The Australian Wine Research Institute

Evaluating non-conventional yeast for the production of wines that contain less alcohol

FINAL REPORT to

WINE AUSTRALIA

Chief Investigator: Cristian Varela

Project Number: AWRI 3.3.1

Research Organisation: The Australian Wine Research Institute

Date: 30/06/2016

Disclaimer

This document has been prepared by The Australian Wine Research Institute ("the AWRI") for a specific purpose and is intended to be used solely for that purpose and unless expressly provided otherwise does not constitute professional, expert or other advice.

The information contained within this document ("Information") is based upon sources, experimentation and methodology which at the time of preparing this document the AWRI believed to be reasonably reliable and the AWRI takes no responsibility for ensuring the accuracy of the Information subsequent to this date. No representation, warranty or undertaking is given or made by the AWRI as to the accuracy or reliability of any opinions, conclusions, recommendations or other information contained herein except as expressly provided within this document. No person should act or fail to act on the basis of the Information alone without prior assessment and verification of the accuracy of the Information.

To the extent permitted by law and except as expressly provided to the contrary in this document all warranties whether express, implied, statutory or otherwise, relating in any way to the Information are expressly excluded and the AWRI, its officer, employees and contractors shall not be liable (whether in contract, tort, under any statute or otherwise) for loss or damage of any kind (including direct, indirect and consequential loss and damage of business revenue, loss or profits, failure to realise expected profits or savings or other commercial or economic loss of any kind), however arising out of or in any way related to the Information, or the act, failure, omission or delay in the completion or delivery of the Information. In the event that any legislation or rule of law implies any condition, warranty or liability with respect to the AWRI or the Information, the AWRI's liability for breach of any condition, warranty or liability shall be limited, at the option of the AWRI, to the re-supply of that Information; the cost of acquiring equivalent Information or the payment of the cost of having the Information re-supplied.

The Information must not be used in a misleading, deceptive, defamatory or inaccurate manner or in any way that may otherwise be prejudicial to the AWRI, including without limitation, in order to imply that the AWRI has endorsed a particular product or service.

Summary

From the 1980s to the mid-2000s, the average ethanol concentration in Australian wine increased, in part reflecting market trends for wine styles associated with increased grape maturity. Since 2005, ethanol levels have trended downwards but are still significantly above those in the 1980s (Godden et al. 2015). High ethanol concentration can, however, adversely affect wine sensory properties, reducing the perceived complexity of flavours and aromas. In addition, for reasons associated with corporate social responsibility and taxation regimes the wine sector is actively seeking technologies that facilitate the production of wines with lower ethanol content. Non-conventional yeasts have shown potential for production of wines with lower alcohol concentration. These yeast species, largely associated with grapes pre-harvest, are usually present in the early stages of fermentation but in general are not capable of completing alcoholic fermentation unaided. Recently, the AWRI identified that a *Metschnikowia pulcherrima* strain was able to produce wine with reduced ethanol concentration when sequentially inoculated with a wine strain of *Saccharomyces cerevisiae*. Later, the AWRI identified a *Saccharomyces uvarum* strain which was also able to produce wine with reduced ethanol concentration. This effect was additive when both strains *M. pulcherrima* and *S. uvarum* were co-inoculated in sterilised or dimethyl dicarbonate (DMDC) treated grape must at laboratory-scale. In freshly prepared grape musts both yeasts were less effective in reducing alcohol concentration due to competition with indigenous yeast populations. Subsequent pilot-scale winemaking was, therefore, performed using DMDC-treated musts. Several treatments produced wines with lower ethanol concentration than control *S. cerevisiae* wines. Formal sensory analysis revealed that while wines fermented with *S. uvarum*, alone or in combination with *M. pulcherrima*, were lower in alcohol concentration, they were associated with negative sensory attributes. Wines fermented with *M. pulcherrima* were associated with positive sensory attributes but were not lower in alcohol concentration than uninoculated controls. Overall the results suggest that further work is required to render low-alcohol non-*Saccharomyces* yeasts more robust, and to ameliorate their potential negative sensory impacts.

Background

There is growing interest from winemakers in being able to produce wines with lower ethanol content that do not have compromised aroma, flavour, and mouth-feel. There are opportunities across the value chain to implement strategies to achieve this, including viticultural practices, pre-fermentation and winemaking practices, microbiological strategies and post-fermentation practices and processing technologies (Varela *et al.* 2015). However, the application of yeast strains that produce less ethanol during fermentation remains a simple and cheap strategy for producers to adopt towards this goal. Unfortunately, all available commercial *S. cerevisiae* wine yeasts are very similar in terms of ethanol yield; a difference of 0.5% v/v has been observed between 'high' and 'low' ethanol producers (Palacios et al. 2007; Varela et al. 2008). In order to reduce ethanol concentration, novel yeast need to be isolated or generated.

A promising strategy with a potentially short path-to-market is the use of non-conventional wine yeasts to produce wine with reduced ethanol concentration. Non-conventional yeasts present during wine fermentation can have a positive effect on wine composition, flavour and aroma (Domizio et al. 2011), and several are now commercially available through major yeast suppliers (Jolly et al. 2014). Work at the AWRI and other international research groups has shown that some non-conventional yeasts differ substantially from *S. cerevisiae* in how they metabolise carbon, and that they may be effective for producing quality wine with lower ethanol content (Comitini et al. 2011; Contreras et al. 2014). Indeed, the AWRI recently demonstrated that a *M. pulcherrima* strain when used in sequential inoculation was able to produce wine with reduced ethanol concentration in both white and red wine compared to wine produced by *S. cerevisiae* alone (Contreras et al. 2014). Population dynamics of Shiraz fermentations revealed the presence of several indigenous yeast species and one of these, a *S. uvarum* strain, was also able to produce wine with reduced ethanol concentration (Contreras et al. 2015). When used in combination and followed by sequential inoculation with *S. cerevisiae*, *M. pulcherrima* and *S. uvarum* enabled an additional reduction of wine ethanol concentration compared to the same must fermented with either strain alone. Analysis of the volatile flavour profile of white and red wines produced with these two strains

indicated the potential of non-conventional yeast for the production of wines with reduced alcohol concentration and presumptive beneficial volatile composition (Varela *et al.* 2016).

This project evaluated the performance of the most promising non-conventional lower-ethanol yeasts identified at the AWRI during wine fermentation at pilot-scale. Particular attention was paid to establishing the required conditions to ensure the production of wines with reduced ethanol content under these conditions. Additionally, the sensory profile of the resulting wines was evaluated in order to determine the strain(s) or combination of strains able to produce reduced-alcohol wine with positive sensory attributes.

Aims

The aims of the project were to conduct proof of performance evaluations at pilot scale on non-conventional strain(s) or a combination of strains with the lowest ethanol yield and to establish the conditions required at pilot-scale to ensure the production of wines with reduced ethanol concentration, whilst not compromising sensory profile and wine quality.

Specific objectives:

- Evaluate the effect of inoculation rate and yeast inoculation ratio for two candidate non-conventional yeasts on lab-scale production of wine with reduced ethanol content.
- Assess competitiveness of these strains in non-sterile fresh grape must.
- Assess the most suitable inoculation protocols at pilot-scale for the production of wines with reduced ethanol concentration using these strains.
- Determine the effect of these non-conventional yeasts on wine sensory properties.

Methodology

Yeast strains. *M. pulcherrima* AWRI1149 was identified as a strain able to produce wine with reduced ethanol concentration when used in sequential inoculation with *S. cerevisiae.* Although this strain grew normally in white must, it flocculated in red must, making yeast quantification and inoculation very difficult and potentially affecting fermentation kinetics. Therefore, a non-flocculent variant of AWRI1149 was evolved and isolated. *M. pulcherrima* AWRI3050 did not flocculate in red must and retained the ability to produce reduced-alcohol

wine. Thus, for white wine fermentation *M. pulcherrima* AWR1149 and *S. uvarum* AWRI 2846 were assessed in sequential fermentations with *S. cerevisiae* AWRI838, while for red wine fermentation *M. pulcherrima* AWR3050 and *S. uvarum* AWRI 2846 were tested in sequential fermentations with *S. cerevisiae* AWRI838.

Lab-scale fermentations in sterile must. Chardonnay must was filter-sterilised (0.2 μm) while Shiraz must was treated with dimethyl dicarbonate (DMDC) to inhibit the growth of native microorganisms present in the must. Five inoculation regimes were performed (Table 1): a control *S. cerevisiae* inoculation (Treatment Sc); a *M. pulcherrima/S. cerevisiae* sequentially inoculated ferment (Treatment Mp); a *S. uvarum/S. cerevisiae* sequential inoculation (Treatment Su); and two sets of *M. pulcherrima/S. uvarum* ferments where *S. uvarum* was inoculated at different cell densities followed by sequential inoculation with *S. cerevisiae* (Treatments MpSu and MpSu2). Different cell densities for Treatments MpSu and MpSu2 were trialled to test the effect of *S. uvarum* on the metabolism of *M. pulcherrima*. Starter cultures of all yeast strains were grown overnight in YM medium (glucose 20 g/L, yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L) under aerobic conditions at 28°C, shaking at 200 rpm. These cultures were then used to inoculate 100 mL of sterile grape must, diluted 1:1 with water, in 250 mL Erlenmeyer flasks. Flasks were incubated overnight at 22°C with shaking (120 rpm) under aerobic conditions and then used to inoculate grape must. Fermentations were performed in triplicate in 250 mL fermentation flasks equipped with fermentation locks and containing 200 mL of DMDC-treated grape must. Ferments were incubated at 22°C (120 rpm). After 50% of sugar was consumed *S. cerevisiae* AWRI838 was inoculated into each flask to ensure completion of fermentation. Samples were taken for chemical analysis after sugar was completely consumed.

Lab-scale fermentations in non-sterile must. Five non-sterile grape musts were used, two Chardonnay, one Semillon and two Cabernet Sauvignon musts. White wine fermentations were performed in triplicate in 1 L fermentation bottles equipped with fermentation locks containing 1 L free run juice with shaking (120 rpm). Red wine fermentations were performed in triplicate in coffee plungers containing 1 kg of red must; these were incubated statically with the solids cap plunged twice daily. Sulfur dioxide was added prior to inoculations at a concentration of 50 mg/L. Six inoculation regimes were performed (Table 1): a control *S.*

cerevisiae inoculation (Treatment Sc); a *M. pulcherrima/S. cerevisiae* sequentially inoculated ferment (Treatment Mp); a *S. uvarum/S. cerevisiae* sequential inoculation (Treatment Su); two sets of *M. pulcherrima/S. uvarum* ferments where *S. uvarum* was inoculated at different cell densities followed by sequential inoculation with *S. cerevisiae* (Treatment MpSu and MpSu2); and an uninoculated control fermentation (Treatment Un). Fermentations were incubated at 22°C and no sequential inoculation with *S. cerevisiae* was performed. Samples were taken for chemical analysis after sugar was completely consumed.

Table 1. Winemaking treatments in grape must. Yeast strains were inoculated at the indicated cell densities (cells/mL).

	M. pulcherrima AWRI1149/AWRI3050	*S. uvarum* AWRI2846	*S. cerevisiae* AWRI838	
Treatment	Inoculation at t_0	Inoculation at t_0	Inoculation at t_0	50% sugar consumption
Sc	-	-	$1x10^6$	-
Mp	$1x10^6$	-	-	$1x10^6$
Su	-	$1x10^6$	-	$1x10^6$
MpSu	$1x10^6$	$1x10^5$	-	$1x10^6$
MpSu2	$1x10^6$	$1x10^4$	-	$1x10^6$
Un	-	-	-	-

M. pulcherrima AWRI1149 was used to inoculate white musts, while *M. pulcherrima* AWRI3050 was used to inoculate red musts.

Pilot-scale fermentations. Semillon and Merlot musts were treated with DMDC to inhibit the growth of native microorganisms present in the must. Semillon fermentations were performed in triplicate in 30 L fermentation vessels equipped with fermentation locks. Merlot fermentations were performed in triplicate in fermentation vessels containing 50 kg of grapes. Five inoculation regimes were performed (Table 1): a control *S. cerevisiae* inoculation (Treatment Sc); a *M. pulcherrima/S. cerevisiae* sequentially inoculated ferment (Treatment Mp); a *S. uvarum/S. cerevisiae* sequential inoculation (Treatment Su); a *M. pulcherrima/S.*

uvarum sequentially inoculated ferment (Treatment MpSu); and an uninoculated control fermentation (Treatment Un). Semillon and Merlot fermentations were incubated statically at 15°C and 22°C respectively. For red ferments the solids cap was plunged twice daily. Samples were taken during fermentation to determine yeast populations and for chemical analysis after sugar was completely consumed. Merlot wines were inoculated for malolactic fermentation with *Oenococcus oeni* (Lalvin VP41, Lallemand) as recommended by the manufacturer.

Sensory analysis. A panel of eight assessors (one male, seven females) with an average age of 45 years (SD = 10.5) was convened to evaluate the Semillon wines and a panel of nine assessors (one male, eight females) with an average age of 46 years (SD = 10.1) was convened to evaluate the Merlot wines. Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24 °C, in isolated booths under daylight-type lighting, with randomised presentation order. All samples were expectorated. Fifteen samples were presented to assessors three times, in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software (version 2.46, Biosystemes, Couternon, France). For both the Semillon and Merlot wines, each replicate was poured from a separate bottle. The intensity of each attribute was rated using an unstructured 15 cm line scale numbered from 0 to 10, with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data was acquired using Fizz sensory software. Panel performance was assessed using Fizz, Senstools (OP&P, The Netherlands) and PanelCheck (Matforsk) software. Analysis of variance (ANOVA) was carried out using Minitab (Minitab Inc., Sydney, NSW). Following ANOVA, Fisher's least significant difference (LSD) value was calculated (P=0.05).

Statistical analyses

Differences between volatile and non-volatile compounds measured in fermentation samples were determined using analysis of variance (ANOVA). Principal component analysis (PCA) was used for reducing the dimensionality of data and for finding the best differentiation among samples. ANOVA analysis was performed as indicated above. PCA was carried out with Unscambler (CAMO ASA, Oslo, Norway).

Results and discussion

Lab-scale fermentations in sterile must. In Chardonnay both *M. pulcherrima* and *S. uvarum* (Treatments Mp and Su) were able to produce wines with lower ethanol concentration than *S. cerevisiae* wines (1.4% v/v less) (Table 2). Wines produced by mixed cultures *M. pulcherrima/S. uvarum* (MpSu and MpSu2) also showed reduced ethanol concentration compared to the control *S. cerevisiae* wines (1.2 – 1.5 % v/v less). In Shiraz, only the single fermentations with *M. pulcherrima* or *S. uvarum* (Mp and Su) produced wines with lower ethanol concentration than *S. cerevisiae* wines (0.6 – 0.7 % v/v less). Mixed cultures *M. pulcherrima/S. uvarum* (MpSu and MpSu2) failed to produce wines with reduced ethanol concentration. In both Chardonnay and Shiraz all treatments produced wines with no residual sugar. These results confirm the potential of both *M. pulcherrima* and *S. uvarum* to produce wines with reduced ethanol concentration. Although no additional ethanol reduction compared to single cultures was observed for mixed cultures *M. pulcherrima/S. uvarum* as reported previously (Varela *et al.* 2016), it is possible that this finding might be influenced by must composition and consequent effects on yeast population dynamics.

Table 2. Ethanol concentrations from fermentations carried out in sterile musts at laboratory scale.

	Chardonnay		**Shiraz**	
Treatment	**Ethanol [% v/v]**	**Consumed sugar [%]**	**Ethanol [% v/v]**	**Consumed sugar [%]**
Sc	15.9 ± 0.1	100	15.5 ± 0.2	100
Mp	14.5 ± 0.1	100	14.8 ± 0.2	100
Su	14.5 ± 0.3	100	14.9 ± 0.2	100
MpSu	14.4 ± 0.2	100	15.3 ± 0.1	100
MpSu2	14.7 ± 0.3	100	15.5 ± 0.1	100

Chardonnay initial sugar concentration 246 g/L
Shiraz initial sugar concentration 233 g/L

Lab-scale fermentations in non-sterile must. While treatments tested in sterile must (inoculation rate, ratio between strains) showed different wine ethanol concentrations, no differences were found between treatments in any of the five fresh grape musts tested (data not shown). This result suggests that *M. pulcherrima* and *S. uvarum* were not able to compete with the indigenous microorganisms present in the musts at the evaluated inoculation rates.

Pilot-scale fermentations. Three of the Semillon treatments: *S. uvarum* (Su), *M. pulcherrima/S. uvarum* (MpSu) and the uninoculated control (Un) showed lower ethanol concentration than *S. cerevisiae* wines. Treatments Su, MpSu and Un had 0.4% v/v, 0.7% v/v and 0.3% v/v less ethanol concentration, respectively (Table 3). Several differences among treatments were observed for volatile flavour compounds, including ethyl- and acetate-esters, higher alcohols and low-molecular weight sulfur compounds (Figure 1). Interestingly, *S. cerevisiae* and *M. pulcherrima* wines were higher in ethyl acetate, ethyl hexanoate, ethyl octanoate and 2-methylbutyl acetate, all compounds associated with fruity sensory descriptors. These wines also contained less 2-methyl propanol and 2&3-methyl butanol than other treatments, compounds which are associated with negative sensory descriptors at elevated concentrations.

Principal component analysis (PCA) enabled visualisation of the key differences between treatments based on volatile composition (Figure 2). Wines from different treatments grouped separate to each other, with wines fermented with *S. cerevisiae* and wines inoculated with *M. pulcherrima* being very different to the wines from other treatments. *S. cerevisiae* and *M. pulcherrima* wines were associated with some ethyl esters (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate) and the acetate esters 2-methylpropyl acetate and 2-methylbutyl acetate. *M. pulcherrima* wines were higher in carbon disulfide than *S. cerevisiae* wines. Uninoculated wines were characterised by higher concentration of the sulfur compounds hydrogen sulfide and methanethiol.

Table 3. Ethanol concentrations from fermentations conducted in pilot-scale trials in DMDC-treated must.

Treatment	Semillon		Merlot	
	Ethanol [% v/v]	**Consumed sugar [%]**	**Ethanol [% v/v]**	**Consumed sugar [%]**
Sc	10.6 ± 0.1[a]	100[a]	14.7 ± 0.1[a]	99.7[a]
Mp	10.5 ± 0.1[a]	100[a]	13.9 ± 0.1[b]	99.7[a]
Su	10.2 ± 0.1[b]	99.9[a]	13.5 ± 0.1[c]	99.6[a]
MpSu	9.9 ± 0.1[c]	99.9[a]	13.9 ± 0.1[b]	99.7[a]
Un	10.3 ± 0.1[b]	100[a]	14.0 ± 0.1[b]	99.7[a]

Semillon initial sugar concentration 150 g/L
Merlot initial sugar concentration 246 g/L

The different volatile composition observed between treatments resulted in distinct wine sensory profiles (Figure 3). *S. cerevisiae* and *M. pulcherrima* wines (Treatments Sc and Mp) showed higher scores for positive sensory descriptors, particularly attributes associated with fruit flavour and aroma. At the same time, these wines showed low scores for negative sensory descriptors such as 'yeasty' and 'sweaty/cheesy'. Conversely, wines fermented with *S. uvarum* (Treatments Su and MpSu) and uninoculated wines (Treatment Un) were characterised by low scores in positive sensory attributes ('overall fruit flavour', 'overall fruit aroma', 'stone fruit flavour', 'citrus aroma' and 'floral'), and high scores in negative sensory attributes ('salt', 'yeasty' and 'sweaty/cheesy').

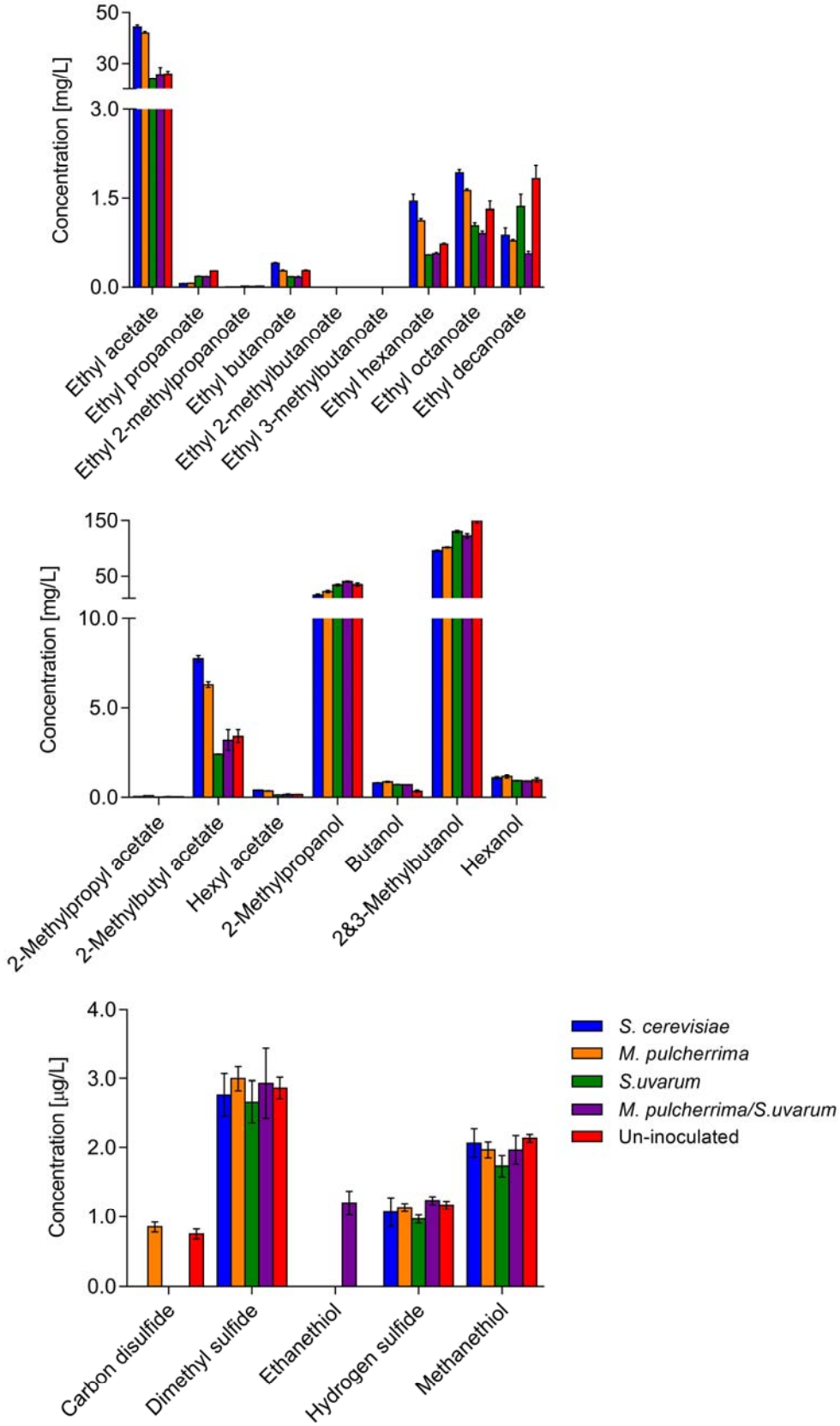


Figure 1. Volatile chemical composition of Semillon wines fermented with non-conventional yeasts in sequential inoculation trials. All ferments were carried out in triplicate in DMDC-treated must at pilot-scale.

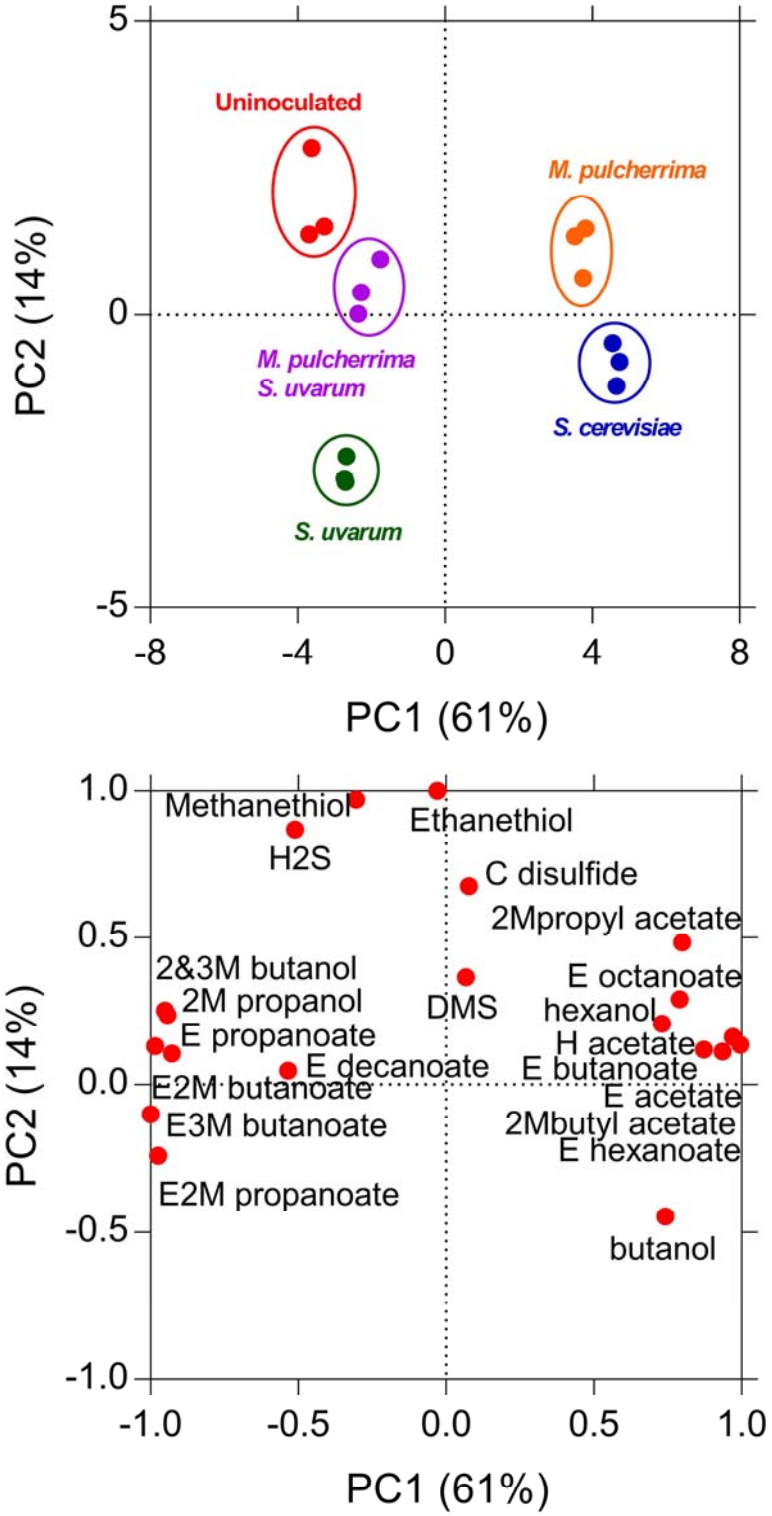


Figure 2. Principal component analysis of volatile fermentation products in Semillon wines. Scores and loading for the first two principal components. All ferments were carried out in triplicate in DMDC-treated must at pilot-scale

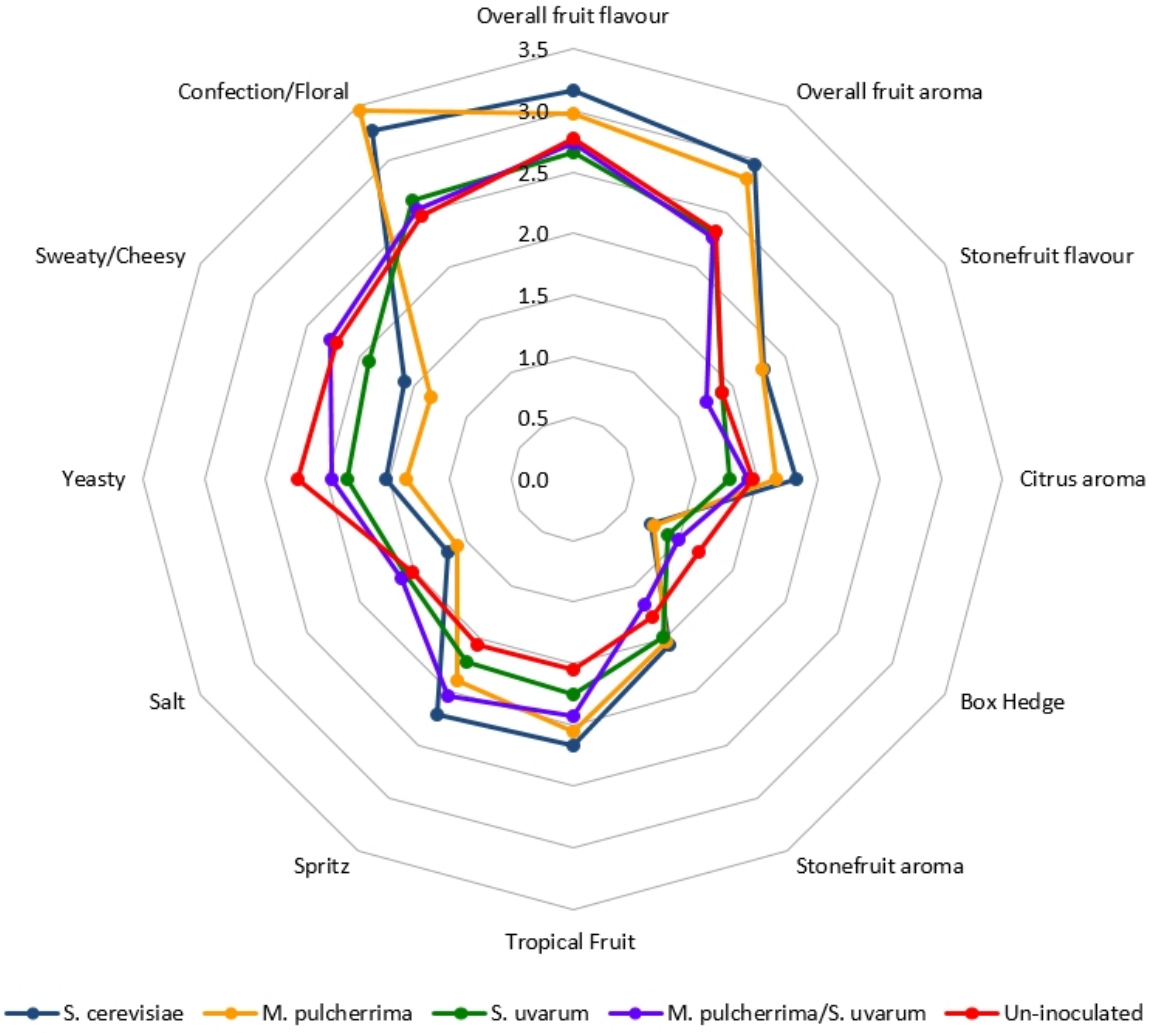


Figure 3. Sensory profile of Semillon wines fermented with non-conventional yeasts in sequential inoculation trials. Only statistically significant sensory descriptors are included. All ferments were carried out in triplicate in DMDC-treated must at pilot-scale.

In Merlot all treatments showed reduced wine ethanol concentration relative to wines made with *S. cerevisiae*. While Treatments Mp, MpSu and Un had 0.8% v/v lower ethanol concentration, Treatment Su showed 1.2% v/v less (Table 3). As was the case for Semillon wines, differences were found for volatile chemical composition among treatments (Figure 4). *S. cerevisiae* wines showed the lowest concentration for ethyl acetate, 2-methylbutyl acetate and hydrogen sulfide; and the highest concentration of ethyl butanoate, ethyl hexanoate and ethyl octanoate. Wines from Treatments Mp, MpSu and Un showed similar

concentration of most volatile compounds, although large differences were found for dimethyl sulfide and hydrogen sulfide which were highest for uninoculated wines; both compounds are associated with negative sensory descriptors. *S. uvarum* wines (Su) were significantly different to other treatments showing the lowest concentrations of ethyl hexanoate, ethyl octanoate, ethyl decanoate and dimethyl sulfide; and the highest concentration of ethyl propanoate, ethyl 2-methyl propanoate, ethyl 3-methyl butanoate and 2&3-methyl butanol.

Differences in volatile aroma compound composition across the treatments were evident when visualised according to their principal components (Figure 5). Wines inoculated with *S. cerevisiae* were associated with the ethyl esters ethyl butanoate, ethyl hexanoate and ethyl octanoate. Wines inoculated with *M. pulcherrima* (Treatments Mp and MpSu) clustered together and were associated with carbon disulfide, methanethiol, dimethyl sulfide and hexyl acetate. Uninoculated wines were associated with hydrogen sulfide and clustered close to *M. pulcherrima* wines. Wines fermented with *S. uvarum* were very different from other wines and were associated with several ethyl esters (ethyl-2-methyl propanoate, ethyl-2-methyl butanoate, ethyl-3-methyl butanoate) and the higher alcohols butanol and 2&3-methyl butanol.

Differences in appearance, aroma and flavour were found among treatments (Figure 6). Wines from Treatments Sc, Mp and Un showed similar sensory profiles characterised with high scores for positive sensory descriptors including ‘purple tinge’, ‘overall fruit flavour’ and ‘overall fruit aroma’, ‘floral’ and ‘red fruit’ attributes; and low scores for negative sensory attributes such as ‘acid’, ‘vegetal’, ‘meaty’ and ‘barnyard’. As observed in the Semillon trial, wines inoculated with *S. uvarum* (Treatments Su and MpSu) were associated with high scores for negative sensory attributes and low scores for positive sensory descriptors. These wines were high in ‘vegetal’, ‘meaty’ and ‘barnyard’; and low in ‘purple tinge, ‘overall fruit aroma, ‘mint aroma’, ‘floral’ and ‘red fruit aroma’.

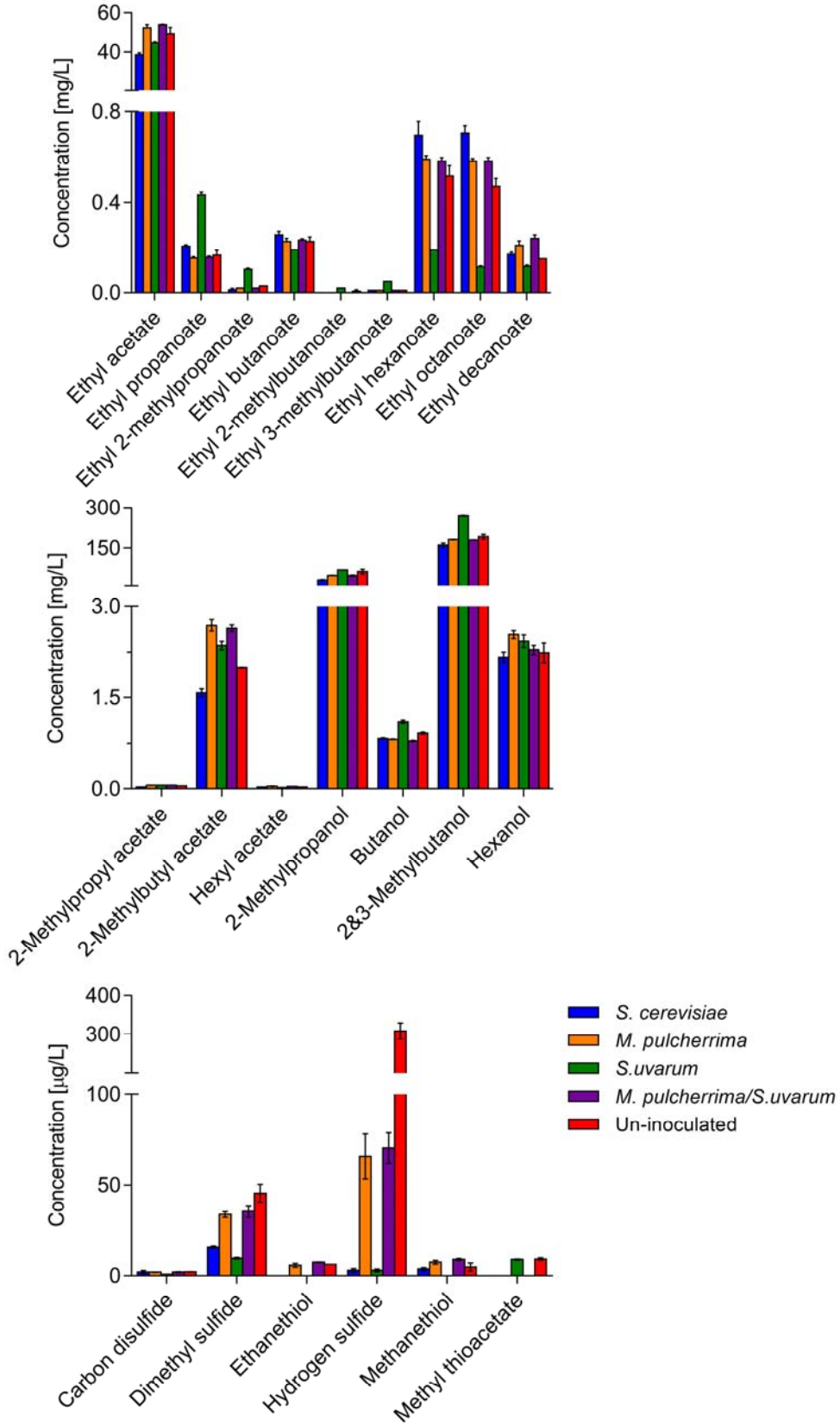


Figure 4. Volatile chemical composition of Merlot wines fermented with non-conventional yeasts in sequential inoculation trials. All ferments were carried out in triplicate in DMDC-treated must at pilot-scale.

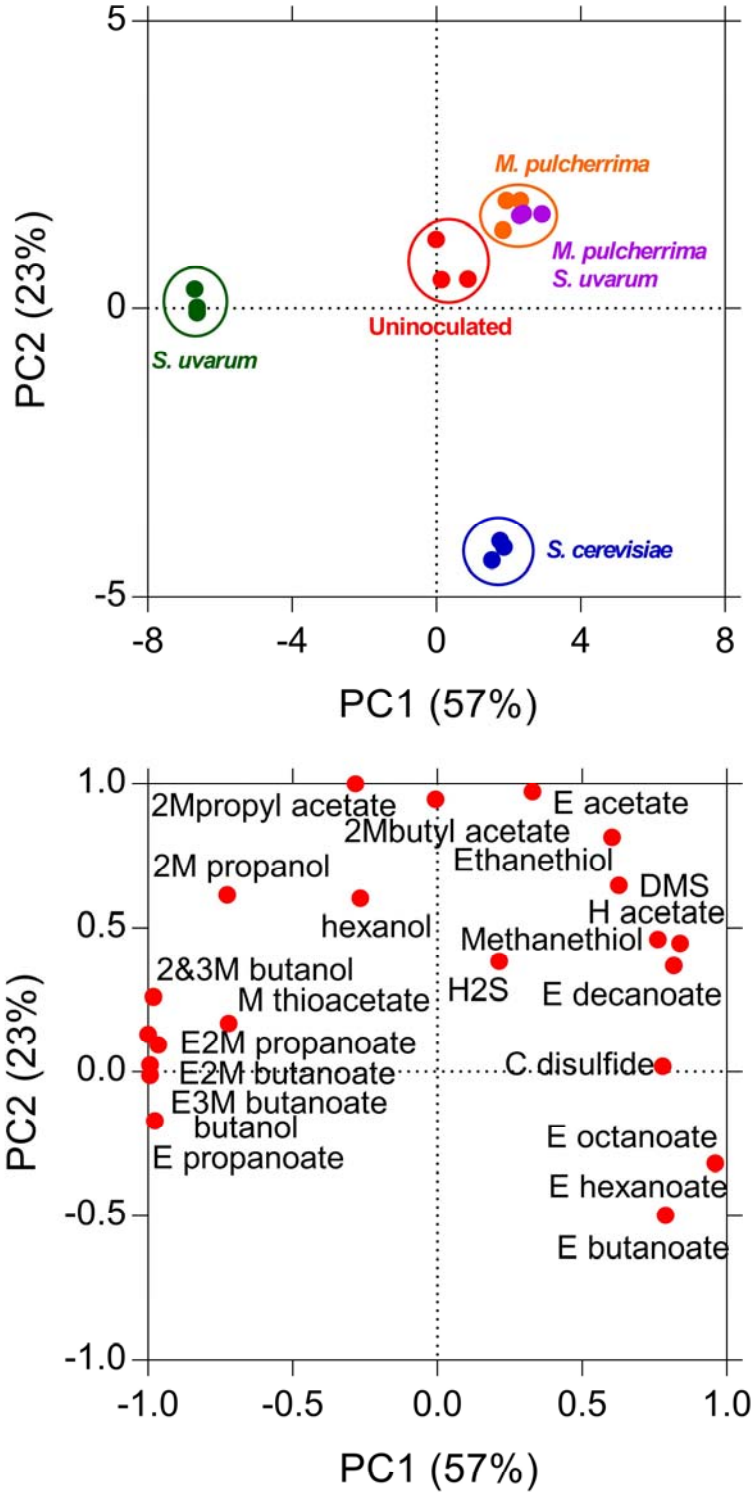


Figure 5. Principal component analysis of volatile fermentation products in Merlot wines. Scores and loading for the first two principal components. All ferments were carried out in triplicate in DMDC-treated must at pilot-scale

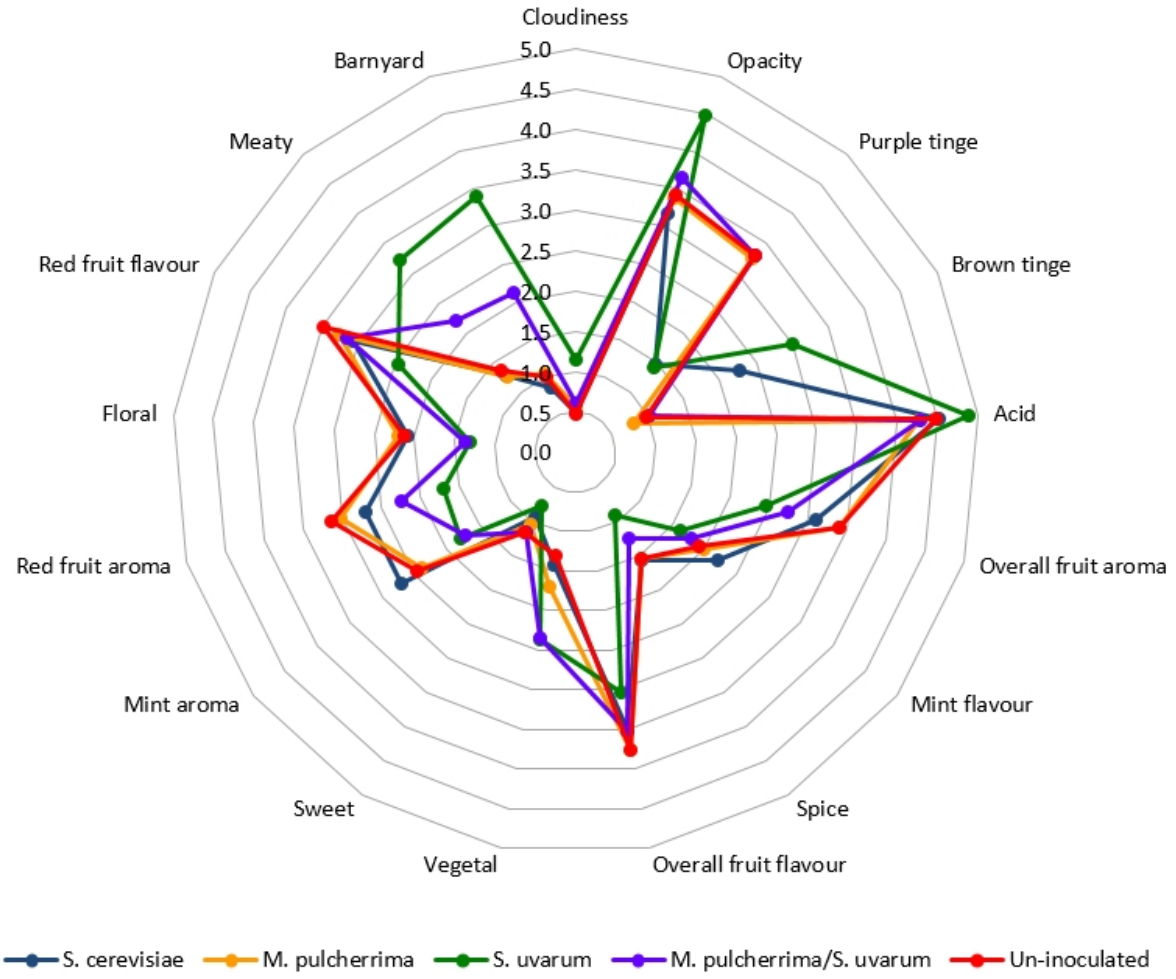


Figure 6. Sensory profile of Merlot wines fermented with non-conventional yeasts in sequential inoculation trials. Only statistically significant sensory descriptors are included. All ferments were carried out in triplicate in DMDC-treated must at pilot-scale.

It is clear from these results that although *S. uvarum* strain AWRI2846 can contribute to wines exhibiting reduced ethanol concentration, the sensory profiles of these wines are dominated by negative sensory attributes. It is interesting to note that the volatile chemical composition of these wines does not indicate any potential negative sensory attributes, suggesting that compounds associated with the negative descriptors ('meaty', 'barnyard', etc.) are not amongst those identified by the standard methods employed, or are the result of interactive

effects between compounds. This is especially relevant giving the increasing interest winemakers are showing in the use of non-conventional yeasts and spontaneous fermentation.

In both pilot-scale trials *M. pulcherrima* AWRI1149/3050 exerted minimal influence on chemical composition or sensory profile. In the Semillon trial *M. pulcherrima* wines were most similar to the *S. cerevisiae* controls, while in the Merlot trial they resembled the non-inoculated wines.

Conclusions and recommendations

Both non-conventional yeasts, *M. pulcherrima* and *S. uvarum,* were able to produce wines with reduced ethanol concentration when inoculated in sterile must. However, they were not able to compete with indigenous microorganisms in grape must and failed to produce reduced-ethanol wines when inoculated in non-sterile must at laboratory-scale

S. uvarum AWRI2846 was effective at producing reduced-alcohol wines at pilot-scale in DMDC-treated must, but unfortunately this strain produced wines characterised by negative sensory descriptors and in its current form appears to be poorly suited for commercial winemaking. Since the volatile profile of wines fermented with this strain did not indicate any potential sensory issues, further work could be performed to investigate compounds associated with negative descriptors such as 'meaty' and 'barnyard', using techniques such as gas-chromatography-olfaction (GCO).

M. pulcherrima, whilst not imparting negative sensory characteristics, was ineffective at producing wines with reduced alcohol concentration at pilot-scale, most likely due to competition from other microorganisms. It is possible that higher inoculation rates (> $1x10^6$ cells/mL) would be required to overcome this lack of competitive fitness in non-sterile musts. Alternatively, adaptive evolution strategies may be applied to increase the competitiveness of these strains. It is advisable that all future work on non-conventional yeasts in winemaking,

regardless of whether targeting reduced alcohol or other desirable traits, includes provision to evaluate and where necessary improve their competitiveness in fresh grape must.

In any future work considering robustness and off-flavours produced by non-conventional yeast, it would be worthwhile to include low-ethanol *S. cerevisiae* mutants obtained at the AWRI which produce reduced-alcohol wines. These conventional strains also impart flavour defects and remain untested in terms of robustness in fresh grape musts. Optimisation of the application of multiple yeasts in parallel will be more efficient and increase the probability of success.

References

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M. 2011. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiol.* 28:873-882.

Contreras, A., Curtin, C., Varela, C. 2015. Yeast population dynamics reveal a potential 'collaboration' between *Metschnikowia pulcherrima* and *Saccharomyces uvarum* for the production of reduced alcohol wines during Shiraz fermentation. *Appl. Microbiol. Biotechnol.* 99:1885-1895.

Contreras, A., Hidalgo, C., Henschke, P.A., Chambers, P.J., Curtin, C., Varela, C. 2014. Evaluation of non-*Saccharomyces y*easts for the reduction of alcohol content in wine. *Appl. Environ. Microbiol.* 80:1670-1678.

Domizio, P., Romani, C., Lencioni, L., Comitini, F., Gobbi, M., Mannazzu, I., Ciani, M. 2011. Outlining a future for non-*Saccharomyces* yeasts: Selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. *Int. J. Food Microbiol.* 147:170-180.

Godden, P., Wilkes, E., Johnson, D. 2015. Trends in the composition of Australian wine 1984-2014. *Aust J Grape Wine Res.* 21:741-753.

Jolly, N.P., Varela, C., Pretorius, I.S. 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res.* 14:215-237.

Palacios, A., Raginel, F., Ortiz-Julien, A. 2007. Can the selection of *Saccharomyces cerevisiae* yeast lead to variations in the final alcohol degree of wines? *Aust. NZ Grapegrower Winemaker* 527:71-75.

Varela, C., Dry, P.R., Kutyna, D.R., Francis, I.L., Henschke, P.A., Curtin, C.D., Chambers, P.J. 2015. Strategies for reducing alcohol concentration in wine. *Aust. J. Grape Wine Res.* 21:670-679.

Varela, C., Kutyna, D., Henschke, P.A., Chambers, P.J., Herderich, M.J., Pretorius, I.S. 2008. Taking control of alcohol. *Aust. NZ Wine Ind. J.* 23:41-43.

Varela, C., Sengler, F., Solomon, M., Curtin, C. 2016. Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. *Food Chem.* 209:57-64.