OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2023.57.2.7306

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Transient acetaldehyde production by SO_2-producing *Saccharomyces cerevisiae* promotes the survival of *Oenococcus oeni* during co-fermentation

Peter J. Costello[1*], Radka Kolouchova[1], Jane M. McCarthy[1], Damian Espinase Nandorfy[1], Desireé Likos[1] and Simon A. Schmidt[1]

[1] The Australian Wine Research Institute, Urrbrae, SA 5064, Australia

*correspondence: peter.costello@awri.com.au

Associate editor: Patrick Lucas

Received: 21 December 2022

Accepted: 25 March 2023

Published: 15 June 2023

This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

ABSTRACT

Stuck or sluggish malolactic fermentation (MLF) can be problematic in stressful wine conditions, particularly white and sparkling base musts/wines. In these cases, knowledge of yeast-bacteria strain compatibility and the amount of sulfur dioxide (SO_2) a yeast strain produces are important considerations for successful MLF. Here, laboratory- and pilot-scale co-fermentations in Chardonnay were used to investigate the effect of yeast-derived SO_2 on *Oenococcus oeni* survival. Although yeast-derived SO_2 is generally inhibitory, we show that SO_2 production (to approximately 65 mg/L) can be uncoupled from *O. oeni* survival in the early stages of co-fermentation. Bacterial survival in the presence of specific SO_2-producing yeast strains was correlated with the early, transient formation of high acetaldehyde concentrations. *Oenococcus oeni* survival coincided with molecular SO_2 concentrations remaining below an extremely low threshold of inhibition, which exponentially increased from approximately 3–6 µg/L in the first three days of co-fermentation. Strain-dependent sensitivity of *O. oeni* to bound SO_2 remains a possibility, although the extent and mechanism of such inhibition by the SO_2 adduct during co-fermentation remain unclear. The choice of co-inoculation yeast strain also influenced wine diacetyl concentration, which was only detected in wines co-inoculated with high SO_2-producing *S. cerevisiae* strains. The wines with high diacetyl concentrations were found to be distinct by a sensory panel, with comparatively high citation frequency for a *buttery* sensory attribute. Both the SO_2- and acetaldehyde production capacity of yeasts are, therefore, seen as meaningful co-inoculation selection criteria. The range of yeast strains suitable for MLF induction by co-inoculation could be widened to include SO_2-producing strains that transiently produce an early, high concentration of acetaldehyde. The effects of low, equilibrium concentrations of molecular SO_2 should also be considered in conjunction with total SO_2 as a measure of SO_2 toxicity towards *O. oeni* following co-inoculation.

Abbreviations: CFU: colony forming unit; LAB: lactic acid bacteria; MLF: malolactic fermentation; SO_2: sulfur dioxide; SD: standard deviation.

KEYWORDS: acetaldehyde, co-inoculation, malolactic fermentation, *Oenococcus oeni*, *Saccharomyces cerevisiae*, sulfur dioxide

INTRODUCTION

Malolactic fermentation (MLF) is an important winemaking process performed by certain wine lactic acid bacteria (LAB), principally *Oenococcus oeni*. The onset of MLF, characterised by the conversion of L-malic acid to L-lactic acid and CO_2, commences after the malolactic bacteria population has grown to approximately 10^6 cells/mL (Wibowo *et al.*, 1985). Consequently, the success or failure of MLF is highly dependent on wine parameters that affect the growth and survival of malolactic bacteria, particularly wine pH, the concentrations of alcohol and sulfur dioxide, and temperature. In general, wine conditions conducive to MLF include < 13 % v/v alcohol, > pH 3.4, < 8 mg/L free SO_2, < 30 mg/L total SO_2, 18–22 °C, while conditions outside of this range can become more challenging for MLF (Krieger-Weber, 2017). Moreover, for any given wine, the ability of wine LAB to grow and conduct MLF is highly dependent on the species and strain of LAB involved and their respective tolerances to the latter parameters.

Another major factor affecting the growth and MLF capability of wine LAB is the potential for interaction with fermentation yeast, which can encompass a diverse range of *Saccharomyces cerevisiae* and also non-*Saccharomyces* strains. Such interactions are complex. MLF outcomes can be affected by metabolite production or physical cellular interactions (Bartle *et al.*, 2019). Inhibitory yeast metabolites include the formation of SO_2 (Henick-Kling and Park, 1994; Larsen *et al.*, 2003; Wells and Osborne, 2011), medium-chain fatty acids (Guilloux-Benatier *et al.*, 1998), inhibitory peptides (Nehme *et al.*, 2010) and succinic acid at concentrations greater than 1 g/L (Torres-Guardado *et al.*, 2022). Further, in a study of the interactions between *Leuconostoc oenos* (*O. oeni*) PSU-1 and *S. cerevisiae* 522 during co-culture, King and Beelman (1986) reported that the greatest inhibition of early bacterial growth was caused not by yeast metabolites but by yeast growth itself.

Of the potential inhibitory yeast metabolites, the formation of yeast-derived SO_2 during fermentation is well recognised (Rauhut, 1993) and can create a significant obstacle for MLF. Although most *S. cerevisiae* strains produce low concentrations (10–30 mg/L) of SO_2, some can produce over 100 mg/L (Eschenbruch, 1974; Rauhut, 1993; Richter *et al.*, 2013). Various compositional and vinification factors can also influence SO_2 production by yeast (Andorrà *et al.*, 2018; Rauhut, 1993).

Whether intrinsically derived during fermentation or added during the winemaking process, SO_2 enters a pH-dependent equilibrium. In an aqueous solution at pH 3.5, only a minor proportion (2 %) of free SO_2 exists in the molecular, and primarily antimicrobial, form (Ribéreau-Gayon *et al.*, 2006). At lower pH, such as the pH of white wine, this proportion increases. For example, at pH 3.2, the proportion of molecular SO_2 in an aqueous solution increases to 3.91 % (Ribéreau-Gayon *et al.*, 2006).

The molecular SO_2 concentrations reported as inhibitory towards *O. oeni* and other wine LAB are varied. Fia *et al.* (2018) reported that molecular SO_2 concentrations between 0.2–0.6 mg/L impeded growth and MLF activity of an *O. oeni* strain in white wine, and Ribéreau-Gayon *et al.* (2006) indicated 0.15–0.4 mg/L molecular SO_2 had varying, wine-dependent, inhibitory effects on LAB survival. Carr *et al.* (1976), however, suggest that much lower molecular SO_2 concentrations (0.032 mg/L) completely suppressed the growth of heterofermentative cocci (isolated from cider) in a nutrient medium, whereas *Lactobacillus plantarum* (currently *Lactiplantibacillus plantarum*) could tolerate up to approximately 1.5 mg/L molecular SO_2.

Bisulfite can also readily form adducts with compounds in wine, particularly carbonyls such as acetaldehyde, pyruvic acid, and 2-ketoglutaric acid (respective K_d at pH 3.3: 1.5×10^{-6}, 5.55×10^{-4} and 1.4×10^{-4}) (Burroughs and Sparks, 1973). Such bound forms of SO_2 may also exhibit inhibitory activity towards wine LAB, with inhibition in wine typically occurring at > 50 mg/L bound SO_2 (Fugelsang, 1997; Wibowo *et al.*, 1985). However, this concentration also appears subject to variability; as little as 5 mg/L acetaldehyde- and pyruvic acid-bound SO_2 was inhibitory to the growth of wine LAB in a semi-defined culture medium (Wells and Osborne, 2012), and 10 mg/L acetaldehyde-bisulfite restricted the growth and MLF activity of some bacterial strains in red wine (Lafon-Lafourcade, 1975). Several authors (Larsen *et al.*, 2003; Osborne and Edwards, 2006; Wells and Osborne, 2011) have demonstrated that wines fermented by yeast strains producing higher total SO_2 concentrations were generally inhibitory to *O. oeni*. However, Larsen *et al*. (2003) and Osborne and Edwards (2006) could not demonstrate a clear relationship between yeast-derived SO_2 production and bacterial inhibition and proposed other inhibitory mechanisms.

White and sparkling-based wines present unique and difficult challenges for MLF. Compared to red wines, white wines destined for MLF generally have higher SO_2 concentration (e.g., up to approximately 50 mg/L total SO_2) and lower pH (e.g., pH 3.1–3.3), which approaches the limits for *O. oeni* survival. In such cases, increases in SO_2 from the fermentation yeast strain can render MLF extremely challenging. Consequently, the tolerance and adaptive capability of the malolactic bacterial strain to these conditions becomes critical to the success or failure of MLF.

Another important factor that can affect MLF outcome is the timing of bacterial inoculation. Of the different inoculation timings, sequential and co-inoculation are considered two major options (Bartowsky *et al.*, 2015; Sumby *et al.*, 2019). Sequential inoculation, whereby the starter culture is inoculated after alcoholic fermentation (AF), reduces the risk of acetic acid accumulation from carbohydrate metabolism by heterofermentative LAB. However, exposure of the starter culture to a high alcohol concentration can be detrimental to bacterial survival and MLF capability. During co-inoculation, the malolactic starter culture is typically inoculated 24 hours or more after yeast, thus enabling the bacteria to acclimatise

TABLE 1. Microorganisms used in studies of the interaction of *Saccharomyces cerevisiae* and *Oenococcus oeni*.

Microorganism Commercial Name	AWMCC ID[a]	Source	Culture type[b]	Experiment[c]	Yeast SO_2 production potential[d]
S. cerevisiae					
796	AWRI796	AWMCC	LLC	IE-1	Non-SOP
Lalvin ICV D254	AWRI1483	AWMCC	LLC	IE-1	Non-SOP
NT 50	AWRI2880	AWMCC	LLC	IE-1, IE-2	SOP
Lalvin T73	AWRI1497	AWMCC	LLC	IE-1, IE-2, Ac.add	SOP
796	na	AB Biotek	ADWY	Winery trial	Non-SOP
CY3079	na	Lallemand Aust.	ADWY	Winery trial	Non-SOP
NT 50	na	Anchor	ADWY	Winery trial	SOP
Actiflore Rosé	na	Laffort Aust.	ADWY	Winery trial	SOP
O. oeni					
YV Select	na	Lallemand Aust.	FD	IE-1, IE-2	
VP41	na	Lallemand Aust.	FD	IE-2, Winery trial	
CH35	na	Chr. Hansen, Aust.	FD	IE-2	

[a] AWMCC ID; AWRI Wine Microorganism Culture Collection identification number, na; not available.
[b] LLC; Laboratory Liquid Culture, ADWY; Commercial active dry wine yeast, FD; Commercial freeze-dried bacteria.
[c] IE-1; Interaction experiment 1, IE-2; Interaction experiment 2, Ac.add; Effect of exogenous acetaldehyde addition.
[d] Non-SOP; limited SO_2 production, SOP; high SO_2 production potential.

to increasing alcohol concentration and, further, allowing greater availability of grape-derived nutrients in the early stages of AF. Importantly, this strategy has been suggested to improve overall MLF efficiency and assist in overcoming difficult wine conditions (Bartowsky *et al.*, 2015).

Some decades ago, the benefits of early inoculation of yeast and malolactic bacteria for rapid MLF induction were recognised (Beelman and Kunkee, 1985). A growing interest in co-inoculation (Bartowsky *et al.*, 2015) has led to its successful application for MLF induction in white wine (Jussier *et al.*, 2006; Sereni *et al.*, 2020; Zapparoli *et al.*, 2006), including white varieties with low pH (Guzzon *et al.*, 2016; Knoll *et al.*, 2012). However, the potential of co-inoculation for MLF induction in white musts/wines with challenging concentrations of SO_2 remains unexplored. Further, since the interactions between yeast and bacteria during co-inoculation are likely more complex than sequential inoculation (Zapparoli *et al.*, 2006), a greater understanding of such interactions is required to improve MLF performance in harsh wine environments.

This study investigates the role of *S. cerevisiae*-derived SO_2 on MLF progress during the co-inoculated fermentation of Chardonnay. Representative yeast strains with varying SO_2 production potential were investigated for their interactions with *O. oeni* during co-fermentation in Chardonnay. The early, transient formation of acetaldehyde by yeast was observed to be an important factor modulating SO_2 resistance in malolactic starter cultures during co-fermentation. Finally, a pilot-scale study further explored the effect of different yeast-bacteria pairings, in which acetaldehyde production varied, on MLF progress and wine sensory properties.

MATERIAL AND METHODS

1. Microorganisms

Experiments investigating the SO_2 production potential of *S. cerevisiae* wine yeast strains utilised isolates of commercial preparations and other wine strains obtained from the Australian Wine Research Institute (AWRI) Wine Microorganism Culture Collection (Figure 1). Some yeast strains are interspecific hybrids in nature (Borneman *et al.*, 2016). In subsequent experiments, representative yeast strains were selected to investigate the interactive effects of yeast strain SO_2 production potential on the survival of *O. oeni* during co-fermentation (see Table 1 for a listing and description of *S. cerevisiae* and *O. oeni* strains used in yeast-bacteria interaction studies). The yeast strains included two non-SO_2-producing yeast strains (AWRI796, AWRI1483) and two SO_2-producing yeast strains (AWRI1497 and AWRI2880). A final pilot-scale yeast-bacteria interaction study utilised commercial active dry wine yeast (ADWY) preparations of two of these strains [Maurivin™ AWRI796 (AB Biotek, Australia) and Anchor NT 50™ (Lallemand Australia)], and an additional non-SO_2-producing strain [(Lalvin CY3079™ (Lallemand Australia)] and an SO_2-producing strain [Actiflore® Rosé (Laffort Australia)]. The latter ADWY strain was chosen since the commercial form of AWRI1497 (Lalvin T73) was not available in Australia. Commercial freeze-dried preparations of *O. oeni* [AWRI YV Select™, Lalvin VP41™ (Lallemand Australia),

and VINIFLORA® CH35 (Chr. Hansen, Australia)] were used in yeast-bacteria interaction studies where indicated.

2. Laboratory-scale studies

2.1 Grape juice

Laboratory-scale studies utilised commercially processed Chardonnay juice (Pernod Ricard Winemakers, Rowland Flat, South Australia, 2017 vintage) stored at –20 °C. Before experimental use, sub-lots were thawed and sterile-filtered (0.2 µm pore size membrane). The chemical composition of this juice is shown in Table 2.

2.2 Yeast and bacterial starter culture preparation

Yeast starter cultures were precultured in YPD (1 % w/v yeast extract, 2 % w/v peptone, and 2 % w/v D-glucose) medium at 28 °C for 24 h with constant agitation. The YPD cultures were then inoculated (1 % v/v) into Chardonnay grape juice (50 % v/v diluted with sterile Milli-Q® purified water). Yeast cells for experimental use were obtained from Chardonnay grape juice starter cultures after aerobic incubation at 27 °C for 24 h with constant agitation. Commercial preparations of *O. oeni* malolactic starter cultures were rehydrated in sterile purified water for approximately 20 min prior to use.

2.3 Fermentation conditions

Laboratory-scale fermentations were conducted in 100 mL or 500 mL in the case of Interaction experiment 1, screw-cap sealed (air-tight) glass (Schott) vessels fitted with a one-way directional-flow gas check valve and sample port assembly described by Schmidt *et al.* (2020). This apparatus prevented air ingress and enabled the rapid formation of inert conditions at the start of AF, which were critical in preventing metabolic interference from oxygen and oxidative reactions with SO_2. The 100 mL and 500 mL vessels were filled with 90–100 mL and 530 mL Chardonnay juice and constantly stirred at 250 rpm and 150 rpm, respectively. AF was undertaken at 17 °C, with minimal foaming in the fermentation headspace. Yeast starter cultures prepared in grape juice were inoculated at an optical density of 0.02 absorbance units (600 nm) (approximately 1×10^6 cells/mL).

In the study of SO_2 production by wine yeast, fermentations were sampled to measure glucose plus fructose. Fermentations were cold settled (4 °C) and clarified by centrifugation and filtration (0.2 µm syringe filters, Millipore, Billerica, M.A., USA) after completion of AF (average glucose plus fructose concentration ≤ 1 g/L). Analysis of total SO_2 was undertaken on completion of clarification operations. All experiments were done in triplicate.

For yeast-bacteria co-fermentations, *O. oeni* starter cultures were inoculated 24 h after yeast inoculation at approximately 5×10^6 cells/mL. Co-fermentations were undertaken in triplicate (three bacteria strain replicates per yeast strain), except for Interaction experiment 2, in which yeast strain was replicated (n = 3) and the bacterial strain was also replicated (n = 3) within yeast strain (Figure S1). Upon completion of AF, wines were incubated statically at 22 °C for the duration of MLF. Samples (1–2 mL) were taken aseptically during fermentation to determine glucose plus fructose, L-malic acid, acetaldehyde, total SO_2, and viable bacteria as required. Samples for chemical analyses were centrifuged (approximately 24,000 × g, 2 min), and supernatants were stored at –20 °C. At MLF completion (average L-malic acid concentration < 0.3 g/L), wines from Interaction experiment 1 were centrifuged (3273 × g, 30 min) and stored at –20 °C for further analyses.

2.3.1. Effect of exogenous acetaldehyde addition

The effects of adding excess acetaldehyde (99 mg/L; molar ratio acetaldehyde:SO_2, 5:1) during co-fermentation of the SO_2-producing yeast *S. cerevisiae* AWRI1497 on the survival of *O. oeni* YV Select was investigated in the same 2017 Chardonnay juice. Acetaldehyde (Sigma-Aldrich, St. Louis, MO) was added to fermentations 4 h prior to bacteria inoculation from a freshly prepared aqueous stock solution (2 % v/v distilled acetaldehyde in Milli-Q H_2O). In preparing the latter solution, losses of acetaldehyde were minimised by liquid handling in a 4 °C cold room, using Milli-Q purified water (49 mL) pre-chilled to 4 °C and dispensing acetaldehyde (stored at –20 °C) under the aqueous surface using a pre-chilled (4 °C) gas-tight glass micro-syringe. The required volume of acetaldehyde stock solution (0.62 mL) was added to 100 mL fermentations via a fitted (Luer) sampling port and sterile syringe.

3. Pilot-scale winemaking study

A pilot-scale study of the interactive effects of two non-SO_2-producing commercial yeast strains [(Maurivin™ AWRI796, Lalvin CY3079™)] and two SO_2-producing

TABLE 2. Composition of Chardonnay grape juice used in this study.

	2017[a]	2021[b]
Density (°Brix)	22.0 (0.2)	20.4
pH	3.34 (0.03)	3.27
Total acidity (g/L)	6.2 (0.5)	7.5
YAN[c] (mg/L)	337 (18)	316
Free SO_2 (mg/L)	6 (4.8)	N.D.[d]
Total SO_2 (mg/L)	40 (2.1)	N.D.

[a]Data are mean (SD) values of several juice sub-lots.

[b]Data from single juice sub-lot. [c]YAN, yeast assimilable nitrogen. [d]N.D. Not determined, non-sulfited juice.

commercial yeast strains [(Actiflore® Rosé, Anchor NT 50)] on the survival and MLF capability of *O. oeni* VP41™ was undertaken during co-fermentation in Chardonnay (2021). Approximately 1 tonne of Chardonnay grapes (Willunga, South Australia) from the 2021 vintage were hand-picked (non-sulfited) and transported to the Hickinbotham Roseworthy Wine Science Laboratory, Waite Campus, The University of Adelaide, South Australia, and stored in a cool room at 0 °C for several days. Grapes (non-sulfited) were crushed, destemmed, pressed and, after the addition of pectinase enzyme (Rohavin L, AB Biotek, 4 mL/100 L), the juice was cold settled (0 °C for approx. 5 days) and acid adjusted with tartaric acid (1.5 g/L). The composition of the 2021 Chardonnay grape juice (non-sulfited) is shown in Table 2. Twelve stainless steel fermentation vessels (50 L) were filled with 35 L of Chardonnay juice and warmed to 18–20 °C before yeast inoculation. AF was initiated by inoculation of the respective yeast strain preparations. These were rehydrated and inoculated (triplicate) according to the manufacturer's recommendations. After 24 h, *O. oeni* VP41 malolactic starter culture was rehydrated according to the manufacturer's recommendations and inoculated into each fermentation at 1.5 times the recommended rate to achieve approximately 2×10^6 CFU/mL. Alcoholic fermentations were maintained at approx. 17–22 °C and, after the active phase, were racked to fill 30 L stainless steel fermentation vessels. After completion of AF, wines were maintained at 20 °C for MLF. Samples were taken aseptically during co-fermentations to determine concentrations of glucose plus fructose, L-malic acid, acetaldehyde, total SO_2, viable bacteria, and other wine components as required. After MLF, wines were incubated for an additional 7 days (approx.) at 20 °C, after which SO_2 was added (80 mg/L). Wines were then settled at 0–4 °C, subsequently racked, free SO_2 concentration adjusted to approximately 40–45 mg/L, filtered (0.45 µm pore size membrane), and bottled with a screw-cap closure. Bottled wines were stored at 15 °C for approximately 7 months prior to sensory and diacetyl analyses.

4. Chemical analyses

Concentrations of residual sugar and L-malic acid were determined enzymatically (Megazyme) utilising 96-well microtiter plates. Methods for quantifying glucose and fructose were based on those described by Hohorst (1965) and Vermeir *et al.* (2007). Reagent and enzyme concentrations used to determine L-malic acid concentration were based on a proprietary (Molecular Devices, MDS Analytical Technologies) microplate method.

Acetaldehyde concentration was determined enzymatically (Megazyme) using 96-well microplates and glycylglycine buffer similar to that described by McCloskey and Mahaney (1981) (15.8 g/L glycylglycine, 7.46 g/L KCl, pH 9, without polyvinylpyrrolidone). Acetaldehyde standards (0–100 mg/L) were prepared from an equimolar aqueous stock solution of acetaldehyde-bisulfite (4.55 mM, 250 mL). The stock solution was prepared from freshly distilled acetaldehyde (250 µL, density 0.801 g/cm^3 at 4 °C) and potassium metabisulfite (Ace Chemical Co., Camden Park, South Australia, or Ajax Finechem, Seven Hills, N.S.W., Australia; 0.5061 g) in MilliQ purified water. During stock solution preparation, losses of acetaldehyde were minimised by using the previously described procedure for the preparation of 2 % v/v aqueous acetaldehyde. After preparation, the stock solution was equilibrated by storage at 4 °C for at least 24 h. Total SO_2 concentration was determined colorimetrically (420 nm) using 5,5´-dithio-bis-2-nitrobenzoic acid (DTNB) reagent prepared in phosphate buffer (pH 8.4) or commercial preparation (Rowe Scientific, Adelaide, Australia). Samples of juice and wines during yeast SO_2 production experiments were analysed using a Discrete Analyser (Thermo Fischer Scientific) by Affinity Labs (previously AWRI Commercial Services) [International Organization for Standardization 17025 accredited laboratory, Adelaide, SA, Australia]. For the remaining studies, SO_2 concentration was analysed using DTNB reagent and phosphate buffer (pH 8.4) in conjunction with 96-well microplates (reaction volume 260 µL). Sulfite standards (0–120 mg/L total SO_2) for microplate assays were prepared from the equimolar stock solution of acetaldehyde-bisulfite (4.55 mM; molecular mass SO_2, 64.1 g/mol) described above for the analysis of acetaldehyde.

Low concentrations of free SO_2 were calculated based on the approach of Hood (1983), using the following formula (assuming acetaldehyde as the major carbonyl contributing to bound SO_2):

$$[S] = K/[X\text{-}x][x]$$

where [S], [X], and [x] are molecular concentrations of, respectively, free SO_2 in any form, (total) carbonyl compound (acetaldehyde) and undissociated carbonyl bisulphite (bound SO_2), and K = equilibrium constant of acetaldehyde bisulphite (1.5×10^{-6} at pH 3.3) (Burroughs and Sparks, 1973). Molecular SO_2 concentration was calculated from the respective free SO_2 concentration and initial Chardonnay juice pH.

The concentration of succinic acid was determined by high-performance liquid chromatography (HPLC) as described by Nissen *et al.* (1997) using a Bio-Rad HPX-87H column. Other wine compositional analyses (pH values and total acidity, ethanol, acetic acid, and diacetyl concentrations) were determined using the National Association of Testing Authorities (NATA) accredited protocols by Affinity Labs (previously AWRI Commercial Services) (Adelaide, Australia). Diacetyl concentration was determined using a stable isotope method using solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC–MS), based on that described by Hayasaka and Bartowsky (1999). Diacetyl analysis was undertaken using bottled wine samples at the time of sensory assessment.

5. Determination of viable bacteria

Viable *O. oeni* cells were enumerated by dispensing 50 µL aliquots of serially diluted (0.1 % w/v bacteriological peptone (Amyl Media, Australia)) fermentation samples onto a modified de Man, Rogosa, and Sharpe (MRS, Amyl or Oxoid) agar using an automated spiral plater (WASP 2, Don Whitley Scientific, Australia). The latter medium was prepared by

supplementing MRS medium with preservative-free clarified apple juice (20 % v/v) and agar (1.5–2.4 % w/v, Amyl or Oxoid); in the first interaction experiment, this medium was further supplemented with preservative-free clarified red grape juice (20 % v/v). Natamycin (50 mg/L, NataP UltraPure, Handary, Alchemy Agencies, Australia) was added to modified MRS agar media to suppress yeast growth. The agar plates were incubated aerobically (or for some experiments in a nitrogen atmosphere) at 27 °C for approximately 7–10 days, and resultant bacterial colonies were enumerated using a Protocol 3 colony counter (Synopsis, Don Whitley Scientific, Australia).

6. Sensory analysis

The projective mapping sensory method of Napping (N) was used to holistically evaluate the treatments and replicate Chardonnay wines from the pilot-scale study based on their perceived similarities and differences. Ultra-Flash Profiling (UFP) was also employed to provide indicative sensory (appearance, aroma, and palate) descriptors of the sample set and groupings (Pagès, 2005). A panel of 9 assessors (5 females, 4 males) with an average age of 48 years (*SD* = 10.0) were convened to evaluate the wine set in duplicate. All panellists were part of the external AWRI-trained descriptive analysis panel, had extensive experience in wine quantitative sensory descriptive analysis (QSDA), and had completed N/UFP tasks previously. Prior to the evaluation, panellists were familiarised with a sub-selection of the samples for tasting and general discussion as a training task. Panellists assessed the wines in duplicate for the formal evaluation, with both sets of 12 wines (4 treatments × 3 replicates) assessed on the same day. Assessors were allowed as long as required to complete their projective maps and to add descriptive attributes, with a minimum 15-minute break between duplicate assessments. Samples were provided to panellists in 30 mL aliquots, in 3-digit-coded, covered, ISO standard wine glasses at 22–24 °C, in a randomised presentation order, but assessors were allowed to revisit samples as needed. The formal evaluation was completed in one two-hour session. Each sample was presented in a randomised William's block design generated by Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada). The samples were assessed, and responses were transferred directly onto a 1.5:1 digital sensory space via a tablet, with panellists able to enter free-choice comments for each sample or group of samples.

7. Statistical analysis

Final wine compositional data were assessed by oneway ANOVA using Minitab Statistical Software 21 (Minitab LLC, PA, USA). Tukey's pairwise comparison post hoc test was used to determine if respective means differed among yeast strain treatments ($\alpha = 0.05$). All sensory data analysis was carried out using R with the SensoMineR (http://sensominer.free.fr) (Le and Husson, 2008), factoextra (http://www.sthda.com/english/rpkgs/factoextra) (Kassambara and Mundt, 2020), reshape2 (https://github.com/hadley/reshape), readxl (https://readxl.tidyverse.org, https://github.com/tidyverse/readxl) (Wickham *et al.*, 2019) and ggrepel (http://github.com/slowkow/ggrepel) (Slowikowski, 2021) packages. Multiple factor analysis (MFA) of the sample coordinates of all panellists' maps was conducted to produce a group consensus map for the 12 wines. Secondarily, UFP descriptors were incorporated as supplementary variables to the MFA consensus map through the ratio scaling of the scores of the correlations between variables and factors to the range of the group factor scores of the wines. The position of the UFP descriptors on the MFA map provides indicative sensory properties of the wines.

For finalising the UFP data, one sensory analyst with wine expertise inspected and consolidated perceptually similar attributes upon discussion with assessors, known attribute correlations, and paying particular attention to the wine descriptive terminology hierarchy proposed in Noble *et al*. (1987). Attributes describing the entire wine set were generally eliminated from the data, while attributes discriminating between wines were retained. Attributes with a low frequency of use were removed. This data processing aided in the clarity of the visualisation of the consensus maps produced, with the resulting maps highlighting only the attributes that discriminated the wines. Panel performance was assessed using the multivariate RV coefficient for each panellist compared to the consensus MFA map produced. All panellists were found to be performing at an acceptable level, given the overall variability in the wine set.

RESULTS

1. Yeast strain SO_2-production potential

The SO_2 production potential of a genetically diverse subset of 94 *S. cerevisiae* strains from within the wine clade (Figure 1) was assessed to identify the potential for positive and negative interactions with *O. oeni*. From the initial SO_2 concentration of 41 mg/L in this Chardonnay (2017) juice batch, the SO_2 concentration after fermentation varied by more than tenfold from 20 to 212 mg/L. Further, approximately half of the strains either increased or decreased the SO_2 concentration relative to the initial concentration of SO_2. The 90th percentile of SO_2 concentration was 63 mg/L, 22 mg/L more than the starting concentration, and the 10th percentile of SO_2 concentration was 29 mg/L, 12 mg/L less than the starting concentration.

2. Effect of yeast strain and SO_2 production on the survival of *O. oeni* during co-fermentation

The effects of two non-SO_2-producing yeast strains (AWRI796, AWRI1483) and two SO_2-producing yeast strains (AWRI1497 and AWRI2880) on the survival, growth and MLF performance of the commercial *O. oeni* strain YV Select was investigated during co-fermentation in Chardonnay. The yeast strains were selected on their ability to modulate SO_2 concentration during fermentation in Chardonnay (Figure 1). The concentrations of total SO_2, L-malic acid, viable bacteria, and the duration of AF during co-fermentation of *O. oeni* YV Select with each yeast strain are shown in Figure 2.

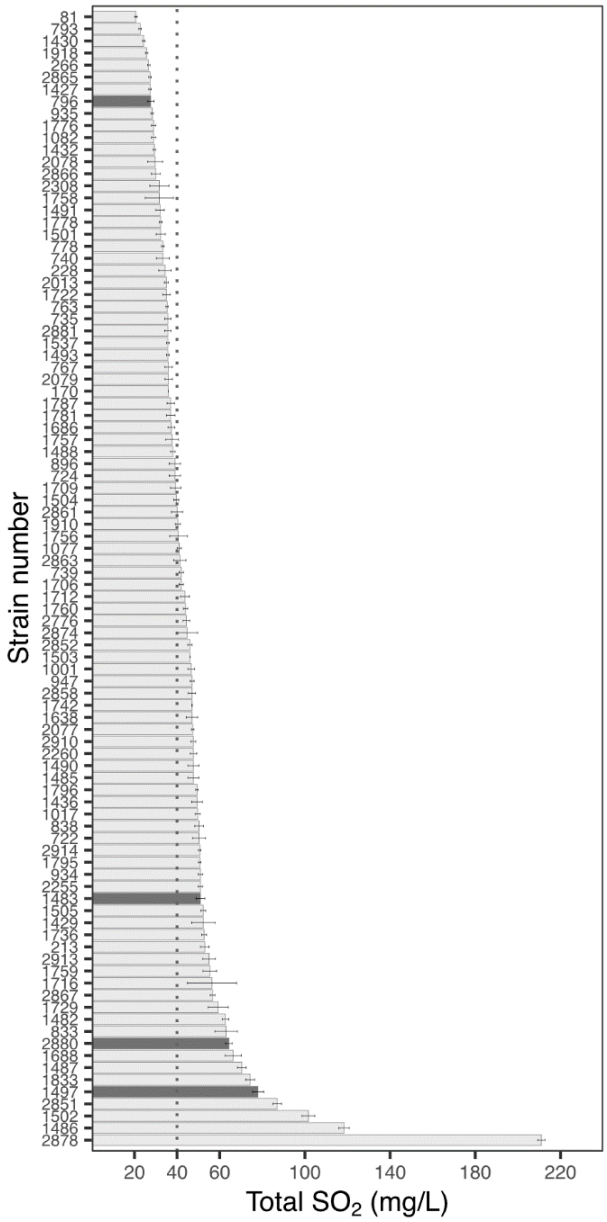


FIGURE 1. Total SO_2 concentration in finished Chardonnay wine following fermentation with different strains of *S. cerevisiae*.

Yeast strains are identified by their AWMCC identification number on the y-axis. The dotted line indicates the total SO_2 concentration of the Chardonnay juice prior to fermentation. Strains marked with dark-coloured bars indicate those used in subsequent work. Bars show the mean total SO_2 concentration, with error bars showing the standard deviation (n = 3).

Bacterial viable cell concentrations in co-fermentations with each *S. cerevisiae* strain remained relatively constant (4.4×10^6 (SD = 1.1×10^6) CFU/mL) over the first 24 h following bacterial inoculation (days 1–2 of AF). However, bacterial viable cell concentrations in all co-fermentations declined over the subsequent 5 days (days 2–7 of AF). The extent of the decrease in bacterial viability varied greatly regardless of whether *O. oeni* was inoculated with SO_2-producing or non-SO_2-producing yeast strains.

For the non-SO_2-producing yeast strains, the bacterial viable cell population within 7 days decreased by $>10^2$- and $>10^4$-fold for AWRI796 and AWRI1483, respectively. The decrease in bacterial cell viability affected MLF duration, which increased from 36 days with AWRI796 to 64 days with AWRI1483. These co-fermentations were associated with decreases in total SO_2 concentration of 14 mg/L and 5 mg/L during AF.

Co-fermentations with the yeast strains AWRI1497 and AWRI2880 substantially increased total SO_2 concentration to 65 mg/L and 63 mg/L after 5 days (Figure 2). However, these yeast strains had different effects on bacterial survival and MLF activity despite producing similar concentrations of SO_2. For *S. cerevisiae* AWRI1497, the viable bacterial population diminished to non-detectable

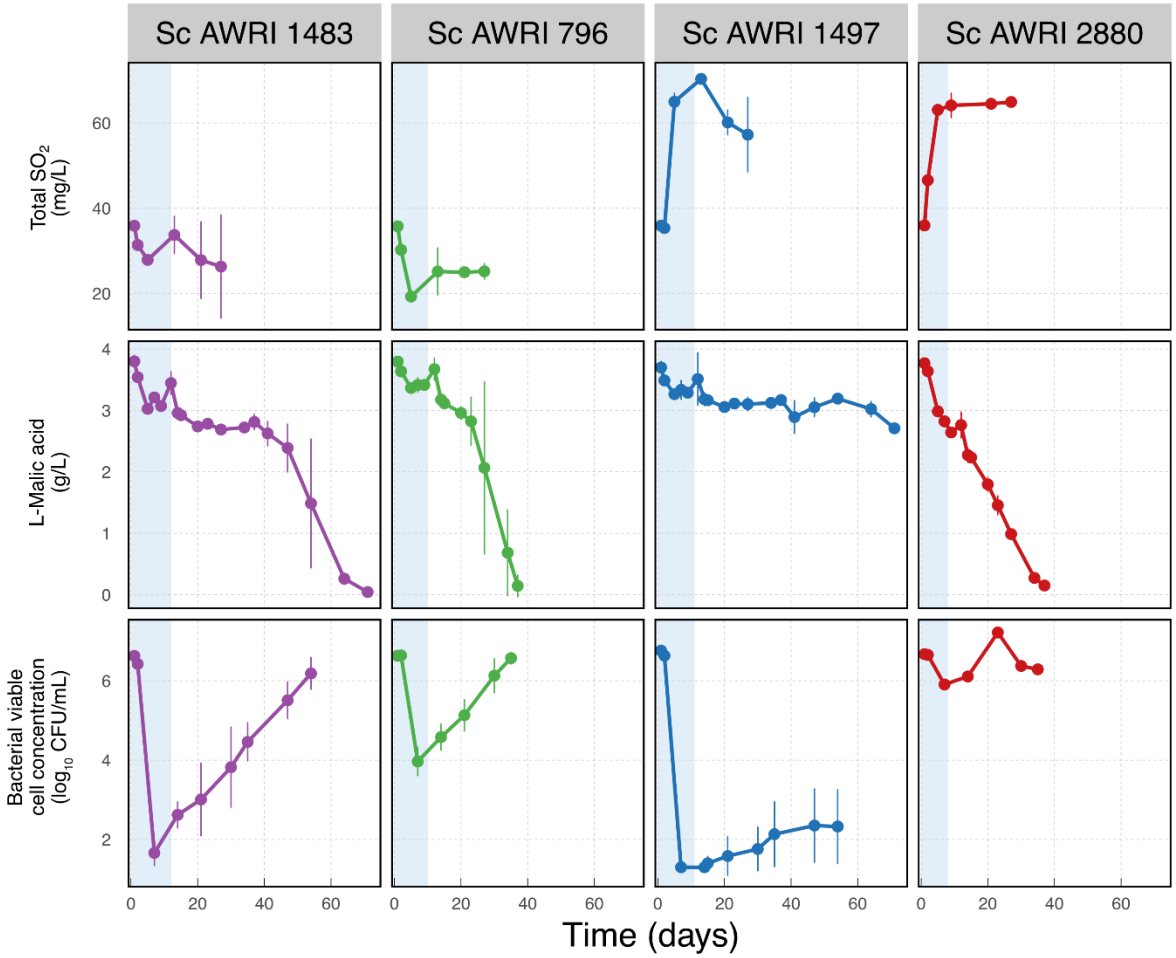


FIGURE 2. Effects of *S. cerevisiae* (Sc) strains AWRI1483, AWRI796, AWRI1497, and AWRI2880 on MLF by *O. oeni* YV Select and bacterial survival following co-inoculation of Chardonnay juice.

Figures show total SO_2, L-malic acid, and bacterial viable cell concentrations for respective yeast-bacteria combinations. *O. oeni* YV Select was inoculated 24 h after inoculation of each yeast strain. Blue shading indicates the time for completion of AF. Data are the average and standard deviation of 3 replicate fermentations. Data points for viable bacterial concentrations on days 7 and 14 in Sc AWRI1497 co-fermentations are below the limit of detection (1.3 $\log_{10}$ CFU/mL).

levels (< 20 CFU/ mL) and failed to recover sufficiently to initiate MLF. Co-fermentations with *S. cerevisiae* AWRI2880 exhibited a comparatively small initial decline in bacterial viability to 8.1×10^5 CFU/mL within the first 7 days, after which it increased to $> 1.0 \times 10^6$ CFU/mL, and ultimately completed MLF within 34 days. Moreover, although there was evidence ($P < 0.05$) for differences in the concentrations of some major final wine parameters (including alcohol, total acidity, acetic and succinic acids, and pH value) (Table 3), the magnitude of those were small and remained within a normal concentration range for wine.

3. Influence of early acetaldehyde production by SO_2-producing yeast on *O. oeni* survival

Variations between yeast strains in the production of acetaldehyde may give rise to subtle variations in free SO_2 concentrations during the early stages of fermentation. Consequently, modulation of free SO_2 concentration during the crucial post-inoculation period may also affect bacterial survival. Whether variation in acetaldehyde formation by SO_2-producing yeast strains could explain the differential post-inoculation survival rates of *O. oeni* was investigated in the laboratory- and pilot-scale studies. We also sought to determine whether the effect of yeast on *O. oeni* survival reported in Figure 2 was specific to the bacterial strain used or whether that observation could be generalised to other bacterial strains.

3.1 Laboratory scale studies

The effects of the two previously studied SO_2-producing yeast strains, *S. cerevisiae* strains AWRI1497 and AWRI2880, on the viability of *O. oeni* YV Select and two other commercial *O. oeni* strains (VP41 and CH35) following co-inoculation into Chardonnay was investigated (Figure 3). These three *O. oeni* strains are commercially available to the Australian wine industry. As with the previous experiment (Figure 2), AWRI1497 and AWRI2880 produced similar concentrations

TABLE 3. Chemical composition of Chardonnay wines after MLF following co-fermentation with different *S. cerevisiae* yeast strains and *O. oeni* YV Select.

		AWRI796	AWRI1483	AWRI1497	AWRI2880
Alcohol	(%v/v)	13.4 (0.00) [a]	13.4 (0.00) [a]	13.4 (0.00) [a]	13.2 (0.06) [b]
pH		3.52 (0.01) [c]	3.57 (0.01) [a]	3.43 (0.01) [d]	3.54 (0.01) [b]
Total acidity	(g/L)	6.0 (0.0) [b]	5.6 (0.1) [c]	7.1 (0.1) [a]	5.7 (0.0) [c]
Malic acid	(g/L)	0.14 (0.18) [b]	0.04 (0.02) [b]	2.71 (0.02) [a]	0.15 (0.05) [b]
Acetic acid	(g/L)	0.40 (0.08) [a]	0.37 (0.09) [a]	0.27 (0.01) [a]	0.37 (0.01) [a]
Succinic acid	(g/L)	3.0 (0.0) [a]	2.8 (0.1) [b]	3.0 (0.1) [a]	2.8 (0.0) [b]
Total SO_2*	(mg/L)	25.1 (5.6) [b]	33.7 (4.4) [b]	70.3 (1.1) [a]	64.1 (2.9) [a]
AF Ferm Time	(days)	10	12	11	8
MLF Ferm Time	(days)	37	64	na**	34

* Total SO_2 determined after completion of AF.

* * na; not applicable, wine treatment did not undergo MLF. Data are the mean (SD) of 3 replicate fermentations. Mean values in rows with different letters are significantly different ($\alpha = 0.05$).

of total SO_2 (25–31 mg/L average maximum increase), but their effect on bacterial survival differed (Figure 3). The variation in bacterial survival was associated with the early transient formation of acetaldehyde and, consequently, MLF duration (Figure 3). Over the first 5 days of co-fermentations with *S. cerevisiae* AWRI1497, each *O. oeni* strain exhibited a similar, linear reduction in viability from approximately 5×10^6 CFU/mL to 6×10^2 CFU/mL (Figure 3), coinciding with a slow increase in acetaldehyde concentration to a peak of 64 mg/L at day 4 (Figure 3). From day 5, recovery of YV Select and VP41 viability was observed, increasing to $> 10^6$ CFU/mL at 44 days, with MLF completion at 58 days (Figure 3). In comparison, the recovery of *O. oeni* CH35 was slower, achieving $> 10^5$ CFU/mL after 65 days (Figure 3), and although initiating L-malic acid degradation, MLF was still incomplete at 74 days (Figure 3).

In contrast to *S. cerevisiae* AWRI1497, the impact of *S. cerevisiae* AWRI2880 on *O. oeni* strains YV Select and VP41 was relatively minor, their viability declining to approximately 2×10^6 CFU/mL after 5 days and remaining at $\geq 1 \times 10^6$ CFU/mL until 23 days (Figure 3). For both *O. oeni* strains, MLF was complete at 26 days (Figure 3). However, for co-fermentations of *S. cerevisiae* AWRI2880 and *O. oeni* CH35, a linear decrease in bacterial viability from 5×10^6 CFU/mL to 3×10^4 CFU/mL occurred during the first 5 days, followed by recovery to $\geq 1 \times 10^6$ CFU/mL at 51 days and completion of MLF at 74 days (Figure 3). The average acetaldehyde concentration in co-fermentations with *S. cerevisiae* AWRI2880 increased rapidly, reaching 95 mg/L on day one and a maximum of 112 mg/L on day 3, ultimately decreasing to 50–53 mg/L on day 8 (Figure 3). Compared to *S. cerevisiae* AWRI1497, it is noteworthy that acetaldehyde production began within the first 24 h for AWRI2880, while SO_2 production was not observed until 2 days after inoculation.

Determination of the molar ratio of acetaldehyde to total SO_2 in the early stages of fermentation revealed these values were considerably higher for AWRI2880 than AWRI1497, particularly at, and immediately following, bacterial inoculation; day one (4.0 vs. 1.6), day two (2.7 vs. 1.9) and day three (2.7 vs. 1.6). At the end of AF, the average molar ratio of acetaldehyde to total SO_2 for each yeast-bacteria strain combination was similar (1.1–1.3). The concentrations of total SO_2 and acetaldehyde were used to calculate the concentration of molecular SO_2 over time (Figure 3). These calculations suggest that the variation in acetaldehyde concentrations produced by the two yeast strains resulted in differences in *O. oeni* molecular SO_2 exposure.

During co-fermentation with *S. cerevisiae* AWRI1497, the calculated molecular SO_2 concentration experienced by bacteria was 3.8–5.8 µg/L at days 1–3 and 7.1–15.6 µg/L at days 4–5. These molecular SO_2 concentrations were associated with a loss in bacterial viability. In contrast, the much lower calculated concentrations of molecular SO_2 during co-fermentation with *S. cerevisiae* AWRI2880 (i.e., approx. 0.9-1.7 µg/L at days 1–3 and 3.6–5.9 µg/L at days 4–5) had a negligible effect on the viability of *O. oeni* strains YV Select and VP41, although were associated with a reduction in viability of approximately 10^2 CFU/mL for *O. oeni* CH35. Interestingly, a subsequent increase in calculated molecular SO_2 concentration to 14.6 µg/L on day 8 in this co-fermentation did not impact bacterial viability further.

3.2 Direct acetaldehyde addition prior to *O. oeni* inoculation does not stimulate bacterial survival

If early rapid acetaldehyde accumulation contributes to *O. oeni* survival through quenching of molecular SO_2 concentration, adding exogenous acetaldehyde might stimulate a similar effect. This idea was tested in fermentations initiated with AWRI1497 by adding excess acetaldehyde

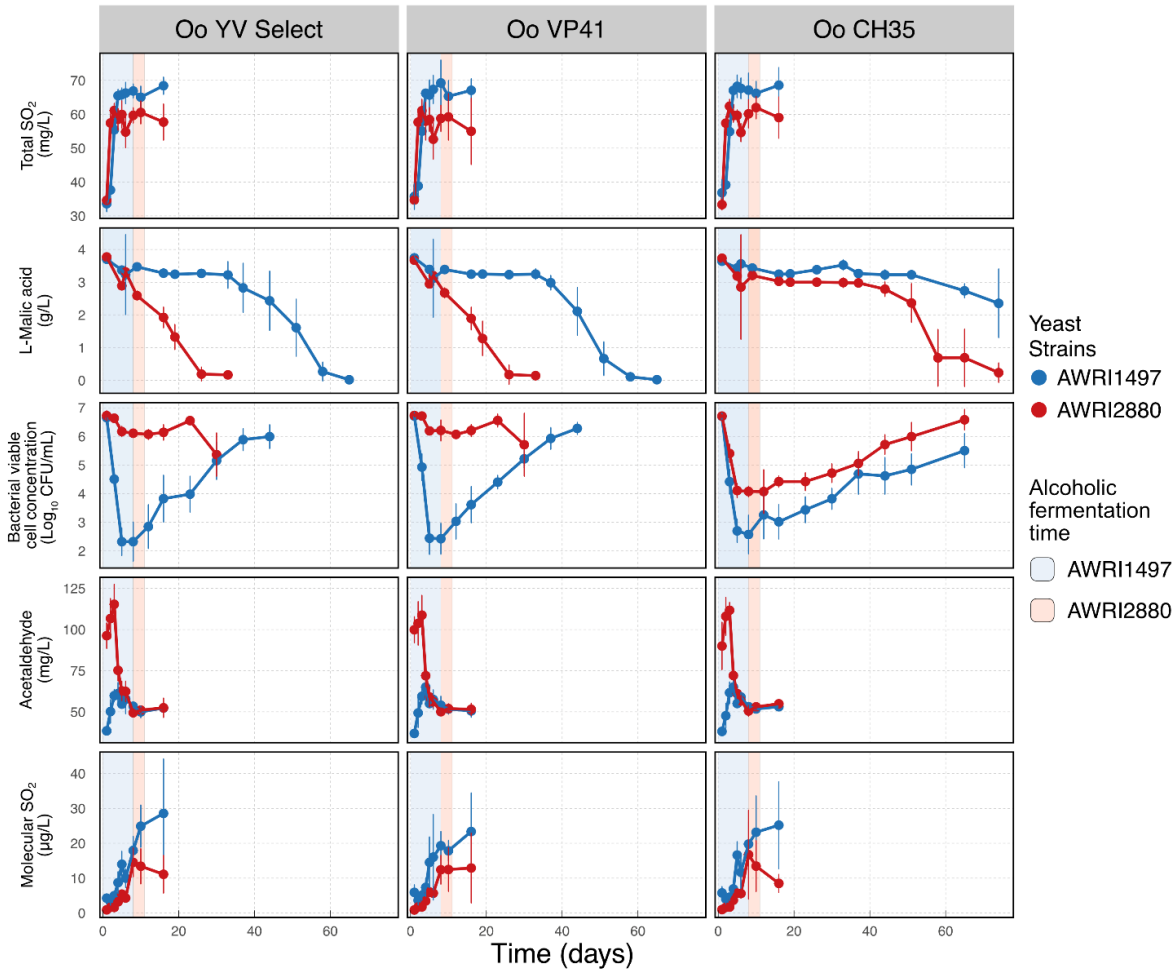


FIGURE 3. Effects of acetaldehyde and SO_2 production by *S. cerevisiae* strains AWRI1497 and AWRI2880 on bacterial survival and MLF duration by *O. oeni* strains YV Select, VP41, and CH35 following co-inoculation of Chardonnay.

Figures show concentrations of total SO_2, L-malic aid, bacterial viable cell concentration, acetaldehyde and calculated molecular SO_2 for respective yeast-bacteria combinations. Shading indicates the time for completion of AF by respective yeast strains. Data are the average and standard deviation of 9 replicate fermentations. For the yeast/bacteria combination AWRI2880 and *O. oeni* CH35, data for 8 replicate fermentations are shown.

(99 mg/L) four hours before inoculation with *O. oeni* YV Select. Bacterial viability was monitored and compared to fermentations without an acetaldehyde addition. The concentration of acetaldehyde in treated co-fermentations decreased rapidly (Figure S2). Within 24 h of acetaldehyde addition, the average concentration of acetaldehyde in treated and untreated ferments was indistinguishable (42–43 mg/L). The control and acetaldehydetreated cofermentations also displayed similar bacterial viability declines to 87–100 CFU/mL at day 4 (Figure S2).

3.3 Pilot-scale study

The relevance of these observations to commercial wine production was demonstrated in a winery-based pilot-scale coinoculation study. This study used commercial ADWY preparations of two non-SO_2-producing and two SO_2-producing yeast strains. Two of these were ADWY preparations of strains used in the first interaction experiment: AWRI796 (i.e., Maurivin AWRI796) and AWRI2880 (i.e., Anchor NT 50). The ADWY strain Actiflore Rosé was included as a second SO_2-producing strain because the commercial form of AWRI1497 (i.e., Lalvin T73) was unavailable in Australia. Lalvin CY3079 was used as the second non-SO_2-producing ADWY strain. The *O. oeni* strain VP41 was selected as a representative commercial malolactic bacterial strain.

Two distinct patterns of total SO_2 formation were evident in the early stages of co-fermentation (Figure 4). Two yeast strains, Actiflore Rosé and NT 50, yielded 56 and 50 mg/L total SO_2 on days 3 and 4, respectively. The other two yeast strains, AWRI796 and CY3079, yielded 18 and 21 mg/L total SO_2 on day 3 (Figure 4). Consistent with laboratory-scale experiments, the effects of the two high SO_2-producing yeast strains on *O. oeni* VP41 were notably different. The bacterial population in co-fermentations with *S. cerevisiae* Actiflore Rosé declined approximately 10-fold to 1.9×10^5 CFU/mL at day 9, recovering to 9.4×10^5 CFU/mL at day 16, after

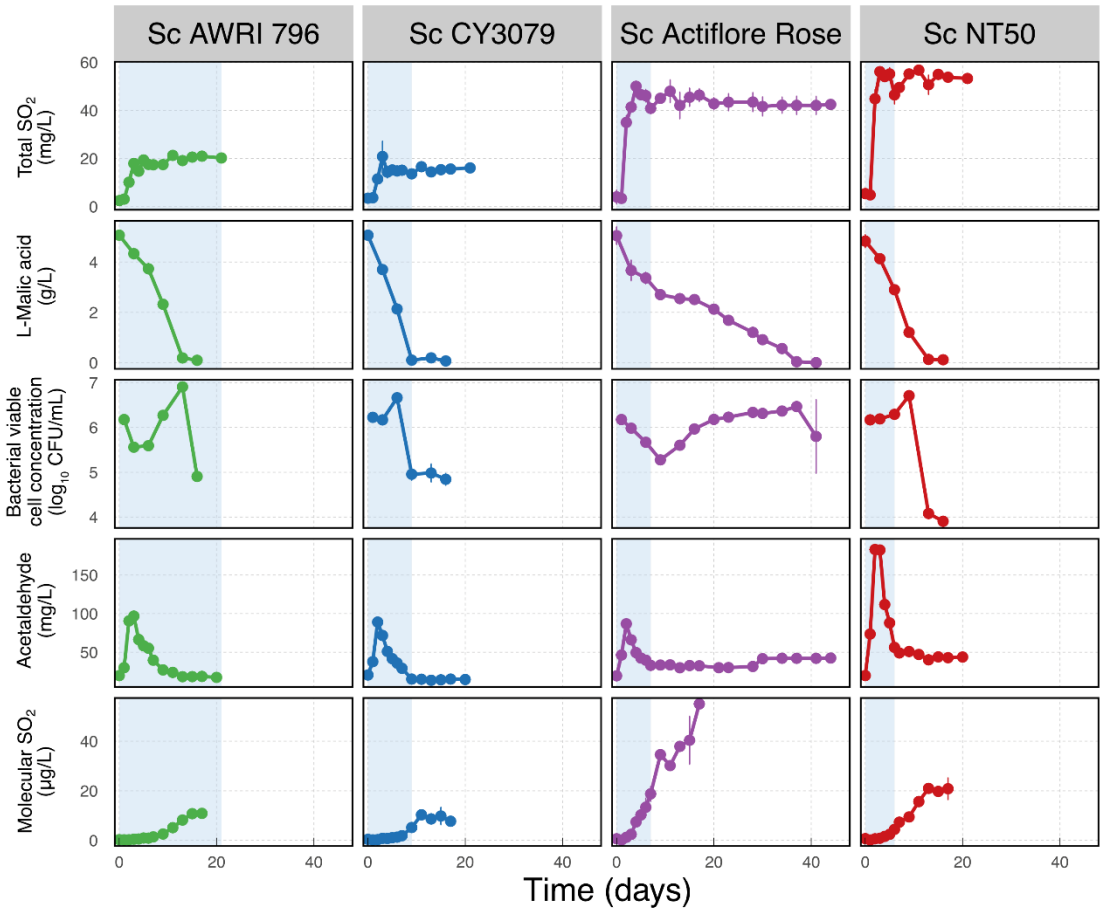


FIGURE 4. Effects of acetaldehyde and SO_2 production by *S. cerevisiae* strains AWRI796, CY3079, Actiflore Rosé and NT 50 on bacterial survival and MLF duration by *O. oeni* VP41 in pilot-scale fermentations of Chardonnay.

Figure panels show concentrations of total SO_2, L-malic acid, bacterial viable cell concentration, acetaldehyde and calculated molecular SO_2 for respective yeast-bacteria combinations. Blue shading indicates the duration of AF. Data are the average and standard deviation of 3 replicate fermentations (3.5 L).

which MLF was complete at day 37 (Figure 4, Table 4). In contrast, co-fermentations with *S. cerevisiae* NT 50 were stimulatory to bacterial growth, yielding a maximum bacterial cell population of 5.1×10^6 CFU/mL at day 9 and rapid completion of MLF at day 13 (Figure 4). A similar pattern of bacterial growth and L-malic acid degradation occurred during co-fermentation with *S. cerevisiae* CY3079 and, to a lesser extent, AWRI796, which only produced maxima of 21 and 19 mg/L total SO_2 within the first five days of AF, respectively (Figure 4).

Consistent with the laboratory-scale interaction experiment 2, the peak acetaldehyde concentration observed during the early stages of co-fermentations with *S. cerevisiae* NT 50 was notably higher (183–182 mg/L maximum at days 2 and 3) compared to the other yeast strains, which produced 87–98 mg/L acetaldehyde at this time (Figure 4).

Further, the calculated molecular SO_2 concentrations during the early stages of co-fermentation (Figure 4) are consistent with laboratory-based work. Higher molecular SO_2 concentrations were observed with *S. cerevisiae* Actiflore Rosé (i.e., approx. 0.2–2.5 µg/L at days 1–3, 7.5–13.4 µg/L at days 4–6 and 18.8–34.6 µg/L at days 7–9) and associated with bacterial inhibition. Lower concentrations were observed with *S. cerevisiae* NT 50 (i.e., approx. ≤ 1.0 µg/L at days 1–3, 1.6–4.4 µg/L at days 4–6 and 7.4–9.5 µg/L at days 7–9) which generally supported bacterial growth.

The concentrations of other chemical components of Chardonnay wines co-fermented with *O. oeni* VP41 and each yeast strain are shown in Table 4. There was evidence ($P < 0.05$) for differences in the concentrations of alcohol, total acidity, acetic acid and in pH value between wines produced by different yeast strains. Notably, acetic acid concentration increased by 0.16 g/L in NT 50 compared to CY3079 wines. Co-fermentations with the different yeast strains had a major effect on wine diacetyl concentration, which was only detected in wines coinoculated with the high SO_2-producing yeast strains. Wines produced with NT 50 (4.9 (*SD* = 0.6) mg/L) contained significantly ($P < 0.05$) greater diacetyl concentrations than wines produced with Actiflore Rosé (1.0 (*SD* = 0.1) mg/L). In contrast, diacetyl was not detected in wines produced from co-fermentations with either of the non-SO_2-producing yeast strains (CY3079 and AWRI796).

TABLE 4. Chemical composition of Chardonnay wines after MLF following pilot-scale co-fermentation with different *S. cerevisiae* yeast strains and *O. oeni* VP41.

		AWRI796	CY3079	Actiflore Rosé	NT 50
Alcohol	(%v/v)	11.9 (0.1) ^{bc}	12.0 (0.0) ^{a}	11.9 (0.0) ^{b}	11.8 (0.0) ^{c}
pH		3.41 (0.01) ^{d}	3.42 (0.00) ^{c}	3.48 (0.01) ^{b}	3.53 (0.01) ^{a}
Total acidity	(g/L)	7.0 (0.0) ^{a}	6.7 (0.0) ^{b}	6.6 (0.1) ^{b}	7.0 (0.1) ^{a}
Acetic acid	(g/L)	0.35 (0.00) ^{b}	0.27 (0.01) ^{c}	0.29 (0.01) ^{c}	0.42 (0.02) ^{a}
Succinic acid	(g/L)	4.8 (0.3) ^{a}	5.1 (0.1) ^{a}	4.8 (0.4) ^{a}	5.0 (0.1) ^{a}
Total SO_2*	(mg/L)	20.2 (0.5) ^{b}	13.6 (0.8) ^{c}	40.8 (2.1) ^{a}	46.5 (3.7) ^{a}
Diacetyl**	(mg/L)	n.d.	n.d.	1.0 (0.1) ^{b}	4.9 (0.6) ^{a}
AF Ferm Time	(days)	21	9	7	6
MLF Ferm Time	(days)	13	9	37	13

*Total SO_2 determined after completion of AF;

**Diacetyl analysis was undertaken at the time of sensory analysis (approx. 7 months after bottling); n.d.: not detected, less than 0.1 mg/L. Data are the mean (SD) of 3 replicate fermentations. Mean values in rows with different letters are significantly different ($\alpha = 0.05$).

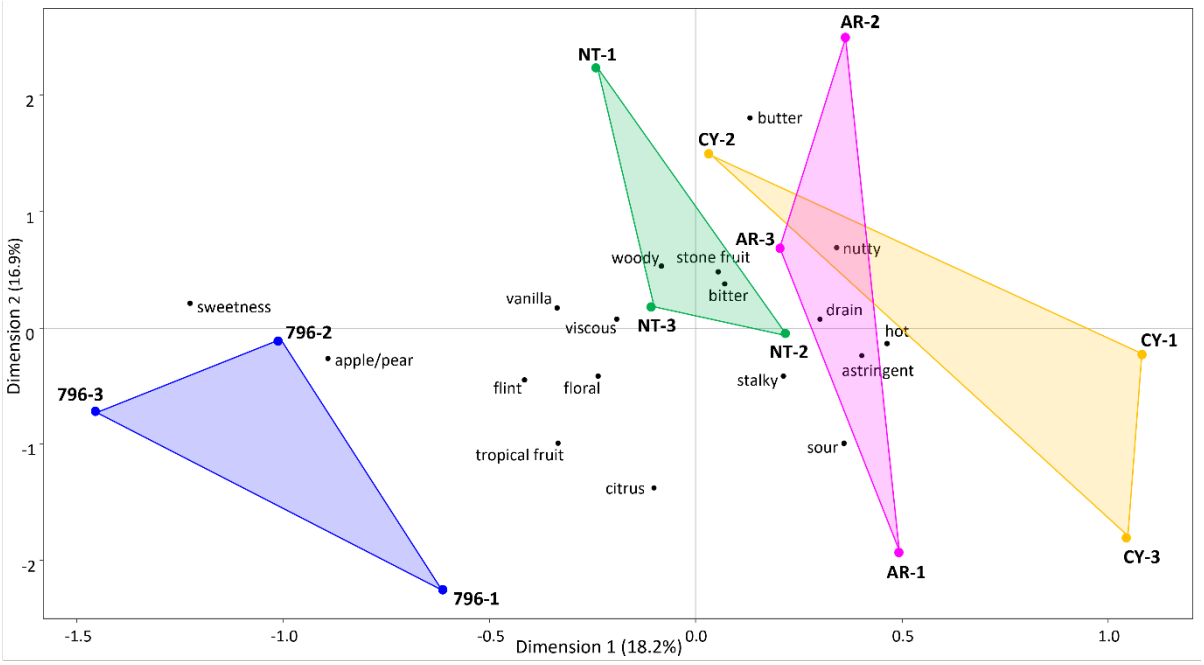


FIGURE 5. Multiple factor analysis (MFA) map from Napping (N) assessment of replicate Chardonnay wines co-inoculated with *O. oeni* VP41 and different *S. cerevisiae* strains.

Saccharomyces cerevisiae strains: AWRI796 (796, purple circles), CY3079 (CY, light brown circles), Actiflore Rosé (AR, pink circles) and NT 50 (NT, green circles). Sensory descriptors from Ultra Flash Profiling (UFP) are superimposed.

Sensory assessment of the Chardonnay wines by Napping combined with UltraFlash Profiling revealed that there were differences related to the co-inoculation yeast strain (Figure 5). The AWRI796 co-inoculated wines were positioned to the left of Figure 5 and were described more often with the terms *citrus, apple/pear, tropical fruit, floral, vanilla, flint, sourness,* and *sweetness*. The CY3079 co-inoculated wines were separated along Dimension 1 from the AWRI796 wines, with a lower citation frequency of *apple/pear* and *floral* terms. The Actiflore Rosé and NT 50 coinoculated wines were also positioned away from the AWRI796 wines, and *stone fruit, woody, butter,* and *bitterness* terms were used more frequently for these wines. The NT 50 coinoculated wines had the highest citation frequency for *butter* aroma and flavour.

DISCUSSION

As a prerequisite to examining the effects of yeast-derived SO_2 on *O. oeni*, the SO_2-producing potential of 94 wine yeast strains was investigated following fermentation in a commercially prepared, pre-sulfited Chardonnay juice (2017). The SO_2-producing capability observed supports other phenotypic surveys (Richter *et al.*, 2013; Suzzi *et al.*, 1985) and reflects the categorisation that most strains exhibit low SO_2-producing ability (an increase of up to 22 mg/L SO_2 at the 90th percentile), while some are high sulfite-producers (Eschenbruch, 1974). Although the fermentation conditions used here may not fully account for possible minor losses in SO_2 through gaseous exchange, the observation that approximately half of the yeast strains exhibited the ability to consume SO_2 in sulfited grape juice is of practical importance, yet such capability has received little attention. Wine yeasts utilise sulfite over sulfate as a source of sulfur, and can oxidise and reduce sulfite added to grape must (Eschenbruch, 1973). Recently, Ochando *et al*. (2020) showed that a winemaking *S. cerevisiae* strain (MC005) could produce or consume SO_2 in a synthetic grape juice medium and suggested that cellular uptake (diffusion) and production of SO_2 by yeast from the sulfate pool was in equilibrium with an initial threshold concentration of SO_2 in the juice. Similarly, for respective yeast strains in the current study, the final total SO_2 concentration of wines fermented using sulfited and non-sulfited juices was also in a similar range. For example, that of Chardonnay wine fermented with *S. cerevisiae* AWRI796 in sulfited (2017) and non-sulfited (2021 pilot-scale study) juices was 28 (*SD* = 1.5) mg/L and 20 (*SD* = 0.5) mg/L, respectively.

Representative yeast strains with differing SO_2 production exhibited wide-ranging impacts on the survival and MLF capability of *O. oeni* following co-inoculation in Chardonnay. This was highlighted in the first interaction experiment in which differential MLF duration was observed in ferments of non-SO_2-producing and SO_2-producing yeast strains. This observation supports the findings of others (Larsen *et al.*, 2003; Osborne and Edwards, 2006) that yeast metabolites other than SO_2 are likely to affect bacterial survival. Of those yeast metabolites with the potential to cause inhibition of *O. oeni*, there were no major differences in the final concentrations of succinic acid (Torres-Guardado *et al.*, 2022). This suggests that metabolites such as toxic fatty acids or bioactive peptides (Bartle *et al.*, 2019) could be involved in bacterial inhibition in wines co-fermented with the non-SO_2-producing yeast strains. Further, a notable observation from this study was the highly divergent effects of the two SO_2-producing yeast strains (AWRI1497 and AWRI2880) on the survival and growth of *O. oeni*. While the accumulation of yeast-derived SO_2 is generally associated with *O. oeni* inhibition (Larsen *et al.*, 2003; Wells and Osborne, 2011), particularly in grape must already containing SO_2 (Henick-Kling and Park, 1994), the enhanced survival of *O. oeni* after co-inoculation with *S. cerevisiae* AWRI2880 compared to AWRI1497 appeared novel, and formed the basis of subsequent investigations.

Of the yeast metabolites capable of modulating yeast-derived SO_2 toxicity towards *O. oeni*, acetaldehyde was considered a primary candidate as it binds strongly with SO_2, especially at low pH ($K = 1.5 \times 10^{-6}$ at pH 3.3). Yeasts exhibit a strain-dependent ability to rapidly produce acetaldehyde during the early stages of AF, which is subsequently re-utilised as fermentation progresses (Cheraiti *et al.*, 2010; Li and Mira de Orduña, 2017; Ochando *et al.*, 2020). In the current study, peak acetaldehyde production was exceptionally high with the SO_2-producing *S. cerevisiae* strain AWRI2880. This phenotypic feature resulted in comparatively high molar ratios of acetaldehyde:SO_2 (up to approximately 4:1) in the first few days following co-inoculation, and is proposed as a key factor contributing to the enhanced survival of *O. oeni*.

These observations support the findings of Carr *et al.* (1976), who reported that the addition of an excess quantity of acetaldehyde (or an equivalent quantity of H_2O_2) alleviated SO_2 inhibition of *L. plantarum*. Similarly, Larsen *et al.* (2003) observed improved growth and MLF rate of *O. oeni* in Chardonnay wine fermented by *S. cerevisiae* V-1116 after treatments with excess amounts (50 mg/L) of acetaldehyde (or H_2O_2) and concluded that inhibition of bacterial growth was due to inhibitory concentrations of free SO_2. Under the co-fermentation conditions of the current study, however, attempts to validate the link between bacterial survival and acetaldehyde:SO_2 ratio were prevented by the rapid utilisation of added acetaldehyde by *S. cerevisiae* AWRI1497 (99 mg/L consumed within ≤ 24 h), resulting in no noticeable impacts on bacterial survival. The utilisation of exogenous acetaldehyde by yeast has been previously described (Ochando *et al.*, 2020) and reflects the tightly regulated and dynamic flux of this intermediate during fermentation.

Further insight into the impact of the relative proportions of acetaldehyde:SO_2 on *O. oeni* survival was obtained by calculating the concentrations of free and molecular SO_2 in fermentation samples using acetaldehyde-bisulfite equilibria. This enabled estimation of free and molecular SO_2 concentration well below the detection limit of most analytical methods (i.e., < 2–3 mg/L free SO_2), which can occur during and after white wine fermentation. Although becoming unreliable as acetaldehyde:SO_2 molar ratios approach unity (approximately < 1.1:1.0), these data revealed that the initial survival of *O. oeni* starter cultures following co-inoculation with SO_2-producing yeast strains could be linked to, and regulated by, a narrow threshold concentration of molecular SO_2 (approximately 1–35 µg/L). In particular, the second interaction experiment demonstrated that following co-inoculation with *S. cerevisiae* AWRI1497, initial exposure of malolactic starter cultures to only 4–6 µg/L molecular SO_2 was associated with considerable reduction in bacterial viability over the first few days of co-fermentation. On the other hand, following co-inoculation with *S. cerevisiae* AWRI2880, negligible loss in viability of *O. oeni* strains YV Select and VP41 occurred after exposure to an initial molecular SO_2 concentration of 1–2 µg/L. These findings suggest that molecular SO_2 concentrations almost 100-fold less than those previously reported by Ribéreau-Gayon *et al.*

(2006) (0.15–0.4 mg/L) may cause inhibition of *O. oeni* in wine. Further, such inhibition appears to be alleviated, and malolactic starter performance optimised, in wine conditions where molecular SO_2 is almost completely absent in the first few days of co-fermentation.

The extremely low concentrations of molecular SO_2 associated with *O. oeni* inhibition observed here support other reports regarding the SO_2 sensitivity of this organism. For example, Carr *et al.* (1976) noted that 0.032 mg/L molecular SO_2 in a culture medium (pH 4) was sufficient to completely suppress and kill 3 of 4 wild heterofermentative cocci (isolated from cider). Similarly, Hood (1983) demonstrated that very low calculated levels of free SO_2 (0.05-0.8 mg/L), which can exist in equilibria with acetaldehyde-bisulfite, caused a dose-response inhibition in *L. brevis* and *Leuc. oenos* growth in a culture medium (pH 4) containing 10, 20, and 30 mg/L total SO_2. This author also reported that a calculated free SO_2 concentration of 0.8 mg/L (equivalent to 5 µg/L molecular SO_2) entirely suppressed the growth of *Leuc. oenos* LN6 in the same medium containing 30 mg/L total SO_2.

The current work has also provided further insight into the adaptive capability of *O. oeni* to SO_2. For example, in the pilot-scale experiment, exposure of *O. oeni* VP41 to an extremely low and incremental rise in molecular SO_2 concentration (up to approximately 10 µg/L) within the first 9 days of co-fermentation with *S. cerevisiae* NT 50 facilitated effective adaptation to molecular and total SO_2 concentrations of approximately 16 µg/L and 57 mg/L, respectively. This suggests that the adaptive capability of *O. oeni* towards SO_2 (Delfini and Morsiani, 1992; Guzzo *et al.*, 1998) encompasses the molecular form of this compound. Overall, the evidence from this work suggests the survival and adaptation of *O. oeni* following co-inoculation with SO_2-producing yeast strains requires that molecular SO_2 concentrations remain below a threshold inhibitory range in the early stages of co-fermentation, which exponentially increases from approximately 3–6 µg/L in the first three days of co-fermentation.

While evidence from the second interaction experiment indicates some strain-dependent reduction in *O. oeni* viability after exposure to ≥ 50–60 mg/L bound SO_2, it is unclear whether such inhibition was caused by bound SO_2 itself, or extremely low concentrations (1–6 µg/L) of molecular SO_2 accumulated over the first 5 days of co-fermentation, or a combination of both. Manufacturer recommendations for *O. oeni* CH35 indicate its suitability for MLF induction in wines of up to 45 mg/L SO_2, and those of *O. oeni* VP41 and YV Select indicate SO_2 tolerances of up to 60 mg/L and < 50 mg/L, respectively. Interestingly, Carr *et al.* (1976) report no indication of toxicity of acetaldehyde-bound SO_2 towards *L. plantarum*. However, further studies are needed to clearly define the tolerance and adaptive capabilities of *O. oeni* strains to molecular and bound forms of SO_2 under wine conditions.

Further insight into the potential effects of acetaldehyde-bound SO_2 on co-inoculated *O. oeni* was indirectly gained from the concentrations of acetaldehyde observed during the latter stages of co-fermentation. The toxicity of acetaldehyde-bound SO_2, at least in part, requires the bacterial utilisation of acetaldehyde, and subsequent release of sufficient free SO_2 to affect growth and survival (Fornachon, 1963; Hood, 1983; Osborne *et al.*, 2006; Wells and Osborne, 2012). Moreover, the ability of wine LAB to degrade acetaldehyde and SO_2-bound acetaldehyde (Jackowetz and Mira de Orduña, 2012; Jackowetz and Mira de Orduña, 2013; Osborne *et al.*, 2000; Pan *et al.*, 2011; Wells and Osborne, 2012) appears to coincide with bacterial growth (Fornachon, 1963; Hood, 1983; Osborne *et al.*, 2006).

In white wine, acetaldehyde catabolism can initiate after the commencement of MLF and continue for up to several weeks after its completion (Jackowetz and Mira de Orduña, 2012). However, in the current pilot-scale study, such metabolism was not evident in post-MLF stages, nor during MLF in wines co-inoculated with *S. cerevisiae* Actiflore Rosé. An absence of acetaldehyde catabolism may reflect differences in wine parameters, such as pH, that are critical to the growth and MLF capability of *O. oeni*. Osborne *et al.* (2006) reported that acetaldehyde degradation by *O. oeni* in white wine containing acetaldehyde-bound SO_2 occurred at pH 3.6 but not at pH 3.3. Similarly, Flamini *et al.* (2002) indicated that following MLF with two different *O. oeni* strains, substantial acetaldehyde degradation occurred in Cabernet-Sauvignon wine (pH 3.55) but not in Chardonnay (pH 3.24). Hence, it appears unlikely that acetaldehyde metabolism is sufficiently active to account for the inhibition of *O. oeni* immediately following co-inoculation in low pH Chardonnay wine observed in the current study.

In addition to affecting the survival and MLF capability of *O. oeni* during co-fermentation, the pilot-scale study demonstrated that the choice of co-inoculation yeast strain also had a major impact on the production of diacetyl and on wine sensory properties. The diketone diacetyl is an important flavour compound imparting buttery aroma and flavour attributes, and can arise from LAB metabolism during MLF (Bartowsky and Henschke, 2004). Yeast can also synthesise and degrade diacetyl (Martineau and Henick-Kling, 1995; Mink *et al.*, 2014) and, although the diacetyl concentration in newly fermented wine is typically low (Bartowsky and Henschke, 2004), some variation may occur depending on factors including yeast lees contact, SO_2 addition and grape variety (Martineau and Henick-Kling, 1995). For Chardonnay wines without MLF, Martineau *et al.* (1995b) reported an average diacetyl concentration of 0.22 (*SD* = 0.20) mg/L. The presence of yeast during and after MLF will also influence diacetyl content (Bartowsky and Henschke, 2004; Martineau and Henick-Kling, 1995).

Since Chardonnay wines in this study were co-fermented and left in contact with yeast lees for one week after MLF, the absence of diacetyl (< 0.1 mg/L) in wines co-inoculated with the low SO_2-producing yeast strains AWRI796 and CY3079 is not unexpected. However, the higher concentrations of diacetyl in wines coinoculated with the high SO_2-producing *S. cerevisiae* strains, particularly NT 50 (4.9

(*SD* = 0.6) mg/L), is surprising, and may relate to diacetyl binding and retention at elevated SO_2 concentration.

The observations regarding diacetyl were also reflected in wine sensory properties, whereby wines co-inoculated with high SO_2-producing yeast strains Actiflore Rosé and NT 50 had higher citation frequency for *butter* and *woody* attributes, and those co-inoculated with NT 50 had the highest citation frequency for *butter*. These results align with the sensory detection threshold for diacetyl in Chardonnay wine (0.2 mg/L, Martineau *et al.* (1995a)). However, while a bacterial origin of accumulated diacetyl is likely, a potential contribution from yeast (Martineau and Henick-Kling, 1995) cannot be discounted and requires investigation.

CONCLUSIONS

This study confirms that *S. cerevisiae* strain choice can affect the survival of *O. oeni* and the conduct of MLF during co-inoculation. Immediately following bacterial inoculation, the early stages of co-fermentation provide a unique and metabolically dynamic environment, the composition of which is critical for bacterial survival and MLF efficiency.

Significantly, for co-inoculation with SO_2-producing yeast strains, laboratory- and pilot-scale studies have demonstrated a direct association between the survival of *O. oeni* and the molar ratio of acetaldehyde:SO_2 in the early stages of AF. Alleviation of SO_2inhibition of *O. oeni* and improved MLF efficiency was associated with an early, transient accumulation of a high concentration of acetaldehyde.

By applying relevant acetaldehyde-bisulfite equilibria, the survival and adaptation to SO_2 by *O. oeni* starter cultures following co-inoculation appear to be regulated by an extremely low concentration range of molecular SO_2 (approximately 1–35 µg/L). Bacterial survival coincided with molecular SO_2 concentrations remaining below a minimum inhibitory range, which increased exponentially from 3–6 µg/L in the first 3 days of co-fermentation. Although some *O. oeni* strains may exhibit sensitivity to bound SO_2, the mechanism of such inhibition during co-fermentation remains unclear.

Moreover, the possible role of other mechanisms, such as medium chain fatty acid formation, should not be discounted to fully explain the complex interactions that may occur between yeast and malolactic bacteria during co-fermentation. Reliance on total/bound SO_2 concentration may only provide an indirect and potentially inaccurate measure of SO_2 toxicity towards *O. oeni* for co-inoculation. Equilibrium concentrations of molecular SO_2 should also be considered. Knowledge of both the SO_2- and acetaldehyde-producing potential of yeast strains are, therefore, important selection criteria for co-inoculation, particularly for white musts/wines in which elevated SO_2 concentrations are anticipated.

The choice of co-inoculation yeast strain also influenced wine diacetyl concentration and sensory properties. Co-inoculation with high SO_2-producing yeast strains produced wines with higher diacetyl concentration. These wines had a higher citation frequency for *butter* attributes.

DATABASE

Access to all data from the study is provided in the following on-line repository file: https://datadryad.org/stash/share/a7q7umBAe6yKC_zHKzd5uJfrWwyUo6c1al0LwCLzakQ

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Pernod Ricard Winemakers for the generous supply of Chardonnay juice. We thank Dr Leigh Francis for helpful discussion with this manuscript, Dr Toni Garcia Cordente for undertaking HPLC analysis and quantification of organic acids, Charlotte Jordans and Mark Rullo for technical assistance, and AWRI sensory panellists for their time and assistance. Alchemy Agencies (Australia) is also thanked for providing NataP UltraPure used to prepare selective agar media. This work was supported by Wine Australia, with levies from Australia's grapegrowers and winemakers and matching funds from the Australian Government. The AWRI is a member of the Wine Innovation Cluster in Adelaide.

REFERENCES

Andorrà, I., Martín, L., Nart, E., Puxeu, M., Hidalgo, C., & Ferrer-Gallego, R. (2018). Effect of grape juice composition and nutrient supplementation on the production of sulfur dioxide and carboxylic compounds by *Saccharomyces cerevisiae*. *Australian Journal of Grape and Wine Research*, ***24***(2), 260-266. https://doi.org/10.1111/ajgw.12325

Bartle, L., Sumby, K., Sundstrom, J., & Jiranek, V. (2019). The microbial challenge of winemaking: yeast-bacteria compatibility. *Fems Yeast Research*, ***19***(4). https://doi.org/10.1093/femsyr/foz040

Bartowsky, E. J., Costello, P. J., & Chambers, P. J. (2015). Emerging trends in the application of malolactic fermentation. *Australian Journal of Grape and Wine Research*, ***21***, 663-669. https://doi.org/10.1111/ajgw.12185

Bartowsky, E. J., & Henschke, P. A. (2004). The 'buttery'attribute of wine—diacetyl—desirability, spoilage and beyond. *International Journal of Food Microbiology*, ***96***(3), 235-252. https://doi.org/10.1016/j.ijfoodmicro.2004.05.013

Beelman, R., & Kunkee, R. (1985). Inducing simultaneous malolactic-alcoholic fermentation in red table wines. In Lee, T. (Ed.), Australian Society for Viticulture and Oenology seminar, Malolactic Fermentation, 16 August 1984, Melbourne, Victoria, (pp. 97-112) The Australian Society for Viticulture and Oenology, Inc.

Borneman, A. R., Forgan, A. H., Kolouchova, R., Fraser, J. A., & Schmidt, S. A. (2016). Whole genome comparison reveals high levels of inbreeding and strain redundancy across the spectrum of commercial wine strains of *Saccharomyces cerevisiae*. *G3: Genes, Genomes, Genetics*, ***6***(4), 957-971. https://doi.org/10.1534/g3.115.025692

Burroughs, L. F., & Sparks, A. H. (1973). Sulphite-binding power of wines and ciders. II. Theoretical consideration and calculation of sulphite-binding equilibra. *Journal of the Science of Food and Agriculture*, ***24***(2), 199-206. https://doi.org/10.1002/jsfa.2740240212

Carr, J. G., Davies, P. A., & Sparks, A. H. (1976). The toxicity of sulphur dioxide towards certain lactic acid bacteria from fermented

apple juice. *Journal of Applied Bacteriology*, ***40***(2), 201-212. https://doi.org/10.1111/j.1365-2672.1976.tb04166.x

Cheraiti, N., Guezenec, S., & Salmon, J.-M. (2010). Very early acetaldehyde production by industrial *Saccharomyces cerevisiae* strains: a new intrinsic character. *Applied Microbiology and Biotechnology*, ***86***(2), 693-700. https://doi.org/10.1007/s00253-009-2337-5

Delfini, C., & Morsiani, M. G. (1992). Resistance to sulfur dioxide of malolactic strains of *Leuconostoc oenos* and *Lactobacillu*s sp. isolated from wine. *Sciences Des Aliments*, ***12***, 493-511.

Eschenbruch, R. (1973). On the sulphite formation and sulphite consumption of wine yeasts. *Wynboer*, ***506***, 42-44.

Eschenbruch, R. (1974). Sulfite and sulfide formation during winemaking - a review. *American Journal of Enology and Viticulture*, ***25***(3), 157-161.

Fia, G., Millarini, V., Granchi, L., Bucalossi, G., Guerrini, S., Zanoni, B., & Rosi, I. (2018). Beta-glucosidase and esterase activity from *Oenococcus oeni*: Screening and evaluation during malolactic fermentation in harsh conditions. *LWT- Food Science and Technology*, ***89***, 262-268. https://doi.org/10.1016/j.lwt.2017.10.060

Flamini, R., De Luca, G., & Di Stefano, R. (2002). Changes in carbonyl compounds in Chardonnay and Cabernet-Sauvignon wines as a consequence of malolactic fermentation. *Vitis*, ***41***(2), 107-112.

Fornachon, J. (1963). Inhibition of certain lactic acid bacteria by free and bound sulphur dioxide. *Journal of the Science of Food and Agriculture*, ***14***(12), 857-862. https://doi.org/doi.org/10.1002/jsfa.2740141202

Fugelsang, K. C. (1997). *Wine microbiology*. Chapman & Hall, International Thomson Publishing.New York https://doi.org/10.1111/j.1574-6968.1998.tb12888.x

Guilloux-Benatier, M., Le Fur, Y., & Feuillat, M. (1998). Influence of fatty acids on the growth of wine microorganisms *Saccharomyces cerevisiae* and *Oenococcus oeni*. *Journal of Industrial Microbiology and Biotechnology*, ***20***(3), 144-149.

Guzzo, J., Jobin, M.-P., & Diviès, C. (1998). Increase of sulfite tolerance in *Oenococcus oeni* by means of acidic adaptation. *Fems Microbiology Letters*, ***160***(1), 43-47. https://doi.org/10.1111/j.1574-6968.1998.tb12888.x

Guzzon, R., Moser, S., Davide, S., Villegas, T. R., Malacarne, M., Larcher, R., Nardi, T., Vagnoli, P., & Krieger-Weber, S. (2016). Exploitation of simultaneous alcoholic and malolactic fermentation of Incrocio Manzoni, a traditional Italian white wine. *South African Journal of Enology and Viticulture*, ***37***(2), 124-131.

Hayasaka, Y., & Bartowsky, E. J. (1999). Analysis of diacetyl in wine using solid-phase microextraction combined with gas chromatography−mass spectrometry. *Journal of Agricultural and Food Chemistry*, ***47***(2), 612-617. https://doi.org/10.1021/jf9807667

Henick-Kling, T., & Park, Y. H. (1994). Considerations for the use of yeast and bacterial Starter cultures: SO_2 and timing of inoculation. *American Journal of Enology and Viticulture*, ***45***(4), 464-469. https://www.ajevonline.org/content/ajev/45/4/464.full.pdf

Hohorst, H.-J. (1965). D-Glucose-6-phosphate and D-fructose-6-phosphate: determination with glucose-6-phosphate dehydrogenase and phosphoglucose isomerase. In Bergmeyer, H.-U. (Ed.), *Methods of enzymatic analysis* (pp. 134-138). Elsevier, https://doi.org/10.1016/B978-0-12-395630-9.50025-6

Hood, A. (1983). Inhibition of growth of wine lactic-acid bacteria by acetaldehyde-bound sulphur dioxide. *Australian Grapegrower and Winemaker*, ***232***, 34-43.

Jackowetz, J. N., & Mira de Orduña, R. (2012). Metabolism of SO_2 binding compounds by *Oenococcus oeni* during and after malolactic fermentation in white wine. *International Journal of Food Microbiology*, ***155***(3), 153-157. https://doi.org/10.1016/j.ijfoodmicro.2012.01.025

Jackowetz, J. N., & Mira de Orduña, R. (2013). Survey of SO_2 binding carbonyls in 237 red and white table wines. *Food Control*, ***32***(2), 687-692. https://doi.org/10.1016/j.foodcont.2013.02.001

Jussier, D., Dube Morneau, A., & Mira de Orduna, R. (2006). Effect of simultaneous inoculation with yeast and bacteria on fermentation kinetics and key wine parameters of cool-climate Chardonnay. *Applied and Environmental Microbiology*, ***72***(1), 221-227. https://doi.org/10.1128/aem.72.1.221-227.2006

Kassambara, A., & Mundt, F. (2020). *factoextra: Extract and visualize the results of multivariate data analyses R package version 1.0.7*. https://CRAN.R-project.org/package=factoextra

King, S., & Beelman, R. (1986). Metabolic interactions between *Saccharomyces cerevisiae* and *Leuconostoc oenos* in a model grape juice/wine system. *American Journal of Enology and Viticulture*, ***37***(1), 53-60.

Knoll, C., Fritsch, S., Schnell, S., Grossmann, M., Krieger-Weber, S., du Toit, M., & Rauhut, D. (2012). Impact of different malolactic fermentation inoculation scenarios on Riesling wine aroma. *World Journal of Microbiology & Biotechnology*, ***28***(3), 1143-1153. https://doi.org/10.1007/s11274-011-0917-x

Krieger-Weber, S. (2017). Application of yeast and bacteria as starter cultures. In König, H., Unden, G., Fröhlich, J. (Eds.), *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 605-634). Springer International Publishing, Cham, Switzerland. https://doi.org/10.1007/978-3-319-60021-5_25

Lafon-Lafourcade, S. (1975). Factors of the malo-lactic fermentation of wines. In Carr, J. G., Cutting, C. V., Whiting, G. C. (Eds.) Lactic acid bacteria in beverages and food, Long Ashton Research Station, University of Bristol, (pp. 43-53) Academic Press, London, New York.

Larsen, J. T., Nielsen, J.-C., Kramp, B., Richelieu, M., Bjerring, P., Riisager, M. J., Arneborg, N., & Edwards, C. G. (2003). Impact of different strains of *Saccharomyces cerevisiae* on malolactic fermentation by *Oenococcus oeni*. *American Journal of Enology and Viticulture*, ***54***(4), 246-251. https://www.ajevonline.org/content/ajev/54/4/246.full.pdf

Le, S., & Husson, F. (2008). Sensominer: A package for sensory data analysis. *Journal of Sensory Studies*, ***23***(1), 14-25. https://doi.org/10.1111/j.1745-459X.2007.00137.x

Li, E., & Mira de Orduña, R. (2017). Acetaldehyde kinetics of enological yeast during alcoholic fermentation in grape must. *Journal of Industrial Microbiology and Biotechnology*, ***44***(2), 229-236. https://doi.org/10.1007/s10295-016-1879-7

Martineau, B., Acree, T. E., & Henick-Kling, T. (1995a). Effect of wine type on the detection threshold for diacetyl. *Food Research International*, ***28***(2), 139-143. https://doi.org/10.1016/0963-9969(95)90797-E

Martineau, B., Henick-Kling, T., & Acree, T. E. (1995b). Reassessment of the influence of malolactic fermentation on the concentration of diacetyl in wines. *American Journal of Enology and Viticulture*, ***46***(3), 385-388.

Martineau, B., & Henick-Kling, T. (1995). Formation and degradation of diacetyl in wine during alcoholic fermentation with *Saccharomyces cerevisiae* strain EC1118 and malolactic fermentation with *Leuconostoc oenos* strain MCW. *American Journal of Enology and Viticulture*, ***46***(4), 442-448.

McCloskey, L., & Mahaney, P. (1981). An enzymatic assay for acetaldehyde in grape juice and wine. *American Journal of Enology and Viticulture*, ***32***(2), 159-162.

Mink, R., Sommer, S., Kölling, R., Schmarr, H. G., Baumbach, L., & Scharfenberger-Schmeer, M. (2014). Diacetyl reduction by commercial *Saccharomyces cerevisiae* strains during vinification. *Journal of the Institute of Brewing*, ***120***(1), 23-26. https://doi.org/10.1002/jib.106

Nehme, N., Mathieu, F., & Taillandier, P. (2010). Impact of the co-culture of *Saccharomyces cerevisiae-Oenococcus oeni* on malolactic fermentation and partial characterization of a yeast-derived inhibitory peptidic fraction. *Food Microbiology*, ***27***(1), 150-157. https://doi.org/10.1016/j.fm.2009.09.008

Nissen, T. L., Schulze, U., Nielsen, J., & Villadsen, J. (1997). Flux distributions in anaerobic, glucose-limited continuous cultures of *Saccharomyces cerevisiae*. *Microbiology*, ***143***, 203-218. https://doi.org/10.1099/00221287-143-1-203

Noble, A. C., Arnold, R. A., Buechsenstein, J., Leach, E. J., Schmidt, J. O., & Stern, P. M. (1987). Modification of a standardized system of wine aroma terminology. *American Journal of Enology and Viticulture*, ***38***(2), 143-146.

Ochando, T., Mouret, J.-R., Humbert-Goffard, A., Aguera, E., Sablayrolles, J.-M., & Farines, V. (2020). Comprehensive study of the dynamic interaction between SO_2 and acetaldehyde during alcoholic fermentation. *Food Research International*, ***136***, 109607. https://doi.org/10.1016/j.foodres.2020.109607

Osborne, J. P., Dubé Morneau, A., & Mira de Orduña, R. (2006). Degradation of free and sulfur-dioxide-bound acetaldehyde by malolactic lactic acid bacteria in white wine. *Journal of Applied Microbiology*, ***101***(2), 474-479. https://doi.org/10.1111/j.1365-2672.2006.02947.x

Osborne, J. P., & Edwards, C. G. (2006). Inhibition of malolactic fermentation by *Saccharomyces* during alcoholic fermentation under low- and high-nitrogen conditions: a study in synthetic media. *Australian Journal of Grape and Wine Research*, ***12***(1), 69-78. https://doi.org/10.1111/j.1755-0238.2006.tb00045.x

Osborne, J. P., Mira de Orduña, R., Pilone, G. J., & Liu, S.-Q. (2000). Acetaldehyde metabolism by wine lactic acid bacteria. *Fems Microbiology Letters*, ***191***(1), 51-55. https://doi.org/10.1111/j.1574-6968.2000.tb09318.x

Pagès, J. (2005). Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. *Food quality and preference*, ***16***(7), 642-649. https://doi.org/10.1016/j.foodqual.2005.01.006

Pan, W., Jussier, D., Terrade, N., Yada, R. Y., & Mira de Orduña, R. (2011). Kinetics of sugars, organic acids and acetaldehyde during simultaneous yeast-bacterial fermentations of white wine at different pH values. *Food Research International*, ***44***(3), 660-666. https://doi.org/10.1016/j.foodres.2010.09.041

Rauhut, D. (1993). Yeasts - production of sulfur compounds. In Fleet, G. H. (Ed.), *Wine microbiology and biotechnology* (pp. 183-224). Harwood Academic Publishers GmbH, London, New York.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). *Handbook of Enology.* (2nd ed., Vol. 1 - The microbiology of wine and vinifications). John Wiley & Sons Ltd Chichester, England.

Richter, C. L., Dunn, B., Sherlock, G., & Pugh, T. (2013). Comparative metabolic footprinting of a large number of commercial wine yeast strains in Chardonnay fermentations. *Fems Yeast Research*, ***13***(4), 394-410. https://doi.org/10.1111/1567-1364.12046

Schmidt, S. A., Kolouchova, R., Forgan, A. H., & Borneman, A. R. (2020). Evaluation of *Saccharomyces cerevisiae* wine yeast competitive fitness in enologically relevant environments by barcode sequencing. *G3: Genes, Genomes, Genetics*, ***10***(2), 591-603. https://doi.org/10.1534/g3.119.400743

Sereni, A., Phan, Q., Osborne, J., & Tomasino, E. (2020). Impact of the timing and temperature of malolactic fermentation on the aroma composition and mouthfeel properties of Chardonnay wine. *Foods*, ***9***(6), 802. https://doi.org/10.3390/foods9060802

Slowikowski, K. (2021). *ggrepel: Automatically Position Non-Overlapping Text Labels with 'ggplot2'* R package version 0.9.1. https://CRAN.R-project.org/package=ggrepel

Sumby, K. M., Bartle, L., Grbin, P. R., & Jiranek, V. (2019). Measures to improve wine malolactic fermentation. *Applied Microbiology and Biotechnology*, ***103***(5), 2033-2051. https://doi.org/10.1007/s00253-018-09608-8

Suzzi, G., Romano, P., & Zambonelli, C. (1985). *Saccharomyces* strain selection in minimizing SO_2 requirement during vinification. *American Journal of Enology and Viticulture*, ***36***(3), 199-202.

Torres-Guardado, R., Rozès, N., Esteve-Zarzoso, B., Reguant, C., & Bordons, A. (2022). Influence of succinic acid on *Oenococcus oeni* and malolactic fermentation. *Oeno One*, ***56***(3), 195-204. https://doi.org/10.20870/oeno-one.2022.56.3.5403

Vermeir, S., Nicolai, B., Jans, K., Maes, G., & Lammertyn, J. (2007). High-throughput microplate enzymatic assays for fast sugar and acid quantification in apple and tomato. *Journal of Agricultural and Food Chemistry*, ***55***(9), 3240-3248. https://doi.org/10.1021/jf0637022

Wells, A., & Osborne, J. P. (2011). Production of SO_2 binding compounds and SO_2 by *Saccharomyces* during alcoholic fermentaton and the impact on malolactic fermentation. *South African Journal of Enology and Viticulture*, ***32***(2), 267-279. https://doi.org/10.21548/32-2-1387

Wells, A., & Osborne, J. P. (2012). Impact of acetaldehyde-and pyruvic acid-bound sulphur dioxide on wine lactic acid bacteria. *Letters in applied microbiology*, ***54***(3), 187-194. https://doi.org/10.1111/j.1472-765X.2011.03193.x

Wibowo, D., Eschenbruch, R., Davis, C. R., Fleet, G. H., & Lee, T. H. (1985). Occurrence and growth of lactic-acid bacteria in wine - a review. *American Journal of Enology and Viticulture*, ***36***(4), 302-313.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., . . . Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, ***4*** (43), 1686. https://doi.org/10.21105/joss.01686

Zapparoli, G., Tosi, E., & Krieger, S. (2006). Influence of the pH of Chardonnay must on malolactic fermentation induced by bacteria co-inoculated with yeasts. *VITIS-Journal of Grapevine Research*, ***45***(4), 197-198.