AB Biotek and the Australian Wine Research Institute (AWRI) collaboration brings two new Solutions for floral Aroma and Flavour in the Maurivin Next Generation wine yeast range, Maurivin Rosa AWRI 2965 and Maurivin Rosa Intense AWRI 2940.

Stop and smell the roses: novel yeast that impart 'floral' aromas in wine

Across the wide range of commercial yeast strains available to winemakers, there is substantial variation in their production of aroma compounds. Some desirable compounds are not typically produced at high enough concentrations to make a difference to wine aroma and flavour. Examples include the yeast-derived compounds 2-phenylethanol (2-PE) and 2-phenylethyl acetate (2-PEA), which are associated with 'rose' and 'floral' aromas in wine, as well as in other fermented foods and beverages (Cordente 2012). Generally, the concentrations of these 'rose' aroma compounds in wines are below their aroma sensory thresholds, particularly in white wines, so their potential contribution to wine aroma is considered to be minimal (de-la-Fuente-Blanco 2016; Vilanova 2013).

Trialling yeast that overproduce 'floral'/'rose' aroma compounds

By using classical yeast strain development techniques, more than forty non-genetically modified (non-GM) yeasts that produce high concentrations of both 'rose' aroma compounds 2-PE and 2-PEA were generated at the AWRI (Cordente 2018). These 'rose' yeasts were isolated from three different parent *Saccharomyces cerevisiae* strains, with each having different fermentation volatile profiles and competitive fitness characteristics that might suit different winemaking styles.

Three of these 'rose' yeasts were extensively trialled at pilot scale over the last four vintages to assess wine styles that may be compatible with enhanced 'rose' aroma characteristics, including white, rosé, red and sparkling wines. In addition, by selecting 'rose' yeast that produce different amounts of 2-PE and 2-PEA (moderate and high), it was possible to fine-tune concentrations for specific wine styles.

Figure 1 shows the 2-PE concentration produced by two of these 'rose' yeasts after alcoholic fermentation of different grape cultivars, after cellaring in bottle between 3 and 15 months. Regardless of the style of wine, the 'rose' yeast produced significantly higher concentrations of 2-PE than commercially available strains (trial controls) widely used in the wine industry. Depending on the variety assessed, these 'rose' yeasts produced between 2 and 12 times more 2-PE than the controls (Figure 1), and well above its sensory threshold (10 mg/L). Similar increases were also observed for 2-PEA, which has an even lower detection threshold (0.25 mg/L).

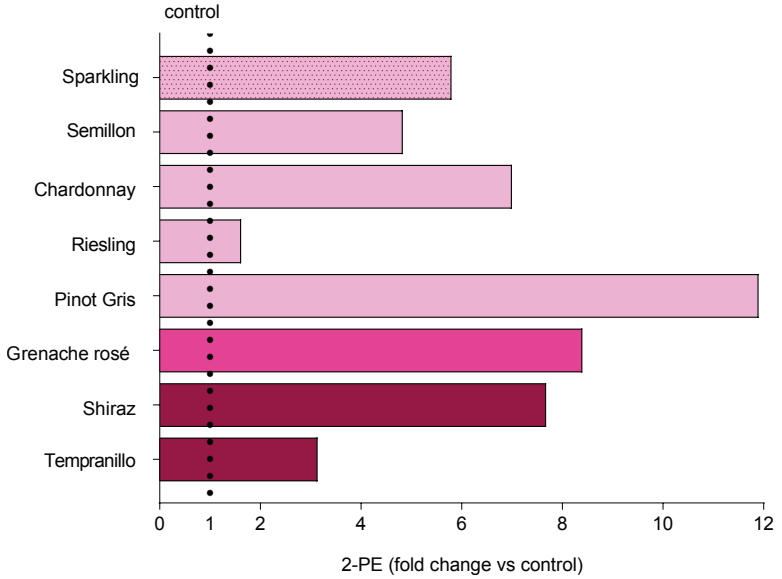

Figure 1. Fold change in 2-PE production between 'rose' yeasts and a control strain in different wine styles. The relative amount of 2-PE produced by the control strain was normalised to 1. The scale of the pilot-scale winemaking trials was 20 litres for the white and rosé wines, and 50 kg for the red wines. White, sparkling and rosé wines were fermented with strain AWRI 2965; while AWRI 2940 was used for the red wines.

Sensory effects

These wines were subjected to either informal sensory evaluation or formal quantitative sensory descriptive analysis using the highly trained AWRI sensory panel, to determine whether differences in wine volatile concentrations resulted in differences in wine aroma and flavour. Wines produced with the 'rose' yeasts were always compared with those made with a commercially available strain as a control. The sensory data demonstrate that the 'rose' yeast had the largest sensory effect in white varieties, particularly those with neutral or less aromatic profiles such as Chardonnay and Pinot Gris.

Case study 1: Chardonnay

In the 2018 vintage the sensory effects of two different 'rose' yeast strains (one moderate-2-PE-producing [AWRI 2965], and one high 2-PE-producing [AWRI 2940]) were assessed in making Chardonnay wine from Adelaide Hills grapes. Both yeasts produced wines that were more highly rated in 'rose' aroma and flavour than the control (Figure 2). The 'rose' aroma intensity scores correlated with the amount of 2-PE produced by each strain. Conversely, the 'rose' yeast wines were rated lower than the control wine for the negative attribute 'cooked vegetable/potato' aroma. Interestingly, the 'rose' aromas were more prominent after 12 months in bottle than at 3 months, highlighting the stability of higher

alcohol-based aromas, as opposed to some 'fruity' or 'estery' aromas that tend to decrease with time in bottle.

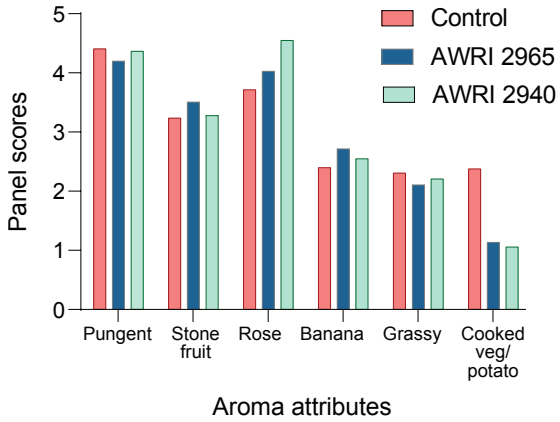

Figure 2. Mean ratings for aroma attributes for 2018 Chardonnay wines (20-litre scale) produced using a control, and a moderate (AWRI2965) and high (AWRI2940) 2-PE-producing yeast. Wines were produced in three fermentation replicates and assessed by a panel of 10 judges.

Case study 2: Shiraz

The use of 'rose' aroma yeasts may not necessarily complement the flavour profile of all varieties, in particular red wines. An example sensory profile of Shiraz wine (50 kg ferments) made using a standard red winemaking strain (control) and the high 2-PE-producing strain (AWRI 2940) is provided in Figure 3.

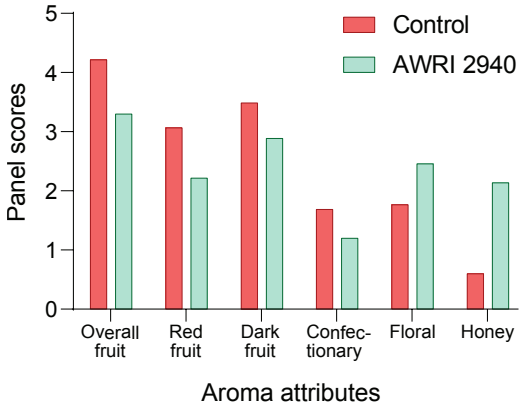

Figure 3. Mean attribute scores for Shiraz wines made using a control yeast and the high PE-producing yeast AWRI 2940. Wines were produced in three fermentation replicates (50 kg each) and assessed by a panel of 10 judges.

Shiraz wines made with AWRI 2940 yeast were rated higher in 'floral' and 'honey' aromas in a formal sensory analysis, and in this sense their sensory profiles were comparable to the Chardonnay wines. However, other aroma attributes typically seen in Shiraz were masked, with the wines scoring lower in 'overall fruit', 'dark fruit' and 'red fruit' aromas, as well as in 'confectionary' (Figure 3). In this study the wines made with the 'rose' yeast were considered unbalanced on the palate and did not meet expectations for the flavour profile of Shiraz wine.

Case study 3: Sparkling wines

In 2019 two 'rose' yeast strains that produce moderate amounts of 2-PE were evaluated for their suitability for Chardonnay sparkling wine production. The sparkling wines in the trial were produced using the traditional method (bottle fermented), with hand-picked Chardonnay grapes from the Adelaide Hills. Different strain combinations were used for the primary and secondary fermentations to discover the stage in which the 'rose' yeast would have the greatest effect on the finished wine. Chardonnay must was partitioned into 30-litre vessels and inoculated with either the control strain or one of the two 'rose' yeasts. After completion of both alcoholic and malolactic fermentations, base wines were cross-flow filtered. Sugar was added to each of the base wines at a level that would result in ~ 6 Bar pressure following secondary fermentation. The base wines were then split and inoculated with either the control strain or one of the 'rose' yeast strains to initiate secondary fermentation, which was completed in bottle. The wines were matured for eight months on yeast lees and disgorged, with sugar added to achieve a style similar to a Brut wine.

The largest effect was achieved when the 'rose' yeasts were used during primary fermentation, with concentrations of 2-PE between five and seven times higher when a 'rose' yeast was used compared to the control strain (Figure 4). The amount of 2-PE produced during secondary fermentation was limited compared to that produced during the primary fermentation, with increases of around 20–25 mg/L compared to the control (Figure 4).

Sensory analysis of the wines revealed that both 'rose' yeasts produced sparkling wines with distinctive sensory properties. In a reflection of the 2-PE production profile, yeast strain choice during primary fermentation had the greatest sensory impact. While the 'rose' yeasts were able to successfully complete secondary fermentation, the sensory attributes of the finished wines were indistinguishable from those in which the control strain was used for secondary fermentation. The use of AWRI 4390 for primary fermentation resulted in sparkling wines with the most interesting attributes, as this strain brought forward more intense 'rose' aromas and flavours than the control, as well as more intense 'apple' aromas (Figure 5, green). Surprisingly, and even though wines fermented with AWRI 2965 had the

highest 2-PE levels overall, these were not characterised by intense 'rose' or 'fruity' aromas, but instead displayed a 'brioche' aroma (Figure 5, blue). In addition, these wines showed some bitterness and were noted as having more foam persistence (crown).

These results showed that AWRI 4390 may offer winemakers an alternative yeast with which to produce a distinctive base wine with desirable 'floral' aroma and low bitterness for fresh and fruity sparkling styles; conversely, AWRI 2965 due to its less fruity and more oxidative aroma profile may be better suited to more austere sparkling wine styles.

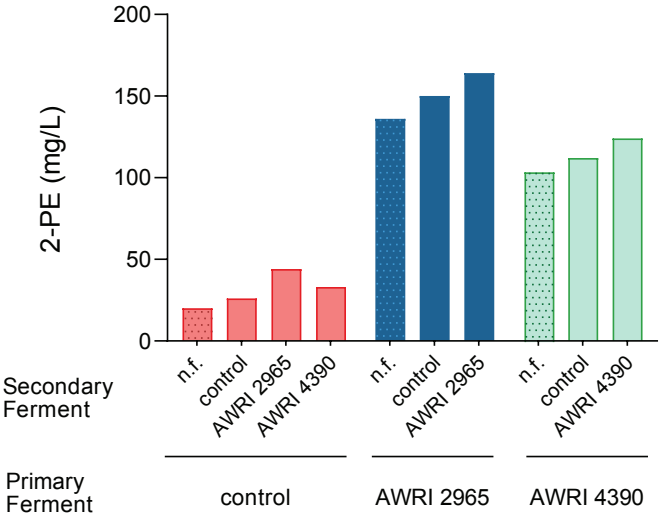

Figure 4. Production of 2-PE (mg/L) at different phases of the sparkling winemaking process by combinations of two moderate 'rose' yeasts (AWRI 2965 and AWRI 4390) and a control strain. Bars with the same colour denote wines produced with the same yeast for primary fermentation (red for control, blue for AWRI 2965 and green for AWRI 4390), while the yeast used for secondary fermentation is also indicated. Base or non-fermented (n.f.) wine is shaded.

Conclusion

Different wine yeast strains produce divergent profiles of aroma compounds, and the choice of strain to conduct the alcoholic fermentation can have a substantial effect on the final aroma and flavour of wine. Trials of newly developed 2-PE overproducing 'rose' yeasts in producing white wines found an especially large effect on Pinot Gris and Chardonnay wines. The use of 'rose' yeast might also be suitable for light rosé styles but seems to be less appropriate for red winemaking.

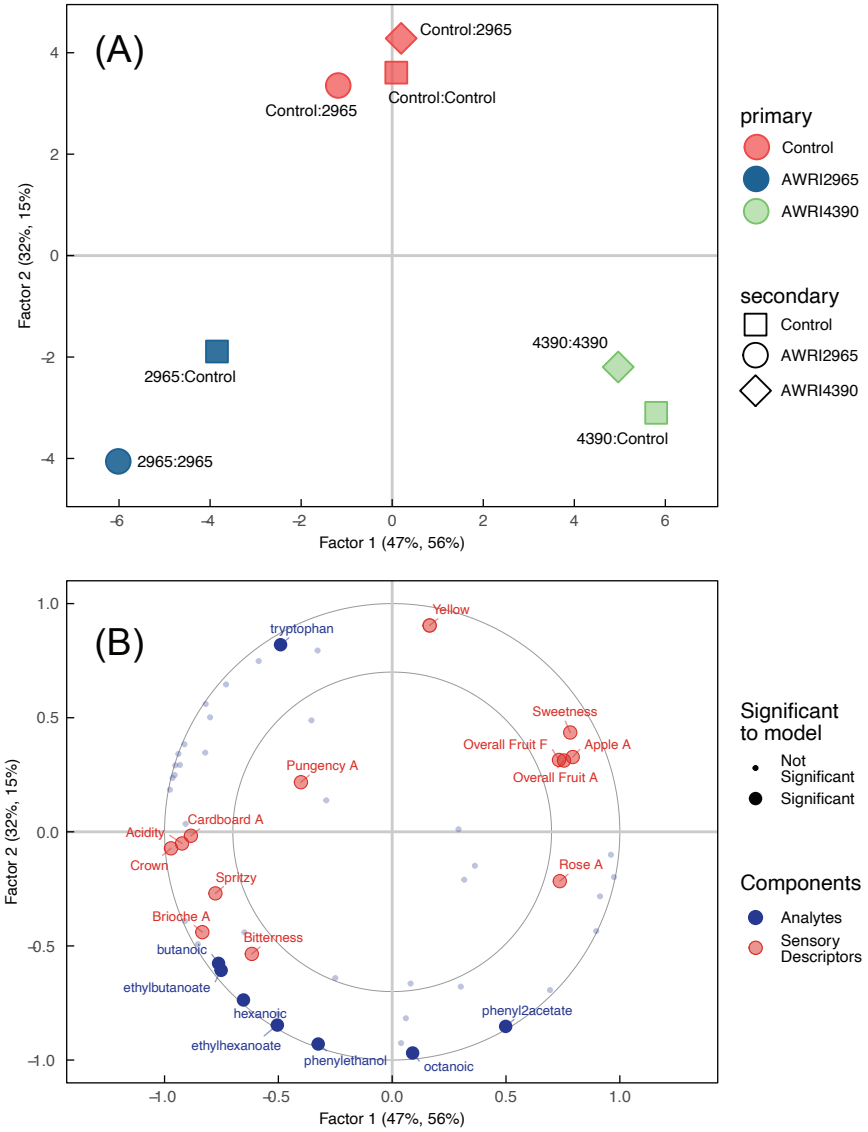

Figure 5. Factors 1 and 2 for the scores (A) and loadings (B) plots from partial least squares regression models for the sparkling wine trial. Compounds important to the model are indicated by large dots with non-significant analytes indicated by small unlabelled dots. Different colours in the scores plot indicate different yeast strains used in primary fermentation and different shapes indicate different yeast strains used in secondary fermentation.

More research is needed to select the right 'rose' yeast for sparkling wine production; although one of the two strains assessed (AWRI 4390) in the work described here was shown to have potential. This strain produced sparkling wines with a distinctive aroma profile while also exhibiting robust fermentation kinetics. Two 'rose' yeast strains have been commercialised by AB Biotek, in association with Wine Australia, with the names AWRI Rosa (AWRI 2965) and AWRI Rosa Intense (AWRI 2940).

Acknowledgements

This work was supported by Australia's grapegrowers and winemakers through their investment body, Wine Australia, with matching funds from the Australian Government. The AWRI is a member of Wine Innovation Cluster in Adelaide, South Australia. The authors thank John Gledhill for his valuable contribution to the winemaking and members of the AWRI's sensory panel for their efforts.

References

Cordente, A.G., Solomon, M., Schulkin, A., Francis, I.L., Barker, A, Borneman, A.R., Curtin, C.D. 2018. Novel wine yeast with *ARO4* and *TYR1* mutations that overproduce 'floral' aroma compounds 2-phenylethanol and 2-phenylethyl acetate. Appl. Microbiol. Biotechnol. 102(14): 5977–5988.

Cordente, A.G., Curtin, C.D., Varela, C., Pretorius, I.S. 2012. Flavour-active wine yeasts. Appl. Microbiol. Biotechnol. 96(3): 601–18.

Vilanova, M., Genisheva, Z., Graña, M., Oliveira, J.M. 2013. Determination of odorants in varietal wines from international grape cultivars (*Vitis vinifera*) grown in NW Spain. S. Afr. J. Enol. Vitic. 34(2): 212–222.

de-la-Fuente-Blanco, A., Saenz-Navajas, M.P., Ferreira, V. 2016. On the effects of higher alcohols on red wine aroma. Food Chem. 210: 107–114.

Toni Cordente, Research Scientist, *toni.garciacordente@awri.com.au*
Simon Schmidt, Research Manager
Damian Espinase Nandorfy, Scientist
Leigh Francis, Research Manager, Sensory and Flavour
Mark Solomon, Senior Scientist
Wes Pearson, Senior Scientist