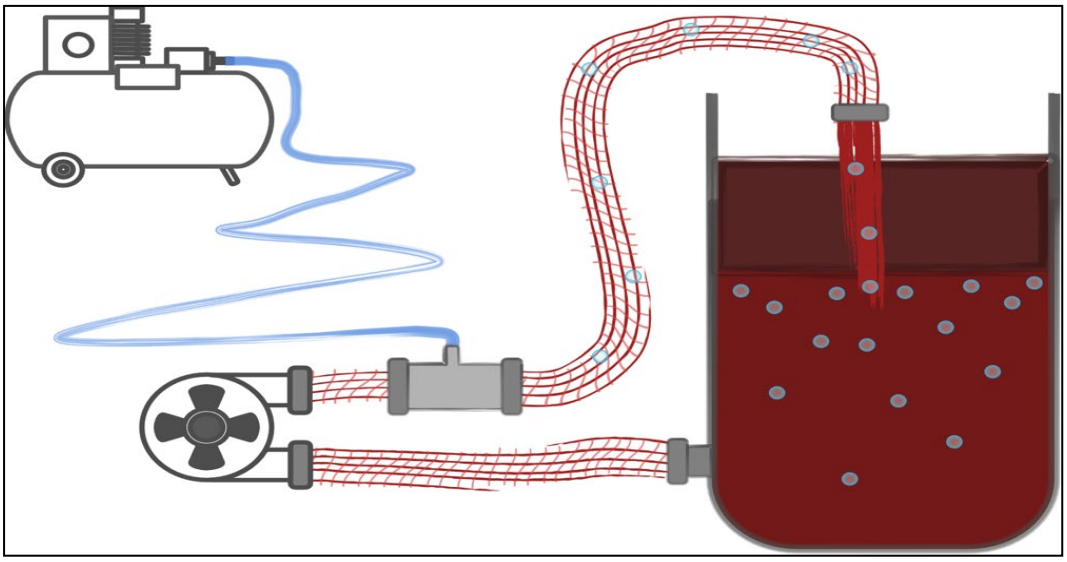
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AWRI Australian Wine Research Institute

Influencing wine style and efficiency through management of oxygen during wine production



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1. Abstract

Aeration applied during active fermentation is known to have effects on fermentation performance and wine style in white and red wines respectively. An understanding of the practical approaches to achieve these effects was lacking at the project outset. A series of pilot-scale trials and collaborations with industry partners was undertaken to refine our understanding of optimal schemes for white and red ferment aeration and to elucidate practical strategies for implementing those schemes. These investigations have informed the development of educational resources that are available to winemakers interested in using aeration during the production of their wines.

1. Executive summary

A major aim of the project team was to focus on aeration solutions that did not require information about dissolved oxygen concentration or redox status during fermentation. Experimentation with various types of equipment for the measurement of dissolved oxygen status indicated that their implementation was likely to be impractical, economically unviable or technically challenging in many instances. Therefore, laboratory-scale and pilot-scale trials were undertaken to define airflow rate and duration metrics that could be applied with minimal investment.

Fermentations of red and white grapes respond very differently to aeration and therefore require different approaches, both in regard to the intent of the application and the practicalities of implementation. For these reasons they are discussed separately in this report.

A compilation and analysis of data across four years of red wine vintage trials showed the limited effect of aeration on the efficiency of red wine alcoholic or malolactic fermentation. The lack of an observable effect on fermentation duration led to a shift in focus to examine its effect on red wine style. It was shown that increasing airflow rate during fermentation resulted in a step-wise increase in the intensity of red fruit attributes and a decrease in attributes related to sulfide compounds compared to the non-aerated wines. The effects on 'viscosity' or 'dark fruit' aroma and flavour were limited.

Aeration applied over an extended period at low intensity, or repeated short-duration high-intensity applications had equivalent compositional and sensorial effects. The red fruit attributes were consistently associated with the enrichment of ethyl-2- and ethyl-3-methylbutanoate in wines that had been aerated during fermentation.

Red wines made without aeration or with limited oxygen exposure were typically more opaque and astringent. Wine colour density and either hue, non-bleachable pigment or total anthocyanin were strongly associated with 'opacity'. Total phenolics and other non-volatile measures such as mean degree of polymerisation of tannin (mDP) and total tannin were positively related to wine 'astringency'. In addition, descriptors such as 'drain' aroma, 'earthy' aroma, 'bitterness' and 'vegetal' were more commonly associated with wines made using little or no aeration. Hydrogen sulfide was strongly implicated in reductive aroma attributes such as 'drain', 'vegetal' and 'earthy' in some cases combined with MeSH or its acetate ester.

Optimal strategies for the aeration of red wine fermentations are those that can be coupled with cap management operations. Effective aeration coincident with cap management requires that these operations are undertaken frequently, at least daily but ideally more often, due to the generally short duration of cap management.

Investigations of the effect of oxygen exposure during the processing of white grapes were completed and published during the project period, showing the possible stylistic potential and limitations of oxygen management during processing. Subtle increases in floral and confection characteristics were demonstrated in white wines with limited oxygen exposure during pressing and handling operations. Strict protection of wine from oxygen exposure also

minimised yellow colour development. However, these wines were more sensitive to post-fermentation oxidative colour development.

In contrast to the effect of aeration on red fermentations, the effect on white wine fermentations was almost entirely related to fermentation efficiency. Aeration intensity beyond that required to stimulate fermentation performance was invariably detrimental to wine quality. Low-intensity aeration sustained over a longer duration was determined to be the optimal approach for white ferments. Attempts to apply shorter aeration treatments at defined times during fermentation were less effective at reducing fermentation times than longer aerations.

The concept of aerating white ferments to reduce fermentation time was extended to non-inoculated ferments, where timing and aeration intensity were explored, mindful of the potential repercussions on microbial community structure. Optimal fermentation performance benefits from aeration of non-inoculated ferments were achieved when aeration coincided with the onset of *S. cerevisiae* ascendency within the microbial community.

Built-in sparger systems lend themselves better to white fermentations due to the absence of a cap and their more uniform nature. Such systems are also less prone to damage during the post-fermentation operations associated with white fermentation.

Knowledge building about the implementation of aeration at scale was undertaken through collaboration with industry partners. These trials included comparisons of aeration using rack and return with inline sparger systems, and initial evaluations of the contribution of Pulse-Air devices to ferment aeration. Whether Pulse-Air-like devices can be configured to be used efficiently for aeration operations remains an open question.

A substantial set of educational resources was generated on the effects of aeration during fermentation in red and white wines. These resources include the different ways of undertaking aeration and the methods available to measure those effects. Extension materials related to aeration were continuously developed as a direct consequence of experimental work undertaken during the project. In the final year of the project, team members provided content for an adoption program across various formats, including webinars, podcasts, demonstration videos and web-accessible fact sheets.

2. Acknowledgements

This work was supported by Australian grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The AWRI is a member of the Wine Innovation Cluster in Adelaide, SA.

The project team would like to acknowledge the sensory team, including Leigh Francis, Desireé Likos, Eleanor Bilogrevic, Damian Espinase Nandorfy and the AWRI's quantitative descriptive analysis panel for their help with sensory analysis of wine. Mark Solomon and Allie Kulcsar from the AWRI are thanked for their assistance in LC-MS, GC-MS and HPLC analyses for analytical chemistry support and Stella Kassara and Keren Bindon are thanked for assistance with phenolic analysis of wine. The AWRI node of Metabolomics Australia provided additional analytical support.

WIC Winemaking Services contributed to the preparation of grape juice and the production of wine across multiple vintages.

The project team would also especially like to thank Treasury Wine Estates for the supply of juice used in pilot-scale work and Yalumba Wines, Cape Jaffa Wines and Richmond Grove for their assistance in the conduct of industry trials.

3. Background

Research conducted prior to this project, indicated that red and white fermentation systems respond very differently to the addition of oxygen. The effects of oxygen addition to white wine fermentations manifest primarily through a decrease in fermentation duration, with limited sensory impacts. In contrast, oxygen additions to red wine fermentations cause relatively little improvement in performance, but can have substantial impacts on the chemical and sensory attributes of the wine. Fruit-driven wines with less reductive character, altered tannin profile and less bitterness, are associated with red fermentations that have been exposed to oxygen.

This project used both model systems and pilot-scale fermentations to investigate these impacts. Shiraz and Chardonnay were the primary varieties investigated prior to the initiation of this project. Therefore the project team continued investigations using Shiraz and Chardonnay to refine our understanding of aeration effects. In addition, it was of interest to understand whether spontaneous fermentations differed in their response to aeration compared with inoculated fermentations.

The project team also monitored wines with known oxygen exposure as they aged, to assess oxygen-related chemical changes which occur after fermentation. Factors that modulate these changes (e.g. metals, pH, time and duration of oxygen exposure) are critical to delivering the best quality product to market.

Finally, practical knowledge was accumulated about appropriate oxygen dose and delivery methods to lower the barriers to uptake by industry. In collaboration with industry partners, this project explored different approaches to oxygen delivery, contributing to specific adoption programs that were delivered to winemakers.

4. Project aims

The key objectives of this project were to:

a) Develop methods to assess and improve the efficiency of fermentation through the use of oxygen during alcoholic and malolactic fermentation.
b) Generate knowledge on the scale-related impacts of processes for wineries by determining the effectiveness and impact of different practices for introducing oxygen at different scales (e.g. barrel stirring, splashy racking).
c) Generate data on the impact of juice exposure to oxygen (timing, point in production process, dose) on final wine composition and sensory characteristics.

Project deliverables were:

Year 3 deliverables

- Methods to assess and improve the efficiency of fermentation through the use of oxygen during alcoholic and malolactic fermentation.
- Knowledge on the scale-related impacts of processes for wineries by determining the effectiveness and impact of different practices for introducing oxygen at different scales (e.g. barrel stirring, splashy racking).
- Data on the impact of juice exposure to oxygen (timing, point in production process, dose) on final wine composition and sensory characteristics.

Year 5 deliverables

- Knowledge on the impact on wine style and sensory properties of key oxygen modulated compounds.
- Practical advice and extension materials about methods to introduce oxygen and the impacts of timing and dose of addition (ongoing).

5. Methods

5.1 Pilot-scale investigation of aeration during fermentation of white wines

Commercially prepared Chardonnay juice was distributed into 12 x 500 L fermenters and inoculated with active dried yeast. In each case three aeration treatments were carried out in triplicate with an additional 'no-treatment' variant also performed in triplicate. These timings and durations were determined based on earlier work in this area. Wines were subsequently handled identically through to bottling.

5.1.1 The effect of aeration intensity on Chardonnay (vintage 2019)

Three variations to aeration intensity were incorporated into the experiment:

a) Control, no aeration provided
b) 'Low intensity' (0.5 L/min/kL),
c) 'Medium intensity' (3.0 L/min/kL)
d) 'High intensity' (10.0 L/min/kL).

All treatments were applied for 48 hours.

Table 1. Basic composition of Chardonnay juice used in the 2019 vintage trial

Analyte	Value	Unit
^{o}Baumé	13.7	
pH	3.28	
TA	6.0	g/L
Malic acid	2.1	g/L
YAN	197	mg/L
Total SO_2	56	mg/L
Free SO_2	17	mg/L

5.1.2 The effect of aeration mode on Chardonnay (vintage 2020)

Variations to the duration and timing of aeration incorporated into the experiment were as follows:

a) Control, no aeration provided
b) 'Long low-intensity' (0.5 L/min/kL) applied for 48 hours
c) 'Short medium-intensity 1' (3.0 L/min/kL) applied for 2.5 h
d) 'Short medium-intensity 2' (3.0 L/min/kL) applied for 2.5 h.

'Short medium-intensity 1' and 'Short medium-intensity 2' were applied 24 and 48 hours after the start of the 'Long low-intensity' treatment.

Table 2. Basic composition of Chardonnay juice used in the 2020 vintage trial

Analyte	Value	Unit
°Baumé	12.9	
pH	3.5	
TA	4.9	g/L
YAN	120	mg/L
Total SO_2	0	mg/L
Free SO_2	0	mg/L

5.2 Pilot-scale investigation of aeration during fermentation of red wines

Pilot-scale (500 L) fermentations of Shiraz grapes were carried out in triplicate using standard pump-over techniques with a fixed irrigator placed just below the opening of the tank. Fixed cross-shaped sparging devices were placed at the bottom of nine tanks and fed with compressed air. In each case three aeration treatments were carried out in triplicate with an additional 'no-treatment' variant also performed in triplicate. Malolactic fermentation was initiated with rehydrated *Oenococcus oeni* bacteria (VP41 Lallemand) simultaneously after the total soluble solids (TSS) had dropped by 1 °Bé. The timings and durations applied during these trials were determined as a result of earlier work in this area. Wines were subsequently handled identically through to bottling. Detailed methods are provided in (Day et al. 2021).

5.2.1 The effect of aeration mode on Shiraz - 1 (Vintage 2018)

Three variations to aeration intensity were incorporated into the experiment:

a) Control, no aeration provided
b) 'Long low-intensity' (1.8 L/min/kL) applied for 40 hours
c) 'Short high-intensity' (18 L/min/kL) applied for 4 hours
d) 'Repeated high-intensity' (18.0 L/min/kL) applied for four repetitions of 1 hour.

Table 3. Basic composition of Shiraz must used in the 2018 vintage trial

Analyte	Value	Unit
°Baumé	12.3	
pH	3.27	
TA	8.1	g/L
YAN	81	mg/L
Total SO_2	0	mg/L
Free SO_2	0	mg/L

5.2.2 The effect of aeration intensity on Shiraz (Vintage 2019)

Three variations to aeration intensity were incorporated into the experiment:

a) Control, no aeration provided
b) 'Low intensity' (0.5 L/min/kL),
c) 'Medium intensity' (3.0 L/min/kL)
d) 'High intensity' (10.0 L/min/kL).

All treatments were applied for 48 hours.

Table 4. Basic composition of Shiraz must used in the 2019 vintage trial

Analyte	Value	Unit
°Baumé	14.8	
pH	4.14	
TA	3.0	g/L
YAN	252	mg/L
Total SO_2	0	mg/L
Free SO_2	0	mg/L

5.2.3 The effect of aeration mode on Shiraz - 2 (Vintage 2020)

Variations to the duration and timing of aeration incorporated into the experiment were as follows:

a) 'Long low-intensity' (0.5 L/min/kL) applied for 48 hours,
b) 'Short medium-intensity 1' (3.0 L/min/kL) applied for 2.5 h
c) 'Short medium-intensity 2' (3.0 L/min/kL) applied for 2.5 h.

'Short medium-intensity 1' and 'Short medium-intensity 2' were applied 24 and 48 hours after the start of the 'Long low-intensity' treatment.

5.3 The effect of aeration timing and intensity of wild ferments on the microbial composition during fermentation and the chemical composition of Chardonnay wine

Commercial, high-solids Chardonnay juice (not pre-treated with SO_2 during harvesting or processing) was obtained directly after destemming and crushing from Yalumba (Barossa Valley, South Australia) during the 2019 and 2020 vintages. The juice was transferred to sterile 2 L Schott bottles, which were sealed with airlocks and incubated at 18°C. Ferments were continuously stirred at 250 rpm using magnetic stirrers. Four different aeration treatments were evaluated in each vintage to assess the impact of aeration on yeast populations. Detailed methods are provided in (Varela et al. 2021)

5.3.1 The effect of aeration timing on wild ferments (Vintage 2019)

Three variations to aeration timing were incorporated into the experiment:

a) Control, no aeration provided
b) Air_24 h, aeration at 6 mL/min 24 h after crushing, aeration continued for 24 h
c) Air_24 h, aeration at 6 mL/min 24 h after crushing, aeration continued for 48 h
d) Air_24 h, aeration at 6 mL/min 24 h after crushing, aeration continued for 72 h

5.3.2 The effect of aeration intensity in wild ferments (Vintage 2020)

Three variations in aeration intensity were incorporated into the experiment

a) Control, no aeration provided
b) Air_low, 24 h after crushing, aeration at 18 mL/min for 48 h
c) Air_medium, 24 h after crushing, aeration at 25 mL/min for 24 h and then 35 mL/min for another 24 h
d) Air_high, 24 h after crushing, aeration at 50 mL/min for 24 h and then 70 mL/min for another 24 h

5.4 Pilot-scale investigation of aeration during fermentation of non-inoculated Chardonnay wines (Vintage 2021)

This industry trial was undertaken at Yalumba (Angaston, South Australia). Commercial, high-solids Chardonnay juice (not pre-treated with SO_2 during harvesting or processing) was destemmed and pressed directly to 1000 L vessels fitted with cross-head spargers. The juice composition immediately post-pressing was: °Baumé; 11.6, pH; 3.27, TA; 8.1 g/L, YAN; 343 mg/L. Aeration was applied for 24 hours at a rate of 0.5 L/min/kL. Dissolved oxygen concentration was measured using fluorescence-based sensors at two positions in each tank.

°Baumé and temperature were measured daily. The temperature was used to control the fermentation rate. Treatments were performed in duplicate. Wines were bottled at Yalumba.

Analysis of higher alcohols and acetate and ethyl esters was performed by Metabolomics SA using gas chromatography-mass spectrometry (GC-MS) with stable isotope dilution analysis (SIDA), as described by Holt et al. (2013). Low molecular weight sulfur compound concentrations (LMWSC) were quantified using headspace cool-on-column gas chromatography coupled with a sulfur chemiluminescence detector (Siebert et al. 2010). Wine sensory properties for each trial (within-trial comparisons only) were evaluated using quantitative sensory descriptive analysis (QSDA) methodology (Lawless and Heymann 2010).

5.5 Studies on the relationship between dissolved oxygen concentration and redox in synthetic medium

The relationship between dissolved oxygen (DO) concentration during fermentation and the redox state of the fermentation was investigated using 0.9 L bioreactors run at a defined temperature (18°C) with slow stirring (100 rpm). Fermentation was conducted using a defined medium (Schmidt et al. 2011) inoculated with 0.2 g/L of EC1118 (Lallemand) rehydrated according to the manufacturer's instructions. The effect of compositional variation on the relationship between DO and redox was also explored by comparing standard defined medium with DO and redox values obtained when copper concentrations were increased to 10 mg/L or white wine phenolic extracts were added to the medium. As previously described, white juice phenolic material was extracted from 5 L of Chardonnay juice using an FPX66 resin (Gawel et al. 2014).

5.6 Studies on the sparger arrangement, gas flow rate and fermenter scale on the effectiveness of aeration treatments

6. Results and discussion

6.1 Use of oxygen to improve the efficiency of fermentation

6.1.1 Aeration of inoculated Chardonnay ferments – pilot-scale trials

Previous work attempting to understand how aeration might be optimally applied during fermentation, predominantly in red fermentations, demonstrated that the most effective way to aerate an active fermentation was to perform continuous aeration for 24 – 48 h. The work described here sought to evaluate whether this was true for white grape fermentations.

Over two pilot-scale (450 L) Chardonnay winemaking trials, the aim was to answer the following two questions.

a) How does aeration intensity (i.e. the airflow rate applied) affect fermentation outcomes during a long-duration aeration treatment?
b) Are short-duration aerations (1-2 hours) an adequate substitute for long-duration aerations?

In the first of the two trials, continuous aeration was applied for 48 h, commencing when 20% of the initial sugar had been consumed. Three treatments of increasing airflow rate (0.5, 3.3 and 33 L/min/kL) were applied and compared to a non-aerated control. These treatments resulted in a cumulative dissolved oxygen exposure of 0.8, 11 and 92 mg/L*h over the life of the ferments (Figure 1C).

The low cumulative dissolved oxygen exposure shows that an airflow rate of 0.5 L/min/kL resulted in negligible accumulation of dissolved oxygen in the bulk of the ferment, indicating that the oxygen transfer rate was being matched by the oxygen uptake rate. At higher airflow rates, oxygen accumulated during treatment, with a persistent non-negligible dissolved oxygen concentration. At the highest airflow rate (33 L/min/kL), the dissolved oxygen concentration was persistently high (20% air saturation). The consequences of this persistent elevated concentration of dissolved oxygen are discussed later.

The effect of these aeration treatments on fermentation duration was profound (Figure 1A). The lowest airflow rate (0.5 L/min/kL) reduced the fermentation time by 14 days. These ferments were complete, with a residual sugar concentration of < 0.2 g/L. Untreated ferments struggled to complete fermentation on day 24, with a mean residual sugar concentration of 3.7 (SD 1.8). The difficulty of this fermentation was likely due to the elevated sugar concentration of the juice (13.7 °Baumé) and sub-optimal nitrogen availability (YAN = 197 mg/L). Nevertheless, the low-intensity aeration treatment over a long duration was sufficient, without any additional intervention, to stimulate a timely completion of this fermentation. Higher air addition rates, with the consequent accumulation of dissolved oxygen, did not further reduce the fermentation time.

As an addendum to this work, the question arose as to whether there was room for operational flexibility in aeration delivery. Were long-duration aerations required, or could short aeration at the right time provide the same fermentation stimulus?

A second trial was undertaken to understand how much room there was to move in the deployment of aeration. This trial used an equivalent cumulative oxygen exposure across three aeration treatments. In each treatment, the duration and timing were altered (Figure 1B and D). As in the previous trial, the experiment included a no-aeration control and a 48-hour aeration with an airflow rate of 0.5 L/min/kL. The two additional treatments were two 2.5-hour aerations with airflow rates of 3.3 L/min/kL initiated 24 and 48 hours after the long aeration. After the second of these two short aerations, the long aeration was also complete. DAP was added to bring the final YAN to 150 mg/L due to this fruit's very low initial YAN; however, no other interventions were undertaken during the production of these wines.

Results showed that the most effective aeration treatment, as indicated by the decrease in fermentation time, was the long-duration aeration, which reached a target sugar concentration of <1 g/L on day 11. Short-duration aerations did affect the duration of fermentation, although the decrease in fermentation time (complete on days 24 and 26) was not as large as that observed in the long-duration fermentation. The non-aerated wines took substantially longer, still having 10-14 g/L residual sugar on day 26.

This second trial demonstrated that short aerations could be used to stimulate fermentation activity and that their precise timing was not critical to their success. However, a single short aeration was not as effective at decreasing fermentation times as a continuous low-intensity aeration.

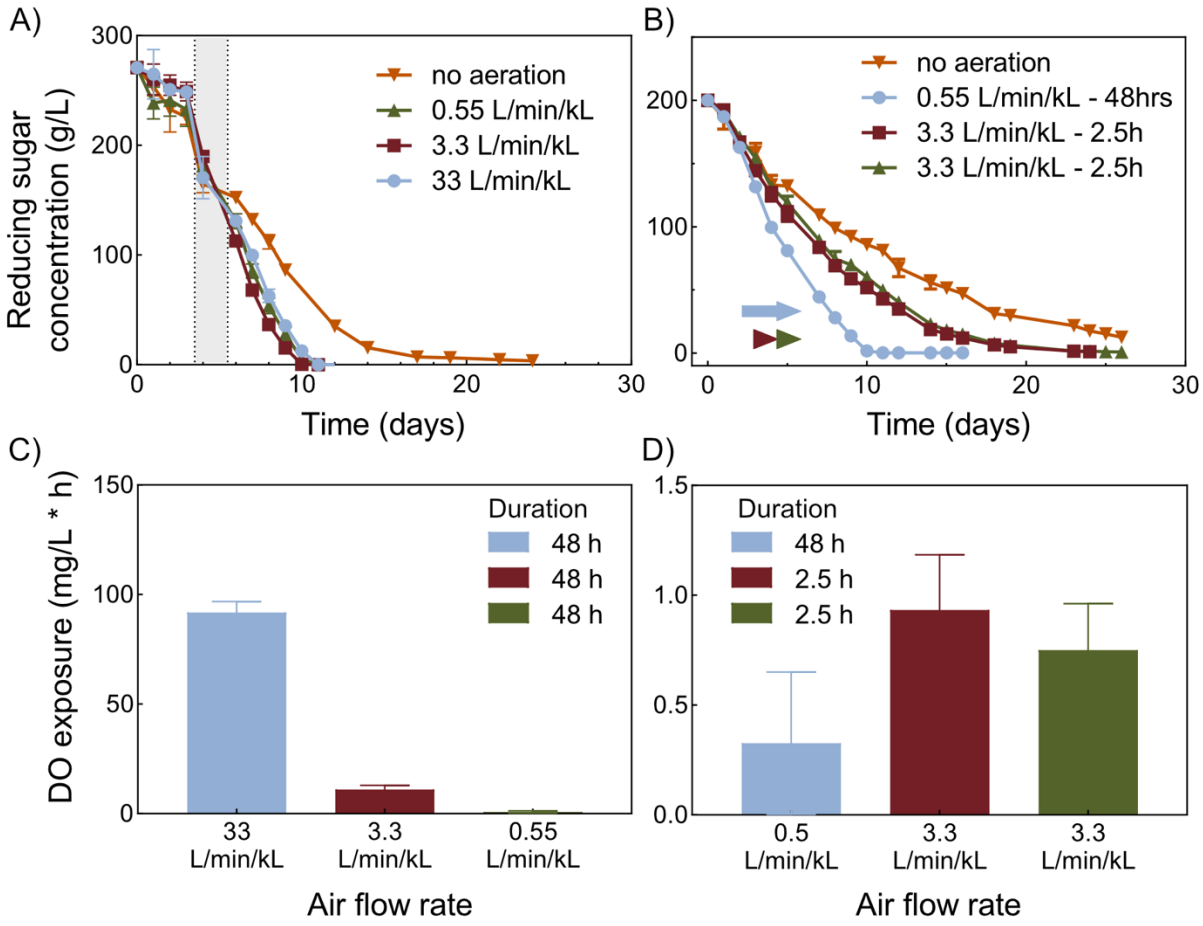


Figure 1. The effect of airflow rate (vintage 2019, A) and aeration timing (vintage 2020, B) on Chardonnay fermentation progress undertaken in 450 L fermenters. (A) The airflow rate was altered by providing adding air at 0.5, 3.3 or 33 L/min/kL for 48 hours. The shaded area in (A) shows when aeration was applied. (B) The timing of aeration was varied as follows: 0.5 L/min/kL continuously for 48 h (blue), 3.3 L/min/kL for 2.5 hours started 72 hours after inoculation (red) or 3.3 L/min/kL for 2.5 hours started 96 hours after inoculation (green). Arrows in (B) show the times the aeration treatments were applied. The cumulative dissolved oxygen exposure of the different aeration treatments is shown in (C) and (D). All symbols show the means of three treatments, with error bars indicating standard deviation.

Several studies suggest that early oxygen addition has the potential to modulate wine chemistry (reviewed in Day et al. 2015). A concern that many winemakers have about aeration of white ferments is the risk of oxidation. To resolve questions about how aeration affects wine composition and to what degree, a comprehensive quantitative compositional analysis was undertaken on the wines discussed above.

In all, 50 compounds associated with yeast metabolism, chemical transformation or oxidation of wine were quantified. There was evidence (ANOVA $P < 0.05$) that the concentration of 35 compounds changed due to one or more of the aeration treatments.

When air was provided at 0.5 L/min/kL, the concentration of 19 compounds differed from the control. Notably, there was no evidence that this treatment increased the concentration of acetic acid or ethyl acetate, two classic markers of oxidation often associated with descriptors such as vinegar or nail polish remover. These wines also exhibited lower concentrations of H_2S (mean decrease of 2 µg/L) and benzaldehyde (mean decrease of 20 µg/L). Consistent with earlier work, the concentration of medium-chain fatty acids decreased (e.g. decanoic acid) and concentrations of small branched-chain compounds increased (e.g. ethyl-2 methyl butanoate) (Figure 2).

Aeration with airflow rates of 3 L/min/kL continued to suppress H_2S and benzaldehyde production but contributed progressively to acetic acid and ethyl acetate formation. With airflow rates of 33 L/min/kL, the concentration of acetic acid approached 1 g/L, and ethyl acetate concentrations exceeded 150 mg/L. These concentrations were 0.8 g/L and 120 mg/L higher respectively than the control, resulting in serious quality issues for these wines.

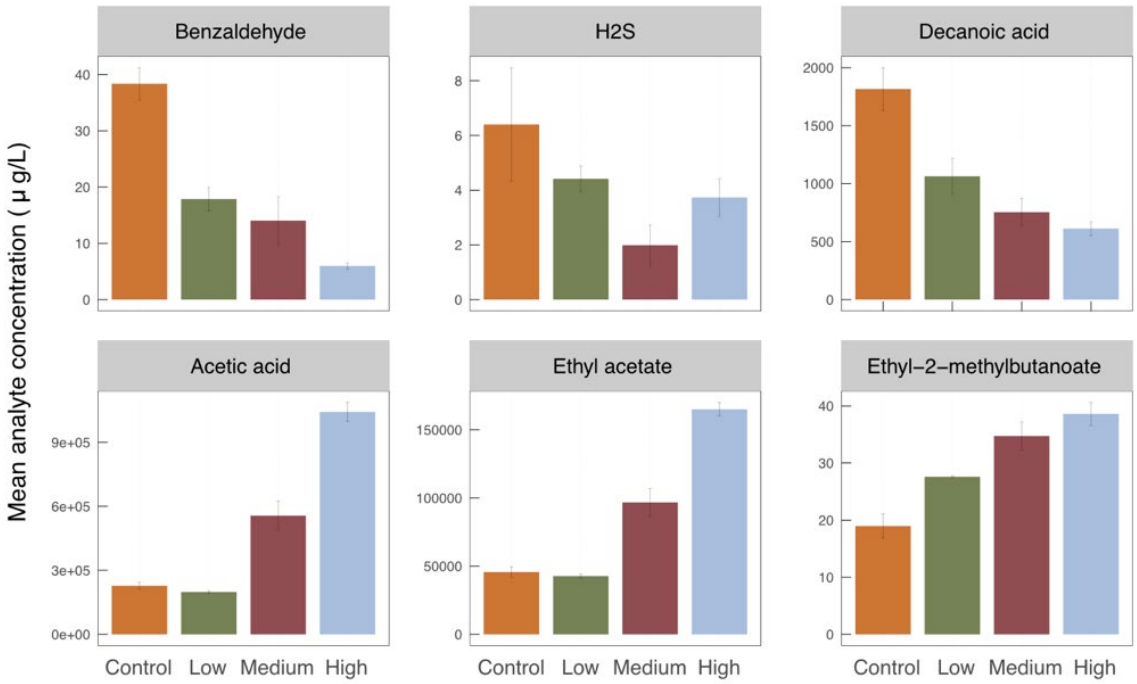


Figure 2. Analytes for which there was evidence of a difference in concentration in finished wine (P < 0.05) in response to aeration during fermentation using different air flow rates. Control (orange), 0.5 L/min/kL (green), 3.3 kL/min/L (red), 33 kL/min/L (blue). Columns show the average value of two replicates, error bars show the standard deviation.

An experiment was conducted to determine whether there was a significant sensory effect of oxygen exposure treatments during fermentation for a 2020 Chardonnay wine. Two oxygen treatments were compared to a standard oxygen treatment (0.25 L/min for 48 hours), which was also compared to an untreated control using a balanced reference triangle test. A preliminary sensory assessment of all replicates was conducted before the difference test with a panel of nine experienced wine sensory assessors. No winemaking faults or off-flavours were evident in any of the wines.

Thirty-one experienced, screened, and qualified assessors evaluated the wines, selecting the wine in the set that was different from the other two. Panellists were also asked to comment on the nature of any differences between the samples if possible. The results (Table 5) showed that panellists could perceive a difference between wine that had been aerated at 0.5 L/min/kL for 48 hours and non-aerated wines (P<0.001). The comments provided by the judges suggested a higher fruit intensity in the non-aerated wine compared to wine that had been aerated at 0.5 L/min/kL for 48 hours, which was indicated by several assessors to be lower in overall fruit and had a more 'dull' aroma. There was no evidence that differences between long aeration and short aeration treatments could be perceived.

Table 5. Triangle test results comparing wines from ferments aerated at 0.5 L/min/kL for 48 h to non-aerated and short aeration ferments. Treatment A was delivered 40 hours after inoculation, Treatment B was delivered 72 hours after inoculation.

Comparison	No. of responses	Correct responses	Significance[1]

0.5 L/min/kL for 48 hours vs non-aerated	31	19	***
0.5 L/min/kL for 48 hours vs 3.3 L/min/kL for 2.5 hours (A)	31	14	ns
0.5 L/min/kL for 48 hours vs 3.3 L/min/kL for 2.5 hours (B)	31	13	ns

These trials showed that aeration could be used to decrease fermentation times and that with the use of conservative airflow rates, aeration could be implemented in a way that would not compromise product quality. However, in white wines, there was a risk that the application of airflow rates beyond the minimum required to support healthy fermentation could have adverse effects on wine quality.

6.1.2 Aeration of inoculated Shiraz ferments – pilot-scale trials

Previous work explored the effects of short high-intensity or repetitive aerations on Shiraz wine style and the impact on MLF performance. Here the project team sought to extend this knowledge to include long-duration aerations. The objective of this work was to understand the effects of aeration timing (when aeration is applied), duration (how long aeration is applied) and air flow rate (the intensity with which aeration is applied) on the fermentation kinetics, chemical composition and sensory properties of Shiraz wines. This section summarises work published in Day et al (2021).

The work on white wine fermentations discussed above, and previous work by other groups, made it clear that aeration can be used to improve the kinetics of fermentation and reduce fermentation times. Previous work with red wine fermentations was ambiguous concerning the effects of aeration on kinetics. Specifically, the effects of aeration during fermentation of fruit with suboptimal composition were unknown.

Figure 3 shows the fermentation profiles of two vintage trials (2018 and 2019). In the first experiment (Figure 3A), different modes of aeration (long, short or repeated) were used during fermentation of a low-YAN must (81 mg/L). Aeration, in this case, did nothing to decrease fermentation times. In all cases, fermentation was protracted due to the minimal nitrogen concentration of the must. In the second experiment, an increasing series of airflow rates was applied, from 0.5 L/min/kL to 10 L/min/kL. These aeration treatments increased the cumulative dissolved oxygen exposure from 1.4 to 52 mg/L*h, showing that these operations achieved an equivalent degree of aeration to those that successfully decreased the fermentation times of white ferments.

Despite the aggressive aeration regimes applied in this work, only a limited decrease in fermentation time was evident. The highest aeration ferment completed fermentation only two days sooner than those managed without aeration. Unlike the 2018 experiment, the 2019 trial was nitrogen sufficient (YAN = 250 mg/L). These data, taken together with data from previous trials, suggest that aeration is not a helpful tool for stimulating fermentation activity in red ferments.

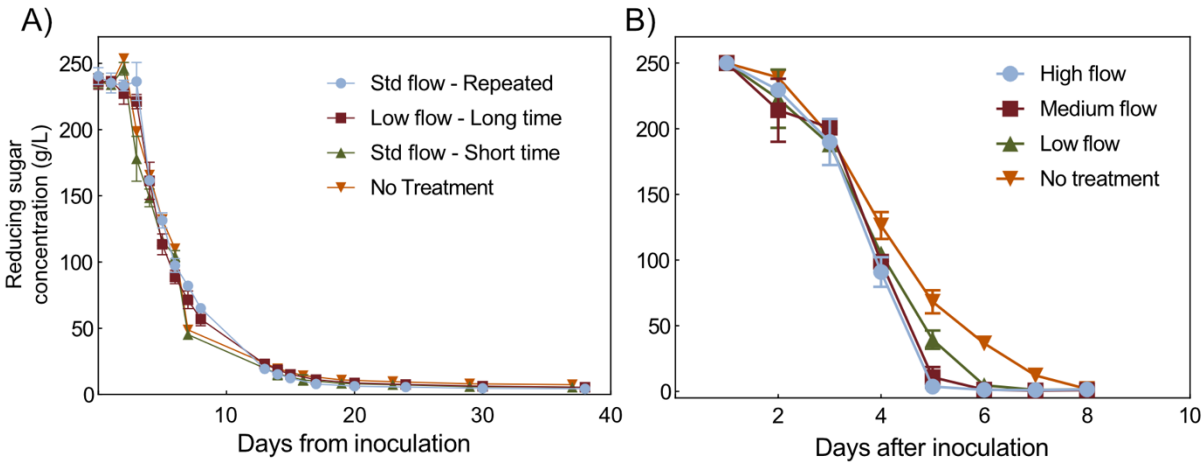


Figure 3. The effect of aeration mode (vintage 2018, A) and airflow rate (vintage 2019, B) on Shiraz fermentation progress undertaken in 450 L fermenters. (A) The airflow rate provided was 1.8 L/min/kL for 48 hours, 18 L/min/kL for 1 hour daily for 4 days or 18 L/min/kL for 4 hours at a single time, (B) The airflow rate provided was 0.5, 3.0 or 10 L/min/kL for 48 hours.

The observed lack of impact of aeration on red wine fermentation kinetics means that stylistic impact is likely to be more pertinent to the adoption of red wine aeration as an oenological tool. Previous work has shown that a variety of compositional effects are stimulated by aeration. Prominent among these is the suppression of low molecular weight volatile sulfur compounds (Bekker et al. 2015) and modification of phenolic material (McRae et al. 2015).

In work reported here, 53 compositional parameters were quantified across the two vintages. Limited changes to primary yeast-derived metabolites were observed in response to aeration. Ethanol concentrations remained largely insensitive to aeration. Volatile acidity, which can be thought of primarily as acetic acid, was not affected by aeration except for the most intense treatments in the 2019 trial. Consistent trends in the production of volatile aroma compounds were observed with decreased concentration of medium-chain fatty acid ethyl esters and increased concentrations of short branched-chain acetates and ethyl esters such as ethyl-2-methylbutanoate. The observations of volatile compound production in aerated red ferments are consistent with observations of these compounds in aerated white ferments.

There was evidence for a decrease in the concentration of low molecular weight sulfur compounds in the 2019 trial, but the size of the effect was small (decrease of 0.5 µg/L). Overall, it was apparent that the effect of aeration on low molecular weight volatile sulfur compounds is vintage-specific and dependent on their production potential in the system in question. Variables influencing this are primarily yeast-derived in the first instance, although their formation and release from other sources (Bekker et al. 2018) may also be affected by aeration.

The effect of aeration on phenolic material in red wine was the most prominent and distinguishing feature evident in these trials. Figure 4 shows the changes in the concentration of total tannin and anthocyanin in response to aeration. For both of these measures, the effect was inversely and linearly related to total oxygen exposure across both vintages. The impact of decreases in anthocyanin concentration is evident in wine colour density. In addition to changes in tannin concentration, changes to tannin structure were also observed, as indicated by the decline in the mean degree of polymerisation (MDP). MDP decreased by 27% with repeated aeration in the 2018 trial and between 25% and 37% with long-duration aerations in 2018 and 2019, respectively. The loss of larger tannin material may partly account for the decreased MDP observed here.

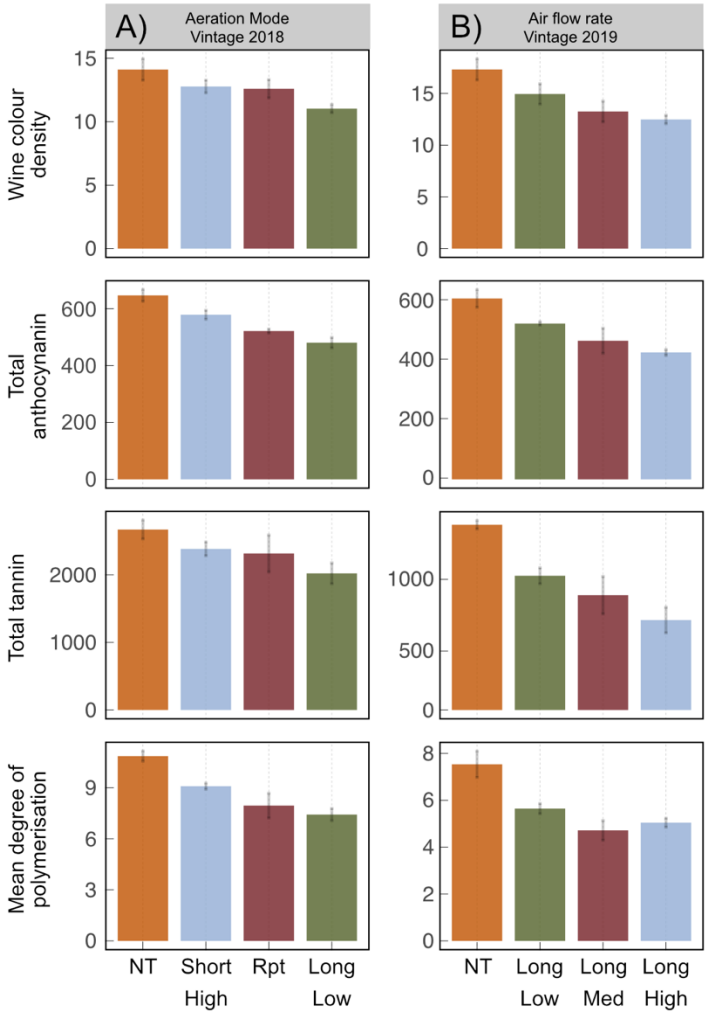


Figure 4. The effect of aeration mode and airflow rate on key phenolic concentration and structure in Shiraz wine over two vintages (2018 and 2019). The airflow rate provided was (A) 1.8 L/min/kL for 48 hours, 18 L/min/kL for 1 hour daily for 4 days or 18 L/min/kL for 4 hours at a single time, (B) 0.5, 3.0 or 10 L/min/kL for 48 hours.

The effect of Shiraz ferment aeration on wine sensory properties was evaluated through independent sensory descriptive analysis. The outcomes of these studies are summarised in Figure 5, which shows the results of a PLS-R analysis exploring the links between wine chemical composition and sensory profiles.

The effect of aeration was consistently and significantly ($p < 0.05$) associated with higher ratings for the descriptors 'red fruit' flavour and 'floral' aroma. Aeration was also associated with 'red fruit' and 'confection' aroma. Generally, four ethyl esters (ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, ethyl decanoate, ethyl octanoate), two fatty acids (octanoic and decanoic acids) and one acetate ester (2-phenylethyl acetate) were consistently significant and highly loaded for models. Ethyl-2-methylbutanoate and ethyl-3-methylbutanoate were consistently located to the left of loadings plots and associated with positive red wine aromas and the most oxygen-exposed samples.

The wines made without aeration or with limited oxygen exposure were rated highly for 'opacity' and 'astringency' across all trials. The regression coefficients for total phenolics and some other non-volatile measures such as MDP and total tannin had significant and highly positive regression coefficients relating to wine 'astringency'. In addition, 'drain' aroma, 'earthy' aroma and 'bitterness' were also more highly rated in wines made with little or no aeration.

Overall, increasing oxygen flow rate during fermentation resulted in a step-wise increase in the intensity of 'positive' red wine attributes. Decreases in attributes related to sulfide compounds were also observed. Aeration had limited and mixed effects on 'viscosity' or 'dark fruit' aroma and flavour, characteristics that could be considered undesirable.

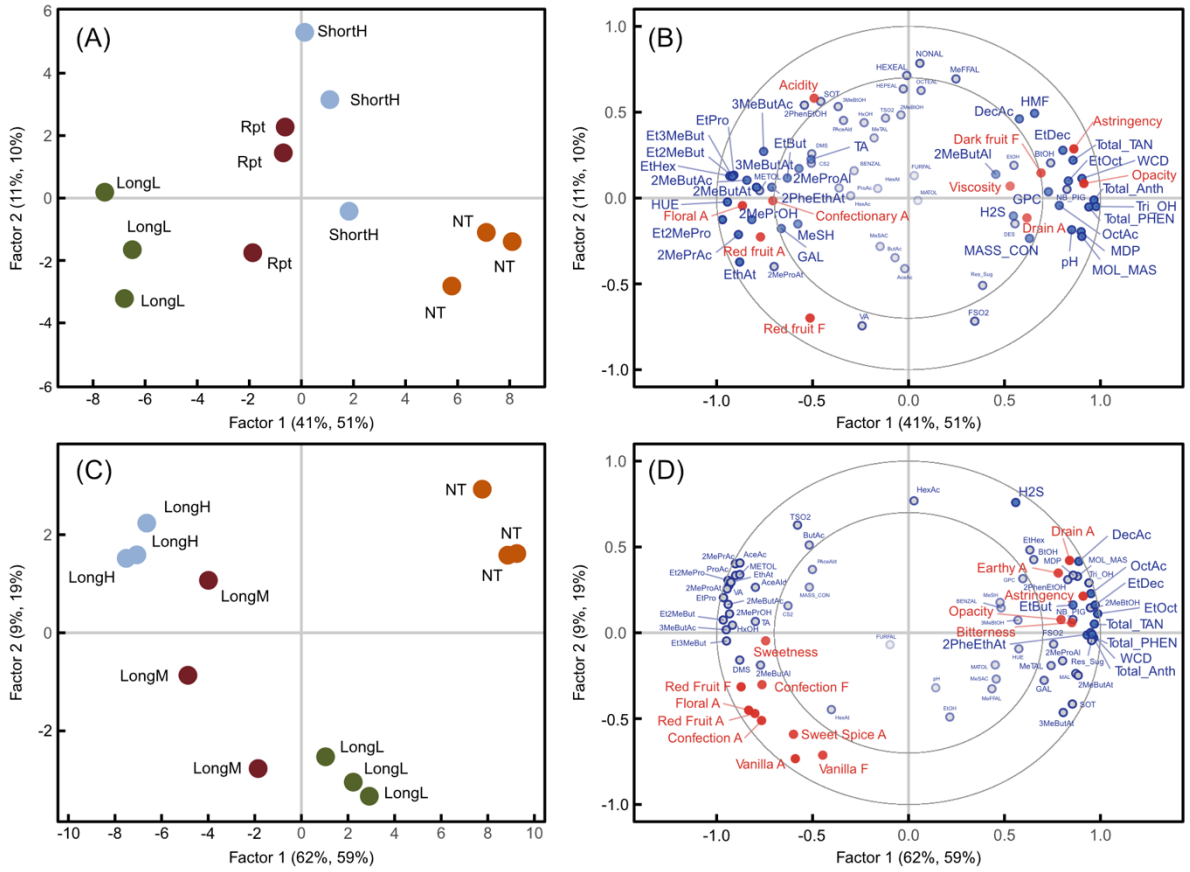


Figure 5. The effects of aeration mode and airflow rate on wine sensory characteristics and the relationship of these with wine composition. Factor 1 and 2 for the scores (a, c) and loadings (b, d) plots from PLS regression models for vintage 2018 (e, f) and vintage 2019 (g, h). Plots were generated using significant ($P < 0.05$) and close to significant ($P < 0.10$) sensory attributes [Y variables (red)] and compositional compounds [X variables (blue)] for each fermentation replicate of the winemaking treatments. The treatments were (A) 1.8 L/min/kL for 48 hours (LongL), 18 L/min/kL for 1 hour daily for 4 days (Rpt) or 18 L/min/kL for 4 hours at a single time (ShortH), (B) The airflow rate provided was 0.5 (LongL), 3.0 (LongM) or 10 L/min/kL (LongH) for 48 hours. NT indicates non-aerated wines.

6.1.3 Aeration of non-inoculated Chardonnay ferments – laboratory trials

The optimal time to aerate a wild ferment may depend on what the winemaker is trying to achieve. With the progression of different microbial species over the first few days of fermentation, aeration may benefit various microbial community members differently depending on when air is provided. In 2019, the effect of aeration timing was evaluated by sparging with air at a fixed flow rate for 24 hours with aeration treatments starting 24, 48 or 72 hours after grapes were pressed.

Aeration timing had a clear effect on fermentation duration (Figure 1), with duration decreasing in all aerated ferments compared to the control and shorter fermentation times when the air was applied later. Non-aerated ferments required 25 days to achieve a sugar concentration < 2 g/L. In contrast, ferments aerated at 72 hours finished after only 13 days and those aerated at 48 hours and 24 hours finished after 15 and 18 days, respectively.

Ferment samples were taken at similar sugar concentrations across the different ferments to quantify total yeast and non-*Saccharomyces* yeast populations at four stages. Aeration enabled a higher total yeast concentration to be sustained in the latter stages of fermentation. After 80% of fermentable sugar had been consumed, the yeast concentration in non-aerated ferments was 2×10^7 CFU/mL. In contrast, a cell concentration of greater than 10^8 CFU/mL was present in the aerated ferments. This increase in microbial abundance could be attributed to higher non-*Saccharomyces* yeast numbers (Figure 6). Similar yeast counts were obtained regardless of aeration timing, with a trend to higher concentrations of non-*Saccharomyces* yeast related to earlier aerations.

Using higher airflow rates did not result in further reductions in fermentation time. The minimum airflow rate used in this work resulted in the shortest fermentation time (Figure 6). All aerated ferments finished in 11-12 days, whereas non-aerated ferments required an additional 3-4 days (15 days total fermentation time). Higher airflow rates did increase total and non-*Saccharomyces* yeast counts with non-aerated, low and medium/high-intensity aerations supporting ever-increasing cell densities. Ferments aerated at low, medium and high flow rates reached 2 x 10^8 CFU/mL, 4 x 10^8 CFU/mL and 5 x 10^8 CFU/mL at 85% sugar consumption, respectively 5-, 10- and 12-times higher than non-aerated ferments.

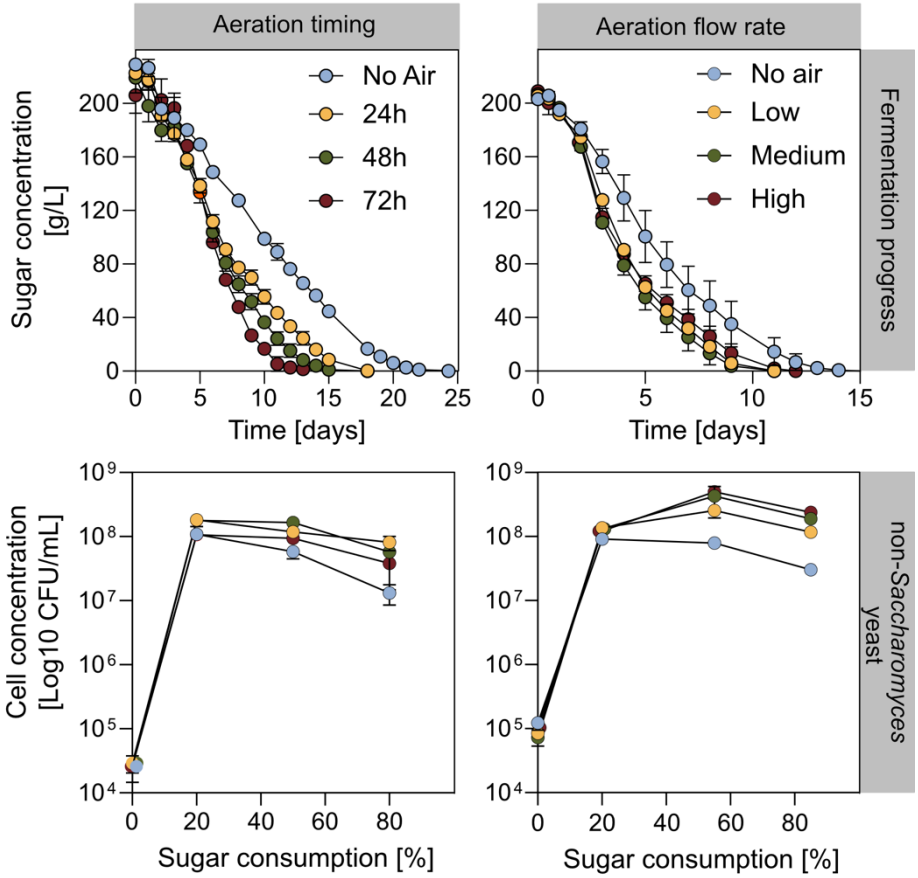


Figure 6. The effect of aeration timing (vintage 2019, left) and aeration flow rate (vintage 2020, right) on fermentation progress (top) and non-*Saccharomyces* viable counts (bottom). All symbols show the means of three treatments, with error bars indicating standard deviation. Timing of aeration was altered in vintage 2019 by providing a fixed flow rate of air to ferments for 24 hours, starting 24 hours, 48 hours, or 72 hours after crushing of grapes. In 2020 the airflow rate was altered by adding air at 7, 17 or 25 L/min/kL for 48 hours starting 24 h after crushing of grapes.

6.1.4 The effect of aeration on yeast community structure

In this work, the concentration of different yeasts at various times throughout fermentation was determined using meta-barcoding. Meta-barcoding uses high-throughput sequencing of a diagnostic fragment from the yeast genome to identify which yeasts are present. The number of

times a sequence is identified in a complex sample is a measure of its abundance relative to the other species in the sample.

Figure 7 shows the aggregated relative abundance data for yeast genera identified in ferments that were aerated at different times. The data for the non-aerated control ferment is typical for a freshly pressed grape juice, with the yeast community dominated by *Aureobasidium*, *Cladosporium*, *Epicoccum* and *Hanseniaspora* genus members and very little *S. cerevisiae* detectable. Once 20% of the sugar is consumed, 75% of the community is comprised of *Hanseniaspora* species, and *S. cerevisiae* has grown to represent nearly 20% of the population. This transition in community structure takes place within the first two days of fermentation and is typical of most non-inoculated fermentations.

Ferments that were aerated within those first two days showed increased growth of non-*Saccharomyces* species, particularly *Hanseniaspora* and *Torulaspora* (Figure 7). By the time ferments progressed to 80% completion, the concentration of *Hanseniaspora*, *Lachancea* and *Torulaspora* was 10-fold greater than non-aerated ferments. Surprisingly the stimulation of non-*Saccharomyces* yeast did not occur at the expense of *S. cerevisiae*. Only minor differences were observed in the total size of the *Saccharomyces* population, with estimated cell numbers correlating with the timing of aeration: control and ferments aerated at 24, 48 and 72 hours reached 7 x 10^6 CFU/mL, 1 x 10^7 CFU/mL, 2 x 10^7 CFU/mL and 3 x 10^7 CFU/mL, respectively. In summary, earlier aeration preferentially benefitted non-*Saccharomyces* yeasts.

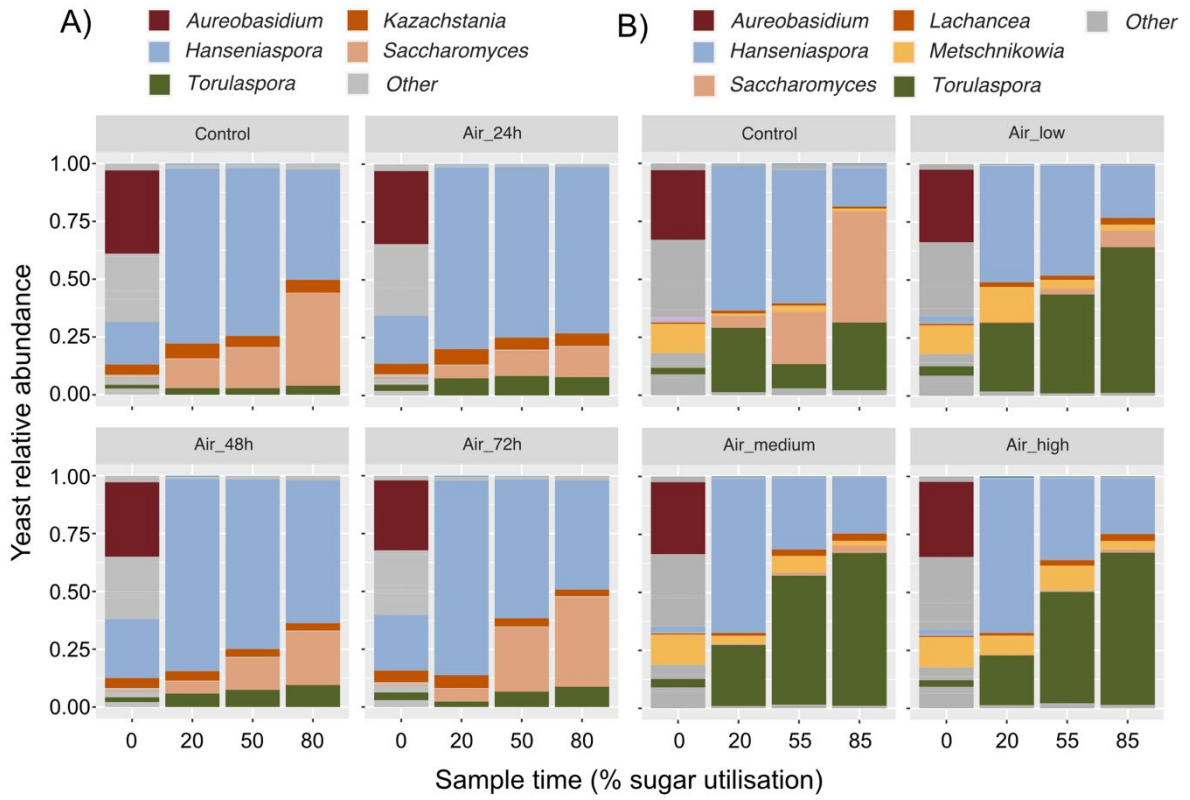


Figure 7. Relative abundance of yeasts in response to aeration timing (A) and intensity (B). The timing of aeration was varied by providing air at 24, 48 and 72 hours post-pressing of grapes for a treatment duration of 24 hours. The intensity of aeration was varied by providing increasing flow rates of air (7, 17 or 25 L/min/kL) for a period of 48 hours starting within 24 hours of pressing. Ferment samples were taken at four time points: 0%, 20%, 50% and 80% of sugar consumption. All sub-columns show mean percentages of three replicates.

6.1.5 Aeration of non-inoculated Chardonnay ferments – industry trial

This study aimed to evaluate the effect of aeration and SO_2 addition separately on the sensory properties of non-inoculated 2021 Chardonnay wines. Aeration of white wines was previously shown to accelerate wine fermentation rates in non-inoculated and inoculated ferments alike

and to promote the growth and persistence of non-*Saccharomyces* yeasts. Previous work had shown later aerations of non-inoculated ferments tended to benefit predominantly *S. cerevisiae* with little discernible compositional difference between late-aerated and non-aerated wines. Therefore, the timing of aeration in this trial (very early) was selected to maximise growth of non-*Saccharomyces* yeast, with the ultimate aim of determining the sensory effect of such a treatment.

SO_2 addition to non-inoculated ferments has been shown to promote the growth of the non-*Saccharomyces* yeast strain *Hanseniaspora*. This strain is naturally abundant in grape musts, can dominate low-sulfite juices, and has been shown to increase fruity and floral notes in the wine. Aeration and SO_2 addition were conducted as part of the same industry trial, in collaboration with Project 4.4.3, at Yalumba in Angaston and are therefore presented together here, as excluding data from one of the treatments would diminish the results.

Aeration immediately affected the sugar consumption rate in pilot-scale non-inoculated ferments (Figure 8). While not immediately evident in the record of total soluble solids (Figure 8A), in which all fermentation finished in 15 – 16 days, it is evident in the temperature record for the fermentations (Figure 8B). In this winery trial, temperature was used to control the fermentation rate. The set temperature for the aerated ferments was reduced from 15°C to 12°C on day 2 of the trial, two full days earlier than the control fermentations and four days earlier than SO_2-treated fermentations. This showed that in regard to fermentation performance, even the least effective aeration timing, based on previous laboratory experiments (Figure 6), still exerted a powerful influence on fermentation rate.

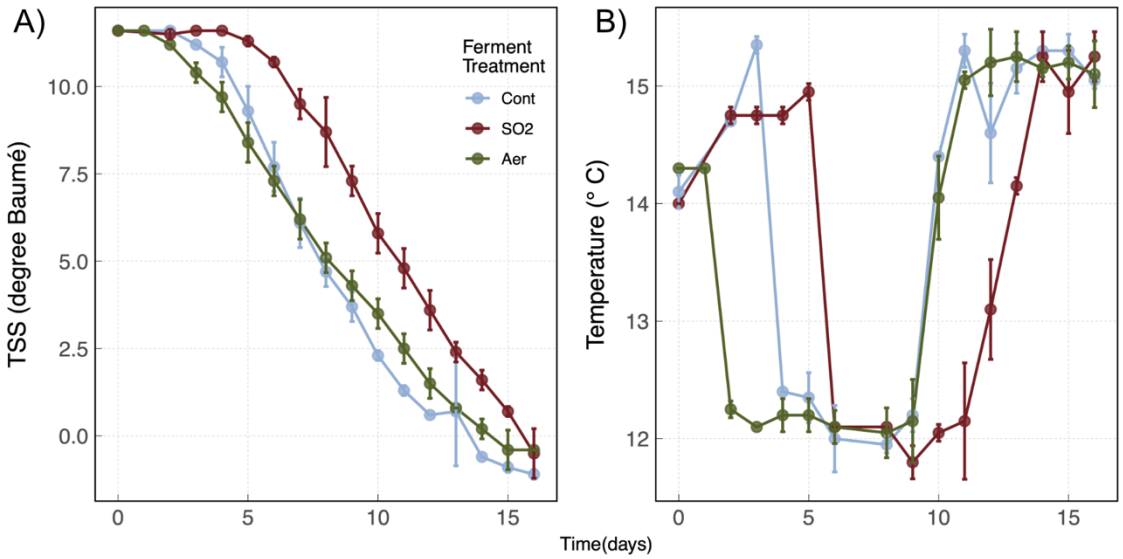


Figure 8. Sugar utilisation (A) and temperature (B) of 1,000 L non-inoculated fermentation that had been aerated at a rate of 0.5 L/min/kL (green) or SO_2 treated at a rate of 40 mg/L (red). Control (untreated) ferments are shown in blue. Points show the average of two ferments, error bars show standard deviation.

The sensory properties of the wines were evaluated using the quantitative sensory descriptive analysis method. An ANOVA assessing the effect of the three treatments showed statistical evidence that nine sensory attributes, yellow (colour intensity), citrus aroma, tropical fruit aroma, stone fruit aroma, apple/pear aroma, floral aroma, nail polish remover aroma, drain aroma, and apple/pear flavour differed among the treatments.

The aerated wines were rated lowest for the intensity of all fruit attributes and were rated highly for drain aroma and nail polish remover aroma (Figure 9). The SO_2-treated wines were generally rated highest for the fruity attributes mentioned above and lowest for drain aroma.

The control wines were similarly rated to the SO_2-treated wines but were scored at lower aroma intensities.

While accentuated nail polish remover and drain attributes were the expected outcome of such an early aeration, it had been hoped that the conservative airflow rate used might have enabled the stimulation of non-*Saccharomyces* yeast without the emergence of these negative attributes. This trial demonstrated that such an outcome is not achievable using aeration as a tool. However, it did not negate the use of aeration at later stages of non-inoculated fermentations to enhance fermentation performance metrics. Aerations during the recommended time (20% fermentation progress) are unlikely to elicit such a response.

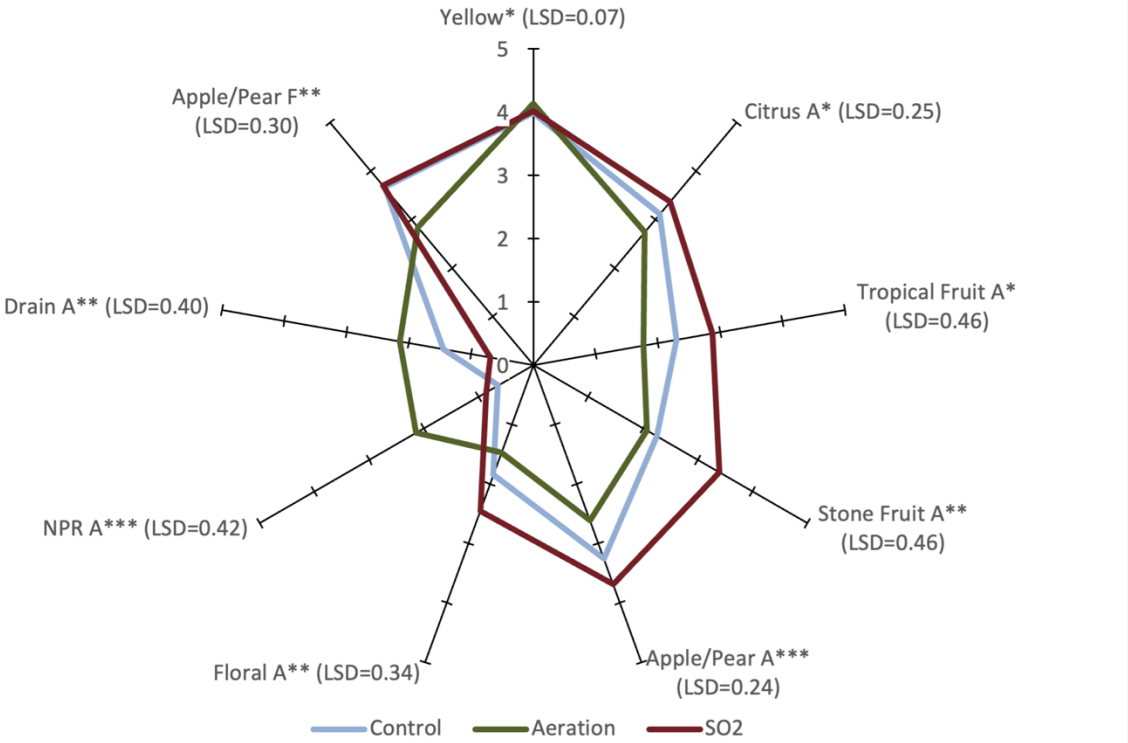


Figure 9. Effects of aeration (green) or SO_2 addition (red) on wine sensory properties as determined by quantitative sensory descriptive analysis. The plot shows mean attribute intensity scores for which there was evidence of a difference between treatments ($P < 0.05$). LSD values at 95% confidence level are shown. A: Aroma, F: Flavour.

Compositional analysis of the wine across a suite of 39 aroma-active compounds found limited evidence for an effect of aeration on the production of yeast-derived volatile compounds. Those for which there was evidence of a treatment-related effect on concentration ($P < 0.05$) are shown in Figure 10. Early aeration was associated with an increased concentration of ethyl acetate and acetic acid, which was consistent with the increased perception of the nail polish remover attribute during sensory analysis of the wines. Increases in the concentration of methanethiol were also observed (~0.5 µg/L) and, while small, were also consistent with the perception of the wines having a drain-like character.

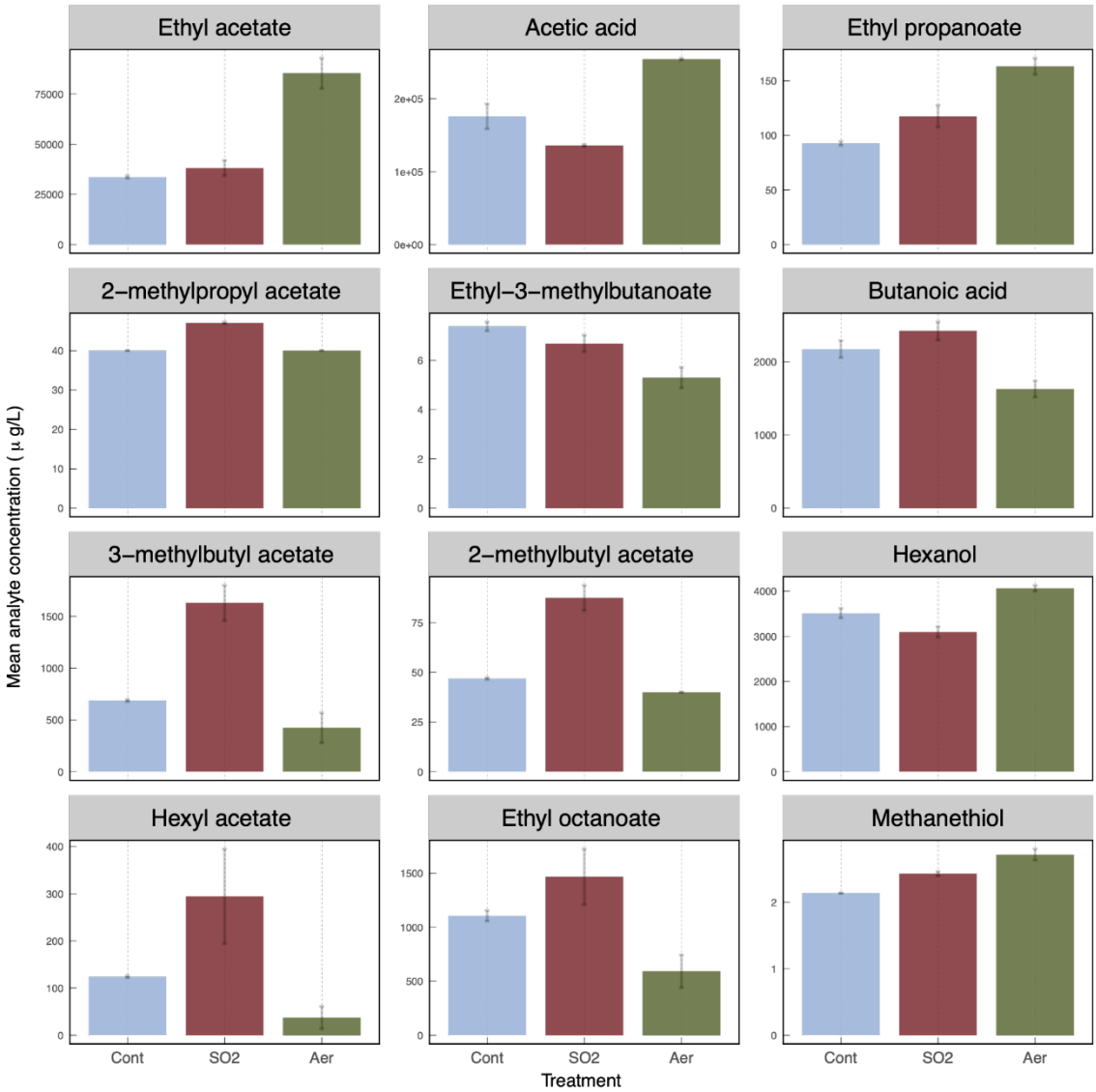


Figure 10. Analytes for which there was evidence of a difference in concentration in finished wine ($P < 0.05$) in response to aeration during fermentation (green) or SO_2 addition prior to fermentation (red). Analytes from untreated control wines are shown in blue. Columns show the average value of two replicates, error bars show standard deviation.

Taken together, this work showed that aeration of non-inoculated ferments could be used to stimulate fermentation activity and reduce the duration of fermentation. Timing of aeration was instrumental in reducing fermentation time, with the fastest ferments achieved when aeration was applied later, at a time when *S. cerevisiae* was emerging as a prominent member of the microbial community. It is therefore likely that the later aeration benefited the emerging *S. cerevisiae* population to a greater degree than the non-*Saccharomyces* population. While earlier aerations still decreased overall fermentation time, the effect was diminished.

Earlier aeration, however, did maximise the reshaping of yeast populations, affecting the growth, viability and likely metabolism of non-*Saccharomyces* yeast species. Overall, wines resulting from aeration had a different chemical composition from those from non-aerated ferments, with the magnitude of the change in the concentration of some compounds suggesting a likely effect on the sensory profile. Higher intensity aeration early during fermentation was detrimental to wine quality, with the appearance of elevated volatile acidity in laboratory experiments.

Industry trials also showed that even conservative aeration rates at the early stages of fermentation were sufficient to elicit ethyl acetate production, with the resulting wines appearing volatile and showing reductive characters. During this fermentation phase, there is limited microbial activity, which would generally help keep the dissolved oxygen concentration low. For this reason, early aeration of ferments in general and non-inoculated fermentation in particular, is not advised. There does not appear to be an airflow rate that is sufficiently low to eliminate the risk of detrimental effects. The risk of increased volatile acidity means winemakers have limited opportunity to influence microbial composition using aeration. However, this work demonstrates that air addition is a tool that winemakers may use, with due consideration to its timing, to improve fermentation performance in uninoculated white wines.

6.1.6 Effectiveness of different aeration practices on oxygen transfer rates

Much of the work described above related to the effects of aeration on fermentation performance and wine style. With a substantial foundation of knowledge about the effects of aeration established, several questions remained about the most effective tools that might aid in the measurement of aeration in the winery and the types of aeration approaches that could be most effective.

With regard to oxygen measurement, two types of devices are most readily available. The first, fluorescent probes such as those used in the work described above, provide a direct measurement of the concentration of oxygen in solution. These come in a variety of formats and have the advantage that the analyte they measure bears an obvious relationship to the treatment being applied. In addition, the structure of the probes themselves is extremely robust, usually constructed from stainless steel. The second device that can be used is the redox or ORP probe. Redox probes are electrochemical in nature and measure the flow of electrons to and from compounds in solution. These probes are typically made of glass.

It was of interest to understand the relationship between dissolved oxygen (DO) concentration and measures of redox, and whether variations in juice composition might alter that relationship. The DO/redox relationship was evaluated in a series of experiments conducted in controlled laboratory fermentations using a defined medium that compositionally resembled Chardonnay juice (Schmidt et al. 2011). Aeration was applied at a fixed airflow rate. The oxygen composition of the gas was varied by mixing air with nitrogen. Five different oxygen compositions (0%, 25%, 50%, 75% and 100% air) were sparged into the fermentation for 2 hours each in a repeating loop, permitting the measurements of variations in dissolved oxygen and redox to be made as fermentation progressed through high to low sugar concentrations.

Three different fermentation media were studied in this way: standard defined medium (SD), SD + 10 mg/L copper, and SD + white grape phenolics. Figure 11 shows the mean dissolved oxygen concentration and redox value in the final 30 min of each two-hour treatment period over the course of an entire fermentation. This shows quite clearly the increased consumption of oxygen as biomass increases over the first 50 hours of fermentation. The oxygen input vs response is then very stable over the remainder of the fermentation period, increasing again only when fermentation is close to complete.

The sensitivity of the probes at different dissolved oxygen concentrations is also striking. Whereas the dissolved oxygen probe has a limited operational sensitivity at low dissolved oxygen concentrations, the redox probe records substantial changes in the matrix. Conversely, the redox probe measurements become compressed at high dissolved oxygen concentrations, whereas the DO probe exhibits a considerable measurement range.

Higher copper concentration and the inclusion of white phenolic material increased the minimum redox potential observed in these fermentations but otherwise did not appear to substantially alter the oxidation/reduction potential of the medium in response to aeration. Interestingly, the addition of white wine phenolics suppressed the dissolved oxygen concentration during the period of maximal fermentation activity. Whether this was due to

inhibition of the oxygen transfer rate or stimulation of yeast oxygen consumption is not evident from the data.

These data do highlight one additional feature of redox as a measure of oxygen activity during aeration of fermentation: its increased sensitivity at low dissolved oxygen concentrations will assist with the automated control of aeration while minimising the accumulation of dissolved oxygen. Minimising dissolved oxygen accumulation is a particularly appealing attribute specifically for the control of aeration of white fermentations. The second feature of redox as a control option is that the decay of redox potential is slower than the decay of the dissolved oxygen concentration. A slower or more stable signal is a useful attribute for a controller function. The merits of redox as a tool for the control of aeration have been strongly argued elsewhere (Killeen et al. 2018).

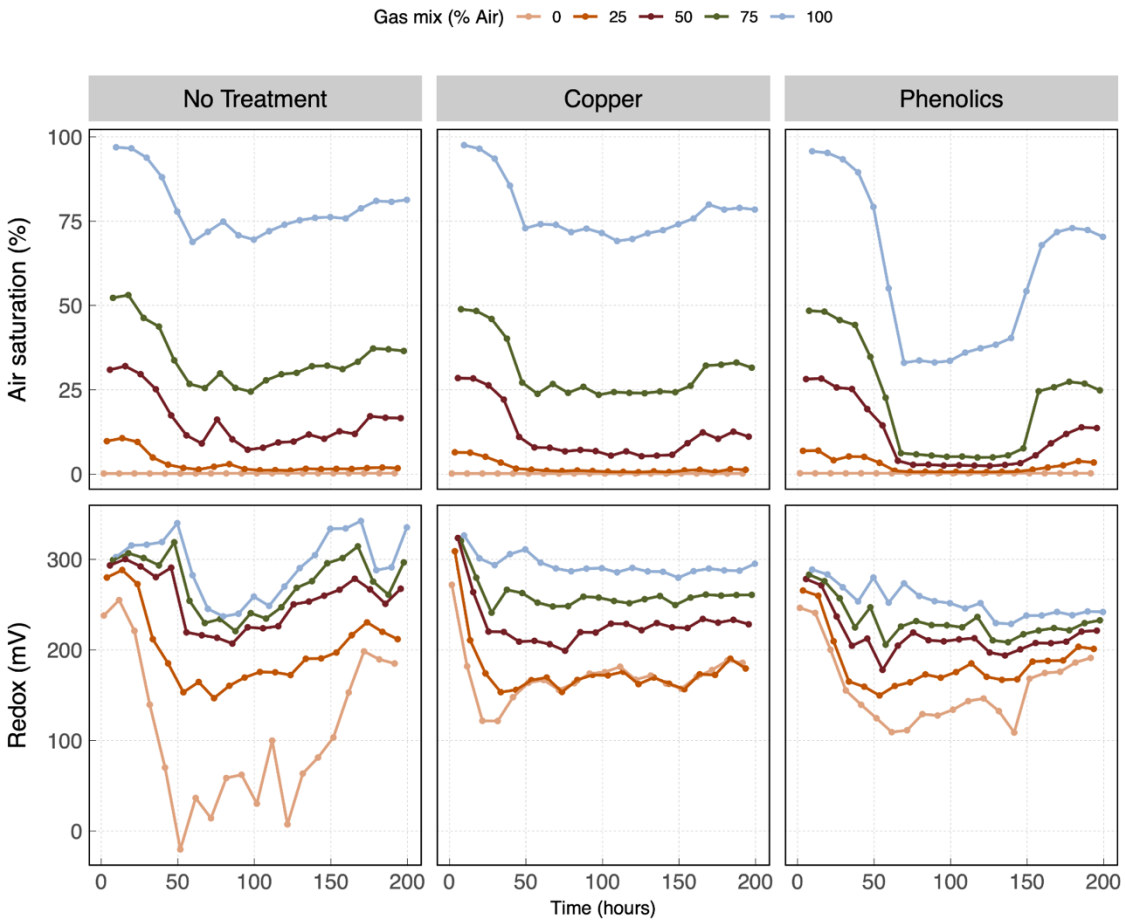


Figure 11. The effect of copper and white wine phenolic material on measures of dissolved oxygen and redox. Each point shows the average dissolved oxygen concentration (% air saturation) or redox value (mV) for the 30 min of each two-hour treatment period in defined medium, defined medium containing 10 mg/L copper, or defined medium containing white grape phenolic material at a concentration equivalent to that found in a standard Chardonnay juice.

Following an examination of the relative merits of DO and redox measurement technologies for aeration control, an investigation was initiated into the methods by which air can be introduced. In almost all of the pilot-scale work undertaken in this project, fermentations have been aerated using spargers installed into the bottom of the fermentation vessels. These spargers were constructed in a cross configuration with a total sparger surface area of 61,575 mm^2. The question of whether sparger surface area has a major influence on the effectiveness of an aeration operation has not previously been addressed.

The aim of the following series of experiments was to determine whether sparger surface area or arrangement was important for the transfer of oxygen into a ferment. The experiment was conducted in a 1,000 L Chardonnay fermentation. Aeration treatments were performed for 30

min each. Each new treatment was initiated after the redox potential had declined to pre-aeration values. Two airflow rates (0.25 L/min/kL and 0.75 L/min/kL) and three sparger arrangements (single-point, two-point or four-point) were used in the trial. The sparger areas were 15,393 mm^2 and 61,575 mm^2.

In this trial, there was a moderate effect of sparger arrangement on the maximum dissolved oxygen concentration achieved, with small increases in the cumulative dissolved oxygen (CDO) concentration observed at a gas flow rate of 0.25 L/min/kL (Figure 12). Dissolved oxygen data obtained at a gas flow rate of 0.75 L/min/kL were inconsistent across the three sparger arrangements. Redox measures gave a similar picture of the effect of sparger arrangement, with increases in the redox potential equivalent between two-point and four-point spargers at gas flow rates of 0.25 and 0.75 L/min/kL (Table 6).

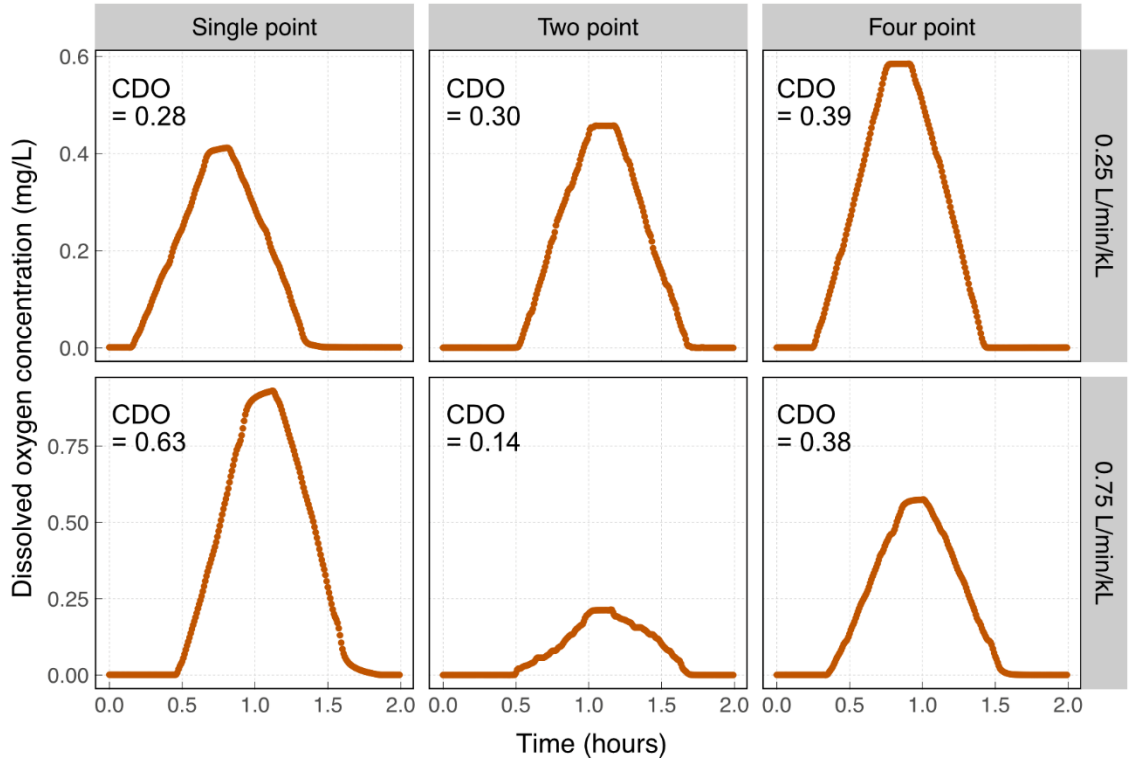


Figure 12. Effect of sparger head configuration and gas flow rate on dissolved oxygen concentration in 1,000 L Chardonnay fermentation. Spargers were single-point, two-point or four-point arrangements, with each arrangement doubling the effective sinter area relative to the previous configuration. Gas was provided as air at 0.25 and 0.75 L/min/kL. CDO is cumulative dissolved oxygen concentration (mg/L*h).

Table 6. Summary of redox potential change during fermentation due to different aeration treatments and sparger head configurations

Sparger	***Flow rate (L/min)***	***Initial redox potential (mV)***	***Final redox potential (mV)***	***Δ Redox potential (mV)***
Single	0.25	-113.9	-81.7	32.2
	0.75	-51.61	-26.04	25.57
2-way	0.25	-140.8	-127.9	12.9
	0.75	-137.2	-106.9	30.3
4-way	0.25	-149.2	-136.5	12.7
	0.75	-150.8	-131.2	19.6

7. Outcome and conclusion

7.1 Performance against planned outputs

7.1.1 Methods to assess and improve the efficiency of fermentation through the use of oxygen during alcoholic and malolactic fermentation

Experimentation with various types of equipment for the measurement of dissolved oxygen indicated that the implementation of aeration practices based on direct measurement of fermentation conditions (i.e. dissolved oxygen measurements or redox measurements) is likely to be impractical, economically unviable or too technically challenging for many wineries. Therefore, the project team focused on solutions that did not require information about the dissolved oxygen concentration or redox status during fermentation. Over a series of years, laboratory-scale and pilot-scale trials were undertaken to define airflow rate and duration metrics that could be applied to red and white fermentations, respectively.

A compilation and analysis across four years of red wine vintage trials showed the limited effect of aeration on the efficiency of red wine fermentation. This analysis also showed that malolactic fermentation, either simultaneously or sequentially, was unaffected by aeration. This finding opened the door to the use of aeration, with the data providing confidence that aeration would not impede MLF. However, the lack of an effect on fermentation duration requires that the motivation for its use as an oenological application shifts to the effect on wine style. The effects of aeration on wine style are discussed in more detail in section 7.1.4.

In contrast to what was observed for red wine fermentations, the effect of aeration on white wine fermentation was almost entirely related to fermentation efficiency. Aeration intensities beyond those required to stimulate fermentation performance were invariably detrimental to wine quality. Low-intensity aerations sustained over longer durations were found to be the optimal approach for white ferments. Attempts to apply shorter aeration treatments at defined times during fermentation were less effective at reducing fermentation times than longer aerations.

The concept of aerating white ferments to reduce fermentation times was extended to non-inoculated ferments, where timing and aeration intensity were explored, mindful of the potential repercussions on microbial community structure. Optimal fermentation performance benefits from aeration of non-inoculated ferments were achieved when aeration coincided with the onset of *S. cerevisiae* ascendency within the microbial community. The dominance of fermentation by *S. cerevisiae* occurs later in non-inoculated fermentations. Therefore, the optimal time to aerate non-inoculated ferments is also a day later than is ideal for inoculated fermentations.

The project team also experimented with different aeration methods in red and white fermentations. While most of the pilot-scale trials were undertaken using spargers built into the fermentation vessels to deliver air during fermentation, this particular implementation of air delivery is best suited to the aeration of white ferments. The suitability of built-in sparger systems to white ferments is almost entirely due to the absence of a cap and their more uniform nature. Such systems are also less prone to damage during post-fermentation operations.

The optimal strategies for the aeration of red wine fermentations are those that can be coupled with cap management operations. Effective aeration coincident with cap management requires that these operations are undertaken frequently, at least daily but ideally more often, due to the generally short duration of cap management. It would not be possible to deliver extended aeration using this approach.

7.1.2 Knowledge of the scale-related impacts of processes for wineries by determining the effectiveness and impact of different practices for introducing oxygen at different scales (e.g. barrel stirring, splashy racking)

Several approaches to understanding how aeration can be applied at different volumetric scales were attempted during the project. While there were inevitably some COVID-related impacts on this aspect of the project, given its dependence on winery access, progress was made on understanding how aeration schemes can be implemented at scale.

Knowledge building about the implementation of aeration at scale was initially achieved through visits to Cape Jaffa winery (Limestone Coast), Richmond Grove (Tanunda) and Tintara Winery (McLaren Vale). The trials were primarily concerned with the effectiveness of introducing air into the bulk of red and white wines. Evaluation of the treatment effects on the compositional or sensory properties of the wines themselves was not attempted.

The industry trials at Cape Jaffa winery compared aeration of red wine via rack and return to aeration using an inline sinter. The practicalities of undertaking each operation were evaluated in addition to aeration efficiency. The trial found that rack and return could be an effective method for introducing air into red ferments but that it was time-consuming and messy. Inline spargers were almost twice as effective at getting air into the ferment and had the advantages of being cleaner and easier to implement and being amenable to automation.

The trial at Richmond Grove explored the potential of Pulse-Air devices for getting air into red ferments during cap management operations. This trial simply monitored the dissolved oxygen concentration at three heights in a 5 m tall fermentation vessel during a series of fermentations in which a Pulse-Air device was used. This work showed that in the upper regions of the tank, just below the cap, dissolved oxygen concentrations approaching 10% air saturation were possible. However, in the lower parts of the tank, very little dissolved oxygen was evident, primarily due to the low levels of mixing within the vessel. These results were intriguing. Whether Pulse-Air-like devices can be configured to be used efficiently for aeration operations remains an open question.

The project team also attempted to determine whether sparger surface area or arrangement was important for transferring oxygen into a ferment. The aim was to optimise the use of a potentially limited resource for some wineries: access to a sufficient supply of air for extended aeration schemes. The trials compared 20 L and 1,000 L Chardonnay ferments to which equivalent aeration treatments were applied. While a moderate effect of sparger arrangement on the maximum dissolved oxygen concentration was observed at a gas flow rate of 0.25 L/min/kL, the variability across the treatments was too great to draw definitive conclusions about the effect of sparger arrangement on aeration efficiency. The experiments were inconclusive.

7.1.3 Data on the impact of juice, must and wine exposure to oxygen (timing, point in production process, dose) on final wine composition and sensory characteristics

Work on the effect of oxygen exposure during the processing of white grapes was completed and published during the project period. This work showed the possible stylistic potential and limitations of oxygen control. Subtle increases in floral and confection characteristics were demonstrated in white wines with limited oxygen exposure during pressing and handling operations. Strict protection of wine from oxygen exposure also minimised yellow colour development. These wines were more sensitive to post-fermentation oxidative colour development.

Extensive work was undertaken to understand the effects of aeration timing and intensity on white and red wine. The effects of increasing aeration intensity with a fixed duration were explored in both systems. Increasing the overall oxygen exposure negatively affected white wine quality without an accompanying improvement in fermentation time. The negative effects of increasing oxygen exposure in red wines were muted relative to the effects on whites (more detail below).

White wine trials showed that aeration could be used to decrease fermentation times. The optimal mode of delivery was an extended period of aeration delivered through spargers installed into the bottom of fermentation vessels. Using conservative airflow rates (< 0.5 L/min/kL) allowed aeration to be implemented in a way that did not compromise product quality. However, at higher airflow rates, there was a risk of adverse effects on wine quality. The effects of high airflow rates on wine quality are discussed in more detail in section 7.1.4.

The effects of aeration during white wine production were also explored through the application of very early low-intensity aeration of non-inoculated white wine fermentations. Early aeration was undertaken to stimulate the persistence of non-*Saccharomyces* yeasts and invariably led to the production of wines with higher concentrations of ethyl acetate and acetic acid. Unsurprisingly, this was associated with an increased perception of aromatic characters described as 'nail polish remover'. Similar effects were observed following high-intensity aeration of inoculated white wine fermentations. These observations underline the point that aeration of white wines beyond that necessary to stimulate fermentation performance will be detrimental to wine quality. The project team's observations suggest that an airflow rate of 0.5 L/min/kL delivered for 24 hours is the upper limit for white ferments, beyond which adverse effects on wine quality are increasingly likely to be observed.

In red wines, increasing airflow rate during fermentation resulted in a step-wise increase in the intensity of red fruit attributes and a decrease in attributes related to sulfide compounds compared to the non-aerated wines. The effects on 'viscosity' or 'dark fruit' aroma and flavour were limited. Aeration applied over an extended period at low intensity, or repeated short-duration high-intensity applications had equivalent compositional and sensorial effects. This finding supports the practical strategy discussed in section 7.1.1 of, where possible, coupling aeration with cap management operations in red winemaking.

7.1.4 Knowledge on the impact of key oxygen-modulated compounds on wine style and sensory properties

This project contributed to knowledge of the effect of oxygen-modulated compounds on wine style and sensory properties, primarily through the work undertaken on red fermentations. As discussed in section 7.1.3 aeration of white ferments should seek to minimise the effect on wine sensory attributes while maximising the effects on fermentation performance. Conversely, the effects of red ferment aeration are predominantly on composition.

Red wine trials over four vintages consistently highlighted the enrichment of ethyl-2- and ethyl-3-methylbutanoate in wines that had been aerated during fermentation. These two compounds had high positive regression coefficients associated with 'overall fruit' aroma, 'red fruit' aroma and 'floral' attributes across the trials. These findings are consistent with the work of de-la-Fuente-Blanco et al. (2019).

Red wines made without aeration or with limited oxygen exposure were typically more opaque and astringent. Wine colour density and either hue, non-bleachable pigment or total anthocyanin were strongly associated with 'opacity'. Total phenolics and other non-volatile measures such as mDP and total tannin were positively related to wine 'astringency'.

In addition, 'drain' aroma, 'earthy' aroma, 'bitterness' and 'vegetal' aroma were more highly rated in wines made with little or no aeration. Hydrogen sulfide was strongly implicated in reductive aroma attributes 'drain', 'vegetal' and 'earthy', in some cases combined with MeSH or its acetate ester. These findings are also consistent with previous observations.

7.1.5 Practical advice and extension materials about methods to introduce oxygen and the impacts of timing and dose of addition

A substantial set of resources was generated on the effects of aeration during fermentation in red and white wines, directly from information generated from experimental work undertaken in the project. These resources include information on different ways of undertaking aeration

and the methods available to measure those effects. Extension materials related to aeration have continuously been developed as a direct consequence of that knowledge generation. Early in the project, content focused on why aeration might be a valuable tool for winemakers. Later in the project, extension materials evolved to include more information about how to implement aeration across various scenarios. Despite some COVID-related interruptions, the project team has been highly motivated to engage in extension activities, which is reflected in the number of in-person seminar presentations delivered in different winemaking regions.

In the last year of the project, team members provided content for an adoption program across various formats, including webinars, podcasts, demonstration videos and web-accessible fact sheets. This information coalesced into a dedicated workshop delivered at the 18th Australian Wine Industry Technical Conference in June 2022.

7.2 Could changing the methodology/technology have improved the outcome?

Several different oxygen measurement devices were trialled throughout the project, all of which had strengths and weaknesses. The oxygen sensors all used fluorescent technology, which is sensitive and rapid, allowing oxygen transfer and consumption rates to be calculated. However, some technologies were insufficiently resilient for fermentation monitoring, and others were difficult to connect to computers or programmable logic controllers (PLCs). Redox probes had similar issues, with modern digital probes often outputting data in proprietary formats that require expensive transmitters to convert and relay the signal. It is likely that many wineries would struggle to easily or economically deploy these devices. That being said, over the course of the project, technology in this area has developed significantly with increasing connection options, including wireless technologies for remotely deployed probes and software solutions for monitoring multiple measurement sources.

7.3 Practical implications

The overriding practical implication for the adoption of aeration as an oenological tool is the clarity in intent when considering white and red wine production. Knowing that aeration of white ferments can be used to improve fermentation performance metrics while being conscious of the associated risks helps to define upper bounds for operational processes. Knowing that aeration of red fermentations can be used to shape wine style while being conscious that there will be a minimal effect on fermentation time, helps wineries to manage resources and infrastructure investments to greater effect. Knowing that red wines are at a massively reduced risk from adverse effects helps to give practitioners confidence to experiment with aeration in their wineries. Beyond that, the project team has been able to provide some basic guidance about how to get started, conservative airflow rates that practitioners can use and ideal timing and methods for implementing aeration schemes for inoculated and non-inoculated fermentations alike.

7.4 Benefits to industry

Knowledge generated during the project and the associated educational resources and materials will help winemakers decide when, where and how to use aeration. More widespread and targeted use of aeration during fermentation will contribute to decreasing the incidence of suboptimal fermentations, a decrease in reductive characteristics of some wines, and allow the production of 'fruit-forward' red wine styles. As with all techniques in winemaking, aeration is not a universal solution to all winemaking issues, but used with discipline it can be a useful addition to the winemaker's toolbox, adding further diversity and control to the art of winemaking.

8. Recommendations

A great deal of progress has been made in delineating when, where and how to use aeration during winemaking, especially with red fermentations. For smaller-scale red ferments where plunging is used for cap management, or for white wine ferments where physical interventions are not a feature of fermentation management, it can make more sense to consider using sinters or diffusers. There are some technical features of these devices that may present a barrier to deployment. These include the potential for fouling, contamination of air lines and higher maintenance requirements. In future it will be necessary to understand and reduce barriers to

entry for winemakers that wish to use aeration in medium to large-scale white fermentations. Subsequent work should aim to find and evaluate cost-effective air delivery options for winemakers seeking to implement aeration strategies at those scales.

A significant investment in the generation of educational resources has been made through this and associated projects. Concluding investment in adoption efforts with the culmination of this project would be counterproductive. To maximise the benefits from the knowledge gained though investment in this project, support for adoption efforts needs to be ongoing. This will assist those who have yet to try aeration in their winemaking as well as those who have started to adopt this practice but for whom additional support is required to overcome early barriers to adoption.

9. Appendix 1: Communication

Outcomes and knowledge generated during the progress of this project have been communicated through peer-reviewed publications and conference presentations. Information has been extended to industry stakeholders through articles in trade journals, presentations in the AWRI roadshow program and webinars. The project team has provided content as part of a separate adoption program reported under Project 2.1.1 entitled 'Use of oxygen during fermentation'. Project communications are listed in detail below.

9.1 Presentations delivered during the conduct of the project

- Schmidt, S.A. 2020. Aerating ferments: why it is useful and how you can do it [Webinar recording]. https://youtu.be/w0QYC4JXoBM
- Schmidt, S.A. 2021. Aeration of red ferments [Webinar recording]. https://youtu.be/RwJeTeph0HY
- Schmidt, S.A. 2021. Oxygen – breathing life into red wine flavour [Podcast]. AWRI Decanted. https://www.awri.com.au/industry_support/courses-seminars-workshops/podcast-awri-decanted/
- Schmidt, S.A. and Holdstock, M. Ways to introduce oxygen into an active red ferment [Fact sheet]. Available from: https://www.awri.com.au/wp-content/uploads/2015/02/introducing_oxygen.pdf
- Schmidt, S.A. 2022. Changing wine with air [Seminar]. 10/5/2022 Stanthorpe seminar series
- Schmidt, S.A. 2022. Changing wine with air [Seminar]. 1/6/2022 Hunter seminar series
- Schmidt, S.A. 2022. Changing wine with air [Seminar]. 2/6/2022 Mudgee seminar series
- Schmidt, S.A. 2018. The style and performance effects of oxygen addition during winemaking [Seminar]. 23/9/2018 Langhorne Creek, SA.
- Schmidt, S.A. 2019. The beneficial style and performance effects of oxygen addition during fermentation [Seminar] 21/2/2019 Stanthorpe, Qld
- Day, M.P. 2019. The beneficial style and performance effects of oxygen addition during fermentation [Seminar] 16/10/2019 Southern Highlands, NSW.
- Day, M.P. 2019. The beneficial style and performance effects of oxygen addition during fermentation [Seminar] 17/10/2019 Canberra, ACT.
- Bekker, M.Z. 2019. Macro-oxygenation during red winemaking: use and impacts [Seminar] 12/12/2019 Clare Valley, SA
- Day, M.P. 2017. The beneficial style and performance effects of oxygen addition during fermentation. [Seminar] 14/9/2017, Clare Valley, SA
- Day, M.P. 2017. The beneficial style and performance effects of oxygen addition during fermentation. [Seminar] 24/10/2017, Rutherglen, Vic.
- Day, M.P. 2017. The beneficial style and performance effects of oxygen addition during fermentation. [Seminar] 26/10/2017, Bendigo, Vic.
- Schmidt, S.A. and Holdstock, M. 2022. Aeration during fermentation [Workshop]. The Australian Wine Industry Technical Conference. Adelaide, 26 June 2022.

- Schmidt, S.A. and Day, M.P. 2019. Macro-oxygenation during red winemaking: use and impacts [Workshop] The Australian Wine Industry Technical Conference. 20/7/2019
- Day, M.P. 2017. Airy thoughts on winemaking. CRUSH Grape and Wine Symposium
- Day, M.P. 2018. Oxygen is not always bad in winemaking. WEA South Australian Conference 26/7/2018
- Day, M.P. 2018. Modern winemaking, or how I learned to stop worrying and love oxygen. ASVO Seminar, 27/8/2018
- Schmidt, S.A. 2020. Modulating red wine composition through aeration during fermentation. ASVO Seminar, 20/10/2020

9.2 Articles written or published during the conduct of the project

Day, M.P., Schmidt, S.A., Pearson, W., Kolouchova, R. and Smith, P.A. 2019 Effect of passive oxygen exposure during pressing and handling on the chemical and sensory attributes of Chardonnay wine. *Aust. J. Grape Wine Res.* 25: 185–200.

Bekker, M.Z., Day, M.P., Smith, P.A. 2019. Changes in metal ion concentrations in a Chardonnay wine related to oxygen exposure during vinification. *Molecules* 24: 1523.

Day, M.P., Espinase Nandorfy, D., Bekker, M.Z., Bindon, K.A., Solomon, M., Smith, P.A., Schmidt, S.A. 2021. Aeration of *Vitis vinifera* Shiraz fermentation and its effect on wine chemical composition and sensory attributes. *Aust. J. Grape Wine Res.* 27: 360–377.

Varela, C., Cuijvers, K., Heuvel, S.V.D., Rullo, M., Solomon, M., Borneman, A., Schmidt, S., 2021. Effect of aeration on yeast community structure and volatile composition in uninoculated Chardonnay wines. *Fermentation.* 7 (2): 97.

Schmidt, S.A., Holdstock, M. 2021. Ask the AWRI: Aerating red ferments. *Aust. N.Z. Grapegrower Winemaker* 695: 54.

9.3 Conference posters

Day, M.P., Barker, A.M., Bekker, M.Z., Bindon, K.A., Smith, P.A., Schmidt, S.A. 2019. Positive sensory and chemical outcomes from oxygen addition during red wine fermentation. 17th Australian Wine Industry Technical Conference 21-24 July.

Day, M.P. and Thoman, B. 2019. Study of the effectiveness of several industry-scale oxygen introduction techniques into red fermenters. 17th Australian Wine Industry Technical Conference 21-24 July.

10. Appendix 2: Intellectual property

Program IP has been communicated through presentations and publications and resides in the public domain. Copyright in any reports, posters or presentations is held by the AWRI. Copyright in publications remains with the publisher. No inventions or patentable discoveries have been identified from the work. No Program IP has been commercialised or identified for commercialisation at the time of writing this report.

11. Appendix 3: References

Bekker, M.Z., Day, M.P., Holt, H., Wilkes, E., Smith, P.A. 2015. Effect of oxygen exposure during fermentation on volatile sulfur compounds in Shiraz wine and a comparison of strategies for remediation of reductive character. *Aust. J. Grape Wine Res.* 22: 24–35.

Bekker, M.Z., Kreitman, G.Y., Jeffery, D.W., Danilewicz, J.C. 2018. Liberation of Hydrogen Sulfide from Dicysteinyl Polysulfanes in Model Wine. *J. Agric. Food Chem.* 66: 13483–13491.

de-la-Fuente-Blanco, A., Sáenz-Navajas, M.-P., Valentin, D., Ferreira, V. 2019. Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration. *Food Chem.* 307: 125553.

Day, M.P., Nandorfy, D.E., Bekker, M.Z., Bindon, K.A., Solomon, M., Smith, P.A., Schmidt, S.A. 2021. Aeration of Vitis vinifera Shiraz fermentation and its effect on wine chemical composition and sensory attributes. *Aust. J. Grape Wine Res.* 27: 360–377.

Day, M.P., Schmidt, S.A., Smith, P.A., Wilkes, E.N. 2015. Use and impact of oxygen during winemaking. *Aust. J. Grape Wine Res.* 21: 693–704.

Gawel, R., Schulkin, A., Smith, P.A., Waters, E.J. 2014. Taste and textural characters of mixtures of caftaric acid and Grape Reaction Product in model wine. *Aust. J. Grape Wine Res.* 20: 25–30.

Holt, H., Cozzolino, D., McCarthy, J., Abrahamse, C., Holt, S., Solomon, M., Smith, P., Chambers, P.J., Curtin, C. 2013. Influence of yeast strain on Shiraz wine quality indicators. *Int. J. Food Microbiol.* 165: 302–311.

Killeen, D.J., Boulton, R., Knoesen, A. 2018. Advanced Monitoring and Control of Redox Potential in Wine Fermentation. *Am. J. Enol. Viticult.* 69: 394–399.

McRae, J.M., Day, M.P., Bindon, K.A., Kassara, S., Schmidt, S.A., Schulkin, A., Kolouchova, R., Smith, P.A. 2015. Effect of early oxygen exposure on red wine colour and tannins. *Tetrahedron* 71: 3131–3137.

Schmidt, S.A., Dillon, S., Kolouchova, R., Henschke, P.A., Chambers, P.J. 2011. Impacts of variations in elemental nutrient concentration of Chardonnay musts on Saccharomyces cerevisiae fermentation kinetics and wine composition. *Appl. Microbiol. Biotechnol.* 91: 365–375.

Siebert, T.E., Solomon, M.R., Pollnitz, A.P., Jeffery, D.W. 2010. Selective Determination of Volatile Sulfur Compounds in Wine by Gas Chromatography with Sulfur Chemiluminescence Detection. *J. Agric. Food Chem.* 58: 9454–9462.

Varela, C., Cuijvers, K., Heuvel, S.V.D., Rullo, M., Solomon, M., Borneman, A., Schmidt, S. 2021. Effect of Aeration on Yeast Community Structure and Volatile Composition in Uninoculated Chardonnay Wines. *Ferment*. 7: 97.

12. Appendix 4: Staff

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13. Appendix 5: Other supporting material (including raw data, if appropriate)

Not applicable