What happens when you aerate a wild Chardonnay ferment?

Introduction

Aeration during fermentation is a winemaking practice whose effects have become increasingly well understood. In white wines, air addition during fermentation positively affects yeast growth, viability and fermentation efficiency (Larue et al. 1980, Mauricio et al. 1997, Blateyron et al. 2001, Fornairon-Bonnefond et al. 2003). The timing of aeration is important for fermentation performance management, with air addition being most beneficial when applied towards the end of the yeast exponential growth phase (Sablayrolles et al. 1996, Blateyron et al. 1998). In addition, aeration can affect the formation of volatile yeast metabolites (Varela et al. 2012), can modify tannin composition in red wines (McRae et al. 2015) and can reduce or eliminate the concentration of volatile sulfur compounds (Bekker et al. 2021).

All of these observations have been made in ferments inoculated with *S. cerevisiae*. In such fermentations, the target of aeration is clear, as is the definition of when the fermentation starts. This makes identifying optimal times to undertake aeration during inoculated ferments more straightforward. However, in the more complex microbial ecology of non-inoculated fermentations, the answers to some of those questions can be more open-ended. For example: When is the start of a wild ferment? Which organism will benefit most from the addition of air? What will be the outcome if aeration helps some microorganisms selectively?

This article reports experimental work, first published by Varela et al. (2021), exploring the effect of aeration on yeast populations during uninoculated Chardonnay fermentation over two successive vintages. In the first vintage (2019), ferments were aerated at different intervals after grapes were crushed. In the second vintage (2020), ferments were aerated concurrently, 24 hours after grape crush, but with a range of airflow rates, changing the total amount of oxygen to which each ferment was exposed. The way that different aeration regimes altered individual yeast species populations in these 2 L ferments was followed using a DNA-based approach called metagenomics. The effect of population shifts on the chemical composition of finished wines was also assessed.

What is the impact of aeration timing?

The optimal time to aerate a wild ferment may depend on what is trying to be achieved through aeration. 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 air at a fixed flow rate for 24 h with aeration treatments starting 24, 48 or 72 h after grapes were pressed.

The timing of aeration 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 air was applied later. Non-aerated ferments required 25 days to achieve a sugar concentration < 2 g/L, whereas ferments aerated at 72 h finished after only 13 days and those aerated at 48 h and 24 h finished after 15 and 18 days, respectively.

Ferment samples taken at similar sugar concentrations across the different ferments were plated to quantify total yeast and non-*Saccharomyces* yeast populations at four stages during fermentation. 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, while 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 1). In general, similar yeast counts were obtained regardless of aeration timing, with a trend to higher concentrations of non-*Saccharomyces* yeast related to earlier aerations.

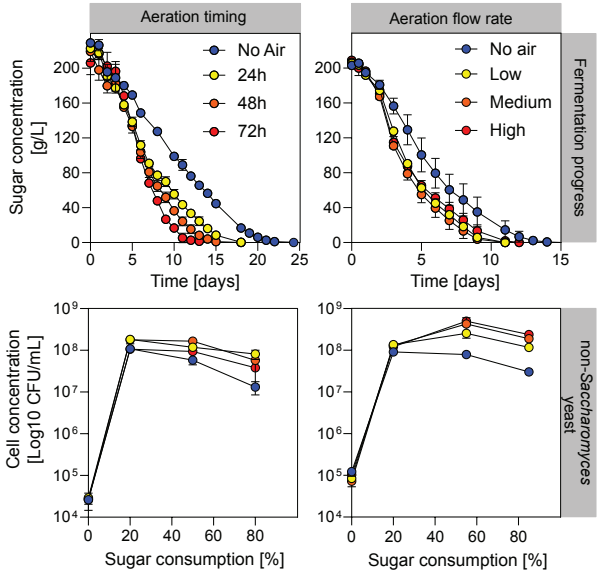

Figure 1. The effect of aeration timing (vintage 2019, left) and aeration flow rate (vintage 2020, right) on fermentation progress (top) and non-*Saccharomyces* viable counts (bottom). All symbols show the means of three treatments, with error bars indicating standard deviation. Timing of aeration was altered in vintage 2019 by providing a fixed flow rate of air to ferments for 24 h, starting 24 h, 48 h, or 72 h after crushing of grapes. In 2020 the airflow rate was altered by providing adding air at 7, 17 or 25 L/min/kL for 48 h starting 24 h after crushing of grapes.

How does airflow rate affect fermentation performance?

If some air can help decrease fermentation times, does more air decrease them further? This question was addressed in 2020 when ferments were sparged at three different airflow rates while keeping aeration timing and duration constant.

In this trial, the minimum airflow rate resulted in the shortest fermentation time. Higher airflow rates did not result in further declines in fermentation duration (Figure 1). 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 2×10^8 CFU/mL, 4×10^8 CFU/mL and 5×10^8 CFU/mL at 85% sugar consumption, respectively 5-, 10- and 12-times higher than non-aerated ferments.

How does the structure of yeast populations during fermentation change in response to aeration timing?

Immediately following the inoculation of a standard fermentation, the concentration of *S. cerevisiae* can be 10-fold greater than the concentration of all other yeasts combined. For this reason, aeration of inoculated ferments predominantly benefits the growth of *S. cerevisiae*, especially once growth commences. However, in the early stages of non-inoculated fermentation, the microbial community is dominated by yeast species other than *S. cerevisiae*. The progression of yeast species in these ferments creates an opportunity to differentially stimulate individuals within that population by aerating ferments at different times.

In this work, the concentration of different yeasts at various times throughout fermentation was determined using a method called meta-barcoding. Meta-barcoding uses high-throughput sequencing of a diagnostic fragment from the genome of yeast 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 2 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. By the time 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.

Ferments that were aerated within those first two days showed increased growth of non-*Saccharomyces* species, particularly *Hanseniaspora* and *Torulaspora* (Figure 2). By the time ferments progressed to 80% completion, the concentration of *Hanseniaspora*, *Lachancea* and *Torulaspora* was 10-fold greater in aerated ferments than in 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 h, 48 h and 72 h reached 7×10^6 CFU/mL, 1×10^7 CFU/mL, 2×10^7 CFU/mL and 3×10^7 CFU/mL, respectively. In summary, earlier aeration preferentially benefitted non-*Saccharomyces* yeasts.

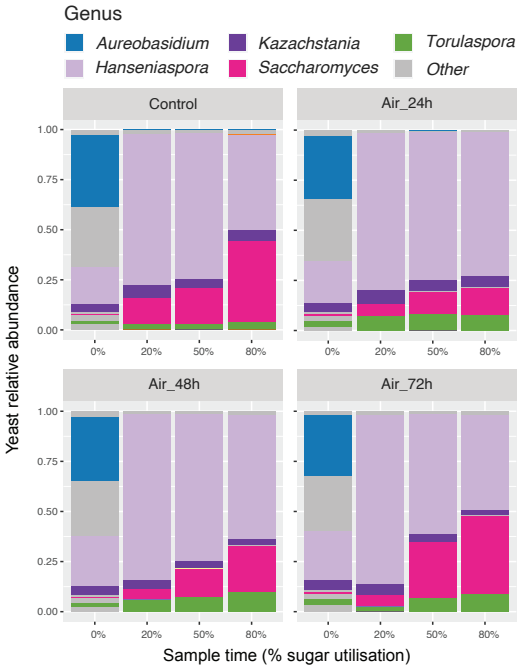

Figure 2. Relative abundance of yeasts in response to aeration at 24 h, 48 h and 72 h post-pressing of grapes for a treatment duration of 24 h in the 2019 vintage. Ferment samples were taken at four time points: 0%, 20%, 50% and 80% of sugar consumption. All subcolumns show mean percentages of three replicates.

Does increasing the airflow rate at the earlier time point increase the benefits to non-*Saccharomyces* yeast?

Increasing the airflow rate in a fermenter with imbedded spargers will increase the dissolved oxygen concentration that can be sustained during aeration. It is possible that some yeasts present during these earlier stages of fermentation are able to utilise the oxygen more effectively when it is present at higher concentrations.

Figure 3 shows the microbial structure of freshly pressed juice in the second vintage trial (2020), which differed from the first in that *Metschnikowia* replaced *Hanseniaspora* in the list of the dominant genera. Nevertheless, by the time 20% of the sugar had been consumed, *Hanseniaspora* again made up more than 50% of the species present. Increasing the airflow rate increased the abundance of *Torulaspora* and *Metschnikowia*, with an increased abundance of *Torulaspora* particularly evident later in fermentation (Figure 3).

The stimulation of non-*Saccharomyces* yeasts by aeration was reflected in the higher estimated cell numbers of the four main non-*Saccharomyces* genera, *Hanseniaspora*, *Lachancea*, *Metschnikowia* and *Torulaspora*, which were higher in aerated ferments, particularly toward the end of fermentation. Unlike the previous vintage, the growth of non-*Saccharomyces* yeasts did appear to come at the expense of delayed *S. cerevisiae* growth.

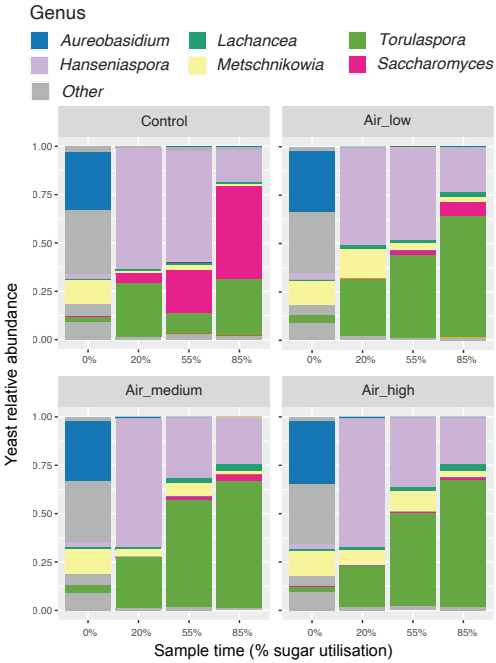

Figure 3. Relative abundance of yeasts in response to aeration at 24 h post pressing of grapes using three different airflow rates for a treatment period of 48 h in the 2020 vintage. Ferment samples were taken at four time points: 0%, 20%, 55% and 85% of sugar consumption. All subcolumns show mean percentages of three replicates.

How is wine composition altered by aeration and the resulting changes in microbial community structure?

Aeration of white wine fermentation has previously been shown to result in changes to wine composition (Bertrand et al. 1984, Varela et al. 2012). Major fermentation products

and volatile compounds, including ethanol, glycerol, lactic acid, ethyl- and acetate esters, medium chain fatty acids and higher alcohols, have been shown to be significantly affected by air supplementation of co-fermentations of *S. cerevisiae* and non-*Saccharomyces* yeast (Shekhawat et al. 2017, Yan et al. 2020). It is possible that modulation of the fermentation microbial community with aeration can change wine composition in ways that are different from the effects of aeration in inoculated ferments.

Figure 4 presents a visual summary of the changes in composition resulting from the aeration of the uninoculated ferments, showing differences between the concentrations of the 38 analytes measured in these wines. The figure shows that early aeration (24 h and 48 h) produces wines with similar compositional profiles, while late aeration produces wines more closely aligned to the non-aerated control (Figure 4A). In general, aeration resulted in a decrease in the concentration of medium-chain fatty acids and their associated esters, an observation consistent with the work undertaken in single inoculum systems.

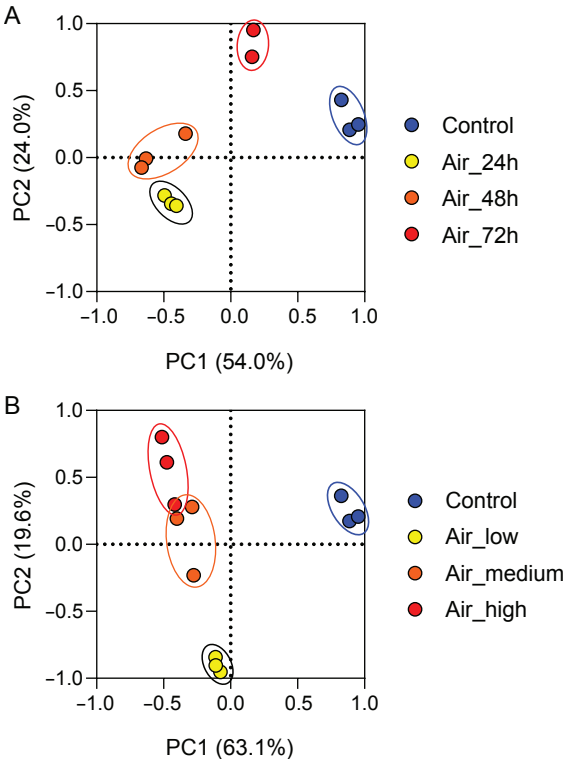

Figure 4. Principal component analysis scored plots based on chemical composition for ferments from the (A) 2019 and (B) 2020 vintages. Individual winemaking replicates are shown, with ellipses drawn to visually indicate the difference between replicates.

The use of higher airflow rates was associated with higher acetic acid, ethyl acetate and propanoic acid concentrations. Low airflow rate aerations were associated with 2-phenyl ethanol and glycerol. Control wines clustered separately from the aeration treatments in the 2020 vintage, a feature that was associated with the medium-chain fatty acids and their ethyl esters (Figure 4B).

Summary

Aeration of non-inoculated ferments can be used to stimulate fermentation activity and reduce the duration of fermentation. Timing of aeration was critical to fermentation duration, with the fastest ferments achieved when aeration was applied later, at a time when *S. cerevisiae* is 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 that 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 than 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. However, higher intensity aeration early during fermentation was detrimental to wine quality with the appearance of elevated volatile acidity. For this reason, lower flow rates are advised when aerating white fermentations. This work demonstrates that air addition is a tool that winemakers may use to improve fermentation performance and shape yeast community structure in uninoculated white wines.

Acknowledgements

The authors would like to thank Renata Kucera for helping with the oxygen measurements. This work was supported by Australia's 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. Metabolomics Australia is funded through the Australian Government National Collaborative Research Infrastructure Strategy (NCRIS).

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