Technical notes

Yeast strain selection – an easy and effective way to drive wine style in Grenache

During alcoholic fermentation of grape juice *Saccharomyces cerevisiae* produces a wide range of volatile compounds such as esters, higher or fusel alcohols, fatty acids and volatile sulfur compounds. These compounds play a crucial role in the aroma and flavour profiles of wines, both in a positive and a negative way. For example, varietal thiols are significant contributors to fruity aromas in Chardonnay and Sauvignon Blanc wines, while other sulfur-containing compounds have negative sensory attributes. The relative balance of yeast-derived flavour compounds can determine whether a wine is perceived as 'confectionary', 'cheesy/sweaty', or 'fruity'.

Different wine yeast strains produce varying amounts of these compounds, which means that choice of wine yeast can be a simple and effective way to optimise and differentiate wine style, while conducting risk-free fermentations. There is not yet a full understanding of the variation in wine sensory profiles produced by different wine yeast strains. This is particularly the case for red winemaking where surprisingly little information is available about the effect of yeast strains on the final flavour and aroma of red wines. A current research project at the AWRI is evaluating a large number of strains, partly from the AWRI's extensive wine microorganism culture collection, to assess the most promising performers for red wine fermentation.

Evaluating the potential of wine yeast strains for fermentation of red grape varieties

A series of laboratory-scale fermentations were conducted with small volumes of Shiraz juice (90 mL), using 100 strains, representative of the broader genetic diversity of wine yeast. Clustering of strains according to their overall volatile aroma profiles revealed six major groups. Replicated pilot-scale winemaking trials were then undertaken to determine whether the volatile profiles observed at laboratory scale would also be observed at a larger scale. Grenache fruit was used, to allow the assessment of the effect of the strains in a relatively light and fruity variety where their influence might be more prominent. Six strains were chosen as representatives of the six groups (Table 1). Four of these strains are commercially available from yeast manufacturers. Strain AWRI2914 was found to be a high ester and fruity thiol producer, currently used mainly for white wine production. This strain was particularly chosen to assess the effect of the production of high levels of aromatic thiols in red wines. AWRI1833 and AWRI2260 can be best characterised as robust yeasts that can ferment

fructose, with a relatively neutral volatile profile. Finally, the two winery isolates, AWRI778 and AWRI1776, are characterised by diverse organic acid profiles.

Small-lot wines were produced at the Hickinbotham-Roseworthy Wine Science laboratory at Urrbrae. Grenache grapes from a high-value McLaren Vale (SA) vineyard were hand picked (total soluble solids 14.7°Baumé, pH 3.64, TA 4.2, YAN 249 mg/L) and crushed with no SO_2 added prior to fermentation. Crushed and destemmed grapes were partitioned into 50 kg lots and inoculated with the six strains in Table 1. Wines were pressed at dryness and seeded for malolactic fermentation (MLF). The TA of the wines was adjusted to a constant value prior to bottling. No oak was used in the winemaking. All the yeast strains fermented at a similar rate and ferments were dry (less than 2 g/L sugar) after 8–9 days, except for winery isolate AWRI1776 which took 13 days (Figure 1).

Basic chemical analysis of the wines after pressing showed a few initial differences between strains. The most obvious was their potential for sulfite production, and while three of the strains produced no detectable SO_2, wines made with AWRI2260 and AWRI2878 had 33 and 71 mg/L of total SO_2, respectively (Table 2). Wines made with AWRI2878 were unable to complete MLF due to the combination of high SO_2 and high ethanol content. Wines made with AWRI2260 took almost four months to finish MLF, while wines made with the other strains took six weeks on average. In addition, wines differed in their organic acid content, as

Table 1. Wine yeast strains used in this study, and brief description of their aromatic/chemical characteristics and their recommended use by yeast manufacturers

AWRI strain number	Origin	Recommended use	Chemical/aromatic profile
AWRI778	Winery isolate (Australia)		High acetic acid, low succinic acid, low fruity acetate ester
AWRI1776	Winery isolate (Australia)		Low acetic acid, high reductive aroma and high fruity acetate ester
AWRI1833	Commercial strain	High sugar musts/restarting stuck fermentations (able to ferment fructose)	Low fruity acetate ester, moderate ethyl ester producer
AWRI2260	Commercial strain	Difficult fermentation conditions, both white and red wines	Neutral aroma
AWRI2878	Commercial strain	Red wine	Low volatile sulfur compound producer
AWRI2914	Commercial strain	White wine	High fruity acetate ester and high aromatic thiol producer

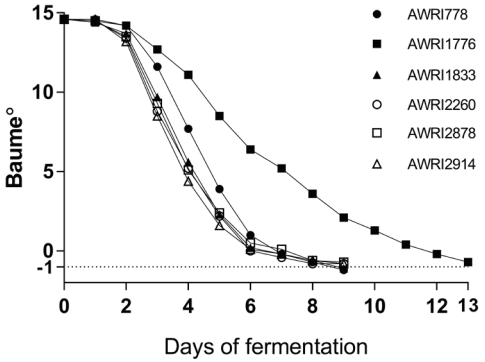

Figure 1. Fermentation kinetics of each of the six wine yeast strains in Grenache ferments (50 kg), as monitored by °Baumé (initial value of 14.7). Fermentations were initially conducted at 15°C, and when °Baumé was below 1 (Day 6), they were transferred to 20°C.

Table 2. Summary of the volatile and non-volatile compounds produced by the six wine yeast strains in Grenache wines. The concentration units, and the aroma descriptors of the individual volatile compounds or the groups of compounds, are indicated in parentheses. The results are the average of triplicate ferments.

Chemical compound	AWRI 778	AWRI 1776	AWRI 1833	AWRI 2260	AWRI 2878	AWRI 2914
Main fermentation compounds (g/L)						
Alcohol (% v/v)	16.1	16.0	16.1	15.9	15.9	15.9
Acetic acid	0.80	0.17	0.31	0.16	0.30	0.23
Succinic acid	0.94	1.46	1.04	1.22	1.30	1.17
Glycerol	7.1	8.3	7.8	7.5	8.2	8.0
SO_2 production (free/total) (mg/L)	0 / 0	0 / 0	0 / 0	0 / 33	6 / 71	0 / 15
Esters (mg/L)						
Ethyl acetate (nail polish remover)	60.6	43.0	32.0	41.4	53.5	53.5
Acetate esters (fruity, banana)	0.45	1.80	0.16	0.91	0.74	1.56
Ethyl esters (fruity, floral)	0.64	0.87	1.11	1.26	1.04	1.30
Higher alcohols (mg/L)						
2-phenylethanol (rose, floral)	17.4	34.5	19.7	35.5	26.0	42.4
Isoamyl alcohol + isobutanol (fusel, spirituous)	192	427	221	308	228	271
Volatile sulfur compounds (µg/L)						
Hydrogen sulfide (rotten egg)	0.9	0.8	0.4	0.4	0.4	0.3
Methanethiol (rotten cabbage)	1.1	2.8	1.3	1.4	1.4	1.1
Methyl thioacetate (cheesy)	0	9.8	0	1.9	0	3.2
3-mercaptohexanol (3-MH) (ng/L)	217	476	461	334	422	668

observed in the laboratory-scale ferments. AWRI778 produced the highest concentrations of acetic acid (0.80 g/L), while strains AWRI1776 and AWRI2260 showed low acetic acid production (0.16 g/L).

Yeast strains driving differences in aroma active compounds

Analysis of volatile aroma compounds was undertaken nine months after the wines had been bottled (Table 2). Sulfur-containing compounds with negative characteristics such as methanethiol ('cabbage') and methyl thioacetate ('cheesy'), were produced at unusually high concentrations by AWRI1776, as observed in the laboratory-scale ferments. Strains also differed greatly in their ability to release the fruity aromatic thiols 3-MH and 3-mercaptohexyl acetate (3-MHA). AWRI2914 produced 668 ng/L of 3-MH, almost twice as much as the average of the other wines in this work (380 ng/L) (Figure 2), and substantially higher than the concentrations of 3-MH found in a recent AWRI survey of thiols in commercially available red wines (average in Grenache ~ 300 ng/L). This strain also produced the highest concentration of the 'rose/floral' compound 2-phenylethanol. Two strains stood out as higher producers of fruity acetate esters (AWRI1776 and 2914), while strain AWRI1833 was characterised by particularly low concentrations of these compounds.

So the wines are chemically very different, how do they taste?

The wines were subjected to sensory descriptive analysis, using the highly trained AWRI sensory panel, to determine whether the differences in wine chemistry resulted in important differences in aroma and flavour. There were large sensory differences in the wines produced

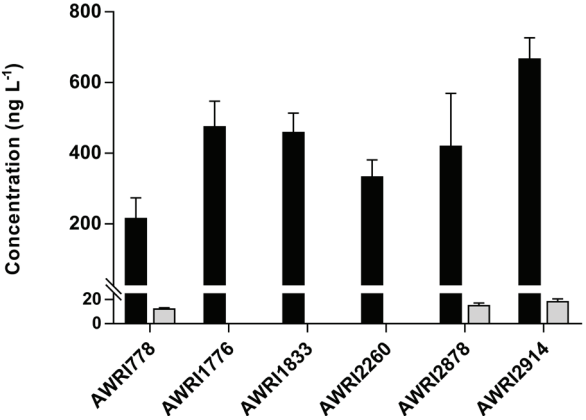

Figure 2. Production of the fruity thiols 3-mercaptohexanol (3-MH) (in black) and 3-mercaptohexyl acetate (3-MHA) (in grey) by each of the strains assessed in Grenache wines. Concentrations of these compounds are the average of triplicate ferments. In aqueous alcohol solution, the odour thresholds of 3-MH and 3-MHA are 60 and 4 ng/L, respectively.

using the six yeast strains (Figure 3). As expected, wines made with AWRI2260, considered a neutral strain, were scored somewhere in the middle for many of the sensory attributes assessed. Even though strains AWRI1833 and AWRI2914 showed very different chemical profiles, they were rated similarly for many of the aroma attributes, particularly in 'overall intensity', 'red fruit', 'floral' and 'confectionary' aroma. AWRI778 was rated highly in 'nail polish remover' (volatile acidity) aroma and flavour, which reflects the high acetic acid and ethyl acetate concentrations produced by this strain. Strain AWRI1776 was rated high in 'earthy' and 'drain' (reductive) aromas, and was also low in 'red fruit' and 'floral' aroma characters despite the high concentrations of esters and 2-phenylethanol. This is likely to be due to the high concentrations of methanethiol and methyl thioacetate in these wines acting to mask the fruit-related aromas.

These results indicate that two of the strains assessed, AWRI1833 and AWRI2914, might be more suitable for red fermentations, particularly those with high sugar content and therefore the potential to produce high ethanol concentrations. Both strains produced low SO_2 concentrations under stressful conditions, which facilitated the completion of MLF in a reasonable time (six weeks), in comparison with the other strains that produced moderate to high levels of SO_2. In addition, wines produced with both these strains were rated highly in the desirable sensory attributes such as 'red fruit' and 'confectionary' aroma, and rated lowest in

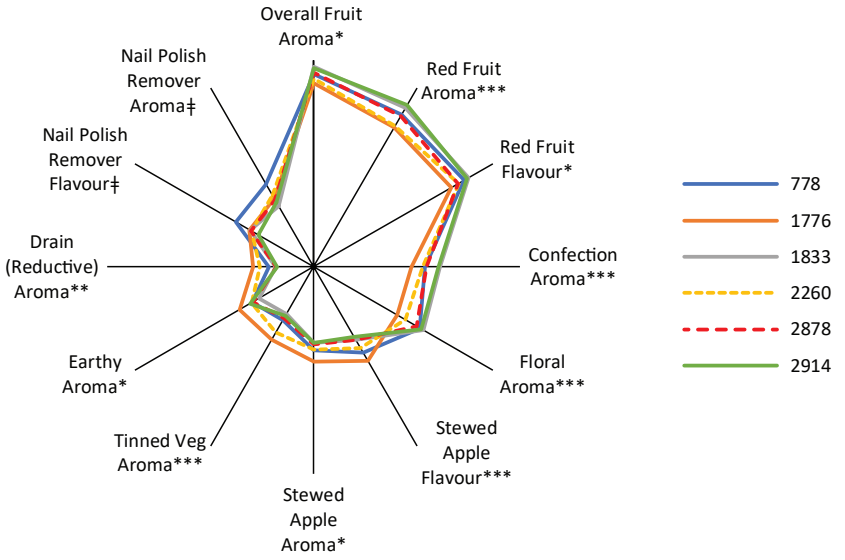

Figure 3. Mean sensory attribute intensity scores for the wines made with the six strains. Significance levels are as follows: P <0.05*, P <0.01**, P <0.001*** ; and approaching significance (‡ P< 0.10).

the undesirable 'nail polish remover' (volatile acidity) and 'drain' (reductive) characters. Interestingly, these strains, and especially AWRI2914, also produced high concentrations of the fruity thiols 3-MH and 3-MHA. These compounds can impart desirable 'tropical fruit' characters, and are important contributors to the flavour of Sauvignon Blanc and Chardonnay wines. Further work is planned to determine whether these compounds have contributed to the 'red fruit' character in these Grenache wines.

Conclusion

The choice of yeast strain for primary fermentation can have a large effect on the final aroma and flavour of Grenache wine. While a strain such as AWRI1776 was found to be not a good choice for red fermentation due to its slow fermentation kinetics and propensity to form reductive aromas, it might prove to be a valuable candidate for research studies to investigate the formation of reductive sulfur compounds and to identify strategies to minimise their formation. Interestingly, a strain commonly used for white winemaking, AWRI2914, was found to be very suitable for ripe Grenache fruit. Further studies into yeast strain effects are currently underway. These studies will help winemakers control the degree to which yeasts contribute to the aromas of their wines, and to choose the right strain depending on their desired style of wine, while avoiding the formation of undesirable compounds.

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