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Sensory profile and volatile aroma composition of reduced alcohol Merlot wines fermented with *Metschnikowia pulcherrima* and *Saccharomyces uvarum*

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ABSTRACT

Strategies for production of wines containing lower alcohol concentrations are in strong demand, for reasons of quality, health, and taxation. Development and application of wine yeasts that are less efficient at transforming grape sugars into ethanol has the potential to allow winemakers the freedom to make lower alcohol wines from grapes harvested at optimal ripeness, without the need for post-fermentation processes aimed at removing ethanol. We have recently shown that two non-conventional wine yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum* were both able to produce wine with reduced alcohol concentration. Both species produced laboratory-scale wines with markedly different volatile aroma compound composition relative to *Saccharomyces cerevisiae*. This work describes the volatile composition and sensory profiles of reduced-alcohol pilot-scale Merlot wines produced with *M. pulcherrima* and *S. uvarum*. Wines fermented with *M. pulcherrima* contained 1.0% v/v less ethanol than *S. cerevisiae* fermented wines, while those fermented with *S. uvarum* showed a 1.7% v/v reduction in ethanol. Compared to *S. cerevisiae* ferments, wines produced with *M. pulcherrima* showed higher concentrations of ethyl acetate, total esters, total higher alcohols and total sulfur compounds, while wines fermented with *S. uvarum* were characterised by the highest total concentration of higher alcohols. Sensorially, *M. pulcherrima* wines received relatively high scores for sensory descriptors such as *red fruit* and *fruit flavour* and overall exhibited a sensory profile similar to that of wine made with *S. cerevisiae*, whereas the main sensory descriptors associated with wines fermented with *S. uvarum* were *barnyard* and *meat*. This work demonstrates the successful application of *M. pulcherrima* AWRI3050 for the production of pilot-scale red wines with reduced alcohol concentration and highlights the need for rigorous evaluation of non-conventional yeasts with regard to their sensory impacts.

1. Introduction

The ethanol content of wine is largely determined by the ripeness, and hence sugar content, of grapes. Market demand for wines containing lower ethanol concentrations (Rowley, 2013) has seen a number of studies into the feasibility of pre- and post-fermentation strategies that reduce the concentration of sugar available for fermentation into ethanol, and reduce the concentration of ethanol in finished wine (Belisario-Sanchez et al., 2009; Bindon et al., 2013; Schmidtke et al., 2012; Stoll et al., 2010; Varela et al., 2015).

Ideally, these strategies should reduce ethanol levels without compromising wine flavour, quality, consumer acceptance or increasing the cost of production. Earlier harvest of grapes shows promise for moderate reductions in final ethanol concentration while not impacting on consumer acceptability (Bindon et al., 2014). Substantial decreases in ethanol concentration can be achieved by post-fermentation processes, however these can substantially alter wine volatile composition and sensory profile (Belisario-Sanchez et al., 2009; King and Heymann, 2014) and also impact on consumer preferences (King and Heymann, 2014; Meillon et al., 2010a, b). Using wine yeast to produce wine with reduced alcohol content remains one of the simplest and least expensive approaches for winemakers to employ, and could be used in combination with other strategies.

Saccharomyces cerevisiae, the principal yeast species used in winemaking, is very efficient at producing ethanol from sugars under most environmental conditions, and although some natural variability can be found among wild isolates of this species, existing *S. cerevisiae* wine strains generate comparable alcohol concentrations when fermenting the same must (Ciani et al., 2016). Research has therefore focused on generating new *S. cerevisiae* strains that produce less alcohol than

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traditional wine yeast during fermentation (Ehsani et al., 2009; Kutyna et al., 2010; Tilloy et al., 2014; Varela et al., 2012); and isolating non-conventional yeasts that metabolise sugar without generating ethanol or do so with less efficiency (Ciani et al., 2016; Varela, 2016).

Non-conventional yeast, which include non-*Saccharomyces* and non-*cerevisiae* yeast, are part of the natural microbiota present on grapes, and harvesting and winemaking equipment, and are present at least during the early stages of fermentation (Fleet and Heard, 1993; Renouf et al., 2006; Renouf and Lonvaud-Funel, 2007). The use of non-conventional yeast is increasingly popular particularly for their effects on wine composition, flavour, aroma and colour (Jolly et al., 2014; Varela, 2016) and for their potential to produce reduced-alcohol wines (Ciani et al., 2016; Varela, 2016). Some of the mechanisms responsible for reduced ethanol yields include altered biomass synthesis, by-product formation and/or alternative regulation of respiration.

While some non-*Saccharomyces* yeast require moderate application of oxygen during fermentation to grow and impact on wine composition (Gonzalez et al., 2013), and indeed to produce wine with reduced ethanol concentration (Contreras et al., 2015a; Quiros et al., 2014), Contreras et al. (2014) identified a *Metschnikowia pulcherrima* strain able to produce wine with reduced ethanol concentration when sequentially inoculated with *S. cerevisiae* without aeration. Furthermore, when studying yeast population dynamics during Shiraz fermentations, an indigenous *Saccharomyces uvarum* yeast strain, which was also able to produce wine with reduced ethanol concentration, was also isolated (Contreras et al., 2015b). When used in combination, these strains of *M. pulcherrima* and *S. uvarum* behaved additively, reducing final ethanol concentrations in both white and red wines to a greater extent than either strain alone (Varela et al., 2016). Preliminary data on volatile aroma composition suggested that some treatments may affect the sensory properties of wine.

Here we describe the sensory profile and volatile aroma composition of pilot-scale Merlot wines produced with *M. pulcherrima* AWRI3050 and *S. uvarum* AWRI2846. While wines fermented with both yeast strains showed reduced ethanol concentration compared to *S. cerevisiae* wines, volatile and sensory profiles differed substantially between wines produced with non-conventional yeasts. This work shows for the first time the use of amplicon-based ITS phylotyping for validating the role of non-conventional yeasts in pilot-scale wine fermentation.

2. Materials and methods

2.1. Microorganisms and media

Saccharomyces cerevisiae AWRI838, *Metschnikowia pulcherrima* AWRI3050 and *Saccharomyces uvarum* AWRI2846 were obtained from the Australian Wine Research Institute (AWRI) Wine Microorganism Culture Collection. *M. pulcherrima* AWRI3050 is a non-flocculant derivative obtained from *M. pulcherrima* AWRI1149. Briefly, a 80 mL sample from the end of fermentation of sterile Shiraz (200 mL) was allowed to settle at room temperature for 20 min. A 1 mL sample was then taken from the top of the settled culture and used to inoculate a flask containing 200 mL of sterile Shiraz. After a third selection step, samples from the top of settled cultures were plated and single colonies isolated. Colonies were identified as *M. pulcherrima* following PCR and RFLP of the 5.8S rRNA gene as described previously (Contreras et al., 2015b). *M. pulcherrima* AWRI3050 showed no significant differences in wine basic composition to *M. pulcherrima* AWRI1149 (Table S1). Cryogenically preserved (− 80 °C) strains were cultured and maintained on YM plates (3 g/L malt extract, 3 g/L yeast extract, 5 g/L peptone, 10 g/L glucose, 16 g/L agar) and stored at 4 °C.

2.2. Pilot-scale fermentations

Unlike *S. uvarum* AWRI2846, *M. pulcherrima* AWRI3050 is not able to finish wine fermentation unaided and requires co- or sequential-inoculation with *S. cerevisiae*. Therefore, four treatments were performed to evaluate *M. pulcherrima* AWRI3050 and *S. uvarum* AWRI2846 in Merlot must: a control *S. cerevisiae* inoculation (AWRI838, 1×10^6 cells/mL); a *M. pulcherrima/S. cerevisiae* co-inoculated ferment (*M. pulcherrima* 1×10^6 cells/mL, *S. cerevisiae* 1×10^5 cells/mL); a *S. uvarum* inoculation (1×10^6 cells/mL); and an uninoculated control fermentation. Merlot must was prepared from grapes obtained from the Riverland region (South Australia) and contained 240 g/L of sugar (equal amounts of glucose and fructose), 190 mg N/L of yeast assimilable nitrogen (YAN) and 6.2 g/L titratable acidity (determined to pH 8.2) with a pH of 3.2. Fermentations were performed in triplicate at 22 °C in fermentation vessels containing 50 kg of randomised grapes and 50 mg/kg potassium metabisulfite, which were stored at 10 °C overnight. Twenty-four hours before inoculation Merlot must was treated with 125 mg/L dimethyl dicarbonate (DMDC) to reduce the population of native microorganisms.

Starter cultures of all yeast strains were grown overnight in YM medium under aerobic conditions at 28 °C, shaking at 120 rpm. These cultures were then used to inoculate 1 L of sterile Merlot, diluted 1:1 with water, in 5 L Erlenmeyer flasks. Flasks were incubated overnight at 22 °C with shaking (120 rpm) under aerobic conditions and then used to inoculate Merlot must. Ferments were incubated at 22 °C and the solids cap was plunged twice daily. Samples were taken during fermentation to determine yeast populations and basic chemical composition. After sugar was completely consumed Merlot wines were inoculated for malolactic fermentation with *Oenococcus oeni* (Lalvin VP41, Lallemand) as recommended by the manufacturer.

2.3. Analytical techniques

Ethanol, glucose, fructose, glycerol and organic acids were quantified by high-performance liquid chromatography (HPLC) using a BioRad HPX87H column as described previously (Varela et al., 2004). Analysis of higher alcohols, acetate-, and ethyl esters was performed by Metabolomics Australia (Adelaide) using gas chromatography–mass spectrometry (GCMS) using a stable isotope dilution analysis (SIDA) as previously described (Bizaj et al., 2012). Yeast-derived sulfur-containing volatiles (carbon disulfide, diethyl disulfide, dimethyl sulfide, ethanethiol, ethyl thioacetate, hydrogen sulfide, methanethiol and methyl thioacetate) were determined by using headspace cool-on-column gas chromatography coupled with sulfur chemiluminescence detection (GC-SCD), with ethylmethyl sulfide and propyl thioacetate as internal standards (Siebert et al., 2010). 4-Ethylphenol and 4-ethylguaiacol were analysed according the method of Pollnitz et al. (2000).

2.4. Determination of microbial populations

Total DNA was isolated from each sample using the PowerFood Microbial DNA isolation kit (Mobio, California, USA). Proportions of each microbial strain were assessed using amplicon-based ITS phylotyping as described previously (Sternes et al., 2017). Briefly, 1.0 ng of total DNA from each sample was subjected to a two-step PCR process that amplifies a portion of the fungal ribosomal ITS region (Bokulich and Mills, 2013) while adding both custom in-line barcodes and sequences necessary for Illumina sequencing (including compatible Illumina dual-indexes). Following sequencing, raw reads were first quality and adaptor trimmed (Trimmomatic (Bolger et al., 2014); Cutadapt (Martin, 2011)), with paired-end reads overlapped (Magoc and Salzberg, 2011) to form a single contiguous ITS synthetic read. Reads were then assigned to samples and timepoints using a combination of both the in-line and Illumina barcodes using custom python scripts. Operational taxonomic units (OTUs) were clustered *de novo* using Swarm v2.0 (Mahe et al., 2014) and taxonomies assigned using the "*assign_taxonomy.py*" functionality of QIIME (Caporaso et al., 2010) in conjunction with a modified version of the QIIME UNITE fungal ITS