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Coinoculated Fermentations Using *Saccharomyces* Yeasts Affect the Volatile Composition and Sensory Properties of *Vitis vinifera* L. cv. Sauvignon Blanc Wines

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Alcoholic fermentation using *Saccharomyces* wine yeast is an effective means of modulating wine aroma. This study investigated the impact of coinoculating commercial yeast strains (Vin7, QA23, Vin13) on the volatile composition and sensory profile of Sauvignon Blanc wines. Small-scale replicated fermentations were conducted using single-strain and coinoculations of Vin7 with QA23 and with Vin13. The results showed that the chemical and sensory profiles of the coinoculated wines were different from both the single-strain wines and equal blends of the single-strain wines. Volatile thiol analysis indicated that the Vin7/QA23 coinoculated wines were highest in 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), although this pattern was not observed for the Vin7/Vin13 yeast combination. The negative *white vinegar* aroma and high volatile acidity measured in the Vin7 single-strain wines were not present in the coinoculated wines. This study demonstrates that coinoculations can modify the aroma profile of wines, when complementary yeasts are used.

KEYWORDS: Wine; yeast; *Saccharomyces cerevisiae*; coinoculation; mixed culture; aroma; sensory; thiols

INTRODUCTION

A vast number of volatile compounds are formed and modulated by yeast during alcoholic fermentation that significantly impact the flavor and overall quality of wines. In this way, controlling alcoholic fermentation is an effective method for modulating wine aroma.

Volatile thiols are a group of aroma compounds whose significance to wine aroma, particularly Sauvignon Blanc wines, has been widely studied (*1–3*). Some of the most important of these are 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), which have aromas described as *box hedge*, *passionfruit*, *grapefruit* and *blackcurrant*. Research has shown that yeasts are responsible for the release and modulation of volatile thiols from grape-derived, nonvolatile cysteinylated precursors during wine fermentation (*4, 5*).

Esters, higher alcohols and volatile acids are also groups of volatile aroma compounds produced by yeast metabolism during fermentation. Esters, specifically acetate esters and fatty acid ethyl esters, are present in all wines and contribute, in general, 'fruity' characters that significantly influence wine aroma and quality (*5*). Ester production in yeast is catalyzed by alcohol acetyltransferase enzymes (*6*). Interestingly, these same enzymes in yeast are also responsible for the modulation of the volatile thiol 3MH to 3MHA during fermentation (*7*). Therefore, there is a link between ester and volatile thiol metabolism in yeast cells.

The use of different *Saccharomyces* strains for wine fermentations has been shown to result in wines with differing volatile profiles, through varied relative concentrations of acetate esters, fatty acid ethyl esters and higher alcohols (*5*) and volatile thiols (*5, 8*). These compositional differences resulted in significant sensory differences among wines fermented using different yeast strains (*5*).

Studies have also investigated the effect of simultaneously inoculating multiple yeast strains to conduct fermentations (*9, 10*). Known as a coinoculation or mixed culture fermentation, this technique has been used to investigate the volatile profiles of wines produced using multiple *Saccharomyces* strains in an attempt to control the production of desirable metabolites and potentially enhance aroma complexity in wines. In all studies it was shown that one yeast strain dominated the yeast population toward the end of fermentation (*9, 10*). Despite these findings, Howell et al. (*9*) and Grossmann et al. (*10*) showed that each of the strains in the coinoculations had an effect on the volatile composition of the coinoculated wines as compared

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with single-strain wines, by modifying the concentration of volatile compounds produced.

It has been hypothesized that distinct volatile profiles of coinoculated wines might be owing to interactions between yeast strains, brought about by the sharing of metabolic intermediates (*9*). Evidence for this hypothesis was provided by the work of Cheraiti et al. (*11*), who found that the redox status of coinoculations differed from that of the single-strain ferments, thereby indicating that the interactions between the yeast strains involved the diffusion of metabolite(s) within the coinoculated fermentations. A comparison of the composition of coinoculated wines and a blend of the single-strain fermentations by Howell et al. (*9*) provided support for this conclusion, as the volatile composition of the coinoculated fermentations could not be replicated by the blended wines. However, the fundamental mechanisms of metabolic yeast interactions remain unknown.

Due to the influence on the volatile profile of wines, coinoculated fermentations have the potential to assist the wine industry to tailor wine to market specifications for increased competitiveness, and provide novel products to increase diversity of wine styles. There remains very limited information, however, on the coinoculation of mixed *Saccharomyces* strains, with as yet no information available on the effect of yeast interactions on the volatile thiol composition and, importantly, on the sensory properties of wines.

In this study we investigated the impact of coinoculating commercial *S. cerevisiae* strains on the volatile composition and sensory profile of Sauvignon Blanc wines.

MATERIALS AND METHODS

Winemaking. Homogenized and unfiltered 2006 Adelaide Hills *Vitis vinifera* L. cv. Sauvignon Blanc juice was fermented using commercial wine yeast strains *S. cerevisiae*/*S. kudriavzevii* Vin7 (Anchor Yeast, Cape Town, South Africa) in combination with either *S. cerevisiae* QA23 (Lalvin, Lallemand, Montreal, Canada) or *S. cerevisiae* Vin13 (Anchor Yeast).

Initial must analysis results were as follows: total soluble solids 231.1 g/L; pH 3.31; titratable acidity 7.0 g/L and yeast assimilable nitrogen 289 mg/L. Fermentations were conducted using the above strains in an active-dried form both singly and in coinoculations (Vin7/QA23 and Vin7/Vin13). The coinoculations were conducted by simultaneously inoculating two yeast strains, each at half the recommended inoculation rate, to achieve approximately 5×10^5 cells/mL. The fermentations were carried out in triplicate in 20-L stainless steel pressure vessels at approximately 15 °C. Must sampling commenced one week after inoculation and continued once per week for analysis of sugar concentrations [refractive index and Clinitest (Bayer, Leverkusen, Germany)]. Diammonium phosphate was added to all fermentations 48 h after inoculation and after five days, at rates of 150 mg/L and 100 mg/L, respectively.

When less than 5 g/L residual sugar remained in the wine, the wines were separated from the gross lees by siphoning the clear wine from the fermentation vessel. Homogenized 10 mL samples were collected from the gross lees and stored at 4 °C for no longer than one week until further analysis. Two replicates of the single-yeast fermentations were randomly selected and mixed at an equal ratio to produce a wine blend consistent with the coinoculated yeast combinations (Vin7+QA23 and Vin7+Vin13), so that three different replicate blends were produced for comparative analysis. Sulfur dioxide was added to all wines in the form of potassium metabisulfite (PMS) to a total concentration of 60 mg/L and the wines were settled by storing them in closed vessels at 0 °C for one month. After a final racking a second sulfur dioxide addition was made to all wines to obtain a final free sulfur dioxide concentration of 25−30 mg/L. The wines were filtered (Z6 pad) and then bottled in 375 mL glass bottles under inert gas and sealed with roll-on tamper evident screw caps. Wines were stored upright at 15 °C until analysis. Twenty-one wines were analyzed in this study, three replicates of seven wine treatments: three single-strain wines Vin7, QA23, Vin13; two coinoculated wines Vin7/QA23, Vin7/Vin13; and two blended wines Vin7+QA23, Vin7+Vin13.

Molecular Quantification Techniques. Serial dilutions of yeast lees samples were plated onto YPD medium [10 g of yeast extract (Difco, Becton, Dickinson and Company, Sparks, MD), 20 g of peptone (Amyl Media, Victoria, Australia), 20 g of dextrose (Sigma, St. Louis, MO), 15 g of agar (Amyl Media), 1 L of sterile water] for identification of microorganisms in all ferments. Colonies from all wines were determined by polymerase chain reaction (PCR)-based methods transposon and internal transcribed spacer (ITS), as detailed below. Thirty-three independent isolates, obtained as colonies from the YPD plates, were analyzed per fermentation replicate. A crude, unsuspended DNA template of all isolates was prepared using the colony-pick technique detailed in Bradbury et al. (*12*). Transposon PCR was used for analyzing yeast colonies taken from the single-strain QA23 and Vin13 wines, as described in Ness et al. (*13*). The yeast isolates from the single-strain Vin7 wines and coinoculated wines were analyzed using ITS region PCR, detailed by Bradbury et al. (*12*), as this PCR technique is capable of distinguishing genetically distinct strains, such as hybrid yeast Vin7, *S. cerevisiae*/*S. kudriavzevii* from nonhybrid *S. cerevisiae* strains (*12*). The yeast isolates obtained from the wine fermentations were compared to reference standards of Vin7, QA23 and Vin13 sourced from frozen glycerol stocks (−80 °C) of the culture collection of The Australian Wine Research Institute (AWRI) (Adelaide, Australia).

Chemical Analyses. All chemical analyses were conducted from two to twelve months after bottling. An analysis of the basic chemical composition of all wines was conducted by the AWRI Analytical Service as detailed in Iland et al. (*14*) prior to bottling. The titratable acidity, volatile acidity and alcohol were measured using Fourier Transfer Infrared WineScan (FOSS, Hillerød, Denmark).

Thirty fermentation-derived volatile compounds—six acetate esters, ten fatty acid ethyl esters, six higher alcohols and eight volatile acids—were measured for each of the 21 wines in duplicate two months after bottling using headspace-solid phase microextraction/stable isotope dilution analysis/gas chromatography/mass spectrometry (HS-SPME/SIDA/GC/MS) with deuterium-labeled analogues as the internal standards (*15*). All solvents were Merck Suprasolv grade (Kilsyth, Victoria, Australia). All solvents and analytical standards were verified for purity by GC/MS prior to use. One compound, 2-methylpropanoic acid was synthesized in-house. Ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, 2-methylbutanol, 3-methylbutanol, 2-methylpropanol, ethyl hexanoate, hexyl acetate, ethyl lactate, hexanol, propanoic acid, ethyl decanoate, ethyl dodecanoate, butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, 2-phenylethanol and decanoic acid were supplied by Sigma-Aldrich Corporation (St Louis, MO). Ethyl acetate, butanol and 2-phenylethyl acetate were supplied by Merck & Co, Inc. (Whitehouse Station, MJ). Ethyl octanoate, hexanoic acid and octanoic acid were supplied by Hopkin and Williams (Essex, England). NMR spectra were acquired with a Varian Gemini Spectrometer, operating at 300 MHz (H) or 75.5 MHz (C). Labeled standards purchased from Sigma-Aldrich were d_8-ethyl acetate, d_{10}-butanol, d_{13}- hexanol, d_5-propanoic acid, d_7-butanoic acid, d_{11}-hexanoic acid, d_{15}-octanoic acid and d_{19}-decanoic acid. All had >98 atom % deuterium. The rest of the polydeuterated standards were prepared as detailed in Seibert et al. (*15*). Peaks on chromatograms were manually integrated using Agilent G1701CA ChemStation software (Agilent, Avondale, PA).

Volatile thiol compounds 4MMP, 3MH and 3MHA were analyzed by SARCO Laboratories (Bordeaux, France) one year after bottling using the method outlined in Tominaga et al. (*16*).

Sensory Descriptive Analysis. A sensory descriptive analysis study was performed on all wines six months after bottling. Eleven assessors were recruited (six female), all with previous experience in wine descriptive analysis studies. The assessors participated in five training sessions: three 90 min discussion sessions to generate attributes and two practice rating sessions in isolated booths, prior to formally rating the wines. The assessors rated 19 aroma attributes and 10 flavor and mouthfeel attributes (**Table 1**). The intensity of each attribute was rated

Table 1. Aroma, Flavor and Mouthfeel Attributes Used in the Sensory Descriptive Analysis, as Rated by the Sensory Panel, and the Reference Standard Composition for Aroma Attributes

attribute	reference standard composition[a]
estery	0.2 mL of "estery" mix stock solution[b]
banana lolly	1/2 banana lolly (Black & Gold)—no base wine
floral/rose	0.2 mL of *cis*-rose oxide[c]
fresh citrus	5 mL of lemon cordial (Bickfords), 1/2 teaspoon of lemon zest and 1/2 teaspoon of grapefruit zest
lime (fresh)	0.5 cm^2 of fresh lime[c]
cooked citrus	10 mL of lime cordial (Bickfords)
pineapple	0.5 cm^2 of canned pineapple (Edgell) and 5 mL of canned pineapple juice (Edgell)[c]
passionfruit	3 fresh passionfruit seeds and 0.5 cm^2 of fresh passionfruit skin—no base wine[c]
apple/pear	20 mL of canned pear juice (SPC), 10 mL of apple juice (Just Juice), 0.5 cm^2 of canned pear (SPC) and 0.5 cm^2 of fresh apple[c]
stonefruit	10 mL of canned peach juice (Goulburn Valley), 10 mL of apricot nectar (Berri) and 0.5 cm^2 of canned peach (Goulburn Valley)[c]
lychee	4 teaspoons of canned lychee juice (UFO)
box hedge	4 fresh box hedge leaves—no base wine[c]
fresh green	1 lantana leaf, 0.5 cm^2 piece of green capsicum and 1 cm of green bean—no base wine[c]
cooked/canned green	2 teaspoons of canned asparagus brine (Edgell) and 4 teaspoons of canned bean brine (Edgell)
sweaty/cheesy	0.3 mL "sweaty" stock solution[d]
yeasty	1/4 teaspoon of active-dried baker's yeast (Tandaco)
nail polish remover	10 μL of ethyl acetate
bruised apple	50 μL of acetaldehyde
white vinegar	1 teaspoon of white vinegar (Anchor)
overall fruit flavor	
overall green flavor	
acidity	
sweet	
viscosity	
drying	
hotness	
bitterness	
metallic	
fruit flavor persistence	

[a] Prepared in 100 mL of Chenin Blanc, 2006, 2-L bag-in-box wine (11%v/v), unless otherwise specified. [b] Estery mix contains 0.5 g of 2-methylpropyl acetate, 0.09 g of ethyl butanoate, 0.2 g of ethyl hexanoate, and 0.2 g of ethyl octanoate in 100 mL of redistilled ethanol. [c] Per glass. [d] Sweaty stock solution contains 6.7 g of hexanoic acid and 3.3 g of 3-methylbutanoic acid in 100 mL of redistilled ethanol.

using an unstructured 15 cm line scale with indented anchor points of "low" and "high" placed at 10% and 90%, respectively.

The samples were assessed under sodium lighting in isolated, ventilated tasting booths at 22−24 °C. Judges were presented with seven wines per session, with wine selection such that one fermentation replicate of each of the seven wine treatments was presented in any one session. Each sample was presented at a constant volume (30 mL) in ISO coded, covered tasting glasses in a random and balanced order across the judges. Two presentation replicates of each of the 21 samples were assessed. FIZZ software (Version 2.1, Biosystemes, France) was used for the collection of all data.

Table 2. Average[a] Yeast Strain Percentages from Molecular Identification of the Five Yeast Treatments[b]

yeast treatments	strain as inoculated	unknown
Vin7	98 (3.8)	2.2 (3.4)
QA23	93 (3.4)	7.1 (1.8)
Vin13	96 (1.8)	4.2 (3.8)
Vin7/QA23		
Vin7	10 (15)	nd[c]
QA23	90 (15)	
Vin7/Vin13		
Vin7	6.1 (3.0)	1.0 (1.7)
Vin13	93 (1.7)	

[a] The percentages indicate counts of each strain either positively identified as one of the inoculated strains or as an unknown. Standard deviations in parentheses. [b] $n = 33$ colonies $\times$ 3 fermentation replicates. [c] Not detected.

Data Analysis. Statistical software (JMP 5.1, SAS Institute, Cary, NC) was used for analyzing all data. Sensory data for each attribute were analyzed using an analysis of variance (ANOVA) testing for the effects of treatment, fermentation replicate nested within treatment, as well as presentation replicate and judge, as detailed in Chapman et al. (*17*). Judge performance was assessed using FIZZ and Senstools (OP&P, Utrecht, Netherlands). A one-way ANOVA was used to analyze the chemical data.

RESULTS

Fermentations. All single-strain and coinoculated fermentations finished successfully. The Vin13 single-strain fermentations had the fastest fermentation rates, finishing 14 days after inoculation (data not shown). The coinoculated fermentations for both yeast combinations showed similar rates of sugar consumption to the QA23 single-strain fermentations, finishing 23 days after inoculation. In contrast, the Vin7 yeast had a slower rate of sugar consumption in the single-strain fermentations, finishing 34 days after inoculation.

Yeast Strain Identification. Molecular quantification analysis of yeast samples taken at the end of fermentations verified that inoculated yeast strains were present at the end of fermentation, shown in **Table 2**. On average, less than 5% unknown/wild yeast colonies were identified in all fermentations, with QA23 single-strain fermentations measuring the highest percentage of unknown yeast colonies. From an initial inoculation of a 1:1 ratio of both yeasts in the coinoculated yeast strain treatments, the Vin7 strain populations were substantially lower than the other strain at the end of fermentation for both treatments.

Basic Chemical Composition (Table 3). The basic composition of the wine treatments was analyzed prior to bottling. An ANOVA of the basic chemical data revealed that glucose + fructose, free sulfur dioxide, pH and volatile acidity were significantly different among the wine treatments ($p < 0.05$), whereas total sulfur dioxide, titratable acidity and alcohol concentrations remained constant for all wines.

The concentrations of residual sugar in **Table 3** (glucose + fructose) differed slightly among the wine treatments, however, all values were very low and considered "dry" from an oenological viewpoint. The free sulfur dioxide concentrations differed significantly among the wine treatments (**Table 3**). This result is probably owing to the addition of sulfur dioxide prior to bottling as a preservative.

The reason for the small but statistically significant differences in pH among the wine treatments is unknown. It might be owing to variability in the rate of tartrate stabilization prior to bottling. For volatile acidity, the Vin7 single-strain wines had the highest

Table 3. Basic Chemical Analysis Results Averaged across Triplicate Fermentations of Vin7, QA23 and Vin13 Single-Strain, Coinoculated and Blended Wines[a]

		SO_2 (mg/L)			acidity (g/L)		
wine treatments	glucose + fructose (g/L)	free	total	pH	titratable	volatile	alcohol (%v/v)
Vin7	0.63 (0.033)	28 (1.2)	106 (0.67)	3.27 (0.0088)	7.55 (0.10)	0.84 (0.012)	12.9 (0.034)
QA23	0.60 (0.0)	30 (0.33)	128 (3.8)	3.28 (0.022)	6.81 (0.16)	0.40 (0.035)	12.9 (0.012)
Vin13	0.47 (0.033)	28 (0.67)	124 (3.8)	3.33 (0.0)	6.64 (0.052)	0.31 (0.015)	12.9 (0.015)
Vin7/QA23	0.60 (0.0)	27 (0.33)	124 (3.4)	3.28 (0.0033)	7.04 (0.037)	0.45 (0.045)	12.9 (0.020)
Vin7/Vin13	0.67 (0.033)	30 (0.33)	119 (0.58)	3.31 (0.0067)	7.12 (0.32)	0.40 (0.0033)	12.9 (0.0088)
Vin7+QA23	0.50 (0.0)	30(0.33)	117 (3.5)	3.25 (0.0088)	7.16 (0.093)	0.53 (0.039)	12.9 (0.048)
Vin7+Vin13	0.93 (0.23)	30 (0.0)	118 (1.7)	3.28 (0.0)	6.85 (0.10)	0.51 (0.019)	12.9 (0.023)
sig[b]	**	*	ns	**	ns	**	ns

[a] One standard error of the mean ($n = 3$) in parentheses. [b] Significance where * $p < 0.05$; ** $p < 0.01$; ns: not significant.

concentrations, followed by the Vin7+QA23 and Vin7+Vin13 blended wines (**Table 3**). Both coinoculated wine treatments and QA23 and Vin13 single-strain wines contained low concentrations of volatile acidity.

Fermentation-Derived Compound Analysis (Table 4). All wines were analyzed for 30 fermentation-derived volatile compounds using GC/MS. These data are shown in **Table 4**.

An ANOVA of the fermentation-derived compounds (**Table 4**) showed that 27 compounds were significantly different among the seven wine treatments ($p < 0.05$). The three compounds not found to be significantly different were ethyl octanoate, 3-methylbutanol and butanoic acid. The data presented in **Table 4** indicate that the acetate ester compounds behaved relatively similarly across the wine treatments. Four of the six acetate esters were above their aroma detection thresholds: ethyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate and 2-phenylethyl acetate. The Vin13 single-strain wines had the highest concentrations of these acetate esters, followed by the Vin7/Vin13 coinoculation and QA23 single-strain wines. The blended wine treatments contained intermediate concentrations of acetate esters. The Vin7 single-strain and Vin7/QA23 coinoculated wines containing the lowest concentrations of acetate esters, although this was not true of ethyl acetate for Vin7 single-strain wines, which contained higher concentrations than the blended wines.

Five fatty acid ethyl esters were measured above their aroma detection thresholds in all wines and had different concentration trends to one another (**Table 4**). Ethyl-2-methylpropanoate was highest in the Vin7 single-strain wines and lowest in the QA23 single-strain wines. In contrast, the QA23 single-strain wines and Vin7/QA23 coinoculated wines had the highest concentrations of ethyl hexanoate, with the Vin13 single-strain and Vin7/Vin13 coinoculated wines the lowest for this compound. Apart from Vin7+Vin13 blended wines which had the highest concentration of ethyl decanoate, the blended wines were intermediate in all fatty acid ethyl ester concentrations.

Ethyl-2-methylbutanoate and ethyl dodecanoate (**Table 4**) were found at concentrations bordering their thresholds. For ethyl dodecanoate it was observed that the Vin13 single-strain, Vin7/Vin13 coinoculated and Vin7+Vin13 blended wines contained concentrations above the aroma detection threshold. The Vin7/Vin13 coinoculated wines contained approximately two, three and ten times more ethyl dodecanoate than the Vin13 single-strain, Vin7+Vin13 blended and Vin7 single-strain wines, respectively. Similarly the Vin7/QA23 coinoculated wines contained approximately two times more ethyl dodecanoate than the Vin7 and QA23 single-strain and Vin7+QA23 blended wines, although the concentrations of these wines were below the aroma detection threshold.

Of all the six higher alcohols measured in **Table 4**, only 2-phenylethanol concentrations were above the aroma detection threshold in all wine treatments. The concentration of 2-phenylethanol was found to be highest in the QA23 single-strain wines, lowest in the Vin7/Vin13 coinoculated wines and relatively equal in concentration for all other wine treatments.

The concentrations of four volatile acids were found above their aroma detection thresholds (**Table 4**). For 3-methylbutanoic acid, the Vin7 single-strain and Vin7+Vin13 blended wines contained the highest concentrations. The lowest concentrations of 3-methylbutanoic acid were found in the QA23 single-strain and Vin7/QA23 coinoculated wines. In contrast, the concentrations of octanoic acid were found to be lowest in the Vin7 single-strain wines and equally highest in the QA23 and Vin13 single-strain wines.

Volatile Thiol Analysis. Analysis of volatile thiol compounds 4MMP, 3MH and 3MHA was carried-out on the seven wine treatments. Clear differences were observed in the concentrations of the volatile thiols (**Figure 1**).

All wine treatments containing detectable concentrations of the volatile thiols measured had concentrations higher than the reported aroma detection thresholds for each compound. The single-strain Vin7 wines contained relatively high concentrations of 4MMP, relatively low concentrations of 3MH and moderate concentrations of 3MHA. The single-strain QA23 wines contained low concentrations of 4MMP, relatively low concentrations of 3MH and moderate concentrations of 3MHA. Vin13 single-strain wines contained relatively high concentrations of 4MMP and 3MH and, in contrast, no 3MHA was detected in these wines.

Not surprisingly, both blended wines contained an intermediate concentration of 3MH, 3MHA and 4MMP compared to the single strain treatments. However, the Vin7+QA23 blended wines contained relatively higher concentrations of 4MMP than would be expected, possibly due to analytical error. It must be noted that one fermentation replicate of the blended Vin7+Vin13 wines was found to contain no detectable 4MMP, which affected the average concentration for this treatment.

For the coinoculated wines, Vin7/QA23 contained moderate concentrations of 4MMP and 3MH and the highest concentration of 3MHA. The Vin7/Vin13 coinoculated wines contained no detectable concentration of 4MMP, relatively high concentrations of 3MH and low concentrations of 3MHA. The coinoculated wines for both yeast combinations contained higher concentrations of 3MH than their single-strain and blended wine components. Similarly, Vin7/QA23 coinoculated wines contained higher 3MHA concentrations than the single-strain and blended wines.

Table 4. Average Concentration (mg/L) of 30 Fermentation-Derived Volatile Compounds for Single-Strain, Coinoculated and Blended Wines Made Using Commercial Wine Yeast Strains Vin7, QA23 and Vin13[a]

	aroma detection threshold[b,c]	Vin7	QA23	Vin13	Vin7/QA23	Vin7/Vin13	Vin7+QA23	Vin7+Vin13
				Acetate Esters				
ethyl acetate	7.5 (*18*)	81 (0.94)	91 (3.1)	94 (2.7)	74 (1.7)	91 (2.1)	75 (2.8)	76 (1.9)
2-methylpropyl acetate	1.6 (*21*)[d]	0.10 (2.8×10^{-3})	0.12 (5.2×10^{-3})	0.19 (7.5×10^{-3})	0.079 (7.3×10^{-4})	0.17 (2.1×10^{-3})	0.10 (1.4×10^{-3})	0.12 (4.0×10^{-3})
2-methylbutyl acetate		0.23 (0.011)	0.38 (0.015)	0.70 (0.021)	0.26 (0.009)	0.60 (0.015)	0.29 (0.011)	0.43 (0.013)
3-methylbutyl acetate	0.030 (*18*)	4.5 (0.67)	8.6 (0.36)	9.3 (0.36)	5.1 (1.2)	8.8 (0.17)	6.8 (1.3)	6.6 (0.11)
hexyl acetate	2.4 (*21*)[e]	0.58 (7.6×10^{-3})	0.86 (0.019)	0.79 (0.015)	0.76 (0.032)	0.80 (0.014)	0.70 (0.016)	0.63 (7.9×10^{-3})
2-phenylethyl acetate	0.25 (*18*)	0.34 (7.9×10^{-3})	0.54 (0.044)	0.51 (0.018)	0.30 (0.016)	0.46 (0.006)	0.43 (0.019)	0.43 (7.2×10^{-3})
				Fatty Acid Ethyl Esters				
ethyl propanoate	1.8 (*21*)[e]	0.065 (1.5×10^{-3})	0.11 (3.4×10^{-3})	0.10 (2.4×10^{-3})	0.10 (4.8×10^{-3})	0.10 (4.0×10^{-3})	0.088 (3.0×10^{-3})	0.083 (2.0×10^{-3})
ethyl-2-methylpropanoate	0.015 (*18*)	0.019 (5.4×10^{-4})	0.011 (4.2×10^{-4})	0.017 (5.4×10^{-4})	0.014 (6.5×10^{-4})	0.018 (6.8×10^{-4})	0.016 (2.3×10^{-4})	0.018 (6.5×10^{-4})
ethyl butanoate	0.020 (*18*)	0.67 (6.4×10^{-3})	0.61 (4.2×10^{-3})	0.52 (7.2×10^{-3})	0.61 (2.0×10^{-2})	0.54 (7.7×10^{-3})	0.64 (1.7×10^{-3})	0.58 (1.1×10^{-3})
ethyl-2-methylbutanoate	0.0010 (*18*)	1.5×10^{-3} (5.6×10^{-5})	9.8×10^{-4} (6.0×10^{-5})	1.9×10^{-3} (6.1×10^{-5})	1.2×10^{-3} (5.2×10^{-5})	2.0×10^{-3} (2.2×10^{-5})	1.4×10^{-3} (4.9×10^{-5})	1.8×10^{-3} (5.2×10^{-5})
ethyl-3-methylbutanoate	0.0030 (*18*)	3.3×10^{-3} (1.3×10^{-4})	5.7×10^{-3} (2.0×10^{-4})	5.5×10^{-3} (2.2×10^{-4})	6.5×10^{-3} (2.8×10^{-4})	4.5×10^{-3} (2.0×10^{-4})	4.6×10^{-3} (1.8×10^{-4})	4.6×10^{-3} (1.8×10^{-4})
ethyl hexanoate	0.0050 (*18*)	1.6 (0.020)	1.7 (0.048)	1.4 (0.015)	1.7 (0.066)	1.4 (0.012)	1.6 (0.023)	1.5 (0.011)
ethyl lactate	14 (*20*)	5.6 (0.083)	4.9 (0.25)	3.4 (0.31)	3.6 (0.15)	4.6 (0.15)	5.1 (0.11)	5.0 (0.34)
ethyl octanoate	0.0020 (*18*)	1.7 (0.028)	1.9 (0.058)	2.1 (0.11)	1.9 (0.074)	1.7 (0.035)	1.8 (0.047)	1.9 (0.038)
ethyl decanoate	0.20 (*19*)	0.89 (0.045)	1.1 (0.066)	1.4 (0.044)	1.2 (0.063)	1.8 (0.17)	0.92 (0.034)	2.1 (0.082)
ethyl dodeconoate	0.64 (*20*)	0.26 (0.013)	0.33 (0.022)	1.2 (0.061)	0.60 (0.042)	2.8 (0.17)	0.30 (0.010)	0.80 (0.059)
				Higher Alcohols				
2-methylpropanol	40 (*18*)	19 (0.85)	13 (0.47)	14 (0.20)	13 (0.51)	15 (0.49)	17 (0.67)	17 (0.65)
butanol	1.5×10^2 (*21*)[e]	0.80 (0.20)	1.0 (0.67)	1.7 (0.066)	1.0 (0.033)	1.4 (0.077)	0.94 (0.022)	1.1 (0.047)
2-methylbutanol	75 (*20*)	22 (0.034)	15 (0.054)	25 (1.4)	18 (0.061)	24 (0.29)	19 (0.018)	22 (0.34)
3-methylbutanol	30 (*18*)	1.2×10^{-2} (2.1)	1.2×10^{-2} (2.9)	1.0×10^{-2} (6.0)	1.2×10^{-2} (5.0)	1.1×10^{-2} (4.4)	1.3×10^{-2} (0.91)	1.2×10^{-2} (5.0)
hexanol	8.0 (*18*)	1.2 (6.2×10^{-3})	1.2 (0.043)	0.95 (9.1×10^{-3})	1.2 (0.041)	0.98 (5.0×10^{-3})	1.2 (0.040)	1.1 (7.1×10^{-3})
2-phenylethanol	10 (*18*)	12 (0.10)	13 (0.11)	12 (0.21)	12 (0.34)	11 (0.14)	12 (0.14)	12 (0.067)
				Volatile Acids				
propanoic acid	20 (*20*)	1.3 (0.037)	1.4 (0.049)	0.91 (0.052)	1.4 (0.062)	1.3 (0.073)	1.3 (0.039)	1.1 (0.036)
2-methylpropanoic acid	2.3 (*19*)	1.3 (0.031)	0.65 (0.017)	1.0 (0.021)	0.98 (0.018)	1.0 (0.058)	0.94 (0.014)	1.1 (0.042)
butanoic acid	0.17 (*19*)	1.5 (0.058)	1.3 (0.043)	1.5 (0.083)	1.4 (0.057)	1.6 (0.074)	1.5 (0.026)	1.6 (0.033)
2-methylbutanoic acid	3.0 (*18*)	0.40 (0.010)	0.24 (0.010)	0.38 (0.012)	0.26 (9.1×10^{-3})	0.39 (0.011)	0.35 (0.013)	0.37 (0.015)
3-methylbutanoic acid	0.033 (*19*)	0.75 (0.049)	0.46 (0.028)	0.55 (0.017)	0.49 (0.028)	0.56 (0.021)	0.62 (0.033)	0.78 (0.035)
hexanoic acid	0.42 (*19*)	7.6 (0.58)	7.6 (0.44)	6.6 (0.16)	6.2 (0.20)	6.4 (0.18)	7.1 (0.039)	7.1 (0.086)
octanoic acid	0.50 (*19*)	8.5 (0.34)	10 (0.40)	10 (0.24)	9.8 (0.40)	8.7 (0.18)	9.5 (0.10)	9.4 (0.14)
decanoic acid	1.0 (*19*)	3.7 (0.15)	4.0 (0.15)	5.0 (0.048)	3.9 (0.10)	4.5 (0.11)	3.9 (0.095)	4.5 (0.044)

[a] One standard error of the mean ($n = 6$) in parentheses. [b] Aroma detection threshold concentrations in mg/L. [c] Reference from which the value has been taken is given in parentheses. In ref ***18*** the matrix was 10%w/w water/ethanol solution; in ref ***19*** the matrix was 11%w/w synthetic wine; in ref ***20*** the matrix was 9.5%w/w ethanol solution. [d] In ref ***21*** the matrix was beer. [e] In ref ***21*** the matrix was white wine.

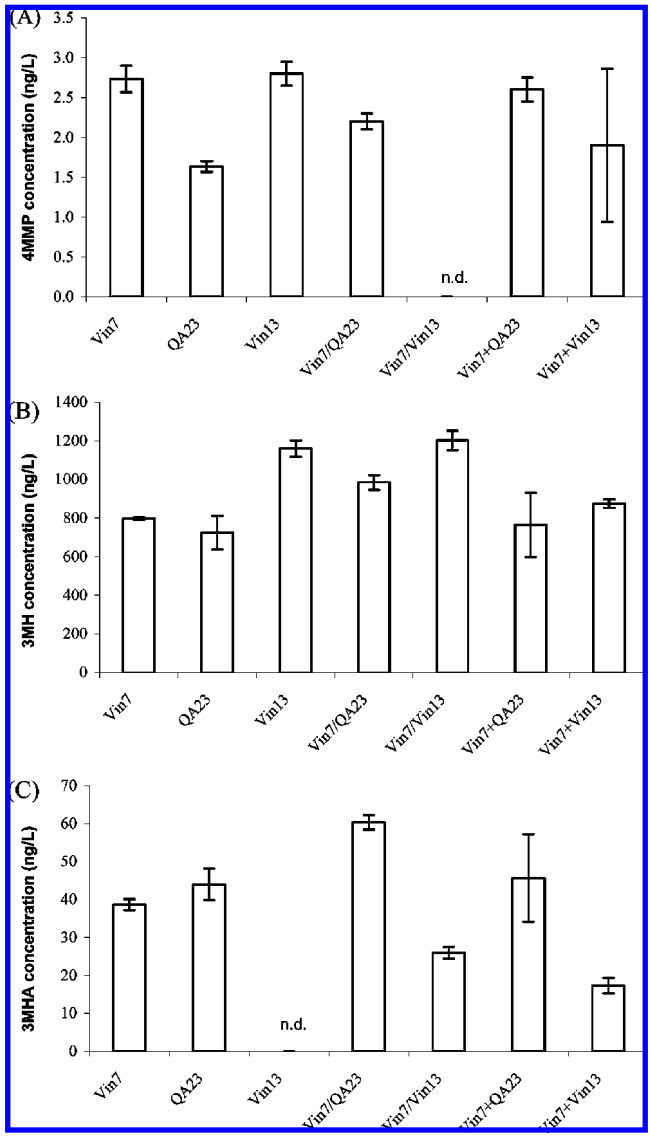

Figure 1. Average volatile thiol concentrations of single-strain, coinoculated (/) and blended wines (+) of wine yeasts Vin7 and QA23 and Vin7 and Vin13. (**A**) 4-Mercapto-4-methylpentan-2-one; (**B**) 3-mercaptohexan-1-ol; (**C**) 3-mercaptohexyl acetate. Error bars represent ± one standard error of the mean ($n = 3$); n.d., compound not detected.

A comparison of the relative concentrations for the three volatile thiol compounds across all seven wine treatments shows that 3MH and 3MHA have a similar pattern, with the coinoculated wines higher in concentration than the blends and most of the single-strain wines, whereas 4MMP has a concentration order unlike the other thiols.

Sensory Analysis. From an analysis of variance there were found to be no significant differences among fermentation replicates for any wine in any attribute, except for *banana lolly*. For this attribute, Vin7/QA23 coinoculation fermentation replicate 2 was rated significantly higher (a mean value of 2.7) than the other two fermentation replicates (mean values of 1.1 and 0.6).

It was found that five attributes were significantly different among the wine treatments ($p < 0.05$). These attributes were *estery*, *floral/rose*, *bruised apple*, *white vinegar* and *acidity*. Six other attributes were significantly different among the wine treatments at $p < 0.15$: *box hedge* ($p = 0.07$), *passionfruit* ($p = 0.12$), *drying* ($p = 0.06$), *bitterness* ($p = 0.07$), *viscosity* ($p = 0.06$) and *sweet* ($p = 0.08$). For the purpose of this study only the aroma attributes are considered further. The mean scores of the significantly different aroma attributes, as well as *passionfruit* and *box hedge*, are shown in the principal component analysis (PCA) biplot in **Figure 2**.

Figure 2 shows that 91% of the total variation is explained by the first two PCs: PC1 explaining the majority of variance in the data set (70%) in the horizontal direction. The Vin7 single-strain wines were situated on the far left side of the biplot, indicating that these wines had the highest ratings for the *white vinegar* and *bruised apple* attributes, and were lowest for *passionfruit*, *estery* and *floral/rose*. The QA23 and Vin13 single-strain wines were located on the right side of the biplot, relatively close to each other. This indicates that these wines had similar ratings for all attributes, although the QA23 wines were generally rated lower for all attributes except *floral/rose* (mean value of 2.5 compared with 2.1). The position of the QA23 and Vin13 single-strain wines also shows that these wines, unlike the Vin7 wines, were relatively low in *white vinegar* and *bruised apple*.

The blended wine treatments were located on the left side of the biplot near the origin, being intermediate in those attributes highly loaded on PC1, compared with their respective single strain components. The Vin7+Vin13 blended wines had a slightly higher intensity of *white vinegar* (mean values of 0.8 compared with 0.5), *floral/rose* (mean value of 2.2 compared with 2.0) and *estery* (means value of 3.5 compared with 3.4), and lower intensity for *bruised apple* (mean value of 0.4 compared with 0.5) than the Vin7+QA23 blended wines.

In contrast, the coinoculated wines for both yeast combinations were located on the right side of the biplot, with the Vin7/Vin13 coinoculated wines in the upper right quadrant similar to the Vin13 single-strain wines. This indicates that wines coinoculated with Vin7 and Vin13 were rated relatively highly in *passionfruit*, highest in *box hedge* aroma, and relatively low for *white vinegar* and *bruised apple*. The Vin7/QA23 coinoculated wines were situated in the lower right quadrant, rated highest in *estery*, *floral/rose* and *passionfruit* and rated lowest in *box hedge*, *white vinegar* and *bruised apple*.

DISCUSSION

This study clearly demonstrates that coinoculations can be used effectively to alter the volatile composition and sensory profile of wines, when complementary yeast strains are combined.

The successful completion of fermentation of all wine treatments, including the coinoculations for both yeast combinations has been reported by others investigating coinoculations of *S. cerevisiae* (*9, 11*). To our knowledge, this is the first report of a successful *S. cerevisiae* coinoculated fermentation outside laboratory conditions.

The strain identification of yeast samples taken at the end of the coinoculated fermentations (**Table 2**) confirmed findings that coinoculations result in changing yeast strain populations throughout the fermentation (*22, 23*). The mechanism for population reduction was not determined in this experiment, however, Howell et al. (*22*) suggested that it was caused by the dominance of one yeast strain, either owing to a higher proportion of viable to nonviable cells of the dominant strain at inoculation or other factors, such as faster growth of the dominant strain favored by conditions of the culture. The initial cell viability was not measured at inoculation, therefore QA23 and Vin13 yeast strains might have contained higher viability compared with Vin7 yeast strain. For Vin 7 coinoculated fermentations, the longer fermentation time and lower viability at the end of fermentation might also be related to the hybrid nature of this strain. It is well-known that *S. cerevisiae*

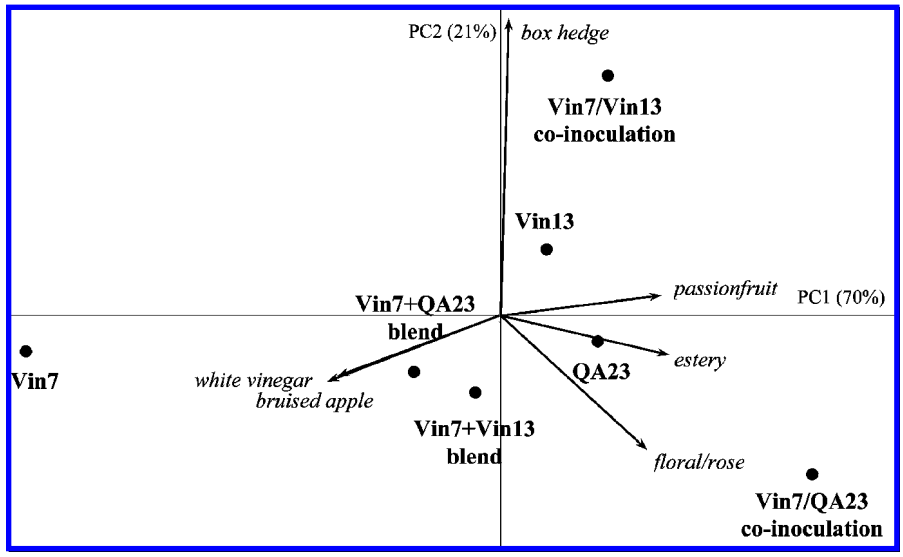

Figure 2. Principal component analysis (PCA) biplot of mean values of six significantly different attributes ($p < 0.15$) for seven wine treatments: single-strain, coinoculated and blended wines for wine yeast combinations Vin7 and QA23, and Vin7 and Vin13.

strains such as QA23 and Vin13 are generally more efficient in wine fermentations than other *Saccharomyces* species (*24*). Alternatively, the reduction in Vin7 yeast population might have been caused by the production of a killer toxin by the partner strain during coinoculated fermentations. Killer activity is reported in both QA23 and Vin13 yeast strains, while Vin7 is killer-sensitive. However Jacobs and van Vuuren (*25*) determined that Vin7 yeast strain has minimal killer sensitivity for K_2 killer toxins of *S. cerevisiae*, therefore killer activity was probably not the only cause of reduced Vin7 yeast populations. Further research is required to understand the population dynamics of coinoculation with specific yeast strains.

Knowledge of yeast populations cannot allow the prediction of the production of volatiles by individual strains in a mixed fermentation (*9*), partly because measures of yeast populations indicate viability, not fermentation capacity. Nevertheless, the results of the chemical and sensory analysis demonstrate that the Vin7 yeast strain contributed to the volatile composition of the coinoculated wines, despite reducing to, on average, less than one-quarter of the overall yeast population at the end of fermentation.

Of the wine parameters measured in the basic chemical composition (**Table 3**), volatile acidity was of most interest in this study. Volatile acidity is naturally present in wine through yeast production during fermentation, but when concentrations exceed the aroma detection threshold 0.72 g/L it is considered detrimental to wine quality. It is well established that volatile acidity production by a particular strain is linked to environmental parameters and can be considered sporadic. The Vin7 single-strain wines were the only wine treatment that contained concentrations of volatile acidity above the aroma detection threshold, which has affected its aroma profile, as was confirmed by the sensory analysis data.

Notably, the Vin7/QA23 and Vin7/Vin13 coinoculated wines contained almost half the concentration of volatile acidity of the Vin7 single-strain wines (**Table 3**). Volatile acidity was also found to correlate positively to the sensory attribute *white vinegar* ($r = 0.82$, $p = 0.02$, $n = 7$). *White vinegar* aroma was also a feature of the Vin7 single-strain wines and was negligible in the coinoculated wines. These findings show that undesirable volatile acidity production in the Vin7 single-strain wines can be largely negated by coinoculation with QA23 or Vin13. Grossmann et al. (*10*) reported a similar outcome for other metabolites and concluded that one yeast strain can compensate for possible negative characters of partner strains in a coinoculation if the strains are well-balanced. Our results support this theory, although this conclusion is based on one volatile compound and one vintage, and should be explored more thoroughly in the future.

All blended wines contained relatively high volatile acidity concentrations (**Table 3**) and high ratings for *white vinegar* aroma (**Figure 2**) relative to the coinoculated wines and QA23 and Vin13 single-strain wines. This suggests that blending does not diminish negative characters in the single strains as effectively as coinoculating does. Although more research is necessary to confirm this hypothesis.

The concentration of acetate esters, fatty acid ethyl esters, higher alcohols and volatile acids shown in **Table 4** differed among the Vin7, QA23 and Vin13 single-strain wines. These results demonstrate that *Saccharomyces* strains produce different chemical profiles during single-strain fermentations, confirming previous work by Swiegers et al. (*5*).

The concentration of fermentation-derived compounds (**Table 4**) also varied among the coinoculated wines. All the acetate esters measured were found at higher concentrations in the Vin7/Vin13 coinoculated wines compared with the Vin7/QA23 coinoculated wines. This indicates that the type of yeast strains used in the coinoculations will affect the volatile composition at the end of fermentation. The data in **Table 4** also indicate that the coinoculated wines contained significantly different concentrations of volatile compounds from the single stain and blended wines.

Consistent with the fermentation-derived data (**Table 4**), the volatile thiol results (**Figure 1**) show that the single-strain wines produced varying concentrations of 4MMP, 3MH and 3MHA. This confirms findings that *Saccharomyces* strains differ in their release and modulation mechanisms of volatile thiol compounds (*5, 8*).

It is surprising that no 4MMP was detected in the Vin7/Vin13 coinoculated wines (**Figure 1A**), as high 4MMP concentrations were measured in the Vin7 and Vin13 single-strain wines. It might be that when Vin7 and Vin13 are present together in fermentation, the releasing mechanism for 4MMP is inhibited or the 4MMP is degraded to other metabolites. This pattern was not observed for the Vin7/QA23 coinoculated wines.

No 3MHA was detected in the single-strain Vin13 wines, unlike the results found by Swiegers et al. (*5*). Since Vin13 contained high concentrations of most acetate esters (**Table 4**), this demonstrates the presence of the alcohol acetyltransferase enzymes which are also involved in 3MHA production (*7*). Therefore, the reason why no 3MHA was present in the Vin13 single-strain wines needs to be determined.

Both coinoculated wine treatments contained higher 3MH concentrations than the single-strain and blended wines. Similarly, the Vin7/QA23 coinoculated wines were higher in 3MHA concentrations than the single-strain and blended components, although this did not hold true for the Vin7/Vin13 coinoculated wines. This demonstrates that coinoculated wines can result in increased concentrations of the volatile thiols when certain yeast combinations are used to conduct alcoholic fermentation.

The coinoculated wines for both yeast combinations contained different chemical profiles to the single-strain and blended wines of their respective components, as can be seen in **Table 4** and **Figure 1**. The results of this study support the previously stated hypothesis that metabolic interactions are occurring between the yeast strains in the coinoculated fermentations (*9, 11*), resulting in a modified volatile composition that cannot be achieved by blending the single-strain wines after fermentation (*9*). Different interactive effects occurring within the coinoculated fermentations might increase or inhibit the production of certain compounds. It is possible that the nature, size or uptake of the metabolites into yeast cells varies between strains. Alternatively, the efficiency of enzymes involved in further processing the metabolites function at different rates depending on the strain. Further research is necessary to investigate metabolic interactions on a molecular level.

The sensory descriptive analysis data in the biplot (**Figure 2**) showed clearly that the single-strain wines differed in their sensory profiles. These results confirm previous research that the *Saccharomyces* strain used during alcoholic fermentation has an effect on the sensory profile (*5*).

The coinoculated wines for both yeast combinations differed in their sensory profiles compared to each other, and to the single-strain wines and blends of the single-strain wines. This suggests that the use of different yeast combinations will result in altered wine sensory properties.

To our knowledge, this study is the first to report the effect of coinoculating *S. cerevisiae* strains on the volatile thiol composition and sensory properties of wines. The concentrations of volatile thiols 3MH and 3MHA increased in the Vin7/QA23 coinoculation, although this pattern was not found for the other yeast combination. Coinoculated wines using Vin7/QA23 were rated highest in *estery*, *floral/rose* and *passionfruit* aromas, while the Vin7/Vin13 coinoculated wines were rated highest in *box hedge*. The high concentrations of volatile acidity, and negative *white vinegar* and *bruised apple* aromas of the Vin7 single-strain and blended wines were not present in the coinoculated fermentations for both yeast combinations, which offers the useful application that yeast strains can compensate for any undesirable characteristics of partner strains in coinoculated fermentations.

Coinoculated fermentations using commercially available yeast strains in the same ferment resulted in Sauvignon Blanc wines with substantially modified chemical and sensory profiles, likely owing to metabolic interactions. This work has demonstrated that coinoculation of wine yeast can be used in order to modulate the volatile composition and sensory profile of wines, when a balanced yeast combination is used. Coinoculated fermentations are a promising tool for the wine industry, allowing winemakers to alter the aroma of wines according to market specifications. Future work at the AWRI will involve determining whether coinoculations have an effect on consumer preference. Such work will enable the wine industry to tailor yeast selection and fermentation control for particular wine styles that are preferred by groups of consumers.

NOMENCLATURE

Vin7/QA23, coinoculation of Vin7 and QA23; Vin7/Vin13, coinoculation of Vin7 and Vin13; Vin7+QA23, blend of Vin7 and QA23 single-strain wines; Vin7+Vin13, blend of Vin7 and Vin13 single-strain wines.

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