantly influenced by the interplay of abiotic factors such as climate and soil (e.g., plant available water throughout the growing period depends on rainfall, evapotranspiration and water holding capacity of the respective soil type, which is directly linked to soil temperatures). Consequently, the accumulation of soluble solids and the volatile and non-volatile composition changes with temperatures, light exposure and water availability (van Leeuwen 2004[62]). Even plain alluvial soils can vary within a few metres in terms of soil type, water capacity and nutritive status. This heterogeneity is then apparent as well in the respective vineyard, where different pruning weights, leaf areas or phenologic development rates can be observed within a plot (King et al. 2014[63]). Managing this heterogeneity by replotting vineyards into homogenous units is suggested as a major approach for quality improvement in viticulture (Trought and Bramley 2011[64]). Research has generated innovative methods to analyse the heterogeneity, giving even more precise maps of vineyard characteristics assessing each plant by optical

chlorophyll measurements (Hall 2002[65]). This enables not only the precise application of irrigation and fertilisers but also the adjustment of pruning levels and yield on a vine-to-vine resolution.

Recent studies have focussed on berry heterogeneity within a bunch, where mechanical properties (firmness, size) are compared to quality attributes, i.e. Brix, water content (and implicit ratio of skin surface to berry volume) (Doumouya 2014[66]). It was found that maturity and softness of berries increased from basipetal towards apical bunch positions. This could bear a potential to decrease bunch heterogeneity and maybe ethanol levels. Instead of removing clusters during cluster thinning, cutting bunches in half, as it is frequently practiced in Europe, may delay overall maturity and give a more homogeneous profile of the berry population. The distribution of total soluble solids concentration, and hence maturity among a population of berries at ripeness, resembles a Gaussian bell shape (Figure 1), starting with a tail of unripe berries, followed by the mature berries and ending with a second tail representing overripe berries.
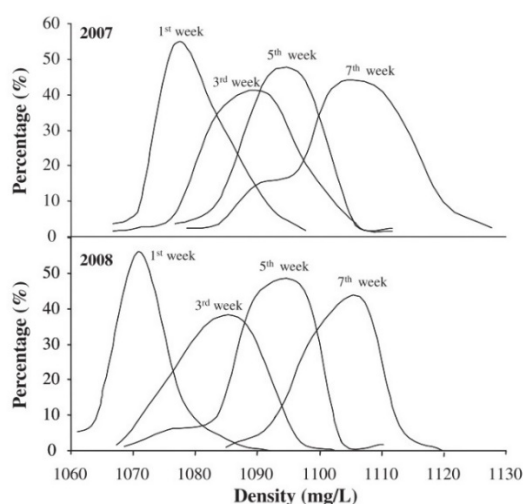

Figure 1 Distribution of grape densities throughout ripening (in weeks after veraison). Reprinted from Food Chemistry, Vol 124, Kontoudakis, N., Esteruelas, M., Fort, F.; Canals, J. M., De Freitas, V., Zamora, F., Influence of heterogeneity of grape phenolic maturity on wine composition and quality, 767-774, Copyright (2010) with permission from Elsevier.

The bell shape is likely to change throughout ripening describing different portions of individual ripening levels. Large proportions of underripe or overripe berries can have a negative impact on wine quality through contribution of 'green'/'herbaceous' or 'cooked'/'jammy' fruit attributes, respectively. Besides the impact on wine aroma, Kontoudakis et al. (2011[67]) have stated that the underripe tail can significantly increase the perception of bitterness and astringency in the wine through increasing the proportion of seed tannins. In order to minimise the impact of unripe aroma (mainly high methoxypyrazine or C6-alcohol concentrations), winemakers tend to delay harvest until all the berries are ripe, accepting that the main population might be shifted into over-ripeness. The resulting wines may consequently be higher in alcohol, pH and overripe fruit flavours and may not meet consumer preferences anymore. The wine industry is therefore interested to know to which extent the 'tails' on the ripeness continuum influence wine quality, and how this heterogeneity evolves throughout the ripening phase and across vintages, as this may offer clues to develop sorting and management approaches.

1.1.8 Current techniques used to decrease ethanol in wines

Several approaches are available in the industry to reduce alcohol levels in wines using physical and microbiological techniques before, during or post fermentation. Reducing the potential alcohol degree in a pre-fermentative state can be realised in several ways and basically consists of reducing the concentration of fermentable sugars. Picking the fruit at an earlier stage of ripeness can be seen as the most obvious method to do so. However picking fruit early may shift grape composition towards green and unripe attributes due to increases in methoxypyrazine, higher levels of C-6 alcohol or aldehyde precursors ('green apple', 'grass') (Kalua and Boss 2010[2]) and less anthocyanin concentration. The evolution of those substances behave differently among vintages, regions and cultivars, which makes it difficult for winemakers to choose the optimum harvest point for this approach without

compromising wine quality and style. Better knowledge of the factors influencing the evolution of those compounds for adaptation to individual vineyard sites is necessary to target certain wine styles and qualities. Therefore, blending approaches using water or partially dealcoholized wines to reduce the sugar concentration in grape juice is more popular in the wine industry (Pickering 2000[61]) as chemical and sensory properties are easier to determine and the resulting qualities are more predictable. Despite the advantages of simplicity and low cost, legal restrictions regarding water addition and the generally associated lower consumer acceptance for the resulting products limits the success of this method. Physical pre-fermentative methods may avoid exogenous additions into the product such as the procedure patented by (Lang and Casimir, 1986[68]) which divides the must into a high and a low sugar fraction through freezing which then can be treated separately, however this method is not common due to the severe impact on aroma quality.

The use of biochemical or microbiological techniques are other possibilities to reduce potential ethanol contents, for instance, the approach patented by (Kappeli 1989[69]) that takes advantage of a continuous yeast culture which converts sugar into CO_2 and water rather than ethanol. In future, the use of enzymes such as glucose oxidase, which lowers the alcohol by volume (ABV) of wines by up to 50% through converting glucose to gluconic acid (Villettaz 1987[70]), or the use of yeast with lower fermentation efficiencies that alter the carbon flux from ethanol to glycerol for instance (Schmidtke et al. 2012[71]), can be promising alternatives. Due to drawbacks foremost in the resulting wine qualities, the industrial application of pre-fermentative ethanol reduction methods is rather limited to low quality wines or so called wine cooler products (Pickering 2000[61]).

On the other hand, post-fermentative alteration of the ethanol concentration is more often implemented in the wine industry, using thermal based methods such as evaporators, distillation columns or spinning cone columns (SCC) (Pickering 2000[61]). A further kind of distillation based process involves a supercritical solvent extraction, where

the distillate consisting of mainly ethanol and volatiles is introduced to liquid carbon dioxide

under high pressure. A sudden pressure drop provokes a phase change of the carbon dioxide

to gaseous CO_2. The gas extracts the volatiles from the remainder which then can be

reintroduced into the wine (Schmidtke et al. 2011[71]). Continuous development and patenting

of the distillation and SCC equipment has improved the resulting product qualities through

lower temperatures and aroma recovery (Pickering 2000[61]). Other widely used processes are

based on membrane technologies, namely reverse osmosis, which enables the reduction of

alcohol to any degree desired, with the advantages of cool process temperatures and

reductive environment that helps preserve the product (Pickering 2000[61]). In comparison to

other physical methods stated above, wines treated with reverse osmosis show least

difference to regular wines (Schmidtke et al. 2011[71]).

1.1.9 Impact of the alcohol reduction processes on wine quality

Alcohol reduction of wines has frequently been associated with inferior product

quality in terms of aroma loss, alteration of mouthfeel and reduced viscosity (Schobinger

and Duerr 1983[72], Schobinger 1986[73], Howley and Young 1992[74]). However, those

references deal with the dealcoholisation of wine to levels of around 0.5% ABV and most

literature concentrates in improving process efficiencies rather than qualifying and

quantifying alterations in volatile and non-volatile composition of wines. In the context of

globally increasing alcohol levels in wines, the wine industry is mainly interested to

compensate those with a partial dealcoholisation of around 2% ABV, for instance. Only little

published material deals with this demand. But even though publications are mostly limited

to few specific alcohol reduction processes and sensory analysis without conducting detailed

volatile or non-volatile analysis, they give already a good understanding about the limits of

physical ethanol reduction methods. For instance, Fedrizzi et al. (2014[75]) investigated the

effect of osmotic distillation via membrane contactor on wine composition and observed an

important loss of esters for an alcohol reduction of 2% ABV, which is consistent with drops

of ethylacetate by 70% in a model wine observed by Varavuth et al. (2009[76]). Sensory analysis however showed no difference in the attribute 'fruity' or 'floral', but a significant depletion of body, persistence and honey scent Fedrizzi et al. (2014[75]). Similar results come from Lisanti et al. (2013[77]) who observed only a slight aroma loss at a 2% ABV reduction level, but almost 100 % of the volatile composition was lost at -5% ABV. Additionally there was a drop in sensory preference with decreasing ethanol levels. Interestingly, the effect on wine aroma at a reduction of 3% ABV depended on the initial ethanol level and less change in composition was noticed with a higher initial alcohol content. Gil et al. (2013[78]) worked on the effect of partial dealcoholisation by reverse osmosis, which, as stated above, is considered as having the least impact on wine quality. Sensory analysis showed that testers could distinguish the wines (Cabernet Sauvignon, Grenache and Carignan) reduced by 1% ABV and 2% ABV, but varietal differences were evident as the wines made of Cabernet Sauvignon were easier differentiated then the wines made from the other two cultivars. Ultimately, the testers preferred the non-treated control over the treated, alcohol reduced ones. The problem of interpreting the changed wine composition and correlate it to the sensory profile is that in fact two mechanisms overlap, the depletion of ethanol which is interacting with the volatile matrix of the wine, and the change of the matrix itself by loss of volatile compounds. Additionally, no work has investigated the eventual change in the non-volatile matrix and the consequence on the perception of the volatile matrix.

Poor quality management throughout all steps of wine production can contribute significantly to unsatisfactory sensory characteristics in alcohol reduced wines, such as the choice of low quality fruit sourced from overcropped vineyards, low in nitrogen and amino acid, or the introduction of faults during winemaking (Pickering 2000[61]). The trend of increasing quality awareness throughout the chain of production which the wine industry has experienced over the recent decades, along with technical improvements of dealcoholisation processes, have increased product quality. However, wine compositional alternations by

physical (aroma loss through evaporation or membranes, temperature impacts) or microbiological (variations of yeast metabolic pathways which not only reduce ethanol but increase undesirable attributes) are still reasons for the wine industry to search for alternatives, especially for premium 'terroir' wines. Where alcohol reduction techniques are already applied, cost reduction and environmental awareness may induce interest for innovative and easier to apply approaches to increase the overall value and possibly the quality of the product.

1.1.10 The concept of an early harvest regime to reduce ethanol content in wine

To decrease alcohol levels in wine, a recently proposed approach is expected to be a simple and easy-to-adopt strategy against the effects of warmer climates on high must sugar concentrations and therefore undesirably high alcohol contents in wines. This approach involves a sequential harvest regime. In a first step, grapes are harvested at an unripe stage resulting in a low alcohol wine. In a second step, this wine will be legitimately incorporated into the must/mash produced from the remainder of the fully ripened crop before fermentation. The early-harvest blending material, which is potentially qualitatively unfavourable, can also be subjected to different fining treatments and dealcoholisation in order to manipulate various compositional and quality aspects as required. The work by Kontoudakis et al. (2011[79]) tested this approach with the varieties Cabernet Sauvignon, Merlot and Bobal and found it to be suitable for partial reduction of alcohol contents. However, different qualititative responses among the cultivars imply further investigation on a variety basis with different target ethanol levels. For instance, testers could not distinguish between the control wine and the alcohol reduced wine in case of Cabernet Sauvignon of Merlot. This goes in hand with observations in the respective phenolic compositions, where no significant differences between the control and alcohol reduced wine was seen for proanthocyanin concentration and mDP levels (Table 9).

In case of Bobal, the quality difference was more evident. Bobal matures at higher Brix levels so the degree of ethanol reduction was more severe for this cultivar than for the other two. The Bobal was the wine least preferred by the testers, which implies a limit for the blending approach.

Table 9 HPLC analysis of total wine proanthocyanidins and related parameters following acid-catalysis in the presence of excess phloroglucinol. Reprinted from The Australian Journal of Grape and Wine Research, Vol 17, Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., Zamora, F., *Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine,* 230-239, Copyright (2011) by John Wiley Sons, Inc. with permission from John Wiley & Sons, Inc. H1: 1st harvest, carried out at potential ABV between 13 and 4%; H2: 2nd harvest at optimum phenolic maturity; RAH2: Blend of H2 and low ethanol wine to target H1 ABV levels; CH2: Control, straight vinification of H2.

Cultivar	Parameter	H1	H2	
			CH2	RAH2
Cabernet Sauvignon	Proanthocyanidins (mg/L)	703.6 ± 31.3^a	1104 ± 183.4^b	940.1 ± 197.8^b
	mDP	4.35 ± 0.09^a	6.54 ± 0.20^b	6.34 ± 0.14^b
	(+)-Catechin (%)	18.6 ± 0.4^a	12.4 ± 0.5^b	13.1 ± 0.4^b
	(−)-Epicatechin (%)	56.2 ± 1.5^a	54.6 ± 1.2^a	55.0 ± 0.7^a
	(−)-Epicatechin-3-O-gallate (%)	4.5 ± 0.1^a	4.8 ± 0.4^a	4.3 ± 0.2^a
	(−)-Epigallocatechin (%)	20.5 ± 0.9^a	28.2 ± 0.1^b	28.4 ± 0.7^b
Merlot	Proanthocyanidins (mg/L)	427.0 ± 115.5^a	1070.1 ± 17.2^b	969.9 ± 41.4^c
	mDP	2.72 ± 0.13^a	4.80 ± 1.84^b	4.43 ± 0.36^b
	(+)-Catechin (%)	26.2 ± 2.2^a	21.8 ± 1.4^b	19.0 ± 2.1^b
	(−)-Epicatechin (%)	57.8 ± 0.7^a	57.6 ± 0.1^a	57.6 ± 1.9^a
	(−)-Epicatechin-3-O-gallate (%)	4.4 ± 0.4^a	4.9 ± 0.2^b	5.7 ± 0.3^c
	(−)-Epigallocatechin (%)	11.6 ± 1.3^a	16.5 ± 0.6^b	17.7 ± 0.7^b
Bobal	Proanthocyanidins (mg/L)	761.4 ± 54.0^a	1648.3 ± 38.0^b	1573.5 ± 78.6^b
	mDP	6.60 ± 0.14^a	9.54 ± 0.30^b	8.77 ± 0.34^c
	(+)-Catechin (%)	18.9 ± 0.3^a	11.3 ± 0.6^b	13.2 ± 1.1^c
	(−)-Epicatechin (%)	54.5 ± 0.8^a	60.4 ± 0.3^b	57.5 ± 1.0^c
	(−)-Epicatechin-3-O-gallate (%)	3.2 ± 0.1^a	3.6 ± 0.3^a	3.6 ± 0.3^a
	(−)-Epigallocatechin (%)	23.3 ± 1.0^a	24.7 ± 0.5^b	25.6 ± 0.2^b

All data are expressed as the average of the three replicates ± standard deviation ($n = 3$). Statistical analysis: one-factor ANOVA and Scheffe's test (both $P < 0.05$). Different letters indicate the existence of statistically significant differences. CH2, control wines of H2; H1, first harvest; H2, second harvest; RAH2, reduced alcohol wines from H2.

In case for Cabernet Sauvignon, testers were actually not able to differentiate between the reduced alcohol wine and the wine made of earlier harvested grapes with a similar ABV. Consequently, for this variety an earlier harvest rather than enologic methods could be considered to lower the ethanol level. A profound investigation of the volatile and non-volatile composition at different ethanol levels is necessary to fully determine the effect on sensory quality. Different ethanol levels established by this method mean different levels of run-offs and low ethanol wine additions, giving distinct settings for the evolution of phenolic composition in the wine that may lead to further insight into their extraction behaviour. Harbertson et al. (2009[80]) for example observed different extraction efficiencies as a function of run-off and maceration time configurations and low levels of juice evacuation showed higher discrepancy between grape and skin tannin than high saignée application.

The work of Bindon et al. (2013[4]) investigated the effect of earlier ripening stages on grape and wine composition and sensory perception. Grapes harvested between 12 and 15.5% ABV were analysed and compared to identify key grape wine compounds associated with the character of Cabernet Sauvignon and consumer preferences. The sensory analysis showed no significant differences of wines harvested between 13% and 15.5% potential ABV. Consequently in this case, any efforts to use ethanol reducing technology would have been unnecessary if all the fruit was picked at 13% potential ABV, without compromising the quality of the reduced alcohol wine. This and other work dealing with sequential harvest and impact on compositional quality of grapes and wines such as Kalua and Boss (2009[81]) and (2010[2]) indicate that the concept of late harvest linked to better wine qualities will evolve and has to be revisited.

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Compared to the ethanol reducing technology mentioned above, the early harvest approach might have marketing advantages, as the products are not manipulated after fermentation and could be branded as a natural and genuine method To justify a profound research effort for optimisation of the blending approach described earlier, the approach should first be chemically and sensorially compared to wines made from grapes picked at different ripening stages. This may reveal the advantages and disadvantages of both methods regarding the reduction of ethanol levels in wines. Taking into consideration the complex behaviour of grape and wine composition, related research should be conducted over a minimum of two vintages with consistent data collection.

1.2   Research questions

The main questions of this project can be regarded as a continuation of the work of Kontoudakis et al. (2011[79]) described above, where the potential to reduce ethanol contents with the means of earlier harvests and subsequent blending has been shown. But with respect to the conclusion of Bindon et al. (2013[36]), where actually no sensory preference could be detected between 13% and 15% ABV in wines made of grapes with different maturity levels, the benefits of reducing ABV levels by blending compared to simply harvesting grapes earlier needs to be shown in order to justify this approach. As the volatile and non-volatile matrix of wines are likely to be significantly different among vintages, what can have an important impact on the qualitative and quantitative limits of ethanol reduction, harvest decisions may need specific adaptation for each vintage. So it remains to be investigated how much the vintage conditions actually interfere in the extent this approach can be used to reduce ethanol in wines. This also implies the need to involve detailed grape and wine composition analysis and sensory evaluation after the example of Bindon et al.

(2013[4]) in order to work out key compounds responsible for positive and negative qualitative impacts on wine to ultimately provide tools for winemakers for decision making. By my current knowledge, literature does not provide any direct comparison in the compositional matrix of wines made of earlier harvested grapes and wines made by blending low alcohol wines into high brix must/mash at different ABV levels. As interaction effects between volatiles and non-volatiles are still not comprehensively understood, the comparison of both approaches could give further insight in the mechanisms of mutual enhancements or reductions of aroma and flavour attributes.

Further, no work has been done so far to investigate the extent in which heterogeneity in berry maturity influences the final brix values of grape juice and ABV levels in wine. Knowledge about the evolution in Brix levels and sizes of a berry population can add to enhance decisions about harvest dates or sorting options and may be a further potential for alcohol reduction in wine.

1.3   Summary of research aims

The main objective of this project was to evaluate the pre-fermentative implementation of green harvest wines and water as approaches to manage red wine alcohol concentrations, while comparing the resulting wine qualities with wines made from earlier harvested grapes. We hereby aimed for covering a range of vintage conditions and two red cultivars of major industrial importance, which was realised as follows (listed in order of the respective manuscripts prepared):

1. Vintage 2015 represented conditions that elicited extreme grape ripening dynamics and berry shrivel. During this first project vintage, Cabernet Sauvignon grapes were picked at various earlier harvest dates, and lower alcohol wines were produced via the pre-fermentative juice substitution with a low alcohol green harvest wine as well as water, based on the overripe crop. Grape and wine non-volatile analysis were performed,

assessing the implications on colour, tannin and polysaccharide characteristics as elicited by harvest date and the juice substitution treatments.

2. Wines made from the 2015 vintage were further analysed for their wine volatile and sensory profiles, allowing for a final evaluation of the resulting wine qualities and respective alcohol management strategies.

3. Under more benign growing conditions with the absence of berry shrivel, the experimental design was reapplied in 2016, but encompassed both Cabernet Sauvignon and Shiraz cultivars to assess cultivar dependent implications on the qualites of the lower alcohol wines. Comprehensive assessments of grape and wine non-volatile characteristics as well as the analysis of wine volatile and sensory profiles were therefore conducted. The suitability of earlier harvests or blending approaches were further discussed in the context of the regulation changes (that applied late 2016) allowing for the pre-fermentative water addition.

4. Given the results from the 2015 and 2016 vintage trials, the third vintage trial in 2017 focussed on assessing the pre-fermentative implementation of water, either through juice substitution (as assessed in 2015 and 2016), as well as through simple dilution, therefore aiming to support informed decision making within the regulations for water addition. Experimental winemaking was conducted using Shiraz, given the importance for the wine industry and particular susceptibility for high alcohol levels. The adapted experimental design aimed for applying pre-fermentative water additions on crop from two distinct harvest dates ('Fresh Fruit' and 'Mature Fruit') to evaluate the effect of grape maturity on the wine qualities. Grape and wine non-volatile analysis as well as the wine sensory profiles were assessed, and results were discussed in the context of the preceding vintage trials.

1.6   References cited

1.   Ryona, I.; Pan, B. S.; Intrigliolo, D. S.; Lakso, A. N.; Sacks, G. L., Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *J. Agric. Food Chem.* **2008**, *56*, 10838-10846.

2.   Kalua, C. M.; Boss, P. K., Comparison of major volatile compounds from Riesling and Cabernet Sauvignon grapes (*Vitis vinifera* L.) from fruitset to harvest. *Aust. J. Grape Wine Res.* **2010**, *16*, 337-348.

3.   Harris, S. A.; Ryona, I.; Sacks, G. L., Behavior of 3-isobutyl-2-hydroxypyrazine (IBHP), a key intermediate in 3-isobutyl-2-methoxypyrazine (IBMP) metabolism, in ripening wine grapes. *J. Agric. Food Chem.* **2012**, *60*, 11901-11908.

4.   Bindon, K.; Varela, C.; Kennedy, J.; Holt, H.; Herderich, M., Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chem.* **2013**, *138*, 1696-1705.

5.   Ebeler, S. E., Analytical chemistry: Unlocking the secrets of wine flavor. *Food Rev. Int.* **2001**, *17*, 45-64.

6.   Wood, C.; Siebert, T. E.; Parker, M.; Capone, D. L.; Elsey, G. M.; Pollnitz, A. P.; Eggers, M.; Meier, M.; Vossing, T.; Widder, S.; Krammer, G.; Sefton, M. A.; Herderich, M. J., From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008**, *56*, 3738-3744.

7.   Siebert, T. E.; Wood, C.; Elsey, G. M.; Pollnitz, A. P., Determination of rotundone, the pepper aroma impact compound, in grapes and wine. *J. Agric. Food Chem.* **2008**, *56*, 3745-3748.

8.   Roujou de Boubee, D.; Van Leeuwen, C.; Dubourdieu, D., Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental

conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830-4834.

9.   Robinson, A. L.; Adams, D. O.; Boss, P. K.; Heymann, H.; Solomon, P. S.; Trengove, R. D., The relationship between sensory attributes and wine composition for Australian Cabernet Sauvignon wines. *Aust. J. Grape Wine Res.* **2011**, *17*, 327-340.

10.   Clarke, R. J.; Bakker, J., Volatile components. In *Wine Flavour Chemistry*, 1 ed.; Blackwell Publishing Ltd: Oxford, UK, 2004; pp 120-188.

11.   Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D., Varietal aroma. In *Handbook of Enology*, John Wiley & Sons, Ltd: West Sussex, UK, 2006; Vol. 2, pp 205-230.

12.   Fischer, U., Wine aroma. In *Flavours and fragrances: chemistry, bioprocessing and sustainability*, Berger, R. G., Ed. Springer-Verlag: Berlin Heidelberg, 2007; pp 241-267.

13.   Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S., Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139-173.

14.   Dennis, E. G.; Keyzers, R. A.; Kalua, C. M.; Maffei, S. M.; Nicholson, E. L.; Boss, P. K., Grape contribution to wine aroma: production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependant upon precursors in the must. *J. Agric. Food Chem.* **2012**, *60*, 2638-2646.

15.   Malcorps, P.; Dufour, J. P., Short-chain and medium-chain aliphatic-ester synthesis in *Saccharomyces cerevisiae. Eur. J. Biochem* **1992**, *210*, 1015-1022.

16.   Rojas, V.; Gil, J. V.; Pinaga, F.; Manzanares, P., Studies on acetate ester production by non-Saccharomyces wine yeasts. *Int. J. Food Microbiol.* **2001**, *70*, 283-289.

17.   Wondra, M.; Beroviv, M., Analyses of aroma components of chardonnay wine fermented by different yeast strains. *Food Technol. Biotechnol.* **2001**, *39*, 141-148.

18.   Perez-Prieto, L. J.; Lopez-Roca, J. M.; Gomez-Plaza, E., Differences in major volatile compounds of red wines according to storage length and storage conditions. *J. Food Compos. Anal.* **2003**, *16*, 697-705.

19.   Ugliano, M.; Bartowsky, E. J.; McCarthy, J.; Moio, L.; Henschke, P. A., Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J. Agric. Food Chem.* **2006**, *54*, 6322-6331.

20.   Loscos, N.; Hernández-Orte, P.; Cacho, J.; Ferreira, V., Evolution of the aroma composition of wines supplemented with grape flavour precursors from different varietals during accelerated wine ageing. *Food Chem.* **2010**, *120*, 205-216.

21.   Pérez-Coello, M. S.; Díaz-Maroto, M. C., Volatile Compounds and Wine Aging. In *Wine Chemistry and Biochemistry*, Moreno-Arribas, M. V.; Polo, M. C., Eds. Springer New York, 2009; pp 295-311.

22.   Garde-Cerdán, T.; Ancín-Azpilicueta, C., Review of quality factors on wine ageing in oak barrels. *Trends Food Sci. Technol.* **2006**, *17*, 438-447.

23.   Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Pons, M., The origin of ethylphenols in wines. *J. Sci. Food Agric.* **1992**, *60*, 165-178.

24.   Rapp, A., Natural flavours of wine: correlation between instrumental analysis and sensory perception. *Fresen. J. Anal. Chem.* **1990**, *337*, 777-785.

25.   Guth, H., Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027-3032.

26.   Ferreira, V.; López, R.; Cacho, J. F., Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659-1667.

27.   Maggu, M.; Winz, R.; Kilmartin, P. A.; Trought, M. C. T.; Nicolau, L., Effect of skin contact and pressure on the composition of Sauvignon Blanc must. *J. Agric. Food Chem.* **2007**, *55*, 10281-10288.

28.   Šuklje, K.; Lisjak, K.; Baša Česnik, H.; Janeš, L.; Du Toit, W.; Coetzee, Z.; Vanzo, A.; Deloire, A., Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon Blanc (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2012**, *60*, 9454-9461.

29.   Šuklje, K.; Antalick, G.; Coetzee, Z.; Schmidtke, L. M.; Baša Česnik, H.; Brandt, J.; du Toit, W. J.; Lisjak, K.; Deloire, A., Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 223-233.

30.   Mayr, C. M.; Geue, J. P.; Holt, H. E.; Pearson, W. P.; Jeffery, D. W.; Francis, I. L., Characterization of the Key Aroma Compounds in Shiraz Wine by Quantitation, Aroma Reconstitution, and Omission Studies. *J. Agric. Food Chem.* **2014**.

31.   Atanasova, B.; Thomas-Danguin, T.; Langlois, D.; Nicklaus, S.; Chabanet, C.; Etievant, P., Perception of wine fruity and woody notes: influence of peri-threshold odorants. *Food Qual. Pref.* **2005**, *16*, 504-510.

32.   Escudero, A.; Campo, E.; Farina, L.; Cacho, J.; Ferreira, V., Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**, *55*, 4501-4510.

33.   Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D., Examples of perceptive interactions involved in specific "red-" and "black-berry" aromas in red wines. *J. Agric. Food Chem.* **2009**, *57*, 3702-3708.

34.   Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D., Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103-4108.

35.   Robinson, A. L.; Ebeler, S. E.; Heymann, H.; Boss, P. K.; Solomon, P. S.; Trengove, R. D., Interactions between wine volatile compounds and grape and wine matrix

components influence aroma compound headspace partitioning. *J. Agric. Food Chem.* **2009**, *57*, 10313-10322.

36.   Sáenz-Navajas, M. a.-P.; Campo, E.; Culleré, L.; Fernández-Zurbano, P. n.; Valentin, D.; Ferreira, V., Effects of the Nonvolatile Matrix on the Aroma Perception of Wine. *J. Agric. Food Chem.* **2010**, *58*, 5574-5585.

37.   C. Dallas, J. M. R.-D.-S. a. O. L., Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation. *Vitis* **1995**, *34*, 51-56.

38.   V. A. P. de Freitas, Y. G. a. A. M., Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *51* **2000**, *4*.

39.   Cheynier, V.; Duenas-Paton, M.; Salas, E.; Maury, C.; Souquet, J. M.; Sarni-Manchado, P.; Fulcrand, H., Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.* **2005**, *57*, 298-305.

40.   Cadot, Y.; Caille, S.; Samson, A.; Barbeau, G.; Cheynier, V., Sensory representation of typicality of Cabernet Franc wines related to phenolic composition: Impact of ripening stage and maceration time. *Anal. Chim. Acta* **2012**, *732*, 91-99.

41.   Ristic, R.; Bindon, K.; Francis, L. I.; Herderich, M. J.; Iland, P. G., Flavonoids and C_{13}-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. *Aust. J. Grape Wine Res.* **2010**, *16*, 369-388.

42.   Mercurio, M. D.; Dambergs, R. G.; Cozzolino, D.; Herderich, M. J.; Smith, P. A., Relationship between red wine grades and phenolics. 1. Tannin and total phenolics concentrations. *J. Agric. Food Chem.* **2010**, *58*, 12313-12319.

43.   Gil, M.; Pascual, O.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I.; Zamora, F.; Canals, J. M., Influence of berry size on red wine colour and composition. *Aust. J. Grape Wine Res.* **2015**, n/a-n/a.

44.   Somers, T. C.; Evans, M. E., Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines. *J. Sci. Food Agric.* **1974**, *25*, 1369-1379.

45.   Perez-Magarino, S.; Gonzalez-San Jose, M. L., Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chem.* **2006**, *96*, 197-208.

46.   Castillo-Sanchez, J. J.; Mejuto, J. C.; Garrido, J.; Garcia-Falcon, S., Influence of wine-making protocol and fining agents on the evolution of the anthocyanin content, colour and general organoleptic quality of Vinhao wines. *Food Chem.* **2006**, *97*, 130-136.

47.   Romero-Cascales, I.; Ortega-Regules, A.; Lopez- Roca, J. M.; Fernandez-Fernandez, J. I.; Gomez-Plaza, E., Differences in anthocyanin extractability from grapes to wines according to variety. *Am. J. Enol. Vitic.* **2005**, *56*, 212-219.

48.   Guidoni, S.; Hunter, J. J., Anthocyanin profile in berry skins and fermenting must/wine, as affected by grape ripeness level of *Vitis vinifera* cv. Shiraz/R99. *Eur. Food Res. Technol.* **2012**, *235*, 397-408.

49.   Bindon, K.; Holt, H.; Williamson, P. O.; Varela, C.; Herderich, M.; Francis, I. L., Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chem.* **2014**, *154*, 90-101.

50.   Kassara, S.; Kennedy, J. A., Relationship between red wine grade and phenolics. 2. Tannin composition and size. *J. Agric. Food Chem.* **2011**, *59*, 8409-8412.

51.   de Freitas, V.; Mateus, N., Structural features of procyanidin interactions with salivary proteins. *J. Agric. Food Chem.* **2001**, *49*, 940-945.

52.   Chira, K.; Pacella, N.; Jourdes, M.; Teissedre, P.-L., Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chem.* **2011**, *126*, 1971-1977.

53.   Goldner, M. C.; Zamora, M. C.; Di Leo Lira, P.; Gianninoto, H.; Bandoni, A., Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *J. Sens. Stud.* **2009**, *24*, 243-257.

54.   Williams, A. A., Flavour effects of ethanol in alcoholic beverages. *The Flavour Industry* **1972**, *3*, 604-607.

55.   Nurgel, C.; Pickering, G., Modeling of sweet, bitter and irritant sensations and their interactions elicited by model ice wines. *J. Sens. Stud.* **2006**, *21*, 505-519.

56.   King, E. S.; Heymann, H., The effect of reduced alcohol on the sensory profiles and consumer preferences of white wine. *J. Sen. Stud.* **2014**, *29*, 33-42.

57.   Meillon, S.; Dugas, V.; Urbano, C.; Schlich, P., Preference and acceptability of partially dealcoholized white and red wines by consumers and professionals. *Am. J. Enol. Vitic.* **2010**, *61*, 42-52.

58.   Godden, P. a. M., Richard, Trends in the composition of Australian wine, 1984-2008. *Aust. N.Z. Grapegrower Winemaker* **2010**, 47-61.

59.   Edder, P.; Ortelli, D.; Viret, O.; Cognard, E.; de Montmollin, A.; Zali, O., Control strategies against grey mould (*Botrytis cinerea* Pers.: Fr) and corresponding fungicide residues in grapes and wines. Food Addit Contam Part A. **2009**, 26 *(5)*, 719-725.

60.   Schultz and Jones, Climate induced historic and future changes in viticulture. *J. Wine Res*. **2010,** *21, (2-3)*, 137-145.

61.   Pickering, G. J., Low- and reduced-alcohol wine: A review. *J. Wine Res.* **2000**, *11*, 129-144.

62.   van Leeuwen, C.; Friant, P.; Choné, X.; Tregoat, O.; Koundouras, S.; Dubourdieu, D., Influence of climate, soil, and cultivar on terroir. *Am. J. Enol. Vitic.* **2004**, *55*, 207-217.

63.   King, P. D.; Smart, R. E.; McClellan, D. J., Within-vineyard variability in vine vegetative growth, yield, and fruit and wine composition of Cabernet Sauvignon in Hawke's Bay, New Zealand. *Aust. J. Grape Wine Res.* **2014**, *20*, 234-246.

64.	Trought, M. C. T.; Bramley, R. G. V., Vineyard variability in Marlborough, New Zealand: characterising spatial and temporal changes in fruit composition and juice quality in the vineyard. *Aust. J. Grape Wine Res.* **2011**, *17*, 72-82.

65.	Hall, A.; Lamb, D. W.; Holzapfel, B.; Louis, J., Optical remote sensing applications in viticulture - a review. *Aust. J. Grape Wine Res.* **2002**, *8*, 36-47.

66.	Doumouya, S.; Lahaye, M.; Maury, C.; Siret, R., Physical and physiological heterogeneity within the grape bunch: Impact on mechanical properties during maturation. *Am. J. Enol. Vitic.* **2014**, *65*, 170-178.

67.	Kontoudakis, N.; Esteruelas, M.; Fort, F.; Canals, J. M.; De Freitas, V.; Zamora, F., Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. *Food Chem.* **2011**, *124*, 767-774.

68.	Lang, T. R.; Saimir, D. J., Low alcohol wine, European Patent Application EP 0 177 282 As. **1986**.

69.	Kappeli, O., Process for manufacture of sugar-free or low-sugar fruit juice, and fruit juice prepared by this process, Swiss Patent CH 668-887 AS. **1989**.

70.	Villettaz, J. C. Method for production of a low alcoholic wine US Patent-4675191. June 23 1987. US 4675191, 1987.

71.	Schmidtke, L. M.; Blackman, J. W.; Agboola, S. O., Production technologies for reduced alcoholic wines. *J. Food Sci.* **2012**, *77*, R25-R41.

72.	Schobinger, U. a. D., P., Diatische und sensorische Aspekte alkoholfreier Weine. *Alimenta* **1983**, 33-36.

73.	Schobinger, U.; Duerr, P.; Waldvogel, R., Die Entalkoholisierung von Wein und Fruchtweinen : Eine neue Moeglichkeit zur Herstellung von zuckerreduzierten Fruchtsaftgetraenken. *Schweizerische Zeitschrift fuer Obst- und Weinbau* **1986**, *122*, 98-110.

74.   Howley, M. a. Y., N. , Low alcohol wines: the consumers choice? *IJVM* **1992**, 45-46.

75.   Fedrizzi, B.; Nicolis, E.; Camin, F.; Bocca, E.; Carbognin, C.; Scholz, M.; Barbieri, P.; Finato, F.; Ferrarini, R., Stable isotope ratios and aroma profile changes induced due to innovative wine dealcoholisation approaches. *Food Bioprocess Technol* **2014**, *7*, 62-70.

76.   Varavuth, S.; Jiraratananon, R.; Atchariyawut, S., Experimental study on dealcoholization of wine by osmotic distillation process. *Sep. Purif. Technol.* **2009**, *66*, 313-321.

77.   Lisanti, M.; Gambuti, A.; Genovese, A.; Piombino, P.; Moio, L., Partial dealcoholization of red Wines by membrane contactor technique: Effect on sensory characteristics and volatile composition. *Food Bioprocess Technol* **2013**, *6*, 2289-2305.

78.   Gil, M.; Estevez, S.; Kontoudakis, N.; Fort, F.; Canals, J. M.; Zamora, F., Influence of partial dealcoholization by reverse osmosis on red wine composition and sensory characteristics. *Eur. Food Res. Technol.* **2013**, *237*, 481-488.

79.   Kontoudakis, N.; Esteruelas, M.; Fort, F.; Canals, J. M.; Zamora, F., Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Aust. J. Grape Wine Res.* **2011**, *17*, 230-238.

80.   Harbertson, J. F.; Mireles, M. S.; Harwood, E. D.; Weller, K. M.; Ross, C. F., Chemical and sensory effects of saignée, water addition, and extended maceration on high Brix must. *Am. J. Enol. Vitic.* **2009**, *60*, 450-460.

81.   Kalua, C. M.; Boss, P. K., Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2009**, *57*, 3818-3830.

Chapter 2

Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition

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Contribution to the Paper	Designed experiments, conducted vineyard monitoring, organised and executed grape harvests and experimental winemaking, conducted basic chemical measurements as well as grape and wine tannin and polysaccharide analysis, performed statistical analyses of the data sets (one-way ANOVA, repeated measures ANOVA, LSD means comparisons), interpreted the data, drafted and constructed the manuscript, revised the manuscript and addressed reviewer comments.
Overall percentage (%)	70
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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By signing the Statement of Authorship, each author certifies that:

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    ii.   permission is granted for the candidate in include the publication in the thesis; and

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Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition

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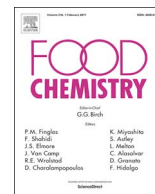
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A B S T R A C T

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.

1. Introduction

Warm and dry weather conditions during grape ripening have been characteristic of a range of viticultural regions in Australia and elsewhere, but the changing climatic conditions have imposed more challenging conditions on the wine industry. The trend of higher daily average temperatures during the vegetative period has led to accelerated phenological development of grapevines, confronting winemakers with increased berry sugar levels at harvest (Schultz & Jones, 2010). Decision-making regarding optimum harvest dates has become difficult as the ripening windows for distinct varieties now frequently overlap. This leads to peaks in harvest activity that may not be manageable in the winery, thereby exposing the unharvested part of the crop to berry shrivel and over-maturity (Suklje et al., 2016).

Furthermore, to account for the heterogeneity inherent in berry ripening, winemakers tend to delay harvest in the search of "flavour ripeness", minimising the contribution of unripe berries. This is of particular importance for the second-most widely grown grape cultivar in Australia, *Vitis vinifera* L. cv. Cabernet Sauvignon, with both "fruity"

and "green" attributes shaping the varietal aroma. The risk of berry shrivel is thus increased for the major proportion of the fruit, which can lead to higher wine alcohol concentrations and altered aroma and flavour profiles of the wines (Suklje et al., 2016). Simultaneously, winemakers may seek to achieve a higher level of grape tannin ripeness ("phenolic maturity") by extending grape maturation time, to minimise the impact of bitter seed tannins and maximise the proportion of skin tannins (Bindon, Varela, Kennedy, Holt, & Herderich, 2013; Heymann et al., 2013). However, the potential benefits of extended ripening in this context are not entirely clear, as few studies have investigated the different sensory properties of wines resulting from harvest dates chosen around an optimum ripeness state (Bindon et al., 2013; Lasanta, Caro, Gomez, & Perez, 2014). A 2014 study showed there was no significant differentiation in consumer liking of wines resulting from different harvest time points and containing 13%–15.5% alcohol by volume (ABV) (Bindon et al., 2014a). Indeed, it appears that the sensory quality of some Cabernet Sauvignon wines changes only marginally with different harvest dates after the grapes have passed a certain level of maturity (Heymann et al., 2013). Therefore, in the context of

compressed vintages and with a view to managing wine alcohol concentrations, the perceived benefit of extended ripening needs further examination.

Unmitigated increases of alcohol levels in wines due to the aforementioned reasons are not only problematic in terms of potential impact on product quality (perception of hotness and bitterness (Heymann et al., 2013)). Higher tax penalties that apply for exports above a certain % ABV, as well as the rising trend of consumers demanding wines with moderate alcohol, imply there are limitations in marketability. Hence, intervention by the winemaker is necessary to counterbalance excessive grape maturity with techniques that can decrease wine alcohol concentration. Pre-fermentative and fermentative approaches to lower the final alcohol content in wine are still limited due to associated quality losses (Longo, Blackman, Torley, Rogiers, & Schmidtke, 2017), and physical processes for dealcoholisation via spinning cone columns or reverse osmosis technologies (Longo et al., 2017) are appreciated by large-scale wineries for their low running costs and versatility. Smaller wineries, however, struggle to benefit from such technologies because of the initial costs of the equipment.

Due to the unpredictable nature of compressed vintages, where the occurrence and severity of heatwaves and harvest pressures may vary annually, winemakers are searching for easy-to-adopt, flexible and cost-effective alternatives to deal with overripe and/or shrivelled crops. An approach tested previously involves a sequential harvest regime, where a portion of the crop is harvested very early (at veraison, when berries start to soften and gain colour) and fermented to a low alcohol blending wine (hereafter defined as "green harvest wine", GHW) that can be incorporated into the wine produced from the overripe or shrivelled remainder (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011), or indeed into any wine that is undesirably high in alcohol. That study showed that the blending approach could be suitable for a partial decrease of alcohol concentration in wines, however different qualitative responses among cultivars (Cabernet Sauvignon, Merlot and Bobal) necessitate further investigation on a variety basis, with different targeted alcohol levels to test the limits for quality wine production. In addition, winemaking legislation in the US and changes to regulations more recently in Australia permit the pre-fermentative incorporation of water into high sugar must under certain conditions to facilitate yeast activity and enable sound fermentation dynamics.

Few studies (Harbertson, Mireles, Harwood, Weller, & Ross, 2009; Kontoudakis et al., 2011) have investigated the impact on wine composition following a manipulation of the pre-fermentative juice matrix via additions of water or GHW, particularly with respect to compositional changes in polyphenols, polysaccharides, volatiles and sensory quality. Water addition (either with an equivalent amount of juice removal [saignée] or simply must dilution) had little effect on polyphenol measures and sensory properties (aroma and flavour), in contrast to a higher ethanol wine that was characterised by having less fresh fruit flavour with a hot/dry mouthfeel (Harbertson et al., 2009). When blending with GHW, there were minimal impacts on polyphenols, a varietally-dependent effect on colour properties (mostly as a function of lower wine pH) and an inability to distinguish between wines from the same harvest stage using sensory assessment, except in the case of one varietal wine that had a greater amount of GHW added and was therefore more acidic (Kontoudakis et al., 2011). Nonetheless, no study investigated these methods in the context of severe berry shrivel and a direct comparison between the different pre-fermentative alcohol adjustment methods has yet to be reported. Thus additional information is required to provide winemakers with tools for adequate decision-making, especially in terms of using water as a means to manage wine alcohol levels.

Given the gaps in knowledge, this work was aimed at investigating the chemical composition resulting from pre-fermentative incorporation of GHW or water into Cabernet Sauvignon must, and evaluating the impact on quality of the resulting wines. To enable a comparison and discussion about potential benefits of each approach, the blended wines were compared to wines of similar targeted alcohol levels made from sequentially-harvested grapes. Berry ripening evolution was monitored and berry ripening heterogeneity was assessed to provide context regarding the vintage conditions and fruit characteristics.

2. Material and methods

2.1. Chemicals

Reagents and reference compounds used for analyses were purchased from Sigma Aldrich (Castle Hill, NSW, Australia) or Alfa Aesar (Ward Hill, MA, USA). Stock solution of standards were prepared volumetrically in redistilled ethanol and stored at $-20\,°C$, and working solutions were stored at $4\,°C$ until required. Analytical grade sodium chloride and HPLC grade solvents were sourced from Chem-Supply (Gillman, SA, Australia) and Merck (Kilsyth, Victoria, Australia), respectively. Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia) for all analyses, and filtered tap water was used for the water blending treatments. Ribose, deoxyglucose and 1-phenyl-3-methyl-5-pyrazolone (PMP) were purchased from Sigma Aldrich. Bentonite (SIHA Active Bentonite G, Eaton Filtration, New Jersey, USA) and activated carbon were purchased from Winequip (Adelaide, SA, Australia). Potassium metabisulfite was sourced from Vebigarden (Padua, Italy).

2.2. Climate data

Daily minimum, maximum and average temperatures, total monthly rainfall, and term averages (Table S1 of the Supporting Information) were sourced from the Bureau of Meteorology (weather station in Noarlunga, SA, at 138.5057°E, 35.1586°S) (Australian Government Bureau of Meteorology, 2017). The Huglin index for the vintage 2014/15 was calculated according to Tonietto and Carbonneau (2004).

2.3. Harvesting and winemaking

Vitis vinifera L. cv. Cabernet Sauvignon grapes were sourced from a commercial vineyard located in McLaren Vale, South Australia (138.521139°E, 35.194167°S). Around 200 kg of grapes were hand-picked on 8 January 2015 (further referred to as H0 or GHW) at approximately 50% veraison and with total soluble solids (TSS) of 8.1 °Brix (potential alcohol of 4.5% ABV) to produce GHW. Subsequent hand harvest of 70–80 kg took place on 3, 9 and 18 February 2015 (further referred to as H1, H2 and H3, respectively) with TSS of 20.5, 23.9 and 27.4 °Brix, respectively. Finally, 350 kg of grapes were hand-picked at commercial ripeness (22 February 2015, designated H4, 30.4 °Brix) and further processed to yield the control wine, a portion of which acted as the base wine for a series of blending treatments. Fig. S1 of the Supporting information outlines the experimental plan.

2.4. Green harvest wine

WIC Winemaking Services (Waite Campus, Urrbrae, SA, Australia) conducted the winemaking. Briefly, grapes were destemmed, crushed and directly pressed. After settling overnight the must was racked and inoculated with EC1118 yeast (Lallemand, Montreal, Canada), and thiamine was added to support fermentation. The winemaking involved applying a fermentation restart protocol (Lallemand, 2011). Once fermentation was complete (tested with a spectrophotometric enzymatic assay (Walker et al., 2014)) the wine was fined with 1 g/L charcoal and 1 g/L bentonite to ensure decolourisation and deodorisation and facilitate settling (Kontoudakis et al., 2011), settled overnight and racked. Potassium metabisulfite (PMS, 10% aqueous solution) was added at 100 mg/L to yield approximately 50 mg/L of total SO_2. The wine (approx. 100 L) was stored in stainless steel kegs at 0 °C until implementation of the blending treatments.

2.5. Consecutive harvest wines

Grapes from each consecutive harvest date (H1–H4, Table S2 of the Supporting information) were randomised, crushed, destemmed and 18–19 kg per ferment (in triplicate) were distributed in 20 L plastic buckets. PMS (100 mg/L) and diammonium phosphate (DAP, 200 mg/L) were added prior to inoculation with EC1118 yeast (Lallemand, Montreal, Canada, 0.3 g/L). Fermentation took place in temperature-controlled rooms at 24 °C. The cap was plunged twice a day (morning and evening) with concomitant TSS monitoring using a digital refractometer (Atago Pal-1). Final sugar content was tested by enzymatic assay (Walker et al., 2014) to ensure that wines had fermented to dryness (< 4 g/L). After seven days of maceration all wines were pressed off with a basket press into 10 L demijohns. Dry ice was used to minimise oxidation at all stages of winemaking. The wine was stored at 0 °C for stabilisation and conservation until bottling.

2.6. Blending treatment wines

Remaining grapes from H4 were processed as described for consecutive harvest wines. Prior to inoculation and in triplicate, proportions of the juice were substituted either with filtered water or with GHW in order to adjust sugar levels of the musts to target different final alcohol concentrations in the wines (matching as best as practicable between GHW, water addition and consecutive harvest wines). The substitution volume was determined with the following equation, as used previously (Kontoudakis et al., 2011).

$$\text{Substitution volume } (L) = Y \times \frac{(G2-G1)}{(G2-GH)}$$

where 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.

After the substitution treatments, winemaking continued as described above for the consecutive harvest wines.

At bottling, wine pH was adjusted to 3.7 (using 500 g/L aqueous tartaric acid solution) and 100 mg/L of PMS was added to all the treatments. Blending treatments yielded two series of wines: B1, B2, and B3 with 43.7%, 27.3% and 13.6% v/v, respectively, of juice substituted with GHW, and Bw1, Bw2 and Bw3 with 32%, 19.9% and 10.1% v/v, respectively, of juice substituted with water (Table S3 and Fig. S1 of the Supporting information).

2.7. Assessment of grape ripening and size variability

For each harvest date, 50–70 grape bunches were sampled and destemmed using precision snips to avoid removing the pedicels from the berries. One thousand berries were taken randomly in 10 batches, with 100 berries being distributed on a tray, and an image taken of each of the batches for subsequent image analysis. Then TSS of each berry was measured via a digital refractometer (expressed in °Brix) in a logical order to enable the relation of berry size and TSS. The images were analysed with Image J open source software using the canny edge detector plugin and cell magic wand tool.

2.8. Analysis of basic wine parameters

The pH and titratable acidity (TA, expressed as g/L equivalents of tartaric acid) were measured using the Mettler Toledo T50 Autotitrator, titrating to an endpoint of pH 8.2 with 0.33 M NaOH solution. Wine alcohol (ethanol) concentrations were determined with an alcolyser (Anton Paar, Graz, Austria). The concentrations of glucose, fructose, glycerol, and malic, tartaric, citric and acetic acids, were analysed by HPLC using the method reported previously (Li, Bindon, Bastian, Jiranek, & Wilkinson, 2017).

2.9. Extraction and isolation of grape and wine components

2.9.1. Wine-like tannin extraction

To estimate extractable tannin content, grapes were extracted according to a previous protocol (Bindon et al., 2014b). Berries (50 g ± 0.5 g) were weighed out in triplicate per harvest date and transferred into zip-lock plastic storage bags and crushed by hand. The must (solids and juice) obtained was transferred into 70 mL plastic screw-cap containers, using a spatula to ensure all berry contents were removed from each bag. Aqueous ethanol (15 mL of 40% adjusted to pH 3.4 with a 10 g/L aqueous tartaric acid solution) was added and the capped containers were gently shaken on their side at 60 rpm on a medium orbital shaker (EOM5, Ratek) for 40 h at room temperature (22 °C). Afterwards, the extracts were pressed through a 1 mm^2 sieve, and the extract volume recorded. The extracts were transferred into 50 mL tubes, centrifuged, and the supernatants were frozen at −80 °C until further analysis. Prior to analysis, the extracts were defrosted at room temperature (22 °C) with continuous mixing on a rotational shaker, without centrifugation.

2.9.2. Grape berry tannin and polysaccharide extraction

For the analysis of skin and seed tannin composition, and skin polysaccharide (cell wall) composition, triplicate samples of 50 frozen berries (−20 °C) were peeled and the skins and seeds were separately transferred to a liquid nitrogen-cooled mortar under liquid nitrogen and ground to a fine powder with a pestle. A sub-sample of 500 mg of either frozen skins or seeds, were transferred into 10 mL centrifuge tubes and extracted for 24 h at room temperature (22 °C) with 10 mL of 70% v/v aqueous acetone containing 0.01% trifluoroacetic acid (TFA), using a rotary suspension mixer (RSM7DC, Ratek). After centrifugation, the supernatant was collected and dried under nitrogen at 30 °C. Dried extract supernatants were reconstituted in 15% v/v ethanol containing 0.01% TFA prior to tannin analysis as described below. The acetone-insoluble pellets of the skin extracts were retained, frozen at −80 °C and the solvent removed under vacuum at −50 °C, after which the recovered dry weight was recorded.

2.9.3. Wine polysaccharide extraction

A 1 mL sample of wine was added to 5 volumes absolute ethanol in a 10 mL screw-cap centrifuge tube and maintained at 4 °C overnight. Precipitates were recovered by centrifugation at 2665 rcf for 5 min (Hettich Universal 32R centrifuge), resuspended in ice-cold 80% aqueous ethanol (wash step) and transferred to a fresh 1.5 mL screw-cap centrifuge tube. Tubes were centrifuged again at 2665 rcf for 5 min, and the supernatant was carefully decanted. The pellets were resuspended in water, frozen, and then freeze-dried (−50 °C) prior to analysis.

2.10. Analysis of tannins and wine colour

Reconstituted skin and seed extracts (15% v/v ethanol), wine-like extracts (extractable tannin), and wines were subjected to the methyl cellulose precipitable tannin assay (MCP tannin) to determine tannin concentration (Mercurio, Dambergs, Herderich, & Smith, 2007). Skin and seed tannin extracts were purified using Toyopearl HW-40 as previously described (McRae et al., 2015). Wine tannin was isolated by solid-phase extraction using a published protocol (Jeffery, Mercurio, Herderich, Hayasaka, & Smith, 2008) with a slight modification to collect tannins in one fraction (Kassara & Kennedy, 2011). Isolated grape and wine tannin were reconstituted in pure methanol and analysed by phloroglucinolysis to determine subunit composition using the method described previously (Kassara & Kennedy, 2011), which enabled calculation of the extraction ratio of tannin from skin and seed (Peyrot des Gachons & Kennedy, 2003). (−)-Epicatechin was used as a standard for quantification in both the MCP tannin and phloroglucinolysis assays. In addition, wine tannin size distribution (molecular mass, MM) was determined by gel permeation chromatography

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(GPC) using the methanolic solutions of purified extracts diluted 1:5 with the HPLC mobile phase prior to injection. Instrument parameters, chromatographic conditions and calibrations for GPC were according to the original method (Kennedy & Taylor, 2003) with the modifications described previously (Bindon, Bacic, & Kennedy, 2012). Wine colour density, anthocyanins and total phenolics were determined via the modified Somers colour assay (Mercurio et al., 2007).

2.11. Analysis of polysaccharides

2.11.1. Grape cell wall polysaccharides

A 10 mg sample of dried acetone-insoluble grape skin material was weighed into a 1.5 mL screw-cap centrifuge tube, and carefully covered with 100 μL of 12 M H_2SO_4, mixed and left to stand for 1 h at room temperature. The sample was diluted by addition of 1100 μL H_2O and hydrolysed for 3 h at 100 °C. The tubes were cooled on ice, centrifuged at 16.1 rcf and aliquots of the supernatant were neutralised with NaOH and diluted 5-fold with H_2O. Samples were mixed 1:1 with an aqueous internal standard solution containing 0.6 mM each of ribose and deoxyglucose. Mixtures were derivatised with PMP and the monosaccharides were quantified by HPLC using the method described previously (Ruiz-Garcia, Smith, & Bindon, 2014).

2.11.2. Wine polysaccharides

Material isolated from wine through precipitation in excess ethanol were dissolved in 300 μL of H_2O, and 100 μL was mixed with 100 μL of 4 M TFA and heated for 3 h at 100 °C. After cooling on ice, the tubes were centrifuged for 2 min at 16.1 rcf, and 200 μL of the supernatant was dried under vacuum (Centrivap concentrator) in a separate 1.5 mL centrifuge tube. The pellet was resuspended in 400 μL of water before being mixed 1:1 with internal standard solution, derivatised and analysed for monosaccharides as described above.

2.12. Data analysis

Means and standard errors were calculated from replicated experiments using Microsoft Excel. One-way analysis of variance (ANOVA) and repeated measures ANOVA were conducted to determine significant differences between treatments and means comparisons were performed by Fisher least significant difference (LSD) multiple comparison test at $p \leq 0.05$ (XLSTAT, version 2015.4.1, Addinsoft, Paris, France).

3. Results and discussion

3.1. Vintage conditions and ripening variability

The climatic conditions throughout the vegetative period were distinct from the long-term average for the McLaren Vale grape growing region in South Australia. The 2014/15 growing season in this area was classified as warm according to its Huglin index of 2416 units (Tonietto & Carbonneau, 2004). Average temperatures in September, October, November and February were considerably higher than the long term average (2000–2016) (Australian Government Bureau of Meteorology, 2017), and this was attributable to elevated minimum and maximum temperatures (Table S1 of the Supporting information). The months from August to December 2014 were particularly dry, and considering the likely higher evapotranspiration (Collatz, Ball, Grivet, & Berry, 1991), this resulted in abnormally low stem water potential values during the early ripening phase (data not shown), putting pressure on irrigation practices. Under these conditions berries had commenced shrivelling, and the ripening process deviated from the anecdotal weekly TSS increments of approximately 1.7 °Brix (potentially 1% ABV), with sugar concentrations increasing more rapidly. This was particularly notable with the increment of 3 °Brix in the last 4 days, culminating with a TSS of 30.4 °Brix (Table S2 of the Supporting
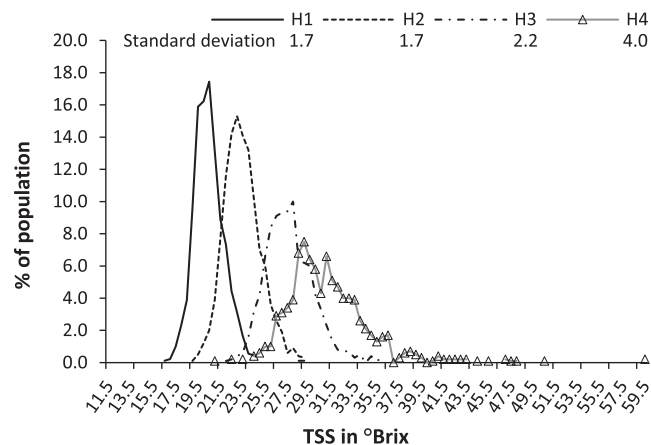
Fig. 1. Berry TSS distribution for H1–H4 and respective standard deviations, showing that as TSS values increase during ripening so too does the variance of those values (increasing heterogeneity).

information). Simultaneously, berry weight decreased and TA concentration increased as a consequence of the grape contents being concentrated due to berry shrivel. This exemplifies the very phenomenon of a compressed vintage due to accelerated ripening, where fruit would need to be picked earlier than projected, potentially resulting in higher TSS levels than desired (and thereby wines with increased alcohol levels).

The results of the TSS analysis of individual berry batches per harvest date are summarised in Fig. 1, showing the ripeness distribution of the berry population for H1–H4. Two observations were characteristic for this vintage: firstly, the proportional increase in the number of berries with high TSS from H3 to H4; secondly, the higher degree of ripeness variation at later harvest dates. Whereas ripeness heterogeneity in H1 and H2 was similar, indicating a relatively even increase in TSS within the berry population, variability increased from H2 to H4. This was concurrent with a steep decline in berry weight at H3 (Table S2 of the Supporting information), and a decrease in berry size determined by image analysis (Figs. S2 and S3 of the Supporting information), which likely resulted from an uneven onset (and progression) of berry shrivel. The formation of an overripe "tail", representing approximately 10% of the berry population with an average TSS higher than 35 °Brix, was evident for H4 (Fig. 1). This class of berries also consisted of the smallest berries that showed obvious signs of shrivel (Fig. S3 of the Supporting information). The tail was likely to contribute considerably to the excessive must TSS of 30.4 °Brix, resulting in 18.2% ABV in the respective wine (Table S2 of the Supporting information). This highlights the need for techniques to effectively deal with a crop such as this without introducing quality losses in the wine, while also achieving target alcohol contents of no more than 15% ABV.

3.2. Basic wine composition

Fermentation of the GHW became stuck at approximately 1.5 °Brix and a fermentation restart protocol (Lallemand, 2011) was executed successfully, resulting in H0 wine containing 4.5% ABV (Table S2 of the Supporting information). Yeast growth and activity were presumably inhibited due to the challenging conditions of the GHW fermentation, with its low pH of 2.76 possibly lowering the tolerance to yeast stressors like ethanol (Narayanan, Sànchez i Nogué, van Niel, & Gorwa-Grauslund, 2016) or medium-chain fatty acids (Fleet, 1990). Table S3 of the Supporting information shows the percentage of juice substituted with GHW (B1–B3) or water (Bw1–Bw3) along with the basic composition of the wines resulting from the treatments. Applying either a consecutive harvest regime or pre-fermentative blending treatment with GHW or water led to wines of lower alcohol content compared to the 18.1% ABV control wine (H4). Consecutive harvesting achieved

dramatic decreases in final alcohol, producing wines at 11.4, 13.4 and 15.1% ABV, whereas blending treatments yielded wines with approximately 14.5, 16, and 17% ABV (B1/Bw1, B2/Bw2 and B3/Bw3, respectively). Ethanol levels as produced in B2/Bw2, B3/Bw3 and H4 wines may have been expected to affect yeast viability, however no sluggish fermentation performance was observed with our choice of yeast strain. As anticipated (Kontoudakis et al., 2011), addition of GHW caused the pH to decrease considerably, in line with increases in malic acid concentrations, whereas pH values remained unchanged for the water blending treatments (Table S3 of the Supporting information). This demonstrates one potential benefit of using GHW wine as a blending material, leading to a lower requirement for tartaric acid to adjust must or wine pH values in comparison with water substitution, where acid addition would be necessary (as is the usual case in warmer grape growing regions) to ensure antimicrobial efficiency of added bisulfite (Jackson, 2008) or to enhance sensory quality (Jackson, 2008) and colour (Somers & Evans, 1974) in red wines. All wines fermented to dryness (i.e., < 4 g/L of total residual sugars), however residual fructose values were noticeably higher with higher initial must sugar concentrations (Tables S2 and S3 of the Supporting information), in line with the glucophilic nature of *Saccharomyces cerevisiae* (Berthels, Cordero Otero, Bauer, Thevelein, & Pretorius, 2004). Interestingly, the water blending treatments tended to slightly inhibit the fermentation efficiency of the yeasts compared to the GHW treatments. Acetic acid and glycerol values increased with the fruit ripeness levels and with higher initial must sugar concentrations among the blended wines. Furthermore, Bw1 and Bw2 wines contained significantly more glycerol than the B1 and B2 counterparts at similar initial must sugar concentrations and final alcohol levels (Table S3 of the Supporting information).

3.3. Changes of grape polysaccharide and phenolic composition during ripening

The analysis of total insoluble skin cell wall polysaccharides showed no statistical differences (p = 0.331) by harvest date (Table S4 of the Supporting information). Similar observations were made previously for Shiraz grapes (Vicens et al., 2009), where it was reasoned that skin polysaccharides are resistant to degradation and solubilisation to ensure their protective properties within the cell wall framework. In terms of absolute polysaccharide concentration, a significant decrease occurred from the first to the last harvest point only for xylose (from 16.15 to 12.13 mg/g of cell wall material, CWM) and galactose (from 20.21 to 16.71 mg/g CWM). Similar observations for galactose have been reported with decreasing values at later harvests (Bindon et al., 2013), but without the notable fluctuation seen here for H2 and H3. Rhamnose, galacturonic acid and arabinose concentrations also varied between harvest points and a downward trend was observed, but in general they were not statistically different.

In contrast to skin cell wall polysaccharides, the results of the grape tannin analyses (Table 1) showed more significant responses to different maturity states and the progressive berry shrivel. As determined by the MCP tannin assay of aqueous acetone extracts, skin tannin content per berry remained steady from H2 and seed tannin content per berry did not change significantly within the sampling period. Skin and seed tannin concentration expressed per g of fruit, however, showed an increase with later harvest dates, revealing a close relationship to the loss in berry fresh weight (Table S2 of the Supporting information). This was particularly evident for skin tannin concentration, which increased significantly from H3 to H4, whereas seed tannin concentration did not change after H3. To assess the impacts of ripening and shrivel on extractable grape tannin, a recently developed "wine-like" extraction protocol (Bindon et al., 2014b) was applied. The changes in tannin concentration of wine-like extracts of crushed berries reflected the increases observed for the skin and seed aqueous acetone extracts also determined by MCP tannin analysis (Table 1). However, the crushed

berry extracts are expected to better represent tannin extractability under winemaking conditions (Bindon et al., 2014b) by approximating the conditions of the grape matrix, i.e., tannin losses due to adsorption to grape cell walls or proteins (hence removal), or enhancement of tannin extraction exerted by anthocyanins (Bindon, Kassara, & Smith, 2017). The data for extractable tannin can be presented in two ways, either as volume-corrected, which accounts for potential differences in juice yield on a mg/g basis, or as uncorrected, which assumes a constant juice yield for a set mass of grapes. The initial study reporting this assay reported no difference in the grape to wine relationship (R^2 values > 0.9) using the respective formulas (Bindon et al., 2014b). The current results showed a two-fold increase in extractable tannin concentration (expressed as mg/g) was observed from H1 to H4, increasing continually (p < 0.02) when the uncorrected measure was applied, which was a steeper increase than the increment observed in skin tannin concentration. When considered using the volume-corrected measure, however, the trend in extractable tannin increase from H1 to H4 was not significant (p = 0.072), but nonetheless showed a reasonable trend. Under our experimental conditions, differences in berry weight (and hence berry volume and juice yield) during the ripening and subsequent shrivel stages were an important consideration; the implications of this are discussed later, in terms of their correlation with the corresponding wine tannin concentrations. Interestingly, on a per berry basis, the extractable tannin seemed to have reached a maximum early in the season such that no further extraction (under wine-like conditions) was evident from H1 onwards, indicating that the effect was primarily berry volume-dependent in this instance.

3.4. Effect of harvest date and shrivel

3.4.1. Wine polysaccharide composition

Polysaccharides are of interest from a wine quality perspective as they are expected to contribute to the perception of 'fullness' and decrease astringency (Vidal et al., 2004). They interact with tannins that cause the sensation of astringency and either inhibit their aggregation, as observed with mannoproteins (MP) or acidic arabinogalactan-proteins, or encourage the formation of larger colloids as in the case of rhamnogalacturonan II (RG-II) dimers (Riou, Vernhet, Doco, & Moutounet, 2002). Our work showed a continuous increase of total polysaccharides from H1 (598 mg/L) to H3 (823 mg/L) (Table 2), in line with previous work that showed increases for polysaccharides rich in arabinose and galactose (PRAG) in wines made from more mature fruit (Gil et al., 2015), and proposed to be as a consequence of pectolytic enzyme activity facilitating pectic polysaccharide release (Cabanne & Doneche, 2001). Polysaccharide increases in the current study from H1 to H3 were principally due to increases in grape-derived galacturonic acid (from 246 mg/L in H1 to 325 mg/L in H3) and neutral monosaccharides such as rhamnose (from 26 to 48 mg/L), arabinose (from 72 to 112 mg/L) and galactose (from 78 to 122 mg/L), and, to a lesser extent, yeast MPs as indicated by an increase in mannose from 109 to 147 mg/L. However, the molar percentages of the monosaccharides within the total polysaccharide concentration from H1 to H3 remained steady, indicating that the earlier stages of ripening were associated with a net increase in total wine polysaccharides (Table 2).

Previous work found contrasting results in their consecutive harvest trial of Cabernet Sauvignon, particularly in terms of grape-derived polysaccharides (Bindon et al., 2013). In that work, as absolute polysaccharide concentrations in wines declined with progressive ripening levels, the proportions of grape-derived polysaccharides like rhamnose, arabinose or glucose also decreased. However, similar to the current results, mannose increased in wines from later harvest dates. Differences between the study of Bindon et al. (2013) and the present one may be attributable to the contrasting ranges in grape maturity levels, hence wine alcohol contents, among other possible factors such as those relating to environmental effects or vineyard practices, and it is likely that in the current context, overripe conditions and berry shrivel helped

Chapter 2   Impact on polysaccharide and tannin content and composition

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Table 1

Grape tannin compositional parameters for consecutive harvest dates (H1–H4).[a]

	Harvest time			
	H1	H2	H3	H4
Extractable tannin[b]				
mg/g berry (vol. corrected)[b]	0.22 ± 0.05	0.27 ± 0.02	0.29 ± 0.06	0.32 ± 0.06
mg/g berry (vol. uncorrected)[b]	0.29 ± 0.04c	0.44 ± 0.01bc	0.52 ± 0.07ab	0.67 ± 0.06a
mg/berry	0.22 ± 0.05	0.25 ± 0.03	0.25 ± 0.05	0.20 ± 0.03
Skin tannin				
mg/g berry[c]	2.33 ± 0.21c	2.83 ± 0.04b	3.09 ± 0.1b	3.45 ± 0.09a
mg/berry[c]	2.38 ± 0.22b	2.50 ± 0.03ab	2.58 ± 0.07ab	2.68 ± 0.09a
Seed tannin				
mg/g berry[c]	5.06 ± 0.08b	5.85 ± 0.61ab	6.44 ± 0.46a	6.36 ± 0.16a
mg/berry[c]	5.17 ± 0.08	5.18 ± 0.54	5.39 ± 0.45	4.94 ± 0.07

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within a row are significantly different ($p \leq .05$, one way ANOVA).

[b] Determined by MCP tannin assay of wine-like extracts for crushed berries, adjusted for relative differences in juice volume (vol. corrected) or unadjusted (vol. uncorrected).

[c] Determined by MCP tannin assay of aqueous acetone extracts for skin and seed.

to provoke different outcomes in terms of wine polysaccharides. Thus, for a clearer understanding of the relative influence of the physiological changes associated with grape ripening on wine polysaccharide composition, further research that accounts not only for seasonal and localised vineyard influences, but also for winemaking approach, is required.

The wine arising from the last (overripe) harvest point (H4) showed a dramatic decrease in total soluble polysaccharide concentration compared to H3, such that it closely resembled H1 (Table 2). This decline was mainly driven by absolute and proportional losses in galacturonic acid which, along with a lower concentration of rhamnose, may be suggestive of a lower RG-II concentration. Arabinose and galactose concentrations also decreased although their relative proportions increased, suggesting a higher contribution of PRAGs to total polysaccharides in H4 relative to the earlier harvest points.

A decrease of acidic polysaccharides and an increase in MPs with delayed harvest has been reported previously (Bindon et al., 2013), albeit across an extended ripening period, whereas our data shows marked changes in wine polysaccharide composition within four days, from H3 to H4. It is important to note that this was also the time when berry shrivel became more apparent, raising the prospect that pectin degradation ensued, thereby impacting wine polysaccharide

composition. Pectinase activity has been investigated in relation to post-ripening dehydration (Vicens et al., 2009; Zoccatelli et al., 2013), but it has not been considered in terms of winemaking outcomes. Severe post-harvest berry shrivel has been associated with an increase in pectin methylesterase and polygalacturonase activities that resulted in an important decline of skin pectin concentration, but apparently in a variety-dependent manner (Zoccatelli et al., 2013). In the case of the observed decline in acidic polysaccharides in wine H4, increased activity of grape pectinases that are transferred into the juice may have led to degradation of pectic polysaccharides that were already released, although the potential that *in situ* degradation occurred in the intact berry cannot be excluded.

3.4.2. Wine phenolic composition

Phenolics measures provide information from which important red wine mouthfeel and colour properties can be inferred. Table 3 shows results related to the phenolic composition of the wines from consecutive harvests. Anthocyanin concentration showed a steep increase at an early ripening stage (from H1 to H2) with only small (non-significant) increases from H2 to H4, similar to previous findings (in the absence of berry shrivel) (Bindon et al., 2013). This suggests that grape anthocyanin content and extractability had approached their peaks at

Table 2

Wine polysaccharide composition presented as monosaccharide units for consecutive harvest dates (H1–H4).[a]

	Harvest time			
	H1	H2	H3	H4 (Control)
[mg/L]				
Total polysaccharides	598 ± 122b	716 ± 26ab	823 ± 20a	581 ± 28b
Rhamnose	26.0 ± 5.1c	37.0 ± 1.7b	47.6 ± 1.8a	36.2 ± 1.2b
Fucose	3.37 ± 1.09c	7.09 ± 0.68b	9.64 ± 0.96a	5.45 ± 0.8b
Arabinose	71.9 ± 13.2c	98.6 ± 2.4b	112.49 ± 7.42a	92.54 ± 2.1b
Xylose	3.35 ± 1.31	2.2 ± 0.25	2.46 ± 0.62	2.12 ± 0.51
Mannose	109.41 ± 25.04b	131.01 ± 5.55ab	147 ± 5a	152 ± 7a
Galactose	78.47 ± 15.2b	102.83 ± 2.88ab	122.04 ± 1.41a	95.7 ± 31.7ab
Glucose	59.41 ± 29.19	51.37 ± 5.6	57.17 ± 5.83	64.59 ± 4.24
Galacturonic acid	246 ± 36b	287 ± 9ab	325 ± 10a	132 ± 3c
[mol%]				
Rhamnose	4.46 ± 0.07c	5.18 ± 0.07b	5.91 ± 0.23a	6.16 ± 0.12a
Fucose	0.63 ± 0.08c	1.12 ± 0.08ab	1.33 ± 0.11a	1.03 ± 0.15b
Arabinose	15.1 ± 0.4c	17.1 ± 0.2b	17.0 ± 1.3b	19.2 ± 0.9a
Xylose	0.67 ± 0.14a	0.38 ± 0.03b	0.37 ± 0.09b	0.44 ± 0.09b
Mannose	18.9 ± 0.5b	18.9 ± 0.4b	18.4 ± 0.2b	26.3 ± 1.9a
Galactose	13.7 ± 0.3c	14.8 ± 0.1b	15.3 ± 0.2b	19.6 ± 0.6a
Glucose	9.77 ± 3.04	7.39 ± 0.54	7.16 ± 0.61	11.2 ± 1.1
Galacturonic acid	36.9 ± 2.9a	35.1 ± 0.8a	34.6 ± 0.5a	19.4 ± 1.4b

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within a row are significantly different ($p \leq 0.05$, one way ANOVA).

Table 3
Wine phenolic and colour measures for consecutive harvest dates determined 3 months and 18 months (colour measures only) after bottling.[a]

	Harvest time			
	H1	H2	H3	H4 (Control)
3 months				
Total anthocyanins (mg/L)	481 ± 5b	754 ± 50a	821 ± 31a	838 ± 76a
SO_2-resistant pigments (au)	1.14 ± 0.04d	1.97 ± 0.11c	3.02 ± 0.05b	4.59 ± 0.14a
Wine colour density (au)	8.56 ± 0.04c	14.96 ± 0.97b	18.69 ± 0.87a	21.50 ± 0.68a
Total phenolics (au)	31.0 ± 1.7c	47.2 ± 3.9b	55.4 ± 1.0b	61.2 ± 0.7a
Wine tannin [mg/L][b]	713 ± 63d	1050 ± 265c	1528 ± 245b	2145 ± 58a
Tannin MM [g/mol][c]	1630 ± 105c	1508 ± 25d	1722 ± 3b	1811 ± 32a
[%] of skin extraction[d]	65.9 ± 1.6	69.6 ± 2.9	67.3 ± 2.3	68.7 ± 2.6
[%] of seed extraction[d]	34.1 ± 1.6	30.4 ± 2.9	32.7 ± 2.3	31.3 ± 2.6
18 months				
Total anthocyanins (mg/L)	265 ± 15d	393 ± 41c	437 ± 17b	461 ± 8a
SO_2-resistant pigments (au)	1.83 ± 0.07d	3.51 ± 0.2c	6.06 ± 0.46b	7.81 ± 0.5a
Wine colour density (au)	6.70 ± 0.19d	12.01 ± 0.78c	17.13 ± 0.60b	20.62 ± 1.01a
Total phenolics (au)	27.7 ± 1.4d	39.3 ± 3.8c	45.5 ± 1.7b	53.2 ± 1.1a

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA or repeated measures ANOVA for analyses conducted again at 18 months).

[b] Determined by MCP tannin assay.

[c] MM, molecular mass determined by gel permeation chromatography at 50% elution.

[d] Calculated using the ratio of [%] epigallocatechin in extension units from skin and wine tannins, determined by phloroglucinolysis.

an earlier time point in our study and no further changes were induced by the effect of berry shrivel (i.e. a concentration of berry contents) when delaying harvest. Constant anthocyanin levels have been described previously during extended maturation, although in that case without onset of berry shrivel (Perez-Magarino & Gonzalez-San Jose, 2006). In contrast, wine colour density, total phenolics and SO_2-resistant pigments in our study increased throughout the four harvest stages, as a result of underlying differences in a range of phenolics contributing to these properties of the wines.

Wine tannin concentrations increased significantly (p = 0.0003) in the wines made from consecutive harvest points, with a particularly steep increment from H3 to H4 (Table 3, 3 months). A strong correlation was observed between wine tannin concentration and both the volume-corrected and uncorrected measures of extractable tannin per gram berry weight, with R^2 values of 0.95 and 0.97, respectively, obtained using linear regression analysis. This observation supports the suitability of the wine-like extraction protocol (Bindon et al., 2014b) to estimate final wine tannin concentrations. However, a similarly strong relationship between wine tannin concentration and acetone-extracted skin tannin concentration was also found (R^2 = 0.97). Such an increase in wine tannin concentrations at later harvest stages has been observed previously, potentially as a result of increased berry tannin concentrations due to shrivel (volume decrease) or due to accumulation within the berry (independent of volume change) (Bindon et al., 2013; Suklje et al., 2016). In our study, berry shrivel is likely to be the primary reason for the elevated tannin concentration, but extraction could have been further facilitated by the increased concentration of alcohol (Table S2 of the Supporting information, H3 = 15.1% and H4 = 18.2% ABV) or other as yet unknown factors.

The evolution of wine tannin MM by GPC followed the trend observed by others (Bindon et al., 2014a), increasing in wines produced from riper grapes (Table 3). Based on the phloroglucinolysis results of the grape skin tannin extracts, the relative proportions of skin and seed tannin extracted from grape into wine were calculated (Peyrot des Gachons et al., 2003), and did not change significantly throughout the consecutive harvest regime. This also likely indicated the dominance of the shrivel effect in inducing the tannin concentration increase, as opposed to relative differences in tannin extractability from the respective grape components.

As they are important measures of red wine ageing potential, colour analyses were repeated after 18 months of bottle ageing to investigate their evolution, whereupon a significant differentiation between

harvest points was observed (Table 3). According to repeated measures ANOVA, the concentrations of anthocyanins (which decreased by about 50% across the board relative to 3 months) were now statistically significantly different (p = 0.00015) and higher for the wines made from later harvest points, with the highest anthocyanin concentration associated with H4. There was approximately a doubling of SO_2-resistant pigments over the ageing period, with the trend following that of anthocyanins (higher for later harvest dates). Wine colour density also started to become more differentiated based on harvest time, with later harvests being higher in colour density, and displaying less of a decrease compared to the values at 3 months, most likely reflecting the greater amounts of anthocyanins remaining in those wines. An important conclusion from these parameters is the indication of higher ageing potential for the wines from later harvests, although wine H1, and especially H2, still had colour parameters that would be consistent with red wine of respectable quality (based on the positive relationship between wine colour density and quality rating reported by Somers and Evans (1974)).

3.5. Impact of blending treatments

3.5.1. Wine polysaccharide composition

Other than the mouthfeel effects described earlier, medium molecular mass polysaccharides could be responsible for altering the perception of hotness and viscosity (Gawel, Smith, & Waters, 2016), which is a factor to consider in the context of warm climate winemaking and managing wine alcohol. The effect of blending treatments on wine polysaccharides was therefore investigated, in parallel to the early harvest regime, to evaluate the effect of initial alternation of the must matrix due to removing must (along with associated colloids, polysaccharides, polyphenols, enzymes) and adding either GHW (containing 4.5% ABV ethanol, organic acids, etc.) or water (effectively diluting the initial must composition).

Total polysaccharide concentrations in wines increased significantly compared to the control (H4) when implementing the GHW (Table 4). Interestingly, despite the large differences in the proportion of juice substituted with GHW (43.7% v/v in B1 vs. 13.6% v/v in B3, Table S3 of the Supporting information) and uncertainty surrounding introduction of polysaccharides with GHW, the polysaccharide concentrations were not statistically different within this blending series. Blending with water at the highest rate (32% v/v in Bw1, Table S3 of the Supporting information) resulted in the highest total polysaccharide

Table 4
Monosaccharide composition of polysaccharides isolated from the blending treatments (GHW addition for B1–B3, water addition for Bw1–Bw3) and the control wine (H4).[a]

	Blending treatment						
	H4 (Control)	B1	B2	B3	Bw1	Bw2	Bw3
[mg/L]							
Total polysaccharides	581 ± 28c	706 ± 49b	717 ± 51b	683 ± 9b	859 ± 20a	514 ± 75 cd	480 ± 7d
Rhamnose	36.2 ± 1.2bc	39.9 ± 4.8b	42.7 ± 3.5b	40.5 ± 0.4b	51.8 ± 0.7a	32.9 ± 5.4c	31.3 ± 1.9c
Fucose	5.45 ± 0.8d	12.6 ± 1.4b	6.88 ± 1.83 cd	8.14 ± 0.27 cd	16.3 ± 0.51a	8.73 ± 1.66c	9.11 ± 0.68c
Arabinose	92.5 ± 2.1b	95.8 ± 7b	101 ± 8b	98.4 ± 2.1b	124 ± 2a	73.9 ± 11.5c	74 ± 1.7c
Xylose	2.12 ± 0.51c	3.09 ± 0.32ab	3.75 ± 0.81a	3.21 ± 0.36ab	3.89 ± 0.29a	2.38 ± 0.25bc	2.04 ± 0.35c
Mannose	153 ± 7a	137 ± 8ab	136 ± 11ab	147 ± 9a	133 ± 6ab	117 ± 21bc	105 ± 3c
Galactose	95.7 ± 31.7ab	104 ± 8ab	111 ± 9ab	113 ± 4ab	120 ± 2a	87.2 ± 13.5b	85.9 ± 2.4b
Glucose	64.6 ± 4.2bc	73.3 ± 4b	73.0 ± 6.1b	75.8 ± 2.3b	96.7 ± 13.9a	52.3 ± 2.1c	51.3 ± 6.8c
Galacturonic acid	133 ± 3d	240 ± 20b	242 ± 14b	197 ± 7c	313 ± 12a	140 ± 23d	121 ± 4d
[mol%]							
Rhamnose	6.16 ± 0.12abc	5.8 ± 0.34c	6.01 ± 0.08abc	5.94 ± 0.03bc	6.1 ± 0.06abc	6.38 ± 0.18ab	6.48 ± 0.47a
Fucose	1.03 ± 0.15b	2 ± 0.08a	1.07 ± 0.25b	1.33 ± 0.04b	2.13 ± 0.08a	1.88 ± 0.27a	2.1 ± 0.18a
Arabinose	19.2 ± 0.9a	16.7 ± 0.5d	17.4 ± 0.3 cd	17.5 ± 0.3 cd	17.8 ± 0.2bc	17.4 ± 0.2 cd	18.6 ± 0.7ab
Xylose	0.44 ± 0.09b	0.54 ± 0.07ab	0.63 ± 0.09a	0.57 ± 0.07ab	0.56 ± 0.03ab	0.57 ± 0.05ab	0.51 ± 0.08ab
Mannose	26.3 ± 1.9a	19.8 ± 0.2c	19.4 ± 0.5c	21.8 ± 1b	15.9 ± 0.4d	22.9 ± 0.8b	22.0 ± 0.4b
Galactose	19.6 ± 0.6a	15.1 ± 0.6ef	15.9 ± 0.4de	16.7 ± 0.4 cd	14.3 ± 0.3f	17.1 ± 0.2bc	18 ± 0.7b
Glucose	11.2 ± 1.1	10.7 ± 0.7	10.4 ± 0.3	11.3 ± 0.4	11.5 ± 1.5	10.5 ± 1.5	10.7 ± 1.3
Galacturonic acid	19.4 ± 1.4e	29.5 ± 0.8b	29.3 ± 0.8b	24.8 ± 1.1c	31.7 ± 1.3a	23.2 ± 0.6 cd	21.6 ± 0.5d

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA).

concentration but in contrast to GHW blends, polysaccharide concentrations were similar for Bw2 and Bw3 and lower than the H4 control. It may be that the substitution of juice with water or GHW diluted the initial colloid concentration, thereby altering the diffusion coefficient of polysaccharides from skin tissues during the early stages of fermentation, which potentially led to the observed increases compared to the control. The increases were mainly caused by increments in rhamnose, galacturonic acid, arabinose and galactose (Table 4), which are monosaccharide residues that can be ascribed to grape-derived polysaccharides.

Compositional analysis showed considerable variation of these grape-derived polysaccharides among the blending treatments (Table 4). Higher substitution of juice with water or GHW led to higher molar proportions and absolute concentrations of galacturonic acid, noting that this effect was more pronounced within the water blends. Compared to the H4 control, all GHW substitution rates caused a higher galacturonic acid proportion and concentration. Within the water treatments, this effect was only seen in Bw1, which had the highest amount of polysaccharide-associated galacturonic acid among the blending series treatments. The results therefore suggest that the substitution rate and the nature of the blending component had a significant effect on the content and composition of grape-derived polysaccharides in the subsequent wines. More specifically, the water treatments seemed to benefit both the extraction and retention of galacturonans and PRAGs more so than the GHW treatments at the high substitution rate.

MPs released by yeasts during fermentation and autolysis are desirable for improving stability and mouthfeel properties of wines (Escot, Feuillat, Dulau, & Charpentier, 2001; Vidal et al., 2004). A previous study (Bindon et al., 2013) showed that the concentration of these mannose-rich glycoproteins may be related to increased yeast turnover during fermentation of higher TSS musts, hence the concentration of MPs increases with higher initial must sugar levels. In our study, the concentration of polysaccharide-derived mannose did not change significantly with different GHW blending proportions compared to the H4 control (Table 4), as well as when compared to H2 and H3 at comparable alcohol levels (Table 2). Interestingly, water incorporation lowered the mannose concentration in Bw2 and Bw3 relative to the control and the GHW treatments. Although the reasons were not entirely apparent, MP concentration differences as a function of the blending material and the substitution rate could potentially be

ascribed to differences in yeast metabolism induced by differences in the fermentation medium. A decrease in suspended grape solids, and in turn a lower must sterol content with water addition relative to GHW addition, may have negatively impacted yeast viability (Delfini, Cocito, Ravaglia, & Conterno, 1993).

3.5.2. Wine colour and tannin composition

There were no statistically significant effects on wine colour density (p = 0.939), total anthocyanin concentration (p = 0.726) and total phenolics (p = 0.805) among the blending treatments, in comparison to the H4 control wine. However, SO_2-resistant pigments decreased in the GHW blended wines compared to the control, particularly in B1, whereas water decreased the SO_2-resistant pigments only in Bw1. However, after 18 months of bottle ageing the level of SO_2-resistant pigments was not significantly different among the control and the blended wines (p = 0.786). These outcomes are particularly important because red wines undergo a period of ageing before being released for sale. The results show promise for wine ageing capability (in terms of the red wine quality parameters measured) when lowering the alcohol content of wines via juice substitution. In addition, compared to the H4 control wine, tannin concentrations did not change with the blending treatments (Table 5) regardless of the substitution rate (p = 0.180) and in general, no differences were apparent among the treatments. In terms of managing wine alcohol without impacting quality, our observations support a blending approach to lower the alcohol level of wine without a significant impact on tannin concentrations.

The impact of tannin on the flavour and mouthfeel properties of wines also depends on the chemical characteristics of the tannin itself, e.g., apparent extent of polymerisation and subunit composition (Bindon et al., 2014a). Subunit data in turn give an indication of the extraction ratio of skin and seed tannins (Peyrot des Gachons et al., 2003). Assessment of the contribution of skin or seed tannin to the wine showed that a higher and a lower proportion of skin tannin was associated with B2 and Bw2, respectively, accounting for the corresponding divergence in MM. However, in general only minor differences between the blending treatments were seen with MM; indeed, only the tannins retained in the water substitution wines Bw1 and Bw2 were of higher MM compared to the control. Whereas skin tannin is more readily extracted, seed tannin extraction is known to be facilitated by higher ethanol concentrations but is also dependent upon the hydration status of the seed itself and does not proceed until after a minimum period of

Table 5

Tannin composition and colour of blending treatment wines determined 3 months and 18 months after bottling.[a]

	Blending treatment						
	H4 (Control)	B1	B2	B3	Bw1	Bw2	Bw3
3 months							
Total anthocyanins (mg/L)	838 ± 76	809 ± 22	788 ± 13	835 ± 23	822 ± 32	792 ± 31	871 ± 36
SO_2-resistant pigments (au)	4.59 ± 0.14a	3.62 ± 0.16e	4.07 ± 0.13bc	4.23 ± 0.14bc	3.85 ± 0.14 cd	4.31 ± 0.17ab	4.54 ± 0.03a
Wine colour density (au)	21.5 ± 0.7	21.1 ± 1.4	22.1 ± 1.8	20.5 ± 1.2	20.7 ± 0.9	20.8 ± 1.2	20.4 ± 2.7
Total phenolics	61.3 ± 0.7	60.6 ± 3.4	63.7 ± 5	61.5 ± 1.7	60.8 ± 0.9	60.6 ± 4.7	64.1 ± 0.4
Wine tannin [mg/L][b]	2144 ± 58ab	2142 ± 205ab	2335 ± 73a	2004 ± 34b	2120 ± 139ab	2041 ± 495b	1996 ± 83b
Tannin MM (g/mol)[c]	1810 ± 32 cd	1816 ± 71 cd	1852 ± 32c	1885 ± 38bc	2054 ± 14a	1989 ± 95ab	1898 ± 21bc
[%] of skin extraction[d]	68.7 ± 2.6ab	69.9 ± 2.9ab	71.7 ± 4.3a	67.9 ± 3.1ab	65.2 ± 3.3b	66.8 ± 1.6b	68.5 ± 2.1ab
[%] of seed extraction[d]	31.3 ± 2.6ab	30.1 ± 2.9ab	28.3 ± 4.3b	32.2 ± 3.1ab	34.8 ± 3.3a	33.2 ± 1.6a	31.6 ± 2.1ab
18 months							
Wine colour density	20.6 ± 1	19.6 ± 1.4	20.7 ± 0.5	20.2 ± 1.1	19.6 ± 0.7	20.3 ± 0.9	20.3 ± 1.4
Total anthocyanins mg/L	461 ± 8	463 ± 30	488 ± 46	494 ± 36	438 ± 14	461 ± 48	470 ± 8
Total phenolics	53.2 ± 1.1	52.7 ± 2.8	55.7 ± 5.3	55.6 ± 3.3	52.5 ± 1.8	54.3 ± 3.3	54.1 ± 1.3
SO_2-resistant pigments (au)	7.8 ± 0.5	7.4 ± 0.5	8.3 ± 0.3	7.6 ± 0.6	7.6 ± 0.4	8 ± 1.1	7.6 ± 0.8

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA or repeated measures ANOVA for analyses conducted again at 18 months).

[b] Determined by MCP tannin assay.

[c] MM, molecular mass determined by gel permeation chromatography at 50% elution.

[d] Calculated using the ratio of [%] epigallocatechin in extension units from skin and wine tannins, determined by phloroglucinolysis.

exposure to the must/wine (Hernández-Jiménez, Kennedy, Bautista-Ortín, & Gómez-Plaza, 2012). As such, it is not immediately evident why higher levels of water addition may have facilitated seed tannin extraction. Nevertheless, the differences in tannin concentration and composition between the blending treatments were minor, and unlikely to impact significantly on sensory outcomes.

4. Conclusions

In the context of compressed vintages, winemakers seek easy-to-adopt solutions to manage alcohol levels resulting from high TSS levels of fruit. Diluting the sugar concentration with low alcohol (green harvest) wine has been previously proposed and the effects on wine non-volatile composition have been thoroughly investigated in our study. Furthermore, a recent change to winemaking regulations in Australia has allowed for the pre-fermentative addition of water to adjust high must sugar concentrations, and this study has provided a timely investigation of the effects of such a manipulation. Given the legislative changes affecting the Australian (and American) wine industries, and in the context of global climate change, it is likely that such developments in regulation will be of interest in other viticultural regions, and the present study could support future endeavours to adapt the proposed alcohol management techniques in their respective region and cultivar contexts. The blending approaches in the presented study were compared to a consecutive harvest regime to produce wines with naturally (and substantially) lower alcohol content (by up to 7% ABV) in a challenging season that saw rapid onset of berry shrivel later in the harvest period. Examination of berry population heterogeneity revealed increases in variability both for berry size and TSS with later harvest dates: at the last harvest time point around 10% of the berries constituted an overripe "tail" that had an average TSS of more than 35 °Brix, thereby substantially impacting overall must TSS and final wine alcohol content.

For the harvest series grapes, tannin per gram of berry increased in line with harvest date and in relation to berry shrivel, and tannin became more extractable. A strong correlation between the concentrations of wine-like extractable grape tannin and final wine tannin was revealed. Delaying the harvest date produced wines that were higher in total anthocyanins and extractable tannins, among other measures such as wine colour that constitute important indicators of red wine quality. Significant differences in wine colour remained after 18 months of ageing in bottle, as a result of the augmented phenolic profile in the

later harvest wines that afforded better ageing potential. Nonetheless, wines from earlier harvest dates were not necessarily lacking in terms of their colour properties.

In contrast to the harvest series, basic wine quality measures including total anthocyanins, wine colour density and total phenolics did not change significantly among the blending treatments, and SO_2-resistant pigments decreased only at the highest substitution rates for water or GHW incorporations. However, after 18 months of bottle ageing these differences were no longer apparent, suggesting no major impact of the treatments on wine composition occurred compared to the control. Both the blending component and proportion of incorporation had a minor impact on tannin and polysaccharide composition, with the effects being associated with increases in polysaccharide concentrations or tannin MM rather than losses of these important macromolecules. Particularly interesting was the apparent absence of dilution effects when using water, which enabled easy moderation of alcohol levels without significantly changing the wine chemistry compared to the control. This could be particularly interesting for winemakers as water is much more convenient than using GHW, and the latter is associated with additional costs and effort. An investigation of the effects of these treatments on wine sensory properties and volatile composition is underway.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.10.024.

References

Australian Government Bureau of Meteorology, Monthly climate statistics: Noarlunga. http://www.bom.gov.au/climate/averages/tables/cw_023885_All.shtml (19 December 2016).

Berthels, N. J., Cordero Otero, R. R., Bauer, F. F., Thevelein, J. M., & Pretorius, I. S. (2004). Discrepancy in glucose and fructose utilisation during fermentation by *Saccharomyces cerevisiae* wine yeast strains. *FEMS Yeast Research, 4*(7), 683–689.

Bindon, K. A., Bacic, A., & Kennedy, J. A. (2012). Tissue-specific and developmental modifications of grape cell walls influence the adsorption of proanthocyanidins. *Journal of Agricultural and Food Chemistry, 60*(36), 9249–9260.

Bindon, K., Holt, H., Williamson, P. O., Varela, C., Herderich, M., & Francis, I. L. (2014a). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry, 154*, 90–101.

Bindon, K. A., Kassara, S., Cynkar, W. U., Robinson, E. M. C., Scrimgeour, N., & Smith, P. A. (2014b). Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in *Vitis vinifera* L. cv. Shiraz and Cabernet Sauvignon grapes. *Journal of Agricultural and Food Chemistry, 62*, 4558–4570.

Bindon, K. A., Kassara, S., & Smith, P. A. (2017). Towards a model of grape tannin extraction under wine-like conditions: The role of suspended mesocarp material and anthocyanin concentration. *Australian Journal of Grape and Wine Research, 23*(1), 22–32.

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry, 138*(1), 1696–1705.

Cabanne, C., & Doneche, B. (2001). Changes in polygalacturonase activity and calcium content during ripening of grape berries. *American Journal of Enology and Viticulture, 52*(4), 331–335.

Collatz, G. J., Ball, J. T., Grivet, C., & Berry, J. A. (1991). Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: A model that includes a laminar boundary layer. *Agricultural and Forest Meteorology, 54*(2), 107–136.

Delfini, C., Cocito, C., Ravaglia, S., & Conterno, L. (1993). Influence of clarification and suspended grape solid materials on sterol content of free run and pressed grape musts in the presence of growing yeast cells. *American Journal of Enology and Viticulture, 44*(4), 452–458.

Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeasts and the influence of released polysaccharides on colour stability and wine astringency. *Australian Journal of Grape and Wine Research, 7*(3), 153–159.

Fleet, G. H. (1990). Growth of yeasts during wine fermentations. *Journal of Wine Research, 1*(3), 211–223.

Gawel, R., Smith, P. A., & Waters, E. J. (2016). Influence of polysaccharides on the taste and mouthfeel of white wine. *Australian Journal of Grape and Wine Research, 22*(3), 350–357.

Gil, M., Quiros, M., Fort, F., Morales, P., Gonzalez, R., Canals, J. M., & Zamora, F. (2015). Influence of grape maturity and maceration length on polysaccharide composition of Cabernet Sauvignon red wines. *American Journal of Enology and Viticulture, 66*(3), 393–397.

Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K. M., & Ross, C. F. (2009). Chemical and sensory effects of saignee, water addition, and extended maceration on high brix must. *American Journal of Enology and Viticulture, 60*(4), 450–460.

Hernández-Jiménez, A., Kennedy, J. A., Bautista-Ortín, A. B., & Gómez-Plaza, E. (2012). Effect of ethanol on grape seed proanthocyanidin extraction. *American Journal of Enology and Viticulture, 63*(1), 57–61.

Heymann, H., LiCalzi, M., Conversano, M. R., Bauer, A., Skogerson, K., & Matthews, M. (2013). Effects of extended grape ripening with or without must and wine alcohol manipulations on cabernet sauvignon wine sensory characteristics. *South African Journal of Enology and Viticulture, 34*(1), 86–99.

Jackson, R. S. (2008). Postfermentation treatments and related topics. *Wine science: Principles and applications* (pp. 418–519). San Diego: Academic Press.

Jeffery, D. W., Mercurio, M. D., Herderich, M. J., Hayasaka, Y., & Smith, P. A. (2008). Rapid isolation of red wine polymeric polyphenols by solid-phase extraction. *Journal*

of Agricultural and Food Chemistry, 56(8), 2571–2580.

Kassara, S., & Kennedy, J. A. (2011). Relationship between red wine grade and phenolics. 2. Tannin composition and size. *Journal of Agricultural and Food Chemistry, 59*(15), 8409–8412.

Kennedy, J. A., & Taylor, A. W. (2003). Analysis of proanthocyanidins by high-performance gel permeation chromatography. *Journal of Chromatography A, 995*(1–2), 99–107.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Australian Journal of Grape and Wine Research, 17*(2), 230–238.

Lallemand, Good practices for restarting a stuck fermentation. http://www.lallemandwine.com/wpcontent/uploads/2013/09/Restarting-Stuck-Fermentation.pdf (22 May 2017).

Lasanta, C., Caro, I., Gomez, J., & Perez, L. (2014). The influence of ripeness grade on the composition of musts and wines from *Vitis vinifera* cv. Tempranillo grown in a warm climate. *Food Research International, 64*, 432–438.

Li, S., Bindon, K., Bastian, S. E., 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.

Longo, R., Blackman, J. W., Torley, P. J., Rogiers, S. Y., & Schmidtke, L. M. (2017). Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *Journal of the Science of Food and Agriculture, 97*(1), 8–16.

McRae, J. M., Day, M. P., Bindon, K. A., Kassara, S., Schmidt, S. A., Schulkin, A., ... Smith, P. A. (2015). Effect of early oxygen exposure on red wine colour and tannins. *Tetrahedron, 71*(20), 3131–3137.

Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007). High throughput analysis of red wine and grape phenolics – Adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. *Journal of Agricultural and Food Chemistry, 55*(12), 4651–4657.

Narayanan, V., Sànchez i Nogué, V., van Niel, E. W. J., & Gorwa-Grauslund, M. F. (2016). Adaptation to low pH and lignocellulosic inhibitors resulting in ethanolic fermentation and growth of *Saccharomyces cerevisiae*. *AMB Express, 6*(1), 59.

Perez-Magarino, S., & Gonzalez-San Jose, M. L. (2006). Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chemistry, 96*(2), 197–208.

Peyrot des Gachons, C., & Kennedy, J. A. (2003). Direct method for determining seed and skin proanthocyanidin extraction into red wine. *Journal of Agricultural and Food Chemistry, 51*(20), 5877–5881.

Riou, V., Vernhet, A., Doco, T., & Moutounet, M. (2002). Aggregation of grape seed tannins in model wine – Effect of wine polysaccharides. *Food Hydrocolloids, 16*(1), 17–23.

Ruiz-Garcia, Y., Smith, P. A., & Bindon, K. A. (2014). Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydrate Polymers, 114*, 102–114.

Schultz, H. R., & Jones, G. V. (2010). Climate induced historic and future changes in viticulture. *Journal of Wine Research, 21*(2–3), 137–145.

Somers, C. T., & Evans, M. E. (1974). Wine quality: Correlations with colour density and anthocyanin equilibra in a group of young red wines. *Journal of Science Food Agriculture, 25*, 1369–1379.

Suklje, K., Zhang, X., Antalick, G., Clark, A. C., Deloire, A., & Schmidtke, L. M. (2016). Berry shriveling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical composition. *Journal of Agricultural and Food Chemistry, 64*(4), 870–880.

Tonietto, J., & Carbonneau, A. (2004). A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest Meteorology, 124*(1–2), 81–97.

Vicens, A., Fournand, D., Williams, P., Sidhoum, L., Moutounet, M., & Doco, T. (2009). Changes in polysaccharide and protein composition of cell walls in grape berry skin (cv. Shiraz) during ripening and over-ripening. *Journal of Agricultural and Food Chemistry, 57*(7), 2955–2960.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry, 85*(4), 519–525.

Walker, M. E., Nguyen, T. D., Liccioli, T., Schmid, F., Kalatzis, N., Sundstrom, J. F., ... Jiranek, V. (2014). Genome-wide identification of the Fermentome; genes required for successful and timely completion of wine-like fermentation by *Saccharomyces cerevisiae*. *BMC Genomics, 15*(1), 552.

Zoccatelli, G., Zenoni, S., Savoi, S., Dal Santo, S., Tononi, P., Zandonà, V., ... Tornielli, G. B. (2013). Skin pectin metabolism during the postharvest dehydration of berries from three distinct grapevine cultivars. *Australian Journal of Grape and Wine Research, 19*(2), 171–179.

SUPPORTING INFORMATION FOR

Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition

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Table S1. Climatic conditions near the McLaren Vale vineyard[a] during growing season 2014/2015 comparing minimum (Ø Temp Min), maximum (Ø Temp Max) and average (Ø Temp) temperatures, and precipitation sums ($\sum$ Rainfall) to the long term values (2000-2016, in parentheses). Bold numbers indicate values above (for temperature) or below (for rainfall) the average values.

month	Ø temp min	Ø temp max	Ø temp	$\sum$ rainfall
Jul '14	**8.9 (+0.2)**	14.6 (-0.2)	11.8 (0.0)	67.6 (+5.4)
Aug '14	7.8 (-1.1)	**16.3 (+0.4)**	12.1 (-0.4)	**4.6 (-44.7)**
Sep '14	**11.2 (+0.8)**	**20 (+1.3)**	**15.6 (+1.1)**	**26.8 (-18.3)**
Oct '14	**12.4 (+1.0)**	**24.8 (+3.4)**	**18.6 (+2.2)**	**3.4 (-27.6)**
Nov '14	**14.4(+0.3)**	**25.4 (+0.5)**	**19.9 (+0.4)**	**16.6 (-5.8)**
Dec '14	14.8 (-0.4)	25.3 (-1.1)	20.1 (-0.7)	**8.8 (-10.5)**
Jan '15	16.6 (-0.5)	27 (-1.7)	21.8 (-1.1)	38.4 (+21.3)
Feb '15	**17.7 (+0.8)**	**30.3 (+2.4)**	**24 (+1.6)**	**0.0 (-20.0)**
Mar '15	14.6 (-0.9)	23.7 (-1.9)	19.2 (-1.4)	**10.4 (-12.0)**
Apr '15	11.7 (-1.9)	19.7 (-2.5)	15.7 (-2.2)	55.8 (+23.4)
May '15	10.9 (-0.5)	17.6 (-0.9)	14.2 (-0.7)	68.4 (+13.7)
Jun '15	8.8 (-0.6)	**15.6 (+0.1)**	12.2 (-0.2)	**12.8 (-51.8)**

[a]Noarlunga weather station (Latitude: 35.16 °S, Longitude 138.51 °E, Elevation: 55 m)

Table S2. Basic grape and wine compositional parameters for the different harvest dates[a]

	Wine				
	H0	H1	H2	H3	H4 (Control)
harvest date	8 Jan	3 Feb	9 Feb	18 Feb	22 Feb
berry weight [g/berry]	–	1.01 ± 0.01a	0.93 ± 0.02b	0.87 ± 0.04b	0.62 ± 0.03c
TSS [°Brix]	11.2	20.5 ± 0.1d	23.9 ± 0.1c	27.4 ± 0.1b	30.4 ± 0.1a
pH before adjustment	2.76	3.03 ± 0.03d	3.75 ± 0.02c	4.02 ± 0.02a	3.85 ± 0.02b
TA before adjustment[b]	20	8.07 ± 0.49b	8.62 ± 0.2b	5.42 ± 0.6c	9.8 ± 0.41a
% ABV [%v/v]	4.5	11.4 ± 0.03d	13.5 ± 0.0c	15.1 ± 0.0b	18.2 ± 0.0a
pH after adjustment	–	3.68 ± 0.01a	3.58 ± 0.01b	3.67 ± 0.02a	3.58 ± 0.00b
TA after adjustment[b]	–	7.06 ± 0.13b	7.98 ± 0.24a	8.05 ± 0.1a	8.13 ± 0.03a
citric acid[c]	–	0.14 ± 0.00d	0.16 ± 0.00c	0.31 ± 0.00b	0.37 ± 0.01a
tartaric acid[c]	–	2.12 ± 0.08ab	2.22 ± 0.05a	2.04 ± 0.00bc	1.98 ± 0.05c
malic acid[c]	–	3.46 ± 0.05a	2.92 ± 0.02b	2.42 ± 0.07c	2.18 ± 0.04d
acetic acid[c]	–	0.33 ± 0.02c	0.34 ± 0.04c	0.48 ± 0.01b	0.58 ± 0.04a
glucose[c]	–	0.00 ± 0.00b	0.00 ± 0.00b	0.04 ± 0.00b	0.14 ± 0.07a
fructose[c]	–	0.00 ± 0.00b	0.00 ± 0.00b	0.30 ± 0.01b	2.67 ± 0.89a
glycerol[c]	–	8.00 ± 0.02c	9.63 ± 0.13b	11.7 ± 0.12a	11.8 ± 0.05a

[a]Values are means of 3 replicates ± standard error (except H0, which was produced without triplicates). [b]Values in [g/L] tartaric acid equivalents. [c]Values in [g/L]. Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA)

Table S3. Blending proportion using green harvest wine (GHW) or water in conjunction with H4 must, and basic wine compositional parameters for the blending treatments[a]

	wine					
	B1	B2	B3	Bw1	Bw2	Bw3
GHW[b]	43.7	27.3	13.6	–	–	–
water[b]	–	–	–	32	19.9	10.1
% ABV[b]	14.4 ± 0.00c	15.8 ± 0.00b	17.0 ± 0.00a	14.7 ± 0.00c	16.0 ± 0.00b	17.4 ± 0.00a
pH before adj.	3.51 ± 0.02e	3.64 ± 0.01d	3.73 ± 0.00c	3.82 ± 0.00b	3.84 ± 0.00ab	3.85 ± 0.00a
pH after adj.	3.67 ± 0.01ab	3.73 ± 0.08a	3.66 ± 0.00ab	3.67 ± 0.02ab	3.65 ± 0.03bc	3.66 ± 0.02ab
TA after adj.[c]	8.54 ± 0.16a	8.13 ± 0.2b	8.23 ± 0.18b	7.34 ± 0.07d	7.79 ± 0.06c	7.78 ± 0.02c
citric acid[d]	0.34 ± 0.02ab	0.33 ± 0.01ab	0.36 ± 0.00a	0.25 ± 0.00c	0.32 ± 0.01b	0.32 ± 0.02b
tartaric acid[d]	2.18 ± 0.03ab	1.91 ± 0.13de	1.84 ± 0.06e	2.31 ± 0.03a	2.12 ± 0.06bc	2.02 ± 0.07cd
malic acid[d]	3.85 ± 0.09a	3.28 ± 0.04b	2.71 ± 0.02c	2.21 ± 0.06d	2.28 ± 0.06d	2.21 ± 0.05d
acetic acid[d]	0.32 ± 0.03bc	0.33 ± 0.05bc	0.41 ± 0.03a	0.28 ± 0.03c	0.36 ± 0.03ab	0.35 ± 0.03abc
glucose[d]	0.00 ± 0.00b	0.00 ± 0.00b	0.02 ± 0.00b	0.00 ± 0.00b	0.01 ± 0.01b	0.14 ± 0.05a
fructose[d]	0.00 ± 0.00b	0.34 ± 0.04b	0.73 ± 0.25ab	0.29 ± 0.00b	0.37 ± 0.03b	1.48 ± 0.86a
glycerol[d]	8.89 ± 0.11e	10.0 ± 0.12c	11.0 ± 0.07a	9.25 ± 0.15d	10.3 ± 0.1b	11.1 ± 0.05a

[a]Values are means of 3 replicates ± standard error. [b]Values in [% v/v]. [c]Values in [g/L] tartaric acid equivalents. [d]Values in [g/L]. Values followed by different letters within rows are significantly different (p≤0.05, one way ANOVA). adj., adjustment

Table S4. Acetone-insoluble grape skin cell wall polysaccharide composition presented as monosaccharide units for consecutive harvest dates (H1–H4)[a]

| | harvest time | | | |
	H1	H2	H3	H4
[mg/g cell wall material]				
total skin polysaccharide	275 ± 13	246 ± 21	271 ± 6	256 ± 20
rhamnose	6.50 ± 1.44	5.18 ± 0.46	5.58 ± 0.26	4.24 ± 1.14
arabinose	31.3 ± 1.0	27.0 ± 3.9	32.9 ± 0.3	28.3 ± 1.8
xylose	16.2 ± 0.7a	12.1 ± 1.5b	13.3 ± 0.5b	12.1 ± 1.2b
mannose	14.4 ± 0.2	11.9 ± 2.0	13.2 ± 0.2	12.1 ± 1.2
galactose	20.2 ± 1.1a	14.9 ± 1.8c	18.3 ± 0.9ab	16.7 ± 1.6bc
glucose	103 ± 8	98.4 ± 9.0	110 ± 2	106 ± 12
galacturonic acid	83.3 ± 6.0	77.0 ± 3.8	78.0 ± 3.0	76.2 ± 4.8
[mol%]				
rhamnose	2.39 ± 0.65	2.11 ± 0.01	2.16 ± 0.08	1.63 ± 0.32
arabinose	13.8 ± 0.2	13.3 ± 0.9	14.7 ± 0.2	13.5 ± 0.6
xylose	7.15 ± 0.59	6.00 ± 0.60	5.94 ± 0.25	5.77 ± 0.52
mannose	5.31 ± 0.18	4.88 ± 0.39	4.91 ± 0.18	4.80 ± 0.10
galactose	7.44 ± 0.42	6.14 ± 0.44	6.80 ± 0.20	6.62 ± 0.53
glucose	37.9 ± 1.2b	40.6 ± 0.6a	40.8 ± 0.6a	42.0 ± 1.4a
galacturonic acid	26.0 ± 0.7	27.0 ± 1.1	24.7 ± 0.5	25.7 ± 1.9

[a]Values are means of 3 replicates ± standard error. Values followed by different letters within a row are significantly different (p≤0.05, one way ANOVA).
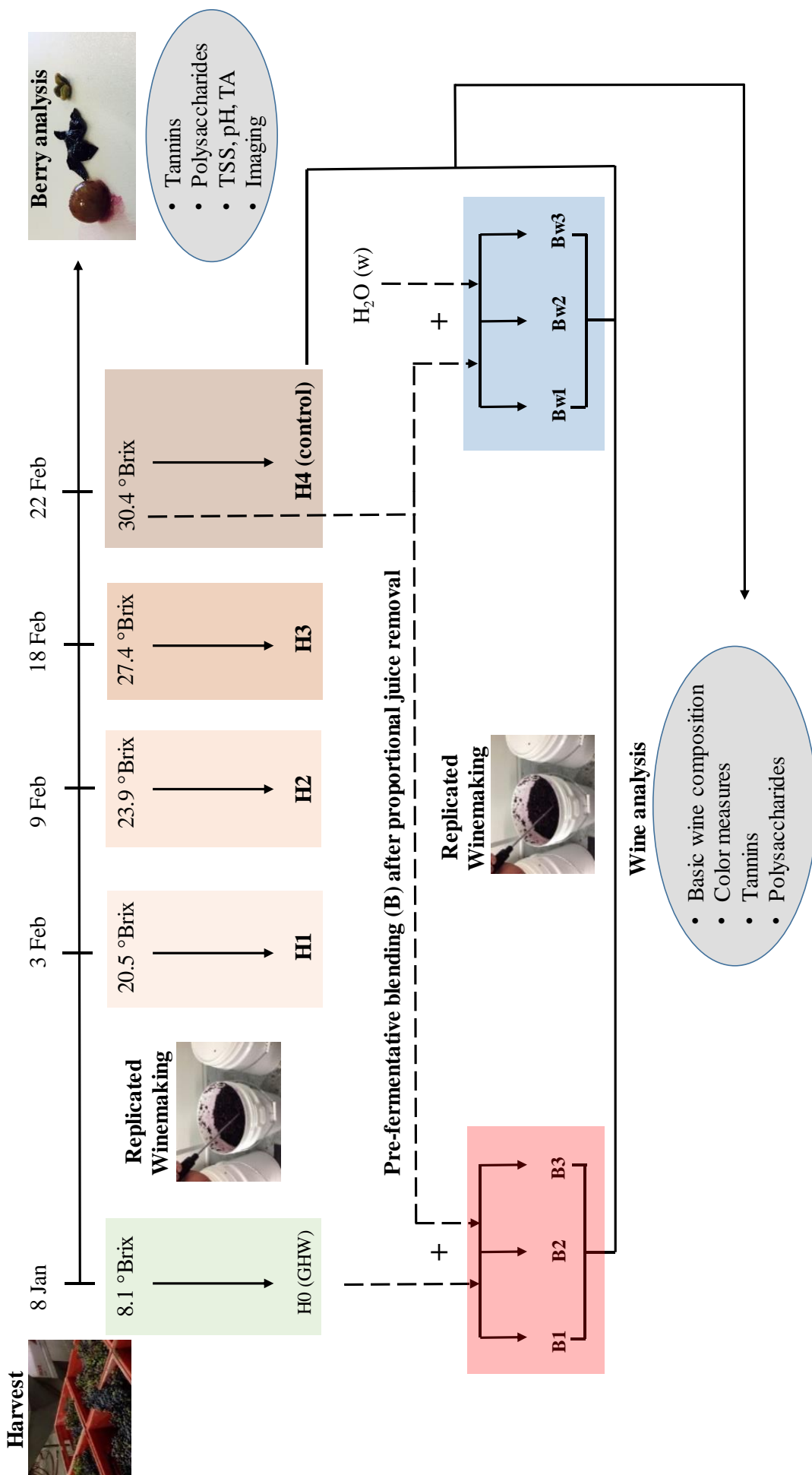
Figure S1. Experimental plan showing harvest dates, winemaking, blending treatments using proportions of green harvest wine (GHW) or water, and grape and wine analyses undertaken.
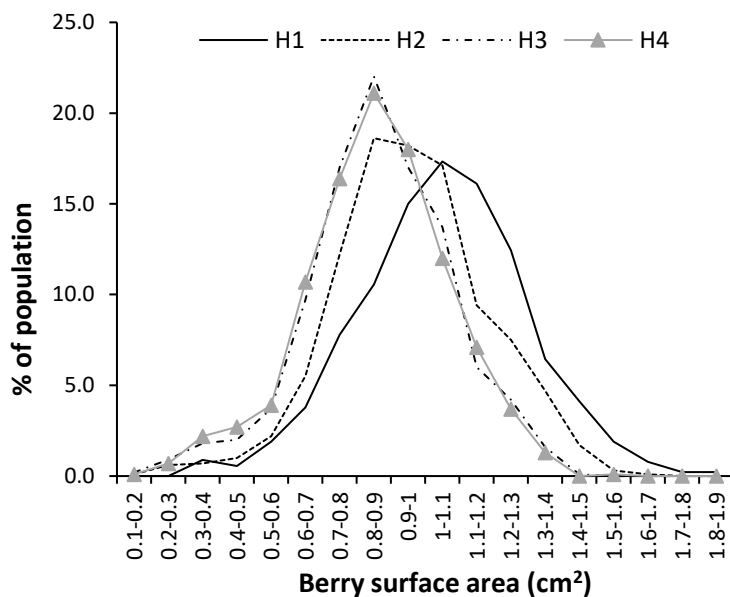
Figure S2. Berry size distribution determined using image analysis for consecutive harvest dates of Cabernet Sauvignon.
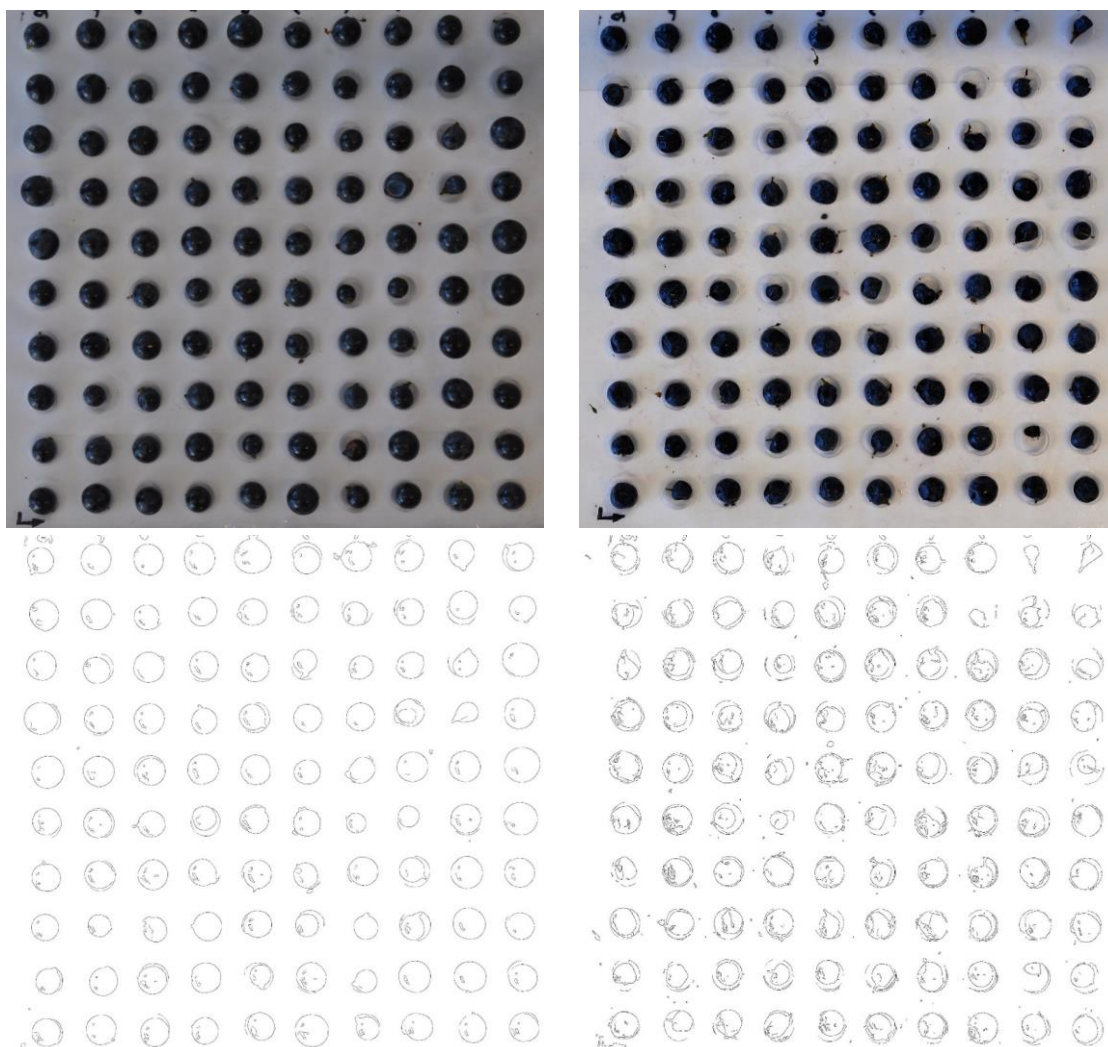
Figure S3. Examples of original (top) and processed (bottom) images for 100 Cabernet Sauvignon berries from H1 (left) and H4 (right). Shriveled berries are clearly evident in both of the images associated with H4, with the apparent smaller berry sizes and loss of the characteristic spherical shapes seen in the images associated with H1.

Chapter 3

Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties

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Name of Principal Author (Candidate)	Olaf J. Schelezki
Contribution to the Paper	Designed experiments, conducted vineyard monitoring, organised and executed grape harvests and experimental winemaking, conducted wine volatile analysis by GC-MS, analysed and interpreted the data, trained a sensory panel and undertook quantitative descriptive analysis of 30 wines, statistically analysed the data sets (one and two-way ANOVA, PCA), interpreted the data, drafted and constructed the manuscript.
Overall percentage (%)	70
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 3/4/2018

Co-Author Contributions

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

     i.   the candidate's stated contribution to the publication is accurate (as detailed above);

     ii.   permission is granted for the candidate in include the publication in the thesis; and

     iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Katja Suklje
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Contribution to the Paper	Supervised the work, contributed to the research idea, experimental design, analysis method and interpretation of the data. Edited the manuscript and acted as the corresponding author.
Signature				Date	5 4 18

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Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties

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A B S T R A C T

This study extends previous work on Cabernet Sauvignon wines of lowered alcohol concentrations produced by pre-fermentatively substituting proportions of juice from an overripe crop with "green harvest wine" or water to adjust initial sugar concentrations. Resulting wines were assessed for their volatile compositions and sensory characteristics to evaluate the suitability of this winemaking approach to managing wine alcohol concentrations in warm viticulture regions. Wines from water or green harvest wine substitution were also compared to wines of similar alcohol content produced from earlier harvested grapes. Implementation of water substitution in particular resulted in minor alterations of wine volatile composition compared to the control, and positive aroma and flavour characteristics were preserved. However, overripe sensory attributes such as 'hotness' and 'port wine' were conserved whereas they were absent in wines of similar alcohol level made from earlier harvested grapes, thereby emphasising the relevance of grape (over)maturity when producing lower alcohol wines.

1. Introduction

Production of quality wine necessarily starts in the vineyard, where one of the most important decisions to be taken is that of harvest timing. However, optimising harvest dates has become more difficult in regions where warm and dry conditions that already prevail are compounded by weather events like severe heatwaves, which are likely to occur more frequently as a result of a changing climate. This becomes problematic when winemakers seek to decrease the proportions of under-ripe grapes to minimise 'green' aroma characteristics while favouring a fuller, riper, fruit-driven aroma spectrum of the wines by delaying harvest dates, thereby risking the occurrence of berry shrivel (Krasnow et al., 2010). Within a very short time, not only may yields decrease significantly but aroma and flavour profiles can also be dramatically altered (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018; Šuklje et al., 2016). Besides an inferior wine quality through more pronounced hotness and bitterness (Heymann et al., 2013), the resulting higher ethanol content means that wines are subject to higher

tax penalties for exports and may fail to meet the rising demand of health-conscious consumers seeking wines with moderate alcohol levels. Even without the influence of berry shrivel, the benefit of extended "hang-time" on wine quality is rather arguable as the sensory quality (Heymann et al., 2013) or consumer preference (Bindon et al., 2014) may only marginally change once grapes have passed a certain level of maturity.

To manage excessive alcohol concentrations in wines, winemakers can choose among a variety of physical dealcoholisation techniques including reverse osmosis, osmotic distillation or vacuum distillation (Longo, Blackman, Torley, Rogiers, & Schmidtke, 2016). However, significant losses in the wine volatile composition (and colour properties) were observed in the past when manipulating wine alcohol levels (Bui, Dick, Moulin, & Galzy, 1986), even if combined with volatile recovery technologies (Medina & Martínez, 1997). Further optimised alternatives have been developed more recently, such as spinning cone column (SCC) technologies, which essentially allow the removal of volatiles in a first step and their re-introduction after the desired

ethanol concentration is established (Schmidtke, Blackman, & Agboola, 2012). Even though the recovery rates of 97–100% suggest a negligible effect on the potential wine aroma profile, the high costs associated with this process limit the application to large-scale bulk wine production.

A potentially more cost-effective and easy-to-apply approach appears to be the pre-fermentative implementation of low alcohol wine produced from unripe grapes (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011) or water (Harbertson, Mireles, Harwood, Weller, & Ross, 2009) into must to lower both the initial sugar levels and final wine alcohol concentrations. Minimal effects on wine phenolics, colour and sensory properties were reported in those studies following the manipulation of the must matrix. However, the lack of comparability in the applied winemaking methods, grape varieties and vintage context makes it difficult to evaluate differences in wine quality according to the chosen blending component (i.e., water or low alcohol wine). Furthermore, it had not been established whether adjusting alcohol levels of an overripe or shrivelled crop (i.e., one that would yield a wine with an excessive alcohol content) through a blending approach would have qualitative benefits compared to simply harvesting earlier (if logistically possible), where grape over-maturity (and berry shrivel) would be avoided.

The first implementation of a pre-fermentative blending approach under conditions of berry shrivel was partly addressed in a recent study by Schelezki et al. (2018). In that work, blending with water or 4.5% alcohol by volume (ABV) wine made from unripe grapes (termed "green harvest wine", GHW) was employed following proportional juice removal, to produce Cabernet Sauvignon wines with different alcohol levels from the same grapes harvested at 30.4 °Brix after the onset of berry shrivel. An assessment was made of non-volatile components, and neither of the blending options affected red wine quality parameters compared to the control, which included total anthocyanin concentrations, colour intensity or total tannin concentrations, and yielded only minor (and most likely positive) differences in polysaccharide and tannin compositions. Even upon lowering the alcohol content by up 3.5% ABV, from over 18% ABV to around 14.5% ABV, the red wine non-volatile parameters remained similar or superior to the untreated control wine.

In a continuation of the study, this report presents and discusses the findings regarding the volatile composition and sensory properties of the wines to i) complete the picture on which qualitative changes to expect when managing alcohol levels via pre-fermentative additions of either GHW or water, ii) verify the more suitable blending component to choose, and iii) assess the sensory quality of the lower alcohol wines resulting from the blending treatments compared to wines made from earlier harvested grapes. The work provides context for optimised harvest decision-making and revisits the presumed benefits of extended grape ripening when seeking riper flavours.

2. Material and methods

2.1. Chemicals

Labelled internal standards for GC–MS analysis included d_{13}-hexanol, d_{11}-hexanoic acid, d_{16}-octanal and d_3-linalool, purchased from C/D/N Isotopes Inc. (Pointe-Claire, Canada), as well as d_5-ethyl nonanoate synthesised previously (Boss et al., 2015).

2.2. Harvest and winemaking

Full details of the experimental design and winemaking conditions were described previously (Schelezki et al., 2018) and are illustrated in Fig. S1 of the Supporting Information, and grape and wine compositional information for the treatments has been provided in Tables S1 and S2. Briefly, *Vitis vinifera* L. cv. Cabernet Sauvignon grapes were sourced from a commercial vineyard located in McLaren Vale, South

Australia, during the 2015 vintage. The first batch of fruit, hand-picked when total soluble solids (TSS) were 8.1 °Brix, was fermented to produce a wine with low values for both alcohol and pH (4.5% ABV, pH = 2.76), further referred to as green harvest wine (GHW, or H0). Decolourisation and deodorisation of the GHW was achieved with charcoal and bentonite (Kontoudakis et al., 2011). In subsequent consecutive harvests, winemaking (in triplicate) was conducted with grapes that were hand-picked at TSS values of 20.5, 23.9, 27.4 and 30.4 °Brix, resulting in wines H1, H2, H3 and H4 (control), respectively, with H4 being the commercial harvest date of this vineyard. Grapes obtained at H4 were also used to produce alcohol-adjusted wines (in triplicate) by substituting proportions of the juice with either filtered water or with GHW prior to fermentation to lower must sugar levels, with the aim of matching the alcohol levels of GHW and water addition treatments with those of the consecutive harvest wines. Finished wines did not undergo malolactic fermentation or oak contact and were bottled in 375 mL bottles and stored at 15 °C until required.

2.3. Analysis of wine volatile composition

Wine volatile composition was determined by headspace solid phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS) according to previous reports (Dennis et al., 2012; Hranilovic et al., 2018) seven months after bottling (at the time of sensory analysis). Analysis of winemaking replicates (n = 3) was conducted using a 7890A gas chromatograph (Agilent, Santa Clara, CA, United States) equipped with an MPS2 autosampler (Gerstel, Mülheim and der Ruhr, Germany) and using an Agilent 5975C mass spectrometer for identification and quantification of analytes. The autosampler was fitted with a 2-cm divinylbenzene-carboxen-polydimethylsiloxane fibre (DVB-CAR-PDMS, 50/30 μm; Supelco, Bellefonte, PA) for extraction of volatiles. Aliquots of wine were analysed after a 1:2 dilution with MilliQ water to a final volume of 10 mL. NaCl (3 g) was added to each SPME vial (20 mL) prior to addition of the diluted sample and subsequent spiking with 10 μL of an ethanolic solution containing the following deuterated internal standards at the specified concentrations: d_{13}-hexanol (920 mg/L); d_{11}-hexanoic acid (930 mg/L); d_{16}-octanal (82.1 mg/L); d_5-ethyl nonanoate (9.2 mg/L), d_3-linalool (1.73 mg/L). Volatile compounds were extracted from the headspace using agitation (250 rpm) at 40 °C for 30 min and desorbed from the fibre in the GC inlet (220 °C) for 1 min. Chromatography was performed using a ZB-Wax column (30 m × 0.25 mm i.d. and film thickness 0.25 μm, Phenomenex, Sydney, Australia) and helium (Ultrahigh Purity, Air Liquide, Adelaide, Australia) as a carrier gas with constant flow of 1.2 mL/min, using the following temperature program: 35 °C for 1.5 min, increasing at 7 °C/min to 245 °C, and holding at 245 °C for 3.5 min. The transfer line was held at 250 °C. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range: m/z 35–350, scan rate: 4.45 scans/s). Authentic standards in model wine (12% aqueous ethanol, pH adjusted to 3.2 with tartaric acid) were prepared in triplicate at five evenly spaced concentrations across the range for quantifying the analytes. The highest standard concentration was approximately 150% of the highest concentration observed in the wines for each analyte. Calibrations were linear throughout the range with R^2 = 0.94–0.99. All calibration samples were prepared and analysed according to the protocol outlined above. 3-Isobutyl-2-methoxypyrazine (IBMP) was quantified by a stable isotope dilution assay using SPME–GC–MS as described previously (Dunlevy et al., 2011). Chromatograms were analysed using Masshunter software (Version B.07.00, Agilent Technologies).

2.4. Sensory analysis

Descriptive analysis (DA) was conducted seven months after bottling the wines. The panel of eleven assessors (seven female and four male) comprised ten wine science researchers from the University of

Adelaide with previous DA experience and one expert panellist. The DA process was structured according to the consensus-based approach (Lawless & Heymann, 2010) and consisted of eight training and three formal sessions. The first two training sessions were aimed at evaluating aroma, flavour and palate characteristics of the wines, and discussing and reaching consensus about the descriptive attributes. In subsequent sessions, the panellists were provided reference standards for aroma attributes, astringency, bitterness and hotness, and evaluated different experimental samples in order to familiarise themselves with the attributes as well as with the rating scales. Wines were rated using Red-Jade online based software, and results during the training sessions were presented to the panellists directly after each session to provide feedback. Descriptive terms were discussed in every session in order to screen out non-discriminating attributes, and the final attribute list elaborated by the panel comprised ten aroma, ten flavour and six mouthfeel attributes (Table S3 of the Supporting Information). During the formal evaluations, the panellists rated the wines on 15-cm unstructured line scales, with anchors at 10%, 50% and 90% of the scale corresponding to 'low', 'medium' and 'high', respectively. During the three formal evaluation sessions to assess all treatment replicates, panellists were presented with ten wine samples (30 mL) in ISO standard (ISO 3951:1977) clear wine glasses coded with four digit numbers and covered with glass lids, in a randomised and balanced order. The evaluations were conducted in a sensory laboratory with isolated booths maintained at 21 °C and under white lighting. Rest breaks of one minute after each sample and five minutes after five samples were imposed on the panellists to avoid fatigue. Panellists were provided with filtered water, 1 g/L pectin solution (pectin from citrus peel, Sigma-Aldrich, NSW, Australia) and plain water crackers to cleanse their palate between samples.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) of the chemical data, principal component analysis (PCA) of normalised chemical and sensory data, and Pearson correlation analysis were conducted using XLSTAT (Version 2015.4.1, Addinsoft, Paris, France). Mean comparisons were performed by Fisher's least significant difference (LSD) multiple comparison test at $p < 0.05$. The panel performance was assessed via PanelCheck (V1.4.2, Nofima Mat) during the DA, and ANOVA and mean comparisons by Fisher's LSD were performed using SENPAQ (Version 6.03, Qi Statistics, Reading, United Kingdom).

3. Results and discussion

3.1. Effect of harvest date and berry shrivel on wine volatile composition

We previously reported on the vintage conditions, basic wine composition (Tables S1 and S2 of the Supporting Information) and non-volatile components of Cabernet Sauvignon grapes and wines arising from consecutive harvests and blending treatments (Schelezki et al., 2018) and now turn our attention to the impacts on volatile composition and sensory profile of those wines. Out of 43 volatile compounds arising from grape or yeast metabolism (Table S4 of the Supporting Information), the concentrations of 34 significantly differed among the harvest dates H1–H4 (Tables 1 and S5 of the Supporting Information).

Amongst the grape-derived volatile compounds in Table 1, methoxypyrazines, especially 3-isobutyl-2-methoxypyrazine (IBMP), are particularly known to shape the sensory profile of Cabernet Sauvignon varietal wines. Whereas concentrations below 15 ng/L are potentially desired for adding complexity and characteristic 'bell pepper' notes to the wine (Roujou de Boubee, Van Leeuwen, & Dubourdieu, 2000), higher levels of this potent odorant (aroma detection threshold of several ng/L) may lead to wines of inferior quality associated with insufficient grape maturity (Sidhu, Lund, Kotseridis, & Saucier, 2015). Reaching maximum levels at veraison, the IBMP content in grapes

decreases during the course of grape ripening, thus the choice of harvest date can have a direct influence on the final wine IBMP concentrations, although the impact of over-ripening and berry shrivel is uncertain.

In accord with the expected degradation during ripening, IBMP concentrations in the consecutive harvest wines significantly decreased from H1 to H3 ($p < 0.0001$) (Table 1) in a similar manner to that observed previously (Bindon, Varela, Kennedy, Holt, & Herderich, 2013). However, with the onset of berry shrivel by the time of the commercial harvest (Schelezki et al., 2018), the concentration of IBMP was found to increase, from 7.9 ng/L in H3 to 10.5 ng/L in H4 ($p < 0.015$), reaching a value similar to H2. Given that methoxypyrazines are primarily located in grape skins (Roujou de Boubee, Cumsille, Pons, & Dubourdieu, 2002) (if only considering the berry and not grape stem), a higher skin to pulp ratio caused by berry shrivel appears to have caused an increase in IBMP concentration in the H4 wine relative to the previous harvest date.

Total concentrations of grape-derived isoprenoids (linalool, β-citronellol, nerolidol and β-damascenone) significantly decreased with maturity from around 8.5 µg/L to 7 µg/L from H3 onwards (Table 1), in line with previously reported trends (Bindon et al., 2013; Šuklje et al., 2016). The occurrence of berry shrivel in H4 did not alter this trend in terms of total isoprenoid concentration, but compound-specific differences were observed. The concentration of linalool tended to decrease and nerolidol increased, as previously observed (Yuan & Qian, 2016), whereas β-citronellol concentration did not change significantly with the last harvest date (Table 1). The respective concentrations were well below the detection thresholds for these compounds (Table S4 of the Supporting Information) so a direct influence of these changes on the varietal aroma spectrum of the wines was deemed unlikely. However, in the case of β-damascenone, with a threshold of 2–7 µg/L in red wines as estimated previously by Pineau, Barbe, Van Leeuwen, and Dubourdieu (2007) (Table S4 of the Supporting Information), those authors argued for an indirect yet significant importance in wine by enhancing fruity aromas. Indeed, this isoprenoid has been ascribed as particularly shaping the aroma (Forde, Cox, Williams, & Boss, 2011) and varietal characteristics of Cabernet Sauvignon wines (Kotseridis & Baumes, 2000). Concentrations of β-damascenone have previously been reported to remain steady throughout the later stages of grape maturity (Bindon et al., 2013), in agreement with our observations for harvests H2 and H3 that were of similar grape maturity. However, other studies involving different varieties and vintage conditions have reported differing results, with concentration increases in Pinot Noir berries (Yuan & Qian, 2016) or decreases in Shiraz wines (Šuklje et al., 2016) with later ripening stages. With the berry shrivel observed in our study, the concentration of β-damascenone declined significantly in the H4 wine, which may have consequences for flavour perception of the wine. Šuklje et al. (2016) also observed lower β-damascenone values in shrivelled berries but the concentrations did not differ significantly from non-shrivelled berries at the late harvest stages, and shrivelled berries at earlier ripening stages even contained higher values than their non-shrivelled counterparts. Other than potential differences in β-damascenone precursor composition in the grapes, the role of the acidic matrix in transforming precursors and liberating β-damascenone during winemaking (and beyond) is likely to contribute to such differences amongst the studies.

As the last group of grape-derived volatiles that were assessed, the total concentrations of four C_6 alcohols decreased by almost 40% in wines between H1 (4142 µg/L) and H4 (2541 µg/L) after showing notable fluctuation among the first three harvest dates, particularly driven by 1-hexanol, but also by changes in (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol and 2-ethyl-1-hexanol (Table 1). Decreasing concentrations of C_6 alcohols and respective aldehydes in grapes towards the end of berry ripening have been reported previously (Canuti et al., 2009; Yuan & Qian, 2016); however, the final amounts in wines can be inconsistent with respect to grape maturity, with either decreasing (Bindon et al.,

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Table 1

List of wine volatile compounds that significantly changed ($p \leq 0.05$) with different harvest dates (H1: 11.4%, H2: 13.5%, H3: 15.1%, H4: 18.2% ABV; see Fig. S1 of the Supporting Information for sample codes).[a]

Volatile compound	Wine from consecutive harvest dates				
	H1	H2	H3	H4 (Control)	*p-value*
Grape-derived					
Methoxypyrazines [ng/L]					
IBMP	15.7 ± 1.4a	11.6 ± 1b	7.88 ± 0.23c	10.5 ± 0.3b	0.0001
Isoprenoids [µg/L]					
Linalool	1.69 ± 0.15ab	1.88 ± 0.04a	1.77 ± 0.05a	1.51 ± 0.05b	0.015
β-Citronellol	4.85 ± 0.63a	4.14 ± 0.24ab	3.35 ± 0.1bc	3.27 ± 0.26c	0.008
Nerolidol	0.69 ± 0.10c	1.50 ± 0.40ab	1.02 ± 0.13bc	1.52 ± 0.05a	0.013
β-Damascenone	1.16 ± 0.10a	0.98 ± 0.07b	0.88 ± 0.03b	0.68 ± 0.03c	0.001
Total isoprenoids	8.39 ± 0.65a	8.5 ± 0.52a	7.01 ± 0.04b	6.98 ± 0.23b	0.010
C_6 alcohols [µg/L]					
1-Hexanol	3971 ± 200a	2828 ± 83b	4190 ± 100a	2463 ± 14c	< 0.0001
(E)-2-Hexen-1-ol	44.6 ± 2.9ab	41.4 ± 4.1bc	48.8 ± 2.1a	38.0 ± 0.7c	0.022
(Z)-3-Hexen-1-ol	119 ± 7a	65.8 ± 5.7b	54.3 ± 2.0c	36.5 ± 1.6d	< 0.0001
2-Ethyl-1-hexanol	5.19 ± 0.36b	4.89 ± 0.33bc	6.20 ± 0.25a	4.50 ± 0.15c	0.002
Total C_6 alcohols	4142 ± 202a	2940 ± 89b	4298 ± 96a	2541 ± 11c	< 0.0001
Fermentation-derived					
Acids [µg/L]					
Hexanoic acid	7776 ± 540a	6822 ± 239b	6242 ± 74b	4725 ± 121c	< 0.0001
Octanoic acid	3171 ± 406a	3063 ± 168a	1008 ± 155b	719 ± 44b	< 0.0001
Total acids	10956 ± 863a	9896 ± 401a	7232 ± 229b	5444 ± 164c	< 0.0001
Higher alcohols [µg/L]					
2-Methyl-1-propanol	64500 ± 5170b	60640 ± 2460b	76961 ± 2568a	76089 ± 84a	0.025
1-Butanol	2.86 ± 0.83c	4.20 ± 0.46bc	5.50 ± 0.10b	8.19 ± 0.91a	0.0003
2-Heptanol	5.17 ± 0.12c	5.65 ± 0.34c	6.52 ± 0.33b	9.25 ± 0.18a	< 0.0001
3-Methyl-1-pentanol	176 ± 14b	258 ± 21a	179 ± 3b	109 ± 0c	< 0.0001
1-Octanol	49.0 ± 4.4a	45.7 ± 3.3a	36.5 ± 2.0b	30.2 ± 1.8b	0.001
1-Nonanol	13.6 ± 0.7b	18.2 ± 1.2a	14.5 ± 0.3b	19.3 ± 1.4a	0.001
3-Methylthio-1-propanol	6488 ± 86a	6032 ± 301b	5234 ± 166c	3484 ± 53d	< 0.0001
Benzyl alcohol	1005 ± 112d	1193 ± 23c	1363 ± 4b	2225 ± 15a	< 0.0001
2-Phenylethanol	159198 ± 9624a	139820 ± 6423b	135998 ± 1559b	91055 ± 6480c	< 0.0001
Total higher alcohols	231346 ± 4930a	208022 ± 8223b	219839 ± 846ab	173008 ± 6468c	< 0.0001
Ethyl esters [µg/L]					
Ethyl acetate	28479 ± 3334c	26378 ± 1631c	39938 ± 1313b	49026 ± 1172a	< 0.0001
Ethyl esters of branched acids [µg/L]					
Ethyl 2-methylpropanoate	59.0 ± 1.0a	40.5 ± 2.1c	49.8 ± 5.1b	40.3 ± 3.4c	0.001
Ethyl 2-methylbutanoate	7.83 ± 0.34a	6.37 ± 0.23bc	7.24 ± 0.63ab	5.70 ± 0.20c	0.003
Ethyl phenylacetate	8.52 ± 1.19a	7.19 ± 0.92ab	8.62 ± 0.54a	5.38 ± 0.29b	0.013
Total ethyl esters of branched acids	75.4 ± 2.3a	54.1 ± 3.0c	65.7 ± 6.3b	51.4 ± 3.3c	0.001
Ethyl esters of fatty acids [µg/L]					
Ethyl hexanoate	1024 ± 150a	853 ± 96a	1061 ± 60a	590 ± 49b	0.005
Ethyl (Z)-3-hexenoate	1.39 ± 0.18a	1.10 ± 0.1b	0.58 ± 0.04c	0.64 ± 0.04c	0.0003
Ethyl octanoate	802 ± 131a	802 ± 111a	723 ± 78a	489 ± 20b	0.032
Total ethyl esters of fatty acids	1827 ± 273a	1656 ± 207a	1785 ± 138a	1080 ± 69b	0.014
HAA from grape lipid degradation [µg/L]					
Hexyl acetate	19.7 ± 1.9bc	14.2 ± 1.5c	30.5 ± 1.5a	11.6 ± 1.2c	< 0.0001
HAA from yeast sugar and N metabolism [µg/L]					
2-Methylpropyl acetate	32.2 ± 2.9a	20.6 ± 1.5b	28.0 ± 1.6a	23.5 ± 0.1b	0.001
3-Methylbutyl acetate	6802 ± 663ab	6084 ± 790b	8251 ± 480a	5652 ± 755b	0.025
2-Phenylethyl acetate	187 ± 25a	158 ± 21ab	192 ± 11a	119 ± 8b	0.011
Total HAA from yeast sugar and N metabolism	7004 ± 691ab	6257 ± 811b	8494 ± 493a	5794 ± 763b	0.022
Other esters [µg/L]					
Ethyl propanoate	261 ± 39bc	252 ± 6c	354 ± 20a	313 ± 16ab	0.009
Methyl octanoate	6.95 ± 1.19a	6.62 ± 0.91a	5.49 ± 0.37a	3.40 ± 0.07b	0.007
3-Methylbutyl hexanoate	2.35 ± 0.28a	1.85 ± 0.22b	2.04 ± 0.19ab	1.19 ± 0.01c	0.003
Total other esters	270 ± 40bc	260 ± 8c	362 ± 20a	318 ± 16ab	0.010

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within a row are significantly different ($p \leq 0.05$, one way ANOVA).

2013) or increasing (Canuti et al., 2009; Šuklje et al., 2016) concentrations when using more mature grapes. On the other hand, a study by Šuklje et al. (2016) showed that wines made from shrivelled fruit contained significantly less C_6 alcohols than wines made from non-shrivelled grapes at the same maturity level, and Franco, Peinado, Medina, and Moreno (2004) reported lower C_6 alcohol concentrations in off-vine dried grapes. This implies that the observed dramatic decline in wine C_6 alcohol concentrations from H3 to H4 in the present study could be directly attributed to the occurrence of berry shrivel at H4. Despite the concentrations shown in Table 1 being below the respective detection thresholds of these C_6 alcohols (Table S4 of the Supporting Information), implications for wine sensory profiles are possible. The concentration of (Z)-3-hexen-1-ol has been negatively associated with flavour impact in Cabernet Sauvignon wines (Forde et al., 2011), and

importantly, several C_6 alcohols are involved in the formation of hexyl acetate upon fermentation (Dennis et al., 2012). This acetate ester may be a driver of berry flavour in Cabernet Sauvignon wines (Forde et al., 2011) but its link with C_6 alcohols in the present study was limited: only hexyl acetate mirrored the trend in 1-hexanol (r = 0.870), which declined with grape maturity (Table 1). The general lack of correlation with C_6 alcohols was also evident among Shiraz wines analysed by Šuklje et al. (2016).

Total higher alcohols were only marginally influenced by the first three harvest dates but declined in the wines produced from the last harvest point (Table 1). The concentrations of nine higher alcohols changed significantly, with a total decrease from H3 to H4 of roughly 21%, which was particularly driven by lower amounts of 3-methyl-1-pentanol, 3-methylthio-1-propanol and especially 2-phenylethanol, and to a lesser extent by 1-octanol and 1-nonanol. In contrast, 2-methyl-1-propanol, 1-butanol, 2-heptanol and benzyl alcohol tended to increase with later harvest points. A limited number of reports were available on the evolution of higher alcohol concentrations in wines made from consecutive harvest points. Bindon, Varela, Kennedy, Holt, and Herderich (2013) reported a general increase of higher alcohol concentrations in Cabernet Sauvignon wines made from later harvest dates, whereas Šuklje et al. (2016) found no effects of grape maturity for Shiraz wines. Furthermore, berry shrivel appeared not to have influenced the concentrations of higher alcohols determined previously in the Shiraz wines (2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol) (Šuklje et al., 2016), in contrast to the present work where severe berry shrivel and a dramatic increase in TSS within four days from H3 (27.4 °Brix) to H4 (30.4 °Brix) appeared to have a significant effect on the final wine higher alcohol composition. The 33% decrease in 2-phenylethanol from above threshold at H3 to subthreshold (Table S4 of the Supporting Information) at H4 is particularly noteworthy. Even though higher alcohols are generally considered as marginal contributors to wine aroma quality (Waterhouse, Sacks, & Jeffery, 2016b), they are substrates for the production of fruity acetate esters that are of much greater consequence.

Fermentation-derived esters play an important role in creating the aroma and flavour profiles of wines. Even when present below their aroma detection thresholds, esters can account for variation in red and black berry aromas through additive and synergistic effects (Sumby, Grbin, & Jiranek, 2010). A number of variables are known to influence their formation, such as the temperature or nutrient availability during alcoholic fermentation (Sumby et al., 2010) and vineyard associated factors like cultivar-dependent precursor variations and grape maturity levels, ultimately affecting the relative abundance of individual esters (Houtman, Marais, & Du Plessis, 1980). The composition of esters analysed in our study changed with higher grape maturity from H1 to H3 depending on the compound group (Table 1). Albeit descending, the total concentrations of ethyl esters of fatty acids were not statistically significant within the first three harvest periods but ethyl (Z)-3-hexenoate consistently decreased. On the other hand, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate (ethyl esters of branched acids), hexyl acetate (higher alcohol acetate (HAA) from grape lipid degradation), total HAA from yeast sugar and nitrogen (N) metabolism, and the total of other esters, decreased in wines from H1 to H2 followed by increments in H3 wines (Table 1). Interestingly, only ethyl acetate concentrations continuously increased from H1 through H4, analogous to previously reported observations for this compound (Bindon et al., 2013), whereas with the exception of ethyl (Z)-3-hexenoate and ethyl propanoate, which remained steady, all other esters decreased significantly in wines from H4 (comprising shrivelled berries) in comparison to H3 (Table 1). The decreases of ethyl hexanoate and ethyl octanoate could be due to the observed lower availabilities of their precursors, hexanoic and octanoic acids (Table 1). Few studies have dealt with the effect of berry dehydration on wine ester concentrations, with work by Franco et al. (2004) on off-vine drying of Pedro Ximenez grapes yielding increases in ester concentrations, and a more recent

report from Šuklje et al. (2016) in line with our observations, with lower values for a range of esters, especially acetate esters like hexyl and phenylethyl acetate, coinciding with berry shrivel.

The compressed grape ripening dynamics in the 2015 vintage for this study exemplified the conditions that wine practitioners are likely to face more frequently in warm climate viticulture, namely prematurely reaching technical grape maturity, when grape sugar levels reach maximum tolerable concentrations while aroma and flavour properties are not yet fully evolved. The often-preferred solution of extending grape hang time to avoid 'green' and unripe sensory attributes in favour of mature fruit and general complexity does not apply entirely if berry shrivel occurs. Although C_6 alcohols that impart 'green' and 'herbaceous' notes decreased, IBMP ('green capsicum') increased, whereas fermentative volatiles like higher alcohols and esters significantly decreased. Hence from a volatile compositional point of view, extended grape maturation did not necessarily increase the wine's aroma potential in terms of absolute concentrations and the suitability of delaying harvest to manage this IBMP concentrations needs to be reconsidered in the context of vintages with a high risk of berry shrivel.

3.2. Effect of harvest date and berry shrivel on wine sensory properties

Previous research has shown that wine sensory quality changes only marginally with further grape maturation after passing a certain level of grape maturity (Heymann et al., 2013). This point accords with the sensory evaluation carried out in the present study, where six out of ten wine aroma attributes changed significantly from the first to the second harvest date but remained unchanged from H2 onwards (13.5% ABV) (Tables 2 and S6 of the Supporting Information), despite the occurrence of berry shrivel at H4 (18.2% ABV) and a difference in alcohol level of almost 5% ABV. Given that the sought-after aroma descriptors of 'aroma intensity' and 'dark fruit' did not significantly change with later harvests and nor did 'green' aroma sensations (Table S6 of the Supporting Information), extending grape hang-time to improve 'fruit' flavour while minimising 'green' characters proved ineffectual in this case. However, the DA panel appeared to rate the H4 wines higher in

Table 2
Average scores for significantly different (p ≤ 0.05) wine sensory descriptors for the harvest series wines (sample codes given in Fig. S1 of the Supporting Information).[a]

Descriptor	Wine from consecutive harvest dates					
	H1	H2	H3	H4 (Control)	LSD	*p-value*
Aroma						
Aroma intensity	51.1b	57.4ab	60.4a	60.3a	7.0	0.0371
Dark fruit	38.3b	50.4a	59.6a	57.5a	10.9	0.0015
Dried fruit/jam	29.8b	43.9a	47.2a	54.6a	11.5	0.0011
Liquorice	20.4c	25.5bc	32.6ab	30.0a	7.5	0.0039
Chocolate	18.5b	26.3ab	28.6a	33.7a	8.8	0.0120
Port wine	15.0b	25.4ab	24.7ab	34.2a	10.7	0.0094
Palate						
Flavour intensity	47.6c	54.3b	63.7a	68.5a	6.1	< 0.0001
Dark fruit	34.9d	48.4c	59.3b	68.2a	8.6	< 0.0001
Dried fruit/jam	24.1c	32.9c	45.6b	64.8a	8.9	< 0.0001
Green	48.6a	47.9a	43.8a	35.7b	7.9	0.0083
Sweet spice	20.6c	26.9c	35.5b	47.1a	6.6	< 0.0001
Confection	22.1b	24.0b	29.0ab	31.9a	7.1	0.0307
Liquorice	16.4c	23.0b	29.1b	39.6a	6.5	< 0.0001
Chocolate	12.9c	17.4bc	24.3b	34.3a	7.7	< 0.0001
Port wine	13.9b	16.4b	19.6b	38.8a	8.6	< 0.0001
Body	31.2d	42.8c	52.5b	64.5a	8.9	< 0.0001
Sweetness	16.0c	20.6c	29.3b	53.6a	7.3	< 0.0001
Astringency	28.9c	49.9b	58.6ab	63.1a	8.7	< 0.0001
Hotness	25.3d	42.9c	55.5b	67.3a	9.0	< 0.0001

[a] Values are means of 3 replicates. Values followed by different letters within a row are significantly different (p ≤ 0.05, one way ANOVA, post hoc Fisher's LSD).

'port wine' aroma compared to the wines made from earlier harvested grapes, which was indicative of an overripe sensory character arising from this last harvest date where berry shrivel was evident.

Regarding palate attributes, greater differences were observed according to the maturity level of the grapes, affecting 13 out of 16 descriptors, particularly with the last harvest date. Noticeably, 'green' sensations significantly dropped in H4 wines after remaining unchanged among the first three harvests, whereas 'dark fruit', 'dried fruit/jam', 'sweet spice', 'confection', 'liquorice', 'chocolate' and 'port wine' gained importance in the wine profile (Table 2). Aside from the changes among different descriptors that may infer a change in the style of the wine, the overall 'flavour intensity' was not affected from H3 to H4 (Table S6 of the Supporting Information). Interestingly, the decrease in 'green' flavour perception was apparently not associated with the IBMP concentration, which in fact increased in the last harvest date, H4 (see Table 1). Rather, the decrease in 'green' flavour could be a consequence of the lower C_6 alcohol concentrations – these are associated with similar sensory characters (Waterhouse et al., 2016b) – and (*Z*)-3-hexen-1-ol in particular significantly correlated with 'green' ratings on the palate (r = 0.691). As for the four remaining palate attributes ('body', 'sweetness', 'astringency' and 'hotness'), which all increased with ripening, 'hotness' positively correlated with wine alcohol concentrations (r = 0.963), even though the DA panel could clearly distinguish the wines produced from shrivelled grapes (emphasising an overripe character) with higher ratings for 'port wine' flavour (rated twice as high as in H3 with only four days of harvest difference). Despite the increments in total tannin concentration and tannin molecular mass in H4 reported previously (Schelezki et al., 2018), which might be expected to influence mouthfeel attributes, astringency perception did not increase significantly from H3 to H4 (Table 2).

3.3. Influence of blending treatments on wine volatile composition

Pre-fermentative incorporation of GHW (wines B1-B3) or water (wines Bw1-Bw3) in different proportions into H4 must after removing a proportionate amount of juice led to wines with different alcohol levels, from a high of 18.2% ABV in H4 to approximately 14.5, 15.9 and 17.2% ABV in B1/Bw1, B2/Bw2, B3/Bw3, respectively (Schelezki et al., 2018). The choice of blending component had different outcomes on wine volatiles: replacement of juice with GHW led to changes in the concentrations of 19 of the 43 assessed volatiles relative to the control (Tables 3 and S7 of the Supporting Information), whereas substitution with water changed only 11 (Tables 4 and S8 of the Supporting Information).

3.3.1. Pre-fermentative implementation of the green harvest wine

Inherent with the pre-fermentative implementation of GHW, higher total concentrations of grape-derived isoprenoids and C_6 alcohols were observed in the respective wines (B1-B3) relative to the H4 control (Table 3). However, after a sharp initial increase with the lowest addition rate, these total concentrations generally did not change further with higher rates of addition of GHW. Taken individually, relationships can been observed between high GHW implementation rates and changes in linalool and β-damascenone (r = 0.667 and −0.640, respectively) as well as in (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol and 2-ethyl-1-hexanol (r = 0.868, 0.981 and 0.872, respectively). In the main, these modifications could likely be attributed to more abundant concentrations of the respective compounds present in the GHW matrix (and no significant change was evident for the water blends, Section 3.3.2). Among the C_6 alcohols, 1-hexanol was the exception, where no change occurred among the B1-B3 wines, although H4 was significantly lower (as it was with the harvest series). Despite the changes being considerable in some cases, all concentrations remained well below the detection thresholds of these compounds (Table S4 of the Supporting Information). Interestingly from a winemaker's perspective, the excessive charcoal treatment of GHW as part of the winemaking process

(Schelezki et al., 2018) did not entirely remove grape-derived volatiles (or precursors) and consequently influenced the grape-derived volatiles of wines B1–B3. Nonetheless, IBMP concentration in the GHW was similar to the levels measured in the H4 wine (Table S5 (and S7) of the Supporting Information), hence GHW implementation did not mediate alterations of this methoxypyrazine in these wines.

Whereas changes in concentration of grape-derived volatiles showed a sound relationship with the amount of GHW employed, only hexanoic and octanoic acids followed a similar trend among the fermentative volatiles (Table 3), with proportional increases according to the amount of GHW added (r = 0.793 and 0.740, respectively). These volatile acids also increased relative to the control when water was applied (Section 3.3.2), but the water blends tended to contain lower concentrations than their GHW counterparts. This discrepancy may be explained by the GHW containing twice the concentration of hexanoic acid and eight times the concentration of octanoic acid compared to the control (Table 3).

In contrast to the acids, levels of higher alcohols and esters did not follow such a pattern (Table 3). Indeed, 3-methyl-1-pentanol and 3-methylthio-1-propanol concentrations tended to be greater, and 1-butanol lower, compared to the control without changing further in wines B1-B3. The total content of ethyl esters of fatty acids and other esters increased as well among the GHW treatments relative to the control, tending towards a higher level in B2 due to fluctuations among the individual compounds. Ethyl esters of branched acids did not differ significantly compared to H4, but noticeable variations of ethyl 2-methylpropanoate and ethyl dodecanoate at different alcohol levels were observed. Solely γ-butyrolactone decreased in a continuous manner with higher added amounts of GHW (in which this compound was minimal), a trend that was also observed among the water blended wines, although it was non-significant with p = 0.058 (see Table S8 of the Supporting Information). The results for γ-butyrolactone may point to the removal of grape-derived precursors (present in H4) with the juice substitution process but the importance of this compound to wine sensory is deemed to be low (Clarke & Bakker, 2004b).

3.3.2. Pre-fermentative implementation of water

Less changes in the wine volatile composition were observed relative to the control when water was employed as blending component, significantly affecting only 11 of 43 analysed compounds (Tables 4 and S8 of the Supporting Information). Interestingly, regarding grape-derived volatiles, only IBMP was significantly different, being lower in wines Bw2 and Bw3 compared to the control. According to Roujou de Boubee, Cumsille, Pons, and Dubourdieu (2002), IBMP is foremost present in the berry skin (aside from stems) and maximum extraction during Cabernet Sauvignon vinification happened during the first 24 h, with almost no subsequent changes despite different cap management techniques. Some extracted IBMP could be removed with a juice during the substitution process, although in our case there was ultimately no practical difference among the treatments in terms of potential aroma impact, with IBMP determined at 9–10 ng/L.

Similarly to that observed for the GHW treatments, concentrations of volatile acids generally increased with the amount of water employed (Tables 4 and S8 of the Supporting Information), with r values of 0.702 for total acids and 0.899 and 0.821 for hexanoic and octanoic acids, respectively. Clarification of juice prior to fermentation and the inherent depletion of sterols or unsaturated fatty acids has been shown to increase medium chain fatty acids present in wines (Waterhouse, Sacks, & Jeffery, 2016a), and the partial removal of grape juice for the substitution treatments could have been responsible for the observed increments in hexanoic and octanoic acids. In the same fashion, total higher alcohol concentrations among the water treatment wines were greater in Bw1–Bw3 compared to the control. Both water and GHW caused significant increases in 3-methylthio-1-propanol but 3-methyl-1-butanol and 2-phenylethanol were only significantly different (and also higher than the control) exclusively in the water treatment wines. 2-

Table 3
List of wine volatile compounds that significantly changed (p ≤ 0.05) with different implementation rates of green harvest wine (GHW, H0) (substitution rates of 43.7, 27.3 and 13.6% v/v resulting in 14.4, 15.8 and 17% ABV in B1, B2 and B3 wines, respectively; sample codes given in Fig. S2 of the Supporting Information).[a]

	GHW blending treatment					
	B1	B2	B3	H4 (Control)	H0	p-value
Grape-derived						
Isoprenoids [µg/L]						
Linalool	2.24 ± 0.14a	1.91 ± 0.13b	1.93 ± 0.10b	1.51 ± 0.05c	25.1	0.001
β-Damascenone	0.57 ± 0.03bc	0.48 ± 0.00c	0.86 ± 0.12a	0.68 ± 0.03b	0.56	0.002
Total isoprenoids	2.91 ± 0.17a	2.39 ± 0.13b	2.78 ± 0.13a	2.19 ± 0.08b		0.003
C6 alcohols [µg/L]						
1-Hexanol	2778 ± 34a	2848 ± 180a	2728 ± 84a	2462 ± 14b	1901	0.023
(E)-2-hexen-1-ol	46.9 ± 1.7a	40.1 ± 1.0b	38.9 ± 1.6b	38.0 ± 0.7b	82.1	0.001
(Z)-3-hexen-1-ol	121 ± 9a	74.8 ± 3.3b	47.1 ± 1.0c	36.5 ± 1.6c	600	< 0.0001
2-Ethyl-1-hexanol	9.61 ± 0.23a	8.66 ± 0.81a	7.14 ± 0.35b	4.50 ± 0.15c	75.9	< 0.0001
Total C6 alcohols	2955 ± 28a	2972 ± 180a	2821 ± 85a	2541 ± 12b		0.009
Fermentation-derived						
Acids[µg/L]						
Hexanoic acid	6955 ± 354a	6543 ± 193ab	6160 ± 153b	4725 ± 121c	11,335	< 0.00001
Octanoic acid	1691 ± 322a	1245 ± 295ab	918 ± 239b	719 ± 44b	5775	0.021
Total acids	8646 ± 670a	7789 ± 254ab	7078 ± 348b	5444 ± 164c		0.0003
Higher alcohols [µg/L]						
1-Butanol	6.11 ± 0.53b	5.83 ± 0.19b	6.94 ± 0.33ab	8.20 ± 0.90a	0.92	0.012
3-Methyl-1-pentanol	134 ± 19ab	145 ± 1a	161 ± 21a	109 ± 1b	12.4	0.036
3-Methylthio-1-propanol	4164 ± 397a	4173 ± 145a	4274 ± 216a	3483 ± 53b	240	0.037
Total higher alcohols	4304 ± 401a	4324 ± 143a	4442 ± 212a	3601 ± 54b		0.029
Ethyl esters of branched acids [µg/L]						
Ethyl 2-methylpropanoate	38.1 ± 6.0a	27.6 ± 1.9b	42.1 ± 3.1a	40.3 ± 3.4a	48.3	0.023
Ethyl dodecanoate	13.0 ± 1.9c	27.0 ± 5.4a	21.9 ± 1.3ab	16.1 ± 2.2bc	3.87	0.004
Total esters of branched acids	51.1 ± 4.4ab	45.6 ± 12.3b	64.1 ± 4.4a	56.4 ± 1.2ab		0.126
Ethyl esters of fatty acids [µg/L]						
Ethyl hexanoate	984 ± 93ab	1250 ± 247a	1086 ± 276a	590 ± 48b	1994	0.045
Ethyl (Z)-3-hexanoate	0.65 ± 0.09b	1.26 ± 0.09a	0.77 ± 0.16b	0.64 ± 0.04b	0.57	0.001
Ethyl octanoate	713 ± 142ab	941 ± 197a	731 ± 27ab	489 ± 20b	419	0.038
Total ethyl esters of fatty acids	1697 ± 234ab	2192 ± 437a	1818 ± 276a	1080 ± 69b		0.027
Other esters [µg/L]						
Methyl octanoate	5.52 ± 0.20ab	7.39 ± 2.02a	5.0 ± 0.2b	3.40 ± 0.07b	n.d.	0.027
3-Methylbutyl hexanoate	1.84 ± 0.17ab	2.20 ± 0.60a	1.67 ± 0.09ab	1.19 ± 0.01b	0.23	0.047
Total other esters	7.37 ± 0.36ab	9.60 ± 2.57a	6.63 ± 0.26ab	4.60 ± 0.07b		0.030
Lactones [µg/L]						
γ-Butyrolactone	7.28 ± 0.21c	8.51 ± 1.48bc	14.9 ± 3.5a	12.6 ± 0.7ab	0.57	0.015

[a] Except for H0, values are means of 3 replicates ± standard error. Values followed by different letters within a row are significantly different (p ≤ 0.05, one way ANOVA).

Phenylethanol is particularly interesting as it has recently been identified as a quorum sensing molecule for *Saccharomyces cerevisiae* (Avbelj, Zupan, & Raspor, 2016), which coordinates the collective adaptation of yeasts to changing environments. Fermentation conditions eliciting the production of 2-phenylethanol might include low yeast available nitrogen concentrations. Indeed, an inhibitory effect on the fermentation efficiency of yeasts was already observed for the water blending treatments in comparison to the GHW treatments (Schelezki et al., 2018) and related to differences in yeast available nitrogen levels according to the blending component. A direct effect of the changing 3-methyl-1-butanol and 2-phenylethanol concentrations (Table 4) on wine quality is unlikely, however, as neither of these higher alcohols were found to be directly involved in determining red wine qualities (Ferreira et al., 2009). Nonetheless, an indirect contribution to the aroma quality could be possible (Waterhouse et al., 2016b), particularly for Cabernet Sauvignon wines (Forde et al., 2011). Finally, the concentrations of five fermentation-derived esters were seen to change when substituting with water compared to the control. Significantly elevated levels of ethyl hexanoate, ethyl (Z)-3-hexenoate, methyl octanoate, 3-methylbutyl hexanoate and diethyl succinate meant that total concentrations of ethyl esters of fatty acids and other esters increased similarly in Bw1 and Bw2, but not in Bw3 with the lowest rate

of water addition. With the exception of diethyl succinate, similar increments of these compounds were also observed among the GHW wines, implying that their formation was rather sensitive to the variation in TSS and final alcohol levels than by the alternation of other juice constituents.

3.4. Influence of blending treatments on wine sensory properties

The pre-fermentative substitution of juice with either GHW or water markedly decreased the alcohol level of an overripe Cabernet Sauvignon crop while only marginally influencing the volatile composition, as demonstrated in the present work. Importantly, the employment of water had the least effect on the volatile profiles of the wines compared to the control, even with substitution rates of 32% v/v. A final aspect was to examine how the sensory qualities of the wines were affected following the different substitution approaches, using a descriptive analysis panel.

According to the panellists, the wines resulting from the blending treatments differed significantly in only three aroma attributes (Tables 5 and S9 of the Supporting Information). The ratings for 'liquorice' and 'port wine' were similar to those perceived in the H4 wine, with the exception being Bw1, which had a significantly lower impact of 'port

Table 4
List of wine volatile compounds that significantly changed (p ≤ 0.05) with different implementation rates of water (substitution rates of 32, 19.9 and 10.1% v/v resulting in 14.7, 16 and 17.4% ABV in Bw1, Bw2 and Bw3 wines, respectively; sample codes given in Fig. S2 of the Supporting Information).[a]

	Water blending treatment				
	Bw1	Bw2	Bw3	H4 (Control)	*p-value*
Grape-derived					
Methoxypyrazines [ng/L]					
IBMP	10.1 ± 0.5ab	9.07 ± 0.55b	8.98 ± 0.67b	10.5 ± 0.3a	0.041
Fermentation-derived					
Acids [µg/L]					
Hexanoic acid	6380 ± 681a	5475 ± 395ab	4593 ± 649b	4725 ± 121b	0.029
Octanoic acid	1676 ± 370a	839 ± 208b	929 ± 61b	719 ± 43b	0.007
Total acids	8056 ± 1046a	6035 ± 815b	5212 ± 1085b	5444 ± 164b	0.039
Higher alcohols [µg/L]					
3-Methyl-1-butanol	446887 ± 6745a	435054 ± 23366a	454585 ± 8197a	397197 ± 2115b	0.009
3-Methylthio-1-propanol	5173 ± 497a	4325 ± 421ab	5317 ± 632a	3483 ± 52b	0.013
2-Phenylethanol	139330 ± 3191a	117480 ± 4093b	129899 ± 4332a	91056 ± 6480c	< 0.0001
Total higher alcohols	591390 ± 8388a	556859 ± 25964b	589801 ± 3825ab	491737 ± 8648c	0.0004
Ethyl esters of fatty acids [µg/L]					
Ethyl hexanoate	1018 ± 204a	1099 ± 131a	651 ± 47b	590 ± 49b	0.008
Ethyl (Z)-3-hexenoate	0.80 ± 0.19ab	1.00 ± 0.18a	0.58 ± 0.09b	0.64 ± 0.04b	0.046
Total ethyl esters of fatty acids	1019 ± 204a	1099 ± 130a	652 ± 47b	591 ± 49b	0.008
Other esters [µg/L]					
Methyl octanoate	7.32 ± 1.64a	6.16 ± 0.77ab	4.23 ± 0.05bc	3.40 ± 0.07c	0.009
3-Methylbutyl hexanoate	2.06 ± 0.47a	2.01 ± 0.22a	1.32 ± 0.10b	1.19 ± 0.01b	0.020
Diethyl succinate	1110 ± 262ab	1483 ± 361a	731 ± 118b	781 ± 92b	0.042
Total other esters	1119 ± 264ab	1491 ± 362a	737 ± 118b	786 ± 92b	0.042

[a] Values are means of 3 replicates ± standard error. Values followed by different letters within a row are significantly different (p ≤ 0.05, one way ANOVA).

wine' aroma. Whereas Bw1 and B1 were not distinguishable, the lower rating for 'port wine' in B1 did not result in a significant difference to the control. The perceptions of 'liquorice' and 'chocolate' differed among the blending treatments but only B1, Bw1 and Bw2 wines were significantly different in 'chocolate' compared to the control (Table 5).

As with the harvest series wines presented in Section 3.2, greater differences were perceived on the palate, significantly affecting 12 out of 16 attributes (Tables 5 and S9 of the Supporting Information). This was almost exclusively confined to the wines with the highest substitution rates, i.e., B1 and Bw1. Regarding 'flavour intensity', a high proportion of GHW was necessary to significantly decrease the rating of this characteristic in the B1 wine, whereas no effect was present in B2, and B3 was not different from B1 or B2. Using water, a significant

decline of this attribute was already perceived in the Bw2 wine compared to the control, but the DA panel could not further distinguish between the two blending components at equal alcohol concentrations. Similarly, the intensities of desirable traits of 'dark fruit', 'sweet spice', 'liquorice' and 'chocolate' decreased (to comparable levels) with the lower wine alcohol concentrations regardless of the blending component employed, with the exception of B2 being lower rated in 'dark fruit' than Bw2 at the same alcohol concentration. These results are somewhat similar to a previous study (Sherman, Greenwood, Villas-Boâs, Heymann, & Harbertson, 2017), in which partial replacement of juice with water lowered the 'fruity' character of Merlot wines.

As much as winemakers would seek the conservation of positive aroma attributes in wines with the treatments assessed in this study,

Table 5
Average scores for significantly different (p ≤ 0.05) wine sensory descriptors for the blending treatment wines compared to the H4 (Control) wine (sample codes given in Fig. S1 of the Supporting Information).[a]

Descriptor	Wines from blending treatments								
	H4 (Control)	B1	B2	B3	Bw1	Bw2	Bw3	LSD	*p-value*
Aroma									
Liquorice	33.0ab	28.2b	37.9a	35.8a	33.9a	28.3b	35.0a	5.4	0.0032
Chocolate	33.7a	25.3b	34.3a	36.2a	26.4b	26.9b	33.6a	6.6	0.0034
Port wine	34.2ab	25.9bc	37.2a	34.0ab	24.3c	33.2ab	30.6abc	8.5	0.0333
Palate									
Flavour intensity	68.5a	59.4c	64.4ab	63.4bc	62.2bc	63.2bc	66.8ab	4.6	0.0062
Dark fruit	68.2a	59.8c	60.1c	66.0ab	61.3bc	61.2bc	65.4ab	5.0	0.0045
Dried fruit/jam	64.8a	42.9c	47.6c	61.5ab	48.1c	56.6b	61.5ab	8.0	< 0.0001
Green	35.7b	46.8a	44.4a	40.5ab	45.2a	43.1a	41.7ab	6.8	0.0451
Sweet spice	47.1a	31.6c	39.1b	38.0bc	35.7bc	36.5bc	40.1b	6.5	0.0011
Liquorice	39.6a	27.4c	35.6ab	39.1a	31.1bc	30.1bc	34.9ab	5.6	0.0001
Chocolate	34.3a	21.7c	28.3abc	29.9ab	24.8bc	26.8bc	31.4ab	6.8	0.0104
Port wine	38.8a	20.6b	32.7a	34.2a	22.5b	31.4a	34.5a	7.5	< 0.0001
Body	64.5a	51.9d	58.2bc	61.3ab	55.3cd	58.8bc	61.9ab	5.7	0.0007
Sweetness	53.6a	25.4cd	31.7bc	35.4b	22.9d	31.4bc	32.7b	6.8	< 0.0001
Acidity	54.0b	63.1a	61.6a	57.0ab	52.0b	57.2ab	57.1ab	6.6	0.0194
Hotness	67.3a	54.1b	64.1a	66.9a	51.3b	63.4a	67.6a	7.4	< 0.0001

[a] Values are means of 3 replicates. Values followed by different letters within a row are significantly different (p ≤ 0.05, one way ANOVA, post hoc Fisher's LSD).

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limiting negative attributes associated with a shrivelled, high sugar crop would be another aim. This, however, was seemingly not an outcome of our study. Only the highest substitution rates with either GHW or water were able to significantly lower the perception of 'hotness' and 'port wine' on the palate, and the DA panel still scored these attributes in wines B1/Bw1 (14.4%/14.7% ABV) similarly to wine H3 (15.1% ABV), which had a slightly higher ethanol concentration. While this is a meaningful result, it remains to further examine wine alcohol management via pre-fermentative juice substitution at different grape maturities without the influence of grape over-maturity.

Interestingly, 'green' attributes were significantly more dominant in the wines B1/Bw1 and B2/Bw2 compared to the control, with similar ratings across all blending treatments and commensurate with wines H1-H3 (Section 3.2, Table 2). The results are reminiscent of the observations made by Sherman et al. (2017), where alcohol-adjusted wines (via water substitution) from overripe Merlot grapes had more pronounced 'vegetal' and 'sour' characters. In that case, a dilution effect or alternated aroma precursor extraction (for volatile compounds that would impart 'fruity' characteristics), implied from the pre-fermentative TSS adjustments, was hypothesised. In the present study, however, no dilution of grape-derived volatiles could be observed: rather, it was the contrary, possibly as a result of changes in the composition of other volatiles.

In line with slight differences in residual sugar levels (Schelezki et al., 2018), wines arising from the blending treatments (less than 1 g/L of residual sugar except for Bw3, which had 1.48 g/L of fructose) were perceived as less 'sweet' compared to H4 (2.67 g/L of residual fructose). Of further note were the decreasing ratings of 'sweetness' in the wines with lower alcohol concentrations (Table 5) despite equal residual sugar levels (Schelezki et al., 2018). This might be a result of the impact of fermentation conditions, particularly the elevated ethanol concentrations, on the expression of *HSP12* (heat-shock protein) genes and/or an effect of yeast lees on the perception of sweetness, as previously reported (Marchal, Marullo, Durand, Moine, & Dubourdieu, 2015). Furthermore, despite equal pH values among the lower alcohol wines, the implementation of GHW had an enhancing effect on perceived 'acidity' in comparison to the wines resulting from water addition, which could be explained by the higher malic acid concentrations (Clarke & Bakker, 2004a) in B1-B3 (3.85–2.71 g/L, respectively) originating from the GHW wine, as opposed to averaging 2.25 g/L in Bw1-Bw3 (Schelezki et al., 2018).

3.5. Principal component analysis of sensory attributes and important wine volatile and non-volatile constituents

Principal component analysis (PCA) was performed on the sensory data of the consecutive harvest and blended wines (Fig. 1 shows mean scores of triplicate wines; see Fig. S2 of the Supporting Information for the respective replicate clustering) supplemented with wine compositional data. This included wine volatiles that were significant for both the consecutive harvest and blending treatment wines, and with non-volatile components presented in a previous publication, namely malic acid, fructose, colour intensity, tannin molecular mass by gel permeation chromatography (MM GPC), total tannin concentration, and total soluble monosaccharide concentration (Schelezki et al., 2018) (Fig. 1). The first two components accounted for 92.82% of total variance, with wines mainly being separated along the first principal component, from left to right according to harvest date. A slight divergence according the treatment may be further ascertained along the second principal component, which mainly separates H1, H4 and B3 from the other wines. Generally, wines from later harvest dates were characterised by increasingly higher 'flavour intensity', 'dark fruit' and 'hotness', whereas the 'green' contribution diminished. Two extremes are formed by H1 and H2 on the left (low ratings in many sensory attributes) and H4 on the right (characterised by higher ratings for 'port wine' and 'confection' flavour and the like), whereas the blending treatments are mainly

grouped between the wines of H3 and H4. With the decreasing alcohol levels, blending treatment wines became less associated with overripe characters such as 'port wine' or 'dried fruit/jam', moving away from H4 towards H3, but only at the highest substitution rates of GHW or water (i.e., B1/Bw1) were there close associations with the H3 wine.

Interestingly, only a few volatiles appeared to be associated with the sensory attributes that differentiated the wines; 1-butanol was closely associated with flavour attributes like 'sweet spice', 'liquorice', and 'chocolate', clearly distinguishing B3/Bw3 and particularly control wine H4 from the remaining treatments. In line with previous associations of ethyl acetate, 2-heptanol, and benzyl alcohol with Cabernet Sauvignon sensory characteristics (Forde et al., 2011), these compounds were closely associated with 'flavour intensity', 'hotness', 'body' and 'dark fruit'; ethyl propanoate, nerolidol and 1-nonanol were further identified as important drivers in the present study. As expected, the total tannin concentration appeared to be a sound predictor of 'bitterness' and 'astringency' (Ma et al., 2014; Ristic, Bindon, Francis, Herderich, & Iland, 2010) and further related closely to colour intensity, as reflected in the changing phenolic composition of harvest series wines whereas these parameters remained unchanged with the substitution treatments. Interestingly, β-damascenone and ethyl 2-methylpropanoate, compounds known to enhance 'aroma' intensities and 'fruity' perceptions (Pineau et al., 2007; Sumby, Grbin, & Jiranek, 2010), were opposite to aroma intensity, which appears counterintuitive. Given the overripe context of the wines in this study, and the apparent importance to fruity characters of other volatiles such as 1-butanol and nerolidol located far to the right of PC1, the relatively small concentration differences of β-damascenone in conjunction with its sub-threshold presence (based on red wine threshold data) may have limited its importance in driving the sensory characteristics in this case.

4. Conclusion

Managing the alcohol content of wines has been a key interest for the wine industry in recent decades, and one obvious way is to limit sugar accumulation in the maturing fruit. However, climatic changes are driving the more frequent occurrence of challenging vintage conditions such as compressed grape ripening behaviour, where grape sugar levels may develop excessively beyond the control of viticulturists and winemakers. This necessitates new solutions to manage wine alcohol content, and has led to changes to winemaking regulations in Australia and elsewhere that allow for the pre-fermentative addition of water to adjust high must sugar concentrations.

Building on previous research into the effects on wine non-volatile composition arising from a sequential harvest series versus pre-fermentative substitution, with GHW or water, of juice arising from overripe and shrivelled Cabernet Sauvignon crop, this follow-up study has provided knowledge of the effects of such manipulations on volatile composition and wine sensory properties of the wines. Grape derived volatiles critical for 'vegetal' sensory characteristics declined with later harvest dates, which was mirrored by lower 'green' ratings in the sensory analysis. In contrast, concentrations of fermentation-derived volatiles generally did not follow a particular trend among the first three harvest dates but declined with the last harvest date, which was affected by severe berry shrivel and over-ripeness. Consequently, 'aroma intensity', 'dark fruit' and other attributes were not perceived higher in wines from H2 (in some cases from H3) on, but H4 was remarkably associated with 'hotness' and 'port wine' characteristics at an alcohol concentration of more than 18% ABV.

Of the options evaluated to manage this overripe crop, employing juice substitution with water had the least effect on the analysed volatiles and any changes were apparently driven by differences in yeast metabolism rather than by grape-derived volatiles. On the other hand, treatments involving GHW appeared to influence the composition of the resultant wines, particularly regarding volatile acids and grape-derived C_6 alcohols, but the charcoal fining applied to the GHW appeared to

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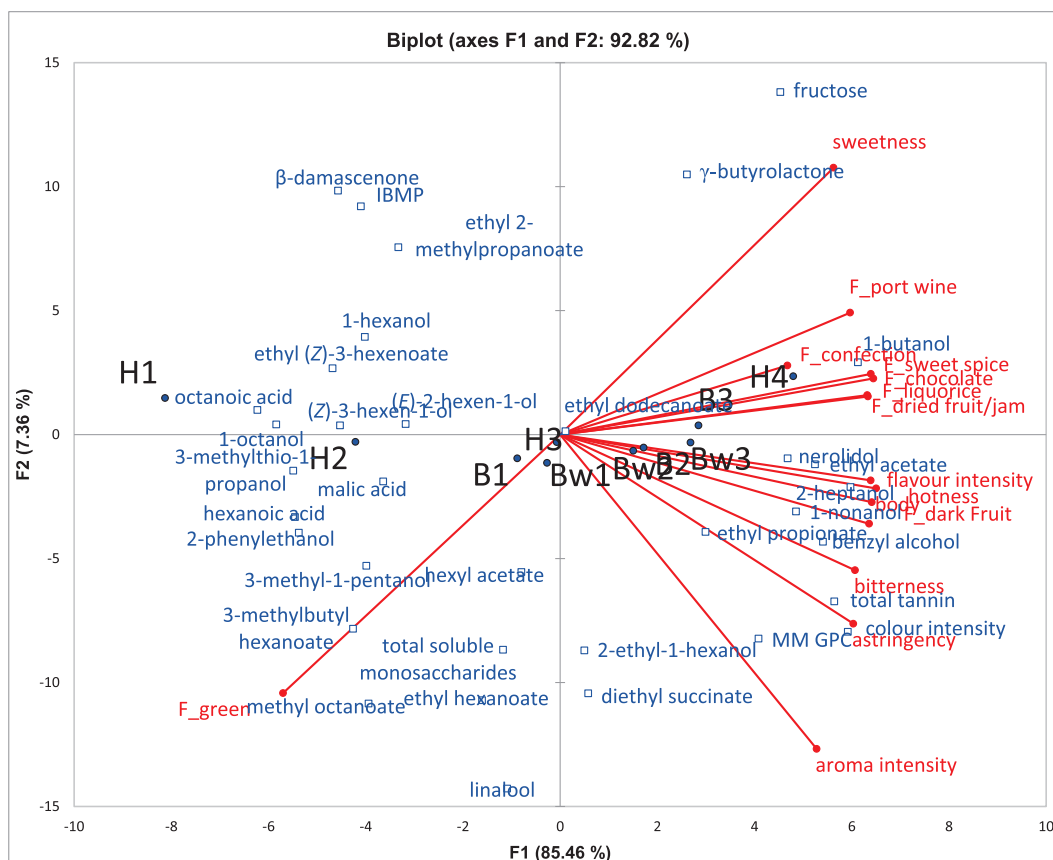
Fig. 1. PCA bi-plot of the significantly different attributes resulting from the descriptive analysis panel (red) and volatile and non-volatile compositional parameters (blue) for harvest series (H) and blending treatment (B for GHW blends, Bw for water blends) wines (sample codes given in Figs. S1 and S2 of the Supporting Information). The F_ prefix designates flavour attributes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

have eliminated excessive 'green' sensory characters. In addition to the maintained tannin concentrations noted previously, and hence high 'astringency' ratings revealed herein, the relatively limited changes in volatile composition have potentially led to overripe sensory characteristics of the last harvest date prevailing within the blending treatments. As such, only the highest substitution rates (leading to alcohol concentrations below those of wine H3) could lessen the 'hotness' perception, leading to the conclusion that harvest date had a more detrimental influence on the wine sensory profiles than the employed blending treatments.

The management of an overripe crop via the pre-fermentative juice substitution with water appeared to be more benign and therefore particularly suitable compared to the use of the GHW, given that the changes in final wine compositional and sensory qualities were less pronounced (and hence predictable), and could be implemented at lower cost than the provision of GHW as blending component (given the additional harvest, winemaking and storage). In the context of overripeness, however, negative sensory characteristics like 'hotness' and 'port wine' (at least in terms of dry table wine) associated with H4 prevailed in the wines with lower alcohol levels produced with the proposed treatments for alcohol management, which is an important consideration as winemakers may still face problems with the marketability of such wines even though the ethanol levels may be successfully adjusted. In comparison, wines from earlier harvested grapes were not necessarily of inferior quality so delaying harvest to seek riper (but not overripe) fruit characters in the context of compressed vintages needs very careful consideration and control. Further studies are still necessary to assess wine quality implications of the presented approaches under less severe vintage conditions (i.e., in the absence of berry

shrivel) and with additional varieties to provide greater understanding about the best way to manage potential wine alcohol content.

Conflict of interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.03.118.

References

Avbelj, M., Zupan, J., & Raspor, P. (2016). Quorum-sensing in yeast and its potential in wine making. *Applied Microbiology and Biotechnology, 100*(18), 7841–7852.

Bindon, K., Holt, H., Williamson, P. O., Varela, C., Herderich, M., & Francis, I. L. (2014). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry, 154*, 90–101.

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry, 138*(1), 1696–1705.

Boss, P. K., Pearce, A. D., Zhao, Y. J., Nicholson, E. L., Dennis, E. G., & Jeffery, D. W. (2015). Potential grape-derived contributions to volatile ester concentrations in wine. *Molecules, 20*(5), 7845–7873.

Bui, K., Dick, R., Moulin, G., & Galzy, P. (1986). A reverse osmosis for the production of low ethanol content wine. *American Journal of Enology and Viticulture, 37*(4), 297–300.

Canuti, V., Conversano, M., Calzi, M. L., Heymann, H., Matthews, M. A., & Ebeler, S. E. (2009). Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. *Journal of Chromatography A, 1216*(15), 3012–3022.

Clarke, R. J., & Bakker, J. (2004a). Basic taste and stimulant components. *Wine Flavour Chemistry* (pp. 66–116). (1st ed.). Oxford, UK: Blackwell Publishing Ltd.

Clarke, R. J., & Bakker, J. (2004b). Volatile components. *Wine Flavour Chemistry* (pp. 120–188). (1st ed.). Oxford, UK: Blackwell Publishing Ltd.

Dennis, E. G., Keyzers, R. A., Kalua, C. M., Maffei, S. M., Nicholson, E. L., & Boss, P. K. (2012). Grape contribution to wine aroma: Production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependant upon precursors in the must. *Journal of Agricultural and Food Chemistry, 60*(10), 2638–2646.

Dunlevy, J., Soole, K., Perkins, M., Dennis, E., Keyzers, R., Kalua, C., & Boss, P. (2011). Two *O*-methyltransferases involved in the biosynthesis of methoxypyrazines: Grape-derived aroma compounds important to wine flavour. *Plant Molecular Biology, 74*(1), 77–89.

Ferreira, V., San Juan, F., Escudero, A., Cullere, L., Fernandez-Zurbano, P., Saenz-Navajas, M. P., & Cacho, J. (2009). Modeling quality of premium spanish red wines from gas chromatography-olfactometry data. *Journal of Agricultural and Food Chemistry, 57*(16), 7490–7498.

Forde, C. G., Cox, A., Williams, E. R., & Boss, P. K. (2011). Associations between the sensory attributes and volatile composition of Cabernet Sauvignon wines and the volatile composition of the grapes used for their production. *Journal of Agricultural and Food Chemistry, 59*(6), 2573–2583.

Franco, M., Peinado, R. A., Medina, M., & Moreno, J. (2004). Off-vine grape drying effect on volatile compounds and aromatic series in must from Pedro Ximénez grape variety. *Journal of Agricultural and Food Chemistry, 52*(12), 3905–3910.

Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K. M., & Ross, C. F. (2009). Chemical and sensory effects of saignée, water addition, and extended maceration on high Brix must. *American Journal of Enology and Viticulture, 60*(4), 450–460.

Heymann, H., LiCalzi, M., Conversano, M. R., Bauer, A., Skogerson, K., & Matthews, M. (2013). Effects of extended grape ripening with or without must and wine alcohol manipulations on Cabernet Sauvignon wine sensory characteristics. *South African Journal of Enology and Viticulture, 34*(1), 86–99.

Houtman, A. C., Marais, J., & Du Plessis, C. S. (1980). Factors affecting the reproducibility of fermentation of grape juice and of the aroma composition of wines. I. Grape maturity, sugar, inoculum concentration, aeration, juice turbidity and ergosterol. *Vitis, 19*, 37–54.

Hranilovic, A., Li, S., Boss, P. K., Bindon, K., Ristic, R., Grbin, P. R., ... Jiranek, V. (2018). Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-*Saccharomyces inocula*. *Australian Journal of Grape and Wine Research, 24*(2), 166–180.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content

and pH of wine. *Australian Journal of Grape and Wine Research, 17*(2), 230–238.

Kotseridis, Y., & Baumes, R. (2000). Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *Journal of Agricultural and Food Chemistry, 48*(2), 400–406.

Krasnow, M. N., Matthews, M. A., Smith, R. J., Benz, J., Weber, E., & Shackel, K. A. (2010). Distinctive symptoms differentiate four common types of berry shrivel disorder in grape. *California Agriculture, 64*(3), 155–159.

Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food.* New York: Springer.

Longo, R., Blackman, J. W., Torley, P. J., Rogiers, S. Y., & Schmidtke, L. M. (2016). Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *Journal of the Science of Food and Agriculture, 97*(1), 8–16.

Ma, W., Guo, A. Q., Zhang, Y. L., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency and bitterness perception of tannins in wine. *Trends in Food Science & Technology, 40*(1), 6–19.

Marchal, A., Marullo, P., Durand, C., Moine, V., & Dubourdieu, D. (2015). Fermentative conditions modulating sweetness in dry wines: Genetics and environmental factors influencing the expression level of the *Saccharomyces cerevisiae HSP12* Gene. *Journal of Agricultural and Food Chemistry, 63*(1), 304–311.

Medina, I., & Martínez, J. L. (1997). Dealcoholisation of cider by supercritical extraction with carbon dioxide. *Journal of Chemical Technology & Biotechnology, 68*(1), 14–18.

Pineau, B., Barbe, J.-C., Van Leeuwen, C., & Dubourdieu, D. (2007). Which impact for β-damascenone on red wines aroma? *Journal of Agricultural and Food Chemistry, 55*(10), 4103–4108.

Ristic, R., Bindon, K., Francis, L. I., Herderich, M. J., & Iland, P. G. (2010). Flavonoids and C_{13}-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: Relationships between grape and wine composition, wine colour and wine sensory properties. *Australian Journal of Grape and Wine Research, 16*(3), 369–388.

Roujou de Boubee, D., Cumsille, A. M., Pons, M., & Dubourdieu, D. (2002). Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *American Journal of Enology and Viticulture, 53*(1), 1–5.

Roujou de Boubee, D., Van Leeuwen, C., & Dubourdieu, D. (2000). Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *Journal of Agricultural and Food Chemistry, 48*(10), 4830–4834.

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.

Schmidtke, L. M., Blackman, J. W., & Agboola, S. O. (2012). Production technologies for reduced alcoholic wines. *Journal of Food Science, 77*(1), R25–R41.

Sherman, E., Greenwood, D. R., Villas-Boâs, S. G., Heymann, H., & Harbertson, J. F. (2017). Impact of grape maturity and ethanol concentration on sensory properties of Washington State Merlot wines. *American Journal of Enology and Viticulture, 68*(3), 344–356.

Sidhu, D., Lund, J., Kotseridis, Y., & Saucier, C. (2015). Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Critical Reviews in Food Science and Nutrition, 55*(4), 485–502.

Šuklje, K., Zhang, X., Antalick, G., Clark, A. C., Deloire, A., & Schmidtke, L. M. (2016). Berry shriveling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical composition. *Journal of Agricultural and Food Chemistry, 64*(4), 870–880.

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry, 121*(1), 1–16.

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016a). Fatty acid metabolism. *Understanding wine chemistry* (pp. 205–2013). (1st ed.). United Kingdom: Wiley.

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016b). Higher alcohols. *Understanding wine chemistry* (pp. 51–55). (1st ed.). United Kingdom: Wiley.

Yuan, F., & Qian, M. C. (2016). Development of C_{13}-norisoprenoids, carotenoids and other volatile compounds in *Vitis vinifera* L. Cv. Pinot Noir grapes. *Food Chemistry, 192*, 633–641.

SUPPORTING INFORMATION FOR

Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties

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Table S1. Basic grape and wine compositional parameters for the different harvest dates[a]

	Wine				
	H0	H1	H2	H3	H4 (Control)
Harvest date	8 Jan	3 Feb	9 Feb	18 Feb	22 Feb
Berry weight [g/berry]	–	$1.01 \pm 0.01a$	$0.93 \pm 0.02b$	$0.87 \pm 0.04b$	$0.62 \pm 0.03c$
TSS [°Brix]	11.2	$20.5 \pm 0.1d$	$23.9 \pm 0.1c$	$27.4 \pm 0.1b$	$30.4 \pm 0.1a$
pH before adjustment	2.76	$3.03 \pm 0.03d$	$3.75 \pm 0.02c$	$4.02 \pm 0.02a$	$3.85 \pm 0.02b$
TA before adjustment[b]	20	$8.07 \pm 0.49b$	$8.62 \pm 0.2b$	$5.42 \pm 0.6c$	$9.8 \pm 0.41a$
% ABV [%v/v]	4.5	$11.4 \pm 0.03d$	$13.5 \pm 0.0c$	$15.1 \pm 0.0b$	$18.2 \pm 0.0a$
pH after adjustment	–	$3.68 \pm 0.01a$	$3.58 \pm 0.01b$	$3.67 \pm 0.02a$	$3.58 \pm 0.00b$
TA after adjustment[b]	–	$7.06 \pm 0.13b$	$7.98 \pm 0.24a$	$8.05 \pm 0.1a$	$8.13 \pm 0.03a$
Citric acid[c]	–	$0.14 \pm 0.00d$	$0.16 \pm 0.00c$	$0.31 \pm 0.00b$	$0.37 \pm 0.01a$
Tartaric acid[c]	–	$2.12 \pm 0.08ab$	$2.22 \pm 0.05a$	$2.04 \pm 0.00bc$	$1.98 \pm 0.05c$
Malic acid[c]	–	$3.46 \pm 0.05a$	$2.92 \pm 0.02b$	$2.42 \pm 0.07c$	$2.18 \pm 0.04d$
Acetic acid[c]	–	$0.33 \pm 0.02c$	$0.34 \pm 0.04c$	$0.48 \pm 0.01b$	$0.58 \pm 0.04a$
Glucose[c]	–	$0.00 \pm 0.00b$	$0.00 \pm 0.00b$	$0.04 \pm 0.00b$	$0.14 \pm 0.07a$
Fructose[c]	–	$0.00 \pm 0.00b$	$0.00 \pm 0.00b$	$0.30 \pm 0.01b$	$2.67 \pm 0.89a$
Glycerol[c]	–	$8.00 \pm 0.02c$	$9.63 \pm 0.13b$	$11.7 \pm 0.12a$	$11.8 \pm 0.05a$

[a]Values are means of 3 replicates ± standard error (except H0, which was produced without triplicates). [b]Values in [g/L] tartaric acid equivalents. [c]Values in [g/L]. Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA). Adapted from Food Chemistry, Vol 244, Olaf J. Schelezki, Paul A. Smith, Ana Hranilovic, Keren A. Bindon, David W. Jeffery, Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition, 50-59, Copyright (2017), with permission from Elsevier.

Table S2. Blending proportion using green harvest wine (GHW) or water in conjunction with H4 must, and basic wine compositional parameters for the blending treatments[a]

	Wine					
	B1	B2	B3	Bw1	Bw2	Bw3
GHW[b]	43.7	27.3	13.6	—	—	—
Water[b]	—	—	—	32	19.9	10.1
% ABV[b]	14.4 ± 0.00c	15.8 ± 0.00b	17.0 ± 0.00a	14.7 ± 0.00c	16.0 ± 0.00b	17.4 ± 0.00a
pH before adj.	3.51 ± 0.02e	3.64 ± 0.01d	3.73 ± 0.00c	3.82 ± 0.00b	3.84 ± 0.00ab	3.85 ± 0.00a
pH after adj.	3.67 ± 0.01ab	3.73 ± 0.08a	3.66 ± 0.00ab	3.67 ± 0.02ab	3.65 ± 0.03bc	3.66 ± 0.02ab
TA after adj.[c]	8.54 ± 0.16a	8.13 ± 0.2b	8.23 ± 0.18b	7.34 ± 0.07d	7.79 ± 0.06c	7.78 ± 0.02c
Citric acid[d]	0.34 ± 0.02ab	0.33 ± 0.01ab	0.36 ± 0.00a	0.25 ± 0.00c	0.32 ± 0.01b	0.32 ± 0.02b
Tartaric acid[d]	2.18 ± 0.03ab	1.91 ± 0.13de	1.84 ± 0.06e	2.31 ± 0.03a	2.12 ± 0.06bc	2.02 ± 0.07cd
Malic acid[d]	3.85 ± 0.09a	3.28 ± 0.04b	2.71 ± 0.02c	2.21 ± 0.06d	2.28 ± 0.06d	2.21 ± 0.05d
Acetic acid[d]	0.32 ± 0.03bc	0.33 ± 0.05bc	0.41 ± 0.03a	0.28 ± 0.03c	0.36 ± 0.03ab	0.35 ± 0.03abc
Glucose[d]	0.00 ± 0.00b	0.00 ± 0.00b	0.02 ± 0.00b	0.00 ± 0.00b	0.01 ± 0.01b	0.14 ± 0.05a
Fructose[d]	0.00 ± 0.00b	0.34 ± 0.04b	0.73 ± 0.25ab	0.29 ± 0.00b	0.37 ± 0.03b	1.48 ± 0.86a
Glycerol[d]	8.89 ± 0.11e	10.0 ± 0.12c	11.0 ± 0.07a	9.25 ± 0.15d	10.3 ± 0.1b	11.1 ± 0.05a

[a]Values are means of 3 replicates ± standard error. [b]Values in [% v/v]. [c]Values in [g/L] tartaric acid equivalents. [d]Values in [g/L]. Values followed by different letters within rows are significantly different (p≤0.05, one way ANOVA). adj., adjustment. Adapted from Food Chemistry, Vol 244, Olaf J. Schelezki, Paul A. Smith, Ana Hranilovic, Keren A. Bindon, David W. Jeffery, Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition, 50–59, Copyright (2017), with permission from Elsevier.

Table S3. List of attributes developed by the descriptive analysis panel to describe the sensory profiles of the wines, with respective definitions and aroma/flavour reference standards.

Descriptor	Type	Definition	Standard, mixed in 30 mL of Cabernet Sauvignon wine
Aroma intensity	Aroma	Overall intensity of the sum of all aroma attributes perceived in the wine	
Flavour intensity	Flavour	Overall intensity of the sum of all flavour attributes perceived in the wine	
Red fruit	Aroma/flavour	Smell/flavour of cherry, raspberry and strawberry	Half a strawberry, 2 canned cherries (Garden Fresh pitted cherries), 1 slice of plum and 2 raspberries
Dark fruit	Aroma/flavour	Smell/flavour of cassis, black berries	Half a tea spoon of forest berry jam (Cottee's), 1 blackberry and 2 blue berries
Dried fruit/jam	Aroma/flavour	Smell/flavour of dried fruit like raisins, dried plums, the smell of jam	1 teaspoon of minced fruit (Robertson's), 2 teaspoon tips of plum jam (Cottee's)
Green	Aroma/flavour	Smell/flavour if green capsicum and/or the smell of fresh cut grass	1 knife tip of fresh green capsicum, 2 blades of grass
Sweet spice	Aroma/flavour	Smell/flavour of cloves, cinnamon, cardamom, mixed oriental spice	Half a clove, a pinch each of mixed spice powder and nutmeg powder (Master Foods)
Confection	Aroma/flavour	Smell/flavour of confectionary	1 cm of bubble gum (Wrigleys Juicy Fruit), half a raspberry cream lolly (Allen's), one quarter of a marshmallow (Allen's), 2.5 cm of red snake lolly (Allen's)
Liquorice	Aroma/flavour	Smell/flavour of liquorice	One quarter of a stick of liquorice (Lyn-Chris Confectionary)
Chocolate	Aroma/flavour	Smell/flavour of dark chocolate	1/3 of a teaspoon chopped dark chocolate (70%, Lindt)
Port wine	Aroma/flavour	Smell/flavour of port wine (oxidised fruits, acetaldehyde, liquor wine)	1 teaspoon of Tawny dessert wine (McLaren Vale)

Body	Palate sensation	Sensation of viscosity density and weight in the mouth
Sweetness	Taste	Perceived sweetness on your tongue
Acidity	Taste	Perceived acidity on your tongue
Astringency	Palate sensation	Astringency, perceived as puckering and drying sensation on the oral mucosa
Bitterness	Taste	Perceived bitterness on the tongue
Hotness	Palate sensation	Perceived hotness that is caused by ethanol

Increase of 2% v/v alcohol by addition of food grade ethanol (98% ABV)

Table S4. Qualitative information about the analysed volatile composition.

Compound	Compound group	LRI[a]	Identifier ion [m/z]	Aroma detection threshold [µg/L][b]	Odour quality[c]
Ethyl acetate	Ethyl ester	877	61	15000 (Moyano, Zea, Moreno, & Medina, 2002)	Nail polish (Swiegers, Bartowsky, Henschke, & Pretorius, 2005)
Ethyl propanoate	Other esters	952	102	900-4500 (Clarke & Bakker, 2004b)	Sweet, ethereal, fruity (Clarke & Bakker, 2004b)
Ethyl 2-methylpropanoate	Ethyl esters of branched acids	960	116	0.01 (Clarke & Bakker, 2004b)	Sweet, rubber
2-Methylpropyl acetate	HAA from yeast sugar and N metabolism	1007	73	1600 (Sumby, Grbin, & Jiranek, 2010)	Fruit, apple, banana
Ethyl butyrate	Ethyl esters of fatty acids	1032	71	1-450 (Clarke & Bakker, 2004a)	Apple (http://www.flavornet.org)
Ethyl 2-methylbutanoate	Ethyl esters of branched acids	1049	102	1 (Guth, 1997)	Fruity, anise, strawberry (Ferreira, San Juan, Escudero, Cullere, Fernandez-Zurbano, Saenz-Navajas, et al., 2009)
Ethyl 3-methylbutanoate	Ethyl esters of branched acids	1067	88	3 (Guth, 1997)	Fruity
2-Methyl-1-propanol	Higher alcohol	1103	74	40000 (Ferreira, López, & Cacho, 2000)	Wine, solvent, bitter
3-Methylbutyl acetate	HAA from yeast sugar and N metabolism	1120	87	30 (Guth, 1997)	Banana
1-Butanol	Higher alcohol	1146	56	150000 (Etiévant, 1991)	Fusel, sweet, balsamic, whiskey (http://www.thegoodscentscompany.com)
3-Methyl-1-butanol	Higher alcohol	1201	70	30000 (Guth, 1997)	Harsh, nail polish(Swiegers, Bartowsky, Henschke, & Pretorius, 2005), fusel (Ferreira, et al., 2009)
Ethyl hexanoate	Ethyl esters of fatty acids	1217	88	14 (Ferreira, López, & Cacho, 2000)	Apple peel, fruit
Hexyl acetate	HAA from grape lipid degradation	1252	84	670 (Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004)	Fruity, floral (Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004)
Ethyl (Z)-3-hexenoate	Ethyl esters of fatty acids	1281	88	n/a	Fruity, green, sweet (Burdock, 2010)
2-Heptanol	Higher alcohol	1297	83	41-81 (Burdock, 2010)	Herbaceous, lemon (Burdock, 2010)
3-Methyl-1-pentanol	Higher alcohol	1303	84	830-1200 (Burdock, 2010)	Fruity, green (Burdock, 2010)
Ethyl heptanoate	Ethyl esters of fatty acids	1313	88	2 (Burdock, 2010)	Fruity, cognac (Burdock, 2010)
1-Hexanol	C6 alcohol	1329	69	8000 (Guth, 1997)	Green, grass (Ferreira, et al., 2009)
(E)-2-hexen-1-ol	C6 alcohol	1337	82	400 (Waterhouse, 2016b)	Green, leafy, unripe banana (http://www.thegoodscentscompany.com)

Compound	Class			Threshold (µg/L)	Aroma descriptors
(Z)-3-hexen-1-ol	C₆ alcohol	1357	82	400 (Waterhouse, 2016b)	Green, grassy, vegetable (http://www.thegoodscentscompany.com)
Methyl octanoate	Other esters	1366	127	n/a	Winy, fruity, orange (Burdock, 2010)
Ethyl octanoate	Ethyl esters of fatty acids	1416	88	20 (Swiegers, Bartowsky, Henschke, & Pretorius, 2005)	Melon, wood (Mayr, Geue, Holt, Pearson, Jeffery, & Francis, 2014)
3-Methylbutyl hexanoate	Other esters	1438	99	n/a	Cooked meat, mushroom (Mayr, Geue, Holt, Pearson, Jeffery, & Francis, 2014)
2-Ethyl-1-hexanol	C₆ alcohol	1459	83	8000 (Buttery, Turnbaugh, & Ling, 1988)	Citrus, green
Linalool	Isoprenoids	1513	93	15 (Guth, 1997)	Flower, lavender
1-Octanol	Higher alcohol	1522	84	0.7 (Clarke & Bakker, 2004b)	Chemical, metal, burnt
γ-Butyrolactone	Lactone	1585	86	35000 (Escudero, Campo, Farina, Cacho, & Ferreira, 2007)	Caramel, sweet
Ethyl decanoate	Ethyl esters of fatty acids	1604	88	200 (Ferreira, López, & Cacho, 2000)(Ferreira et al., 2000)	Floral, soap (Swiegers, Bartowsky, Henschke, & Pretorius, 2005)
1-Nonanol	Higher alcohol	1616	70	57-600 (Burdock, 2010)	Waxy citrus, earthy mushroom, creamy milk (Burdock, 2010)
Diethyl succinate	Other esters	1631	129	1250000 (Moyano, Zea, Moreno, & Medina, 2002)	Wine, fruit
3-Methylthio-1-propanol (methionol)	Higher alcohol	1665	106	1000 (Ferreira, López, & Cacho, 2000)	Sweet, potato
β-Citronellol	Isoprenoids	1715	81	100 (Guth, 1997)	Citrus
Ethylphenyl acetate	HAA from yeast sugar and N metabolism	1758	91	650 (Burdock, 2010)	Fruit, sweet
2-Phenylethyl acetate	HAA from yeast sugar and N metabolism	1758	104	250 (Guth, 1997)	Jammy, plum, floral, fruity (Mayr, Geue, Holt, Pearson, Jeffery, & Francis, 2014; Swiegers, Bartowsky, Henschke, & Pretorius, 2005)
β-Damascenone	Isoprenoids	1764	121	0.05 (Guth, 1997), 2-7 (Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2007)	Apple, rose, honey (http://www.flavornet.org)
Hexanoic acid	Acids	1787	60	420 (Ferreira, López, & Cacho, 2000)	Leafy, wood, varnish (Mayr, Geue, Holt, Pearson, Jeffery, & Francis, 2014)
Ethyl dodecanoate	Ethyl esters of branched acids	1798	88	500 (Moyano, Zea, Moreno, & Medina, 2002)	Fruity, floral (Moyano, Zea, Moreno, & Medina, 2002)
Benzyl alcohol	Higher alcohol	1811	107	1.2-1000 (Burdock, 2010)	Chemical, fruity, balsamic (Burdock, 2010)

Compound	Class	LRI[a]		Threshold[b]	Odour descriptor[c]
Phenylethyl alcohol	Higher alcohol	1844	122	14000 (Ferreira, López, & Cacho, 2000)	Floral, rose (Mayr, Geue, Holt, Pearson, Jeffery, & Francis, 2014; Swiegers, Bartowsky, Henschke, & Pretorius, 2005)
Nerolidol	Isoprenoids	1958	93	n/a	Sweet floral similar to rose and apple (Burdock, 2010)
Octanoic acid	Acids	1973	60	500 (Ferreira, López, & Cacho, 2000)	Butter, almond (Mayr, Geue, Holt, Pearson, Jeffery, & Francis, 2014)
IBMP[d] [ng/L]	Methoxypyrazines	1521	94, 151	8-16 [ng/L](Waterhouse, 2016a)	Bell pepper, vegetal (Waterhouse, 2016a)

[a] LRI, linear retention index (calculated)

[b] Thresholds for solutions in aqueous ethanol except for (Swiegers, Bartowsky, Henschke, & Pretorius, 2005), (Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2007) and (Ferreira, López, & Cacho, 2000), which were in wine matrix.

[c] If not otherwise specified, odour quality descriptors were obtained from http://www.flavornet.org

[d] Analysed using a separate method as described under Section 2.3 in the Material and Methods.

Table S5. Quantitative information for the volatiles analysed in the harvest series wines H1-H4 (Control) given in [μg/L][a], except where specified.

Compound	Harvest time			
	H1	H2	H3	H4 (Control)
Ethyl acetate	28479 ± 3334c	26378 ± 1631c	39938 ± 1313b	49026 ± 1172a
Ethyl propanoate	261 ± 39bc	252 ± 6c	354 ± 20a	313 ± 16ab
Ethyl 2-methylpropanoate	59.0 ± 1.0a	40.5 ± 2.1c	49.8 ± 5.1b	40.3 ± 3.4c
2-Methylpropyl acetate	32.2 ± 2.9a	20.6 ± 1.5b	28.0 ± 1.6a	23.5 ± 0.1b
Ethyl butyrate	145 ± 21	132 ± 19	155 ± 9	124 ± 0
Ethyl 2-methylbutanoate	7.83 ± 0.34a	6.37 ± 0.23bc	7.24 ± 0.63ab	5.70 ± 0.20c
Ethyl 3-methylbutanoate	36.5 ± 2.3	31.5 ± 0.7	37 ± 3.7	31.2 ± 2.2
2-Methyl-1-propanol	64406 ± 5169b	60602 ± 2460b	77095 ± 2567a	76085 ± 83a
3-Methylbutyl acetate	6802 ± 663ab	6084 ± 790b	8251 ± 480a	5652 ± 755b
1-Butanol	2.86 ± 0.83c	4.20 ± 0.46bc	5.50 ± 0.10b	8.19 ± 0.91a
3-Methyl-1-butanol	431036 ± 22729	422191 ± 9927	429622 ± 776	397197 ± 2115
Ethyl hexanoate	1024 ± 150a	853 ± 96a	1061 ± 60a	590 ± 49b
Hexyl acetate	19.7 ± 1.9bc	14.2 ± 1.5c	30.5 ± 1.5a	11.6 ± 1.2c
Ethyl (Z)-3-hexenoate	1.39 ± 0.18a	1.10 ± 0.11b	0.58 ± 0.04c	0.64 ± 0.04c
2-Heptanol	5.17 ± 0.12c	5.65 ± 0.34c	6.52 ± 0.33b	9.25 ± 0.18a
3-Methyl-1-pentanol	176 ± 14b	258 ± 21a	179 ± 3b	109 ± 0c
Ethyl heptanoate	1.83 ± 0.2	1.38 ± 0.18	1.64 ± 0.16	1.35 ± 0.05
1-Hexanol	3971 ± 200a	2828 ± 83b	4190 ± 100a	2463 ± 14c
(E)-2-hexen-1-ol	44.6 ± 2.9ab	41.4 ± 4.1bc	48.8 ± 2.1a	38.0 ± 0.7c
(Z)-3-hexen-1-ol	119 ± 7a	65.8 ± 5.7b	54.3 ± 2.0c	36.5 ± 1.6d
Methyl octanoate	6.95 ± 1.19a	6.62 ± 0.91a	5.49 ± 0.37a	3.40 ± 0.07b
Ethyl octanoate	802 ± 131a	802 ± 111a	723 ± 78a	489 ± 20b
3-Methylbutyl hexanoate	2.35 ± 0.28a	1.85 ± 0.22b	2.04 ± 0.19ab	1.19 ± 0.01c
2-Ethyl-1-hexanol	5.19 ± 0.36b	4.89 ± 0.33bc	6.20 ± 0.25a	4.50 ± 0.15c

Linalool	1.69 ± 0.15ab	1.88 ± 0.04a	1.77 ± 0.05a	1.51 ± 0.05b
1-Octanol	49.0 ± 4.4a	45.7 ± 3.3a	36.5 ± 2.0b	30.2 ± 1.8b
γ-Butyrolactone	9.39 ± 1.44	11.5 ± 1.8	10.8 ± 0.2	12.6 ± 0.7
Ethyl decanoate	180 ± 37	183 ± 29	172 ± 21	168 ± 6
1-Nonanol	13.6 ± 0.7b	18.2 ± 1.2a	14.5 ± 0.3b	19.3 ± 1.4a
Diethyl succinate	763 ± 115	968 ± 94	1004 ± 33	781 ± 92
3-Methylthio-1-propanol	6488 ± 86a	6032 ± 301b	5234 ± 166c	3484 ± 53d
β-Citronellol	4.85 ± 0.63a	4.14 ± 0.24ab	3.35 ± 0.1bc	3.27 ± 0.26c
Ethyl phenylacetate	8.52 ± 1.19a	7.19 ± 0.92ab	8.62 ± 0.54a	5.38 ± 0.29b
2-Phenylethyl acetate	187 ± 25a	158 ± 21ab	192 ± 11a	119 ± 8b
β-Damascenone	1.16 ± 0.10a	0.98 ± 0.07b	0.88 ± 0.03b	0.68 ± 0.03c
Hexanoic acid	7776 ± 540a	6822 ± 239b	6242 ± 74b	4725 ± 121c
Ethyl dodecanoate	20.5 ± 0.1	19.2 ± 5.0	12.2 ± 2.1	16.1 ± 2.2
Benzyl alcohol	1005 ± 112d	1193 ± 23c	1363 ± 4b	2225 ± 15a
2-Phenylethanol	159198 ± 9624a	139820 ± 6423b	135998 ± 1559b	91055 ± 6480c
Nerolidol	0.69 ± 0.1c	1.50 ± 0.40ab	1.02 ± 0.13bc	1.52 ± 0.05a
Octanoic acid	3171 ± 406a	3063 ± 168a	1008 ± 155b	719 ± 44b
IBMP [ng/L]	15.7 ± 1.4a	11.6 ± 1b	7.86 ± 0.23c	10.5 ± 0.3b

[a] 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).

Table S6. Average scores for all wine sensory descriptors used by the descriptive analysis panel for the harvest series wines.[a]

Descriptor	Wines from consecutive harvest dates				LSD
	H1	H2	H3	H4 (Control)	
Aroma					
Aroma Intensity	51.1b	57.4ab	60.4a	60.3a	7.0
Red fruit	48.8a	53.7a	51.8a	50.0a	8.1
Dark fruit	38.3b	50.4a	59.6a	57.5a	10.9
Dried fruit/jam	29.8b	43.9a	47.2a	54.6a	11.5
Green	49.2a	44.4ab	40.9b	39.7b	7.7
Sweet spice	26.1b	35.1a	33.1ab	37.9a	8.8
Confection	25.4a	30.2a	29.6a	25.6a	6.2
Liquorice	20.4c	25.5bc	32.6ab	33.0a	7.5
Chocolate	18.5b	26.3ab	28.6a	33.7a	8.8
Port wine	15.0b	25.4ab	24.7ab	34.2a	10.7
Palate					
Flavour intensity	47.6c	54.3b	63.7a	68.5a	6.1
Red fruit	50.2a	56.8a	56.8a	53.8a	11.1
Dark fruit	34.9d	48.4c	59.3b	68.2a	8.6
Dried fruit/jam	24.1c	32.9c	45.6b	64.8a	8.9
Green	48.6a	47.9a	43.8a	35.7b	7.9
Sweet spice	20.6c	26.9c	35.5b	47.1a	6.6
Confection	22.1b	24.0b	29.0ab	31.9a	7.1
Liquorice	16.4c	23.0b	29.1b	39.6a	6.5
Chocolate	12.9c	17.4bc	24.3b	34.3a	7.7
Port wine	13.9b	16.4b	19.6b	38.8a	8.7
Body	31.2d	42.8c	52.5b	64.5a	8.9

Sweetness	16.0c	20.6c	29.3b	53.6a	7.3
Acidity	61.8a	61.8a	55.2a	54.0a	10.4
Astringency	28.9c	49.9b	58.6ab	63.1a	8.7
Bitterness	34.6b	40.7ab	48.1a	48.9a	12.0
Hotness	25.3d	42.9c	55.5b	67.3a	9.0

[a] Values are means of 3 replicates. Values followed by different letters within a row are significantly different ($p \leq 0.05$, one way ANOVA, post hoc Fisher's LSD).

Table S7. Quantitative information for the volatiles analysed in the green harvest wine H0, the respective blended wines B1-B3 and the H4 (Control) given in [μg/L][a] except where specified.

Compound	GHW blended wine			H4 (Control)	H0
	B1	B2	B3		
Ethyl acetate	40536 ± 3109	45434 ± 13539	47129 ± 10428	49036 ± 1172	20023
Ethyl propanoate	342 ± 66	272 ± 18	359 ± 35	313 ± 16	164
Ethyl 2-methylpropanoate	38.1 ± 6.0a	27.6 ± 1.9b	42.1 ± 3.1a	40.3 ± 3.4a	48.3
2-Methylpropyl acetate	26.8 ± 3.2	29.9 ± 8.9	25.3 ± 5.4	23.5 ± 0.1	20.2
Ethyl butyrate	168 ± 23	192 ± 41	175 ± 46	124 ± 0	235
Ethyl 2-methylbutanoate	5.22 ± 0.57	5.8 ± 1.7	6.8 ± 1.11	5.7 ± 0.19	2.25
Ethyl 3-methylbutanoate	29.3 ± 4	30.2 ± 8	36.5 ± 5.4	31.2 ± 2.2	14.6
2-Methyl-1-propanol	66906 ± 6991	65093 ± 9446	72788 ± 2479	76086 ± 84	7182
3-Methylbutyl acetate	7676 ± 2182	9626 ± 2835	5355 ± 610	5652 ± 755	5333
1-Butanol	6.11 ± 0.53b	5.83 ± 0.19b	6.94 ± 0.33ab	8.20 ± 0.90a	0.92
3-Methyl-1-butanol	396247 ± 7638	409649 ± 37035	427390 ± 29517	397197 ± 2115	32084
Ethyl hexanoate	984 ± 93ab	1250 ± 247a	1086 ± 276a	590 ± 48b	1994
Hexyl acetate	18 ± 3.8	24 ± 5.5	18.3 ± 6.3	11.6 ± 1.3	668
Ethyl (Z)-3-hexenoate	0.65 ± 0.09b	1.26 ± 0.09a	0.77 ± 0.16b	0.64 ± 0.04b	0.60
2-Heptanol	7.65 ± 0.62	9.38 ± 0.66	8.78 ± 0.65	9.25 ± 0.18	17.8
3-Methyl-1-pentanol	134 ± 19ab	145 ± 1a	161 ± 21a	109 ± 1b	12.4
Ethyl heptanoate	1.4 ± 0.07	1.25 ± 0.10	1.68 ± 0.26	1.35 ± 0.05	0.53
1-Hexanol	2778 ± 34a	2848 ± 180a	2728 ± 84a	2462 ± 14b	1901
(E)-2-hexen-1-ol	46.9 ± 1.7a	40.1 ± 1.0b	38.9 ± 1.6b	38.0 ± 0.7b	82.1
(Z)-3-hexen-1-ol	121 ± 9a	74.8 ± 3.3b	47.1 ± 1.0c	36.5 ± 1.6c	600
Methyl octanoate	5.52 ± 0.20ab	7.39 ± 2.02a	5.00 ± 0.20b	3.40 ± 0.07b	n.d.
Ethyl octanoate	713 ± 142ab	941 ± 197a	731 ± 27ab	489 ± 20b	419
3-Methylbutyl hexanoate	1.84 ± 0.17ab	2.20 ± 0.60a	1.67 ± 0.09ab	1.19 ± 0.01b	0.23

2-Ethyl-1-hexanol	$9.61 \pm 0.23a$	$8.66 \pm 0.81a$	$7.14 \pm 0.35b$	$4.50 \pm 0.15c$	75.9
Linalool	$2.24 \pm 0.14a$	$1.91 \pm 0.13b$	$1.93 \pm 0.10b$	$1.51 \pm 0.05c$	25.1
1-Octanol	37.8 ± 4.5	40.0 ± 4.0	38 ± 1.4	30.2 ± 1.8	13.8
γ-Butyrolactone	$7.28 \pm 0.21c$	$8.51 \pm 1.48bc$	$14.9 \pm 3.5a$	$12.6 \pm 0.7ab$	0.57
Ethyl decanoate	147 ± 27	224 ± 667	206 ± 21	168 ± 6	38.8
1-Nonanol	17.0 ± 0.8	19.4 ± 1.7	21.4 ± 1.1	19.3 ± 1.4	0.52
Diethyl succinate	905 ± 58	753 ± 34	978 ± 181	781 ± 92	64.7
3-Methylthio-1-propanol	$4164 \pm 397a$	$4173 \pm 145a$	$4274 \pm 216a$	$3483 \pm 53b$	240
β-Citronellol	3.57 ± 0.27	3.27 ± 0.97	3.86 ± 0.57	3.27 ± 0.26	2.33
Ethyl phenylacetate	6.24 ± 0.46	8.88 ± 4.03	5.81 ± 1.21	5.38 ± 0.29	8.62
2-Phenylethyl acetate	140 ± 10	198 ± 90	129 ± 28	119 ± 8	192
β-Damascenone	$0.57 \pm 0.03bc$	$0.48 \pm 0.00c$	$0.86 \pm 0.12a$	$0.68 \pm 0.03b$	76.9
Hexanoic acid	$6955 \pm 354a$	$6543 \pm 193ab$	$6160 \pm 153b$	$4725 \pm 121c$	11335
Ethyl dodecanoate	$13.0 \pm 1.9c$	$27.0 \pm 5.4a$	$21.9 \pm 1.3ab$	$16.1 \pm 2.2bc$	3.87
Benzyl alcohol	1796 ± 71	1902 ± 420	2166 ± 113	2226 ± 15	25.2
2-Phenylethanol	113239 ± 12829	113703 ± 26883	121472 ± 7695	91056 ± 6480	2866
Nerolidol	1.23 ± 0.16	1.23 ± 0.21	1.62 ± 0.38	1.52 ± 0.05	0.64
Octanoic acid	$1691 \pm 322a$	$1245 \pm 295ab$	$918 \pm 239b$	$719 \pm 44b$	5775
IBMP [ng/L]	9.75 ± 0.7	13.1 ± 1.8	10.6 ± 0.64	10.5 ± 0.3	11.7

[a] 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), except for H0 which results from one value.

Table S8. Quantitative information for the volatiles analysed in the water blended wines Bw1-Bw3 and H4 (Control) given in [μg/L][a] except where specified.

Compound	Water blended wine			H4 (Control)
	Bw1	Bw2	Bw3	
Ethyl acetate	41037 ± 4181	56025 ± 11516	39023 ± 4291	49036 ± 1172
Ethyl propanoate	292 ± 13	427 ± 125	282 ± 42	313 ± 16
Ethyl 2-methylpropanoate	35.5 ± 2.6	54.9 ± 15.6	33.2 ± 5.3	40.3 ± 3.4
2-Methylpropyl acetate	26.3 ± 1.6	35.4 ± 8.5	21.9 ± 2.5	23.5 ± 0.1
Ethyl butyrate	160 ± 31	201 ± 41	127 ± 11	124 ± 0
Ethyl 2-methylbutanoate	6.66 ± 0.06	8.09 ± 1.98	5.76 ± 0.55	5.7 ± 0.19
Ethyl 3-methylbutanoate	33.9 ± 0.23	41.9 ± 10.1	28.6 ± 3.2	31.2 ± 2.2
2-Methyl-1-propanol	70680 ± 8723	74276 ± 11394	71163 ± 8530	76086 ± 84
3-Methylbutyl acetate	7505 ± 1788	10536 ± 2621	6978 ± 1011	5652 ± 755
1-Butanol	5.71 ± 0.85	5.25 ± 0.95	7.05 ± 1.45	8.19 ± 0.92
3-Methyl-1-butanol	446887 ± 6745a	435054 ± 23366a	454585 ± 8197a	397197 ± 2115b
Ethyl hexanoate	1018 ± 204a	1099 ± 131a	651 ± 47b	590 ± 49b
Hexyl acetate	14.1 ± 2.8	19.8 ± 4.3	14.4 ± 2.8	11.6 ± 1.3
Ethyl (Z)-3-hexenoate	0.80 ± 0.19ab	1.00 ± 0.18a	0.58 ± 0.09b	0.64 ± 0.04b
2-Heptanol	8.5 ± 0.93	8.22 ± 2.51	9.22 ± 1.00	9.25 ± 0.18
3-Methyl-1-pentanol	159 ± 17	154 ± 21	158 ± 23	109 ± 1
Ethyl heptanoate	1.52 ± 0.10	1.79 ± 0.47	1.26 ± 0.04	1.35 ± 0.05
1-Hexanol	2567 ± 58.6	2622 ± 147	2476 ± 126	2462 ± 14
(E)-2-hexen-1-ol	34.9 ± 1.3	38.2 ± 3.2	35.3 ± 1.9	38 ± 0.7
(Z)-3-hexen-1-ol	37.3 ± 4.3	42.3 ± 2.8	35.7 ± 1.3	36.5 ± 1.6
Methyl octanoate	7.32 ± 1.64a	6.16 ± 0.77ab	4.23 ± 0.05bc	3.40 ± 0.07c
Ethyl octanoate	805 ± 232	834 ± 138	556 ± 13	489 ± 20
3-Methylbutyl hexanoate	2.06 ± 0.47a	2.01 ± 0.22a	1.32 ± 0.1b	1.19 ± 0.01b
2-Ethyl-1-hexanol	4.93 ± 0.26	5.32 ± 0.35	4.77 ± 0.60	4.50 ± 0.15

Linalool	1.80 ± 0.17	1.80 ± 0.20	1.65 ± 0.12	1.51 ± 0.05
1-Octanol	35.2 ± 2.0	36.9 ± 2.7	32.0 ± 2.2	30.2 ± 1.8
γ-Butyrolactone	7.51 ± 0.65	11.4 ± 3.1	11.7 ± 0.4	12.6 ± 0.7
Ethyl decanoate	187 ± 57	216 ± 53	165 ± 5.95	168 ± 6
1-Nonanol	18.5 ± 1.4	19.3 ± 1.7	19.3 ± 0.9	19.3 ± 1.4
Diethyl succinate	$1110 \pm 262ab$	$1483 \pm 361a$	$731 \pm 118b$	$781 \pm 92b$
3-Methylthio-1-propanol	$5173 \pm 497a$	$4325 \pm 421ab$	$5317 \pm 632a$	$3483 \pm 52b$
β-Citronellol	4.11 ± 0.39	4.03 ± 1	3.44 ± 0.32	3.27 ± 0.26
Ethylphenyl acetate	8.51 ± 1.93	10.2 ± 2.7	8.31 ± 0.85	5.38 ± 0.29
2-Phenylethyl acetate	185 ± 40	222 ± 59	183 ± 18	119 ± 8
β-Damascenone	0.60 ± 0.07	0.75 ± 0.15	0.68 ± 0.03	0.68 ± 0.03
Hexanoic acid	$6380 \pm 681a$	$5475 \pm 395ab$	$4593 \pm 649b$	$4725 \pm 121b$
Ethyl dodecanoate	14.0 ± 0.8	25.8 ± 7.0	16.3 ± 1.3	16.1 ± 2.2
Benzyl alcohol	2331 ± 107	2464 ± 261	2237 ± 16	2226 ± 15
2-Phenylethanol	$139330 \pm 3191a$	$117480 \pm 4093b$	$129899 \pm 4332a$	$91056 \pm 6480c$
Nerolidol	1.13 ± 0.18	$1.41 \pm 0.2.0$	1.63 ± 0.45	1.52 ± 0.05
Octanoic acid	$1676 \pm 370a$	$839 \pm 208b$	$929 \pm 61b$	$719 \pm 43b$
IBMP [ng/L]	$10.1 \pm 0.5ab$	$9.07 \pm 0.55b$	$8.98 \pm 0.67b$	$10.5 \pm 0.3a$

[a] 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).

Table S9. Average scores for all wine sensory descriptors used by the descriptive analysis panel for GHW blended wines B1-B3 and the water blended wines Bw1-Bw3.[a]

Descriptor		Wines from blending treatments						
	H4 (Control)	B1	B2	B3	Bw1	Bw2	Bw3	LSD
Aroma								
Aroma Intensity	60.3a	62.7a	61.9a	61.4a	62.6a	63.5a	63.0a	5.7
Red fruit	50.0a	52.3	50.2a	52.6a	53.2a	49.2a	47.9a	6.8
Dark fruit	57.5b	61.4ab	62.4ab	62.2ab	60.8ab	63.1a	63.1a	5.1
Dried fruit/jam	54.6ab	49.8b	53.6ab	59.9a	53.9ab	56.4ab	54.6ab	8.1
Green	39.7a	40.1a	39.6a	35.4a	40.2a	40.4a	37.3a	6.9
Sweet spice	37.9a	33.4a	38.7a	34.3a	39.7a	36.1a	38.3a	7.1
Confection	25.6a	31.4a	27.4a	28.3a	28.0a	29.6a	28.6a	7.5
Liquorice	33.0ab	28.2b	37.9a	35.8a	33.9a	28.3b	35.0a	5.4
Chocolate	33.7a	25.3b	34.3a	36.2a	26.4b	26.9b	33.6a	6.6
Port wine	34.2ab	25.9bc	37.2a	34.0ab	24.3c	33.2ab	30.6abc	8.5
Palate								
Flavour intensity	68.5a	59.4c	64.4ab	63.4bc	62.2bc	63.2bc	66.8ab	4.6
Red fruit	53.8ab	55.1ab	59.3a	55.6ab	53.4ab	52.9ab	52.1b	6.7
Dark fruit	68.2a	59.8c	60.1c	66.0ab	61.3bc	61.2bc	65.4ab	5.0
Dried fruit/jam	64.8a	42.9c	47.6c	61.5ab	48.1c	56.6b	61.5ab	8.0
Green	35.7b	46.8a	44.4a	40.5ab	45.2a	43.1a	41.7ab	6.8
Sweet spice	47.1a	31.6c	39.1b	38.0bc	35.7bc	36.5bc	40.1b	6.5
Confection	31.9a	31.1a	27.9ab	29.4ab	24.9b	27.1ab	25.4b	5.6
Liquorice	39.6a	27.4c	35.6ab	39.1a	31.1bc	30.1bc	34.9ab	5.6
Chocolate	34.3a	21.7c	28.3abc	29.9ab	24.8bc	26.8bc	31.4ab	6.8
Port wine	38.8a	20.6b	32.7a	34.2a	22.5b	31.4a	34.5a	7.5

Body	64.5a	51.9d	58.2bc	61.3ab	55.3cd	58.8bc	61.9ab	5.7
Sweetness	53.6a	25.4cd	31.7bc	35.4b	22.9d	31.4bc	32.7b	6.8
Acidity	54.0b	63.1a	61.6a	57.0ab	52.0b	57.2ab	57.1ab	6.6
Astringency	63.1a	56.1a	62.7a	60.0a	60.1a	63.1a	62.0a	7.5
Bitterness	48.9a	45.0a	52.8a	52.6a	46.3a	50.5a	51.1a	8.0
Hotness	67.3a	54.1b	64.1a	66.9a	51.3b	63.4a	67.6a	7.4

[a] Values are means of 3 replicates. Values followed by different letters within a row are significantly different (p ≤ 0.05, one way ANOVA, post hoc Fisher's LSD).
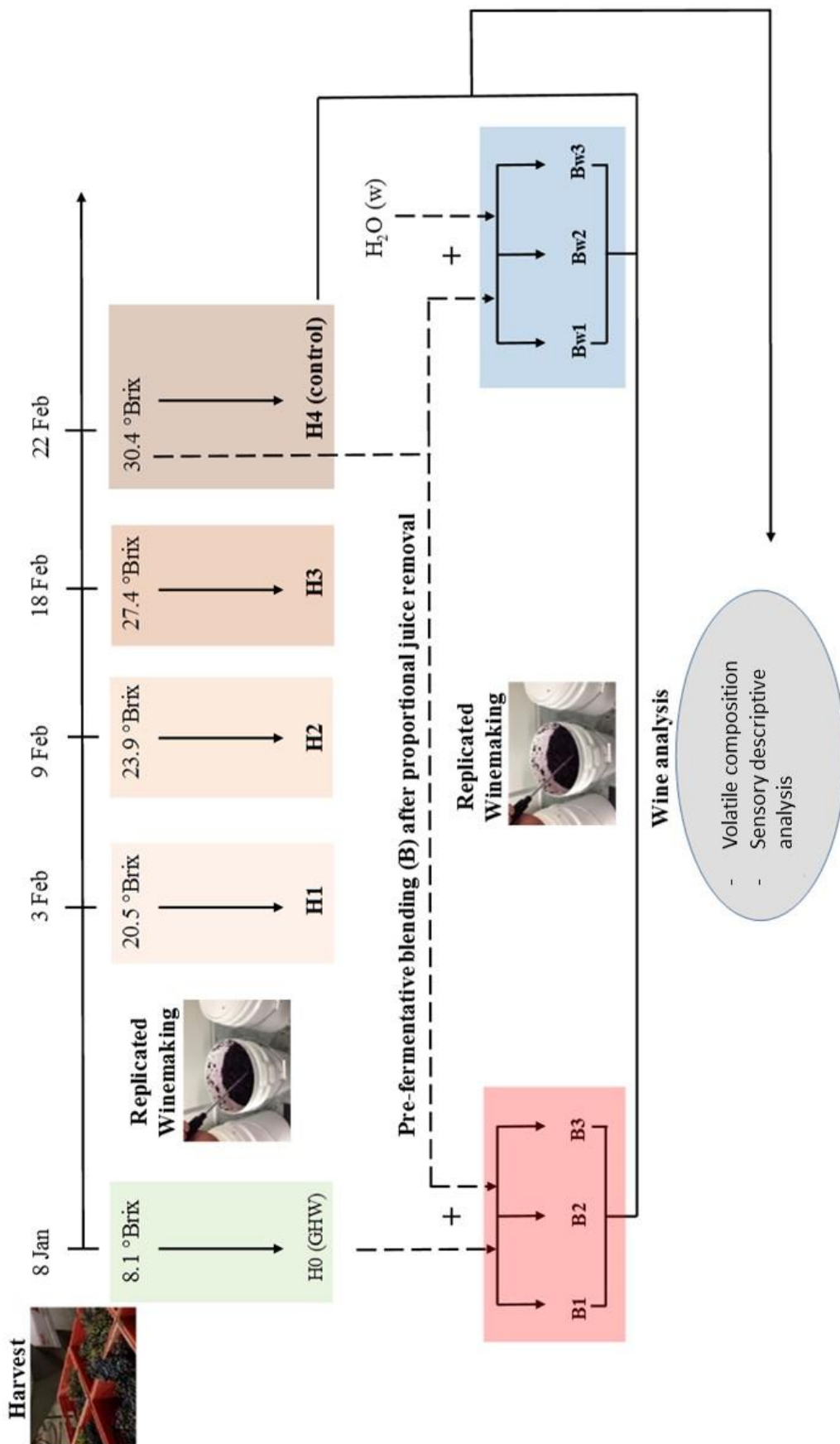
Figure S1. Experimental plan showing harvest dates, winemaking, blending treatments using proportions of green harvest wine (GHW) or water, and grape and wine analyses undertaken. Adapted from Food Chemistry, Vol 244, Olaf J. Schelezki, Paul A. Smith, Ana Hranilovic, Keren A. Bindon, David W. Jeffery, Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition, 50-59, Copyright (2017), with permission from Elsevier.
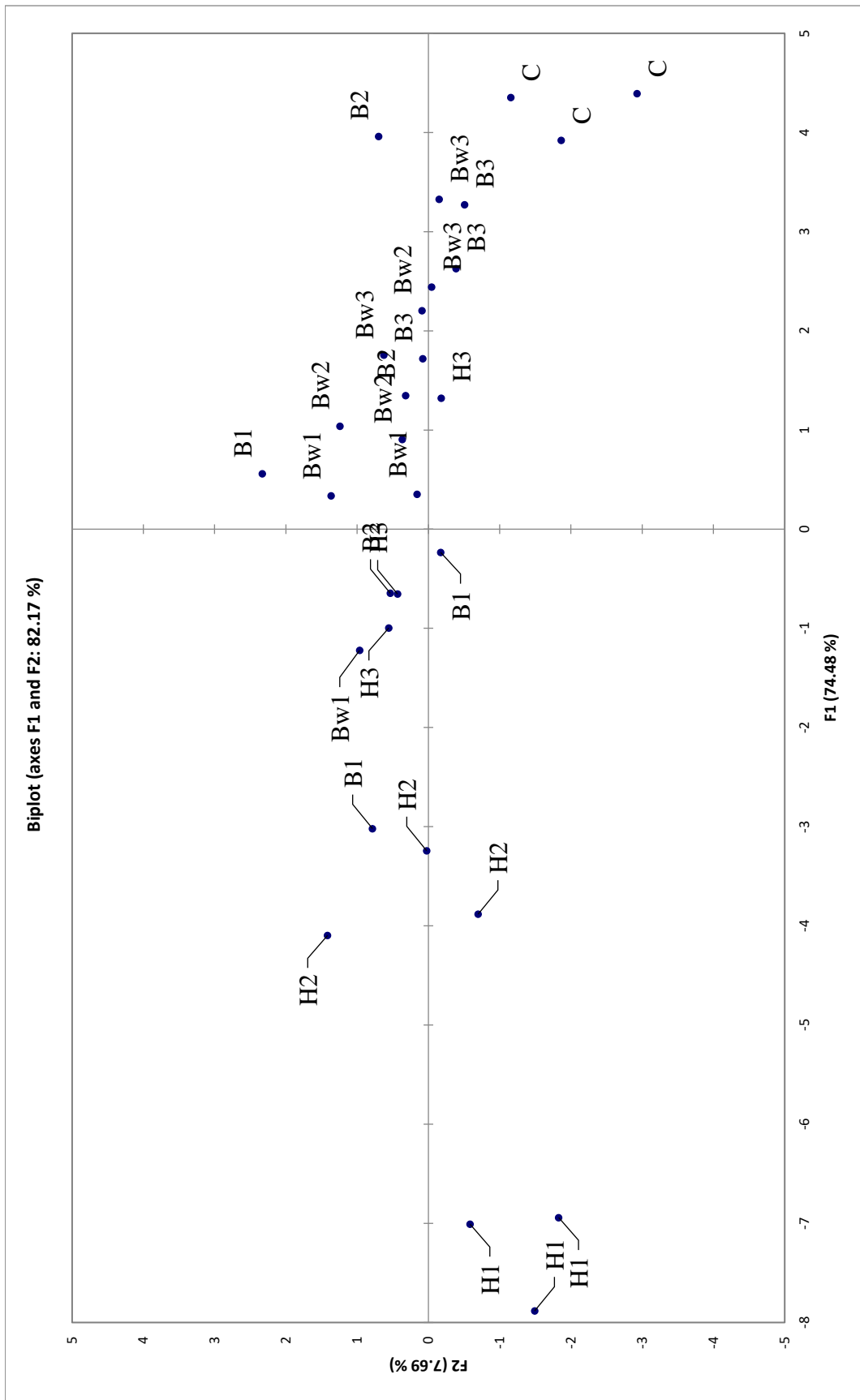
Figure S2. PCA bi-plot showing the replicate clustering according to the significantly different descriptive analysis attributes used in Figure 1, including the harvest series wines (H), GHW blended wines (B), water blended wines (Bw) and controls (C).

References

Burdock, G. A. (2010). *Fenaroli's Handbook of Flavor Ingredients* (6th ed.). Boca Raton, USA: CRC Press.

Buttery, R. G., Turnbaugh, J. G., Ling, L. C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry, 36*(5), 1006-1009.

Clarke, R. J., & Bakker, J. (2004a). Sherry and port. In *Wine Flavour Chemistry,* (pp. 230-261). Oxford, UK: Blackwell Publishing Ltd.

Clarke, R. J., & Bakker, J. (2004b). Volatile components. In *Wine Flavour Chemistry* 1 ed., (pp. 120-188). Oxford, UK: Blackwell Publishing Ltd.

Escudero, A., Campo, E., Farina, L., Cacho, J., Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry, 55*(11), 4501-4510.

Etiévant, P. X. (1991). Wine. In H. Maarse (Ed.), *Volatile Compounds in Foods and Beverages* 1st ed., vol. 44 (pp. 483-546). New York: Marcel Dekker.

Ferreira, V., López, R., Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*(11), 1659-1667.

Ferreira, V., San Juan, F., Escudero, A., Cullere, L., Fernandez-Zurbano, P., Saenz-Navajas, M. P., Cacho, J. (2009). Modeling quality of premium spanish red wines from gas chromatography-olfactometry data. *Journal of Agricultural and Food Chemistry, 57*(16), 7490-7498.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry, 45*(8), 3027-3032.

Mayr, C. M., Geue, J. P., Holt, H. E., Pearson, W. P., Jeffery, D. W., Francis, I. L. (2014). Characterization of the key aroma compounds in Shiraz wine by quantitation, aoma reconstitution, and omission studies. *Journal of Agricultural and Food Chemistry, 62*(20), 4528-4536.

Moyano, L., Zea, L., Moreno, J., Medina, M. (2002). Analytical study of aromatic series in sherry wines subjected to biological aging. *Journal of Agricultural and Food Chemistry, 50*(25), 7356-7361.

Peinado, R. A., Moreno, J., Bueno, J. E., Moreno, J. A., Mauricio, J. C. (2004). Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry, 84*(4), 585-590.

Pineau, B., Barbe, J.-C., Van Leeuwen, C., Dubourdieu, D. (2007). Which impact for β-damascenone on red wines aroma? *Journal of Agricultural and Food Chemistry, 55*(10), 4103-4108.

Sumby, K. M., Grbin, P. R., Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: current knowledge and future prospects. *Food Chemistry, 121*(1), 1-16.

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research, 11*(2), 139-173.

Waterhouse, A. L., Sacks, G. L., Jeffery, D. W. (2016a). Amines, amino acids and proteins. In *Understanding Wine Chemistry* 1 ed., (pp. 40-50). United Kingdom: Wiley.

Waterhouse, A. L., Sacks, G. L., Jeffery, D. W. (2016b). Higher alcohols. In *Understanding Wine Chemistry* 1 ed., (pp. 51-55). United Kingdom: Wiley.

Chapter 4

Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis.

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Name of Principal Author (Candidate)	Olaf J. Schelezki
Contribution to the Paper	Designed experiments, conducted vineyard monitoring, organised and executed grape harvests and experimental winemaking, conducted wine non-volatile as well as volatile analysis (by GC-MS), analysed and interpreted the data, trained a sensory panel and undertook quantitative descriptive analysis of 60 wines, statistically analysed the data sets (one and two-way ANOVA, PCA), interpreted the data, drafted and constructed the manuscript.
Overall percentage (%)	70
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 7/06/2018

Co-Author Contributions

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

     i.   the candidate's stated contribution to the publication is accurate (as detailed above);

     ii.   permission is granted for the candidate in include the publication in the thesis; and

     iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Katja Suklje
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Signature	95	Date	07/06/2018

Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis.

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ABSTRACT

Recent studies have shown that the pre-fermentative juice substitution with water or a very low alcohol wine has potential to produce lower alcohol wines from excessively overripe Cabernet Sauvignon grapes without modifying colour or tannin properties and only marginally changing the volatile and sensory profiles. Whether this approach was also suited to producing lower alcohol wines in the absence of excessive grape ripeness remained to be determined. The current study extends on pre-fermentative approaches to alcohol management under milder grape ripening conditions and builds on the pre-existing experimental design with McLaren Vale Cabernet Sauvignon fruit, allowing for a direct comparison under two distinct vintage conditions. Given its major importance for the Australian wine industry, Shiraz was also included and underwent the same treatments. Cultivar dependant implications on wine chemical properties were apparent and declines in wine colour and tannin were particularly evident in Shiraz wines, although impacts on wine quality were minor when adjusting musts to 13.5 °Baumé.

Keywords

Alcohol management, water addition, wine colour, tannin, volatiles, wine aroma, sensory analysis

1   **1. Introduction**

2   Optimising and managing wine alcohol levels has become a focus in recent decades in

3   response to increasing average wine ethanol concentrations (Godden, Wilkes, & Johnson, 2015), as

4   a result of higher grape sugar levels, mainly driven by rising temperatures during the growing season

5   (Schultz, 2016). Among the various alcohol management approaches, which include physical

6   dealcoholisation methods (Longo, Blackman, Torley, Rogiers, & Schmidtke, 2017), attention has

7   recently focused on a more flexible and easy-implementable approach involving pre-fermentative

8   juice modification (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011; Schelezki, Smith,

9   Hranilovic, Bindon, & Jeffery, 2018; Schelezki, Suklje, Boss, & Jeffery, 2018) to decrease potential

10   wine alcohol while maintaining wine quality. Several studies have investigated the production of red

11   wine using addition of either water or low alcohol wine (described hereafter as green harvest wine,

12   GHW) to substitute a proportion of juice, hence decreasing the must total soluble solids (TSS)

13   concentrations and final wine alcohol levels without greatly "diluting" important wine quality

14   components (such as anthocyanins and tannins, which would be barely extracted) or sensory

15   characteristics (that had not yet formed from grape precursors or fermentation) (Harbertson, Mireles,

16   Harwood, Weller, & Ross, 2009; Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011; Schelezki,

17   Smith, Hranilovic, Bindon, & Jeffery, 2018; Schelezki, Suklje, Boss, & Jeffery, 2018).

18   The present authors determined in preceding studies with Cabernet Sauvignon that wine

19   colour and tannin properties, volatile profiles and sensory characters were only marginally altered

20   when high substitution rates of water or GHW were applied (lowering the alcohol by volume (ABV)

21   from 18.2% to 14.5%). Water substitution was deemed to be particularly benign in terms of wine

22   quality, having preserved the chemical compositions and sensory characteristics as determined by the

23   harvest date (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018; Schelezki, Suklje, Boss, &

24   Jeffery, 2018). However, the importance of grape quality and vintage context were highlighted in that

25   work, with negative sensory characters like 'hotness' and overripe 'port wine aroma' also being

26   retained in the treatment wines, which were produced in a hot season with evident berry shrivel at

27   commercial harvest. As such, grape over-ripeness should probably be avoided in the first place, where

28   possible, by employing an earlier harvest date.

29   Any perceived benefit of prolonging harvest when chasing riper fruit characters may be in

30   doubt given that only minor differences in sensory quality were evident (Heymann, LiCalzi,

31   Conversano, Bauer, Skogerson, & Matthews, 2013) and no consumer preference was observed

32   (Bindon, Holt, Williamson, Varela, Herderich, & Francis, 2014) once grapes had ripened past certain

33   potential alcohol concentrations as low as 13.5% ABV. This value approximately coincides with that

34   described in recent changes in Australian wine regulations (FSANZ, 2016) to facilitate fermentation

35   of high sugar grape musts, whereby pre-fermentative water addition to musts is permitted to decrease

36   TSS to a minimum of 13.5 Bé (i.e., a potential alcohol content of 13.5% ABV). Although our previous

37   studies pre-empted this change in regulation in Australia, water addition remain to be further

38   evaluated, both in terms of its apparently benign nature for pre-fermentative alcohol management and

39   under milder vintage conditions that did not lead to excessively ripe grapes.

40   Extending on previous research, this study aimed to i) reassess the implications on Cabernet

41   Sauvignon wine colour, tannins, volatile compounds and sensory characteristics of the pre-

42   fermentative alcohol management approaches under less severe vintage conditions, using a

43   comparable experimental set up and identical vineyard as in our preceding work ii) extend the

44   approach to Shiraz, owing to its importance as the most widely grown cultivar in Australia as well as

45   to its susceptibility to berry shrivel and iii) assessing for both varieties the suitability of earlier

46   harvests or proportional GHW substitution to produce lower alcohol wines in comparison to water

47   addition to must at around the 13.5 Bé regulated limit.

48   **2. Material and methods**

49   *2.1 Chemicals*

50   Reagents and reference standards used for analyses were purchased from Sigma Aldrich

51   (Castle Hill, NSW, Australia) or Alfa Aesar (Ward Hill, MA, USA). Stock solutions of standards

52   were prepared volumetrically in redistilled ethanol and stored at −20 ℃, and working solutions were

53   stored at 4 ℃ until required. HPLC grade solvents and analytical grade sodium chloride were sourced

54   from Merck (Kilsyth, Victoria, Australia) and Chem-Supply (Gillman, SA, Australia), respectively.

55   Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia) for

56   experimental work, and filtered tap water was used for the water blending treatments. Bentonite

57   (SIHA Active Bentonite G, Eaton Filtration, New Jersey, USA) and activated carbon were purchased

58   from Winequip (Adelaide, SA, Australia), and potassium metabisulfite was sourced from Vebigarden

59   (Padua, Italy).

60   *2.2 Climate data*

61   Daily minimum, maximum and average temperatures, total monthly rainfall, and long-term

62   averages (Table S1 of the Supporting Information) were obtained from the Bureau of Meteorology

63   (weather station in Noarlunga, SA, at 138.5057 ℃E, 35.1586℃S (Australian Government Bureau of

64   Meteorology, 2018)), as in the previous study (Schelezki, Smith, Hranilovic, Bindon, & Jeffery,

65   2018). The Huglin index for vintage 2015/16 was calculated according to Tonietto and Carbonneau

66   (2004).

67   *2.3 Harvesting and winemaking*

68   *Vitis vinifera* L. cv Shiraz and Cabernet Sauvignon grapes were sourced from two adjacent

69   commercial vineyards located in McLaren Vale (138.521139℃E, 35.194167℃S and 138.521016℃E,

70   35.192774℃S, respectively). The Cabernet Sauvignon grapes were sourced from the same vineyard

71   used in the preceding study (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). Approximately

72   200 kg of Shiraz (8 January 2016) and Cabernet Sauvignon (11 January 2016) grapes were harvested

73   around 50% veraison with total soluble solids (TSS) of 14.8 and 13.3 ℃Brix, respectively, and used to

74   separately produce the green harvest wines (GHW). In subsequent harvests (1, 8 and 17 February

75   2016 for Shiraz; 1, 9 and 18 February 2016 for Cabernet Sauvignon), 70-80 kg of grapes were hand-

76   picked at 20.4, 23.4 and 24.9 ℃Brix for Shiraz, and at 19.9, 22.1 and 23.6 ℃Brix for Cabernet Sauvignon

77   (further referred to as H1, H2 and H3, respectively for each cultivar). At commercial ripeness,

78   approximately 450 kg of grapes were hand-picked for each cultivar and processed to obtain the

79   control wines (Shiraz on 21 February at 26.3 ºBrix, and Cabernet Sauvignon on 29 February at 26.2

80   ºBrix (both approximately 14.6º Baumé (Bé), each further referred to as H4) in the same manner as

81   described in Section 2.5 as well as the for the pre-fermentative blending treatments (Fig. S1 of the

82   Supporting Information).

83   *2.4 Assessment of grape ripening and size variability*

84   For each cultivar and for each harvest date, 50–70 grape bunches were sampled, carefully

85   destemmed with precision snips, and a random subsample of 1000 berries was taken to assess grape

86   TSS and size distribution as previously described by Schelezki, Smith, Hranilovic, Bindon, and

87   Jeffery (2018). Briefly, 10 batches of 100 berries were photographed for subsequent image analysis,

88   then TSS was measured for each berry following a logical order, which enabled the relation of TSS

89   to berry size.

90   *2.5 Green harvest wine*

91   Winemaking was conducted by WIC Winemaking Services (Waite Campus, Urrbrae, SA,

92   Australia) according to a protocol used previously (Schelezki, Smith, Hranilovic, Bindon, & Jeffery,

93   2018) but without the need for fermentation restart. Briefly, grapes harvested at approximately 50%

94   veraison were destemmed, crushed, and pressed, and the juice was settled overnight (at 10 °C) before

95   racking. After fermentation (EC1118 yeast, Lallemand, Montreal, Canada), the separate wines were

96   fined at a rate of 1 g/L with each of charcoal and bentonite to achieve decolourisation, deodorisation

97   and to facilitate settling (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011). Potassium

98   metabisulfite (PMS, 10% aqueous solution) was added at 100 mg/L to give an approximate total SO_2

99   concentration of 50 mg/L. The wines (approximately 100 litres of each cultivar) were stored in

100   stainless steel kegs at 0 ºC until required for the blending treatments.

101

102   *2.6 Consecutive harvest wines*

103   Grapes obtained at each consecutive harvest date (H1-H4) for each cultivar were randomised,

104   crushed, destemmed and triplicate lots of 18 to 19 kg were fermented in 20 L plastic buckets under

105   the same conditions as previously described (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018).

106   After a maceration period of seven days, all wines were pressed off with a basket press (20 L Cage

107   Idropress System, Home Make It Pty Ltd, Campbellfield, VIC, Australia), transferred into 10 L glass

108   demijohns, and stored at 0 ºC for stabilisation and conservation until bottling. At bottling (in 375 mL

109   bottles under screw caps), wine pH was adjusted to 3.6 (using 500 g/L aqueous tartaric acid solution),

110   PMS was added at a rate of 100 mg/L, and bottles were stored at 15 ºC until analysis.

111   *2.7 Blending treatment wines*

112   The remaining H4 grapes of each cultivar were destemmed, crushed and 18-19 kg lots were

113   distributed in 20 L plastic buckets analogously to the consecutive harvest wines. Before inoculation

114   and in triplicate, proportions of the juice were removed and substituted either with filtered water or

115   with the associated GHW for each cultivar, to adjust the must TSS levels, targeting as best as

116   practicable the potential alcohol concentrations of the consecutive harvest wines. The substitution

117   volume was determined as previously stated (Esteruelas, Gonzalez-Royo, Kontoudakis, Orte, Cantos,

118   Canals, et al., 2015; Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). In this way, two blending

119   series per variety (B and Bw for GHW and water additions, respectively) were formed. For Cabernet

120   Sauvignon, CS_B1, CS_B2 and CS_B3 refer to treatments with 40%, 26% and 16% v/v substitution

121   with GHW, and CS_Bw1, CS_Bw2 and CS_Bw3 refer to treatments with 25%, 16%, 10% v/v

122   substitution with water. In the case of Shiraz, 40%, 20% and 9% v/v substitution with GHW are

123   designated SH_B1, SH_B2 and SH_B3, and 25%, 12% and 6% v/v substitution with water are

124   referred to as SH_Bw1, SH_Bw2 and SH_Bw3, respectively). Winemaking, bottling and storage

125   conditions were the same as described for the consecutive harvest wines in Section 2.5.

126

127   *2.8 Analysis of basic wine parameters*

128   Wine ethanol concentrations were analysed using an alcolyser (Anton Paar, Graz, Austria).

129   The pH and titratable acitidy (TA, expressed as g/L equivalents of tartaric acid) were measured with

130   a Mettler Toledo T50 Autotitrator, titrating to an endpoint of pH 8.2 with 0.33 NaOH solution.

131   Glucose, fructose, glycerol, and malic, tartaric, citric and acetic acids were analysed by HPLC

132   according a previously reported method (Li, Bindon, Bastian, Jiranek, & Wilkinson, 2017).

133   *2.9 Extraction and isolation of grape and wine tannin*

134   *2.9.1 Wine-like tannin extraction*

135   Grapes were analysed according to a previously reported protocol (Bindon, Kassara, Cynkar,

136   Robinson, Scrimgeour, & Smith, 2014; Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018) in

137   order to estimate extractable tannin content.

138   *2.9.2 Isolation of wine tannins*

139   Wine tannins were isolated by solid phase extraction (SPE) using a previously published

140   method (Jeffery, Mercurio, Herderich, Hayasaka, & Smith, 2008) with a slight modification to collect

141   tannins as one fraction (Kassara & Kennedy, 2011).

142   *2.10 Analysis of tannins and wine colour*

143   Tannin concentrations of wine-like extracts and wines were analysed via the methyl cellulose

144   precipitable tannin assay (MCP tannin) (Mercurio, Dambergs, Herderich, & Smith, 2007). To

145   determine wine tannin subunit composition, wine tannins isolated by solid phase extraction were

146   reconstituted in pure methanol and analysed by phloroglucinolysis using a previously published

147   method (Kassara & Kennedy, 2011). In addition, wine tannin size distribution (molecular mass, MM)

148   was determined by gel permeation chromatography (GPC), for which the methanolic solutions of

149   isolated tannins were diluted 1:5 with the HPLC mobile phase prior to injection. The instrument

150   configuration, chromatographic conditions and calibrations for GPC were as previously described

151   (Kennedy & Taylor, 2003) with modifications (Bindon, Bacic, & Kennedy, 2012). Wine colour

152   density, anthocyanin concentrations and total phenolics were determined via a modified Somers

153   colour assay (Mercurio, Dambergs, Herderich, & Smith, 2007).

154   *2.11 Analysis of the wine volatile composition*

155   A group of 45 wine volatile compounds, which included isoprenoids and a wide range of

156   fermentation-derived metabolites, were analysed in wines six weeks after bottling by HS-SPME-GC-

157   MS according to an adapted version of previously published methods (Antalick, Šuklje, Blackman,

158   Meeks, Deloire, & Schmidtke, 2015; Suklje, Zhang, Antalick, Clark, Deloire, & Schmidtke, 2016)

159   using the same instrumentation. A stock solution (20 μL) of octan-2-ol, $[^2H_3]$-linalool and $[^2H_5]$-ethyl

160   cinnamate (internal standards) at 5 mg/L in absolute methanol was added to 10 mL of wine, of which

161   5 mL was transferred to a 20 mL headspace vial containing 3 g of NaCl, and 5 mL of deionised water

162   were added. The vial was tightly sealed with a PTFE lined cap and vortexed. The extraction consisted

163   of pre-incubating the vial with agitation (at 500 rpm) for 10 min at 40 °C, then extracting the

164   headspace with a 1 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30

165   μm fibre (Supelco, Bellefonte, PA, U.S.A.) for 30 min at 40 °C with agitation. The fibre was desorbed

166   in the injector (fitted with 2 mm i.d. borosilicate liner (SGE)) at 250 °C for 1 min in splitless mode,

167   and then baked in a second injector set at 270 °C with a 50:1 split for 10 min with a 10 mL/min purge

168   flow to clean the fibre prior to extraction of the next sample. A DB-WAXetr capillary column (60 m,

169   0.25 mm, 0.25 μm film thickness, J&W Scientific, Folsom, CA) was used for compound separation

170   by gas-chromatography. The oven temperature program commenced at 40 °C for 5 min; increased to

171   200 °C at a rate of 3 °C/min; and had a final increase to 240 °C at a rate of 10 °C/min, where is was

172   held for 1 min. Ultra-high purity helium gas (BOC, North Ryde, NSW, Australia) was used as carrier

173   gas with a constant flow of 1.5 mL/min. The MS source, quadrupole and transfer line temperatures

174   were set to 230, 150, and 260 °C, respectively. Ions 45, 74, and 181 were used for octan-2-ol, $[^2H_3]$-

175   linalool and $[^2H_5]$-ethyl cinnamate, respectively, for selected ion monitoring (SIM). Peaks were

176   simultaneously sampled in scan mode, in seven segments based on retention time, covering ions at *m/z*

177   40-300 and with dwell times of 30 to 100 ms. The ions, acquisition mode and the internal standards

178   used to perform semi-quantitation of wine volatiles are displayed in Table S2 of the Supporting

179   Information.

180   *2.12 Sensory analysis*

181   Descriptive analysis (DA) was conducted one year after bottling the wines. A total of eleven

182   assessors were recruited, comprising eight female and three male researchers and students from the

183   University of Adelaide with previous DA experience. The DA followed the consensus-based

184   approach (Lawless & Heymann, 2010) and consisted of eight training and four formal sessions.

185   During the first two sessions, aroma, flavour and palate characteristics of the wines were evaluated

186   and discussed to reach consensus about the descriptive attributes. In the following sessions, reference

187   standards (Table S3 of the Supporting Information) agreed upon by the panellists were used to

188   familiarise them with the aroma attributes as well as for astringency, bitterness and hotness, and

189   different experimental samples were served to train panellists in the appropriate usage of the scale

190   and aroma attributes. Wines were rated using RedJade online based software and results during the

191   training sessions were presented to the panellists directly after each session to provide feedback and

192   to discuss and screen out non-discriminating attributes. This led to a final attribute list comprising

193   thirteen aroma, thirteen flavour and five mouthfeel attributes (Table S3 of the Supporting

194   Information) to describe the wines during the formal evaluation sessions. Formal sessions were

195   conducted under conditions and with ratings scales as described previously (Schelezki, Suklje, Boss,

196   & Jeffery, 2018).

197   *2.13 Statistical analysis*

198   One-way and repeated measures analysis of variance (ANOVA) of the chemical data,

199   principal component analysis (PCA) of normalised chemical and sensory data were conducted using

200   XLSTAT (Version 2015.4.1, Addinsoft, Paris, France). Coefficients of determination were calculated

201   using Microsoft Excel. Mean comparisons were performed using Fisher's least significant difference

202   (LSD) multiple comparison test at $p < 0.05$. Agglomerative hierarchical clustering (AHC, Euclidian

203   distance proximity type and Ward's agglomeration method) was applied to the wine volatile data to

204   assess overall differences between treatments using XLSTAT. The panel performance was assessed

205   via PanelCheck (V1.4.2, Nofima Mat) during the DA, and ANOVA and differences in sensory

206   attributes were determined by mixed model ANOVA, including Fisher's LSD post-hoc ($p < 0.05$) for

207   pairwise comparisons using SENPAQ (Version 6.03, Qi Statistics, Reading, United Kingdom).

208   Relative concentration differences of volatile compounds between treatments and control were

209   calculated, and presented as average per compound group in heatmaps.

210   **3. Results and Discussion**

211   *3.1 Vintage conditions*

212   The initial part of the 2015/16 vegetative period in McLaren Vale was remarkably warmer

213   than the long-term average (2000-2016, Table S1 of the Supporting Information). Like the previous

214   year this growing season was classified as warm (Tonietto & Carbonneau, 2004) but at 2627 Huglin

215   index units it slightly surpassed the 2416 units of the preceding season (Schelezki, Smith, Hranilovic,

216   Bindon, & Jeffery, 2018). Higher day and night temperatures in October and December 2015

217   prompted substantial increases in average temperatures by 2.9 and 3.4 °C, respectively, and these

218   warm conditions became further compounded by the below-average monthly rainfall from July to

219   December 2015. However, higher-than-average rainfall occurred from January onwards, entering the

220   main harvest period in February with slightly cooler temperatures. Despite the overall weather

221   conditions translating into a similarly advanced state of plant physiological progression as observed

222   during the 2014/15 season, berry shrivel was far less evident in 2016. This could be partially

223   attributable to the rainfall during the months after veraison (January and February), as well as

224   significantly less days with average temperatures above 35 °C and 40 °C, which was the case during

225   the 2014/15 harvest (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018).
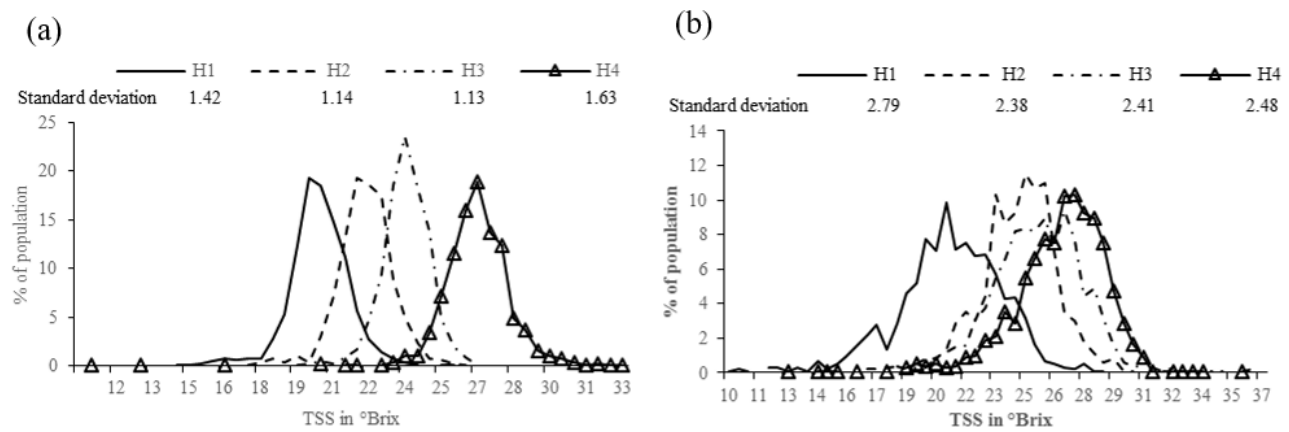
Fig. 1. Berry TSS distribution for H1–H4 and respective standard deviations, showing distinct magnitudes and progression of berry ripening variability with later harvest dates for (a) Cabernet Sauvignon and (b) Shiraz.

Grape ripeness distribution differed between Cabernet Sauvignon (Fig. 1a) and Shiraz (Fig. 1b) for the different harvest dates. The variation around mean TSS levels (given by the standard variation, SD), initially decreased for both varieties and remained unaltered until H4, however, with twice the SD observed with Shiraz than in Cabernet Sauvignon (Fig. 1b). With the commercial harvest approaching, the SD for Cabernet Sauvignon ascended from 1.13 (H3) to 1.63 (H4) in line with the formation of an overripe "head" (Fig 1a) (and a slight but insignificant decline in berry weight (Table S3 of the Supporting Information)), which was not the case for Shiraz. These observations describe a grape ripening progression leading to TSS levels exemplary for warm climate viticulture, yet with absence of berry shrivel, providing a context relevant to the wine industry to test the pre-fermentative alcohol management approaches (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018; Schelezki, Suklje, Boss, & Jeffery, 2018). At similar harvest points and TSS levels, the variability measured in the Shiraz vineyard followed initially a similar pattern (Fig. 1a and 1b) with, however, twice the SD observed in the Cabernet Sauvignon vineyard. Besides a peak at H2, Shiraz berry weights were similar to H1 in the remaining harvests which nonetheless was characterised by an extending unripe "tail" and a wide range of ripeness levels within the population at a similar average TSS as Cabernet Sauvignon.

247   *3.2 Basic wine composition*

248   Ethanol concentrations in the wines from consecutively harvested grapes increased from

249   11.4% /11.8% ABV at H1 to 15.5% /15.4% ABV at H4) for Cabernet Sauvignon and Shiraz,

250   respectively (Table S4 of the Supporting Information). Grapes from H4 used in pre-fermentative

251   substitution treatments (Fig. S1 and Table S5 of the Supporting Information) with water or GHW

252   produced wines of distinctively lower alcohol concentrations compared to the respective H4 controls,

253   achieving decreases in the order of 1-3% ABV in the Cabernet Sauvignon wines and 0.5-2% ABV in

254   the Shiraz wines, and falling into ABV ranges similar to H2 and H3 wines from the earlier harvests.

255   All wines fermented to dryness (i.e. < 1 g/L glucose and fructose, data not shown) regardless

256   of the blending component. The GHW treatments were characterised by significantly higher TA and

257   lower pH levels in wines before adjustment were carried out for both varieties whereas water

258   additions had little (Shiraz) or no (Cabernet Sauvignon) effect on these parameters relative to the

259   control (Table S5 of the Supporting Information). Wines did not undergo malolactic fermentation and

260   consequently, malic acid concentrations were higher as more juice was substituted pre-fermentatively

261   with GHW wine whereas the highest water substitution (CS_Bw1, insignificantly for SH_Bw1)

262   diluted the malic acid concentrations. Interestingly, higher glycerol levels were observed in the water

263   treatments than in the respective GHW counterparts (Bw1/B1 and Bw2/B2 in Cabernet Sauvignon,

264   Bw1/B1, Bw2/B2 and Bw3/B3 in Shiraz), which was also the case in the previous study of Cabernet

265   Sauvignon (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). This could be related to the lower

266   must substitution rates with water and consequently higher fermentable sugar concentrations than

267   were present in the GHW treatments (Yanniotis, Kotseridis, Orfanidou, & Petraki, 2007). Glycerol

268   forms part of the wine dry extract, which is understood to influence mouthfeel properties such as

269   viscosity and astringency, but given the low concentrations in the present case, any perceived

270   differences in these attributes are more likely related to the differences in wine alcohol concentrations

271   (Yanniotis, Kotseridis, Orfanidou, & Petraki, 2007).

272   *3.3 Implications of harvest date on wine colour and tannin properties*

273   In addition to grape TSS and acidity levels, which are mostly used to monitor grape ripening

274   and determine harvest dates, red grape polyphenols including anthocyanins and tannins are highly

275   relevant to red winemaking, as they are inherently associated with variations in wine style and quality

276   (Kassara & Kennedy, 2011; Mercurio, Dambergs, Cozzolino, Herderich, & Smith, 2010). Grape

277   phenolic composition and their extractability changes with fruit maturity, such that higher

278   anthocyanin (closely connected to berry sugar loading) and tannin concentrations are associated with

279   longer grape hang-times (Bindon, Varela, Kennedy, Holt, & Herderich, 2013; Sherman, Greenwood,

280   Villas-Boâs, Heymann, & Harbertson, 2017), and are therefore considered by winemakers when

281   determining optimal harvest dates.

282   Anthocyanin concentrations of Cabernet Sauvignon wines at three months increased

283   moderately from CS_H1 to CS_H2 (Table 1), stabilised at a similar level at CS_H3 but was higher

284   in CS_H4, a pattern that has been observed previously (Bindon, Varela, Kennedy, Holt, & Herderich,

285   2013), and was evident even under severe vintage conditions with berry shrivel (Schelezki, Smith,

286   Hranilovic, Bindon, & Jeffery, 2018). Wine colour density, SO_2-resistant pigments and total

287   phenolics increased as a result of later harvest stages with continuous increments throughout the

288   Cabernet Sauvignon sequential harvests. Wine MCP tannin concentrations remained steady in CS_H1

289   and CS_H3 (around 860 mg/L) and were slightly lower concentration in CS_H2, followed, by an

290   important increase with the last harvest date (CS_H4, 1297 mg/L), which was mirrored by an increase

291   in extractable grape tannin (mg/g berry), whereas values remained similar among the first three

292   harvests. An earlier harvest with the objective to produce a wine lower in alcohol by 1.5% ABV (as

293   in CS_H3, Table S4 of the Supporting Information) was therefore likely to have a significant

294   implication on the perception of wine astringency (Ma, Guo, Zhang, Wang, Liu, & Li, 2014)

295   (discussed under 3.5.1). Similarly to the preceding study (Schelezki, Smith, Hranilovic, Bindon, &

296   Jeffery, 2018), wine tannin MM decreased from the first to the second harvest (CS_H1 to CS_H2)

297   and only ascended again with the last harvest (CS_H4). In fact, the tannin MM were identical at

298 commercial harvest H4 in both the 2015 and 2016 vintages (~1800 g/mol) whereas the tannin

299 concentration was around 1.6-fold higher in 2015 (2145 mg/L) as a result of the berry shrivel that

300 occurred (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018).

301   The anthocyanin concentrations of the Shiraz wines at 3 months after bottling trended

302 similarly to those of the Cabernet Sauvignon wines as a function of harvest date, except for a notable

303 decline between SH_H3 and SH_H4 (Table 1). There were linear increments in wine colour density,

304 total phenolics and SO_2-resistant pigments up to SH_H4, reaching similar levels as in CS_H4 wine

305 (except for SO_2-resistant pigments). In fact, it appeared that both cultivars gained similar ripeness

306 levels, not only in terms of TSS and colour parameters, but also regarding the final tannin

307 concentrations in these wines. However, in wines of similarly lower alcohol levels in relation to the

308 respective controls (i.e., CS_H3 and SH_H2 at approximately 13.8% ABV compared to

309 approximately 15.5% in their controls), the advanced harvest date necessary to approximate 13.5 °Bé

310 resulted in 40% lower values in colour density and SO_2-resistant pigments in SH_H2 (compared to

311 the control), whereas higher levels (as a proportion of the control) were present in the aforementioned

312 Cabernet Sauvignon wine CS_H3 (Table1). This may be important when considering an earlier

313 harvest for alcohol management as the impact on colour properties might compromise red wine

314 quality parameters, particularly in Shiraz. Distinct from the initially steady progression seen in the

315 Cabernet Sauvignon wines, however, were the large increments in wine tannin concentrations,

316 quadrupling from SH_H1 to SH_H4 with comparable alcohol concentrations as in the CS_H1 and

317 CS_H4 counterparts (Table 1). The respective extractable grape tannin (mg/g berry) did not reflect

318 this trend and only increased with the last harvest date.

319   Colour parameter measurements were repeated 12 months after bottling to assess potential

320 difference in ageing abilities of wines from advanced harvest dates. The additional time in bottle

321 resulted in significantly lower anthocyanin concentrations in the Cabernet Sauvignon wines, which

322 decreased by 50% or more across all harvest dates. This was concurrent with a positive effect on wine

323 colour density in line with significantly increased SO_2-resistant pigments (stable forms of wine

324   colour) as the anthocyanins reacted, which is comparable to previous observations (Schelezki, Smith,

325   Hranilovic, Bindon, & Jeffery, 2018). Total phenolics on the other hand did not change significantly

326   during this 12 months period. The effect of ageing on Shiraz colour properties was similarly positive,

327   and the benefits of a later harvest on colour quality parameters remained evident with bottle aging.

328   *3.4 Variety-dependent implications of blending treatments on wine colour and tannin properties*

329   Variety-dependent modifications of the colour properties were observed in the wines resulting

330   from substitution treatments. In accordance with previous observations (Schelezki, Smith, Hranilovic,

331   Bindon, & Jeffery, 2018; Sherman, Greenwood, Villas-Boâs, Heymann, & Harbertson, 2017), total

332   anthocyanins and total phenolics determined at 3 months did not change significantly when

333   employing either water or GHW to manage the Cabernet Sauvignon alcohol levels under the

334   presented vintage conditions (Table 2). However, significantly enhanced wine colour densities were

335   observed following the substitution treatments in CS_Bw2, CS_Bw3) compared to the control, and

336   in CS_Bw2 compared to the GHW counterpart CS_B2. Additionally, the formation of SO_2-resistant

337   pigments was seemingly enhanced with an intermediate water addition (CS_Bw2), and the lowest

338   and highest implementation rates (CS_Bw3 and CS_Bw1) resulted in values that did not differ

339   significantly from each other or CS_H4. Among the GHW treatments, a colour density higher than

340   the control was only measured in the CS_B3 wines, and values remained similar to the control in

341   CS_B1 and CS_B2. At similar alcohol levels, the intermediate and highest GHW addition rates

342   resulted in lower amounts of stable pigments compared to the respective water treatments, and to the

343   CS_H4 control in the case of CS_B1, whereas total phenolics and anthocyanins did not change. As

344   red wines usually undergo some ageing before being released on the market, an important observation

345   arose from the re-analysis of colour parameters after 12 months of time in bottle, revealing

346   significantly higher levels of SO_2-resistant pigments with all water substitution rates (25%, 16% and

347   10% in CS_Bw1, CS_Bw2 and CS_Bw3, respectively) in comparison to the control wine (Table 2),

348   in accord with higher colour densities. Using GHW did not negatively impact the ageing ability of

349   the resulting wines either, resulting in equal (CS_B1, CS_B2) or higher (CS_B3) levels of SO_2-

350   resistant pigments and colour densities in relation to the control. With ageing, a loss in total

351   anthocyanins was evident with both blending components, whereas total phenolics remained

352   relatively static.

353   A diverging prospective impact of the blending component on wine quality was considerably

354   more obvious in terms of tannin concentrations at 3 months (Table 2). Employing water in the

355   winemaking process resulted in similar tannin levels to the control wines whereas the use of GHW

356   resulted in significantly less tannins in CS_B1 and CS_B2 compared to their water-substitution

357   counterparts (however, remaining similar to the control). Furthermore, in contrast to the results of the

358   preceding study, where water implementation led to slightly increased average MM of wine tannins,

359   the MM in the present study remained similar to the control, and decreased only with the highest

360   GHW addition in CS_B1 (Table 2). These observations could be indicative for a possibly accelerated

361   polymerisation of tannins catalysed by the lower pH and higher ethanol presence following GHW

362   implementation, so that these tannins are more likely to either precipitate, or to re-associate to grape

363   cell wall material (Kontoudakis, González, Gil, Esteruelas, Fort, Canals, et al., 2011; Ruiz-Garcia,

364   Smith, & Bindon, 2014), leading to the slight concentration differences between the blending

365   components. This was further, and to a greater extend, observed with Shiraz (mentioned further

366   below).

367   Generally, according to the colour and tannin parameters, aspects associated with wine quality

368   resulting from the grapes at commercial harvest (CS_H4) were retained among the lower alcohol

369   wines from pre-fermentative juice substitution, and particularly so with water, while also yielding

370   ethanol concentrations similar to the consecutive harvest wines.

371 **Table 1** Colour measures (determined after 3 and 12 months after bottling) and tannin properties of the Cabernet Sauvignon and Shiraz wines resulting

372 from consecutive harvests.[a]

Colour and tannin measures	Cabernet Sauvignon				Shiraz			
	CS_H1	CS_H2	CS_H3	CS_H4	SH_H1	SH_H2	SH_H3	SH_H4
3 Months								
MCP tannin [mg/L]	863 ± 61b	728 ± 28c	864 ± 19b	1297 ± 54a	303 ± 42d	497 ± 62c	942 ± 97b	1256 ± 85a
Wine-like MCP tannin [mg/g berry]	0.10 ± 0.03b	0.13 ± 0.04ab	0.11 ± 0.04b	0.20 ± 0.03a	0.23 ± 0.04b	0.24 ± 0.03b	0.25 ± 0.03b	0.39 ± 0.04a
MM by GPC 50% [g/mol]	1819 ± 18a	1663 ± 26b	1656 ± 10b	1806 ± 31a	1448 ± 13b	1503 ± 20b	1541 ± 25ab	1648 ± 88a
Wine colour density [au]	6.61 ± 0.28d	7.26 ± 0.23c	9.45 ± 0.12b	11.7 ± 0.1a	5.58 ± 0.32d	6.96 ± 0.19c	10.3 ± 0.1b	11.8 ± 0.1a
Total anthocyanins [mg/L]	437 ± 38c	522 ± 14b	582 ± 13ab	595 ± 34a	463 ± 22c	555 ± 28b	706 ± 0a	590 ± 7b
Total phenolics [au]	31.5 ± 2.8c	33.5 ± 1.3c	38.7 ± 1.0b	45.0 ± 1.3a	30.2 ± 1.5c	35.3 ± 2.2b	46.3 ± 0.7a	45.6 ± 0.7a
SO_2- resistant pigments [au]	1.29 ± 0.04d	1.46 ± 0.04c	1.97 ± 0.04b	2.84 ± 0.04a	0.86 ± 0.03d	1.19 ± 0.01c	1.73 ± 0.05b	2.59 ± 0.02a
12 Months								
Wine colour density [au]	7.95 ± 0.42d	9.07 ± 0.27c	11.9 ± 0.3b	14.6 ± 0.4a	6.04 ± 0.27d	8.00 ± 1.04c	10.9 ± 0.2b	13.5 ± 0.3a
Total anthocyanins [mg/L]	166 ± 22b	222 ± 3ab	244 ± 11a	193 ± 48ab	283 ± 14c	341 ± 9b	369 ± 10a	200 ± 11d
Total phenolics [au]	29.7 ± 2.5c	31.0 ± 0.9c	37.7 ± 1.8b	43.1 ± 2.7a	29.3 ± 1.4c	34.7 ± 1.3b	43.9 ± 0.7a	43.6 ± 0.9a
SO_2- resistant pigments [au]	2.1 ± 0.1c	2.24 ± 0.07c	3.20 ± 0.07b	4.70 ± 0.29a	1.33 ± 0.06d	1.76 ± 0.07c	2.86 ± 0.14b	4.62 ± 0.07a

373 [a] Values are means of 3 replicates ± standard error. Values followed by different letters within a row and per variety are significantly different ($p < 0.05$,

374 one way (3 months) or repeated measures (12 months) ANOVA with post hoc Fisher's LSD).

375 **Table 2** Colour measures (determined after 3 and 12 months after bottling) and tannin properties of the Cabernet Sauvignon and Shiraz wines

376 following the blending treatments.[a]

Wine	Wine colour density [au]	Total anthocyanins [mg/L]	Total phenolics [au]	SO_2-resistant pigments [au]	MCP Tannin	MM by GPC 50% [g/mol]	Wine colour density [au]	Total anthocyanins [mg/L]	Total phenolics [au]	SO_2- resistant pigments [au]
Cabernet Sauvignon	*3 Months*						*12 Months*			
CS_H4 (Control)	11.7 ± 0.1b	595 ± 34	45.0 ± 1.3	2.84 ± 0.04cd	1297 ± 54ab	1806 ± 31ab	14.6 ± 0.4bc	226 ± 8a	43.1 ± 2.7	4.70 ± 0.29b
CS_B1	11.3 ± 0.3b	588 ± 25	40.3 ± 1.7	2.34 ± 0.02e	1042 ± 100b	1718 ± 4b	13.4 ± 0.2c	202 ± 18abc	38.8 ± 1.2	4.06 ± 0.20b
CS_B2	11.5 ± 0.7b	592 ± 25	43.0 ± 1.0	2.62 ± 0.19de	1165 ± 83b	1803 ± 17ab	13.8 ± 0.4c	207 ± 32ab	42.0 ± 0.2	4.32 ± 0.47b
CS_B3	13.0 ± 0.9a	607 ± 18	45.7 ± 1.1	3.17 ± 0.31b	1257 ± 67ab	1820 ± 81a	16.1 ± 0.7a	156 ± 24de	45.3 ± 0.8	5.83 ± 0.44a
CS_Bw1	12.4 ± 0.2ab	610 ± 43	45.9 ± 2.3	2.84 ± 0.09cd	1481 ± 76a	1812 ± 29ab	15.6 ± 6.0ab	185 ± 9bcd	44.8 ± 3.0	5.50 ± 0.41a
CS_Bw2	13.6 ± 0.2a	543 ± 4	45.1 ± 2.2	3.52 ± 0.03a	1433 ± 275a	1890 ± 45a	16.2 ± 0.8a	166 ± 6cde	45.0 ± 2.4	6.10 ± 0.49a
CS_Bw3	13.4 ± 0.8a	596 ± 46	47.0 ± 3.6	3.05 ± 0.04bc	1501 ± 18a	1869 ± 53a	16.6 ± 0.9a	131 ± 15e	43.4 ± 1.4	5.83 ± 0.01a
Shiraz										
SH_H4 (Control)	11.8 ± 0.1a	590 ± 7	45.6 ± 0.7abc	2.59 ± 0.02a	1256 ± 85a	1648 ± 88b	13.5 ± 0.3a	200 ± 11c	43.6 ± 0.9ab	4.62 ± 0.07a
SH_B1	8.39 ± 0.41e	621 ± 17	40.7 ± 1.2d	1.51 ± 0.05d	708 ± 61d	1551 ± 36b	9.09 ± 0.23e	346 ± 13ab	39.1 ± 1.7c	2.17 ± 0.12e
SH_B2	9.24 ± 0.26cde	640 ± 27	43.6 ± 1.8bcd	1.71 ± 0.03cd	964 ± 39c	1543 ± 21b	10.3 ± 0.1cde	354 ± 24a	42.2 ± 2.2bc	2.47 ± 0.00de
SH_B3	9.59 ± 0.36cd	653 ± 19	46.2 ± 0.8ab	1.76 ± 0.04cd	958 ± 42c	1592 ± 30b	10.7 ± 0.8bcd	340 ± 5ab	44.3 ± 0.7ab	2.79 ± 0.14cd
SH_Bw1	9.06 ± 0.97de	597 ± 24	42.7 ± 1.0cd	1.70 ± 0.26d	994 ± 123bc	1647 ± 71b	9.86 ± 0.95de	314 ± 47ab	41.4 ± 0.6bc	2.65 ± 0.54cde
SH_Bw2	10.2 ± 0.5bc	641 ± 40	46.8 ± 2.0ab	1.99 ± 0.18bc	1193 ± 39ab	1767 ± 20a	11.1 ± 0.5bc	324 ± 53ab	46 .0± 2.3a	3.12 ± 0.38bc
SH_Bw3	10.9 ± 0.6ab	624 ± 38	47.5 ± 2.4a	2.24 ± 0.17b	1273 ± 252a	1661 ± 56ab	11.9 ± 0.5b	272 ± 49bc	45.3 ± 2.4ab	3.60 ± 0.34b

377 [a] Values are means of 3 replicates ± standard error. Values followed by different letters within a column and per variety are significantly different ($p < 0.05$,

378 one way (3 months) or repeated measures (12 months) ANOVA with post hoc Fisher's LSD).

379   When producing lower alcohol wines with Shiraz fruit, the implications on colour and tannin

380   parameters at 3 months appeared to be more decisive than observed in the Cabernet Sauvignon wines

381   (Table 2). This is evident with significantly decreased colour densities (SH_Bw1/Bw2) and SO$_2$-

382   resistant pigment values with higher amounts of water added. Given a similar result among the GHW

383   Shiraz wines, there was no difference between the two highest substitution rates with water or GHW

384   (i.e., SH_B1/Bw1 and SH_B2/Bw2). However, SH_Bw3 remained superior in wine colour density

385   and SO$_2$-resistant pigments compared to SH_B3 (Table 2). In contrast, total anthocyanin

386   concentrations and total phenolics did not change significantly among the treatments, with the

387   exception of a significant decline in total phenolics in SH_B1 compared to the control.

388   Notably, the effects on colour density and SO$_2$-resistant pigments were apparent despite

389   marginal differences in alcohol level: SH_B3 at 15.0% ABV had 20-30% lower values in these

390   parameters compared to the Shiraz control wine at 15.4% ABV (SH_H4). Albeit a less severe effect

391   when substituting with water, only the lowest implementation rate (6% water in SH_Bw3) preserved

392   a similar wine colour density to the control wine (Table 2). This outcome was enlightening in terms

393   of potential cultivar differences, given the equal (water) or even smaller (GHW) substitution rates

394   applied with Shiraz compared to the Cabernet Sauvignon wines (see substitution rates under section

395   2.7, Fig. S1 and Table S5 of the Supporting Information)), and given that control wines of each

396   cultivar were initially similar in colour parameters and tannin concentrations. An explanation could

397   be a higher initial extractability of anthocyanins as well as phenolics directly after crushing, which

398   shows in higher extractable grape tannin content per berry in the Shiraz grapes, compared to Cabernet

399   Sauvignon (Table 1), so that higher proportions of these compounds were removed with the juice.

400   After 12 months of ageing in the bottle, the initial relative differences in Shiraz wine colour

401   parameters largely remained (Table 2) and point to a substantial loss in colour quality. This is

402   particularly stark when comparing colour density and SO$_2$-resistant pigment levels of the substitution

403   treatments to the Shiraz control wine SH_H4, which in contrast attained values similar to the control

404   Cabernet Sauvignon CS_H4.

405   Remarkable effects on the Shiraz wine MCP tannin concentrations were seen when employing

406   GHW, with concentrations that were 44% lower in SH_B1 and 25% lower in both SH_B2 and SH_B3

407   in comparison to the SH_H4 control (Table 2) (concurrent with being slightly lower in GHW wines

408   than the control wine but this trend was not statistically significant). A main factor eliciting this

409   decline in tannin concentration, which was also noticeable albeit non-significant in the Cabernet

410   Sauvignon wines (Table 2) may be the pre-fermentative modification of pH and ethanol levels

411   associated with the GHW addition. Commencing the fermentation in the presence of some ethanol

412   and at lower pH values could have favoured an initially higher extraction of tannin from grape solids

413   (Canals, Llaudy, Valls, Canals, & Zamora, 2005) and accelerated modifications in tannin structure

414   (Kontoudakis, et al., 2011). This would lead to more polymerised tannins, which are more likely to

415   be re-adsorbed by grape cell wall material (Ruiz-Garcia, Smith, & Bindon, 2014) (hence removed

416   from the wine) or potentially precipitated during the winemaking process. This outcome differed to

417   the berry shrivel conditions experienced in 2015, where no treatment-related impacts on tannin

418   concentration were observed (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). In addition,

419   varietal differences in tannin extraction could also play a role, i.e., tannin removed with juice

420   substitution was not replaced at the same rate in Shiraz wines compared to the possible case in the

421   Cabernet Sauvignon wines. This warrants further investigation, to assess how colour and tannin

422   extraction dynamics change as a function of the pre-fermentative blending treatment upon interaction

423   with grape maturity and variety. Using water instead, however, resulted in wine tannin levels close

424   to those of SH_H4, yet a significant decline was noted with the highest water addition rate in SH_Bw1

425   (Table 2). Interestingly, tannin MM appeared to be marginally elevated in comparison to their

426   respective GHW counterparts, becoming significant for the SH_Bw2 treatment.

427   Putting the overall observations at 3 and 12 months into perspective, colour parameters and

428   tannin concentrations resulting from all water blending treatments were similar to, or slightly higher

429   than, those measured for the harvest series SH_H3 wine (Table 1 and 2), whereas this was only true

430   for GHW implementations in SH_B2 and SH_B3. However, the pre-fermentative alcohol

431   management approach (with both water and GHW) in Shiraz failed to retain the wine colour density

432   and colour stability that was seen with the later harvest date (control wine SH_H4). Still, the resulting

433   values may be within an acceptable range for quality red wine production that will come down to

434   subjective evaluations of the winemaker. Of higher concern could be the important decline in tannin

435   concentrations among the GHW wines, raising questions this particular approach with respect to

436   detrimental consequences on mouthfeel or ageing potential. Despite the lower impact compared to

437   GHW, pre-fermentatively adjusting the potential wine alcohol level with water from a relatively

438   common 15.4% ABV to around 13.5% ABV, thus working within current regulations for water

439   addition (FSANZ, 2016) provoked potentially detrimental changes to Shiraz wine quality that were

440   not evident in the Cabernet Sauvignon wines of the current or previous studies (Schelezki, Smith,

441   Hranilovic, Bindon, & Jeffery, 2018). Evidently, application of the chosen approaches to pre-

442   fermentative blending to decrease must sugar concentrations and manage alcohol levels in Shiraz

443   wines needs further careful evaluation to better ascertain the potential for inadvertently changing wine

444   style or quality.

445   *3.4 Impact of the blending treatments on volatiles profiles*

446   *3.4.1 Cabernet Sauvignon*

447   Winemakers may choose among various levels of fruit ripeness to target aroma profiles for

448   certain wine styles, for instance, increasing 'ripe fruit' and decreasing 'green' characteristics with

449   later harvests (Bindon, Holt, Williamson, Varela, Herderich, & Francis, 2014). Once fruit is picked,

450   it is desired to carry this aroma potential through the winemaking process, which could include the

451   necessity for alcohol management. The present study investigated the consequences on wine volatile

452   composition of the proposed pre-fermentative alcohol management approaches, applied under

453   moderate grape ripening conditions, to produce lower alcohol wines in response to the growing

454   demand (Longo, Blackman, Torley, Rogiers, & Schmidtke, 2017) rather than purely mitigating

455   extreme harvest conditions (Schelezki, Suklje, Boss, & Jeffery, 2018). Indeed, results from the 2016

456   vintage revealed different patterns of change in the volatile composition of the wines in response to

457   the blending treatments when assessed using agglomerative hierarchical clustering (AHC) (Fig. 2a).
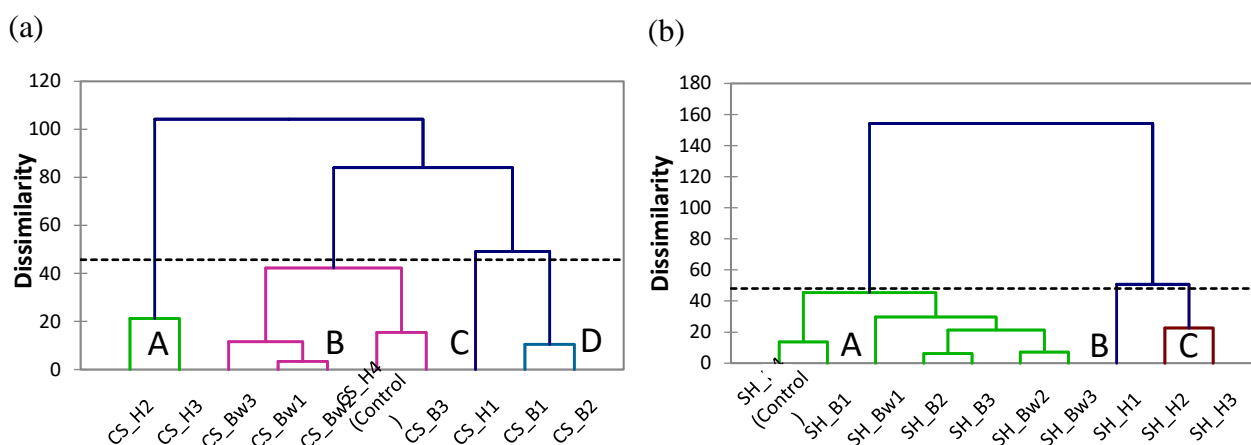
458   **Fig. 2** Agglomerative hierarchal clustering (AHC) based on volatile composition for (a) Cabernet

459   Sauvignon wines and (b) Shiraz wines arising from the different treatments (refer to Material and

460   Methods and Fig. S1 of the Supporting Information for details of the treatments/wine codes). The

461   dotted line represents an automatic truncation into homogenous groups, which are assigned A, B, C

462   (and D).

463   The Cabernet Sauvignon wines were clustered into four groups according to their relative

464   similarities. The higher addition rate of GHW resulted in the separation of CS_B1 and CS_B2

465   treatments (group D) from the control (CS_H4, group B), whereas the lowest addition of the GHW

466   (i.e., 16% juice substitution in CS_B3, affording 14.5% ABV) had the most resemblance to the

467   control wine (15.5% ABV) (Fig. 2a). Indeed, only three compound groups were particularly affected

468   (acids, methyl esters of fatty acids and HAA from yeast sugar and N metabolism) compared to the

469   control (Fig. 3a and Table S6 of the Supporting Information). CS_B1 and CS_B2 were most similar

470   to the earliest harvest series wine CS_H1 (group C) and separated from the remaining wines,

471   coinciding with increments in grape-derived 1-hexanol (significant for CS_B1) and decreases in

472   fermentation-derived compounds (i.e., ethyl esters of branched, odd carbon and straight chain fatty

473   acids, higher alcohols and particularly HAA of yeast sugar and N metabolism) relative to the control

474   (Fig. 3a and Table S6 of the Supporting Information). At the highest GHW implementation rate the

475   level of isoprenoids markedly increased, driven by significant increments in 8 out of 11 individual

476   compounds (e.g., β-damascenone, linalool and vitispirane 1) (Table S6 of the Supporting

477   Information), underlining the divergence between varietal and fermentative compounds.
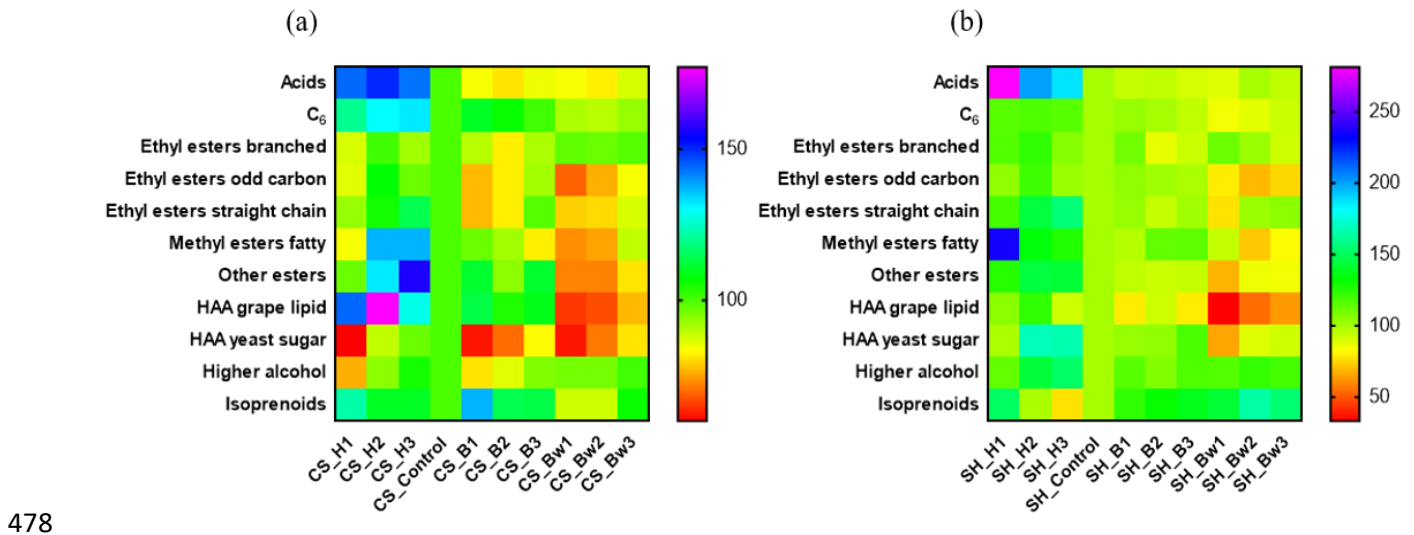
479   **Fig. 3** Heatmap highlighting changes of semi-quantitative data for volatile compound groups in (a)

480   Cabernet Sauvignon wines and (b) Shiraz wines, based on average relative concentration differences

481   of compounds in treatments compared to the controls as presented in Table S6 and S7 of the

482   Supporting Information (refer to Material and Methods and Fig. S1 of the Supporting Information for

483   details of the treatments/wine codes). Values are normalised in relation to the control wines (i.e.,

484   CS_H4, 100%, and SH_H4, 100%). Relative to the control, increasing concentrations are indicated

485   by darker green, blue and purple fill colour, while concentrations decrease with more yellow and red

486   fill colour according to the legends. HAA refers to higher alcohol acetates.

487   The increases in grape-derived compounds with use of GHW were in accord with previous

488   observations for Cabernet Sauvignon (Schelezki, Suklje, Boss, & Jeffery, 2018) but in that previous

489   work the control wines also contained lower levels of esters and higher alcohols. Under the vintage

490   and winemaking conditions presented here, levels of esters showed strong negative correlations with

491   the proportion of juice substitution ($R^2 = 0.99$ for ethyl esters of odd carbon number fatty acids, $R^2 =$

492   0.91 for ethyl esters of straight-chain fatty acids, $R^2 = 0.99$ for HAA from yeast sugar and N

493   metabolism, and $R^2 = 0.93$ for higher alcohols, and $R^2 = 0.72$ for ethyl esters of branched acids), which

494   indicated a decisive effect of the treatments on the juice precursor matrix (Sumby, Grbin, & Jiranek,

495   2010).

496   Water substitution treatments were also clustered in group B with CS_H4 and CS_B3. In that

497   case, CS_Bw3 was grouped second closest to the control wine (Fig. 2a) with noticeable changes in 8

498   of 11 compound groups (acids, C_6-alcohols, ethyl esters of odd carbon, straight chain and methyl fatty

499   acids, other esters, and HAA of grape lipid degradation and yeast sugar and N metabolism), leading

500   to a lower degree of similarity to the control as was the case for the GHW counterpart CS_B3 (Fig.

501   2a and 3a, Table S6 of the Supporting Information). However, in contrast to using GHW, the use of

502   water caused a more homogenous decline among grape-derived and fermentative volatiles alike (Fig

503   2a, Table S6 of the Supporting Information) compared to the control CS_H4. Declines in 1-hexanol

504   and sum of isoprenoids (particularly driven by lower values of citronellol and vitispirane 1) were

505   evident with lower established alcohol levels (higher rate of water substitution, decreasing from

506   CS_Bw1 to CS_Bw3) in comparison to the control (Table S6 of the Supporting Information). In

507   contrast, such grape-derived volatile compounds were unaffected in a previous study of analogously-

508   produced lower alcohol Cabernet Sauvignon wines but that was in the context of highly mature,

509   shrivelled grapes (Schelezki, Suklje, Boss, & Jeffery, 2018). However, in both studies β-damascenone

510   remained unchanged in wines following the water treatments, which can have important implications

511   for overall wine aroma despite the general decrease in some aroma compounds, given the potential

512   of β-damascenone to enhance 'fruity' attributes in wines (Pineau, Barbe, Van Leeuwen, &

513   Dubourdieu, 2007).

514   Generally, fermentation-derived compound groups also decreased with lower established

515   alcohol levels from CS_Bw3 to CS_Bw1, more so as noted for the GHW wines (Fig. 3a). In particular,

516   strong negative correlations in relation to the water implementation rates were determined for ethyl

517   esters of odd carbon number fatty acids ($R^2 = 0.96$), ethyl esters of straight fatty acids ($R^2 = 0.84$),

518   HAA from yeast sugar and N metabolism ($R^2 = 0.95$), HAA of lipid degradation ($R^2 = 0.87$), methyl

519   fatty esters ($R^2 = 0.91$), other esters ($R^2 = 0.99$), and higher alcohols ($R^2 = 0.99$). Interestingly, the

520   concentration declines of HAA from yeast sugar and N appeared to be only affected by the must sugar

521   adjustment than by the blending component used given the similar pattern of decrease. Regarding

522   higher alcohols, concentrations remained higher in the water substitution treatments compared to the

523   respective GHW wines (particularly driven by 3-methyl-1-butanol, benzyl alcohol, 2-phenylethanol)

524   (Fig. 3a, Table S6 of the Supporting Information), which seemed to be the only constant finding to

525   the observations of the preceding work (Schelezki, Suklje, Boss, & Jeffery, 2018). In the previous

526   study, only a small fraction of fermentative wine volatiles was affected, whereas their general dilution

527   in the present experiments appeared to clearly distinguish the two vintage conditions and maturity

528   levels of the grapes harvested in each year. Nonetheless, given the homogeneous nature of the

529   concentration decreases across compound groups, the resulting water substitution wines may be more

530   likely to remain a certain similarity to the control wine even with the lowest final alcohol levels (as

531   opposed to the GHW counterparts CS_B1 and CS_B2, in particular), and were in fact grouped

532   together with the CS_H4 control (group B) according to the AHC (Fig. 2a). Thus, despite the volatile

533   profile being markedly more affected than previously reported (Schelezki, Suklje, Boss, & Jeffery,

534   2018), the least effect was again associated with water as blending component rather than GHW.

535   Harvesting earlier to yield lower wine alcohol concentrations had a greater influence on the

536   volatile composition compared to the control, and CS_H1 (group C) and CS_H2/CS_H3 (group A)

537   wines were clearly distinguished from the lower alcohol wines made via the juice substitution

538   approaches (Fig 1a and 2a). Particularly outstanding were the higher concentrations of volatile acids,

539   C_6-alcohols, other esters and HAA from lipid degradation at earlier harvests, compared to the control

540   and blending treatments, followed by dramatic declines from CS_H3 to CS_H4 (Fig. 3a, Table S6 of

541   the Supporting Information). Given that fermentation-derived compound groups were at similar

542   concentrations to the control (ethyl esters of odd carbon number fatty acids or straight chain fatty

543   acids) or even higher (other esters, HAA from lipid degradation) (Fig. 3a and S6 of the Supporting

544   Information), the increased potential for 'fruity' perceptions may mitigate any negative implications

545   of the C$_6$-alcohol ('grassy', 'green') (Bindon, Holt, Williamson, Varela, Herderich, & Francis, 2014)

546   with an earlier harvest date, as further discussed in Section 3.5.1.

547   *3.4.2 Shiraz*

548   The implications for wine volatile profiles were somewhat different when producing lower

549   alcohol Shiraz wines. According to the heatmap in Fig. 3b, the impact of water substitution treatments

550   on wine volatiles was more decisive than observed for the GHW treatments, affecting a higher

551   number of compound groups in relation to the control wine SH_H4 (Table S7 of the Supporting

552   Information). In fact, the volatile profile of SH_B1 resembled most closely the Shiraz control wine

553   according to AHC (group A, Fig. 2b), differing mostly in lower concentration of HAA of grape lipid

554   degradation. Lower concentrations of ethyl esters of branched acids and methyl esters of fatty acids

555   in the other GHW treatments (SH_B2 and SH_B3, which were most similar to each other but also in

556   group A) distinguished them from SH_B1 (Fig. 2b, Fig.3b and Table S7 of the Supporting

557   Information), but grape-derived volatiles, as well as the remaining compound groups, were still

558   unaffected regardless of the proportion of GHW added (however, levels if isoprenoids were slightly

559   elevated across the GHW treatments, and significantly higher citronellol levels are standing out).

560   Consequently, the characteristic impact on grape- and fermentation-derived volatile constituents seen

561   at the highest GHW implementation rate for the respective lower alcohol Cabernet Sauvignon wines

562   did not occur among the Shiraz wines. Indeed, the resulting volatile profiles of SH_B1-B3 wines were

563   generally reminiscent of the control wine according to the AHC (Fig. 2b) and the heatmap (Fig 2b),

564   despite pre-fermentative GHW substitution yielding wines that were up to 2.4% ABV lower.

565   On the other hand, with higher water substitution rates, total concentrations of 1-hexanol (R^2

566   $= 0.97$), HAA from grape lipid degradation ($R^2 = 0.94$), HAA from yeast sugar and N metabolism (R^2

567   $= 0.88$), ethyl esters of straight-chain fatty acids ($R^2 = 0.38$, lower only in SH_Bw1) and other esters

568   ($R^2 = 0.93$) declined linearly whereas ethyl esters of odd carbon number fatty acids ($R^2 = 0.79$) and

569   methyl fatty acid esters ($R^2 = 0.71$) decreased nearly equally across this treatment series. These losses

570   stand in contrast to higher total isoprenoid levels relative to the control (in particularly elevated

571   concentrations of trans-geraniol and citronellol, which outweigh decreasing other isoprenoids such

572   as linalool and α-terpineol), slightly elevated ethyl esters of branched acids (due to significantly

573   increasing phenyl ethyl acetate) and higher alcohol (octan-1-ol, benzyl alcohol and 2-phenylethanol)

574   levels and unchanged volatile acids upon water addition (Fig. 3b and Table S7 of the Supporting

575   Information). Owing to the decreased concentration of volatiles occurring linearly among the majority

576   of compound groups, the ratios between these groups remained somewhat steady so that the

577   treatments SH_Bw2 and SH_Bw3 showed similarities with the GHW counterparts SH_B2 and

578   SH_B3 (see grouping in Fig. 2b). However, the discrepancy between grape- and fermentation-derived

579   volatiles in SH_Bw1 might have resulted in a separation relative to the other water substitution

580   treatments, and increased the dissimilarity to SH_H4 and SH_B1 (Fig. 2b, Fig. 3b).

581   A difference between varietal and fermentative wine volatiles appeared to be primarily driven

582   by a decline in various esters (esters of odd carbon number fatty acids, esters of straight chain fatty

583   acids, methyl fatty acid esters, other esters and HAA from grape lipid degradation) with higher

584   additions of water (but not with GHW) in relation to the sum of isoprenoids or 1-hexanol (Table S7

585   of the Supporting Information). Still, water implementation altered the profile of isoprenoids on an

586   individual level, with the majority found to decrease (except for trans-geraniol and citronellol as

587   mentioned above, which increments in relation to the control appeared to outweigh the decreasing

588   trend of the remaining isoprenoids, leading to an overall ascending proportion). Of the affected esters,

589   the concentrations of hexyl acetate ('fruity') seemed to be particularly sensitive to water substitutions,

590   with a value for the SH_Bw1 wine that was only 33% that of the control wine. According to a previous

591   study (Keyzers & Boss, 2010), this could be indicative of the lower proportion of grape juice at the

592   start of fermentation. Other notable changes were the step-wise lower concentrations of 3-methylbutyl

593   acetate and phenylethyl acetate ('fruity' and 'floral' HAA from yeast sugar and N metabolism) with

594   higher rates of water implementation, possibly explained by a dilution in the respective amino acid

595   precursors leucine and phenylalanine (Styger, Prior, & Bauer, 2011), whereas sufficient amounts of

596   these precursors may have been replaced with the GHW, therefore preventing any concentration

597   changes (Table S7 of the Supporting Information). Similarly to the Cabernet Sauvignon wines

598   discussed above, these relative changes in grape- and fermentation-derived compound groups were

599   suggestive of a greater prominence of varietal volatiles in these Shiraz wines substituted with water,

600   in contrast to the GHW wines.

601   As an alternative way of producing lower alcohol Shiraz wines, earlier harvest dates were

602   characterised particularly by elevated acid, and methyl esters of fatty acids concentrations, decreasing

603   from SH_H1 to SH_H3, compared to the control SH_H4 and substitution treatment wines (Fig. 3b

604   and Table S7 of the Supporting Information). Ethyl esters of straight chain fatty acids and HAA from

605   yeast sugar and N metabolism even tended to increase with later harvest before dropping at

606   commercial harvest. Also, the remaining compound groups seemed present at higher concentrations

607   than the control, but did not show particular patterns with harvest date, for instance HAA from grape

608   lipid degradation, other esters, or ethyl esters of branched acids. Notably, delaying the harvest by

609   further four days (as in SH_H3 to SH_H4) did not increase wine alcohol levels significantly, but

610   resulted in important decreases in an array of fermentation volatiles, including acids, esters and higher

611   alcohols, whereas grape-derived isoprenoids tended to increase. As discussed above, colour and

612   tannin parameters improved with prolonged fruit hang-time but that was apparently at the expense of

613   aroma potential.

614   *3.5 Influence of blending treatments on wine sensory properties*

615   *3.5.1 Cabernet Sauvignon*

616   It has now been observed over two distinct vintages and similar experimental designs that

617   colour and tannin properties, as defined by harvest date, were mostly retained in Cabernet Sauvignon

618   wines following the proposed pre-fermentative juice manipulations. However, wine volatile

619   composition appeared to be more sensitive to the changing vintage conditions, as discussed above. A

620   last aspect to consider was the relevance of these changes to the wine sensory properties, which was

621   determined by descriptive analysis (DA). According to the DA, producing Cabernet Sauvignon wines

622   that were up to 2.6% ABV lower than the control wine by employing the blending treatments did not

623   cause significant changes in the perceptions of the two aroma terms 'dark fruit' and 'green' (Table 5

624   and Table S8 of the Supporting Information), despite the decreased concentrations of a range of

625   volatiles, particularly among the water substituted wines (given the time separation of both analysis

626   however, a further studies are required to confirm this observation). This was similar to the preceding

627   study, in which the wines were not distinguishable in aroma attributes such as 'aroma intensity', 'red

628   fruit' or 'dark fruit' (Schelezki, Suklje, Boss, & Jeffery, 2018), which are attributes appreciated by

629   consumers and therefore sought by winemakers.

630   Previous differences were more noticeable among the wine flavour attributes, in relation to

631   both the alcohol levels as well as blending component, which appeared to be consistent with the

632   current study. For instance, with higher GHW substitution rates as in CS_B1 and CS_B2, 'flavour

633   intensity', 'red fruit' and 'dark fruit' decreased and were rated similarly to the earlier harvested

634   CS_H2 wine, whereas substitution with water seemed to retain the intensities of these attributes

635   (except for a lower 'dark fruit perception in CS_Bw1, Table 5). Besides the minimal impact on 'red

636   fruit' and 'dark fruit' characters, the use of water did not alter the perception of 'dried fruit' in contrast

637   to the significantly lower ratings among the GHW treatments compared to the control. Sherman,

638   Greenwood, Villas-Boâs, Heymann, and Harbertson (2017) observed a decrease in 'fruity' characters

639   when employing water substitution treatments to produce wines that were lower in alcohol by up to

640   4.2% ABV compared to the respective control (16% ABV), but this was not the case in our study

641   involving a decrease of up to 2.9% ABV in relation to the 15.5% ABV control CS_H4. This

642   potentially reveals the suitability of a pre-fermentative approach using water substitution to adjust

643   must sugar levels (thus potential wine alcohol levels) close to the legally allowed limit in Australia

644   of 13.5 °Bé (approximately 13.5% ABV) without a significant loss of important attributes such as

645   'red fruit' or 'dark fruit'. Interestingly, despite a noticeable downward trend with higher GHW

646   substitution rates, 'confection' did not change significantly compared to the control, but water

647   appeared to accentuate this attribute, particularly in the CS_Bw2 wines (Table 5).

648   Amongst the mouthfeel properties, 'astringency' and 'hotness' were found to differentiate the

649   wines. Compared to the control CS_H4 wine, 'astringency' was lower with the highest water

650   implementation rate (CS_Bw1, Table 5) despite MCP tannin not being significantly different (Table

651   2). According to previous studies, wine 'astringency' is rather exacerbated by lower alcohol

652   concentrations as interactions between tannins and salivary proteins increase (McRae, Ziora, Kassara,

653   Cooper, & Smith, 2015). However, all GHW wines were significantly less astringent than both the

654   CS_H4 and respective water counterparts, which could be a consequence of lower tannin

655   concentrations in those wines (Ma, Guo, Zhang, Wang, Liu, & Li, 2014). Ratings for 'hotness'

656   (tending to be an indicator of alcohol content) followed a similar trend to astringency but only

657   CS_Bw2 remained similar to the control wine (Table 5). It is interesting that only a small substitution

658   rate with water (10% v/v) or GHW (16% v/v) was necessary to lower the perception of 'hotness' in

659   wines in relation to the control without significantly changing 'fruity' attributes, for example.

660   Given that 6 out of 9 attributes were already peaking by harvest point CS_H3, and that the

661   pre-fermentative alcohol management approaches resulted in most of these attributes being rated

662   similarly to this wine, an earlier harvest could have arguably been a valid option to produce a lower

663   alcohol wine in this instance. If picked at the later stage, however, water or GHW were appropriate

664   choices considering the similar sensory profiles at each alcohol level. Of the two options, water

665   addition (within the regulated limits) would obviously be simpler to implement.

666   **Table 3** Average scores for significantly different (p<0.05) wine sensory descriptors for the Cabernet

667   Sauvignon consecutive harvest and blending treatment wines.[a]

Sample	CS_H1	CS_H2	CS_H3	CS_H4 (Control)	CS_B1	CS_B2	CS_B3	CS_Bw1	CS_Bw2	CS_Bw3	LSD
Aroma											
Dark Fruit	34.2c	40.5ab	42.8ab	45.6a	38.5bc	38.7bc	41.4ab	44.1ab	41.2ab	42.3ab	6.3
Green	40.8a	32.3ab	29.3b	30.9ab	23.3b	22.3b	24.8b	26.4b	24.3b	32.5ab	11.1
Flavour											
Intensity	49.9d	51.3cd	54.7abc	57.7a	51.2cd	53.7bcd	56.7ab	55.3ab	56.7ab	57.5a	3.8
Red Fruit	30.9c	34.0bc	36.1ab	40.0a	33.9bc	37.1ab	38.7ab	38.2ab	40.9a	37.5ab	5.1
Dark Fruit	37.7c	43.7abc	47.0a	47.1a	38.3bc	45.0ab	41.8abc	39.5bc	46.8a	43.1abc	6.9
Dried Fruit	15.5c	15.8c	18.9bc	24.5a	18.5c	17.9c	19.0bc	19.8abc	23.8ab	19.5abc	5.2

Confection	6.2c	10.0bc	9.5bc	9.3bc	7.6bc	8.9bc	10.2b	10.5b	14.5a	7.9bc	3.9
Mouthfeel											
Astringency	47.7cde	42.2e	46.4cde	57.4a	44.7de	49.7bcd	48.7cde	49.2bcd	55.7ab	51.9abc	6.8
Hotness	30.5f	41.6e	43.8de	59.5a	44.2cde	46.1bcde	51.2bc	45.6bcde	52.7ab	49.5bcd	7.3

668   [a] Values are means of 3 replicates. Values followed by different letters within a row are significantly

669   different ($p < 0.05$, one way ANOVA, post hoc Fisher's LSD).

670   *3.5.2 Shiraz*

671   Significant sensory attributes used to describe the Shiraz wines are presented in Table 6, with

672   some differences to the ones determined for Cabernet Sauvignon. As with that cultivar, an effect on

673   wine sensory properties of the alcohol management approach in Shiraz was only evident for flavour

674   and mouthfeel attributes, with aroma profiles being no different to the control wine (Table S9 of the

675   Supporting Information). However, unlike Cabernet Sauvignon, the 'flavour intensity' of the Shiraz

676   substitution treatments wines did not decrease in comparison to the control wine SH_H4. 'Dark fruit'

677   flavour tended to diminish with decreasing alcohol levels, although less so in the water blends than

678   in the respective GHW wines, as observed amongst the Cabernet Sauvignon wines, but only SH_B1

679   was significantly different to the control. Pre-fermentative substitution with water appeared to

680   accentuate 'liquorice' flavour in SH_Bw1 and SH_Bw3 wines whereas a higher implementation of

681   GHW lowered the perception of 'chocolate', particularly for SH_B1 wine (Table 6).

682   Four mouthfeel properties were modified with the alcohol management strategy in the Shiraz

683   wines in comparison to two for Cabernet Sauvignon. Interestingly, SH_B1 was rated highest in

684   'acidity', which coincides with the highest malic acid concentration (Table S5 of the Supporting

685   Information). Regarding 'bitterness', only the significantly higher rating of SH_Bw2 stood out (Table

686   6), which was somewhat curious given the significantly higher tannin molecular mass determined for

687   this wine (Table 2). The implications for Shiraz wine 'astringency' were comparable to those

688   observed among the Cabernet Sauvignon wines, with lower ratings in wines made with higher

689   substitution rates. The water substitution treatments were rated more highly for 'astringency' levels

690 than their respective GHW counterparts (Table 6), in line with observed differences in tannin

691 concentrations (Table 2).

692   Interestingly, the 'flavour intensity' attribute had already reached maximum ratings in the

693 wines resulting from the second harvest date (SH_H2), as had 'liquorice', 'chocolate' and

694 'astringency', but additional grape hang-time was necessary to produce a wine with markedly higher

695 'dark fruit' characteristics as in the commercially-ripe SH_H4 (Table 6). In contrast to the Cabernet

696 Sauvignon wines discussed above, 'dried fruit' did not seem to be an indicator of increased fruit

697 ripeness for Shiraz wines, given the absence of significant changes among the consecutive harvest

698 wines SH_H1 to SH_H4. The majority of flavour and mouthfeel attributes peaked in SH_H2 (13.8%

699 ABV), resulting in similar sensory profiles to the control (15.4% ABV) and SH_Bw1 (13.2% ABV)

700 wines, except for liquorice (Table 6). In this case, longer grape ripening did not seem to markedly

701 change the sensory quality of the Shiraz wines as determined by others (Bindon, Holt, Williamson,

702 Varela, Herderich, & Francis, 2014; Heymann, LiCalzi, Conversano, Bauer, Skogerson, & Matthews,

703 2013). Thus, to produce lower alcohol wines approximating alcohol levels equivalent to an initial

704 must TSS of 13.5 °Bé, an earlier harvest could have been the simpler approach under the vintage

705 conditions, yielding similar sensory profiles without the risks and additional vineyard input (i.e.,

706 irrigation) associated with longer fruit hang-times.

707 **Table 3** Average scores for significantly different (p<0.05) wine sensory descriptors for the Shiraz

708 consecutive harvest and blending treatment wines.[a]

Sample	SH_H1	SH_H2	SH_H3	SH_H4 (Control)	SH_B1	SH_B2	SH_B3	SH_Bw1	SH_Bw2	SH_Bw3	LSD
Flavour											
Intensity	48.9b	55.0a	55.9a	57.5a	53.7a	54.6a	54.1a	54.0ab	55.7a	53.6a	4.6
Dark Fruit	38.5c	42.8bc	44.7bc	53.1a	40.9bc	44.9abc	45.7abc	46.8ab	48.0ab	46.8ab	7.9
Liquorice	14.0c	17.3bc	17.2bc	16.5bc	15.8bc	16.6bc	22.6ab	23.5a	18.3abc	23.7a	6.6
Chocolate	8.8c	10.4bc	16.3a	15.5ab	9.8c	12.8abc	13.9abc	13.5abc	15.3ab	13.8abc	5.3
Mouthfeel											
Acidity	57.2b	52.6b	50.8b	56.4b	64.5a	55.1b	57.4ab	52.1b	52.2b	54.7b	7.1
Bitterness	24.5cd	34.8ab	32.2ab	28.7bcd	28.0bcd	22.5d	30.6abcd	31.0abc	36.8a	29.8bcd	7.5
Astringency	38.6c	47.8ab	51.2ab	54.4a	45.2b	44.5bc	49.8ab	45.8b	52.8a	51.0ab	6.6
Hotness	35.8d	49.0bc	53.4ab	57.3a	42.9cd	42.9cd	49.2abc	44.0c	51.7ab	53.8ab	7.8

709   [a] Values are means of 3 replicates. Values followed by different letters within a row are significantly

710   different (p<0.05, one way ANOVA, post hoc Fisher's LSD).

711   **4. Conclusion**

712   In a continuation of a pre-existing experimental design, this study has evaluated the suitability

713   of a pre-fermentative juice substitution with either GHW or water to produce lower alcohol Cabernet

714   Sauvignon wines under milder vintage conditions, this time approximating the recently amended

715   Australian legal limit of 13.5 °Bé for water additions. The approach was further extended by including

716   Shiraz as an additional variety. The benign nature of water or GHW on Cabernet Sauvignon was

717   confirmed, with the use of water led to enhanced (lowest and intermediate substitution rate) or equal

718   (highest water addition rate) colour stability than measured in the control, while tannin concentrations

719   were superior compared to the GHW treatments at similar alcohol levels. Wine volatile profiles were

720   markedly affected, however, either by a general concentration decline with higher water substitution

721   rates, or by accentuating grape-derived constituents with increasing GHW implementations. When

722   working within the legal limit for water addition, the choice of water or GHW resulted in similar wine

723   sensory profiles, but so did an earlier harvest of the Cabernet Sauvignon grapes.

724   More decisive effects were seen on the Shiraz non-volatile characteristics. The

725   implementation of water (up to 25%) and GHW (up to 40%, andto a greater extent) diminished colour

726   stability, and tannins were depleted particularly with higher GHW addition rates. This has significant

727   implications on the ageing potential and 'astringency' perceptions in the resulting wines.

728   Interestingly, the largest effects on the wine volatile profile were observed in the Shiraz wines

729   following water implementation, which elicited declines in an array of fermentative aroma

730   compounds relative to grape-derived compounds, while GHW did not yield such a divergence. Even

731   more pronounced than seen in the Cabernet Sauvignon wines, ratings for the majority of sensory

732   attributes had peaked in wine produced about two weeks before the main harvest date, and were

733   matched with the sensory profile resulting from the highest water addition rate. Therefore, an earlier

734   harvest appeared to be the more sensible approach to manage wine alcohol levels in this case of

735   Shiraz, given the additional costs associated with longer grape hang-times and pre-fermentative juice

736   substitution.

737   The apparent varietally-dependent differences in extraction of non-volatile grape constituents

738   and precursors to wine volatiles with the applied substitution process warrants further research to

739   potentially limit any observed differences to the control wine noted in the present study. Furthermore,

740   research involving consumer studies could be conducted to clarify the implications on the perceived

741   quality of the lower alcohol products. In addition, it remains to be clarified whether juice substitution

742   to maintain must liquid-to-solid ratios is necessary, or whether a simple pre-fermentative net addition

743   of water could be an alternative, given the simpler application.

744   **Abbreviations used**

745   ABV, alcohol by volume; AHC, Agglomerative hierarchical clustering; Bé, Baumé; DA, descriptive

746   analysis; GHW, green harvest wine; HAA, higher alcohol acetate; MM GPC, tannin molecular mass

747   by gel permeation chromatography; TSS, total soluble solids; PMS, potassium metabisulfite.

748   **Conflict of interest**

749   The authors declare no competing financial interest.

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References

759

760 Antalick, G., Šuklje, K., Blackman, J. W., Meeks, C., Deloire, A., & Schmidtke, L. M. (2015).

761   Influence of grape composition on red wine ester profile: Comparison between Cabernet

762   Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and*

763   *Food Chemistry, 63*(18), 4664-4672.

764 Australian Government Bureau of Meteorology. (2018). Montly climate statistics, Noarlunga, 2018

765 Bindon, K., Holt, H., Williamson, P. O., Varela, C., Herderich, M., & Francis, I. L. (2014).

766   Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet

767   Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry, 154*, 90-

768   101.

769 Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest

770   time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine

771   chemistry. *Food Chemistry, 138*(1), 1696-1705.

772 Bindon, K. A., Bacic, A., & Kennedy, J. A. (2012). Tissue-specific and developmental modifications

773   of grape cell walls influence the adsorption of proanthocyanidins. *Journal of Agricultural and*

774   *Food Chemistry, 60*(36), 9249-9260.

775 Bindon, K. A., Kassara, S., Cynkar, W. U., Robinson, E., M. C.,, Scrimgeour, N., & Smith, P. A.

776   (2014). Comparison of extraction protocols to determine differences in wine-extractable

777   tannin and anthocyanin in *Vitis vinifera* L. cv. Shiraz and Cabernet Sauvignon grapes. *Journal*

778   *of Agricultural and Food Chemistry, 62*(20), 4558-4570.

779 Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol

780   concentration on the extraction of color and phenolic compounds from the skin and seeds of

781   tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food*

782   *Chemistry, 53*(10), 4019-4025.

783 Esteruelas, M., Gonzalez-Royo, E., Kontoudakis, N., Orte, A., Cantos, A., Canals, J. M., & Zamora,

784   F. (2015). Influence of grape maturity on the foaming properties of base wines and sparkling

785   wines (Cava). *Journal of the Science of Food and Agriculture, 95*(10), 2071-2080.

786 FSANZ. (2016). Food Standards Australia New Zealand, Application A1119 – Addition of water to

787   facilitate wine fermentation. *2018*.

788 Godden, P., Wilkes, E., & Johnson, D. (2015). Trends in the composition of Australian wine 1984–

789   2014. *Australian Journal of Grape and Wine Research, 21*(S1), 741-753.

790 Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K. M., & Ross, C. F. (2009). Chemical and

791   sensory effects of saignée, water addition, and extended maceration on high Brix must.

792   *American Journal of Enology and Viticulture, 60*(4), 450-460.

793 Heymann, H., LiCalzi, M., Conversano, M. R., Bauer, A., Skogerson, K., & Matthews, M. (2013).

794   Effects of extended grape ripening with or without must and wine alcohol manipulations on

795   Cabernet Sauvignon wine sensory characteristics. *South African Journal of Enology and*

796   *Viticulture, 34*(1), 86-99.

797 Jeffery, D. W., Mercurio, M. D., Herderich, M. J., Hayasaka, Y., & Smith, P. A. (2008). Rapid

798   isolation of red wine polymeric polyphenols by solid-phase extraction. *Journal of Agricultural*

799   *and Food Chemistry, 56*(8), 2571-2580.

800 Kassara, S., & Kennedy, J. A. (2011). Relationship between red wine grade and phenolics. 2. Tannin

801   composition and size. *Journal of Agricultural and Food Chemistry, 59*(15), 8409-8412.

802 Kennedy, J. A., & Taylor, A. W. (2003). Analysis of proanthocyanidins by high-performance gel

803   permeation chromatography. *Journal of Chromatography A, 995*(1–2), 99-107.

804 Keyzers, R. A., & Boss, P. K. (2010). Changes in the volatile compound production of fermentations

805   made from musts with increasing grape content. *Journal of Agricultural and Food Chemistry,*

806   *58*(2), 1153-1164.

807   Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes

808   harvested during cluster thinning as a method for reducing alcohol content and pH of wine.

809   *Australian Journal of Grape and Wine Research, 17*(2), 230-238.

810   Kontoudakis, N., González, E., Gil, M., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011).

811   Influence of wine pH on changes in color and polyphenol composition induced by micro-

812   oxygenation. *Journal of Agricultural and Food Chemistry, 59*(5), 1974-1984.

813   Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food*. New York: Springer

814   Li, S., Bindon, K., Bastian, S. E., Jiranek, V., & Wilkinson, K. L. (2017). Use of winemaking

815   supplements to modify the composition and sensory properties of Shiraz wine. *J Agric Food*

816   *Chem, 65*(7), 1353-1364.

817   Longo, R., Blackman, J. W., Torley, P. J., Rogiers, S. Y., & Schmidtke, L. M. (2017). Changes in

818   volatile composition and sensory attributes of wines during alcohol content reduction. *Journal*

819   *of the Science of Food and Agriculture, 97*(1), 8-16.

820   Ma, W., Guo, A. Q., Zhang, Y. L., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency and

821   bitterness perception of tannins in wine. *Trends in Food Science & Technology, 40*(1), 6-19.

822   McRae, J. M., Ziora, Z. M., Kassara, S., Cooper, M. A., & Smith, P. A. (2015). Ethanol concentration

823   influences the mechanisms of wine tannin interactions with poly($_L$-proline) in model wine.

824   *Journal of Agricultural and Food Chemistry, 63*(17), 4345-4352.

825   Mercurio, M. D., Dambergs, R. G., Cozzolino, D., Herderich, M. J., & Smith, P. A. (2010).

826   Relationship between red wine grades and phenolics. 1. Tannin and total phenolics

827   concentrations. *Journal of Agricultural and Food Chemistry, 58*(23), 12313-12319.

828   Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007). High throughput analysis

829   of red wine and grape phenolics - Adaptation and validation of methyl cellulose precipitable

830   tannin assay and modified Somers color assay to a rapid 96 well plate format. *Journal of*

831   *Agricultural and Food Chemistry, 55*(12), 4651-4657.

832   Pineau, B., Barbe, J.-C., Van Leeuwen, C., & Dubourdieu, D. (2007). Which impact for β-

833   damascenone on red wines aroma? *Journal of Agricultural and Food Chemistry, 55*(10),

834   4103-4108.

835   Ruiz-Garcia, Y., Smith, P. A., & Bindon, K. A. (2014). Selective extraction of polysaccharide affects

836   the adsorption of proanthocyanidin by grape cell walls. *Carbohydrate Polymers, 114*, 102-

837   114.

838   Schelezki, O. J., Smith, P. A., Hranilovic, A., Bindon, K. A., & Jeffery, D. W. (2018). Comparison

839   of consecutive harvests versus blending treatments to produce lower alcohol wines from

840   Cabernet Sauvignon grapes: impact on polysaccharide and tannin content and composition.

841   *Food Chemistry, 244*, 50-59.

842   Schelezki, O. J., Suklje, K., Boss, P. K., & Jeffery, D. W. (2018). Comparison of consecutive harvests

843   versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes:

844   Impact on wine volatile composition and sensory properties. *Food Chemistry*.

845   Schultz, H. R. (2016). Global climate change, sustainability, and some challenges for grape and wine

846   production. *Journal of Wine Economics, 11*(1), 181-200.

847   Sherman, E., Greenwood, D. R., Villas-Boâs, S. G., Heymann, H., & Harbertson, J. F. (2017). Impact

848   of grape maturity and ethanol concentration on sensory properties of Washington State Merlot

849   wines. *American Journal of Enology and Viticulture, 68*(3), 344-356.

850   Styger, G., Prior, B., & Bauer, F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology

851   & Biotechnology, 38*(9), 1145-1159.

852   Suklje, K., Zhang, X., Antalick, G., Clark, A. C., Deloire, A., & Schmidtke, L. M. (2016). Berry

853   shriveling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical composition.

854   *Journal of Agricultural and Food Chemistry, 64*(4), 870-880.

855   Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine:

856   current knowledge and future prospects. *Food Chemistry, 121*(1), 1-16.

857   Tonietto, J., & Carbonneau, A. (2004). A multicriteria climatic classification system for grape-

858   growing regions worldwide. *Agric. For. Meteorol., 124*(1–2), 81-97.

859   Yanniotis, S., Kotseridis, G., Orfanidou, A., & Petraki, A. (2007). Effect of ethanol, dry extract and

860   glycerol on the viscosity of wine. *Journal of Food Engineering, 81*(2), 399-403.

SUPPORTING INFORMATION FOR

Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis.

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Fig. S1 Schematic diagram illustrating the work flow of producing the different treatments. Values in brackets are the juice substitution rates as % v/v.

Refer to Table S4 and S5 for basic chemical composition of the resulting wines.

Table S1 Climatic conditions near the McLaren Vale vineyard[a] during growing season 2015/2016 comparing minimum (Ø Temp Min), maximum (Ø Temp Max) and average (Ø Temp) temperatures, and precipitation sums (∑ Rainfall) to the long-term values (2000-2016, in parentheses). Bold numbers indicate values above (for temperature) or below (for rainfall) the average values

Month	Ø Temp min	Ø Temp max	Ø Temp	∑ Rainfall
Jul '15	7.6 (-1.1)	14.1 (-0.7)	10.9 (-0.9)	**28.6 (-38)**
Aug '15	8.4 (-0.4)	15.1 (-0.8)	11.8 (-0.6)	**41.8 (-11.9)**
Sep '15	9.6 (-0.8)	18.1 (-0.5)	13.9 (-0.6)	**25.2 (-20.7)**
Oct '15	**13.5 (+2.0)**	**25.2 (+3.8)**	**19.4 (+2.9)**	**5.4 (-26.6)**
Nov '15	13.9 (-0.2)	**25.1 (+0.2)**	19.5 (0.0)	**13.6 (-7.5)**
Dec '15	**18.1 (+2.7)**	**30.4 (+4.0)**	**24.3 (+3.4)**	**19.8 (-2.1)**
Jan '16	**17.9 (+0.8)**	**28.9 (+0.2)**	**23.4 (+0.5)**	26.2 (+8.1)
Feb '16	16.3 (-0.6)	26.6 (-1.1)	21.5 (-0.8)	34.6 (+15)
Mar '16	**16.6 (+1.0)**	**26.9 (+1.2)**	**21.8 (+1.1)**	23.0 (+1.4)
Apr '16	**13.9 (+0.3)**	**23.3 (+1.0)**	**18.6 (+0.6)**	**9.8 (-23.6)**
May '16	**13.3 (+1.8)**	**19 (+0.5)**	**16.2 (+1.2)**	66.4 (+12.7)
Jun '16	**9.7 (+0.4)**	**15.7 (+0.1)**	**12.7 (+0.2)**	77.8 (+15.1)

[a]Noarlunga weather station (Latitude: 35.16 °S, Longitude 138.51 °E, Elevation: 55 m)

Table S2 Overview of the ions, acquisition mode and the internal standards used to perform semi-quantitation of wine volatiles, in order as presented in Tables S6 and S7

Compound name	Acquisition mode	Identifier ion [m/z]	Internal standard
Acids			
Hexanoic acid	Scan	59.9	Octan-2-ol
Octanoic acid	Scan	59.9	Octan-2-ol
Decanoic acid	Scan	129	Octan-2-ol
C₆-alcohol			
1-Hexanol	Scan	56	Octan-2-ol
Ethyl esters of branched acids			
Ethyl 2-methylpropanoate	Scan	71	Octan-2-ol
Ethyl 2-methylbutyrate	Scan	102	Octan-2-ol
Ethyl 3-methylbutanoate	Scan	87.9	Octan-2-ol
Ethylphenyl acetate	Scan	164	Octan-2-ol
Ethyl esters of odd carbon number fatty acids			
Ethyl heptanoate	Scan	113	Octan-2-ol
Ethyl nonanoate	Scan	141	Octan-2-ol
Ethyl esters of straight-chain fatty acids			
Ethyl acetate	Scan	70	Octan-2-ol
Ethyl butyrate	Scan	71	Octan-2-ol
Ethyl hexanoate	Scan	88	Octan-2-ol
Ethyl 2-hexenoate	Scan	97	Octan-2-ol
Ethyl octanoate	Scan	88	Octan-2-ol
Ethyl decanoate	Scan	157	Octan-2-ol
Ethyl dodecanoate	Scan	87.9	Octan-2-ol
HAA from grape lipid degradation			
Hexyl acetate	Scan	56	Octan-2-ol
HAA from yeast sugar and N metabolism			
3-Methylbutyl acetate	Scan	70	Octan-2-ol
Phenylethyl acetate	Scan	104	Octan-2-ol
Higher alcohols			
2-Methyl-1-propanol	Scan	43	Octan-2-ol
3-Methyl-1-butanol	Scan	55	Octan-2-ol
Octan-1-ol	Scan	84	Octan-2-ol
3-(Methylthio)propanol	Scan	105.9	Octan-2-ol
Benzyl alcohol	Scan	107.9	Octan-2-ol
2-Phenylethanol	Scan	91	Octan-2-ol
1-Dodecanol	Scan	83	Octan-2-ol
Methyl esters of fatty acid			
Methyl octanoate	Scan	74	Octan-2-ol
Methyl decanoate	Scan	143	Octan-2-ol

3-Methylbutyl octanoate	Scan	70	Octan-2-ol
Diethyl succinate	Scan	101	Octan-2-ol
Ethyl 9-decenoate	Scan	110	Octan-2-ol
Ethyl tetradecanoate	Scan	157	Octan-2-ol
Ethyl hexadecanoate	Scan	157	Octan-2-ol
Isoprenoids			
Vitispirane 1	SIM	192	Ethyl cinnamate-d_5
Vitispirane 2	SIM	192	Ethyl cinnamate-d_5
Linalool	SIM	93	Linalool-d_3
4-Terpineol	SIM	111	Linalool-d_3
α-Terpineol	SIM	136	Linalool-d_3
TDN	SIM	157	Ethyl cinnamate-d_5
Citronellol	SIM	69	Linalool-d_3
β-Damascenone	SIM	190	Ethyl cinnamate-d_5
trans-Geraniol	SIM	93	Linalool-d_3
α-Ionone	SIM	121	Ethyl cinnamate-d_5
trans-Nerolidol	SIM	69	Linalool-d_3

Table S3 Attribute list as developed by the descriptive analysis panel to describe the sensory profiles of the wines, comprising respective definitions and aroma/flavour reference standards.

Descriptor	Type	Definition	Standard, mixed in 30 mL of Cabernet Sauvignon wine
Aroma intensity	aroma	the overall intensity of the sum of all aroma attributes perceived in the wine	
Flavour intensity	flavour	the overall intensity of the sum of all flavour attributes perceived in the wine	
Red fruit	aroma/flavour	the smell/flavour of cherry, raspberry and strawberry	half a strawberry, 2 canned cherries (Garden Fresh pitted cherries), 1 slice of plum and 2 raspberries
Dark fruit	aroma/flavour	the smell/flavour of cassis, black berries	half a tea spoon of forest berry jam (Cottee's), 1 blackberry and 2 blue berries
Dried fruit	aroma/flavour	the smell/flavour of dried fruit like raisins, dried plums, the smell of jam	1 teaspoon of minced fruit (Robertson's), 2 teaspoon tips of plum jam (Cottee's)
Green	aroma/flavour	the smell/flavour if green capsicum and/or the smell of fresh cut grass	1 knife tip of fresh green capsicum, 2 blades of grass
Pepper	aroma/flavour	the smell of crushed pepper	1 knife tip of ground black pepper (Master Foods)
Sweet spice	aroma/flavour	the smell/flavour of cloves, cinnamon, cardamon, mixed oriental spice	half a clove, a pinch each of mixed spice powder and nutmeg powder (Master Foods)
Liquorice	aroma/flavour	the smell/flavour of liquorice	one quarter of a stick of liquorice (Lyn-Chris Confectionery)
Chocolate	aroma/flavour	the smell/flavour of dark chocolate	⅓ of a teaspoon chopped dark chocolate (70%, Lindt)
Confection	aroma/flavour	the smell/flavour of confectionery	1 cm of bubble gum (Wrigleys Juicy Fruit), half a raspberry cream lolly (Allen's), one quarter of a marshmallow (Allen's), 2.5 cm of red snake lolly (Allen's)
Savoury	aroma/flavour	the smell of soy sauce, oyster sauce	½ teaspoon of soy sauce, ½ teaspoon of oyster sauce
Smoke	aroma/flavour	the smell/flavour of smoke, burnt bread	⅓ teaspoon of burnt white bread
Earthy	aroma/flavour	the smell/flavour of soil, dust	½ teaspoon of dry soil material
Acidity	taste	the perceived acidity on your tongue	
Sweetness	taste	the perceived sweetness on your tongue	
Bitterness	taste	the perceived bitterness on the tongue	
Astringency	palate sensation	the astringency, perceived as puckering and drying sensation on the oral mucosa	
Hotness	palate sensation	the perceived hotness that is caused by ethanol	increase of 2% v/v alcohol by addition of food grade ethanol (98% ABV)

Table S4 Basic grape and wine compositional parameters of the different 2016 harvest dates for Cabernet Sauvignon and Shiraz.[a]

Basic grape and wine measures	Cabernet Sauvignon				Shiraz			
	H1	H2	H3	H4	H1	H2	H3	H4
Harvest date	01-Feb	09-Feb	18-Feb	29 Feb	01-Feb	08-Feb	17-Feb	21-Feb
Berry weight [g/berry]	0.79 ± 0.03b	0.93 ± 0.08a	0.93 ± 0.05a	0.87 ± 0.01ab	1.06 ± 0.04b	1.25 ± 0.10a	1.09 ± 0.07ab	1.01 ± 0.04b
TSS [°Brix]	19.9 ± 0.1d	22.1 ± 0.1c	23.6 ± 0.05b	26.2 ± 0.12a	20.4 ± 0.33d	23.4 ± 0.2c	24.9 ± 0.2b	26.3 ± 0.0a
pH at harvest date	3.73 ± 0.03d	3.86 ± 0.02c	3.94 ± 0.01b	4.11 ± 0.04a	3.75 ± 0.05d	3.87 ± 0.01c	3.97 ± 0.01b	4.10 ± 0.00a
TA at harvest date[b]	6.87 ± 0.21	6.56 ± 0.21	6.69 ± 0.03	6.42 ± 0.08	6.73 ± 0.09a	6.45 ± 0.04b	6.36 ± 0.03b	6.20 ± 0.00c
% ABV	11.4 ± 0.12d	12.7 ± 0.1b	13.9 ± 0.1b	15.5 ± 0.2a	11.8 ± 0.3c	13.8 ± 0.0b	15.1 ± 0.0a	15.4 ± 0.0a
pH after adjustment	3.63 ± 0.03b	3.56 ± 0.02c	3.70 ± 0.00a	3.65 ± 0.00b	3.63 ± 0.04b	3.72 ± 0.00a	3.70 ± 0.0a	3.68 ± 0.03ab
TA after adjustment[b]	7.07 ± 0.26	7.46 ± 0.09	7.07 ± 0.02	7.34 ± 0.02	6.60 ± 0.10d	6.73 ± 0.02c	6.88 ± 0.05b	7.63 ± 0.01a
Malic acid[c]	3.31 ± 0.06a	2.99 ± 0.06b	2.99 ± 0.10b	2.27 ± 0.04c	3.46 ± 0.04a	3.49 ± 0.02a	3.22 ± 0.07b	2.93 ± 0.05c
Acetic acid[c]	0.54 ± 0.08b	0.60 ± 0.0b	0.48 ± 0.12b	1.05 ± 0.14a	0.25 ± 0.06b	0.29 ± 0.02b	0.28 ± 0.03b	0.39 ± 0.00a
Glycerol[c]	8.48 ± 0.11d	9.34 ± 0.15c	10.5 ± 0.4b	11.5 ± 0.1a	8.94 ± 0.34d	10.2 ± 0.1c	11.3 ± 0.0b	11.7 ± 0.1a

[a]Values are means of 3 replicates ± standard error (except H0, which was produced without triplicates). [b]Values in [g/L] tartaric acid equivalents. [c]Values in [g/L]. Values followed by different letters within rows for a variety are significantly different ($p \leq 0.05$, one way ANOVA)

Table S5 Basic wine compositional parameters of the GHW (CS_B1-B3 for Cabernet Sauvignon, SH_B1-B3 for Shiraz) and water (CS_Bw1-3 for Cabernet Sauvignon and SH_Bw1-3 for Shiraz).[a]

	Substitution volume (v/v)	% ABV	TA before adjustment[b]	pH before adjustment	TA after adjustment[b]	pH after adjustment	Malic acid[c]	Acetic acid[c]	Glycerol[c]
Cabernet Sauvignon									
CS_H4 (Control)		15.5 ± 0.2a	6.42 ± 0.08d	4.11 ± 0.04a	7.34 ± 0.02ab	3.65 ± 0.00cd	2.27 ± 0.04d	1.05 ± 0.14a	11.5 ± 0.1a
CS_B1	40%	12.6 ± 0.3e	8.80 ± 0.27a	3.64 ± 0.05d	7.84 ± 0.62a	3.62 ± 0.04d	4.31 ± 0.06a	0.68 ± 0.08b	8.35 ± 0.33f
CS_B2	26%	13.8 ± 0.1c	7.88 ± 0.15b	3.79 ± 0.04c	7.62 ± 0.12a	3.73 ± 0.02a	3.71 ± 0.02b	0.78 ± 0.12b	9.35 ± 0.14e
CS_B3	16%	14.5 ± 0.1b	7.19 ± 0.10c	3.94 ± 0.01b	7.61 ± 0.04a	3.69 ± 0.01b	3.1 ± 0.04c	0.75 ± 0.05b	10.1 ± 0.1bc
CS_Bw1	25%	12.9 ± 0.1d	6.02 ± 0.05d	4.06 ± 0.00a	6.85 ± 0.01b	3.63 ± 0.00cd	2.06 ± 0.07e	0.82 ± 0.12ab	9.57 ± 0.29de
CS_Bw2	16%	13.8 ± 0.1c	6.50 ± 0.53d	4.06 ± 0.04a	6.93 ± 0.02b	3.66 ± 0.01bc	2.19 ± 0.01d	1.04 ± 0.15a	9.83 ± 0.13cd
CS_Bw3	10%	14.6 ± 0.1b	6.21 ± 0.02d	4.10 ± 0.00a	7.08 ± 0.03b	3.65 ± 0.01cd	2.22 ± 0.05d	1.04 ± 0.07a	10.4 ± 0.1b
Shiraz									
SH_H4 (Control)		15.4 ± 0.0a	6.20 ± 0.04d	4.10 ± 0.03a	7.63 ± 0.01a	3.68 ± 0.03a	2.93 ± 0.05de	0.39 ± 0.00c	11.7 ± 0.1a
SH_B1	40%	13.1 ± 0.1e	8.25 ± 0.07a	3.63 ± 0.05e	7.78 ± 0.06a	3.38 ± 0.01d	4.55 ± 0.09a	0.26 ± 0.03d	9.08 ± 0.10e
SH_B2	20%	14.3 ± 0.1c	7.18 ± 0.06b	3.86 ± 0.00d	7.27 ± 0.06ab	3.52 ± 0.00bc	3.88 ± 0b	0.30 ± 0.02cd	9.83 ± 0.05d
SH_B3	9%	15.0 ± 0.1b	6.59 ± 0.02c	3.96 ± 0.00c	7.04 ± 0.03bc	3.51 ± 0.01bc	3.36 ± 0.01c	0.55 ± 0.04b	10.6 ± 0.1c
SH_Bw1	25%	13.2 ± 0.1d	6.02 ± 0.03e	3.99 ± 0.01bc	6.21 ± 0.62d	3.54 ± 0.07b	2.88 ± 0.03e	0.74 ± 0.08a	9.23 ± 0.17e
SH_Bw2	12%	14.4 ± 0.0c	6.00 ± 0.07e	4.03 ± 0.02cd	6.56 ± 0.01cd	3.47 ± 0.02c	2.98 ± 0.03d	0.32 ± 0.05cd	10.7 ± 0.1c
SH_Bw3	6%	15.0 ± 0.1b	6.04 ± 0.03e	4.06 ± 0.01cd	6.67 ± 0.05bcd	3.47 ± 0.01c	2.99 ± 0.04d	0.36 ± 0.05cd	11.3 ± 0.0b

[a]Values are means of 3 replicates ± standard error (except H0, which was produced without triplicates). [b]Values in [g/L] tartaric acid equivalents.

[c]Values in [g/L]. Values followed by different letters within columns for a variety are significantly different ($p \leq 0.05$, one way ANOVA).

Table S6 Semi-quantitative data (presented as peak area ratio against internal standard) the volatiles analysed in the Cabernet Sauvignon harvest series (CS_H1-H4), GHW (CS_B1-B3) and water blended wines (CS_Bw1-Bw3).[a]

		Harvest series wines				GHW blended wines			Water blended wines			
	CS_H0	CS_H1	CS_H2	CS_H3	CS_H4 (Control)	CS_B1	CS_B2	CS_B3	CS_Bw1	CS_Bw2	CS_Bw3	p-value
Acids												
Hexanoic acid	8.45	5.59±0.07a	5.87±0.14a	5.86±0.31a	4.07±0.17bc	4.21±0.17b	3.98±0.05bcd	3.84±0.14cd	3.67±0.06d	3.70±0.26d	3.86±0.07cd	0.0000
Octanoic acid	33.1	8.64±1.05a	8.59±0.21a	8.01±0.57a	5.37±0.71b	4.92±0.33b	4.67±0.32b	4.76±0.24b	4.66±0.26b	4.53±0.32b	4.76±0.61b	0.0000
Decanoic acid[b]	8.65	1.11±0.30ab	1.21±0.05a	1.11±0.05ab	0.83±0.29bc	0.47±0.02d	0.49±0.04d	0.59±0.07cd	0.63±0.05cd	0.60±0.05cd	0.64±0.13cd	0.0001
Δ in % to control[b]		144	150	143	100	84	81	85	84	82	87	
C₆-alcohols												
1-Hexanol	8.55	16.8±0.8b	18.2±0.5a	18.5±0.2a	14.0±0.2d	15.4±0.3c	14.9±0.3cd	14.1±0.4d	12.8±0.1e	12.7±0.5e	13.1±0.6e	0.0000
Δ in % to control[b]		120	130	132	100	110	106	101	91	90	93	
Ethyl esters of branched acids												
Ethyl 2-methylpropanoate	0.40	0.29±0.05bcd	0.34±0.02a	0.26±0.00d	0.32±0.04abc	0.33±0.01ab	0.28±0.02cd	0.30±0.01abcd	0.30±0.01abcd	0.29±0.00bcd	0.30±0.02abcd	0.0438
Ethyl 2-methylbutyrate	0.12	0.33±0.03e	0.40±0.01ab	0.35±0.01de	0.37±0.01cd	0.30±0.01f	0.28±0.00f	0.34±0.01e	0.42±0.01ab	0.40±0.00abc	0.38±0.01bc	0.0000
Ethyl 3-methylbutanoate	0.30	0.37±0.03f	0.49±0.02cd	0.46±0.02de	0.56±0.02a	0.47±0.01d	0.43±0.01e	0.51±0.01bc	0.52±0.02bc	0.52±0.00bc	0.54±0.01ab	0.0000
Ethylphenyl acetate	0.03	0.18±0.01bc	0.18±0.01bc	0.20±0.01a	0.18±0.00bc	0.16±0.01de	0.16±0.00e	0.16±0.00e	0.17±0.01cde	0.18±0.01bcd	0.19±0.01ab	0.0001
Δ in % to control[b]		87	101	92	100	90	82	91	98	97	99	
Ethyl esters of odd carbon number fatty acids												
Ethyl heptanoate	0.08	0.40±0.07b	0.49±0.04a	0.40±0.05b	0.37±0.02bc	0.35±0.02bcd	0.36±0.01bcd	0.38±0.01bc	0.29±0.03d	0.32±0.04cd	0.33±0.04bcd	0.0022
Ethyl nonanoate	0.02	0.19±0.00c	0.24±0.01b	0.25±0.00b	0.29±0.02a	0.17±0.01c	0.19±0.02c	0.24±0.01b	0.18±0.01c	0.2±0.01c	0.23±0.01b	0.0000
Δ in % to control[b]		86	107	97	100	77	82	92	69	76	84	
Ethyl esters of straight-chain fatty acids												
Ethyl acetate	1.56	3.89±0.25f	5.37±0.16cd	5.58±0.18bc	6.27±0.12a	4.51±0.13e	4.98±0.32d	5.84±0.15ab	4.98±0.29d	5.23±0.18cd	5.58±0.23bc	0.0000
Ethyl butyrate	1.11	1.57±0.11d	2.22±0.03a	2.22±0.08a	2.31±0.13a	1.71±0.08cd	1.76±0.06cd	2.00±0.10b	1.65±0.04cd	1.71±0.08cd	1.82±0.06cd	0.0000
Ethyl hexanoate	34.9	68.1±5.2c	90.0±2.0a	81.3±4.0b	68.9±2.4c	61.8±1.7de	61.2±1.3de	66.0±2.7cd	56.8±1.8e	57.2±4.4e	60.1±1.2de	0.0000
Ethyl 2-hexenoate	0.35	0.42±0.04d	0.50±0.02cd	0.65±0.04b	0.76±0.12a	0.65±0.04b	0.65±0.02b	0.66±0.03b	0.46±0.00d	0.51±0.01cd	0.57±0.02bc	0.0000
Ethyl octanoate	143	153±2a	66.6±11.0d	129±53abc	148±15abc	101±2cd	113±8bc	146±7ab	112±7bc	113±7bc	123±4abc	0.0052
Ethyl decanoate	9.19	8.82±0.26b	10.6±0.38a	11.2±0.33a	8.45±0.79b	4.21±0.08e	5.61±0.83d	9.17±0.58b	5.78±0.42d	5.66±0.26d	6.75±0.70c	0.0000
Ethyl dodecanoate	1.91	2.63±0.42b	3.16±0.09a	3.21±0.21a	1.69±0.32c	1.68±0.26c	1.71±0.21c	2.12±0.30c	1.96±0.05c	1.79±0.12c	1.99±0.15c	0.0000
Δ in % to control[b]		93	105	114	100	77	82	99	79	80	87	
HAA from grape lipid degradation												
Hexyl acetate	7.16	2.80±0.20b	3.43±0.11a	2.48±0.21bc	1.94±0.25d	2.20±0.21cd	2.01±0.17d	2.12±0.13d	1.26±0.07e	1.29±0.08e	1.50±0.01e	0.0000
Δ in % to control[b]		144	177	128	100	113	104	109	65	67	77	
HAA from yeast sugar and N metabolism												
3-Methylbutyl acetate	6.51	23.4±0.3f	36.4±1.0ab	35.7±1.7ab	37.6±5.0ab	26.7±1.9ef	29.9±3.7cde	34.6±1.4abc	26.8±1.2ef	28.8±0.9de	31.8±1.1bcd	0.0000
Phenylethyl acetate[b]	1.70	4.68±0.32ef	6.56±0.21bc	8.01±0.09ab	8.12±1.77a	4.27±0.14f	4.87±0.53def	5.99±0.44cde	4.28±0.31f	5.24±0.32cdef	6.31±0.94cd	0.0000
Δ in % to control[b]		60	89	97	100	62	70	83	62	71	81	

												p
Higher alcohols												
2-Methyl-1-propanol	1.01	3.35 ± 0.13e	4.07 ± 0.20d	4.29 ± 0.15cd	5.02 ± 0.40a	4.06 ± 0.30d	4.37 ± 0.17cd	4.43 ± 0.11cd	4.48 ± 0.13bcd	4.94 ± 0.34ab	4.71 ± 0.03abc	0.0000
3-Methyl-1-butanol	28.2	89 ± 3g	115 ± 4de	135 ± 3a	132 ± 5ab	100 ± 4f	109 ± 4e	117 ± 3cd	117 ± 1d	125 ± 3b	125 ± 4bc	0.0000
Octan-1-ol	0.17	0.33 ± 0.03	0.35 ± 0.02	0.37 ± 0.03	0.34 ± 0.02	0.38 ± 0.02	0.38 ± 0.03	0.41 ± 0.01	0.35 ± 0.02	0.33 ± 0.04	0.37 ± 0.05	0.1519
3-(Methylthio)propanol	0.05	0.34 ± 0.02c	0.46 ± 0.01ab	0.51 ± 0.03a	0.39 ± 0.07bc	0.31 ± 0.01c	0.30 ± 0.03c	0.37 ± 0.06bc	0.45 ± 0.1ab	0.37 ± 0.07bc	0.45 ± 0.05ab	0.0081
Benzyl alcohol	0.02	0.32 ± 0.01g	0.4 ± 0.03f	0.55 ± 0.02de	0.82 ± 0.03a	0.39 ± 0.03f	0.50 ± 0.01e	0.57 ± 0.04cd	0.62 ± 0.01c	0.67 ± 0.02b	0.78 ± 0.05a	0.0000
2-Phenylethanol	23.1	173 ± 9efg	227 ± 8b	276 ± 2a	227 ± 17b	158 ± 4g	171 ± 7fg	184 ± 5ef	189 ± 6de	202 ± 4cd	212 ± 7bc	0.0000
1-Dodecanol	1.82	1.44 ± 0.05d	1.73 ± 0.08	1.71 ± 0.10	1.47 ± 0.18	1.46 ± 0.07	1.55 ± 0.20	1.77 ± 0.05	1.69 ± 0.05	1.64 ± 0.03	1.56 ± 0.21	0.0798
Δ in % to control[b]		**76**	**94**	**105**	**100**	**81**	**86**	**95**	**96**	**96**	**101**	
Methyl esters of fatty acid												
Methyl octanoate	0.52	0.39 ± 0.02d	0.61 ± 0.03a	0.59 ± 0.01a	0.56 ± 0.05ab	0.52 ± 0.01b	0.51 ± 0.06bc	0.52 ± 0.01b	0.46 ± 0.03c	0.46 ± 0.02c	0.51 ± 0.01bc	0.0000
Methyl decanoate	0.10	0.07 ± 0.01bc	0.12 ± 0.01a	0.12 ± 0.01a	0.07 ± 0.01b	0.07 ± 0b	0.07 ± 0.03bcd	0.05 ± 0.01cd	0.05 ± 0.00d	0.05 ± 0.00d	0.06 ± 0.00bcd	0.0000
Δ in % to control[b]		**84**	**137**	**137**	**100**	**97**	**92**	**82**	**73**	**75**	**89**	
Other esters												
3-Methylbutyl octanoate	0.78	1.80 ± 0.05b	1.95 ± 0.1ab	2.07 ± 0.04a	1.47 ± 0.13cd	1.02 ± 0.02g	1.20 ± 0.12f	1.60 ± 0.07c	1.29 ± 0.04ef	1.25 ± 0.07ef	1.39 ± 0.10de	0.0000
Diethyl succinate	0.48	2.33 ± 0.09b	3.20 ± 0.10a	3.19 ± 0.17a	2.18 ± 0.13bc	2.22 ± 0.11bc	2.10 ± 0.10cd	2.30 ± 0.03bc	1.65 ± 0.06e	1.95 ± 0.14d	2.14 ± 0.05bcd	0.0000
Ethyl 9-decenoate	5.02	0.83 ± 0.03cd	1.17 ± 0.05a	1.07 ± 0.08ab	0.74 ± 0.05de	0.67 ± 0.06ef	0.74 ± 0.14de	0.94 ± 0.05bc	0.61 ± 0.07ef	0.57 ± 0.09f	0.58 ± 0.04f	0.0000
Ethyl tetradecanoate	0.01	0.02 ± 0.00c	0.03 ± 0.00bc	0.05 ± 0.01a	0.03 ± 0.00bcd	0.04 ± 0.01b	0.02 ± 0.01bcd	0.02 ± 0.01bcd	0.01 ± 0.00d	0.01 ± 0.00d	0.02 ± 0.00d	0.0005
Ethyl hexadecanoate	0.02	0.06 ± 0.01cde	0.09 ± 0.00abc	0.13 ± 0.02a	0.09 ± 0.02bcd	0.13 ± 0.02a	0.09 ± 0.03bcd	0.10 ± 0.03ab	0.05 ± 0.00de	0.05 ± 0.00de	0.06 ± 0.01cde	0.0006
Δ in % to control[b]		**97**	**132**	**156**	**100**	**111**	**94**	**111**	**72**	**72**	**81**	
Isoprenoids												
Vitispirane 1	1.09	0.22 ± 0.02b	0.21 ± 0.01b	0.17 ± 0.01cd	0.15 ± 0.01de	0.28 ± 0.02a	0.2 ± 0.01bc	0.21 ± 0.02b	0.1 ± 0.02f	0.13 ± 0.01ef	0.16 ± 0.01d	0.0000
Vitispirane 2	0.53	0.12 ± 0.01ab	0.14 ± 0.01ab	0.12 ± 0.00ab	0.11 ± 0.01b	0.15 ± 0.02a	0.13 ± 0.01ab	0.12 ± 0.02ab	0.06 ± 0.03c	0.06 ± 0.02c	0.11 ± 0.03b	0.0017
Linalool	0.86	1.14 ± 0.02c	1.04 ± 0.06d	0.95 ± 0.03e	1.00 ± 0.02de	1.63 ± 0.09a	1.38 ± 0.04b	1.30 ± 0.03b	1.05 ± 0.01d	1.07 ± 0.03cd	1.05 ± 0.02d	0.0000
4-Terpineol	0.08	0.04 ± 0.00bc	0.03 ± 0.00bc	0.03 ± 0.01bc	0.02 ± 0.00c	0.05 ± 0.00a	0.04 ± 0.00bc	0.04 ± 0.01ab	0.03 ± 0.01bc	0.04 ± 0.02bc	0.04 ± 0.00ab	0.0219
α-Terpineol	0.36	0.14 ± 0.01d	0.14 ± 0.01c	0.16 ± 0.01c	0.14 ± 0.00cd	0.21 ± 0.01a	0.18 ± 0.01b	0.18 ± 0.00b	0.15 ± 0.00cd	0.15 ± 0.00cd	0.16 ± 0.00c	0.0000
TDN	1.45	0.53 ± 0.05c	0.69 ± 0.07a	0.66 ± 0.02ab	0.65 ± 0.06ab	0.63 ± 0.03ab	0.53 ± 0.04c	0.71 ± 0.03a	0.57 ± 0.02bc	0.51 ± 0.03c	0.67 ± 0.06a	0.0009
Citronellol	0.74	3.38 ± 0.19f	4.69 ± 0.14ab	5.02 ± 0.15a	4.88 ± 0.27a	3.61 ± 0.14ef	4.08 ± 0.06cd	3.92 ± 0.08cde	3.71 ± 0.22def	3.93 ± 0.06cde	4.34 ± 0.41bc	0.0000
β-Damascenone	4.80	3.77 ± 0.11ab	3.75 ± 0.06abc	3.33 ± 0.17c	3.58 ± 0.42bc	4.02 ± 0.11a	3.43 ± 0.24bc	3.55 ± 0.06bc	3.36 ± 0.13bc	3.37 ± 0.31bc	3.79 ± 0.18ab	0.0470
trans-Geraniol	0.03	0.04 ± 0.01c	0.09 ± 0.05ab	0.12 ± 0.02a	0.06 ± 0.01bc	0.04 ± 0.00c	0.06 ± 0.02bc	0.07 ± 0.01bc	0.06 ± 0.03bc	0.04 ± 0.01c	0.09 ± 0.00ab	0.0074
α-Ionone	0.02	0.12 ± 0.03a	0.03 ± 0.01b	0.04 ± 0.02b	0.05 ± 0.02b	0.09 ± 0.01a	0.05 ± 0.03b	0.02 ± 0.00b	0.02 ± 0.00b	0.02 ± 0.00b	0.02 ± 0.00b	0.0002
trans-Nerolidol	1.06	0.62 ± 0.06a	0.38 ± 0.02bc	0.31 ± 0.04c	0.41 ± 0.10bc	0.44 ± 0.14bc	0.41 ± 0.09bc	0.46 ± 0.03b	0.38 ± 0.02bc	0.37 ± 0.07bc	0.31 ± 0.02c	0.0189
Δ in % to control[b]		**122**	**110**	**110**	**100**	**137**	**114**	**113**	**88**	**88**	**106**	

[a]Values are means of 3 replicates ± standard error (except H0, which was produced without triplicates). [b]Proportions of accumulated semi-quantitative data of compounds within compound group relative to the control (100%). Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA).

Table S7 Semi-quantitative information of the volatiles (presented as peak area ratio against internal standard) analysed in the Shiraz harvest series (SH_H1-H4), GHW (SH_B1-B3) and water blended wines (SH_Bw1-Bw3).[a] Green and blue colouring indicate significant difference of the GHW and water treatments, respectively, to the SH_H4 control wine. Bold numbers indicate non-significance to the control wine, and are identical to Table 4 of the main document.

| | SH_HO | Harvest series wines | | | | GHW blended wines | | | Water blended wines | | | Pr > F |
		SH_H1	SH_H2	SH_H3	SH_H4 (Control)	SH_B1	SH_B2	SH_B3	SH_Bw1	SH_Bw2	SH_Bw3	
Acids												
Hexanoic acid	7.70	8.00 ± 0.25a	6.74 ± 0.36b	6.27 ± 0.58b	4.04 ± 0.07c	4.04 ± 0.22c	3.85 ± 0.05c	3.84 ± 0.25c	3.66 ± 0.14c	3.91 ± 0.17c	3.7 ± 0.16c	0.000
Octanoic acid	28.20	13.8 ± 1.4a	10.6 ± 0.6b	9.95 ± 1.40b	5.52 ± 0.25c	5.13 ± 0.28c	5.21 ± 0.16c	5.09 ± 0.41c	4.97 ± 0.41c	5.40 ± 0.43c	5.17 ± 0.53c	0.000
Decanoic acid	6.17	2.81 ± 1.25a	1.71 ± 0.20b	1.60 ± 0.25bc	0.71 ± 0.01d	0.64 ± 0.05d	0.67 ± 0.00d	0.62 ± 0.06d	0.61 ± 0.02d	0.76 ± 0.10cd	0.71 ± 0.08d	0.000
Δ in % to control[b]		**281**	**200**	**187**	**100**	**94**	**95**	**91**	**89**	**100**	**95**	
C₆-alcohols												
1-Hexanol	6.96	22.4 ± 2.0a	22.9 ± 0.4a	22.4 ± 0.6a	19.5 ± 0.1bc	20.1 ± 0.4b	19.5 ± 0.1bc	18.5 ± 0.3bcd	16.5 ± 0.8e	17.2 ± 0.5de	18.2 ± 0.4cd	0.000
Δ in % to control[b]		**115**	**117**	**115**	**100**	**103**	**100**	**95**	**85**	**88**	**93**	
Ethyl esters of branched acids												
Ethyl 2-methylpropanoate	0.35	0.27 ± 0.01bc	0.29 ± 0.02ab	0.21 ± 0.01f	0.30 ± 0.02a	0.29 ± 0.03ab	0.24 ± 0.00de	0.23 ± 0.00ef	0.27 ± 0.01bc	0.26 ± 0.00cd	0.25 ± 0.00cde	0.000
Ethyl 2-methylbutyrate	0.11	0.22 ± 0.00b	0.25 ± 0.01a	0.21 ± 0.01bc	0.20 ± 0.01bc	0.22 ± 0.02b	0.17 ± 0.00e	0.18 ± 0.00de	0.22 ± 0.01b	0.20 ± 0.01bc	0.19 ± 0.00cd	0.000
Ethyl 3-methylbutanoate	0.26	0.31 ± 0.01bc	0.37 ± 0.02a	0.31 ± 0.01bc	0.34 ± 0.01ab	0.36 ± 0.03a	0.28 ± 0.01d	0.30 ± 0.00cd	0.32 ± 0.01bc	0.32 ± 0.01bc	0.29 ± 0.00cd	0.000
Ethyl phenyl acetate	0.02	0.09 ± 0.01a	0.09 ± 0.01ab	0.09 ± 0.00ab	0.05 ± 0.01e	0.07 ± 0.00cd	0.06 ± 0.00e	0.07 ± 0.00de	0.08 ± 0.01bc	0.07 ± 0.01cd	0.06 ± 0.00e	0.000
Δ in % to control[b]		**116**	**123**	**106**	**100**	**110**	**87**	**93**	**111**	**102**	**92**	
Ethyl esters of odd carbon number fatty acids												
Ethyl heptanoate	0.06	0.27 ± 0.03bc	0.39 ± 0.05a	0.27 ± 0bc	0.29 ± 0.01b	0.29 ± 0.02b	0.25 ± 0.01bc	0.25 ± 0bc	0.24 ± 0.02c	0.24 ± 0.02c	0.24 ± 0.02c	0.000
Ethyl nonanoate	0.03	0.28 ± 0.04a	0.26 ± 0.01a	0.27 ± 0.02a	0.25 ± 0.00a	0.26 ± 0.01a	0.28 ± 0.00a	0.28 ± 0.01a	0.19 ± 0.06b	0.14 ± 0.01b	0.16 ± 0.00b	0.000
Δ in % to control[b]		**104**	**120**	**102**	**100**	**104**	**101**	**99**	**79**	**69**	**75**	
Ethyl esters of straight-chain fatty acids												
Ethyl acetate	2.04	5.21 ± 0.36d	7.06 ± 0.28a	7.17 ± 0.12a	6.23 ± 0.13bc	6.93 ± 0.60ab	5.99 ± 0.38c	6.58 ± 0.52abc	4.93 ± 0.09d	5.98 ± 0.28c	6.95 ± 0.43ab	0.000
Ethyl butyrate	0.92	2.22 ± 0.08c	2.98 ± 0.09a	3.02 ± 0.21a	2.30 ± 0.05bc	2.48 ± 0.19bc	2.24 ± 0.16bc	2.41 ± 0.23bc	1.80 ± 0.06d	2.34 ± 0.19bc	2.57 ± 0.21b	0.000
Ethyl hexanoate	30.40	80.8 ± 1.4c	97.5 ± 1.2a	89.2 ± 7.0b	66.4 ± 0.9d	65.7 ± 1.0de	58.6 ± 2.2f	59.7 ± 4.0ef	49.5 ± 2.5g	61.4 ± 3.0def	63.4 ± 3.0def	0.000
Ethyl 2-hexenoate	0.22	0.38 ± 0.01a	0.33 ± 0.01b	0.31 ± 0.02b	0.28 ± 0.00c	0.26 ± 0.04cd	0.24 ± 0.00de	0.21 ± 0.00ef	0.19 ± 0.02f	0.19 ± 0.02f	0.18 ± 0.01f	0.000
Ethyl octanoate	135.00	177 ± 3b	255 ± 4a	267 ± 32a	141 ± 5cd	148 ± 11cd	128 ± 7de	145 ± 15cd	107 ± 7e	162 ± 10bc	164 ± 15bc	0.000
Ethyl decanoate	8.06	11.7 ± 1.1b	15.6 ± 0.5a	17.1 ± 2.2a	7.31 ± 0.22de	8.07 ± 1.08cde	7.49 ± 0.28de	8.84 ± 0.97cd	6.55 ± 0.22e	9.71 ± 0.71bc	9.32 ± 0.91cd	0.000
Ethyl dodecanoate	1.39	6.17 ± 0.52b	6.87 ± 0.10ab	7.54 ± 0.69a	4.42 ± 0.19c	4.00 ± 0.42cd	4.25 ± 0.21c	4.72 ± 0.73c	3.24 ± 0.05d	4.81 ± 0.56c	4.87 ± 0.33c	0.000
Δ in % to control[b]		**119**	**144**	**155**	**100**	**103**	**94**	**101**	**77**	**102**	**105**	
HAA from grape lipid degradation												
Hexyl acetate	4.22	7.44 ± 0.51b	8.77 ± 0.34a	6.50 ± 0.91bc	7.10 ± 0.04b	5.59 ± 0.77c	6.57 ± 0.13bc	5.59 ± 0.58c	2.37 ± 0.08e	3.80 ± 0.14d	4.44 ± 0.12d	0.000

Compound		105	123	92	100	79	92	79	33	54	63	p
Δ in % to control[b]												
HAA from yeast sugar and N metabolism												
3-Methylbutyl acetate	7.05	38.6 ± 1.7e	64.2 ± 2.8a	57.5 ± 4.2ab	48.2 ± 0.4cd	48.3 ± 4.6cd	47.9 ± 3.4cd	53.8 ± 5.8bc	31.3 ± 0.5f	42.4 ± 0.2de	44.9 ± 4.2de	0.000
Phenylethyl acetate	2.01	5.97 ± 0.33b	10.3 ± 0.8a	10.7 ± 1.5a	5.06 ± 0.14bc	5.37 ± 0.21bc	5.50 ± 0.57b	6.29 ± 0.57b	3.24 ± 0.28d	4.55 ± 0.12cd	4.61 ± 0.38c	0.000
Δ in % to control[b]		**99**	**168**	**166**	**100**	**103**	**104**	**118**	**65**	**89**	**92**	
Higher alcohol												
2-Methyl-1-propanol	1.14	3.79 ± 0.14de	4.39 ± 0.13a	4.15 ± 0.08abc	3.98 ± 0.05cd	4.02 ± 0.06bcd	3.84 ± 0.12cde	3.54 ± 0.32e	3.85 ± 0.13cde	4.00 ± 0.18cd	4.33 ± 0.13ab	0.001
3-Methyl-1-butanol	25.90	92.4 ± 3.2cd	113 ± 4b	122 ± 4a	89.1 ± 0.3d	94.7 ± 5.5c	86.9 ± 3.8d	93.4 ± 2.7cd	86.2 ± 2.5d	94.6 ± 2.9c	95.5 ± 4.0c	0.000
Octan-1-ol	0.19	0.43 ± 0.04f	0.54 ± 0.01e	0.74 ± 0.04bc	0.59 ± 0.02de	0.70 ± 0.01bc	0.69 ± 0.01bcd	0.76 ± 0.06ab	0.64 ± 0.07cde	0.76 ± 0.05ab	0.84 ± 0.02a	0.000
3-(Methylthio)propanol	0.04	0.42 ± 0.07b	0.49 ± 0.03a	0.51 ± 0.02a	0.25 ± 0.00e	0.25 ± 0.01e	0.26 ± 0.02de	0.30 ± 0.01cde	0.34 ± 0.02c	0.32 ± 0.03cd	0.25 ± 0.04e	0.000
Benzyl alcohol	0.01	0.25 ± 0.03c	0.32 ± 0.01b	0.4 ± 0.01a	0.24 ± 0.00c	0.37 ± 0.08ab	0.32 ± 0.00b	0.38 ± 0.00ab	0.36 ± 0.03ab	0.38 ± 0.01ab	0.41 ± 0.02a	0.000
2-Phenylethanol	18.90	141 ± 9b	174 ± 7a	187 ± 6a	95.1 ± 2.5d	117 ± 16c	99.3 ± 2.3d	117 ± 4c	116 ± 4c	120 ± 4c	110 ± 3cd	0.000
1-Dodecanol	1.88	1.49 ± 0.09cd	2.33 ± 0.14a	1.91 ± 0.15b	1.41 ± 0.02cd	1.52 ± 0.06c	1.41 ± 0.01cd	1.46 ± 0.13cd	1.48 ± 0.06cd	1.56 ± 0.08c	1.32 ± 0.05d	0.000
Δ in % to control[b]		**113**	**143**	**152**	**100**	**115**	**107**	**117**	**116**	**122**	**119**	
Methyl esters of fatty acids												
Methyl octanoate	0.44	1.36 ± 0.05a	1.02 ± 0.05b	0.93 ± 0.06b	0.75 ± 0.03c	0.69 ± 0.07cd	0.74 ± 0.04c	0.75 ± 0.08c	0.63 ± 0.14cd	0.6 ± 0.06d	0.63 ± 0.04cd	0.000
Methyl decanoate	0.06	0.36 ± 0.02a	0.17 ± 0.02b	0.16 ± 0.01b	0.12 ± 0.01bc	0.13 ± 0.01bc	0.16 ± 0.02b	0.16 ± 0.02b	0.13 ± 0.06bc	0.08 ± 0.02d	0.10 ± 0.01cd	0.000
Δ in % to control[b]		**235**	**134**	**126**	**100**	**97**	**113**	**114**	**94**	**72**	**82**	
Other esters												
3-Methylbutyl octanoate	0.66	2.23 ± 0.21a	2.20 ± 0.03a	2.31 ± 0.23a	1.14 ± 0.03b	1.14 ± 0.08b	1.08 ± 0.05b	1.25 ± 0.09b	1.12 ± 0.07b	1.32 ± 0.07b	1.23 ± 0.07b	0.000
Diethyl succinate	0.42	3.61 ± 0.35cd	4.84 ± 0.14a	4.24 ± 0.12b	2.99 ± 0.04e	3.75 ± 0.45bc	3.27 ± 0.07cdef	3.49 ± 0.3cde	2.97 ± 0.36f	3.09 ± 0.21def	3.22 ± 0.04def	0.000
Ethyl 9-decenoate	4.28	0.76 ± 0.01bc	1.08 ± 0.11a	0.82 ± 0.06b	0.79 ± 0.01b	0.73 ± 0.06bc	0.63 ± 0.06cd	0.50 ± 0.05de	0.41 ± 0.04e	0.45 ± 0.10e	0.47 ± 0.05e	0.000
Ethyl tetradecanoate	0.01	0.04 ± 0.01ab	0.04 ± 0.00a	0.04 ± 0.00a	0.03 ± 0.00abc	0.03 ± 0.00d	0.03 ± 0.00cd	0.03 ± 0.00cd	0.02 ± 0.00e	0.03 ± 0.01cd	0.03 ± 0.00d	0.000
Ethyl hexadecanoate	0.01	0.13 ± 0.03b	0.13 ± 0.01ab	0.17 ± 0.02ab	0.12 ± 0.01bc	0.10 ± 0.02bc	0.11 ± 0.00bc	0.12 ± 0.02bc	0.05 ± 0.01d	0.08 ± 0.02cd	0.09 ± 0.02cd	0.000
Δ in % to control[b]		**125**	**144**	**142**	**100**	**95**	**93**	**94**	**68**	**86**	**85**	
Isoprenoids												
Vitispirane 1	1.71	0.47 ± 0.03cd	0.36 ± 0.02e	0.35 ± 0.04e	0.80 ± 0.00a	0.58 ± 0.13b	0.46 ± 0.02cd	0.44 ± 0.02cde	0.39 ± 0.00de	0.46 ± 0.01cd	0.49 ± 0.03bc	0.000
Vitispirane 2	0.99	0.33 ± 0.02cd	0.27 ± 0.02d	0.27 ± 0.07d	0.54 ± 0.03a	0.44 ± 0.07b	0.32 ± 0.03cd	0.33 ± 0.04cd	0.30 ± 0.02cd	0.34 ± 0.02cd	0.36 ± 0.04bc	0.000
Linalool	1.11	1.23 ± 0.03bcde	1.14 ± 0.03de	1.08 ± 0.04e	1.68 ± 0.04e	1.34 ± 0.22b	1.33 ± 0.01bc	1.26 ± 0.02de	1.12 ± 0.02de	1.17 ± 0.01cde	1.19 ± 0.02bcde	0.000
4-Terpineol	0.03	0.01 ± 0.00cd	0.03 ± 0.01de	0.06 ± 0.02bcde	0.1 ± 0.02bc	0.11 ± 0.05bc	0.05 ± 0.01cde	0.12 ± 0.03b	0.11 ± 0.04bc	0.08 ± 0.03bcd	0.19 ± 0.06a	0.001
α-Terpineol	0.33	0.15 ± 0.00cd	0.15 ± 0.00cd	0.15 ± 0.00cd	0.22 ± 0.00a	0.19 ± 0.02b	0.18 ± 0.00b	0.18 ± 0.01b	0.16 ± 0.01c	0.14 ± 0.01d	0.14 ± 0.01cd	0.001
TDN	1.25	0.58 ± 0.02g	0.67 ± 0.02fg	0.72 ± 0.06ef	1.03 ± 0.02a	0.95 ± 0.06ab	0.8 ± 0.02de	0.87 ± 0.03bcd	0.69 ± 0.04f	0.83 ± 0.04cd	0.92 ± 0.11bc	0.000
Citronellol	0.29	3.87 ± 0.33e	4.62 ± 0.10cd	4.94 ± 0.35bc	4.23 ± 0.11de	5.32 ± 0.65ab	4.68 ± 0.19cd	5.34 ± 0.01ab	5.76 ± 0.17a	4.85 ± 0.22bcd	4.88 ± 0.34bc	0.000
β-Damascenone	2.58	2.62 ± 0.08ab	2.00 ± 0.01e	2.04 ± 0.01de	2.65 ± 0.04a	2.74 ± 0.24a	2.34 ± 0.11abc	2.48 ± 0.11abc	2.30 ± 0.16cd	2.27 ± 0.22cde	2.35 ± 0.17bc	0.000
trans Geraniol	0.03	0.03 ± 0.00c	0.02 ± 0.00c	0.02 ± 0.00c	0.02 ± 0.00c	0.07 ± 0.08bc	0.12 ± 0.08bc	0.11 ± 0.07bc	0.17 ± 0.01ab	0.23 ± 0.03a	0.16 ± 0.1ab	0.004
α-Ionone	0.01	0.04 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.123
trans-Nerolidol	0.51	0.27 ± 0.10a	0.14 ± 0.02b	0.05 ± 0.01c	0.03 ± 0.01c	0.05 ± 0.01c	0.06 ± 0.00c	0.04 ± 0.00c	0.01 ± 0.00c	0.03 ± 0.00c	0.04 ± 0.01c	0.000
Δ in % to control[b]		**151**	**99**	**77**	**100**	**123**	**131**	**137**	**142**	**164**	**154**	

[a]Values are means of 3 replicates ± standard error (except H0, which was produced without triplicates). [b]Proportions of accumulated semi quantitative data of compounds within compound group relative to the control (100%). Values followed by different letters within rows are significantly different (p ≤ 0.05, one way ANOVA).

Table S8 Results of the descriptive analysis for the Cabernet Sauvignon wines. Values results from ratings on a 100 point scale.[a]

	CS_H1	CS_H2	CS_H3	CS_H4 (Control)	CS_B1	CS_B2	CS_B3	CS_Bw1	CS_Bw2	CS_Bw3	p-value	LSD
Aroma												
Aroma Intensity	52.1bc	50.7c	53.0abc	54.3abc	53.8abc	51.7bc	53.6abc	54.3abc	55.8ab	57.8a	0.231	5.0
Red Fruit	29.2c	31.2bc	33.2abc	36.6ab	33.5abc	37.2a	34.6abc	37ab	32.5abc	36.5ab	0.114	6.0
Dark Fruit	34.2c	40.5ab	42.8ab	45.6a	38.5bc	38.7bc	41.4ab	44.1ab	41.2ab	42.3ab	0.050	6.3
Dried Fruit	21.2abc	16.8c	18.8bc	23.3ab	20.7abc	20.8abc	21.5abc	25.3a	24.9ab	20.8abc	0.223	6.3
Green	40.8a	32.3ab	29.3b	30.9ab	23.3b	22.3b	24.8b	26.4b	24.3b	32.5ab	0.044	11.1
Pepper	14.5a	12.5a	16.1a	16.6a	16.5a	11.9a	13.8a	15.0a	15.0a	12.0a	0.664	5.7
Sweet Spice	13.3a	17.9a	18.0a	14.8a	17.2a	14.6a	14.8a	13.8a	18.0a	14.7a	0.638	5.6
Liquorice	11.3b	13.7ab	18.6a	16ab	17.5a	13.5ab	15.4ab	16.9a	15.5ab	14.8ab	0.295	5.4
Chocolate	10.6b	13.1ab	14.6ab	13.9ab	14.6ab	12.7ab	17.9a	15.0ab	14.6ab	14.4ab	0.729	6.4
Confection	8.3ab	11.2ab	7.4b	8.5ab	8.7ab	10.1ab	9.6ab	12.0ab	12.3a	9.3ab	0.535	4.9
Savoury	10.9b	9.9b	15.4ab	12.8ab	18.3a	14.2ab	14.2ab	13.8ab	13.0ab	10.0b	0.349	6.8
Smoke	15.3abc	13.8bc	17.7ab	14.0bc	15.3abc	19.9a	15.1bc	13.8bc	14.9bc	12.7c	0.138	4.7
Earthy	16.9a	12.2ab	13.6ab	13.0ab	10.7b	13.2ab	10.1b	11.9ab	12.2ab	12.2ab	0.540	5.5
Flavour												
Flavour Intensity	49.9d	51.3cd	54.7abc	57.7a	51.2cd	53.7bcd	56.7ab	55.3ab	56.7ab	57.5a	0.000	3.8
Red Fruit	30.9c	34.0bc	36.1ab	40.0a	33.9bc	37.1ab	38.7ab	38.2ab	40.9a	37.5ab	0.007	5.1
Dark Fruit	37.7c	43.7abc	47.0a	47.1a	38.3bc	45.0ab	41.8abc	39.5bc	46.8a	43.1abc	0.044	6.9
Dried Fruit	15.5c	15.8c	18.9bc	24.5a	18.5c	17.9c	19bc	19.8abc	23.8ab	19.5abc	0.016	5.2
Green	34.0a	28.8a	31.5a	29.3a	29.8a	28.4a	29a	33.4a	27.8a	31.8a	0.601	6.8
Pepper	15.6a	14.2a	17.0a	19.5a	16.7a	15.3a	14.6a	17.3a	18.9a	15.1a	0.545	5.3
Sweet Spice	14.9ab	15.0ab	18.4ab	18.8ab	14.4b	16.5ab	17.3ab	15.0ab	20.0a	17.0ab	0.465	5.3
Liquorice	6.2c	10.0bc	9.5bc	9.3bc	7.6bc	8.9bc	10.2b	10.5b	14.5a	7.9bc	0.011	3.9
Chocolate	13.2b	12.8b	18.7a	17.3ab	17.8a	15.8ab	17.2ab	15.2ab	17.3ab	16.5ab	0.232	4.6
Confection	7.8c	11.0abc	12.2abc	15.1a	9.6bc	11.1abc	13.5ab	12.8ab	12.7ab	15.3a	0.072	4.8
Savoury	14.1ab	11.1b	15.3ab	19.2a	14.9ab	14.1ab	13.5b	14.8ab	15.3ab	13.0b	0.298	5.3

Smoke	16.8ab	14.2b	16.1ab	17.9ab	13.5b	17.5ab	15.7ab	15.2ab	20.0a	16.1ab	0.577	5.8
Earthy	16.7a	12.0bc	13.7abc	12.3abc	10.7c	12.9abc	13.3abc	15.8ab	12.3bc	13.7abc	0.246	4.4
Mouthfeel												
Acidity	56.1abc	55.2abc	53.4abc	53.3abc	59.2a	51.8c	58.6ab	52.1bc	52.2bc	53.6abc	0.249	6.6
Sweetness	7.5a	9.4a	8.4a	10.5a	10.0a	9.4a	11.1a	8.9a	7.9a	9.8a	0.679	3.8
Bitterness	30.0ab	26.8bc	30.4ab	30.5ab	23.9c	28.3abc	30.0ab	26.8bc	33.3a	32.4ab	0.066	5.8
Astringency	47.7cde	42.2e	46.4cde	57.4a	44.7de	49.7bcd	48.7cde	49.2bcd	55.7ab	51.9abc	0.001	6.8
Hotness	30.5f	41.6e	43.8de	59.5a	44.2cde	46.1bcde	51.2bc	45.6bcde	52.7ab	49.5bcd	<.0001	7.3

[a]Values are means of 3 replicates. Different letters within rows are significantly different if $p \leq 0.05$, as determined by one way ANOVA

Table S9 Results of the descriptive analysis for the Shiraz wines. Values results from ratings on a 100 point scale.[a]

	SH_H1	SH_H2	SH_H3	SH_H4 (Control)	SH_B1	SH_B2	SH_B3	SH_Bw1	SH_Bw2	SH_Bw3	p - value	LSD
Aroma												
Aroma Intensity	55.2ab	55.5ab	58.9a	57.4ab	55.3ab	53.1ab	53.3ab	51.6b	52.0b	54.5ab	0.261	6.0
Red Fruit	28.0ab	26.5b	29.9ab	33.1ab	32.7ab	29.8ab	29.6ab	31.5ab	34.6a	33.0ab	0.525	7.9
Dark Fruit	38.5a	40.8a	41.0a	45.3a	41.2a	44.4a	40.6a	44.2a	46.2a	45.6a	0.624	8.7
Dried Fruit	23.5b	21.3b	21.3b	26.0ab	22.6b	30.8a	22.0b	24.5ab	22.5b	27.0ab	0.219	6.9
Green	14.3a	20.1a	17.0a	15.7a	16.3a	19.8a	19.9a	15.3a	17.1a	18.6a	0.685	6.9
Pepper	16.8a	20.4a	14.7a	12.8a	16.8a	19.2a	15.4a	17.8a	19.6a	18.7a	0.863	9.0
Sweet Spice	17.3ab	22.6a	11.8b	16.1ab	19.5ab	21.4a	21.8a	19.7a	18.9ab	23.9a	0.218	8.3
Liquorice	14.7bc	16.9bc	15.1bc	17.9abc	12.5c	16.6abc	17.8abc	18.9ab	19.1abc	23.1a	0.086	6.3
Chocolate	10.0a	13.0a	16.1a	17.0a	12.9a	15.2a	13.4a	16.9a	14.7a	16.2a	0.627	7.4
Confection	6.4b	9.5ab	7.7b	9.4ab	11.0ab	6.2b	6.3b	10.0ab	13.0a	9.3ab	0.033	4.2
Savoury	32.1a	27.0ab	28.5ab	21.1ab	23.3ab	18.9b	24.6ab	18.7b	19.7b	17.9b	0.263	12.5
Smoke	19.0ab	25.4a	22.2ab	26.8a	24.3ab	19.4ab	23.0ab	20.2ab	17.7ab	16.5b	0.360	9.3
Earthy	12.5b	12.4b	11.9b	9.3b	13.7ab	13.7ab	10.3b	18.2a	13.5ab	12.8b	0.149	5.4
Flavour]												
Flavour Intensity	48.9b	55.0a	55.9a	57.5a	53.7a	54.6a	54.1a	53.0ab	55.7a	53.6a	0.043	4.6
Red Fruit	32.8ab	34.3ab	33.5ab	33.3ab	35.0ab	30.7b	38.6a	34.0ab	38.0a	35.2ab	0.595	6.8
Dark Fruit	38.5c	42.8bc	44.7bc	53.1a	40.9bc	44.9abc	45.7abc	46.8ab	48.0ab	46.8ab	0.055	7.9
Dried Fruit	18.0b	20.8ab	21.7ab	24.7ab	20.9ab	24.8ab	22.1ab	22.9ab	23.5ab	26.9a	0.389	7.1
Green	22.9a	22.4a	22.2a	21.8a	23.1a	26.8a	21.5a	19.8a	20.2a	24.3a	0.732	6.7
Pepper	16.6a	18.7a	18.1a	17.8a	20.5a	19.7a	20.0a	18.5a	19.1a	20.8a	0.986	7.5
Sweet Spice	15.1a	18.9a	15.7a	18.6a	17.3a	16.3a	20.4a	15.2a	20.4a	20.1a	0.593	6.6
Confection	9.3ab	12.1a	7.4b	7.4b	9.0ab	7.6ab	5.6b	10.0ab	8.8ab	8.4ab	0.270	4.4
Liquorice	14.0c	17.3bc	17.2bc	16.5bc	15.8bc	16.6bc	22.6ab	34.5a	18.3abc	23.7a	0.025	6.6
Chocolate	8.8c	10.4bc	16.3a	15.5ab	9.79c	12.8abc	13.9abc	13.5abc	15.3ab	13.8abc	0.043	5.3
Savoury	23.0a	24.1a	25.2a	21.9a	21.0a	16.8a	17.5a	17.6a	19.3a	16.8a	0.572	9.9

Smoke	14.7c	23.9ab	22.7abc	27.1a	19.4abc	20.8abc	20.1abc	19.2abc	17.8bc	17.9abc	0.289	8.9
Earthy	11.5b	14.7ab	13.3ab	12.9ab	15.7ab	12.9ab	13.8ab	14.5ab	14.6ab	16.2a	0.709	5.0
Mouthfeel												
Acidity	57.2b	52.6b	50.8b	56.4b	64.5a	55.1b	57.4ab	52.1b	52.2b	54.7b	0.007	7.1
Sweetness	7.3b	8.6ab	9.1ab	10.1ab	8.4ab	10.3ab	9.2ab	11.8a	10.6ab	9.7ab	0.584	4.2
Bitterness	24.5cd	34.8ab	32.2ab	28.7bcd	28.0bcd	22.5d	30.6abcd	31.0abc	36.8a	29.8bcd	0.012	7.5
Astringency	38.6c	47.8ab	51.2ab	54.4a	45.2b	44.5bc	49.8ab	45.8b	52.8a	51.0ab	0.000	6.6
Hotness	35.8d	49bc	53.4ab	57.4a	42.9cd	42.9cd	49.2abc	44.0c	51.7ab	53.8ab	<.0001	7.8

[a]Values are means of 3 replicates. Different letters within rows are significantly different if p ≤ 0.05, as determined by one way ANOVA

Chapter 5

Substitution or dilution? Assessing pre-fermentative water implementation to produce lower alcohol Shiraz wines.

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Preface

Chapter 5 reports on results from the analysis of non-volatile wine components and

descriptive sensory analysis, and was included to complete the comprehensive assessment of

three vintages and two cultivars. This allowed for even more informed evaluations to be

made regarding the pre-fermentative water implementation and early harvest regimes.

However, in addition to the presented results, the analysis of wine volatile profiles is

currently under way and will be included in the manuscript for journal submission.

Statement of Authorship

Title of Paper	Substitution or dilution? Assessing pre-fermentative water implementation to produce lower alcohol Shiraz wines.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Olaf J. Schelezki
Contribution to the Paper	Designed experiments, conducted vineyard monitoring, organised and executed grape harvests and experimental winemaking, conducted non-volatile wine analysis, trained a sensory panel and undertook quantitative descriptive analysis of 42 wines, statistically analysed the data sets (one-way ANOVA, PCA), interpreted the data, drafted and constructed the manuscript.
Overall percentage (%)	70
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature		Date	6 / 06 /2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Alain Deloire
Contribution to the Paper	Contributed to the research idea and experimental design, provided assistance regarding analysis methods and edited the manuscript..
Signature		Date	07/06/2018

Name of Co-Author	David W. Jeffery
Contribution to the Paper	Supervised the work, contributed to the research idea, experimental design, analysis method and interpretation of the data. Edited the manuscript and acted as the corresponding author.

Signature			Date	07/06/2018

Substitution or dilution? Assessing pre-fermentative water implementation to produce lower alcohol Shiraz wines.

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1 **Abstract**

2 Recent changes by the Food Standards Australia New Zealand, permitted the adjustent must sugar

3 levels with the addition of water in order to ensure a sound fermentation progress as well as mitigating

4 excessive wine alcohol levels. For the first time, this study assessed the implications on wine quality

5 following a pre-fermentative must dilution (changing liquid-to-solid ratios) of Shiraz wines, hereby

6 comparing this approach with a juice substitution with water (constant liquid-to-solid ratios), that has

7 previously been deemed a promising way to adjust wine alcohol levels.

8 While working within the legal limit of water addition, the effect of both alternatives on

9 wine quality parameters and sensory characteristics were rather similar, and of negligible nature.

10 However, different implications between substitution and dilution appeared to be driven by grape

11 maturity. In line with previous observations, longer hang-times followed by alcohol adjustments were

12 of limited merit compared to simply picking grapes earlier. This work provided further knowledge

13 that support an informed decision making regarding this recent winemaking approach.

14   **1. Introduction**

15   Among the options to mitigate elevated alcohol levels in red wines as a result of climate

16   change related shifts in grapevine phenology and the trend toward increased grape total soluble solids

17   (TSS) levels at harvest, pre-fermentative winemaking interventions have drawn some attention in

18   recent years (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011; Longo, Blackman, Antalick,

19   Torley, Rogiers, & Schmidtke, 2018; Schelezki, Antalick, Šuklje, & Jeffery, 2018; Schelezki, Smith,

20   Hranilovic, Bindon, & Jeffery, 2018; Schelezki, Suklje, Boss, & Jeffery, 2018). Specifically, after

21   removing certain amounts of juice (saignée) from a red grape must, equal proportions of a very low

22   alcohol wine (green harvest wine (GHW), resulting from fruit harvested at veraison) or water were

23   added to dilute must TSS concentrations and yield wines with decreased alcohol levels. Those studies

24   variously assessed the implications on wine colour, tannin and volatile compositions as well as the

25   effects on wine sensory profiles. In case of Cabernet Sauvignon, pre-fermentative substitution did not

26   have any adverse effects on parameters such as anthocyanin concentration, colour intensity, the

27   proportion of stable pigments, or tannin concentration and molecular mass (MM), across different

28   vintage conditions (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011; Schelezki, Antalick,

29   Šuklje, & Jeffery, 2018; Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). In one case, lower

30   alcohol Cabernet Sauvignon wines resulting from water implementation were superior in colour

31   intensity, stable pigment formation and tannin concentration (Schelezki, Antalick, Šuklje, & Jeffery,

32   2018) compared to the control .

33   Volatile profiles and sensory characteristics of the lower alcohol Cabernet Sauvignon wines

34   seemed to be reflective of the traits determined by the fruit harvested at commercial maturity when

35   using pre-fermentative substitution with water but differences according to the grape maturity were

36   apparent (Schelezki, Antalick, Šuklje, & Jeffery, 2018; Schelezki, Suklje, Boss, & Jeffery, 2018).

37   That is, in contrast to a season with evident berry shrivel where over-ripe sensory characters were

38   maintained in the water treatment wines, a "normal" grape maturity context (milder ripening season)

39   showed a general decline in concentration and higher proportion of affected volatile compounds with

40   higher levels of water implementation (Schelezki, Antalick, Šuklje, & Jeffery, 2018). Nonetheless,

41   other than potentially harvesting grapes earlier, water was identified being the more favourable choice

42   in comparison to producing a GHW to manage wine alcohol levels, especially given the lower costs

43   and simplicity afforded by water. More research is required, however, in light of previous findings

44   indicating that the apparently benign observations mentioned above do not necessarily apply with

45   cultivars other than Cabernet Sauvignon. For instance, Sherman, Greenwood, Villas-Boâs, Heymann,

46   and Harbertson (2017) and Schelezki, Antalick, Šuklje, and Jeffery (2018) demonstrated decreasing

47   tannin concentrations with higher water (and more so with GHW (Schelezki, Antalick, Šuklje, &

48   Jeffery, 2018)) substitution rates in Merlot and Shiraz wines, respectively, in hand with negative

49   impacts on colour density and stability in the Shiraz wines, which remained evident after 12 months

50   of bottle aging.

51   As the most widely grown cultivar in Australia (Wine Australia, 2017), Shiraz is of significant

52   value to the wine industry but it is also a variety that is particularly susceptible to berry shrivel (Suklje,

53   Zhang, Antalick, Clark, Deloire, & Schmidtke, 2016), which results in higher berry sugar

54   concentrations and increased wine alcohol levels. This implies a prospective role of pre-fermentative

55   alcohol management for Shiraz in Australia (and likely elsewhere) but studies that build on the

56   previous findings are required. Furthermore, according to the Food Standards Australia New Zealand,

57   water can be added to grape must before commencing fermentation to yield a minimum of 13.5

58   °Baumé (Bé) without the need of removing juice (FSANZ, 2016). Thus, in contrast to the previous

59   approach of proportional substitution of juice with water so as not to alter the solid-liquid ratio

60   (Schelezki, Antalick, Šuklje, & Jeffery, 2018), the effectively higher wine volumes and easier

61   implementation of adding water but not removing juice could be more favourable for wine producers.

62   However, the consequences for wine quality of changing the solid-liquid ratio by diluting the must

63   with water remained to be investigated.

64   This study therefore aimed to i) provide a direct comparison between the two variants of pre-

65   fermentative water addition to manage wine alcohol levels through evaluation of wine colour and

66   tannin parameters and wine sensory attributes of lower alcohol Shiraz wines, and ii) assess the

67   implications for wine quality as a result of grape maturity by harvesting Shiraz grapes at two distinct

68   maturity levels and undertaking pre-fermentative water implementation regimes.

69   **2. Material and methods**

70   *2.1 Chemicals*

71   Reagents and reference compounds used for analysis were sourced from Sigma Aldrich

72   (Castle Hill, NSW, Australia) or Alfa Aesar (Ward Hill, MA, USA). Stock solutions of standards

73   were prepared volumetrically in redistilled ethanol and stored at -20 ºC, and working solutions were

74   kept at 4 ºC until required. HPLC grade solvents and analytical grade sodium chloride were sourced

75   from Merck (Kilsyth, Victoria, Australia) and Chem-Supply (Gillman, SA, Australia), respectively.

76   Water for analyses was obtained from a Milli-Q purification system (Millipore, north Ryde, NSW,

77   Australia), and filtered tap water was used for the water blending treatments. Potassium metabisulfite

78   was sourced from Vebigarden (Padua, Italy).

79   *2.2 Climate data*

80   This study was undertaken during the 2017 vintage in South Australia. Daily minimum,

81   maximum and average temperatures, total monthly rainfall, and long-term averages (Table 1) were

82   obtained from the Australian Bureau of Meteorology (Australian Government Bureau of

83   Meteorology, 2018), as used in preceding studies (Schelezki, Antalick, Šuklje, & Jeffery, 2018;

84   Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). Based on this data, the Huglin index for the

85   vintage 2016/17 was calculated as stated in Tonietto and Carbonneau (2004).

86   *2.3 Harvest and winemaking*

87   *Vitis vinifera* L. cv. Shiraz grapes were sourced from a commercial vineyard in McLaren Vale,

88   South Australia (138.521139ºE, 35.194167ºS), using an identical plot to that used previously

89   (Schelezki, Antalick, Šuklje, & Jeffery, 2018). Starting from veraison (i.e., 50% of coloured berries),

90   and twice a week, triplicate lots of 200 berries were randomly sampled from both sides of the canopy

91   to monitor the evolution of grape ripening, targeting two distinct grape maturities once berry sugar

92   accumulation had reached a plateau (increase lower than 3 mg sugar/berry/day, according to the

93   model by Deloire (2013)). The first batch of grapes for winemaking, subsequently referred to as Fresh

94   Fruit (FF), was harvested on 8 March at 22.7 ºBrix, twelve days after the plateau was reached on 24

95   February. A second harvest occurred twelve days later than the first, on 20 March at 25.5 ºBrix, with

96   this stage being designated Mature Fruit (MF).

97   At each harvest, approximately 400 kg of grapes were collected, destemmed, crushed and

98   distributed in 20 L plastic buckets. Prior to inoculation and in triplicate, must TSS concentrations

99   were diluted either by substituting proportions of juice with water, or by directly adding water, to

100   target similar wine alcohol levels. Substitution and dilution rates were calculated as previously

101   reported (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011). All buckets were of similar mass

102   after implementing the treatments, at approximately 18-19 kg. The wines originating from the

103   substitution treatments (i.e., maintaining the original solid-liquid ratio) are further referred to as

104   FF_S1-S3 (from the Fresh Fruit harvest) and MF_S1-S3 (from the Mature Fruit harvest), whereas

105   wines resulting from simple dilution with water are further designated FF_D1-D3 and MF_D1-D3 in

106   the same manner. FF_Control and MF_Control designations refer to untreated control wines for each

107   harvest date, which were also prepared in triplicate (Fig. S1 of the Supporting Information).

108   Subsequent inoculation and winemaking procedures were the same as previously applied (Schelezki,

109   Antalick, Šuklje, & Jeffery, 2018; Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018). The dry

110   wines (< 1 g/L residual sugar) were pressed with a basket press, transferred into 10 L glass demijohns,

111   and stored at 0 ºC for stabilisation and conservation until bottling. At bottling, wine pH was adjusted

112   to 3.5 (using 500 g/L aqueous tartaric acid solution) and potassium metabisulfite (PMS) was added

113   at a rate of 100 mg/L. The bottles were stored at 15 ºC until analysis.

114   *2.4 Analysis of basic chemical parameters*

115   Wine ethanol concentration was measured using an alcolyser (Anton Paar, Graz, Austria).

116   Juice and wine pH and titratable acidity (TA), expressed as g/L equivalents of tartaric acid, were

117   analysed with the Mettler Toledo T50 Autotitrator, with a titration endpoint of pH 8.2 using a 0.33

118   NaOH solution. Glucose, fructose, glycerol, and malic, tartaric, citric and acetic acids were analysed

119   by HPLC using a previously reported method (Li, Bindon, Bastian, Jiranek, & Wilkinson, 2017).

120   *2.5 Extraction and isolation of grape and wine tannin*

121   *2.5.1 Wine-like extraction*

122   Grapes were extracted as previously reported (Bindon, Kassara, Cynkar, Robinson,

123   Scrimgeour, & Smith, 2014; Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018) to estimate

124   extractable tannin content.

125   *2.5.2 Isolation of wine tannins*

126   Wine tannins were isolated by solid-phase extraction using a previously published method

127   (Jeffery, Mercurio, Herderich, Hayasaka, & Smith, 2008) with a slight modification to collect tannins

128   as one fraction (Kassara & Kennedy, 2011).

129   *2.6 Analysis of tannins and wine colour*

130   Tannin concentrations of the wine-like extracts and wines were assessed using the methyl

131   cellulose precipitable tannin assay (MCP tannin) (Mercurio, Dambergs, Herderich, & Smith, 2007).

132   Colour density, anthocyanin concentration and total phenolics of wines were analysed using the

133   modified Somers colour assay (Mercurio, Dambergs, Herderich, & Smith, 2007). Wine tannin size

134   distribution (molecular mass, MM) was determined by gel permeation chromatography (GPC) using

135   methanolic solutions of isolated tannins diluted 1:5 with the HPLC mobile phase prior to injection.

136   Instrument parameters, chromatographic conditions and calibrations for GPC were adapted from

137   Kennedy and Taylor (2003) with modifications according to Bindon, Bacic, and Kennedy (2012)

138   *2.7 Sensory analysis*

139   Wine sensory assessment was conducted ten months after bottling via a descriptive analysis

140   (DA). The DA panel comprised seven female and two male students and researchers from The

141   University of Adelaide that were recruited as assessors because of their previous DA experience.

142   Following the consensus-based approach (Lawless & Heymann, 2010), the DA consisted of nine

143   training and three formal sessions. The panel initially evaluated aroma, flavour and palate

144   characteristics of a subset of wines, which were discussed during two sessions to reach consensus

145   about the descriptive attributes. In subsequent sessions, panellists were given reference standards,

146   that they tried and agreed upon, to familiarise themselves with the aroma attributes as well as

147   mouthfeel characteristics (alcohol, acidity, astringency, bitterness), and further practised with

148   different experimental wines. The wines were rated using RedJade online based software and the

149   results obtained from the training sessions were presented to the panellists to provide feedback and

150   screen out non-discriminating attributes. The final attributes list (Table S1 of the Supporting

151   information) included ten aroma, ten flavour and three mouthfeel attributes, which were rated on 15-

152   cm unstructured line scales, with anchors at 10%, 50% and 90% of the scale corresponding to 'low',

153   'medium' and 'high', respectively. During the formal assessments, panellists were presented 14 wine

154   samples (30 mL) served in ISO standard (ISO 3951:1977) black wine glasses coded with four-digit

155   numbers and covered with glass lids in a randomised and balanced order. The evaluations were held

156   in a sensory laboratory equipped with isolated booths, under an ambient temperature of 21 ºC, and

157   with white lighting. One-minute rest breaks after each sample and five minutes after seven samples

158   were imposed on the panellists to avoid fatigue. Pectin solution (1 g/L, pectin from citrus peel, Sigma-

159   Aldrich, Castle Hill, NSW, Australia), plain water crackers and filtered tap water were provided to

160   the panellists for palate cleansing.

161   *2.8 Statistical analysis*

162   One-way analysis of variance (ANOVA) of the chemical data in combination with mean

163   comparisons via Fisher's least significant difference (LSD) multiple comparison test at $p < 0.05$, and

164   principal component analysis (PCA) of normalised sensory data were performed using XLSTAT

165   (Version 2015.4.1, Addinsoft, Paris, France). PanelCheck (V1.4.2, Nofima Mat) was used to assess

166   panel performance during the DA, and the final results were analysed via ANOVA and mean

167   comparisons by Fisher's LSD using SENPAQ (Version 6.03, Qi Statistics, Reading, United

168   Kingdom).

169   **3. Results and discussion**

170   *3.1 Vintage conditions and fruit parameters*

171   With 2323 Hugling index units, the 2016/17 growing season of McLaren Vale was classified

172   as temperate warm (Tonietto & Carbonneau, 2004), and was therefore significantly cooler as in the

173   preceding vintage 2015/16 (Schelezki, Antalick, Šuklje, & Jeffery, 2018). Lower than average

174   temperatures from September to December 2016 in line with above average monthly rainfall from

175   the beginning of the season (except for a drier November, Table 1) caused a lagging phenological

176   development that delayed the commercial harvest date by almost a month compared to the previous

177   year (Schelezki, Antalick, Šuklje, & Jeffery, 2018).

178   The average berry weight remained constant between FF to MF harvests (Table 2),

179   indicating the absence of vine water constraint until commercial harvest (Triolo, Roby, Plaia, Hilbert,

180   Buscemi, Di Lorenzo, et al., 2017). However, given that grape TSS levels increased from 22.7 ºBrix

181   in FF to 25.5 ºBrix in MF despite the constant berry weight and plateau of sugar content per berry, an

182   asynchronous ripening development within the population led to a diverging result compared to the

183   per berry analysis. This is exemplary for the general problem of working with a grape population with

184   such asynchronous development, as relationships between primary and secondary fruit metabolisms

185   are difficult to capture. The grape TSS increments were in line with rising pH and decreasing TA

186   concentrations. Grapes from the later harvest point were further characterised by higher extractable

187   tannin (expressed in mg/g berry, Table 1), similarly to what was observed previously for the same

188   vineyard under warmer and drier vintage conditions (Schelezki, Antalick, Šuklje, & Jeffery, 2018).

189   The higher amount of extractable tannin per berry in the present study was likely deemed to result

190   from accumulation within the berry (Suklje, Zhang, Antalick, Clark, Deloire, & Schmidtke, 2016)

191   rather than a concentration effect (Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018).

192   **Table 1** Weather conditions near the McLaren Vale[a] vineyard during the growing season 2016/17.

193   Minimum (Ø Temp Min), maximum (Ø Temp Max) and average (Ø Temp) temperatures, as well as

194   precipitation sums (∑ Rainfall) are compared to the long-term values (2000-2017, in parentheses).

195   Numbers in bold indicate values above (for temperature) or below (for rainfall) the average values.

Month	Ø Temp min	Ø Temp max	Ø Temp	∑ Rainfall
Jul '16	**9.0 (+0.3)**	14.5 (-0.3)	11.8 (0.0)	118.4 (+51.8)
Aug '16	8.8 (0.0)	**16.5 (+0.6)**	**12.7 (+0.3)**	68.6 (+14.9)
Sep '16	9.8 (-0.6)	16.6 (-2.0)	13.2 (-1.3)	64.0 (+18.1)
Oct '16	10.4 (-1.1)	19.9 (-1.5)	15.2 (-1.3)	65.8 (+33.8)
Nov '16	12.0 (-2.1)	22.8 (-2.1)	17.4 (-2.1)	**16.0 (-5.1)**
Dec '16	15.2 (-0.2)	**26.6 (+0.2)**	20.9 (0.0)	48.0 (+26.1)
Jan '17	**17.6 (+0.5)**	28.5 (-0.2)	**23.1 (+0.2)**	34.2 (+16.1)
Feb '17	16.5 (-0.4)	26.8 (-0.9)	21.7 (-0.6)	**14.2 (-5.4)**
Mar '17	**17.5 (+1.9)**	**27.7 (+2.0)**	**22.6 (+1.9)**	**9.2 (-12.4)**
Apr '17	**14.1 (+0.5)**	**22.5 (+0.2)**	**18.3 (+0.3)**	50.0 (+16.6)
May '17	11.1 (-0.4)	18.3 (-0.2)	14.7 (-0.3)	**25.8 (-27.9)**
Jun '17	7.7 (-1.6)	**16.2 (+0.6)**	12.0 (-0.5)	**21.8 (-40.9)**

196   [a] Noarlunga weather station (Latitude: 35.16 °S, Longitude 138.51 °E, Elevation: 55 m).

197

198   **Table 2** Basic grape compositional parameters at two distinct harvest dates (Fresh Fruit and Mature

199   Fruit) and grape extractable tannin.[a]

	Fresh Fruit	Mature Fruit
Harvest date	8 March 2017	20 March 2017
TSS [ºBrix]	22.7 ± 0.1b	25.5 ± 0.0a
Berry weight [g/berry]	0.89 ± 0.02	0.90 ± 0.02
TA [g/L][b]	5.75 ± 0.08a	4.53 ± 0.11b
pH	3.82 ± 0.00b	4.03 ± 0.00a
Extractable tannin[c]		
mg/g berry	0.36 ± 0.01b	0.53 ± 0.02a
mg/berry	0.31 ± 0.01b	0.38 ± 0.03a

200   [a]Values are means of 3 replicates ± standard error. [b]Values in tartaric acid equivalents. [c]Determined

201   by MCP tannin assay of wine-like extracts for crushed berries. Values followed by different letters

202   within rows are significantly different (p ≤ 0.05, one way ANOVA)

203   *3.2 Basic wine composition*

204   All wines fermented to dryness (<1 g/L of total sugar, Table 3). The FF_Control and

205   MF_Control wines yielded 13.6% and 15.5% alcohol by volume (ABV), respectively. For the FF

206   harvest, water implementation rates of 41.0, 26.6 and 11.6% v/v resulted in lower alcohol wines with

207   9.6/9.0% ABV in FF_S1/D1, 11.1/10.8% ABV in FF_S2/D2 and 12.6/12.6% ABV in FF_S3/D3,

208   respectively (Table 3, Fig. S1 of the Supporting Information). With the MF fruit, implementation

209   rates of 47.2, 34.0 and 10.2% v/v with water produced lower alcohol wines with 10.6/9.6%,

210   12.0/11.7% and 14.5/14.4% ABV, respectively. Acetic acid concentration tended to remain constant

211   across the wines, except for lower values in FF_S3/D3 compared to the control. A decline in malic

212   acid concentration was further notable, however became only significant in FF_D1 and MF_D1/S1

213   wines. Further, while the pH within each of the substitution treatments (FF_S1-S3 and MF_S1-S3)

214   were of similar levels, identical addition rates of the tartaric acid solution in the wines resulting from

215   the dilution treatments provoked significant declines in pH, likely due to a decreased buffer

216   capacity(Waterhouse, 2016). Acetaldehyde and glycerol levels decreased with lower established

217   alcohol levels and to similar extents regardless of whether substitution or dilution was employed

218   (Table 3). As glycerol forms part of the dry extract of wines, the lower levels resulting from the water

219   implementations could translate into differing mouthfeel properties, such as viscosity or astringency

220   (Yanniotis, Kotseridis, Orfanidou, & Petraki, 2007).

221 **Table 3** Water addition rates and basic parameters for wines resulting from the Fresh Fruit (FF_Control, FF_S and FF_D series) and Mature Fruit

222 (MF_Control, MF_S and MF_D series) harvests.[a]

Wine	Water addition rate	Alcohol level	TA [g/L][b]	pH	Malic acid [g/L]	Acetic acid [g/L]	Glycerol [g/L]	Acetaldehyde [g/L]	Fructose [g/L][c]
Fresh Fruit									
FF Control	n/a	13.6 ± 0.1d	7.14 ± 0.17abc	3.48 ± 0.05de	3.05 ± 0.65abc	0.37 ± 0.04abcd	9.03 ± 1.22cd	0.46 ± 0.17cd	0.11 ± 0.16e
FF_S1	41.0	9.60 ± 0.10k	6.48 ± 0.08cde	3.53 ± 0.01bcd	2.83 ± 0.08cd	0.42 ± 0.02a	7.17 ± 0.08gh	0.26 ± 0.01fgh	0.10 ± 0.14e
FF_S2	26.3	11.1 ± 0.1h	6.74 ± 0.07bcde	3.58 ± 0.05ab	3.07 ± 0.03abc	0.36 ± 0.01bcde	8.14 ± 0.15def	0.32 ± 0.01efgh	0.22 ± 0.16cde
FF_S3	11.6	12.6 ± 0.0e	7.03 ± 0.01bcde	3.57 ± 0.01abc	3.36 ± 0.17a	0.32 ± 0.01efg	9.24 ± 0.22c	0.40 ± 0.06de	0.12 ± 0.18de
FF_D1	41.0	9.00 ± 0.10l	6.25 ± 0.03e	3.39 ± 0.03f	2.52 ± 0.03de	0.39 ± 0.00ab	6.66 ± 0.09h	0.21 ± 0.01h	0.08 ± 0.11e
FF_D2	26.3	10.8 ± 0.1i	6.57 ± 0.06bcde	3.53 ± 0.03cd	2.94 ± 0.06bc	0.34 ± 0.04bcdefg	7.95 ± 0.20efg	0.28 ± 0.03efgh	0.20 ± 0.14cde
FF_D3	11.6	12.6 ± 0.1e	7.86 ± 1.37a	3.62 ± 0.02a	3.25 ± 0.06ab	0.30 ± 0.02fg	9.07 ± 0.08cd	0.38 ± 0.03def	0.12 ± 0.17de
p value		<0.0001	0.12	<0.0001	0.087	0.0036	0.0004	0.039	0.95
Mature Fruit									
MF Control	n/a	15.5 ± 0.1a	7.02 ± 0.02bcde	3.52 ± 0.01cd	2.86 ± 0.02bcd	0.33 ± 0.03cdefg	11.6 ± 0.1a	0.75 ± 0.00a	0.90 ± 0.29a
MF_S1	47.2	10.6 ± 0.1j	6.57 ± 0.17bcde	3.50 ± 0.00de	2.28 ± 0.07e	0.38 ± 0.01abc	7.65 ± 0.12fg	0.33 ± 0.03defgh	0.39 ± 0.01bcd
MF_S2	34.0	12.0 ± 0.0f	6.66 ± 0.05bcde	3.49 ± 0.02de	2.47 ± 0.05de	0.35 ± 0.04bcdef	8.62 ± 0.20cde	0.36 ± 0.02defg	0.44 ± 0.01bc
MF_S3	10.2	14.5 ± 0.0b	7.31 ± 0.21ab	3.52 ± 0.02cd	2.76 ± 0.02cd	0.32 ± 0.0defg	10.5 ± 0.1b	0.61 ± 0.03b	0.58 ± 0.01b
MF_D1	47.2	9.60 ± 0.20k	6.29 ± 0.02de	3.31 ± 0.02g	2.17 ± 0.19e	0.30 ± 0.02g	7.84 ± 0.54efg	0.23 ± 0.04gh	0.35 ± 0.05bcde
MF_D2	34.0	11.7 ± 0.1g	6.52 ± 0.03cde	3.38 ± 0.00f	2.47 ± 0.24de	0.31 ± 0.05efg	9.12 ± 1.04c	0.36 ± 0.14defg	0.46 ± 0.08bc
MF_D3	10.2	14.4 ± 0.0c	7.04 ± 0.11bcd	3.46 ± 0.02e	2.78 ± 0.01cd	0.29 ± 0.01g	10.7 ± 0.2ab	0.55 ± 0.02bc	0.54 ± 0.00b
p value		<0.0001	<0.0001	<0.0001	0.0003	0.074	<0.0001	<0.0001	0.006

223 [a]Values are means of 3 replicates ± standard error. [b]Values in tartaric acid equivalents. [c]Values also correspond to total sugars as glucose was entirely

224 consumed. Values followed by different letters within columns are significantly different (p ≤ 0.05, one way ANOVA) across all treatments for a given

225 maturity stage (i.e., separately for FF and MF wines).

226   *3.3 Colour and tannin properties*

227   The perceived quality and value of red wines critically relates to the sensory attributes that

228   result from their colour and tannin properties (Mercurio, Dambergs, Cozzolino, Herderich, & Smith,

229   2010). Later harvest dates have been associated with higher colour intensities and tannin

230   concentrations (Bindon, Varela, Kennedy, Holt, & Herderich, 2013; Perez-Magarino & Gonzalez-

231   San Jose, 2006; Schelezki, Antalick, Šuklje, & Jeffery, 2018), but longer grape hang-times may be

232   associated with additional vineyard costs (i.e., irrigation) or added risk of berry shrivel. Ideally, the

233   implications on colour and tannin parameters following pre-fermentative water addition to manage

234   alcohol levels should be minimal to retain the wine style as determined by harvest date, so that taking

235   the risk of later harvest remains justifiable.

236   With a later harvest date (FF_Control to MF_Control wines), there were increases in wine

237   colour intensity, total anthocyanins, total phenolics, SO_2-resistant pigments, and tannin concentration

238   and MM, (Table 4), which was consistent with previous observations in the same vineyard (Schelezki,

239   Antalick, Šuklje, & Jeffery, 2018). Notably, despite being of comparable alcohol levels resulting from

240   comparable grape TSS concentrations, anthocyanin concentrations in the MF_Control (944 mg/L)

241   exceeded the level at commercial harvest reported in the study for the preceding vintage (590 mg/L),

242   which was characterised by significantly warmer growing conditions (Schelezki, Antalick, Šuklje, &

243   Jeffery, 2018). A study by Sadras and Moran (2012) suggested that anthocyanin - TSS ratios are

244   likely disrupted by elevated growing season temperatures, which delay the onset of anthocyanin

245   accumulation in the berry skin. Under the milder vintage conditions of the present study versus our

246   former one, the absence of such a disruption due to temperature could account for the higher

247   anthocyanin levels despite the similar grape TSS and wine alcohol levels of the two vintages. On the

248   other hand, lower wine tannin concentration was determined in the MF_Control wine (917 mg/L) in

249   comparison to the preceding vintage (1256 mg/L), albeit of comparable MM values (1640 g/mol).

250   The lower tannin concentration could be hereby attributable to the lower growing season temperature,

251   similarly as observed previously (Pastor del Rio & Kennedy, 2006).

252   The tannin concentration increment from the FF_Control to the MF_Control wine (Table 2)

253   was reflective of the increasing grape extractable tannins determined with the wine-like extraction

254   protocol, which was also the case previously (although less obvious) (Schelezki, Antalick, Šuklje, &

255   Jeffery, 2018). However, the observed difference in tannin concentration between the commercial

256   harvest (i.e., last harvest) wines of both vintages (yielding similar TSS and ABV levels) was not

257   represented by the wine-like assay (0.53 mg/g berry in MF_Control with 917 mg/L wine tannin (Table

258   2 and 4), but 0.39 mg/g berry in the preceding commercial harvest wine with 1256 mg/L wine tannin

259   (Schelezki, Antalick, Šuklje, & Jeffery, 2018))). This might be indicative of differences in tannin

260   extraction dynamics during winemaking as a function of the vintage conditions and grape ripening

261   phenomena, which is not mirrored by the wine-like extracts. In particular, it was previously shown

262   that grape skin cell walls become more porous with longer grape hang-times (Bindon, Bacic, &

263   Kennedy, 2012), which increases their affinity to bind tannins and remove them from the wine

264   solution (Bindon, Madani, Pendleton, Smith, & Kennedy, 2014). Further, differences in seed tannin

265   extractability between these vintages may have been present (Bautista-Ortin, Rodriguez-Rodriguez,

266   Gil-Munoz, Jimenez-Pascual, Busse-Valverde, Martinez-Cutillas, et al., 2012), despite the similar

267   TSS concentrations, given that harvest was significantly delayed in the present study compared to the

268   previous one (Schelezki, Antalick, Šuklje, & Jeffery, 2018). This could be indicative of the

269   decoupling between primary and secondary grape metabolites. Thus, an enhanced seed tannin

270   extractability may have resulted in final higher tannin concentrations in the 2016 commercial harvest.

271   Substituting 11.6% v/v of FF juice with water (FF_S3), affording a decrease of 1% ABV in

272   the wine compared to FF_Control, did not significantly affect wine colour parameters and phenolics

273   measures (Table 4). However, adding the same proportion of water without conducting saignée (i.e.,

274   juice run-off) provoked a lower level of total anthocyanins, total phenolics and stable pigments in the

275   respective wine (FF_D3), albeit without an impact on wine colour density (Table 4).

276   **Table 4** Colour and tannin parameters of wines resulting from Fresh Fruit (FF_Control, FF_S and

277   FF_D series) and Mature Fruit (MF_Control, MF_S and MF_D series) harvests.[a]

	Colour density [au]	Total anthocyanin [mg/L]	Total phenolics [au]	SO_2 resistant pigments [au]	Tannin MM [g/mol][c]	MCP tannin [mg/L][b]
Fresh Fruit						
FF Control	$8.69 \pm 0.17f$	$693 \pm 11f$	$42.8 \pm 0.8e$	$1.42 \pm 0.06f$	$1544 \pm 22bc$	$595 \pm 48cd$
FF_S1	$7.73 \pm 0.17h$	$598 \pm 12h$	$36.0 \pm 0.8h$	$1.16 \pm 0.02h$	$1489 \pm 4d$	$463 \pm 37de$
FF_S2	$8.21 \pm 0.35fgh$	$654 \pm 15g$	$39.4 \pm 0.8g$	$1.25 \pm 0.03g$	$1516 \pm 13bcd$	$399 \pm 23ef$
FF_S3	$8.91 \pm 0.17f$	$688 \pm 17f$	$41.9 \pm 0.7ef$	$1.39 \pm 0.04f$	$1534 \pm 18bc$	$651 \pm 1bc$
FF_D1	$5.80 \pm 0.13j$	$468 \pm 9j$	$27.8 \pm 0.9j$	$0.89 \pm 0.01j$	$1421 \pm 18e$	$231 \pm 17g$
FF_D2	$6.80 \pm 0.08i$	$540 \pm 20i$	$32.7 \pm 0.8i$	$1.08 \pm 0.01i$	$1477 \pm 31d$	$455 \pm 80e$
FF_D3	$8.58 \pm 0.12fg$	$647 \pm 12g$	$40.1 \pm 1.0fg$	$1.30 \pm 0.02g$	$1537 \pm 10bc$	$618 \pm 148bc$
p value	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0003
Mature Fruit						
MF Control	$15.4 \pm 0.5a$	$944 \pm 33a$	$61.0 \pm 2.0a$	$2.30 \pm 0.05a$	$1640 \pm 47a$	$917 \pm 61a$
MF_S1	$10.2 \pm 0.3e$	$727 \pm 10e$	$42.6 \pm 0.8e$	$1.52 \pm 0.03e$	$1509 \pm 10cd$	$680 \pm 62bc$
MF_S2	$11.7 \pm 0.1d$	$784 \pm 10d$	$47.0 \pm 0.7d$	$1.71 \pm 0.01d$	$1551 \pm 11b$	$734 \pm 62b$
MF_S3	$14.2 \pm 0.2b$	$905 \pm 19b$	$57.2 \pm 0.6b$	$2.16 \pm 0.03b$	$1639 \pm 18a$	$1011 \pm 67a$
MF_D1	$7.80 \pm 0.19gh$	$565 \pm 4i$	$33.5 \pm 0.3i$	$1.12 \pm 0.01hi$	$1492 \pm 3d$	$286 \pm 20fg$
MF_D2	$11.1 \pm 1.2d$	$673 \pm 18fg$	$42.0 \pm 0.7e$	$1.50 \pm 0.07e$	$1536 \pm 6bc$	$598 \pm 109bcd$
MF_D3	$12.8 \pm 0.3c$	$821 \pm 6c$	$50.4 \pm 0.5c$	$1.93 \pm 0.03c$	$1633 \pm 16a$	$951 \pm 43a$
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

278   [a]Values are means of 3 replicates ± standard error. [b]Determined by MCP tannin assay. [c] Molecular

279   mass determined by gel permeation chromatography at 50% elution. Values followed by different

280   letters within columns are significantly different ($p \leq 0.05$, one way ANOVA) across all treatments

281   for a given maturity stage (i.e., separately for FF and MF wines).

282   This divergence between the mode of water implementation becomes increasingly evident in

283   wines with lower established alcohol levels. The dilution treatments (FF_D1 & D2) resulted in

284   inferior colour parameters than in the respective substitution treatment (FF_S2 & S1) and FF Control

285   wines. At a dilution rate of 41.0% v/v, colour intensity, anthocyanin concentration, SO_2-resistant

286   pigments and total phenolics decreased in the order of 32–35%, whereas substitution with equivalent

287   volumes lowered these colour and phenolics parameters by only 11–18% (Table 4). Although less

288   pronounced, tannin concentrations followed a similar trend but a divergence only became significant

289   in the lowest alcohol wine (i.e., FF_D1/S1), where the must dilution with 41% v/v of water in FF_D1

290   resulted in 61% less tannin compared to the control, whereas the juice substitution counterpart FF_S1

291   did not significantly differ from the control (however, while not different to FF_S1, FF_S2 resulted

292   in a significantly lower tannin concentration level compared to the control, similar as in FF_D2).

293   The tannin results may indicate that at least up to a dilution level as applied in FF_D2 of

294   around 26% v/v, enough tannin was extractable and retained in the wine to establish an equilibrium

295   similar to the FF_S2 treatment that had a constant solid-liquid ratio (Bindon, Kassara, & Smith, 2017).

296   Additionally, tannin MM results appeared to be more sensitive to the dilution treatments, as a lower

297   MM was already noticeable by FF_D2 (11% ABV) compared to the control, whereas substituting

298   juice with the equivalent amount of water retained the tannin MM as defined by the FF harvest date

299   (i.e., as in FF Control). However, at the highest substitution rate in FF_S1, the tannin MM was

300   significantly lower in relation to FF_Control, and similar to the FF_D2 treatment (which also had the

301   same tannin concentration, Table 4). It could be possible that a proportion of the observed decline in

302   tannin concentration with water implementation was due to the loss of higher MM tannin, given a

303   higher binding affinity with wine matrix constituents (i.e. proteins or polysaccharides), or grape cell

304   walls with lower alcohol concentrations (McRae, Ziora, Kassara, Cooper, & Smith, 2015; Ruiz-

305   Garcia, Smith, & Bindon, 2014), but ultimately the cause or relevance to wine chemical and sensory

306   properties was unresolved.

307   Interestingly, among the water implementation treatments for the MF harvest, lower values

308   for colour density, anthocyanins, phenolics and SO_2-resistant pigments were already evident in wines

309   that were lower in alcohol by only 1% ABV, resulting from either dilution or substitution with 10.2%

310   v/v of water (Table 3 and 4). In terms of substitution, this finding accorded with the preceding study

311   (Schelezki, Antalick, Šuklje, & Jeffery, 2018), where a substitution rate of 12% v/v at comparable

312   grape TSS levels resulted in inferior colour parameters, and seems to confirm the implication of a

313   higher sensitivity of Shiraz wine colour to this alcohol management approach, at least in comparison

314   to Cabernet Sauvignon (Schelezki, Antalick, Šuklje, & Jeffery, 2018; Schelezki, Smith, Hranilovic,

315   Bindon, & Jeffery, 2018). These losses in colour measures were especially apparent when diluting

316   the MF musts (MF_D1-D3) to reach similar alcohol levels as in the MF substitution treatments

317   (MF_S1-S3) (Table 4). At the maximum dilution rate in MF_D1 (47.2% v/v), colour density,

318   anthocyanin concentration, SO_2-resistant pigments and total phenolics were lower by 40–51%

319   compared to the control, whereas the respective substitution treatments resulted in 23–34% lower

320   values (Table 4). Comparing these percentages versus the control to those presented for the FF wines,

321   it is evident that water implementation had a greater negative impact on wine colour density and

322   formation of stable pigments with fruit of higher maturity. Contrarily, Cabernet Sauvignon colour

323   parameters were found not to be affected by water substitution treatments (Schelezki, Smith,

324   Hranilovic, Bindon, & Jeffery, 2018), or were even enhanced under lower grape ripeness conditions

325   (Schelezki, Antalick, Šuklje, & Jeffery, 2018).

326   As observed among the FF wines, the lowest addition/substitution did not significantly change

327   the tannin concentration in the MF wines, but the higher rates significantly decreased the tannin levels

328   compared to the control (Table 4). This was also evident among the dilution treatments compared to

329   the respective substitution counterparts at similar alcohol levels, and the effect became significant

330   with the highest dilution/substitution rate (MF_D1/S1). The non-significant difference in MF_S2/D2

331   could again be indicative of an enhanced tannin extraction in the dilution treatment driven by the

332   lower solid-liquid ratio, as explained for the FF treatments. Similarly to the observations in the FF

333   wines, MF_D2 and MF_S1 wines were equal in tannin concentration and size.

334   *3.4 Implications for wine sensory quality*

335   Aside from the incorporation of additives, water implementation into the winemaking

336   process has generally been viewed with scepticism within the wine industry and among consumers,

337   mainly for preconceived associations with poorer wine quality and dilution of important constituents.

338   As elaborated in the current and previous studies, certain changes in wine chemical composition may

339   occur according to the cultivar, such as less favourable colour characteristics, decrease in tannin

340   concentration, and changes to volatile composition (Schelezki, Antalick, Šuklje, & Jeffery, 2018)).

341   However, it was also apparent that the overall impact on wine sensory profiles was not as stark as the

342   wine compositional modifications may have suggested, for Cabernet Sauvignon and Shiraz wines

343   arising from pre-fermentative substitution with water (Schelezki, Antalick, Šuklje, & Jeffery, 2018;

344   Schelezki, Suklje, Boss, & Jeffery, 2018).

345   That prior work was extended upon to examine whether a simple must dilution with water

346   was comparable to the juice substitution option. Sensory DA revealed a total of 17 significantly

347   different attributes, comprising 6 aroma, 9 flavour, and 2 mouthfeel terms (Table 5, Table S2 of the

348   Supporting Information). These attributes were assessed via PCA, with the first two principal

349   components presented in the bi-plot in Fig. 1 explaining almost 90% of the total variance. Separation

350   occurred mainly along F1, which accounted for 81.69% of the variation, with 'sweaty' aroma, and

351   'sweaty', 'green' and 'sour fruit' flavours being located opposite to the remaining attributes such as

352   'alcohol', and 'dark fruit' aromas, 'confectionery' aroma and flavour, and 'flavour intensity'. The

353   control wines made from Fresh Fruit (FF) and Mature Fruit (MF) harvests were separated according

354   to both F1 and, by a larger extend, F2. Following the additional twelve days of ripening after the FF

355   harvest date, the MF_Control wine differed in 8 out of 17 attributes compared to the FF_Control

356   wine, with higher ratings in 'aroma intensity', 'alcohol' aroma and flavour, 'dried fruit' and 'mixed

357   spice' flavour, as well as 'body' and 'astringency' (Fig.1, Table 5), whereas 'sour fruit' flavour

358   declined with the later harvest. This aroma evolution is exemplary for what is usually thought after

359   by winemakers and consumers (Zamora, 2016). The higher 'astringency' and 'body' perceptions

360   coincided with increments in tannin and glycerol concentrations (Tables 3 and 4), which could be

361   expected according to previous findings (Gawel, Sluyter, & Waters, 2007; Ma, Guo, Zhang, Wang,

362   Liu, & Li, 2014), whereas the diminishing perception of 'sour fruit' aroma (defined as under ripe

363   fruit, Table S1 of the Supporting Information) and flavour was reminiscent of decreasing 'green'

364   characteristics in the preceding study (Schelezki, Antalick, Šuklje, & Jeffery, 2018). No changes were

365   observed among the majority of sensory descriptors, however, including positive attributes such as

366   'confectionery', 'red fruit', or 'dark fruit'. Thus, even without intervention in the winery by way of

367 water addition, an acceptable wine style might well have been achievable at the FF harvest date in

368 this case, providing a wine with 13.6% ABV compared to the 15.5% ABV wine at the MF stage.
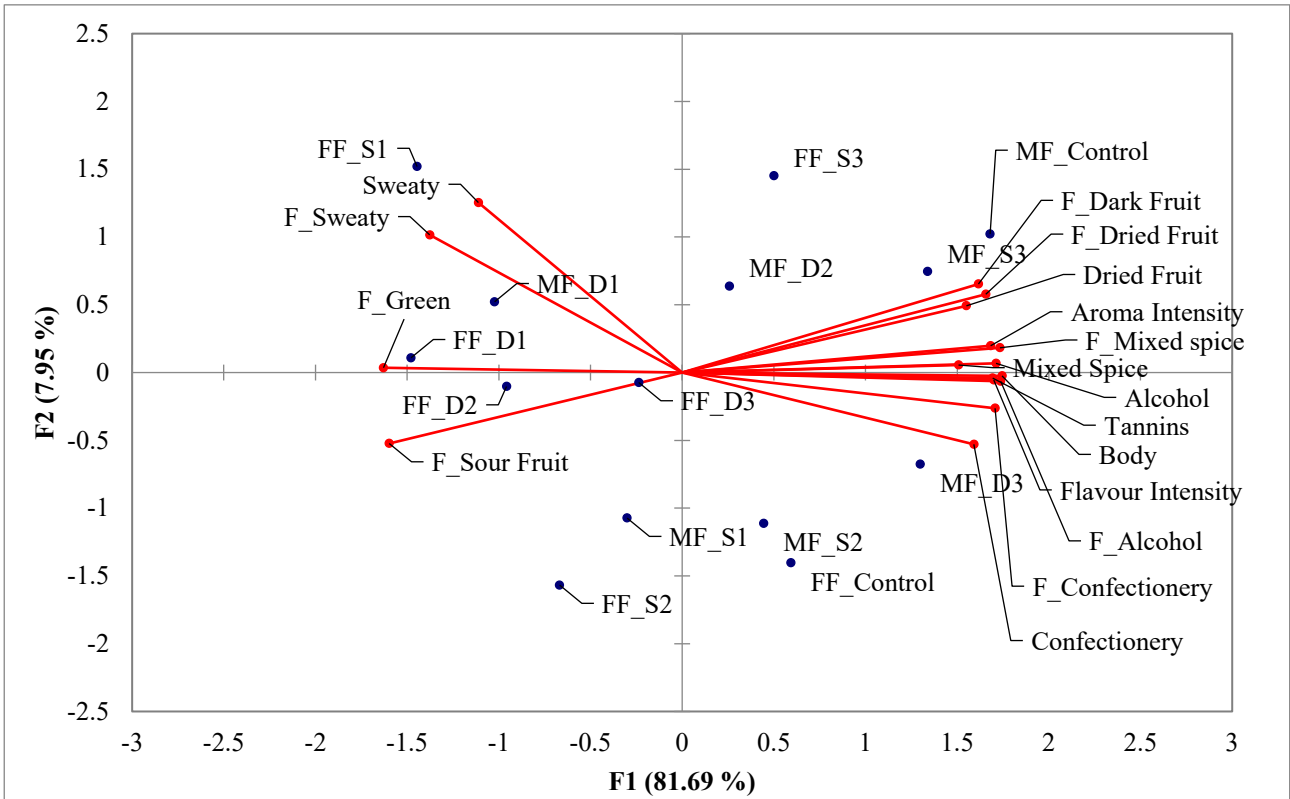
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370 **Fig. 1** PCA bi-plot of significantly different attributes resulting from the sensory DAs panel. Sample

371 codes are detailed in Fig. S1 of the Supporting Information. The 'F' prefix designates flavour

372 attributes.

373   **Table 5** Average scores for significantly different (p<0.05) wine sensory attributes for wines based on Fresh Fruit (FF) and Mature Fruit (MF)

374   harvests.[a]

	Fresh Fruit							Mature Fruit							p-value	pearson corr.[b]
	FF Control	FF_D1	FF_D2	FF_D3	FF_S1	FF_S2	FF_S3	MF Control	MF_D1	MF_D2	MF_D3	MF_S1	MF_S2	MF_S3		
% ABV	*13.6*	*9.0*	*10.8*	*12.6*	*9.6*	*11.1*	*12.6*	*15.5*	*9.6*	*11.7*	*14.4*	*10.6*	*12.0*	*14.5*		
Aroma																
Aroma intensity	58.1cd	52.5d	55.5cd	56.6cd	51.6d	55.8cd	60.6bcd	71.3a	52.5d	62.8abc	69.7ab	58.4cd	59.0cd	69.0ab	0.0001	0.653
Dried fruit	42.1abcde	26.2e	33.6cde	48.0abc	29.0de	29.3de	50.4abc	52.2ab	40.0bcde	47.9abc	52.5ab	30.0de	44.8abcd	58.2a	0.0010	0.683
Confectionery	49.2a	27.1cd	31.6bcd	37.7abc	20.5d	40.3abc	46.3ab	50.8a	28.5cd	40.0abc	46.9ab	40.8abc	42.6abc	42.5abc	0.0054	0.737
Mixed spice	45.2ab	22.8d	23.4d	38.6abc	27.1cd	29.3cd	35.3bcd	50.8a	30.0cd	34.9bcd	35.1bcd	37.7abc	33.3bcd	46.3ab	0.0006	0.699
Sweaty	26.7de	47.1abc	44.0abc	34.8bcde	57.0a	36.2bcde	50.2ab	36.7bcde	43.2abcd	40.2abcd	26.2de	36.1bcde	22.0e	32.3cde	0.0051	0.401
Alcohol	51.3bc	29.2f	35.0ef	36.1ef	30.0f	43.8cde	51.1bc	69.5a	29.8f	49.8bcd	65.0a	37.5def	44.4cde	61.6ab	<0.0001	0.766
Flavour																
Flavour intensity	65.1abc	35.3g	51.8e	52.3e	47.7ef	51.0ef	63.8bcd	75.3a	40.9fg	56.5cde	71.7ab	54.5de	63.9bcd	67.5ab	<0.0001	0.762
Sour fruit	48.3bcdef	57.3abc	66.7a	54.8abcd	63.4ab	67.0a	40.2defg	28.0g	53.5abcde	37.8efg	36.5fg	51.3abcdef	45.8cdef	29.0g	<0.0001	0.524
Dried fruit	33.8cde	20.1e	25.1de	31.6cde	21.5e	19.6e	45.3abc	58.5a	30.4cde	45.0abc	50.9ab	25.9de	38.8bcd	54.8a	<0.0001	0.635
Dark fruit	52.1abcde	35.8ef	39.7def	48.5bcdef	41.0def	33.0f	63.6ab	68.5a	45.9cdef	53.3abcd	60.8abc	40.9def	55.4abcd	66.0a	0.0001	0.670
Confectionery	47.4ab	16.8fg	26.3defg	29.7cdef	15.8g	32.6cde	40.4abc	49.8a	20.5efg	33.8cde	47.5a	34.2cde	34.0bcd	50.8a	<0.0001	0.800
Mixed spice	36.6bc	18.8e	21.5de	32.8cd	23.5de	21.9de	36.1bc	50.4a	22.4de	33.0cd	42.0abc	31.6cd	38.4abc	46.2ab	<0.0001	0.703
Sweaty	25.6bc	38.0ab	37.9ab	34.4ab	47.1a	31.4bc	35.1ab	29.9bc	36.2ab	31.0bc	19.6c	28.5bc	25.6bc	27.3bc	0.0477	0.457
Green	29.8bcde	44.9a	42.5ab	39.0ab	48.3a	38.3abc	23.3de	19.2e	34.5abcd	23.1de	18.6e	30.4bcde	23.0de	24.2cde	0.0001	0.484
Alcohol	62.8bc	23.8gh	35.3fg	47.5def	26.6gh	41.6ef	55.3cd	83.0a	19.8h	48.6de	74.5ab	36.3efg	57.3cd	72.9ab	<0.0001	0.840
Mouthfeel																
Body	53.3cd	20.4f	29.3ef	37.3e	25.3f	37.0e	50.8d	74.7a	21.8f	38.8e	63.3bc	38.3e	50.0d	64.3ab	<0.0001	0.791
Astringency	53.5bc	22.0g	38.3ef	43cde	30.5fg	40.9def	52.8bc	66.5a	20.0g	44.1cde	60.9ab	35.8ef	50.1bcd	60.6ab	<0.0001	0.846

375   [a]Values are means of 3 replicates. Values followed by different letters within a row are significantly different (p<0.05, one way ANOVA, post hoc

376   Fisher's LSD).[b]Pearson correlation of wine sensory attribute scores and water implementation rates, with bolded values representing significance at $\alpha =$

377   *0.05*

378   Substituting 11.6% v/v of FF juice with water to produce the FF_S3 wine at 12.6% ABV

379   resulted in a sensory profile that did not significantly differ from the 13.5% ABV FF Control except

380   for a higher rating in 'sweaty', but diluting the juice with an equal amount of water to derive FF_D3

381   significantly decreased the aroma and flavour perception of 'alcohol', flavour attributes 'intensity'

382   and 'confectionery', and 'body', compared to both the control and to FF_S3 (except for

383   'confectionery', Table 5). Interestingly, the perception of 'green' flavour in FF_D3 was significantly

384   enhanced in comparison to its substitution counterpart. Upon adjusting the alcohol level from 13.5%

385   ABV to around 11% ABV (FF_D2/S2) and further to 9.0% ABV (FF_D1/S1) (Tables 3 and 5), the

386   wines separated from the FF_Control wine and were generally characterised by more intense 'green'

387   flavour and 'sweaty' aroma characteristics, while decreasing in desirable attributes like 'dried fruit'

388   aroma, 'mixed spice' aroma and flavour, 'aroma' and 'flavour' intensities, and 'body' (Fig. 1 and

389   Table 5). In addition, 'dried fruit' and 'dark fruit' flavours, and 'astringency' decreased concurrently

390   with enhanced 'sour fruit' flavour, whereas the sensory profiles of FF_D2 and FF_S2 were similarly

391   perceived (except for a lower 'alcohol' aroma rating in FF_D2, Table 5).

392   The relative sensory similarity between water substitution and dilution treatments remained

393   evident especially at the highest rate as in FF_D1/S1 (Fig. 1), with the exception that the dilution

394   treatment resulted in significantly lower 'flavour intensity', whereas this attribute remained similar

395   from FF_S2 to FF_S1 (Table 5). The increased substitution rate from FF_S2 to FF_S1 corresponded

396   to lower 'confectionery' and 'alcohol' flavour, and 'body' and 'astringency' ratings, but fruity

397   characters like 'red fruit' or 'dark fruit' remained similar (Table S2 of the Supporting Information).

398   In contrast, a further dilution from FF_D2 to FF_D1 markedly lowered the 'flavour intensity'.

399   For the riper MF crop, the MF_S3 water substitution treatment affording 14.5% ABV wine

400   did not significantly differ in sensory quality compared to the MF Control wine (15.5% ABV), and

401   neither did dilution with water for MF_D3 (14.4% ABV), which contrasted with the observed decline

402   in parameters such as 'flavour intensity' in the FF equivalent (FF_D3) compared to the FF Control

403   wine (Table 5). With a decrease in alcohol by approximately 3.5% ABV in wines MF_D2 and MF_S2

404   (11.7% and 12.0% ABV, respectively), both treatments continued to have similar sensory profiles,

405   with the exception of increased 'sweaty' aroma and lower 'body' ratings when dilution was

406   employed, which, seemed to drive a separation along component F2 (Fig. 1), leaving MF_S2 closely

407   associated with the FF_Control wine. Elevated 'sweaty' aroma or flavour characteristics upon water

408   implementation appeared to be a reoccurring theme for the lower alcohol wine sensory profiles,

409   showing reasonable correlations with the water implementations ($r = 0.401$ for aroma, $r = 0.457$ for

410   flavour, Table 5). Notably, MF_D2 and MF_S2 wines diverged from the MF_Control due to lower

411   ratings for 'aroma intensity' (only significant for MF_S2), 'mixed spice' and 'alcohol' aroma and

412   flavour, 'flavour intensity', 'dried fruit' flavour, 'body' and 'astringency'.

413   Sensory profiles for the highest water implementation rate in MF_D1/S1 that afforded

414   wines with 9.6%/10.6% ABV largely remained similar to each other, except for lower perceived

415   'flavour intensity', 'alcohol' flavour, 'body' and 'astringency' in the MF_D1 wine, which might be

416   at least partially attributed to the alcohol concentration difference of 1% ABV between the treatments

417   (Table 5). Consequently, there was clear separation of both treatments along F1 and F2, positioning

418   MF_D1 closely to FF_D1 (similar % ABV), whereas MF_S1 was more like MF_S2 and FF_Control

419   (Fig. 1). Except for a lower 'alcohol' flavour perception in MF_S1, the remaining sensory attributes

420   were similar to MF_S2, revealing that the additional decrease of 1.4% ABV from MF_S2 to MF_S1

421   resulted in a marginal impact on sensory profile). It is somewhat remarkable that 'red fruit' and 'dark

422   fruit' perceptions did not change significantly when diluting the must with 47% v/v of water, and

423   ratings for 'aroma' and 'flavour' intensities seemed not to decline to an extent that this treatment

424   would suggest. The impending assessment of the wine volatile profiles of these wines should provide

425   better understanding of the compounds that might be responsible for providing an aroma foundation

426   that may have buffered against a more severe 'dilution' effect (Ferreira, 2007).

427   The highest extent of water substitution in the preceding study (Schelezki, Antalick, Šuklje,

428   & Jeffery, 2018) was 25% v/v for Shiraz grapes from the same vineyard of a similar sugar ripeness

429   to the present MF harvest. In line with that study, water substitution at 10.2% v/v in MF_S3 was

430 inconsequential to wine sensory properties, a finding that also applied to dilution giving MF_D3

431 (Tables 3 and 5). However, instituting water at 34% v/v as in MF_S2/D2 markedly changed the

432 sensory profiles compared to the control – again almost equally for substitution and dilution – except

433 for the notable difference in 'body' with dilution (showing strong correlations with decreasing levels

434 of % ABV, tannins, glycerol and total phenolics, with r = 0.96, 0.92, 0.91, 0.95, respectively) (Table

435 5). Although the evidence is limited, taken together it could be that a sweet spot exists between 25%

436 v/v and 34% v/v water implementation before a significant impact on wine sensory profile becomes

437 discernible. When considering the lower technological maturity of the FF treatments (i.e., lower grape

438 TSS and pH levels, higher grape malic acid concentrations and lower wine glycerol content), 26.3%

439 v/v juice substitution with water for FF_S2 was already eliciting lower ratings for important attributes

440 like 'flavour intensity' and 'dark fruit' flavour as well as 'body' and 'astringency', which perhaps

441 implies a water substitution sweet spot between 26.3% v/v and 11.6% v/v (FF_S3) for the less ripe

442 treatments. However, this does not hold true for the dilution series, as the same attributes were

443 significantly lower in FF_D3 with only 11.6% v/v water addition. Therefore, in a lower grape

444 maturity context, water implementation, and dilution in particular, seem to be less likely to maintain

445 a sensory profile as defined by harvest date as when applied with more mature grapes. In addition,

446 the sensory analysis also shows that 13 of 17 attributes were of similar ratings when comparing

447 MF_D3/S3 (lowest water addition level) with the earlier harvested FF_Control, which reinforces the

448 rather limited benefit of the longer grape hang-time of 12 days, particularly if the resulting crop is

449 meant to be alcohol adjusted. So despite the slightly lower intensities in 'body' and higher 'sour fruit'

450 perceptions, which are likely to elicit a shift in wine style, an earlier harvest may have been a more

451 sensible option to adjust the wine alcohol level (i.e., within the legal limit for water addition).

452 **4. Conclusions**

453   This study builds on preceding observations that pre-fermentative juice substitution with a

454 proportionate amount of water (within certain limits) appears to be a suitable approach to controlling

455 alcohol concentrations in red wines (two seasons each of Cabernet Sauvignon and Shiraz) while

456   maintaining wine chemical and sensory characteristics as defined by the harvest date. We furthered

457   the approach to the simple dilution of must with water, which is likely to be a preferred method given

458   the easier implementation (while accounting for potential volume losses through berry shrivel).

459   Furthermore, the implications of grape technological maturity were considered with the blending

460   treatments on Shiraz wine quality. At a lower grape maturity as in the FF treatments, juice substitution

461   of 11.6% v/v with water did not change colour properties in contrast to analogous treatment involving

462   dilution with water, which revealed declines in colour intensity and stability in line with total

463   phenolics and tannin concentrations. The impact of dilution was further mirrored in a decline in

464   important sensory attributes such as 'flavour intensity' and 'body' and the divergence between pre-

465   fermentative substitution and dilution became more obvious with lower established alcohol levels

466   (i.e., more water added). With riper grapes used for the MF treatments, substituting or diluting the

467   must with 10.2% v/v water decreased wine colour properties but substitution treatments appeared to

468   have a greater effect than with the less ripe fruit. Tannin concentration remained stable with low

469   substitution and dilution rates alike although notable declines were seen with the higher dilution rates.

470   Nonetheless, wine sensory qualities as determined by the harvest date were more or less maintained,

471   although with higher water implementation rates the decline in an array of attributes was noticeable.

472   Despite this, the difference between dilution and substitution was less pronounced compared to the

473   case for the grapes picked at a lower technological maturity. Ultimately, managing the alcohol level

474   based on the mature crop (within the legal limit) resulted in a sensory profile reminiscent of the wines

475   from the earlier harvest, negating presumed benefits of a longer hang-time if alcohol adjustment is

476   desired regardless. In this case, harvesting earlier could decompress the harvest process and reduce

477   winemaking input, i.e. post-harvest alcohol management.

478   Overall, the knowledge generated in this study contributes to the ability of winemakers to

479   make informed decisions about the most suitable way for pre-fermentative water addition according

480   to desired wine styles (while working within the relevant regulations). Analysis of the volatile

481   composition of the wines will follow and that may help explain the changes observed among the

482   sensory attributes in these Shiraz wines, and in particular could shed light on the rather benign sensory

483   effects of the extreme dilution treatments. In addition, this experimental design could be applied to

484   other cultivars (especially in warm climate viticultural regions), given the apparent dependence of

485   variety on the suitability of this approach, as observed across a number of studies.

486   **Abbreviations used**

487   ABV, alcohol by volume; DA, descriptive analysis; FF, Fresh Fruit; MF, Mature Fruit; MM GPC,

488   tannin molecular mass by gel permeation chromatography; TA, titratable acidity; TSS, total soluble

489   solids; PMS, potassium metabisulfite

490   **Conflict of interest**

491   The authors declare no competing financial interest.

492   **Acknowledgements**

501   **References**

502   Australian Government Bureau of Meteorology. (2018). Montly climate statistics, Noarlunga

503   Bautista-Ortin, A. B., Rodriguez-Rodriguez, P., Gil-Munoz, R., Jimenez-Pascual, E., Busse-

504   Valverde, N., Martinez-Cutillas, A., Lopez-Roca, J. M., & Gomez-Plaza, E. (2012).

505   Influence of berry ripeness on concentration, qualitative composition and extractability of

506   grape seed tannins. *Australian Journal of Grape and Wine Research, 18*(2), 123-130.

507   Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between

508   harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and

509   wine chemistry. *Food Chemistry, 138*(1), 1696-1705.

510   Bindon, K. A., Bacic, A., & Kennedy, J. A. (2012). Tissue-specific and developmental

511   modifications of grape cell walls influence the adsorption of proanthocyanidins. *Journal of*

512   *Agricultural and Food Chemistry, 60*(36), 9249-9260.

513   Bindon, K. A., Kassara, S., Cynkar, W. U., Robinson, E., M. C.,, Scrimgeour, N., & Smith, P. A.

514   (2014). Comparison of extraction protocols to determine differences in wine-extractable

515   tannin and anthocyanin in Vitis vinifera L. cv. Shiraz and Cabernet Sauvignon grapes.

516   *Journal of Agricultural and Food Chemistry, 62*(20), 4558-4570.

517   Bindon, K. A., Kassara, S., & Smith, P. A. (2017). Towards a model of grape tannin extraction

518   under wine-like conditions: the role of suspended mesocarp material and anthocyanin

519   concentration. *Australian Journal of Grape and Wine Research, 23*(1), 22-32.

520   Bindon, K. A., Madani, S. H., Pendleton, P., Smith, P. A., & Kennedy, J. A. (2014). Factors

521   affecting skin tannin extractability in ripening grapes. *Journal of Agricultural and Food*

522   *Chemistry, 62*(5), 1130-1141.

523   Deloire, A. (2013). Predicting harvest date using berry sugar accumulation. *PW Journal*(2 Spring

524   2013).

525   Ferreira, V. E., A.; Campo, E.; Cacho, J. (2007). The chemical foundations of wine aroma: A role

526   game aiming at wine quality, personality and varietal expression. In *Thirteenth Australian*

527   *Wine Industry Technical Conference, Adelaide, 2007*).

528   FSANZ. (2016). Food Standards Australia New Zealand, Application A1119 – Addition of water to

529   facilitate wine fermentation. *2018*.

530   Gawel, R., Sluyter, S. V., & Waters, E. J. (2007). The effects of ethanol and glycerol on the body

531   and other sensory characteristics of Riesling wines. *Australian Journal of Grape and Wine*

532   *Research, 13*(1), 38-45.

533   Jeffery, D. W., Mercurio, M. D., Herderich, M. J., Hayasaka, Y., & Smith, P. A. (2008). Rapid

534   isolation of red wine polymeric polyphenols by solid-phase extraction. *Journal of*

535   *Agricultural and Food Chemistry, 56*(8), 2571-2580.

536   Kassara, S., & Kennedy, J. A. (2011). Relationship between red wine grade and phenolics. 2.

537   Tannin composition and size. *Journal of Agricultural and Food Chemistry, 59*(15), 8409-

538   8412.

539   Kennedy, J. A., & Taylor, A. W. (2003). Analysis of proanthocyanidins by high-performance gel

540   permeation chromatography. *Journal of Chromatography A, 995*(1–2), 99-107.

541   Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes

542   harvested during cluster thinning as a method for reducing alcohol content and pH of wine.

543   *Australian Journal of Grape and Wine Research, 17*(2), 230-238.

544   Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food*. New York: Springer

545   Li, S., Bindon, K., Bastian, S. E., Jiranek, V., & Wilkinson, K. L. (2017). Use of winemaking

546   supplements to modify the composition and sensory properties of Shiraz wine. *J Agric Food*

547   *Chem, 65*(7), 1353-1364.

548   Longo, R., Blackman, J. W., Antalick, G., Torley, P. J., Rogiers, S. Y., & Schmidtke, L. M. (2018).

549   Volatile and sensory profiling of Shiraz wine in response to alcohol management:

550   comparison of harvest timing versus technological approaches. *Food Research*

551   *International, 109*, 561-571.

552   Ma, W., Guo, A. Q., Zhang, Y. L., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency and

553   bitterness perception of tannins in wine. *Trends in Food Science & Technology, 40*(1), 6-19.

554   McRae, J. M., Ziora, Z. M., Kassara, S., Cooper, M. A., & Smith, P. A. (2015). Ethanol

555   concentration influences the mechanisms of wine tannin interactions with poly(L-proline) in

556   model wine. *Journal of Agricultural and Food Chemistry, 63*(17), 4345-4352.

557   Mercurio, M. D., Dambergs, R. G., Cozzolino, D., Herderich, M. J., & Smith, P. A. (2010).

558   Relationship between red wine grades and phenolics. 1. Tannin and total phenolics

559   concentrations. *Journal of Agricultural and Food Chemistry, 58*(23), 12313-12319.

560   Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007). High throughput

561   analysis of red wine and grape phenolics - Adaptation and validation of methyl cellulose

562   precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format.

563   *Journal of Agricultural and Food Chemistry, 55*(12), 4651-4657.

564   Pastor del Rio, J. L., & Kennedy, J. A. (2006). Development of proanthocyanidins in *Vitis vinifera*

565   L. cv. Pinot Noir grapes and extraction into wine. *American Journal of Enology and*

566   *Viticulture, 57*(2), 125-132.

567   Perez-Magarino, S., & Gonzalez-San Jose, M. L. (2006). Polyphenols and colour variability of red

568   wines made from grapes harvested at different ripeness grade. *Food Chemistry, 96*(2), 197-

569   208.

570   Ruiz-Garcia, Y., Smith, P. A., & Bindon, K. A. (2014). Selective extraction of polysaccharide

571   affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydr. Polym., 114*,

572   102-114.

573   Sadras, V. O., & Moran, M. A. (2012). Elevated temperature decouples anthocyanins and sugars in

574   berries of Shiraz and Cabernet Franc. *Australian Journal of Grape and Wine Research,*

575   *18*(2), 115-122.

576   Schelezki, O. J., Antalick, G., Šuklje, K., & Jeffery, D. W. (2018). Thesis Chapter 4: Pre-

577   fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and

578   Shiraz: Implications for wine quality based on chemical and sensory analysis.

579   Schelezki, O. J., Smith, P. A., Hranilovic, A., Bindon, K. A., & Jeffery, D. W. (2018). Comparison

580   of consecutive harvests versus blending treatments to produce lower alcohol wines from

581   Cabernet Sauvignon grapes: impact on polysaccharide and tannin content and composition.

582   *Food Chemistry, 244*, 50-59.

583   Schelezki, O. J., Suklje, K., Boss, P. K., & Jeffery, D. W. (2018). Comparison of consecutive

584   harvests versus blending treatments to produce lower alcohol wines from Cabernet

585   Sauvignon grapes: Impact on wine volatile composition and sensory properties. *Food*

586   *Chemistry*.

587   Sherman, E., Greenwood, D. R., Villas-Boâs, S. G., Heymann, H., & Harbertson, J. F. (2017).

588   Impact of grape maturity and ethanol concentration on sensory properties of Washington

589   State Merlot wines. *American Journal of Enology and Viticulture, 68*(3), 344-356.

590   Suklje, K., Zhang, X., Antalick, G., Clark, A. C., Deloire, A., & Schmidtke, L. M. (2016). Berry

591   shriveling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical

592   composition. *Journal of Agricultural and Food Chemistry, 64*(4), 870-880.

593   Tonietto, J., & Carbonneau, A. (2004). A multicriteria climatic classification system for grape-

594   growing regions worldwide. *Agriculture and Forest Meteorology., 124*(1–2), 81-97.

595   Triolo, R., Roby, J. P., Plaia, A., Hilbert, G., Buscemi, S., Di Lorenzo, R., & van Leeuwen, C.

596   (2017). Hierarchy of factors impacting grape berry mass. Separation of direct and indirect

597   effects on major berry metabolites. *American Journal of Enology and Viticulture*.

598   Waterhouse, A. L. S., Gavin L.; Jeffery, David W. (2016). Acids. In *Understanding Wine*

599   *Chemistry* 1 ed.), 19-33: Wiley.

600   Wine Australia. (2017). National vintage report, https://www.wineaustralia.com/getmedia/12acee4d-

601   d39f-475c-9fac-bff5674f2f28/NationalVintageReport_2017_withappendix, assessed 2018.

602   Yanniotis, S., Kotseridis, G., Orfanidou, A., & Petraki, A. (2007). Effect of ethanol, dry extract and

603   glycerol on the viscosity of wine. *Journal of Food Engineering, 81*(2), 399-403.

604   Zamora, F. (2016). Dealcoholised wines and low-alcohol wines . In M. V. S. Moreno-Arribas,

605   Begona Bartolome (Ed.), *Wine safety, consumer preference, and human health*). Madrid:

606   Springer.

SUPPORTING INFORMATION FOR

Substitution or dilution? Assessing pre-fermentative water implementation to produce lower alcohol Shiraz wines.

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Table of Contents

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Table S1 Attribute list developed by the descriptive analysis panel to describe and rate the wine sensory profiles, showing respective definitions and aroma/flavour reference standards.

| Descriptor	Type	Definition	Standard mixed in 30 mL of Shiraz cask wine (Yalumba, 2017)
Dark fruit	Aroma/Flavour	Fresh blackcurrants, mulberry, blueberry	2 pieces of crushed blackberry (Sunnyside); 4 fresh blueberries, cut; 7 mL blackcurrant cordial (Bickford's), 7 mL blackcurrant juice (Ribena)
Red fruit	Aroma/Flavour	Fresh raspberry, strawberry, red currents	1 cm cube of fresh strawberry and 1 cm cube of fresh raspberry
Confectionery	Aroma/Flavour	Sweet lolly	1 strawberry and cream lolly, cut (5 g) (Allens)
Sour fruit	Aroma/Flavour	Under ripe fruit, fresh tart plum; sour cherries	2 cm fresh plum, cut, 4 crushed pitted sour cherries and 6 mL of cherry juice (Takoland)
Dried fruit	Aroma/Flavour	Over ripe fruit or cooked fruit, plum jam, prune, raisins	1 tablespoon plum jam (Cottee's), 7 dried sultanas, cut (Nature Delight), 1 pitted prune, crushed (Nature Delight)
Mixed spice	Aroma/Flavour	Sweet spice, including cinnamon, nutmeg, cloves and liquorice	¼ teaspoon of ground allspice (Coles) + 1 piece liquorice, cut (Marco Polo)
Savoury	Aroma/Flavour	Salty; soy sauce; medicinal	5 mL of tamari oyster sauce (Chang's)
Green	Aroma/Flavour	Stalky, grassy, granny smith apple peel	2 cm fresh grapevine stalks and ½ grape vine leaf, cut
Sweaty	Aroma/Flavour	Sweat horse, dirty, dusty, earthy, 'bretty' (odour description associated with *Brettanomyces bruxellensis*)	5 mL of Merlot wine inoculated with *Brettanomyces bruxellensis* (AWRI 1499)
Alcohol	Aroma/Flavour	Level of alcohol perceived	3 mL of 70% ethanol
Acidity	Mouthfeel	Level of acid perceived	Tartaric acid (low, 0.5 g/L; high, 2 g/L)
Bitterness	Mouthfeel	Perception of bitterness	quinine sulfate (low, 5 mg/L; high, 20 mg/L)
Astringency	Mouthfeel	Perception of drying or puckering sensation	Felt material (low), sandpaper (high)
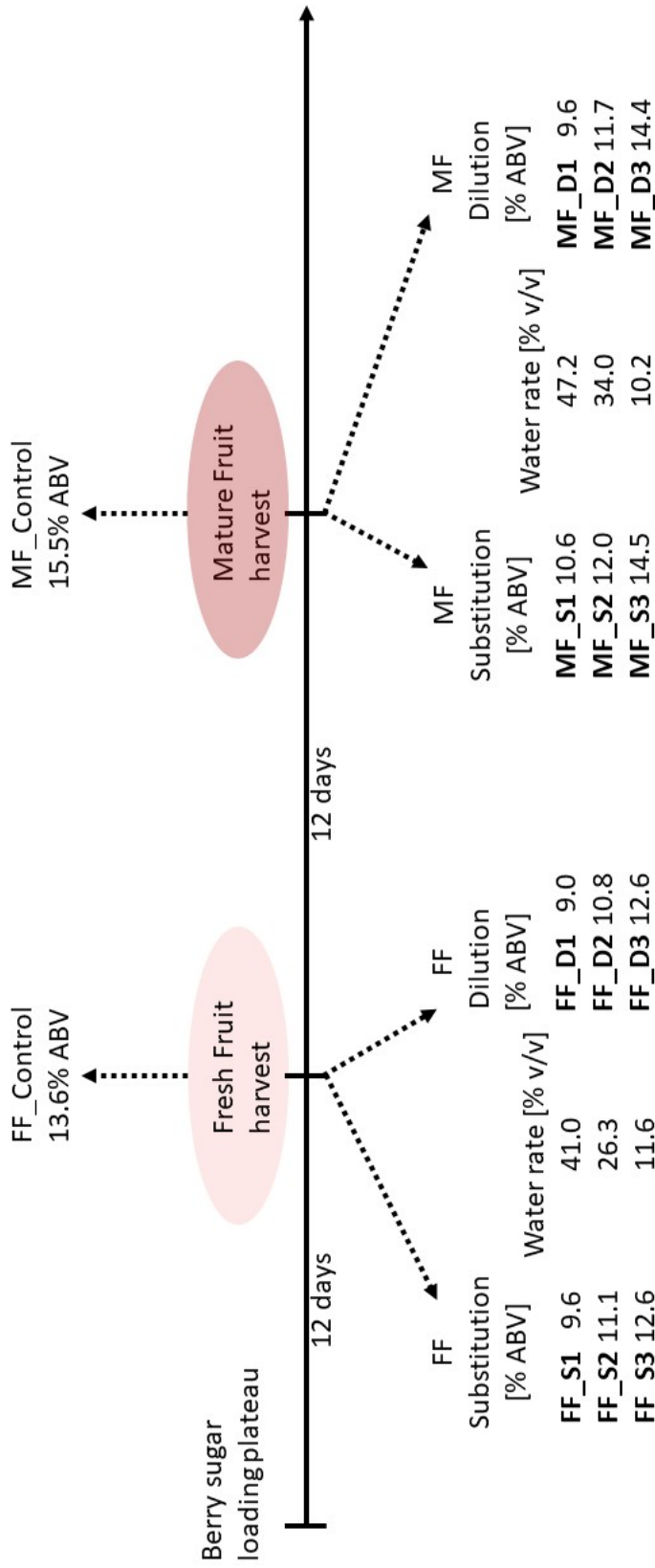
Fig. S1 Flowchart presenting the main wine production steps and resulting treatment definitions, water implementation rates and final wine alcohol levels.

Table S2 All attributes that were rated by the DA panel including insignificant attributes (p > 0.05), that were excluded from Table 5 of the manuscript).[a]

	Fresh Fruit							Mature Fruit								
	FF Control	FF_D1	FF_D2	FF_D3	FF_S1	FF_S2	FF_S3	MF Control	MF_D1	MF_D2	MF_D3	MF_S1	MF_S2	MF_S3	p-value[b]	LSD
%ABV	13.6	9	10.8	12.6	9.6	11.1	12.6	15.5	9.6	11.7	14.4	10.6	12	14.5		
Aroma																
Aroma intensity	58.1cd	52.5d	55.5cd	56.6cd	51.6d	55.8cd	60.6bcd	71.3a	52.5d	62.8abc	69.7ab	58.4cd	59cd	69ab	0.0001	9.5
Sour fruit	**42.0**	**45.3**	**47.3**	**32.5**	**46.4**	**53.5**	**32.1**	**31.1**	**38.0**	**33.1**	**30.5**	**46.7**	**36.1**	**32.3**	**0.0980**	**16.9**
Dried fruit	42.1abcde	26.2e	33.6cde	48abc	29de	29.3de	50.4abc	52.2ab	40bcde	47.9abc	52.5ab	30de	44.8abcd	58.2a	0.0010	16.9
Red fruit	**48.8**	**38.8**	**41.1**	**45.5**	**28.2**	**52.7**	**35.8**	**44.1**	**37.9**	**41.5**	**37.7**	**44.2**	**39.8**	**40.c**	**0.3297**	**15.5**
Dark fruit	**43.9**	**37.1**	**41.5**	**48.8**	**39.3**	**36.1**	**52.5**	**60.5**	**51.0**	**49.4**	**62.4**	**50**	**50**	**54.1**	**0.0660**	**17.1**
Confectionery	49.2a	27.1cd	31.6bcd	37.7abc	20.5d	40.3abc	46.3ab	50.8a	28.5cd	40abc	46.9ab	40.8abc	42.6abc	42.5abc	0.0054	15.8
Mixed spice	45.2ab	22.8d	23.4d	38.6abc	27.1cd	29.3cd	35.3bcd	50.8a	30cd	34.9bcd	35.1abc	37.7abc	33.3bcd	46.3ab	0.0006	13.2
Savoury	**29.5**	**43.3**	**36.8**	**34.5**	**41.4**	**33.8**	**32.6**	**39.1**	**33.2**	**36.5**	**29.4**	**32.4**	**26.8**	**35.9**	**0.7854**	**15.8**
Green	**62.5**	**50.3**	**59.3**	**66.7**	**61.6**	**57.4**	**54.8**	**55.2**	**46.5**	**48.8**	**54.1**	**38**	**51**	**50.7**	**0.0585**	**17.2**
Sweaty	26.7de	47.1abc	44abc	34.8bcde	57a	36.2bcde	50.2ab	36.7bcde	43.2abcd	40.2abcd	26.2de	36.1bcde	22e	32.3cde	0.0051	12.8
Alcohol	51.3bc	29.2f	35ef	36.1ef	30f	43.8cde	51.1bc	69.5a	29.8f	49.8bcd	65a	37.5def	44.4cde	61.6ab	<0.0001	13.3
Flavour																
Flavour intensity	65.1abc	35.3g	51.8e	52.3e	47.7ef	51ef	63.8bcd	75.3a	40.9fg	56.5cde	71.7ab	54.5de	63.9bcd	67.5ab	<0.0001	10.6
Sour fruit	48.3bcdef	57.3abc	66.7a	54.8abcd	63.4ab	67a	40.2defg	28g	53.5abcde	37.8efg	36.5fg	51.3abcdef	45.8cdef	29g	<0.0001	15.7
Dried fruit	33.8cde	20.1e	25.1de	31.6cde	21.5e	19.6e	45.3abc	58.5a	30.4cde	45abc	50.9ab	25.9de	38.8bcd	54.8a	<0.0001	15.5
Red fruit	**46.0**	**35.5**	**39.5**	**43.5**	**25.3**	**53.1**	**32.4**	**34.1**	**30.0**	**38.2**	**39.0**	**39.5**	**40.5**	**37.3**	**0.1105**	**15.4**
Dark fruit	52.1abcde	35.8ef	39.7def	48.5bcdef	41def	33f	63.6ab	68.5a	45.9cdef	53.3abcd	60.8abc	40.9def	55.4abcd	66a	0.0001	16.6
Confectionery	47.4ab	16.8fg	26.3defg	29.7cdef	15.8g	32.6cde	40.4abc	49.8a	20.5efg	33.8cde	47.5a	34.2cde	34bcd	50.8a	<0.0001	13.4
Mixed spice	36.6bc	18.8e	21.5de	32.8cd	23.5de	21.9de	36.1bc	50.4a	22.4de	33cd	42abc	31.6cd	38.4abc	46.2ab	<0.0001	12.4
Savoury	**28.9**	**32.7**	**35.7**	**27.3**	**33.4**	**29.7**	**42.1**	**34.6**	**30.3**	**33.6**	**30.1**	**25.7**	**30.7**	**35.7**	**0.7806**	**14.2**
Sweaty	25.6bc	38ab	37.9ab	34.4ab	47.1a	31.4bc	35.1ab	29.9bc	36.2ab	31bc	19.6c	28.5bc	25.6bc	27.3bc	0.0477	14.1
Green	29.8bcde	44.9a	42.5ab	39ab	48.3a	38.3abc	23.3de	19.2e	34.5abcd	23.1de	18.6e	30.4bcde	23de	24.2cde	0.0001	14.2
Alcohol	62.8bc	23.8gh	35.3fg	47.5def	26.6gh	41.6ef	55.3cd	83a	19.8h	48.6de	74.5ab	36.3efg	57.3cd	72.9ab	<0.0001	12.6
Mouthfeel																
Acidity	**62.5**	**50.3**	**59.3**	**66.7**	**61.6**	**57.4**	**54.8**	**55.2**	**46.5**	**48.8**	**54.1**	**38.0**	**51**	**50.7**	**0.1387**	**17.0**
Body	53.3cd	20.4f	29.3ef	37.3e	25.3f	37e	50.8d	74.7a	21.8f	38.8e	63.3bc	38.3e	50d	64.3ab	<0.0001	10.8
Astringency	53.5bc	22g	38.3ef	43cde	30.5fg	40.9def	52.8bc	66.5a	20g	44.1cde	60.9ab	35.8ef	50.1bcd	60.6ab	<0.0001	11.3

[a]Values are means of 3 replicates. Values followed by different letters within a row are significantly different (p<0.05, one way ANOVA[b], post hoc Fisher's LSD).

Chapter 6

Concluding remarks and future directions

More than two centuries have passed since Frenchman Jean-Antoine Chaptal popularised the pre-fermentative addition of sugar to augment wine alcohol levels and develop more palatable aromas and flavours of then notoriously under-ripe grapes. This technique, since then referred to as 'chaptalisation', revolutionised winemaking and helped sustain viticulture in rather unsuitable growing conditions of northern Europe, and diffused from there to other parts of the world.

Two centuries later, wine alcohol levels have been rising, particular so in warm climate viticulture regions. On the one hand, this has been attributed to winemakers pursuing ripe fruit characteristics by delaying grape harvest times (prolonging ripening) to meet the consumer demand of fuller flavoured wines. On the other hand, concomitant increasing average temperatures or extreme weather events, such as heatwaves and drought periods, have led to advanced vine phenology and higher grape total soluble solids (TSS) levels at harvest. The combination of these two trends has been playing a major role in exacerbating so-called compressed vintage situations, hindering the harvest of grapes at desired quality levels while increasing the likelihood of berry shrivel and excessive wine ethanol concentrations. Trending against this development has been an increasing health awareness among consumers and a consequent preference realignment towards lower alcohol wines. In addition, substantial tax penalties may be imposed in some markets for higher wine alcohol concentrations, overall creating significant pressure on the marketability of such wines (e.g., >14 % alcohol by volume, ABV).

Given that compressed vintages are of rather unpredictable nature, and do not necessarily occur consistently across vintages, winemakers seek easy to apply, flexible solutions to mitigate the implications of grape over-ripeness, ideally involving pre-fermentative applications (as opposed to post-fermentation approaches involving physical alcohol removal, for example) to minimise the loss of wine quality and style. Previous studies indicated that one such approach could be the pre-fermentative substitution of juice

with either a low alcohol wine or water to dilute must sugar concentrations, hereby maintaining a constant liquid-to-solid ratio during red winemaking. However, no comprehensive assessment of the suitability to produce high quality lower alcohol wines was available to help winemakers make informed decisions, despite the need of the wine industry and the easing of regulations, which permit the pre-fermentative water in the USA, and more recently, in Australia.

As mentioned, prolonging the grape ripening time to yield fuller wine styles is one of the factors driving higher alcohol levels. However, a few studies have cast doubt on the benefit of this preconceived practice in terms of consumer preference. The quest for riper grapes may not necessarily be justified given the risk of compressed vintages, and an advanced harvest date could be a valid strategy to mitigate increasing grape TSS and controlling wine alcohol levels. It was therefore considered essential to assess lower alcohol wines resulting from pre-fermentative alcohol management in the context of wines resulting from earlier harvest dates, thus facilitating an evaluation of resulting wine styles and qualities and informing the most appropriate approach. Further, it was important to account for year-to-year differences in grape ripening conditions, so that three distinct vintages were included in this project, and the alcohol management strategies were tested on Cabernet Sauvignon and Shiraz, which are the most widely grown red cultivars in Australia.

6.1 Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition.

Extreme weather conditions (i.e., above average temperatures, heatwaves and below average rainfall) that prevailed during the 2015 vintage of this project led to an exemplary compression of grape ripening at harvest. This provided a context suitable for our aim to investigate the alcohol management strategies under conditions that winemakers may face more frequently in future. In only four days, severe berry shrivel occurred and

dramatically increased the potential alcohol of the Cabernet Sauvignon grapes, and thus the final wine alcohol levels from 15.1% ABV to 18.2% ABV, in line with significant yield losses. With respect to important wine quality measures, tannin per gram of berry increased, and became more extractable with later harvest dates, which was particularly exacerbated by the berry shrivel. This translated into higher wine tannin concentrations with later harvests, in line with enhanced colour measures, particularly with the last, overripe harvest point. We concluded, however, that the wines resulting from the earlier harvest dates were not necessarily lacking in any of those important red wine quality parameters, which is an important consideration given the negative implications of berry shrivel on the wine sensory profiles, as discussed below under section 6.2.

Employing pre-fermentative juice substitution with water or GHW (final wine alcohol levels ranging between 14.5% and 17% ABV) to mitigate the excessive alcohol levels of the overripe crop did not negatively affect important wine colour parameters, such as total anthocyanin concentrations, colour intensity, SO_2-resistant pigments (except in the case of water) or total phenolics, even with the highest implementation rates that decreased the alcohol level by 4% ABV compared to the control. An initially-observed lower content of SO_2-resistant pigments with the highest GHW implementation had vanished after 18 months of bottle ageing. Additionally, wines resulting from the pre-fermentative water addition treatments did not differ in tannin concentration or composition in comparison to the control, but were observed to retain tannins of higher molecular mass with higher substitution rates (and lower alcohol levels) than the respective GHW counterparts. A differentiation between the two blending components was particularly evident with changes in polysaccharide concentrations, which increased rather linearly with higher rates of water usage but were equally higher among the GHW treatments regardless of the substitution rate. These changes were particular ascribed to grape-derived polysaccharides and we suggested that a modified diffusion coefficient following the juice replacement could have provoked

the additional extraction. Water particularly benefited the retention of galacturonans and polysaccharides rich in arabinose and galactose (PRAGs), which indicated a compositional differentiation of wine polysaccharides as a function of the blending component.

The entirety of these observations have potentially allayed concerns that such pre-fermentative juice matrix manipulations could jeopardise the non-volatile wine quality, at least in this vintage context. In fact, the alcohol management approaches generally preserved the non-volatile quality parameters as determined by the last harvest date, such that these lower alcohol wines were superior in colour and tannin characteristics compared to wines of similar alcohol levels arising from earlier harvest dates. However, given that the last (commercial) harvest date came at the cost of a substantial yield loss due to berry shrivel, the relative merits of enhanced non-volatile quality parameters and the resulting wine style required a critical evaluation. In fact, the wine volatile and sensory assessment (summarised in section 6.2) strongly supported the prevention of grape over-ripeness in the first place, favouring an earlier harvest.

6.2 Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties.

In a continuation of the assessment of 2015 trial wines, comprehensive volatile and sensory analyses were undertaken as we aimed for a more complete understanding of the qualitative changes to expect when decreasing alcohol levels in the context of berry shrivel via pre-fermentative blending approaches. Further, the analyses facilitated the direct comparison of those wines substituted with water or GHW with the lower alcohol wines resulting from earlier harvested grapes.

Volatiles associated with 'vegetal' or 'green' characteristics, such as C_6 alcohols and 3-isobutyl-2-methoxypyrazines (IBMP) decreased in wines made from more mature

fruit, in line with lower perceived intensities in 'green' sensory characteristics. On the other hand, fermentative volatiles did not show a particular pattern across the earlier harvest dates, although significant declines of various higher alcohols and esters were concomitant with the berry shrivel at commercial harvest. Important aroma and flavour attributes like 'aroma intensity' and 'dark fruit', among others, were not further enhanced past the second harvest point (13.4% ABV), i.e., thirteen days before the commercial harvest date, with the latter yielding a wine alcohol content of more than 18% ABV. The berry shrivel occurring at commercial harvest remarkably altered the sensory profile, intensifying 'hotness' and 'port wine' characteristics in line with increased 'sweetness' sensations, which might not be appreciated and may negatively impact the 'wine balance'.

In line with the benign nature of the alcohol management approach on non-volatile constituents, the wine volatile composition was only marginally affected, especially with water as the blending component. The differences appeared to mainly originate from yeast metabolic changes, which for instance elicited increased concentrations of higher alcohols and ethyl esters of fatty acids with higher water implementation rates. Although a similar response was evident with the GHW treatments (with differences in individual compounds likely due to a difference in precursor matrix), a differentiation between the two blending components became evident particularly with increased grape-derived volatiles. Isoprenoids and C_6 alcohols tended to increase with higher GHW proportions, and albeit not exceeding the individual detection thresholds, we suggested that the charcoal treatment applied to the GHW wine to limit any negative impact that could be associated with such low grape maturity did not entirely remove these grape-derived volatiles or their precursors. Regarding IBMP, known to markedly shape the 'vegetal' perceptions in Cabernet Sauvignon wines, the implementation of GHW did not mediate any changes, and concentrations remained similar to the control. Indeed, 'green' sensory characteristics did not differ according to the blending component chosen. Further, it appeared that the elevated fatty acid concentrations in the

GHW matrix directly translated into increments of those compounds in the treated wines. In addition, the general trend of increased fatty acid levels, observed in both the water and GHW treatments, was suggested to be due to a depletion of sterols or unsaturated fatty acids with the juice removal.

Compared to the blending treatments, the choice of harvest date had a more significant impact not only on the wine volatile composition but also on the wine sensory profiles. When aiming to adjust wine alcohol levels post-harvest, the more benign nature of the blending approach could be seen quite positively given the substantial pre-fermentative juice substitution (up to 4% ABV lower alcohol concentration) necessary to elicit lower perceptions of 'flavour intensity', 'dark fruit', 'sweet spice' or 'chocolate' flavours, which are desirable sensory attributes. Juice substitution with water appeared to be the most suitable approach, given the lower changes to wine sensory characteristics that were seemingly dictated by harvest date. However, this also applied to attributes that might not be so favourable, such as 'port wine' and 'hotness', which prevailed even with pre-fermentative substitutions yielding wines of similar alcohol levels to those arising from an earlier harvest. This provides good insight into the pre-fermentative alcohol management strategy, which may not mitigate undesirable wine styles that follow from a suboptimal harvest decision (i.e., trying to recover from harvesting overripe fruit). Given these results, the importance of informed harvest decisions remained clear, and an earlier harvest would have resulted in a lower alcohol wine with more favourable sensory characteristics while avoiding severe yield loss due to berry shrivel in the case of the 2015 vintage. Nonetheless, this vintage also created curiosity as to whether (and to which extent) wine alcohol levels could be adjusted under vintage conditions that produce more favourable fruit characteristics, without the impact of berry shrivel or over-ripeness.

6.3 Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis.

Berry shrivel and excessive grape sugar levels are not specific requisites for winemakers to consider managing wine alcohol concentrations, and initial potential alcohol levels may be well below >18% ABV that was experienced in 2015. The pre-fermentative strategy could be applied to different grape ripeness grades (while staying within the bounds of relevant regulations), which could change extraction behaviour of important non-volatile or volatile compounds, such that different outcomes on wine chemistry and sensory properties could be expected under altered vintage conditions. This further leads to a different perspective for advanced harvest dates as an alternative to produce lower alcohol wines, which might be less explicitly recommended in contrast to the case during the 2015 vintage trial.

These considerations therefore related to our aims for the subsequent winemaking trials, which built upon our previous experimental design. Studies during the 2016 vintage were conducted under more benign weather conditions that prevented the excessive occurrence of berry shrivel, and the commercial harvests took place at grape TSS concentrations that would not necessarily require intervention. In addition to further exploring the pre-fermentative juice substitution with GHW and water (in particular) as a suitable post-harvest alcohol management option for Cabernet Sauvignon in a different vintage context, we extended this approach to Shiraz, because of its significance as the most widely grown cultivar in Australia, and its general importance in other viticultural regions that might share similar climatic conditions. Given the close proximity of the vineyards for these two varietals and the identical winemaking conditions, a direct assessment of cultivar-specific implications on wine quality was possible.

Alcohol levels of the wines resulting from the commercial harvest dates were around 15.5% ABV for both varieties, and were adjusted with our substitution strategy to a minimum of 11.5% ABV, while also encompassing the legal limit of must dilution to 13.5 °Baumé (i.e., potential alcohol of 13.5% ABV) according to recent changes in Australian wine regulations that permit the pre-fermentative water addition to a must of 15 °Baumé or more. Regarding Cabernet Sauvignon, the wine non-volatile analysis confirmed the previous observations, showing the absence of negative implications on wine colour quality, total phenolics or tannin concentrations compared to the control. The use of water appeared to retain higher tannin concentrations compared to the GHW counterparts at similar alcohol levels. In this less extreme vintage context, however, wine volatile profiles were markedly modified in the lower alcohol wines compared to the control. The implementation of water resulted in general concentration declines of an array of volatile constituents, both grape-derived and fermentative alike, but were particularly pronounced in ethyl esters and higher alcohol acetates. Although a decline in those compound groups was also evident when using GHW, increments of grape-derived constituents, i.e., 1-hexanol and isoprenoids, were apparent and particularly differentiated this treatment series from the water-substituted Cabernet Sauvignon wines.

In accordance with the preceding study, changes in the wine sensory profiles were mostly noticeable among the flavour attributes, and the water treatments in particular preserved 'dark fruit' aroma even with the highest substitution rate. This further applied to the perceptions of 'flavour intensity', 'red fruit' and 'dark fruit' flavour, which remained unchanged relative to the control, whereas high levels of GHW implementation lowered the ratings of these important attributes to levels comparable to a much earlier harvest date. However, the majority of wine sensory attributes were rated similarly between both treatment series at low or intermediate addition rates, so that from a sensory point of view,

both blending components would be valid choices, in line with the conclusion drawn in the preceding vintage.

Interestingly, only a small implementation rate of either water or GHW was necessary to mitigate the 'hotness' perception while not modifying 'fruity' attributes. Even so, it was suggested that under these vintage conditions (i.e., absence of berry shrivel), an earlier harvest could still have been a valid option to target a lower alcohol wine, given that the majority of important sensory characteristics seemed to peak at a point that was 11 days earlier than the commercial harvest. This is especially the case in light of the risks and additional costs (berry shrivel, irrigation, etc.) associated with longer grape hang-times, particularly if it helped with the logistics of processing many parcels of fruit during the peak of vintage.

In contrast to Cabernet Sauvignon, the non-volatile characteristics of the Shiraz wines were found to be jeopardised with increasing implementation rates of water and GHW. Wine colour stability declined and tannin concentrations were depleted, particularly among the GHW treatment series, which modified the astringency perception and was likely to have negative implications on wine ageing capability. Changes in wine volatile composition further opposed the observations for the Cabernet Sauvignon wines produced within this study and those from the previous vintage trial, with water implementation eliciting notable declines in various fermentative aroma compounds relative to grape-derived constituents (isoprenoids), resulting in a divergence from the control wine and the GHW treatments. Changes among the Shiraz GHW treatment series did not follow a particular pattern with increasing substitution rates, and the relative proportions of compound groups were little affected compared to both the respective lower alcohol Shiraz wines following water addition and to the Cabernet Sauvignon wines.

Regarding the resulting sensory profiles, an impact on aroma attributes was not observed, and among the flavour attributes, only 'dark fruit' and 'chocolate' decreased with

the highest GHW implementation, but not when using water (which appeared to accentuate 'liquorice' sensations). Unlike the observations with Cabernet Sauvignon, the 'flavour intensity' remained similar to the control regardless of the chosen substitution rate and blending component. The limited modifications in sensory profiles did not particularly mirror the trends observed in terms of the volatile composition, which agrees with recent postulations that only a minority of volatile wine constituents detrimentally influence wine sensory characteristics. Wine 'astringency' perceptions followed a similar trend to the Cabernet Sauvignon wines, diminishing with higher implementation rates, whereas higher ratings with the water implementation treatments relative to their GHW counterparts were in line with the higher tannin concentrations.

Similar to the results regarding Cabernet Sauvignon, the majority of flavour and mouthfeel attributes had already reached maximum intensities when using grapes harvested 13 days before the commercial harvest, although some differentiation in wine style was likely with the additional grape ripening time. For instance, 'flavour intensity' did not further change but 'dark fruit' became more dominant in the sensory profile. Nonetheless, given that the alcohol management approaches resulted in similar sensory profiles to wines from earlier harvests, it could be considered better to target lower wine alcohol concentrations through modified picking dates, thus avoiding the costs and risks involved in longer grape ripening. If harvest infrastructure does not allow for an earlier harvest of certain vineyards, winemakers may favour the water implementation option (again within the regulations) to modify the final wine alcohol concentration due to its lower costs and easier adoption, given that the GHW treatments did not show any particular merits with respect to wine quality.

6.4 Substitution or dilution? Assessing pre-fermentative water implementation to produce lower alcohol Shiraz wines.

From the 2015 and 2016 winemaking trials it could be concluded that pre-fermentative water addition may be a valid choice to produce lower alcohol wines without

dramatically changing the wine sensory perceptions as determined by the harvest date, particularly if moderate water substitution rates are applied. Furthermore, Food Standards Australia New Zealand (Application A1119 to amend Standard 2.7.4 and 4.5.1) allows the simple addition of water without prior removal of juice and this may be more convenient and the preferred choice for winemakers. However, no studies have assessed the implications on wine quality following the increase of the liquid-to-solid ratio of a red wine fermentation upon exploiting the maximum allowed water addition to must down to the minimum of 13.5 Baumé (and indeed, its limitations beyond this point). Therefore, the last vintage trial conducted in 2017 aimed to compare the pre-fermentative juice substitution approach against straight dilution. Further, given the indications of the previous vintage trials that grape maturity might affect the extent of changes in wine constituents following the must TSS adjustments, the alcohol adjustment treatments were based on crop harvested at two distinct maturity levels (known as 'Fresh Fruit' (FF) and 'Mature Fruit' (MF) harvest dates). Shiraz was chosen for this component of the project to provide a vintage repetition on this cultivar, as well as due to its major importance in the Australian wine industry, and its particular susceptibility to berry shrivel.

Alcohol concentrations for the earlier harvest wines ranged from 13.6% ABV (FF control) to 9% ABV, and from 15.5% ABV (MF control) to 9.6% ABV for a harvest point 12 days later. With respect to the lower grape maturity for FF wines, implications of an increasing liquid proportion already became evident at the lowest dilution rate employed in this study (yielding a wine with 11.6% v/v), with significant declines in colour intensity and stability, as well as in total phenolics and tannin concentrations in comparison to the control. In contrast, the juice substitution with the same amount of water did not elicit any changes to those parameters. A differentiation became further obvious in the resulting sensory profiles, where the water dilution diminished 'flavour intensity', and 'body', 'alcohol' and 'confectionery' ratings, among other attributes, yet 'green' sensations became more

pronounced as more water was added. At a higher technological grape maturity (MF), however, both the substitution and dilution treatments decreased colour properties, and it appeared that the substitution treatments had a more substantial effect than observed with the less ripe (FF) grapes. Wine tannin concentrations did not follow this trend and were stable with low water implementation rates but decreased markedly when diluted with higher proportions of water. In terms of final sensory profiles, a decline in an array of sensory attributes was noticeable with high water implementation rates, such as perception of 'flavour intensity' or 'dark fruit', however the difference between dilution and substitution appeared smaller than observed with the less ripe fruit.

Ultimately, working within the legal limit of water implementation (i.e., decreasing alcohol level from 15.5% to 13.5% ABV), the difference between dilution and substitution could be regarded as rather negligible so that simple water addition could be a valid option. However, the fruit harvested at an earlier technological ripeness (FF) did not result in a wine lacking in important characteristics, such as 'aroma' or 'flavour' intensities, 'fruit' attributes or even 'body' or 'astringency', and differences in remaining attributes could be associated with FF wines being different in style rather than being of a lower quality level, as already concluded during the 2016 vintage.

6.5. Future directions

Pre-fermentative must substitution with water has been shown to only marginally alter wine qualities, but also dilution is suggested to be a valid and easily applicable approach to manage wine alcohol levels when working within the legal limit. Implications on wine quality were rather benign, particularly under low to moderate water implementation rates that could be associated with adjustments in wine alcohol balance, more than with producing lower alcohol wines in particular. However, utilising unripe grapes collected at veraison, such as those that could be considered as waste material resulting from grape thinning, is a considerable alternative and could be suitable for wineries that promote a sustainable

business model and the minimisation of waste, while rationalising water usage. Given the low pH of this low alcohol wine, it is easily storable and once produced in bulk, could possibly be used over several vintages. The acidification arising from using GHW is another advantage in comparison to water, however, further assessment of the techno-economic feasibility would be required.

Small changes in wine attribute ratings according to the descriptive analysis panel could have consequences for wine style so it would be valuable to extend research to include consumer preferences and expert quality ratings, thereby assessing the attitude of consumers towards this winemaking practice as well as the perceived quality of the products. Such a study could further aim to assess the preference for wine styles as defined by an earlier harvest in comparison to an alcohol-adjusted later harvest. Furthermore, potential drawbacks in wine sensory quality could be addressed with available winemaking practices, which warrants further investigation. For instance, the partial declines in 'body' and 'astringency' perceptions could be mitigated with winemaking supplements such as tannins. Similarly, the use of alternative yeast strains could complement the water addition to suit targeted wine styles and mitigate lower intensities in desirable aroma attributes. In addition, efforts should be extended to include other varieties of both red and white cultivars, as different outcomes on wine qualities could be expected. To account for cultivar-differences in grape physiological ripening, it would enhance the comparability (for instance, of extractability of phenolic compounds, etc.) if harvest dates are planned and standardised according to sugar loading per berry rather than concentration. Last but not least, given that the water addition approach is now included amongst other permitted dealcoholisation techniques such as reverse osmosis, comparisons of these technologies with respect to wine quality and to economics could be carried out to establish better guidelines for producers, to informed decision-making about alcohol management techniques.

Finally, it is worth noting that pre-fermentative water additions might be used to the disadvantage of growers. That is, deliberate delays to harvest date could provoke dramatic yield (and quality losses) for growers due to berry shrivel but afford potential savings for the buyers, who may address lower grape volumes with water addition in the winery. This seems to be at odds with the purpose of the changes in regulation and impacts not only the integrity of grower-buyer relationships, but also goes against principles of environmental and social responsibilities. It also ignores the results arising from the present study that berry shrivel and excessive grape maturity do not necessarily yield satisfactory wine qualities even after wine alcohol adjustment. To avoid this from becoming an entrenched practice, it is recommended to review and amend the respective standards as required to safeguard against potential misuse of water addition in the future. One such measure could involve obligatory financial concessions of buyers towards contracted growers that account for yield loss caused by berry shrivel beyond pre-defined grape TSS levels. However, to ensure compliance, objective and transparent vineyard observations would need to be established and controlled by regulatory bodies, and constructive discussions between the involved parties should be initiated in a timely fashion.

List of abbreviations

ABV	alcohol by volume
AHC	agglomerative hierachical clustering
Bé	baumé
CWM	cell wall material
DA	descriptive analysis
DAP	diammonium phosphate
FF	fresh fruit
MF	mature fruit
GHW	green harvest wine
GPC	gel permeation chromatography
HAA	higher alcohol acetate
IBMP	3-isobutyl-2-methoxypyrazine
MCP	methyl cellulose precipitable
MM	molecular mass
mol%	molar percentage
MP	mannoprotein
PMS	potassium metabisulfite
PRAG	polysaccharides rich in arabinose and galactose
RG-II	rhamnogalacturonan II
TA	titratable acidity
TSS	total soluble solids

Appendices

Appendix 1

A proceedings manuscript was prepared in context of my presentation given at the Australian Society of Viticulture and Oenology (ASVO) Seminar 'Earlier, shorter, hotter – Managing compressed vintages', 19 November 2015, Adelaide, Australia, titled 'Berry heterogeneity and wine quality'.

Appendix 2

Following the first publication (*Food Chemistry*, 2018. 244: p. 50-59), a technical note was prepared titled 'Water into wine: Pre-fermentation strategies for producing lower alcohol wines'.

Berry Heterogeneity and wine quality: A review and outlook

Proceedings manuscript for the Australian Society of Viticulture and Oenology Seminar 2015, Adelaide, Australia.

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Heterogeneity – An introduction

Vineyards are not homogeneous but succumb to spatial and temporal differences in yield and quality. Despite regional inter vineyard variability due to climatic and topographic differences, differences in quality and yield can also be observed within a vineyard on a vine-to-vine (yield and quality variability due to spatial changes in soil type, virus infections, etc.), bunch-to-bunch (physiological differences in function of bunch position on cane, and cane position on vine, sun exposure, etc.) or even berry-to-berry scale (microclimate). Viticulturists have been aware of vineyard variability since the early beginnings of winemaking but it was accepted as an unchangeable fact given the lack of knowledge about the nature behind it and the absence of technical solutions (Bramley & Hamilton, 2004).

Today viticulturists have a more sophisticated understanding about the nature of their vineyards which allows them to identify and manage differences in yield and quality. Dependant on the nature of the variability, viticulturists have the tools to avoid post-planting

variability in the vineyards with the appropriate choice of cultivar and rootstock, to adapt block location, design and irrigation infrastructure or to manage the variability at harvest through split picking and sorting. However, for the latter, detailed knowledge is required regarding the berry population at harvest stage, the magnitude of variation and the quality attribute that needs adjustment in order to appropriately use techniques such as berry sorting. As a consequence, sorting options have not been frequently adapted in the Australian wine industry.

Managing berry heterogeneity in order to improve crop quality has been under discussion for several years, however its application has not been followed up very enthusiastically even though the advantages are evident. With the context of earlier, shorter and hotter vintages, and higher demand in economic efficiency, the time is now for winemakers to evaluate the potential, especially as the required technology is already available. Preliminary results of this work indicate that through targeting berry heterogeneity (size and/or TSS) a powerful tool may be given to the winemaker to adapt to compressed vintages and to give back control over the crop quality when 'optimum' harvest dates are no longer available. This project aims to describe the impact of berry ripening variability (and its alternation through sorting) on wine quality and to give an incentive for the industrial adaptation.

Getting the vineyard homogeneous

Technical innovations in the 20th century increased vineyard efficiency by replacing manual labour with machines and increasing the output of production. The automation of vineyard work was introduced under the assumption of the vineyard being homogenous, so vineyard mechanisation firstly improved economic efficiency of production but not necessarily product quality. The first improvements in wine quality were rather due to progress in oenology, with the introduction of selected yeast strains, temperature and pH control etc. It was not until the late 20th / early 21st century that broad awareness arose to the fact that wine quality

could be improved by understanding the drivers that influence grape quality. In order to increase vineyard performance, research efforts focused on developing methods, like the grape yield monitor introduced in 1999, to assess yield levels of vineyards to characterise spatial differences in yield levels (Bramley & Hamilton, 2004). This knowledge allows the viticulturist to adapt harvest decisions accordingly or to spatially adjust irrigation or fertilisation treatments to decrease the yield heterogeneity, and hence optimise resource input and wine quality. The management of fertilisation is still rarely applied but recent studies aiming to determine the key drivers for vigour differences, for instance, could change that. Work conducted by (King, Smart, & McClellan, 2014) for example showed that differences in yield and vigour are rather controlled by soil driven differences in nitrogen uptake and not directly by plant water status. Adapted nitrogen application could hence equilibrate vineyard ripening dynamics and increase harvest efficiency, while minimising fertiliser input.

Further more (van Leeuwen, Friant, Choné, Tregoat, Koundouras, & Dubourdieu, 2004) determined that differences in soil types with variations in water capacity influence vine vigour and phenological development, suggesting the adaptation of rootstock-cultivar combinations to soil conditions. The identification of homogeneous zones of soil has been a tool for viticulturists to avoid vine heterogeneity and poor yield and quality performance before planting (van Leeuwen, Friant, Choné, Tregoat, Koundouras, & Dubourdieu, 2004) and (Nascimento, Silva, Costa, & Bassoi, 2014). This precision viticulture significantly increased the input and output efficiency of vineyards and enhanced wine quality by producing more homogeneous and predictable fruit quality, matching the increasing quality demand of wineries. The economic benefit was shown in a real case study by (Bramley, Pearse, B. and Chamberlain, P. , 2003), where the commercial value of a vineyard could be increased significantly, simply by selectively harvesting different identified zones of a vineyard as a function of vigour and yield. These researchers also showed that the identified magnitude of

heterogeneity was consistent enough over vintages to permanently adapt zonal management strategies and justify long term investments (Bramley & Hamilton, 2004). The adaptation of precision viticulture by the wine industry has increased profitability and sustainability of wineries in economic and ecological terms.

Despite reducing the vine-to-vine variability in yield and vigour through precision viticultural management as explained, a residual heterogeneity remains on a bunch-to-bunch and berry-to-berry scale. This is caused by differences in microclimate and bunch position rather than by soil or climate characteristics. A significant variation in berry composition within a bunch was observed by (Trought & Tannock, 1997), with smaller berries being higher in total soluble solids (TSS) than larger ones, but same sized berries in a bunch on a more apical position will have lower TSS levels. (Doumouya, Lahaye, Maury, & Siret, 2014) showed there are berry ripening differences according to their positions on the bunch, and berries at distal parts of the bunch are more advanced in ripening than those close to the pedicel.

Higher berry density levels seem to come with higher total polyphenol index (TPI), colour intensity, pH and proanthocyanidin concentration in the respective wine and low density berries contribute with a higher proportion of seed tannin and less polymerized skin tannins (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011). Consequently, a low proportion of less ripe berries within a berry population can have a significant influence on the final wine quality, potentially imparting bitterness and green attributes. Berry size variability appears to play an important role as well, considering the high magnitude of diameter distribution at different sampling points throughout ripening (Šuklje, Lisjak, Baša Česnik, Janeš, Du Toit, Coetzee, et al., 2012), given that anthocyanin content in Cabernet Sauvignon (Gil, Pascual, Gómez-Alonso, García-Romero, Hermosín-Gutiérrez, Zamora, et al., 2015) increases or IBMP concentrations in Sauvignon Blanc (Šuklje, et al., 2012) decrease with larger berry diameter. These findings suggest that berry heterogeneity, in terms of size and density can have a

significant impact on wine quality and that the application of sorting techniques could enhance product quality. To justify the implementation of sorting options to account for the drawbacks of heterogeneity and increase overall production value, more information is required regarding the proportion of undesirable berries and the change throughout grape ripening. The evolution of berry heterogeneity needs to be assessed for several vintages and varieties, as literature showed differences in variability among vintages (Edo-Roca, Sanchez-Ortiz, Nadal, Lampreave, & Valls, 2014) and cultivars (Edo-Roca, Nadal, & Lampreave, 2013). For instance, (Doumouya, Lahaye, Maury, & Siret, 2014) linked cold temperatures at flowering with an increase in ripening heterogeneity and larger proportions of unripe berries within a bunch.

The significance of berry ripening variability in compressed vintages

As mentioned above, berries do not evolve evenly within a vineyard's population. In fact, the TSS distribution of a berry population can be described with a Gaussian normal distribution curve (Singleton, Ough, & Nelson, 1966) meaning that minor portions of the crop are less or more advanced in ripening than the majority of the population (Figure 1).
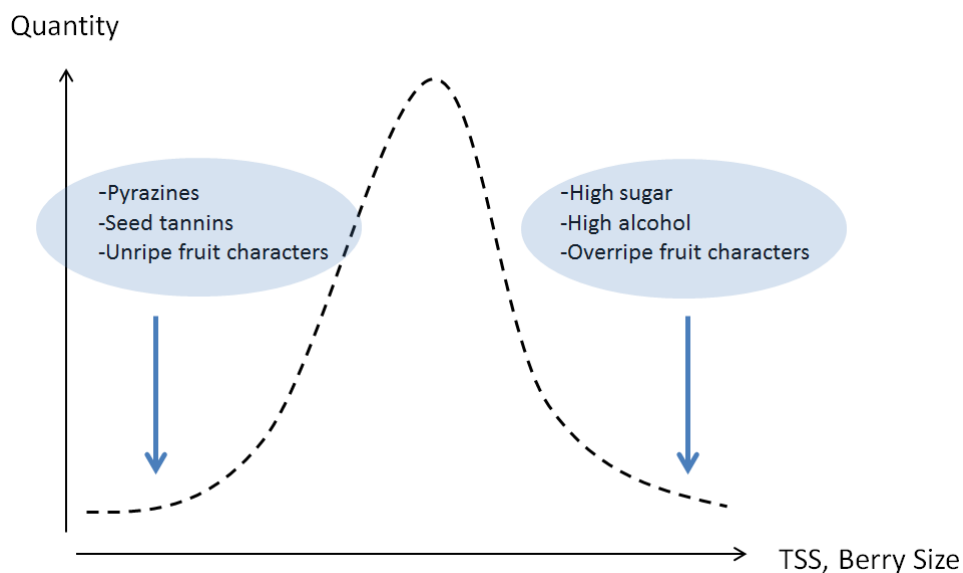
Figure 1: Schematic representation of TSS or Berry Size variability among a berry population, highlighting the presence of unripe or overripe tails with different chemical properties. Unripe

berries may affect the wine with higher pyrazine or higher seed tannin concentrations. Higher TSS values of overripe berries can contribute to higher alcohol levels of the wine.

Dependent on the characteristics of the unripe portion of the crop, a significant impact on the overall quality can be expected. In fact this is often a reason for delaying harvest dates to avoid the contribution of unripe sensory attributes in the final wine. This is particularly the case for varieties impacted by the content of methoxypyrazines such as Sauvignon Blanc, Cabernet Sauvignon or Cabernet Franc, and where winemakers seek to meet the consumer demand for full bodied, soft tannins and ripe fruit characters (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011). However, with ripening periods being more compressed in warm regions and being characterised by water constraints and higher temperatures, a proportion of the fruit can rapidly dehydrate and the delayed harvest time can result in increasing TSS concentrations and alternation of the aroma profile of the majority of the population (Bonada, Sadras, Moran, & Fuentes, 2013). Given the increasing pressure on harvest infrastructure due to accelerated ripening, the crop can be shifted to over-ripeness with an increasing portion of shrivelled berries.

Berry sorting could be used to manage two scenarios: 1) by encouraging earlier harvests through eliminating the unripe tail and hence avoiding high alcohol content, and 2) by sorting out overripe and shrivelled berries, thus reducing quality drawbacks of unintentionally late harvested fruit. Earlier, shorter and hotter vintages put increasing pressure on harvest infrastructure, which results in vineyards being harvested beyond the optimum maturity state. Targeting characteristics of berry heterogeneity by sorting could support the winemaker by improving flexibility in harvest management and providing another opportunity to control wine ethanol content and aroma profile. So far literature dealing with heterogeneity on a berry-to-berry scale is scarce, and mostly deals with distribution of TSS levels and berry sizes within

bunches. Berry ripening heterogeneity within a vineyard, and its evolution throughout consecutive ripening stages and several vintages, has not yet been assessed.

Method

The assessment of berry ripening heterogeneity complements the main focus of a PhD project within the ARC Training Centre for Innovative Wine Production. This project aims to evaluate an early harvest regime for its potential to lower alcohol content in wine and counter the trend of increasing alcohol contents through climatic change. A part of this project includes consecutive harvests with progressive ripening levels, with extensive chemical analysis of the grapes and respective wines arising from a Cabernet Sauvignon vineyard in McLaren Vale. For each harvest point, a sample of at least 1000 berries was taken and distributed into groups of 100 berries. Pictures were taken of each of the groups to assess the berry size via image analysis, followed by measuring the Brix of each individual berry with a digital refractometer in a way that Brix value and berry size can be related. This allowed the berry population characteristic to be presented in TSS and size in a histogram and to show the evolution in heterogeneity for different harvest stages.

Preliminary results

Preliminary results show an increase in berry ripening heterogeneity with an increasing proportion of overripe and shrivelled berries that have the potential to contribute to both excessive Brix values of the crop and high alcohol levels of the resulting wine (Figure 2). Given that the high Brix berries are smaller than the average, removing them by sorting may have lowered the final alcohol level and altered the aroma profile. However, the implementation of sorting machines to decrease alcohol concentrations of wines is associated with costs that need to be justified by a value increase in the production. There are mainly two possibilities where this could be the case. Firstly, removing a small portion of unripe crop in order to harvest the

vast majority at an optimum ripening stage, to avoid subsequent dealcoholisation treatments and to obtain sound fermentation conditions. Secondly, given an overripe crop with a high portion of shrivelled berries, sorting could be used to decrease the proportion of high Brix berries, hence lowering the loss in quality and value due to late harvests. Both scenarios have their limits. On one hand, sorting of grapes harvested too early (to remove the larger berries?) could lead to excessive loss of yield. On the other hand, the sorting of excessively matured (smaller) grapes would still result in high alcohol levels with likely poor quality, so the value loss could not be ruled out. Our initial data supports this theory (Table 1, Figure 3) indicating that the removal of shrivelled berries from the commercial harvest point (H4) would have still resulted in a wine with alcohol levels higher than 16% v/v (after removing 9.9% of the total crop with an average Brix of 35.6) whereas harvesting four days earlier (H3) would have yielded a wine closer to 14% (after removing 6% of the total crop with 31.7 Brix average) with significantly less yield loss after application of sorting.
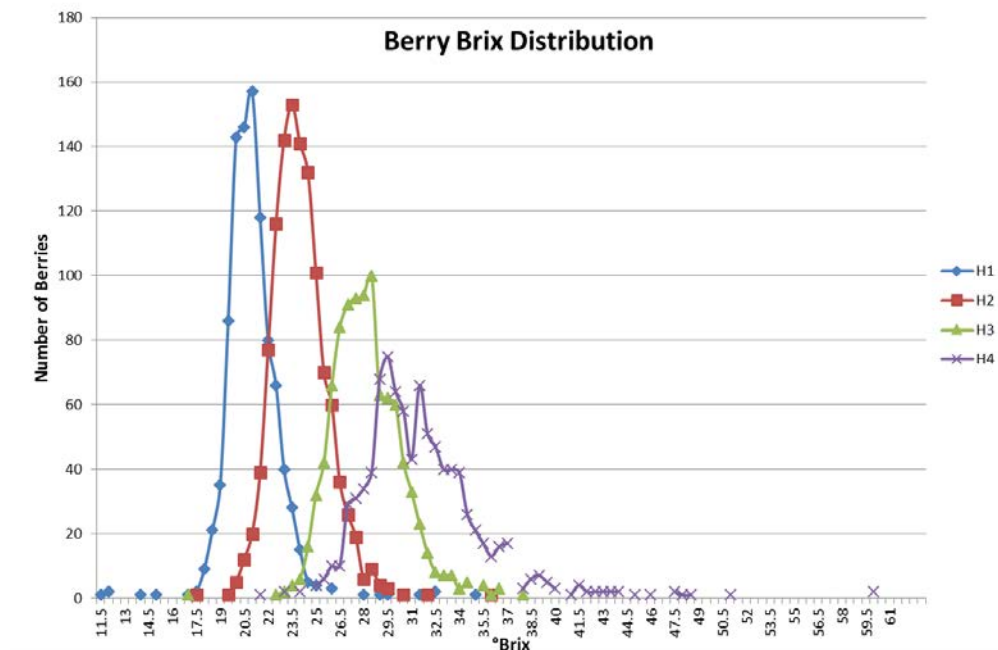
Figure 2: Berry TSS distribution (°Brix) for four consecutive harvest points in 2015 (H1: 3 Feb, H2: 9 Feb, H3: 18 Feb, H4: 22 Feb).
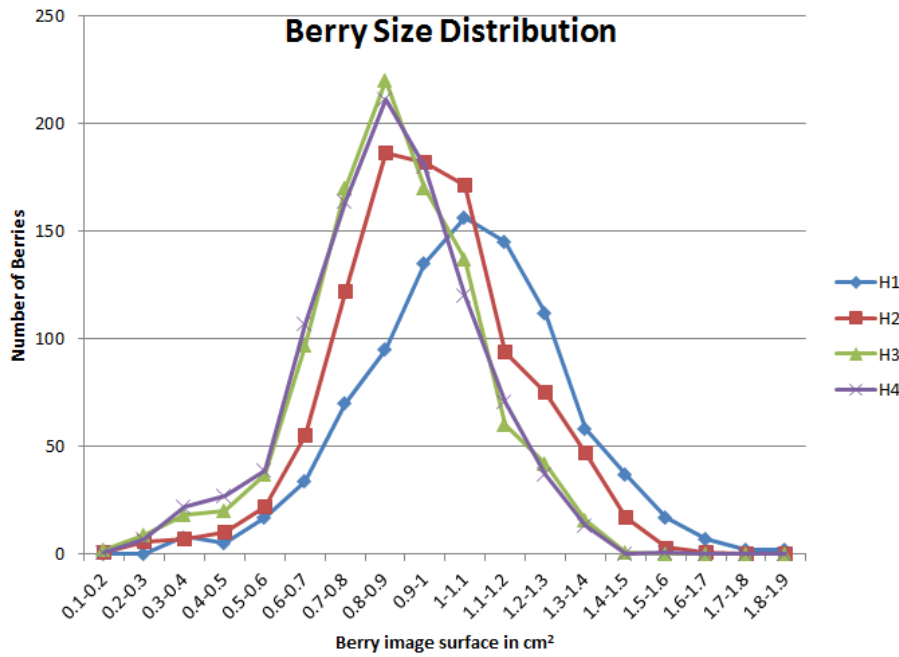
Figure 3: Berry size distribution for four consecutive harvest points in 2015 (H1: 3 Feb, H2: 9 Feb, H3: 18 Feb, H4: 22 Feb). Berry size is given in cm^2 resulting from image analysis (Digital single-lens reflex camera in combination with Image J).

	Harvest			
	H1	**H2**	**H3**	**H4**
	0.1-0.2 to 0.5-0.6			
Brix	21.2	27	31.7	35.6
%total	3.2	3.3	6	4.8
	0.6-0.7 to 1.2-1.3			
	21.4	24.3	28.3	32.2
	83.1	89.8	92.2	93.7
	1.3-1.4 to rest			
	21.2	22.4	25.9	28.6
	13.8	6.9	1.9	1.5

Table 1 Three berry size categories (small, medium and large) representing the two tails and the majority of the berry population at a given harvest date (H1: 3 Feb, H2: 9 Feb, H3: 18 Feb,

H4: 22 Feb). The contribution of berry size categories is given for each harvest point, and the average Brix of the respective size. The berry size is expressed in cm^2 resulting from image analysis.

Further studies

To understand the scope in which sorting could be an option to deal with compressed vintages and the high alcohol content of the resulting wine with consideration for peak work periods, further knowledge of berry ripening heterogeneity is required, especially throughout different vintages and different varieties. Future work needs to focus on three main objectives:

1. To determine if the patterns of unripe and overripe tails are stable and predictable enough to justify a long-term investment in a sorting machine.
2. To determine if targeting the unripe and overripe tails would result in the desired change in wine chemical and sensory properties
3. To show that managing the crop according to its berry variability increases product value and hence gives economic benefits.

Acknowledgements

References

Bonada, M., Sadras, V., Moran, M., & Fuentes, S. (2013). Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrigation Science, 31*(6), 1317-1331.

Bramley, R. G. V., & Hamilton, R. P. (2004). Understanding variability in winegrape production systems 1. Within vineyard variation in yield over several vintages. *Australian Journal of Grape and Wine Research, 10*(1), 32-45.

Bramley, R. G. V., Pearse, B. and Chamberlain, P. . (2003). Being profitable precisely – A case study of precision viticulture from Margaret River. *Australian Grapegrower and Winemaker, 473a*, 84–87.

Doumouya, S., Lahaye, M., Maury, C., & Siret, R. (2014). Physical and physiological heterogeneity within the grape bunch: Impact on mechanical properties during maturation. *American Journal of Enology and Viticulture, 65*(2), 170-178.

Edo-Roca, M., Nadal, M., & Lampreave, M. (2013). How terroir affects bunch uniformity, ripening and berry composition in *Vitis Vinifera* cvs. Carignan and Grenache. *Journal International Des Sciences De La Vigne Et Du Vin, 47*(1), 1-20.

Edo-Roca, M., Sanchez-Ortiz, A., Nadal, M., Lampreave, M., & Valls, J. (2014). Vine vigor and cluster uniformity on *Vitis vinifera* L. seed procyanidin composition in a warm Mediterranean climate. *Spanish Journal of Agricultural Research, 12*(3), 772-786.

Gil, M., Pascual, O., Gómez-Alonso, S., García-Romero, E., Hermosín-Gutiérrez, I., Zamora, F., & Canals, J. M. (2015). Influence of berry size on red wine colour and composition. *Australian Journal of Grape and Wine Research*, n/a-n/a.

King, P. D., Smart, R. E., & McClellan, D. J. (2014). Within-vineyard variability in vine vegetative growth, yield, and fruit and wine composition of Cabernet Sauvignon in

Hawke's Bay, New Zealand. *Australian Journal of Grape and Wine Research, 20*(2), 234-246.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Australian Journal of Grape and Wine Research, 17*(2), 230-238.

Nascimento, P. D., Silva, J. A., Costa, B. R. S., & Bassoi, L. H. (2014). Homogeneous zones of soil properties for irrigation management in a vineyard. *Revista Brasileira De Ciencia Do Solo, 38*(4), 1101-1113.

Singleton, V. L., Ough, C. S., & Nelson, K. E. (1966). Density separations of wine grape berries and ripeness distribution. *American Journal of Enology and Viticulture, 17*, 95-105.

Šuklje, K., Lisjak, K., Baša Česnik, H., Janeš, L., Du Toit, W., Coetzee, Z., Vanzo, A., & Deloire, A. (2012). Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon Blanc (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry, 60*(37), 9454-9461.

Trought, M. C. T., & Tannock, S. J. C. (1997). Berry size and soluble solids variation within a bunch of grapes. *Proceedings of the fourth international symposium on cool climate viticulture & enology, Rochester, New York, USA, 16-20 July 1996.*, V-70-V-73.

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., & Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *American Journal of Enology and Viticulture, 55*(3), 207-217.

ARC Training Centre for Innovative Wine Production

Technical note

WATER INTO WINE: PRE-FERMENTATION STRATEGIES FOR PRODUCING LOWER ALCOHOL WINES

Introduction

Warmer grape ripening periods as a result of a changing climate can pose considerable challenges for Australia's winemakers. Logistical pressures due to a compressed vintage season can lead to delays in harvesting that further increase berry sugar levels, yielding wines with elevated alcohol concentrations. Trying to manage heterogeneous berry ripening, by delaying harvest while waiting for flavour and phenolic ripeness, can also contribute to increases in wine alcohol concentration as a result of increased berry shrivel.

Winemakers often need to moderate the alcohol content of their wines for a number of reasons, including to enhance wine quality and balance, meet consumer demand, and avoid paying higher taxes or duties on exports. Various approaches are available for lowering wine alcohol content, either before, during or after fermentation [1], and we sought one that would be easy to adopt. As a result we investigated an early harvest regime and a blending approach for Cabernet Sauvignon using either "green harvest wine" (GHW) or water to substitute for some juice

prior to fermentation [2]. The inclusion of water to dilute initial sugar must levels was very timely in light of the recent decision [3] to permit the addition of water to high sugar musts to facilitate fermentation [4].

The key outcomes

The 2015 growing season in McLaren Vale was warmer than the long-term average and berry shrivel was evident, making it a very representative season in terms of compressed grape ripening. Berries picked at later time points for the harvest series wines revealed a proportional increase in the number of berries with high sugar and a greater degree of ripeness heterogeneity (with around 10% of berries having > 35 °Brix for the commercial harvest time point, which acted as the control). Water and GHW (4.5% abv and pH 2.76), produced from grapes picked at about 8 °Brix, were also used to blend with the must arising from grapes harvested at commercial maturity, after running off a proportional amount of juice. The harvest series experiments produced wines ranging from 11.4% abv for the first harvest to 18.2% abv for the control, whereas blending yielded wines with around 14.5% abv for treatments containing 30-40% GHW or water by volume, and up to 17% abv for wines made with 10-14% GHW or water. A range of grape and wine compositional measures that are important to red wine quality were undertaken during this study, including colour, phenolics and polysaccharides.

Polysaccharides that contribute to fullness and decrease astringency were found to increase with maturity for wines from the first three harvest time points but declined markedly by the time of commercial harvest, when berry shrivel was more evident. Wine anthocyanin concentrations had a sharp increase for the earlier harvests and then stayed reasonably stable whereas wine colour density, total phenolics and stable pigments continued to increase in line with the ripeness of the grapes. The effect of the treatments was magnified after 18 months in bottle, with the later harvest points potentially showing better ageing potential by having higher levels of anthocyanins, stable pigments and colour density. Wine tannins, which were very well-correlated with grape extractable tannin determined using a wine-like extraction protocol developed at the Australian Wine Research Institute (AWRI), showed an increasing trend with ripeness and a considerable spike in concentration for the last harvest time point.

Blending the must with water or GHW in high proportions resulted in increased polysaccharides in the wines, although polysaccharide sugar composition was affected by the blending material and the amount incorporated. There were no differences in wine colour density, total anthocyanin concentration, total phenolics or wine tannins for the blending treatments compared to the control, but a high proportion of GHW or water decreased stable pigments. However, after 18 months in bottle there were no differences in stable pigments among the treatments, which makes the blending approach promising from a wine ageing perspective.

Recommendations

Overall, large decreases in final wine alcohol concentration can be readily achieved purely through a pre-fermentation approach. 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.

What's next?

A more complete evaluation of the influence of these treatments on the style and quality of wines made in 2015 has been undertaken, with the analysis of volatile composition and sensory profiles to be reported in a subsequent publication. Whereas the first vintage only comprised Cabernet Sauvignon, the experimental winemaking was repeated in 2016 with both Cabernet Sauvignon and Shiraz. In that follow-up work, there was a focus on evaluating the suitability of GHW or water blending approaches to manage wine alcohol concentrations under more moderate harvest conditions with the absence of severe berry shrivel, therefore accounting for year-to-year variations winemakers frequently face in light of climatic changes. Supporting the observations made during the 2015 trials, the outcomes in 2016 strongly underscore the advantages of water implementation, but also raise more questions about the best way to do so. A more convenient way for winemakers to dilute must sugar concentrations is the simple addition of water without running off juice, hence in 2017 the experimental setup was designed to evaluate dilution or substitution with water as tools to manage wine alcohol levels while retaining optimum wine quality. In addition, the 2017 trial sought to understand how the stage of grape maturity may influence attempts to lower wine alcohol concentrations, by picking fruit at two distinct maturity levels described as 'fresh fruit' and 'mature fruit', thus completing these investigations of an early harvest regime and blending approaches to manage alcohol levels in red wines.

Acknowledgements

References

1.   Schmidtke, L.M., J.W. Blackman, and S.O. Agboola, *Production technologies for reduced alcoholic wines.* Journal of Food Science, 2012. **77**(1): p. R25-R41.
2.   Schelezki, O.J., et al., *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, 2018. **244**: p. 50-59.
3.   FSANZ. *Approval report – Application A1119: Addition of Water to Facilitate Wine fermentation*. 2016 [cited 2017 12 October]; Available from: http://www.foodstandards.gov.au/code/applications/Documents/A1119%20Addition%20of%20water%20to%20wine%20AppR.pdf.
4.   Office of Parliamentary Counsel. *Standard 4.5.1 - Wine Production Requirements*. 2017 [cited 2017 12 October]; Available from: https://www.legislation.gov.au/Details/F2017C00713.

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