Enhancing red wine complexity using novel yeast blends

The influence of yeast on wine composition has been well established, particularly for white grape varieties such as Sauvignon Blanc where key aroma compounds are released from flavourless precursors during fermentation. Different yeast species, and indeed different strains of the same species, produce varying amounts of important flavour-active compounds such as esters, higher alcohols, volatile fatty acids and volatile sulfur compounds. This means that commercially available *Saccharomyces cerevisiae* wine strains can be used to impart aroma profiles consistent with specific wine styles (AWRI publication #1514), and some wines made by spontaneous fermentation can contain a more complex mix of flavour compounds (AWRI publication #1123).

What options are there if a winemaker is aiming for 'complexity' but doesn't want the risks associated with spontaneous fermentation? And how can a winemaker optimise the production of flavour-active metabolites that require pathways present in more than one yeast? *Saccharomyces* interspecific hybrids are providing options to enhance wine diversity while retaining fermentation performance (AWRI publications #1304, #1527). Additionally there are various non-*Saccharomyces* yeasts associated with spontaneous fermentation that are now commercially available. Often these are intended for use in sequential inoculation where the non-*Saccharomyces* strain is allowed a period of time to impart its metabolic imprint on the ferment before inoculation with a dominant *Saccharomyces cerevisiae* strain that will complete fermentation. Some yeasts are also made available in blends for co-inoculation (Table 1). Controlled yeast co-inoculation has been shown to produce Sauvignon Blanc wines perceived as higher in desirable 'fruity' attributes and lower in undesirable 'faulty' attributes (AWRI publication #1002) that are preferred by consumers (AWRI publication #1199). In another study 'unique' and 'more complex' Chardonnay wines were produced by co-inoculation of Burgundian yeast isolates compared with single industrial yeast strains (Saberi et al. 2012).

Table 1. Examples of blended yeast products

Product	Supplier	Contains	Purpose of blend
Alchemy I & II	Anchor Wine Yeast	Three different *Saccharomyces* strains	Release and conversion of volatile thiol precursors, ester production
Melody.nsac	Chr. Hansen	*Kluyveromyces thermolerans, Torulaspora delbrueckii, Saccharomyces cerevisiae*	Enhance complexity through action of non-*Saccharomyces* yeasts
Unity	AB Mauri	Two different *Saccharomyces cerevisiae* strains	Production of high quality Chardonnay

Choice of yeast is also important for fermentation of red grape varieties. Yeast-derived esters have a strong influence on red- and blackberry-fruit aromas (Lytra et al. 2013), and tannin concentration in wine can vary by up to 25-30% depending on the yeast strain used for fermentation (AWRI publication #1542). Recent work at the AWRI aimed to generate novel variants of a commercial wine strain (Anchor N96) with divergent flavour profiles, and then combine these novel strains in blends designed to enhance complexity of red wine aroma.

Generating novel strains with divergent flavour profiles using non-GM methods

Variants of Anchor N96 were isolated using two inhibitors that target the enzymes involved in production of flavour-active metabolites. Variants able to grow on these inhibitors should have changes in the sequence of genes encoding the flavour-related enzymes that alter their activity. Yeasts selected on one of these inhibitors were found to produce very high levels of certain volatile fatty acids and their ethyl esters, while yeasts selected on the second inhibitor produced high levels of acetate esters. The impact of these mutations on wine flavour profile was evaluated by fermenting 20 litres of a Chardonnay juice and performing sensory and chemical analyses. The flavour attributes that were most influenced are summarised in Figure 1.

Strain AWRI2362 imparted 'fennel', 'pineapple' and 'sweaty-cheesy' aromas in addition to very high 'overall fruit flavour', while strain AWRI2394 imparted 'confectionary' and 'nail-polish remover' aromas. Given that these two strains were derived from the same original parent strain, these differences illustrate the exceptional metabolic malleability of *Saccharomyces cerevisiae*.

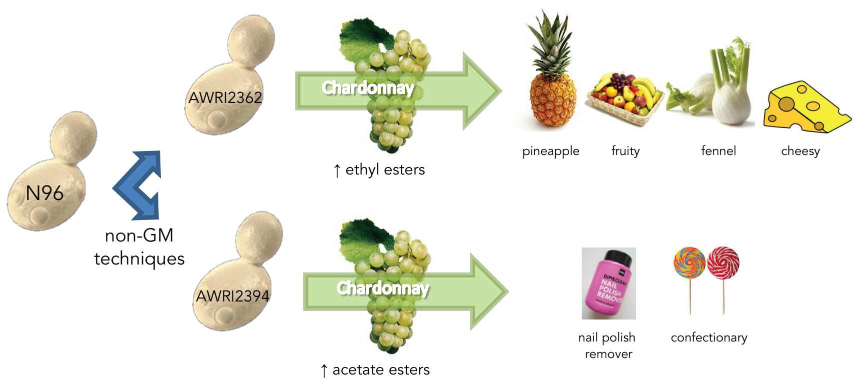


Figure 1. Descriptors associated with N96-derived isolates AWRI 2362 and 2394 after fermentation of a Chardonnay grape juice (20 L)

Evaluating the potential of yeast blends for fermentation of red grape varieties

The next goal was to see if red wines with high levels of 'fruit flavour' could be made with enhanced 'complexity' by introducing more 'savoury' characters through co-inoculation with the two new strains blended at varying ratios with commercially available red wine strains of *Saccharomyces cerevisiae*. Ferments of Merlot grapes (40 kg) were conducted with three co-inoculations and three single strain commercial wine yeasts. The co-inoculated 40 kg Merlot ferments proceeded at similar rates to the individual commercial wine strains included in each co-inoculation (Figure 2), indicating their compatibility, and the survival of AWRI2362 and AWRI2394 in the ferments was tracked by selective plating. In each case the proportions of strains in the co-inoculations were maintained throughout fermentation.

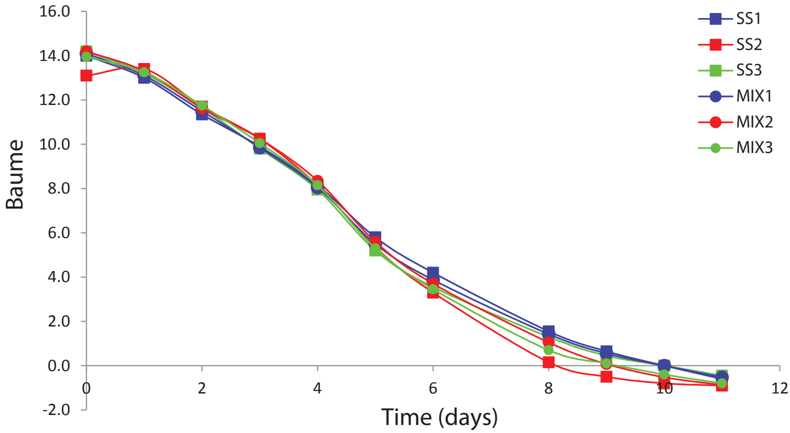


Figure 2. Fermentation kinetics monitored by sugar consumption over time for three single strains and three co-inoculated ferments (SS1,2,3 – single strain ferments; MIX1,2,3 – co-inoculated ferments)

Each co-inoculation produced different profiles of volatile metabolites, and the levels of key marker compounds ethyl hexanoate (green apple, fennel) and ethyl octanoate (apricot, floral) were correlated with the amount of strain AWRI2362 in the co-inoculation (Figure 3). In a similar fashion, the levels of 2&3-methylbutyl acetate (banana) were also correlated with the percentage of strain AWRI2394 in the co-inoculation. In addition, increasing the proportion of strain AWRI2394 in the co-inoculations (Mix 2 and 3), resulted in a reduction in the levels of hydrogen sulfide when compared to the commercially available red wine strains (SS2 and SS3, respectively).

As an example of sensory impact, Merlot wines made with Mix 3 were described as 'fruity', 'floral', 'blackcurrant' and 'savoury', while the dominant 'reductive' and 'hydrogen sulfide' characters of the corresponding single inoculated strain (SS3) were minimised (Figure 4).

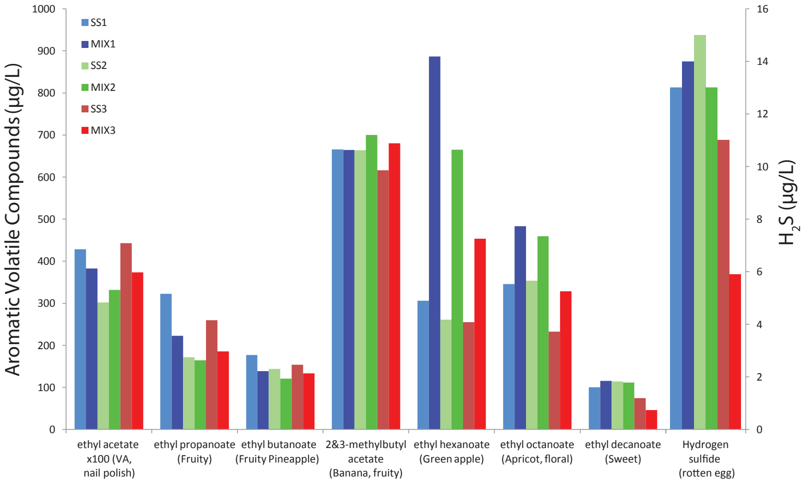
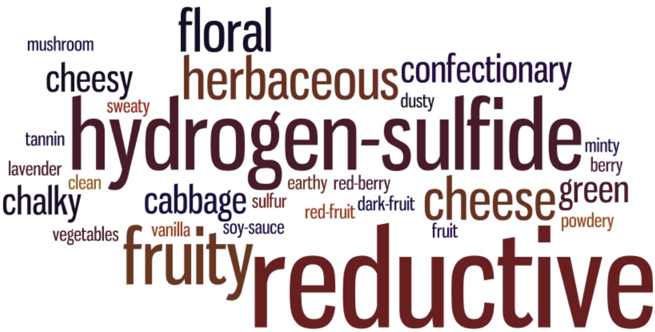


Figure 3. Aroma profiles of Merlot wines including standard fermentation volatiles and hydrogen sulfide concentrations (SS1,2,3 – single strain ferments; MIX1,2,3 – co-inoculated ferments)

SS3



MIX 3

Figure 4. Word cloud images of descriptors used by panellists to describe Merlot wines made by SS3 and Mix 3. The larger the word, the more frequently it was used by panellists to describe the wine

Conclusion

Novel variants of Anchor N96 have been generated that produce divergent profiles of aroma compounds. Novel yeast blends based upon this work are currently being optimised and are expected to be available in active-dry form for trial in vintage 2015. Please contact the AWRI if you are interested in trialling them.

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