Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Influencing wine style through management of oxygen during winemaking



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWRI 3.3.2**

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Research Organisation:

The Australian Wine Research Institute

Date: **22 September 2017**

Project 3.3.2 – Influencing wine style through management of oxygen during winemaking

Abstract

Effective management of oxygen during winemaking can help create diverse wine styles. Oxygen exposure can be readily modulated throughout the winemaking process and a range of approaches are available to manage it. However, many of these are not based on scientific knowledge of their effects on fermentation and wine style, or are not underpinned by a clear and holistic understanding of the benefits and financial impacts across the entire wine production chain.

The aim of this project was to establish the impact of early use of oxygen at crushing or during fermentation on wine style, and on the efficiency of malolactic fermentation, using both model systems and pilot-scale fermentations. In addressing these questions this research also improved understanding of how oxygen management during processing and fermentation impacts on fermentation efficiency and fast track ageing of wine. Adoption of the outcomes from this research represent a significant opportunity for the Australian wine sector to manage oxygen exposure effectively, enhance stylistic diversity, improve fermentation efficiency and reduce costs derived from excessively reductive handling of wines.

Five pilot-scale vintage trials and numerous controlled laboratory experiments were carried out during this investment period. In parallel, several industry partners trialled the use of air additions at small, medium and large-scale wineries across the country.

The benefits of adding sizeable amounts of oxygen to red ferments include a reduction in the need for adding nitrogen supplements (a significant cost saving in itself) and prevention of low levels of sulfidic off-odours, thus bringing bright fruit characters to the forefront of the wine bouquet. In addition, softening of tannins during fermentation may reduce maturation time before bottling and make the wine available for market several months earlier.

In white winemaking, the research showed that oxygen additions can increase fermentation efficiency without having negative effects on sensory outcomes. This kinetic rather than stylistic effect could have a major impact on the efficiency of fermentation by allowing a wine to finish fermentation several days earlier than normal while maintaining style through unaltered fermentation temperatures. This is a particularly valuable outcome considering the growing need to manage fermentations in compressed vintages.

Executive summary

Effective management of oxygen during winemaking can help create diverse styles that are attractive to a range of different consumers. Many approaches to oxygen management are currently practised and oxygen management has predominantly been focused on: post-fermentation treatments; management during bottling; and the effects of closure selection on post-bottling development. However, the effects of oxygen management during the process of winemaking (from crushing through fermentation) are not well understood. The limited information at hand is mostly

about the management of fermentation efficiency and reliability. Oxygen exposure is a valuable and readily available management option throughout the winemaking process and many practical approaches are available to manage it. However, many of these are not based on scientific knowledge of their effects on wine style, or are not underpinned by a clear and holistic understanding of the benefits and financial impacts across the entire wine production chain. For example, managing oxygen exposure at crushing and juice stage may be very capital-intensive and expensive (e.g. inert crushers), whereas management during fermentation can be achieved with modest capital modifications.

The aim of this project was to establish the impact of early use of oxygen at crushing or during fermentation on wine style, and on the efficiency of malolactic fermentation using both model systems and pilot-scale fermentations. These are critical to delivering the best quality product possible. Questions at the outset of this project included: how much oxygen (O_2) gets into juice through production? What does juice exposure to oxygen do to final wine style/composition? What does oxygen exposure during fermentation do to wine style? Can oxygen measurement be improved during winemaking or can markers for exposure be found?

This project focused on influencing fermentation efficiency and/or wine style through management of oxygen during winemaking. Five pilot-scale vintage trials and numerous controlled laboratory experiments were carried out during this investment period. In parallel, several industry partners have trialled the use of air additions at small, medium and large-scale wineries across the country.

The benefits of adding appreciable amounts of oxygen to red ferments have been demonstrated to remove the need for adding nitrogen supplements (a significant cost saving in itself) and prevent low levels of sulfidic off-odours, bringing 'bright fruit' characters to the forefront of the wine bouquet. In addition, softening of tannins may reduce maturation time before bottling and make the wine available for market several months earlier.

In wines made in 2012, greater oxygen exposure during fermentation produced wines with more 'aged' characteristics with respect to greater hue, fewer anthocyanins, lower tannin concentrations and smaller tannins with more modified structure. These changes were similar to those induced by 12 months of bottle-ageing in wines deprived of oxygen during fermentation. Treatments with 40% O2 and air scored lowest for 'bitter' and for 'astringency', while the protective N2 treatment scored highest for 'astringency'. This suggests that increased oxygen exposure during winemaking may reduce the need for extended wine ageing, saving winemakers costs associated with tannin fining and extended storage, and possibly increasing consumer preferences. Recent research on white wine phenolics adds to the body of evidence that oxygen is likely to impact wine texture: these results established that two of the major phenolics in wine (grape reaction product [GRP] and caftaric acid) that are influenced by oxygen exposure also modulate the perception of astringency and oiliness.

Additional beneficial effects of oxygen additions to ferments included decreased metal concentrations in wine post-ferment which may benefit the wine's shelf life and evolution, and significantly faster rates of malolactic fermentation which might provide a practical tool to assist in the reliable completion of malolactic fermentation.

In white winemaking, the research showed that oxygen additions can increase fermentation efficiency without having negative effects on sensory outcomes. Modulating the extent of oxygen exposure at the very earliest stages of juice preparation has been an important tool in understanding the effect of oxygen in white winemaking. Although the project did not set out to assess the merits of inert pressing, experiments highlighted some subtle effects that can be achieved from pressing under low oxygen conditions, if not totally inert environments. This is an area that should receive some further investigation, particularly looking at must from a range of grape varieties.

In a vintage 2014 experiment different oxygen levels that could be achieved simply through pressing and handling operations were investigated in Chardonnay, without further oxygen additions being conducted. The choice of pressing mode and the extent to which juice or wine was protected from oxygen during handling were both shown to affect a wine's final chemical composition and sensory characteristics, in this particular case potentially affecting 'floral' and 'citrus' characters. For juices prepared through normal (i.e. aerobic) pressing, no significant differences were introduced through the choice of handling method. This seems to suggest that, at least for Chardonnay, there is little need to invest too much time and money protecting juice and fermenting wine from oxygen, if it has been produced through aerobic pressing. However, other white varieties may behave differently so caution should be used before dispensing with inert gas blanketing altogether! On the other hand, if a juice is produced by inert pressing it was shown that sufficient phenolics remain to be affected by further oxygen exposure during normal handling. Inertly pressed juices therefore need continued protection through reductive handling, if oxidation is to be avoided.

Having observed and quantified the chemical and sensory differences that occur through passive oxygen exposure in this study, trials during the 2015 vintage focused on making deliberate but controlled oxygen additions during fermentation which have potential for greater impact on wine style. These experiments demonstrated that addition of oxygen during white wine fermentation has positive benefits, with the main impact on the kinetics of fermentation rather than style of wine. This could lead to significant improvements in the efficiency of fermentation by allowing a wine to finish fermentation several days earlier than normal, while maintaining style through unaltered fermentation temperatures. This is a particularly valuable outcome considering the growing need to generate fermentation efficiencies during compressed vintages.

The sensory effects of adding oxygen in the 2015 experiments were minimal. The preferred timing of oxygen addition appeared to be in the first half of fermentation when sugars had dropped by 20% of the starting concentration. It was still beneficial, however, to make a late addition, even once the sugar concentration had dropped by 80 %. Although this did not give a considerable boost to the fermentation, it ensured that the ferment achieved dryness safely. The sensory analysis confirmed that there were no negatives issues associated with using a reasonable amount of oxygen.

The positive impacts of adding oxygen during red wine fermentation was initially explored in 2012 and are detailed above. During the 2016 vintage trials, the type of fermenter used and the way the aeration was carried out was modified to demonstrate how this could be achieved in wineries not equipped with rotary fermenters and with minimal capital outlay. The timings used in the 2015 trial were replicated with additional treatments of a daily dose and a post-press addition. In order to achieve the positive benefits of enhancing the 'bright red fruit' attributes through suppression of low-level reductive aromas, it was shown to be important to use an early aeration during the first few days of active fermentation. In addition, to achieve a decrease in astringency by softening the tannin, a repeated exposure may be necessary.

In summary, by adopting the outcomes from this research significant opportunities can be realised to manage oxygen exposure effectively, enhance stylistic diversity, improve fermentation efficiency and reduce the costs derived from excessively reductive handling of wines.

Background

Effective management of oxygen during winemaking can help create diverse styles that are attractive to a range of different consumers. Many approaches to oxygen management are currently practised but knowledge of oxygen management has predominantly been focused on post-fermentation treatments; management during bottling; and the effects of closure selection on post-bottling development. The effects of oxygen management during the process of winemaking (from crushing through fermentation) are not well understood. The limited information that exists is mostly on the management of fermentation efficiency and reliability.

However, the role of oxygen during winemaking is likely to have a profound effect on the final wine, and thus a significant opportunity exists for winemakers to use oxygen management before or during fermentation to impact on critical aspects of winemaking such as aroma, texture and post-bottling stability; in particular, to remediate or prevent the formation of reductive aromas during fermentation and possibly minimise the risk of reductive aroma formation post-bottling. The aim of this project was to establish the impact of early use of oxygen at crushing or during fermentation on wine style, and on the efficiency of malolactic fermentation using both model systems and pilot-scale fermentations. Furthermore, strategies were assessed for prevention of oxygen-related quality loss after fermentation. These areas are critical to delivering the best quality product possible.

Questions at the outset of this project included: how much oxygen (O_2) gets into juice through production? What does juice exposure to oxygen do to final wine style/composition? What does oxygen exposure during fermentation do to wine style? Can oxygen be better measured during the process or markers for exposure be identified? This project focused on influencing wine style through management of oxygen during winemaking.

The practice of winemaking in Australia has a tendency to be reductive in nature. Grapes are protected during crushing and pressing, and juices during transfer, with the liberal use of dry ice and/or early and frequent use of SO_2. Combined with the increasing availability of inert presses and inert crushers, the move to screw-caps and total package oxygen management post-production may increase the risk of 'in bottle' reductive characters, depending on the wine composition. While serving to protect against the negative effects of oxidation, blanket application of these approaches may also unnecessarily limit the tools available to winemakers to manipulate wine style.

Consultation with industry indicates sporadic and dispersed use of oxygen during primary fermentation, especially with red musts. Such treatment is carried out in a couple of the bigger wineries who use fixed air sparging systems in rotary or static fermenters, and in smaller boutique wineries where *ad hoc* solutions are employed. In the case of the rotary fermenters, air is used to minimise sulfidic (or 'reductive') aroma formation but in smaller wineries oxygen (sometimes 100%) may be used for colour stabilisation. There are limitations in the understanding of how these practices affect wine, or their efficiency in O_2 transfer, and very little scientific research has been reported on the effects on wine composition or sensory properties.

Oxygen exposure occurs to varying degrees during production of grape juice. Practices such as mechanical harvesting, crushing and pressing all contribute to oxygen contact with grape components, but it is unclear how much exposure occurs and what is the effect on the wine. However, studies of inert juice pressing (using nitrogen gas blanketing during the operation of a tank/membrane press) and hyperoxidation (an extreme example of juice oxidation which has the main goal of phenolic stabilisation by exposure to very large amounts of oxygen after pressing) representing both extremes have been carried out (Boselli et al. 2010, Cejudo-Bastante et al. 2011).These studies represent extremes and the work undertaken at AWRI aimed to study careful dose-controlled additions of oxygen and monitor effects on fermentation efficiency and wine composition.

In terms of fermentation efficiency, the role of oxygen in the stimulation of fermentation rates and assisting in the completion of difficult fermentations has been well described previously. Based on this work, the established time for the addition of oxygen to fermentation is at the end of exponential growth, 36 to 48 hours into fermentation. While fermentation efficiency has been a key driver of much work on oxygen use during fermentation, the sensory effects on the finished wine of oxygen exposure during fermentation have not been thoroughly investigated.

In terms of wine composition, the limited literature available on compositional effects from oxygen exposure shows that single dose oxygen exposure (added with the intention to manage efficiency) during fermentation can alter the ester profile, increase the production of higher alcohols and alter the composition of the volatile fatty acid pool compared to strictly anaerobic fermentations. Limited compositional effects have been reported, oxygen dose or duration has not been explored, and no sensory evaluations have accompanied existing literature.

Highlights

Measuring oxygen in the winery

Five different sensors were evaluated for their ability to measure oxygen in must and wine. Results showed that chemo-luminescence probes were suited to in-line dissolved oxygen (DO) measurements during must transfer or pump-over. ‘Mini-DOT’ sensors were suitable for monitoring DO inside a press or tank.

Practical tools to aerate ferments

Aeration of must is widely used to enhance fermentation performance, especially in red wine fermentations. A Venturi injector was trialled in a medium-sized winery and was shown to be very effective at high pump-over flow rates, giving up to 40% air saturation directly after the device. On a smaller scale, air-draw tubes gave constant and low dissolved oxygen (DO) pick-up, achieving 2-9% air saturation. Both approaches present viable alternatives to the classic method of aeration through ‘cracking the fitting’ which may cause pump cavitation and potentially burn-out the pump rotor.

Impacts of oxygen in white wine production can be positive

In continuing work on the effects of oxygen during winemaking, oxygen concentration in Chardonnay must at the time of inoculation was found to have no impact on fermentation duration

or the concentration of yeast-derived aroma compounds in wine. However, aeration of fermentation later than typical practice still had a stimulatory effect on fermentation performance without negative consequences for wine sensory attributes. This suggests that, if required (e.g. for stimulation of a sluggish/stuck ferment), the use of oxygen outside the previously defined narrow window (24 – 72 hours post-inoculation) can be considered beneficial for ferment performance with limited risk to sensory outcomes.

Impacts of oxygen in red wine production can provide beneficial tannin softening and curtail reductive aromas

By adding oxygen during red fermentations on skins it is possible to modify the tannin composition of wine in ways that equate to several years of post-bottle ageing. Aeration during normal pump-overs is easily achieved if sub-cap recirculation is carried out as well. Alternatively, aeration devices using a sinter fed by compressed air sources may be used. Other equipment is also available for aerating red ferments without recourse to compressed gas, although care must be taken in the choice of pump.

Project objectives

Key objectives are to:

- determine how much oxygen gets into juice through production and what juice exposure to oxygen does to final wine style/composition;
- define key grape and yeast derived volatile and non-volatile compounds, which are affected by oxygen management during winemaking;
- determine the impact on wine style and sensory properties of key oxygen modulated compounds;
- develop improved measurement tools for monitoring oxygen exposure during wine production;
- provide practical advice about ways to introduce oxygen and the impacts of timing and dose of addition; and
- develop methods to improve the efficiency of malolactic fermentation through the use of oxygen during alcoholic fermentation.

Methods

Laboratory-scale fermentation suite

A laboratory-scale fermentation suite was assembled to investigate oxygen uptake rate, with the ability to supply input gas with variable O_2 content and measure outgas flow rates. The set-up used 15 custom-made glass fermenters fitted with dissolved oxygen (DO) probes, gas spargers, outgas flow rate monitoring and a sampling port. A standard fermentation volume of 250 mL agitated at 250 rpm and incubated at 17°C was used throughout.

The investigation into the effect of dissolved oxygen concentration at time of inoculation used Chardonnay juice which prior to inoculation was sparged with air to achieve three different levels of DO: 100 mg/L, 500 mg/L and 2,500 mg/L.

The effect of timing and dose of oxygen during fermentation was investigated using similar juice with gas of differing O_2 content sparged into the active ferment. Prior to inoculation all ferments were adjusted to a dissolved oxygen concentration of 2,500 mg/L. Two basic regimes of aeration during fermentation were applied. In the first, ferments were treated with nitrogen containing different concentrations of oxygen (0.1%, 1%, 10.5%, 21% v/v) or different flow rates of gas 48 hours post inoculation. The second regime fixed the concentration at 0.1% oxygen in nitrogen, delivered at a flow rate of 5 mL/min but varied the duration from 2 – 72 hours. When the effect of oxygen treatment timing was evaluated, a fixed dose of 10.5% oxygen in nitrogen was applied for 2 hours at 5 mL/min.

Pilot-scale investigation of passive oxygen exposure during juice preparation and primary fermentation in white winemaking (vintage 2014)

This trial was carried out using hand-picked Chardonnay grapes which were whole-bunch pressed using a Bucher-Vaslin Inertys press before transportation to the Hickinbotham-Roseworthy Wine Science laboratory at the University of Adelaide. Juice was cold settled then racked into 500 L temperature-controlled tanks before inoculation. Ferments for each treatment in the trial were conducted in triplicate.

Pressing: The first two juice lots were made in 'inert mode' in which the membrane press was configured to draw in nitrogen gas rather than air as the membrane deflated; the press was also sparged with CO_2 gas, prior to and during loading. The other two juice lots were made in 'aerobic (or normal) mode' allowing ambient air to be drawn in as the press membrane deflated. Dissolved oxygen (DO) measurements were made using a PME 'miniDOT' datalogger temporarily fixed near to the press's juice channels. This type of device is typically used by hydrographers gathering data from the ocean, rivers or even waste-water lagoons.

Handling: After pressing, one of each type of juice (inertly pressed or aerobically pressed) was handled reductively and the other two juices were handled oxidatively. Overall, this resulted in four different treatments, from the four possible combinations of pressing and handling: inert-oxidative, inert-reductive, aerobic-oxidative and aerobic-reductive.

Reductive handling was achieved by blanketing the source and receival tanks during transfer or racking operations with either dry ice or an inert gas mixture. This handling regime was continued from the initial filling of the press holding tank until after post-fermentation racking when SO_2 was added. The ullage was minimal but the tank headspace was regularly sparged. Conversely, the oxidative handling regime did not involve any use of inert gas until after the post-ferment racking. After post-ferment racking the four treatments were handled identically, with all subsequent operations carried out in a standard reductive manner under inert gas cover.

Investigation to assess different O_2 concentrations during pressing

A commercial-scale trial was carried out to assess the chemical and sensory characterisation of different O_2 concentrations only during the pressing stage, with subsequent fermentation under laboratory conditions. Handpicked Pinot Gris grapes were whole-bunch pressed using a Bucher XPlus 30 Inertys with five different O_2 exposure regimes: N_2 (99.9%), 5, 10, 15% O_2 and air (20.9 % O_2). The juice was fermented at laboratory scale (5 L) and subsequently bottled for sensory and chemical analysis.

Pilot-scale investigation of validation of aeration during fermentation of white wines (vintage 2015)

Commercially prepared Chardonnay juice was distributed into 12 x 500 L fermenters and inoculated with active dried yeast. Three aeration treatments were carried out in triplicate with an additional 'no-treatment' variant also performed in triplicate. Two timing variants were incorporated into the experiment: 'early' treatment started once 20% total soluble sugars (TSS) had been consumed by the yeast and 'late' when 80% of initial sugars had been consumed. Two oxygenation durations were carried out at the 'early' time point lasting two hours ('early-short', ES) and 20 hours ('early-long', EL). The 'late' treatment lasted 20 hours. These timings and durations were determined from the laboratory experiments described above. Wines were subsequently handled identically through to bottling.

Pilot-scale investigation of the effect of timing and dose of oxygen addition in red wine (vintage 2016)

The effect of timing of oxygen addition in red wine, determined by laboratory experiment, was verified at pilot-scale using donated commercial Shiraz grapes (Langhorne Creek), fermented in pilot-scale (500 L) fermentation vessels using standard pump-over techniques with a fixed irrigator placed just below the opening of the tank. Sub-cap recirculation was carried out during which time air was introduced in the flow of must. One treatment received a one-off addition during pump-over to saturate the fermenting must with air when sugars had dropped by 20% ('Early') while another treatment consisted of repeated aerations to achieve air saturation repeatedly over five consecutive days ('Daily'). Another single treatment occurred later when the initial concertation had dropped by 80% (Late) and a final treatment on previously unaerated wines was carried out after pressing (Post-Press). All wines were then handled in a standard manner until bottling. Sensory analysis was carried one year after vintage.

Pilot-scale investigation of the effects of aeration of red ferments in closed rotary fermenters (vintage 2012)

Donated hand-harvested Shiraz grapes (Barossa Valley) were crushed into rotary fermenters (730 kg each) fitted with three stainless steel sintered sparging heads (2 µm frit size) and fed with three separate gas treatments (10 mL/min at 200 kPa): 40% O_2/N_2 ($O_2$40), air (containing approximately 21% O_2) or pure N_2, applied for 60 min every 12 h starting 24 h after inoculation. The treatments were compared with a 'no treatment' control. After fermentation, the wines were drained, the marc pressed and the wines settled for 24 h, prior to undergoing malolactic fermentation (MLF) in 200 L drums. Tartaric acid (1.5 g/L) wasadded prior to inoculation with the malolactic bacteria (VP41, 10 mg/L, 20 °C). Finished wines were bottled in 375 mL antique green bottles and sealed under screw cap with Saran Tin™ or Saranex™ liners. Samples were analysed for tannin and colour at time 0 (finished wine, samples taken after MLF), at 8 months (2 months post-bottling), and 18 months (12 months post-bottling).

Pilot-scale investigation of the effect on malolactic fermentation of oxygen addition in red wine (vintage 2017)

The effect of oxygen addition during red wine fermentation on simultaneous and sequential malolactic fermentation (MLF) was verified at pilot-scale in a 2 x 2 factorial experimental design. Using commercial Shiraz grapes (McLaren Vale), pilot-scale (500 L) fermentations 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 six tanks and fed with compressed air (5 L/min). Half of the tanks were inoculated with commercial *Oenococcus oeni* bacteria two days after the addition of rehydrated yeast while the remainder were left uninoculated until they were sugar dry. Half of the simultaneously malolactic inoculated ferments and half of the uninoculated ferments received five additions of air nominally every 12 hours when the total soluble solids were between 11 Bé and 4 Bé. Dissolve oxygen was monitored in tank using oxoluminescent probes. All tanks were pressed on day 10 and after settling were transferred to 50 L stainless steel kegs. SO_2 was added to the co-inoculated wines when the residual L-malic acid was consumed; commercial *Oenococcus oeni* bacteria was added to the remaining wine previously uninoculated for MLF. Wines were bottled three months after the end of fermentation.

Results and discussion

Measuring oxygen exposure in the winery during white winemaking (vintage 2014)

The first stage of this project involved investigating the effects of oxygen during standard types of processes undertaken during production. The primary aim of this work was to determine how much oxygen gets into juice through production and specifically to investigate if passive oxygenation – that amount of O_2 which is introduced during winemaking by virtue of the choices a winemaker makes – can have a measurable difference in terms of both wine chemistry and sensory impact. As soon as a grape is crushed the enzyme polyphenol oxidase (PPO) is activated and O_2 will react with phenolic material (Macheix 1991). The extent of that phenolic oxidation may influence the aroma precursors and aroma compounds (Patel et al. 2010) as well as how the yeast metabolise their nutrient source (Salmon 2006).

The way in which the must/juice is prepared (mechanical harvesting, crushing, whole-bunch press) determines the window of first exposure of O_2. This is obviously very difficult to control in scientific experiments so a proxy technology was employed. If whole bunch pressing is used then the oxygenation that occurs is only from the air that is pulled into the press as the press membrane deflates for crumbling; the extent of this is obviously influenced by the press program as well: more frequent deflate cycles increase the overall O_2 exposure. It is for this reason that the development of the inert press (Ardilouze 2006) has given more control to the winemaker over O_2 inputs to grape must. It is because of this ability to control the O_2 atmosphere in which a grape is initially crushed that this trial has used whole bunch pressing in an oxygen-controlled environment to regulate O_2 exposure.

Some authors (Boselli et al. 2010, Motta et al. 2014) have characterised the composition of juice made using inert-gas cover during pressing while others (Antonelli et al. 2010, Mattivi et al. 2012, Motta et al. 2014) have analysed wine made using reductive handling techniques. However, the relative effect of using reductive handling compared to early protection during pressing has not been assessed within the same experiment. The first winery-scale experiments in the 2014 vintage involved using two pressing techniques on white fruit (inert and aerobic pressing) followed by two common handling methods for transfer to tank (reductive and oxidative handling), resulting in four very different final wines which reflect the extremes of oxygen use under 'usual' production conditions.

A 2 x 2 factorial design allowed the relative merits of each technique to be assessed. To assess the oxygen inputs during juice preparation several DO measuring devices were employed at different stages of the winemaking process. The PME 'miniDOT' datalogger was ideal to measure the oxygen environment inside a modern membrane press. The dissolved oxygen (DO) profiles inside the press during the two modes of operation are shown in Figure 1. In the normal/aerobic mode each time the membrane is deflated prior to crumbling a spike in the DO is observed. As this occurs multiple times during a press cycle juice from partially pressed grapes can be exposed to a considerable amount of oxygen. Conversely once the press chamber is flushed of air, the environment remains anoxic until the end of the cycle and the press doors are opened.

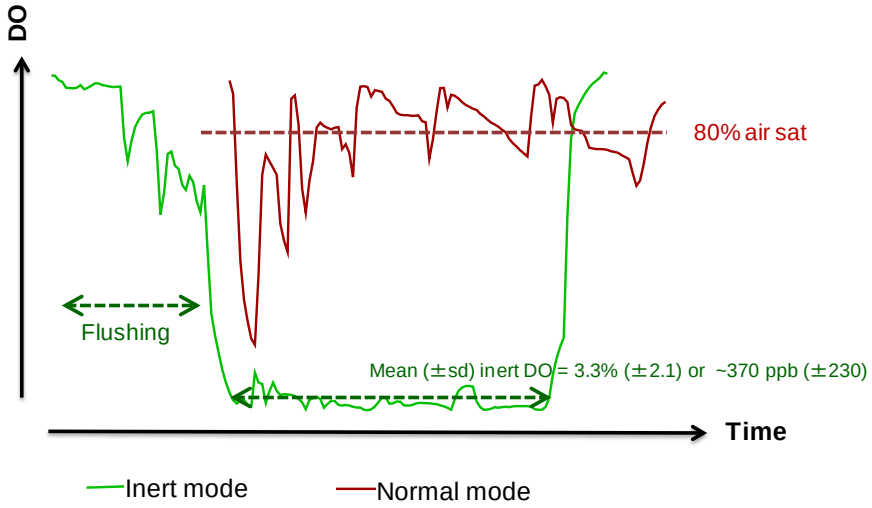


Figure 1. Dissolved oxygen during an inert and an aerobic (or normal) press run

To assess the amount of exposure experienced by a juice or wine, the DO was measured before and after cellar operations. Each time juice or wine was moved from one tank to another, usually for racking to remove supernatant liquid from juice or yeast solids, a probe integrated into the transfer line after the pump allowed the DO to be measured or a handheld DO meter was used to measure headspace O_2 (HSO) concentration. Juice and wine racking was carried out with a variable speed displacement pump. A 'tee-piece' with an appropriate fitting on the side arm allowed an optical process-grade DO to be placed on the delivery side of the pump, positioned tangentially to the flow. During each transfer or racking, the DO was recorded manually. *Ad hoc* measurements of tank headspace were made using a hand-held DO meter.

During the racking operations, juice or wine was moved from the fermentation tank into a temporary buffer tank which had either been inerted with sublimed solid CO_2 during 'reductive' handling or left unprotected for the 'oxidative' handling. The effectiveness of these operations is demonstrated by HSO concentration. During inerted racking operations, mixed gas flowed onto the surface of the juice or wine using a floating gas diffuser to prevent surface gas exchange. As juice or wine moved out of the source tank, the DO was measured in the transfer line and averaged out over the few minutes the operation took; it was measured on its return to the same source tank. The success of this reductive handling is indicated by +9% change in DO for the Inert-Reductive juice and a 16% decrease for Aerobic-Reductive juice; the margins of error in measuring the DO within a commercial pilot-scale winery are likely to account for these negative changes. In contrast, the DO increased 37% and 59% when oxidative handling was carried out. During post-fermentation wine racking (B), however, there is discrepancy in the DO for the reductively handled juices compared to the previous juice-racking operation since the DO increased by 71% and 132%, although HSO conditions in the temporary buffer tank were similar. This may be because wine is more sensitive to O_2 than recently pressed juice.

Assessment of the effect of O_2 on final wine style with identification of key grape and yeast-derived sensory impact compounds

Effect of passive oxygen exposure during juice preparation and primary fermentation in white winemaking on chemical composition and sensory outcomes (vintage 2014)

The first pilot-scale winery trial in 2014 investigated the effect of passive oxygen additions during white winemaking; that is the oxygen that gets into wine during pressing and handling but is not actively bubbled into the juice or ferment. By separating the very early oxygen exposure that occurs at pressing from the later exposure which happens through different ways of handling juice or wine after pressing until the end of fermentation, it was possible to find out at which stage oxygen has the greatest effect. In the trial two pressing modes (inert and aerobic) and two forms of post-pressing handling (reductive or oxidative) were used to create four distinct Chardonnay wines, allowing the effects of oxygen timing to be closely examined. Figure 2 outlines the trial design.

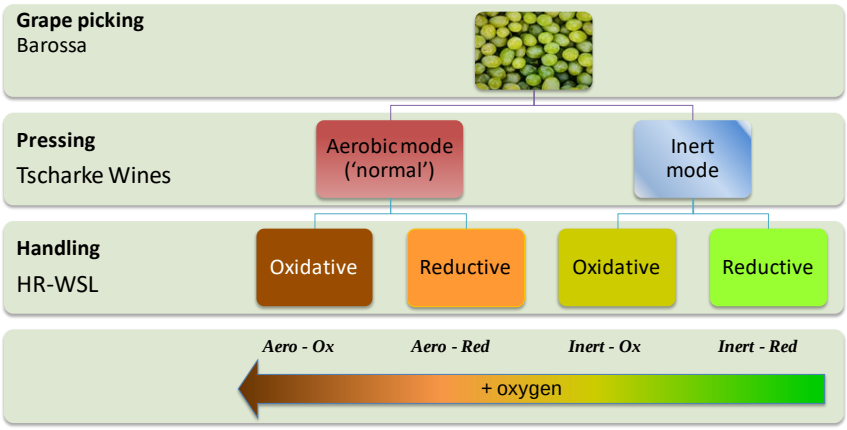


Figure 2. Flow chart of experimental pilot-scale set-up (vintage 2014)

Analysis of aromatic compounds and phenolic composition showed that oxygen exposure during the phase when grapes are first burst open by pressing (as a controlled proxy for mechanical harvesting or crushing) is significantly greater than the effect of oxygen exposure during post-pressing handling. The large amounts of oxygen to which white grapes are exposed during pressing (in this case whole-bunch) resulted in a juice with lower phenolic load, increased higher alcohols, and modified fermentation esters, amino acids and volatile organic acids.

Compositional differences, resulting from either pressing mode (particularly for total phenolics) were far greater than the differences brought about by using reductive handling techniques (with extensive dry ice cover) compared to passive oxidative techniques. This was particularly the case for aerobically pressed juice where the chemical differences between handling techniques were not statistically valid. There were, however, subtle differences between reductive and oxidative handling techniques.

The first indication that there were real differences in this experiment came from Somers' white wine phenolic measures, which showed that the total phenolics and total hydroxycinnamic acids were highest in the inert press treatments and lowest for the aerobic press treatment (Figure 3).

Wines with higher levels of phenolics and hydroxycinnamic acids may have potential for improved texture, as a recent study demonstrated that wines with added phenolics received higher sensory scores for texture (Gawel et al. 2013). Even more interesting is that the type of handling had an influence on the phenolic content of the inertly pressed juices but not the aerobically pressed juices.

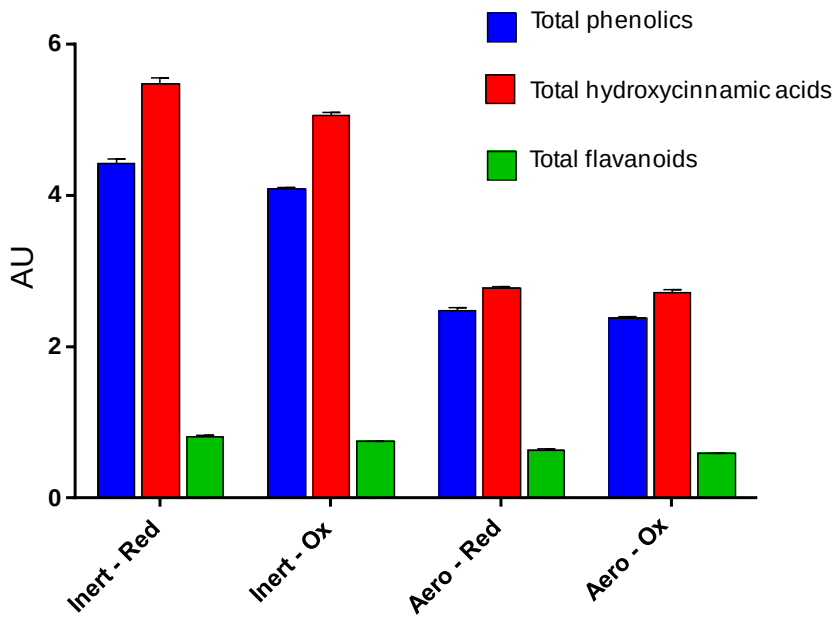


Figure 3 Somers' white wine phenolic indices for the four wines. Treatment codes: Inert-Red and Inert-Ox are inertly pressed and reductively or oxidatively handled. Aero-Red or Aero-Ox are aerobically/normally pressed and reductively or oxidatively handled

Accelerated browning test

Another difference between the wines was found after the assessment of their tendency to undergo oxidative browning. This was done using a simple accelerated browning test (Singleton and Kramling 1976) which compares the absorbance at 420 nm (A_{420}) between wines which are either saturated with air or flushed with nitrogen gas and then stored at 55°C for eight days and assessed using simple colour measures. The results of this test on the four wines are shown in Figure 4, which plots the percent increase in A_{420} caused by the excess of oxygen. It can be seen that wines made from inertly pressed juices have a greater potential to brown than normally pressed juices. This highlights the balance that must be considered in protecting a juice to retain fresh aromas versus the increased potential for browning.

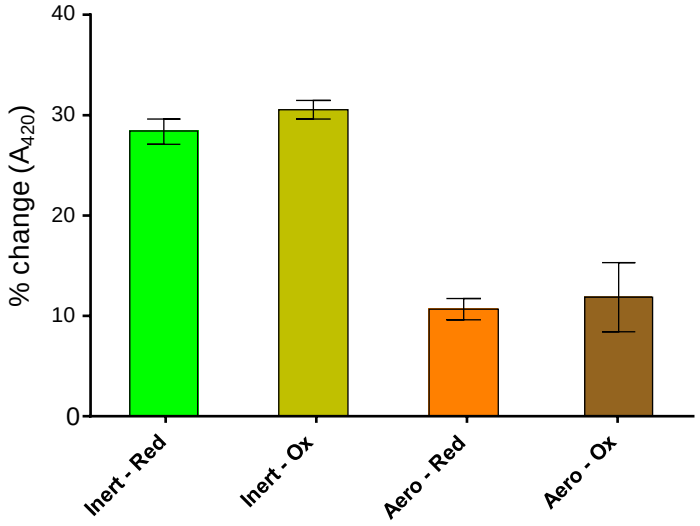


Figure 4. Accelerated browning test (55°C for 8 days) measured with A420, higher values indicate higher potential for browning

Analysis of chemical data for the wines indicated variations in certain aroma compounds (methanethiol, methional, furfural, benzaldehyde, ethyl propanoate and ethyl octanoate) were significantly impacted only through the very early oxygen exposure during pressing, while other compounds (glycine, glutamic acid, tyrosine, 2-methylpropyl acetate, and hexyl acetate) were only affected by oxygen introduced through oxidative handling. A number of other amino acids and volatile esters were influenced by both pressing mode and handling.

Sensory outcomes

The wines were bottled under controlled DO pick-up conditions six months after fermentation and descriptive sensory analysis was conducted by a trained panel of tasters at the AWRI six weeks after bottling. Attributes where the panel found the most significant differences among the wines are shown in Figure 5. The results show that the inert-reductive treatment was significantly higher in 'floral' and 'confection' aroma, and lowest in 'yellow colour' compared to the other three treatments which did not differ significantly from each other in the two aroma attributes. However, for 'acid' taste, the aerobic-oxidative treatment was rated lowest, while for 'yellow colour' the inert-oxidative and the aerobic-oxidative treatments were rated significantly higher than aerobic-reductive, and significantly lower than inert-reductive.

The sensory data were also analysed statistically to separate out the effects of press mode and handling mode. This showed that pressing had a larger effect on the sensory perception of the attribute 'confection' while the attributes 'acid' and 'citrus' were more affected by the handling treatments. While the sensory differences observed at this time point are relatively small, when considered in conjunction with differences found in the chemistry of the wines, they suggest that more significant differences may appear as the wines develop.

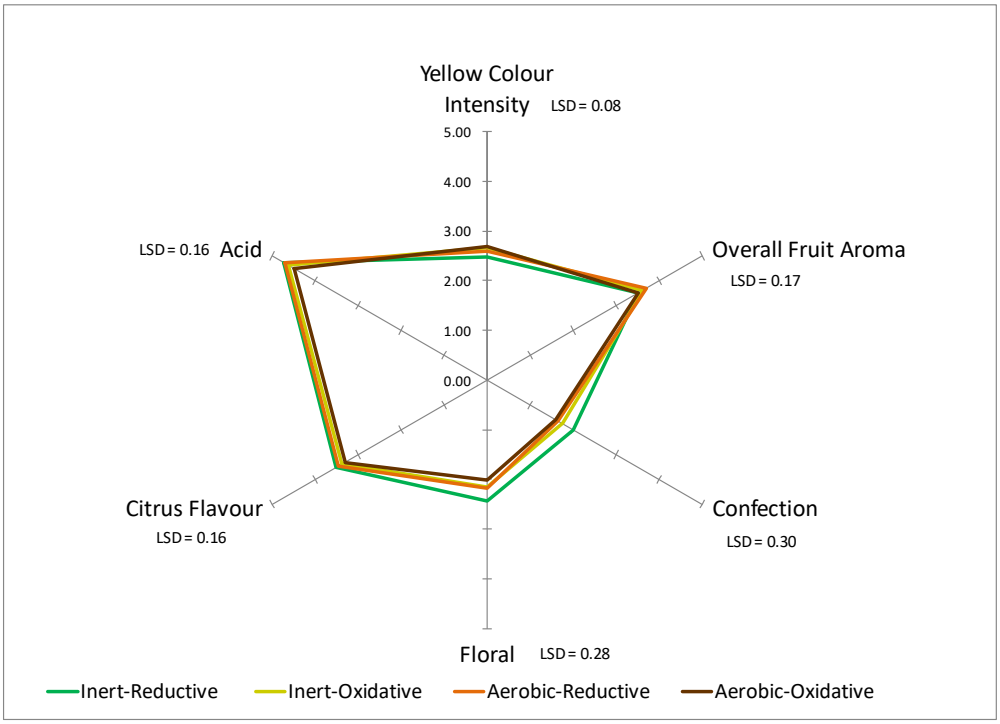


Figure 5. Plots of significant sensory attributes ($P < 0.1$). (Least Significant Difference values (LSD) are the smallest difference between treatments for them to be noticeable with a probability of 95%.)

Linking aroma compounds to sensory attributes

Aroma compounds which contribute positive characters, such as the fruity and floral esters, the varietal thiols and volatile acids, were analysed after fermentation and stabilisation. A series of aldehydes and other aromatic compounds that contribute to the 'oxidised' aroma of wine were also analysed along with amino acids which are potentially their precursors, giving a total of 72 analytical parameters and eight sensory attributes to analyse. A data reduction technique known as partial least squares analysis (PLS) was used to understand both sets of data, and the results are shown in Figure 6. Looking at how the individuals from each treatment group together and their relative positions on the plot, it is possible to describe the horizontal axis, Factor-1, as the 'pressing axis' and the vertical axis as the 'handling axis'. A lot more of the initial variance is represented by horizontal axis, showing that pressing has a bigger impact on both chemistry and sensory characteristics than handling.

Wines from inertly pressed grapes showed higher concentrations in some ethyl esters and medium-chain volatile acids as well as total phenolics. These wines should display some fruiter notes and may develop enhanced texture. The wines from aerobically pressed grapes had higher concentrations in the varietal thiols, different medium-chain volatile acids and ethyl esters. The volatile sulfur compounds hydrogen sulfide (H_2S) and methanethiol (MeSH) were also higher in wine from aerobically pressed grapes giving them a potentially more reductive character. This is directly opposite to the situation seen in red wines (Day et al. 2013) and has been confirmed in laboratory experiments.

Fewer chemical attributes were affected by the handling mode of wines. For wines handled oxidatively there was greater influence from 2- and 3-methylbutanoic acid ('sweaty/cheesy') and

hexyl acetate ('sweet/perfume') while for reductively handled wines there was more benzaldehyde ('marzipan') and benzylmethanethiol ('struck flint'). Sensory results, however, do not reflect a higher 'flint' character in the reductively handled wines, most likely because the levels of benzylmethanethiol are very close to the aroma threshold.

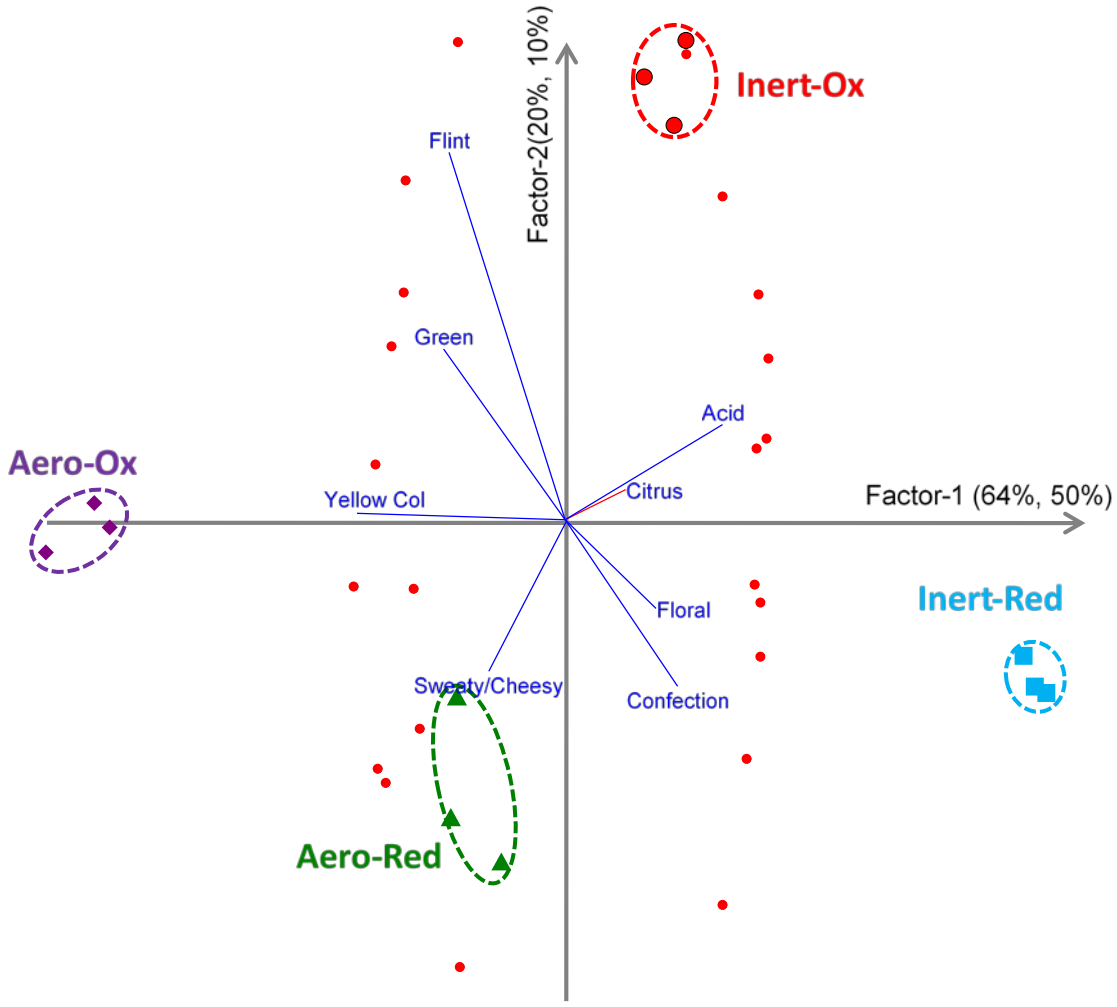


Figure 6. Partial Least Squares analysis (PLS) biplot of significant volatile compounds (red dots) and sensory attributes (blue text)

Effect of different O_2 concentrations on grape crushing/pressing

As demonstrated above, the impact of very early O_2 exposure had the most impact during winemaking. There were clear chemical markers which differentiated wines made from normally pressed juice and wines made in an entirely inert atmosphere. In the case presented above, this occurred during pressing of whole bunches. The extreme represented by totally inert-pressed whole bunches led to a higher browning potential because of the higher residual phenolic matter and tendency to 'pink'. It may be possible to modulate these effects by having a less-inert pressing environment that minimises oxidation of varietal aromas (Makhotkina et al. 2014). This hypothesis was tested by whole-bunch pressing Pinot Gris grapes with different gas compositions. Three gas blends of O_2 in N_2 (5%, 10% and 15%) along with 100% N_2 and air (21% O_2) were introduced into the previously evacuated/deflated gas reservoir of a Bucher-Vaslin XPlus 30 Inertys. A small portion of the juice produced from 1,500 kg grapes was transferred to a 30 L keg and transported back to the AWRI laboratories where the juice was settled, fermented, racked and bottled under strict inert conditions. An operational incident at the winery resulted in the 0% O_2 treatment having juice-

clarifying enzymes added inadvertently and therefore any comparison with other O_2 treatments needs to be treated with caution.

The phenolic content of the wine resulting from pressing with different O_2 concentrations is shown in Figure 7. There is an exponential decrease in the total phenolic content with increasing O_2 concentration ($y = 2.07e^{-0.075x}$; $r^2 = 0.989$). This would indicate that, in the presence of excess PPO, the rate limiting step is based on the O_2 concentration at the time of initial grape rupture and that modulation of phenolic content can be carefully controlled with O_2 environment. Another observation from this experiment is that the concentrations of the haze-forming proteins, chitinase and thaumatin-like protein, decrease with increasing O_2 concentration exposure when the grape PPO-mediated oxidation first occurs Figure 8. The increased quinone activity with higher O_2 concentrations leads to the increased aggregation of polyphenolics which can bind grape proteins (Poncet-Legrand et al. 2007) and eventually precipitate out of solution during fermentation. The effect to the winemaker of pressing in a diminished O_2 environment will be an increase in the need for protein fining agents such as bentonite (Sauvage et al. 2010).

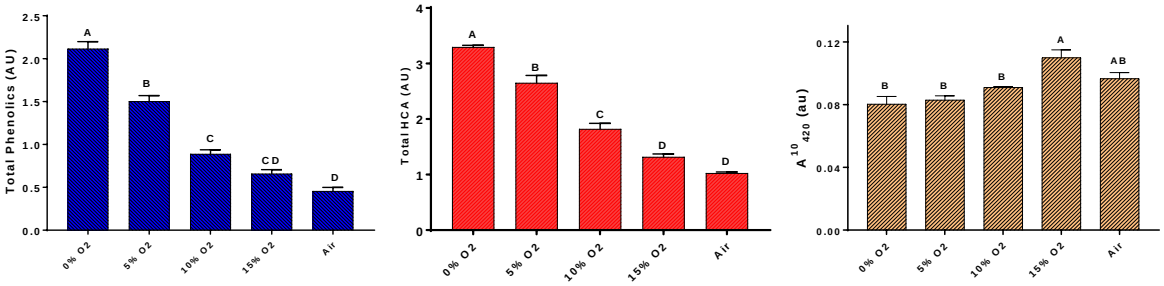


Figure 7. Comparison of phenolic indices with O_2 concentration at pressing

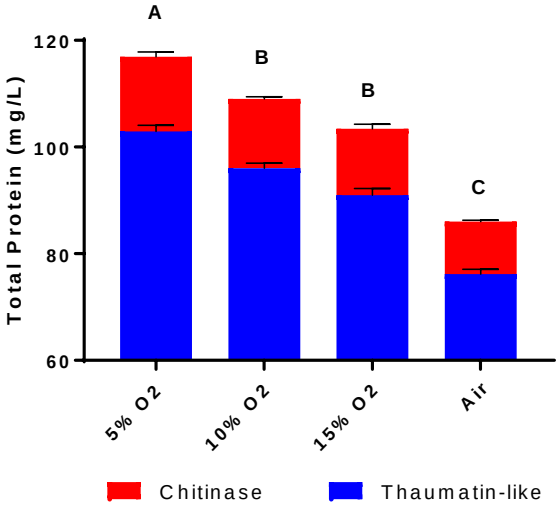


Figure 8. Effect of O_2 concentration at pressing on concentration of haze-forming proteins

The wines were bottled in the laboratory soon after post-fermentation clarification. Fermentation-derived acids, alcohols and esters were analysed, within a few weeks of bottling, to observe any aroma differences arising from early O_2 exposure. Because of the inadvertent addition of pectolytic enzymes to the 0% O_2 treatment with fully inert pressing, it is possible that aroma precursors would have been released from the grape skins and pulp before fermentation, making any comparison of the aroma profile of these wines not possible and therefore these data were excluded from analysis. Data from the remaining treatments were mean-centred and scaled by standard deviation before a PCA was performed. Of the original variance in the data, 62% is represented in the first two principal components (Figure 9). In this plot the replicate fermentations from the 5% O_2 treatment are clearly separate from the other groups of samples, with the air treatment also being slightly separate from the 10% and 15% O_2. The parameters that associate with the 5% O_2 samples are: butanol, acetic, propanoic, butanoic and decanoic acids, ethyl acetate, 2-phenylethyl acetate, 2-methylbutyl acetate. Those that are associated with air are: 2- and 3-methyl butanol, 2- and 3-methyl butanoic acid, 2-methyl propanol and ethyl 2-methyl propanoate. The samples arising from pressing with 10% and 15% O_2 associate with ethyl octanoate and ethyl decanoate.

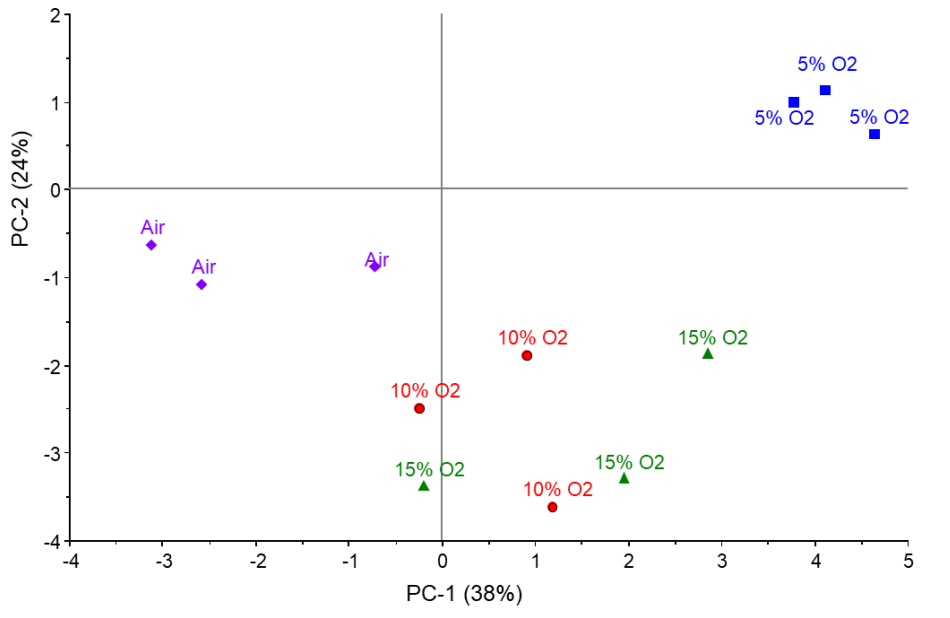


Figure 9. PCA scores plot of fermentation volatile compounds grouped by O_2 concentration at pressing

Sensory analysis was carried out using a full descriptive panel on all the treatments carried out. Of the 3 appearance terms, 13 aroma terms and 16 palate descriptors, only the attributes 'yellow colour intensity', 'pink tinge', 'sweaty aroma', 'apple/pear flavour' and 'hotness' were significant at $P < 0.05$. Calculation of the 95% least significant difference (LSD) indicated that these statistically significant differences were driven by the 0% treatment samples in which a low yellow colour intensity and observable pink tinge was seen; all other aroma or flavour descriptors were maximum for this treatment too. As explained above, these differences should be discarded because of the accidental use of pectolytic enzyme in this treatment.

Laboratory investigation of the effect of DO at inoculation

Must oxygen concentration at the time of inoculation had no impact on fermentation duration or the concentration of yeast-derived aroma compounds in wine. However, aeration of ferments later than might normally be recommended still had a stimulatory effect on fermentation performance without negative consequences for wine sensory attributes. As such, if required (e.g. for stimulation of sluggish/stuck ferments), the use of oxygen outside the previously defined narrow window (24-72 hours post-inoculation) could be considered beneficial for ferment performance with limited risk to the sensory outcome.

Impact of rate, length and timing of oxygen addition during fermentation

Oxygen is a key nutrient in the context of fermentation despite wine fermentation being conducted largely anaerobically. Supplementation of ferments with oxygen has been shown to be beneficial to fermentation progress, especially if added at key growth stages. The effects of oxygen addition during white wine fermentation outside of these narrowly defined time points were examined, looking particularly at efficiency and chemistry impacts. Small (250 mL) and winery-scale (500 L) fermentations were used to evaluate the impacts of oxygen addition on fermentation performance and sensory characteristics of Chardonnay.

Laboratory-scale exploration

The effect of oxygen addition, both its quantity and timing, was explored in a series of laboratory fermentation trials. The primary finding was that fermentation performance and wine chemistry were predominantly influenced by the **total amount of oxygen** consumed by the fermentation, **not the duration** over which it was delivered. Ferments that received a large amount of oxygen in a short period and those that received a small amount over a longer period, with equivalent overall consumption, exhibited similar performance and chemical profiles.

Specifically, it was observed that:

- volatile acids such as acetic, octanoic, and decanoic acids, normally associated with negative sensory attributes in wine, decreased with increasing oxygen dose
- branch chain acids and their associated esters, such as 2-methyl butanol and ethyl-2-methyl butanoate increased proportionally with oxygen treatment
- in particular, the concentrations of branch chain esters were modulated around their aroma thresholds and therefore may change sufficiently to influence sensory qualities
- significant stripping of oxygen by CO_2 occurred, with oxygen uptake rates inversely proportional to CO_2 production rates, at least at low oxygen input concentrations.

The extent of must aeration at the time of inoculation, which can be influenced by tank filling operations, had minimal impact on fermentation performance and production of yeast-derived volatile compounds. This suggests that variations in must oxygen concentration at the time of inoculation are unlikely to influence wine sensory attributes.

In addition to investigations on the effects of total oxygen consumption, the effect of oxygen addition timing was also explored. From a fermentation performance perspective, oxygen additions when ferments had reached 80% to 60% of initial sugar had the biggest impact, which is consistent with the work of others. Fermentation duration was still reduced by treatment at 40% initial sugar, but was substantially longer than observed for the earlier treatments. No difference in fermentation

duration was found between ferments treated at 20% initial sugar or no oxygen treatment. The effects on wine chemistry largely mirrored that of fermentation performance, with exposure of fermentations to oxygen at 80% of initial sugar having lower concentrations of medium chain fatty acids and later additions showing increasing concentrations of these acids.

In summary, laboratory experiments demonstrated that oxygen addition between 80 to 60% of initial sugar for a period of 2 to 48 hours, depending on the concentration and flow rate of gas used, had maximal impact on fermentation outcomes and that these parameters can be modulated to shape the extent of the effect.

Pilot-scale validation (vintage 2015)

The vintage 2015 trial examined the effect of 'actively' adding oxygen during active fermentation. Short (2 hours) and long exposure (20 hours) treatments were applied when sugars had dropped to 80% of their initial concentration, designated as Early-short (ES) and Early-long (EL); a third treatment, long exposure, was applied when sugars had dropped to 20% of initial level, designated as Late-long (LL).

The progress over time of the fermentations was followed by measuring the sum of the concentrations of glucose and fructose and is shown in Figure 10. The 'early' treatments (ES and EL) started on the afternoons of day 4 and the 'late' (LL) on day 11, although samples were taken for sugar concentration determination several hours prior to the aeration treatments. It was not until day 6 that significant differences occurred, with only EL being statistically lower. By day 8, ES and EL were significantly different from each other until day 13 when there was no significant difference between the treatments. Although no statistical differences were calculated in the sugar concentrations between LL and the control, the LL ferments finished by day 15 at which time there was still residual sugar in the control.

The Somers' phenolic indices were measured directly after post-ferment cold setting and are shown in Figure 11 (Tukey *post hoc* pairwise comparisons). The increased exposure to oxygen during the EL treatment significantly decreased the total phenolic content when compared to wine which did not receive any aeration. This treatment also significantly increased the yellow colour. This rise in yellow colour may be due to an increased generation of the xanthylium ion from enhanced quinone production when oxygen and PPO are at their peak activity (Guo et al. 2017).

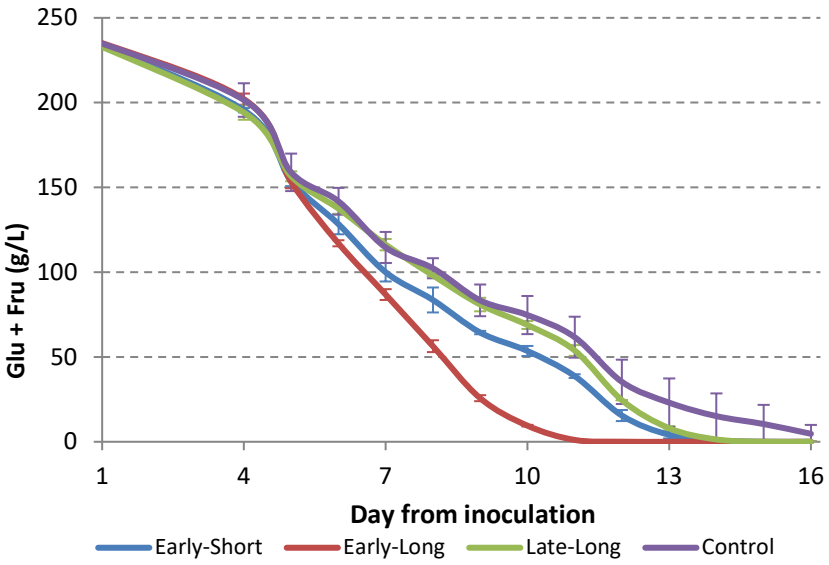
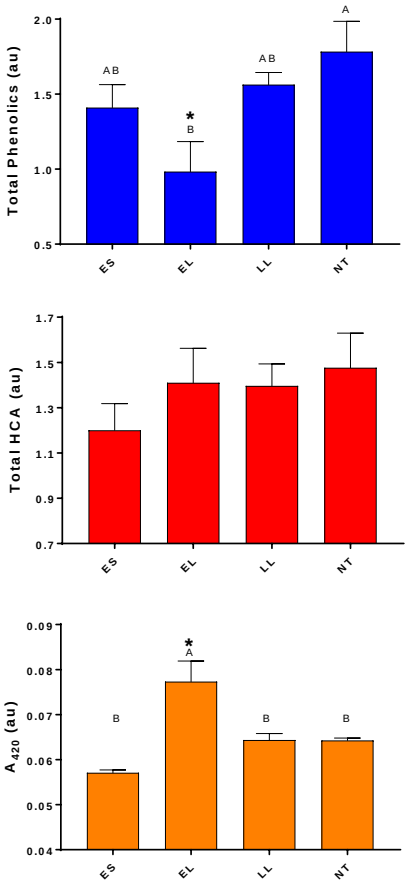


Figure 10. Sugar consumption (glucose + fructose) as a function of time in Chardonnay juice subjected to aeration during fermentation for different timing and duration



* indicates significant difference compared to 'No treatment' using Dunnett t (P = 0.05) *post-hoc* test

Figure 11. Spectral indices indicating phenolics contents as a function of timing and duration of aeration of Chardonnay fermentations

The chemical aroma profiles of the finished wines were determined six months after bottling, at the same time as sensory analysis. Mean concentrations of the fermentation-derived alcohols, organic acids and esters were subjected to ANOVA along with significant treatment differences using the Tukey *post hoc* test at 95% confidence level. All compounds analysed showed significant differences between the treatments ($P < 0.005$) with over 50% being present above the odour threshold values given by Siebert et al. (2005).

Sensory analysis was carried out after the wine had been stored in bottle for exactly six months to replicate a reasonable parallel with industry. Following the last training session, the descriptive terms were finalised as containing one 'appearance' term, twelve 'aroma' terms (eleven defined and 'other') and fifteen 'palate' terms. The ANOVA indicated the attributes 'green aroma', 'green flavour' and 'sweet' differed significantly between treatments ($P < 0.05$), as did 'stone fruit aroma' and 'chemical' at a lower significance level ($P < 0.1$). There were no significant differences among ferment replications for any attribute, signifying consistency in the winemaking replication.

The mean data for each treatment are shown Figure 12. The LL treatments gave rise to wines with significantly higher ratings for 'green' aroma and flavour, and slightly lower ratings for sweetness, compared to the control, with the EL also being rated somewhat higher than the control in 'green' aroma. The EL and ES treatments showed a trend for a lower 'stone fruit' aroma, indicating the application of oxygen early in the fermentation gave a slight less fruity wine.

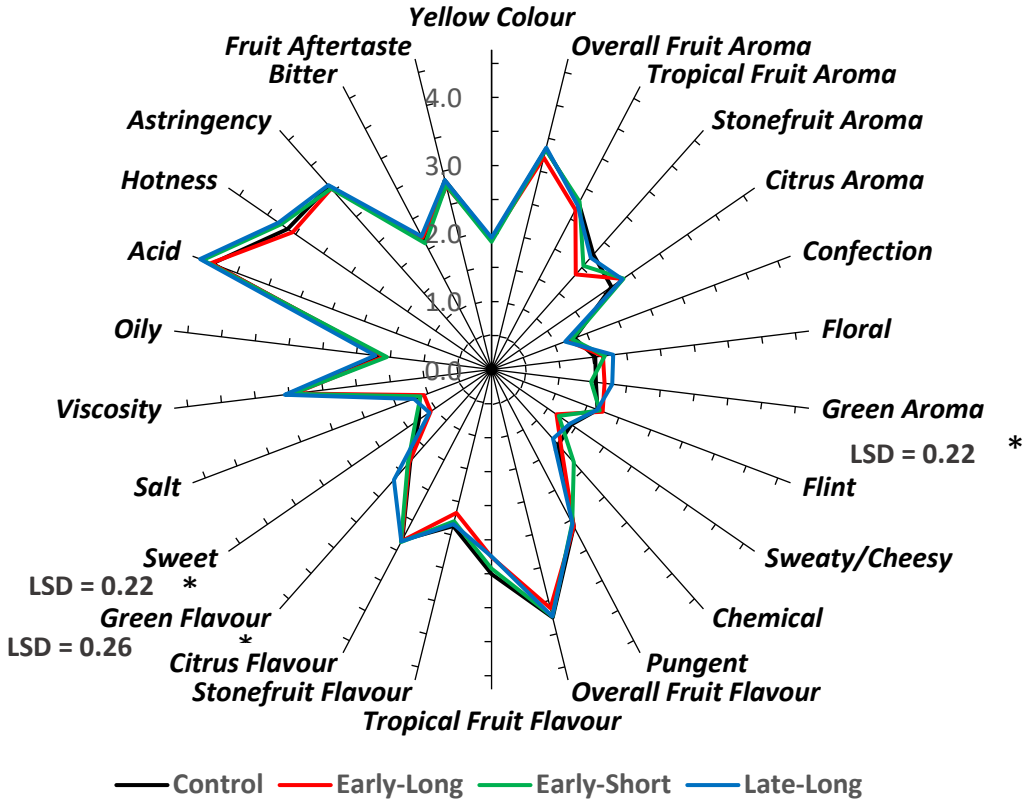


Figure 12. Radar plot of the mean scores for the Chardonnay wines made with varied timing and duration of air sparging during active primary fermentation

Effect of timing and dose of oxygen addition in red wine

Pilot-scale validation using pump-overs and a sub-cap aeration (vintage 2016)

Having established that the timing of oxygen additions during primary fermentation was crucial for maximum advantage in efficiency, aroma and palate structure in white wines, focus was shifted to red fermentations to see if similar effects occurred. In the initial foray into red winemaking with O_2 at the AWRI in 2012, it was established that stylistically diverse red wines could be created by use of 'macro-oxygenation' (introducing large volumes of oxygen) during fermentation in rotary tanks using air (21% oxygen) or 40% oxygen. These wines showed bright red fruit characters, softer astringency, no reductive aromas and much lower residual metal concentrations than the wines made without oxygen. In vintage 2016 macro-oxygenation was further explored using air in red fermentations (during pump-overs), examining the timing of air addition as well as the duration or treatment.

Optimum timing of oxygen addition (from a performance gain perspective) was compared to later addition and repeated aerations in pilot-scale (500 L) Shiraz fermentations. One treatment received a one-off addition during pump-over to saturate the fermenting must with air when sugars had dropped by 20% ('Early') while another treatment consisted of repeated aerations to achieve air saturation over five consecutive days ('Daily'). Another single treatment occurred later when the initial concertation had dropped by 80% ('Late') and a final treatment on previously unaerated wines was carried out after pressing ('Post-Press').

All on-skins aeration treatments showed lower H_2S concentrations post-MLF and at sensory; the 'Daily' treatment showed significantly lower methanethiol (MeSH) than 'No Treatment' with methanethioacetate (MeSAc) being totally absent for this treatment. The differences in chemical aroma composition were a lot subtler. Only half of the 15 significant aroma compounds were above odour thresholds (Siebert 2005) but were characterised by descriptors of 'berry', 'fruit' or 'sweet' in aeration treatment. The concentration of the only varietal thiol to show significant differences (3-MH) was suppressed during aeration treatments on skins although the trends in the precursor hexenal were not as clear cut. Textural descriptors from sensory analysis also showed there were differences in astringency, viscosity, hotness and acidity due to the treatments which were not correlated with the aroma characteristics. Only the 'Daily' treatment had significant effects on the tannin composition: total phenolics were lower, tannin polymer length was shorter (mDP of 8 vs 10), skin-like tannins were reduced with a corresponding increase in wine/seed-like tannins. The convoluted or knotted character (inverse of % mass conversion) of tannins was measured to be statistically lower in the 'Early' treatment compared to no oxygen exposure. Wine colour density and total anthocyanins were depressed and hue was increased with less obvious corresponding trends in improvements to stabilised colour (Figure 13). Sensory analysis one year after vintage showed 'Early' and 'Daily' treatments were found to be higher in 'fruity' and 'floral' characters in comparison to the 'No Treatment', 'Late' and 'Post-Press' treatments which were found to possess higher levels of reductive 'vegetal', 'earthy' and 'black olive' characters and lower intensity of fruit characters.

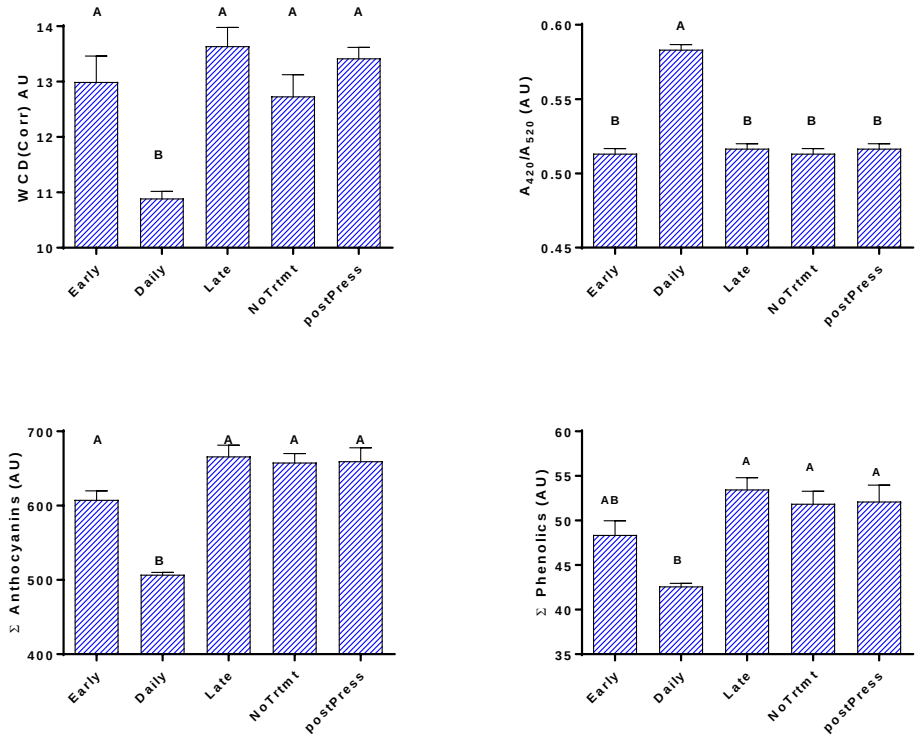


Figure 13. Somers' phenolic indices, prior to inoculation for MLF

Further understanding on the addition of oxygen during red fermentation (vintage 2012).
The effects of oxygen treatment during red wine fermentations on phenolics, metals and malolactic fermentation were determined during this project, stemming from a vintage trial in 2012. In this 2012 vintage experiment, Shiraz wines were made in triplicate using rotary fermenters and treated with different levels of O_2 exposure during fermentation, namely air (containing approximately 20% O_2) and 40% O_2/60% N_2. The impact of the physical displacement of volatile compounds and mixing effects by gas was assessed using gas injections of pure N_2, and the controls were fermented without any gas addition. Wine colour and tannin characteristics were measured after fermentation (Time 0), and after 2 and 12 months of bottle ageing under two different screw cap liners, Saran-Tin and Saranex.

Different levels of O_2 exposure can dramatically alter the colour and texture of red wines. Gradual exposure to O_2 over many months, for example during barrel ageing, can impart a softer mouth-feel to the wine and a more reddish, rather than purple, hue (Cheynier et al. 2006). With O_2 exposure, the purple, monomeric anthocyanins become more stable and resistant to SO_2 bleaching by directly or indirectly forming polymers with condensed tannins as well as acetaldehyde-mediated derivatives such as pyranoanthocyanins (Bakker and Timberlake 1997).This induces a change to red-orange hues and can lower wine astringency, as anthocyanin-bound tannins are less astringent than non-pigmented tannins (Weber et al. 2012). O_2 exposure also alters the structure of wine tannins by decreasing the proportion of acid-labile interflavan bonds, which is related to a decrease in percent conversion yield in depolymerisation reactions, as calculated from the molar mass of cleaved tannin subunits relative to the mass of tannin used in the reaction (Sauvage et al. 2010). Lower percent tannin yield also decreases the extent of protein binding (McRae et al. 2010) and may lead to a less intense wine astringency. Ageing of wines under bottle closures with high oxygen transfer rates

(OTRs), such as cork or Saranex lined screw caps, can modify wine colour and tannin structures to a greater extent than closures with lower OTRs, such as SaranTin screw caps. This effect is particularly enhanced for wines at lower pH (McRae et al. 2010). The importance of O_2 exposure on colour stability and mouthfeel without the use of extended barrel ageing has led to the development of microoxygenation (MOX) techniques in red wines although there is still significant debate about the best time to apply MOX and the ideal dosage rate (Schmidtke et al. 2011).

As an alternative to MOX, changing the level of O_2 exposure during fermentation may alter the colour and mouth-feel of the resulting wine by inducing chemical and enzymatic oxidation of polyphenols, including catechin and caffeic acid, and potentially modifying the extraction of tannin from grape cells. In the production of red wine, O_2 exposure may occur whenever the ferment is plunged or pumped over but the level of O_2 exposure can vary significantly, depending on the number, duration and modality of pump-overs. As an alternative to pump-overs or cap plunging, appropriate levels of grape skin contact during fermentation can be achieved during winemaking with the use of rotary fermenters. These fermenters are horizontal tanks that rotate axially and, while effective in improving skin contact, the vessels are enclosed and can produce reductive conditions. Grape tannins extracted from skins and seeds during winemaking may be directly impacted by chemical oxidation, as well as enzymatic oxidation via such enzymes as laccase and polyphenol oxidases (PPO). PPO can polymerise flavan-3-ols and smaller phenolics such as caffeic acid which may react with tannin to form complex structures. The production of acetaldehyde as a fermentation product as well as from oxidation of ethanol may also increase with greater O_2 exposure during fermentation, changing the structures of extracted tannins. Oxidation reactions at the grape cell surface (i.e. cell wall-bound PPO) may also potentially enhance the retention of tannins and anthocyanins by grape solids during winemaking (Bindon et al. 2010).This may reduce the amount of tannin extracted into the wine and thus the intensity of wine astringency.

Colour and tannin effects

The O_2-treated (40% O_2 and Air) wines were significantly lower in total anthocyanin than the control or N_2-treated wines, and were slightly higher in non-bleachable pigments. This suggested that the pigments formed in the presence of higher O_2 concentrations were more stable. The wine colour density (WCD) remained relatively consistent regardless of fermentation treatment. Tannin composition was significantly different between the wines produced by the O_2-treated ferments (40% O_2 and Air) and those produced from the control or N_2-treated ferments. The tannins from the 40% O_2 and Air wines were less susceptible to depolymerisation reactions (more 'cross-linked'), were more coloured and smaller (McRae et al. 2015). In terms of sensory effects, the 40% O_2 and Air treatments scored lowest for 'bitter' and for 'astringency', while the N_2 treatment was scored highest for 'astringency' emphasising the anecdote that oxygen diminishes the astringency of red wines.

Impact on metal concentrations

After bottling, the O_2-treated wines contained significantly lower concentrations of iron, copper and zinc than the control or N_2-treated wines (McRae et al. 2014). Recent studies have shown the particular importance of iron and copper, as well as others, in the formation of both positive and negative flavour and texture compounds, and on the shelf life of wine (Viviers et al. 2013). The impacts of different metal ions on wine flavour and aroma remains an ongoing area of research at the AWRI and this research indicates that early oxygen exposure in red wines is likely to influence metal-catalysed changes in composition.

Impact on 'sulfidic' or 'reduced' aromas.

The evolution of H_2S was monitored during fermentation; after only two days of treatment, the amount of H_2S gas generated by the fermentations treated with O_2 (40% O_2 and Air) was significantly decreased and after a further two days all production had stopped with no subsequent evolution, even when O_2 treatment stopped. Analysis of volatile sulfur compounds (VSCs) after pressing showed that O_2-treated wines had much lower EtSH and MeSAc and a complete absence of EtSAc. The data demonstrated that the O_2 treatment during fermentation created an environment that favoured VSC removal or modification, either through increased yeast activity or potentially through the incorporation of VSCs into other wine compounds. Comparison of the O_2-treated wines with the no treatment/control showed that there were no statistically significant differences in VSCs, thus indicating that 'splashing' or other aerative cellar operations do not physically displace H_2S gas. The VSCs of the wines sealed under two OTR closures were monitored over 12 months; although there were variations during extended bottle maturation the O_2-treated wines showed consistently lower VSC concentrations (Bekker et al. 2016). Oxygenated handling during fermentation produced wines with desirable 'red' and 'dark fruit' aromas, and correspondingly, the lack of O_2 during fermentation resulted in sensory characters associated with unpleasant 'reductive' aromas, such as 'sewage', 'rotten egg' and 'rubber' aromas

Impact of wine ageing and closure type on wine composition

After 12 months of bottle ageing of the control and N_2 wines, the colour measures and two tannin compositional characteristics associated with ageing (specifically size and degree of 'cross-linkage') were similar to those of the Air and 40% O_2 wines as measured after 2 months of bottle ageing. This highlights the effect of O_2 during fermentation in producing aged wine-like characteristics. Thus the level of O_2 exposure during fermentation may also improve the mouth-feel of young red wines through the influence of modified tannins.

The effect of O_2 exposure during fermentation was compared with the impact of relatively limited O_2 exposure during bottle ageing with two different screw caps: Saranex, which allows a reasonable O_2 ingress, and Saran Tin, which restricts O_2 ingress. The impact of closure type on colour measures and tannin composition after 12 months in bottle was greater in the wines fermented in the absence of O_2 than the O_2-treated wines. The reason that the tannins from the control and N_2-treated ferments were susceptible to this change more so than the O_2-treated wines may relate to differences in the formation of tannin under each treatment type. Greater O_2 exposure during fermentation may increase the oxidised proportion of tannins, resulting in the formation of stabilised tannins with modified interflavan bonds and increased intramolecular interactions to such an extent as to restrict further oxidation due to O_2 ingress through bottle closures. Thus, the oxidation induced by slight O_2 ingress through the Saranex closures was more pronounced in the control and N_2-treated wines than in the air/$O_2$40 wines. Increased O_2 exposure during wine fermentation had a much greater impact on tannin structure in the resulting wine than closure type and this highlights the significance of O_2 exposure during fermentation to tannin formation, development and stabilisation.

Effect of oxygen addition on malolactic fermentation efficiency in red wine
One of the serendipitous observations from the 2012 pilot-scale vintage trial was that aeration into red Shiraz fermentations in vinimatic tanks had a marked effect on the speed of subsequent malolactic fermentation (inoculated after post-press settling). MLF was completed after 8 days for the air and $O_2$40-treated wines and after 17 days for the control and N_2-treated wines. This effect has been examined several times in the laboratory with no confirmations of this acceleration effect having been made.

An investigation into the potential negative effects of fermentation oxygenation on effectiveness of co-inoculation of malolactic acid bacteria or sequential inoculation was performed in Shiraz wines in 2017. Aeration was carried out with distributed sinter points across the bottom of the tank. DO values approaching 50 % air saturation were achieved. Malic acid consumption showed no overall differences between co-inoculated ferments whether aerated or not, nor for sequentially inoculated which had previously received aeration or not. Sensory and chemical analysis will occur at the end of the calendar year. However, the VSCs were analysed at the end of MLF once all treatments had received an addition of SO_2. ANOVA indicated that there were no statistical interactions between the use of oxygen and timing of inoculation for the detected VSCs: MeSH, DMS, CS_2 or MeSAc. A low-level interaction ($P = 0.075$) was observed for H_2S. There were significant differences for the timing of inoculation for MeSH, DMS and MeSAc. Further analyses will be carried out after 4-5 months in bottle.

Practical measurement tools and oxygenation equipment for use in the winery
One aspect of this project was to develop practical experience using tools available for monitoring oxygen exposure during different stages of wine production. In brief, several optical-based dissolved oxygen (DO) measurement tools were used in both the pilot-scale experiments and a large commercial winery. It was found that process-grade probes in specialist housings were best suited for DO measurement during pump-overs or transfers due to their fast equilibration and response time and that they had appropriate configuration for use in a commercial winery. Hand-held meters were equally adaptable to measuring in-tank DO during racking operations. Techniques for introducing oxygen into an active ferment were also assessed. A Venturi injector was trialled in industry and proved a simple and effective device.

There are various analytical technologies that allow the determination of dissolved oxygen (DO) and or headspace oxygen (HSO) in grape and wine products. The industry-standard for many years has been the Clark electrode which contains an oxygen-permeable membrane linked with an electrochemical detector. These are known popularly as the ‘Orbisphere‘ and are mostly adapted for use in a production laboratory or bottling line. They require careful maintenance and fouling of the membrane is a common problem in the routine use of such devices. A more robust technology makes use of the quenching effect of oxygen on the chemiluminescent properties of certain ruthenium diimide complexes or similar compounds and work by measuring the phase-angle shift of an exciting light source. This technology was initially used for good effect in the Presens or Nomasense devices using in determining DO inside wine bottles (O'Brien et al. 2009). The technology has now been transferred to more traditional style immersion probes as well as process-suitable probes. Because of increased robustness and ease of use, only devices using chemiluminescent DO determination were assessed. This technology is often referred to as LDO.

A number of devices were trialled throughout the project, including:

- Presens ‘Fibox Trace [sensor spot/dipping probe]
- Pyroscience Firesting [sensor spot/dipping probe]

- Hach HQ 30d [handheld DO meter]
- Mettler-Toledo 6870i + M400 Controller [process probe]
- PME miniDOT [deploy and forget data logger]

Some observations of experiences with these tools are outlined below.

The AWRI has been using Presens devices for in-bottle measurements for a number of years and has used this as a useability benchmark, based on experience from several vintages in industry settings as well as the Hickinbotham-Roseworthy Wine Science Laboratory's pilot winery. In addition to in-bottle measurements which employ a chemiluminescent sensor spot glued to the bottle wall, it is possible to measure DO in the winery by attaching a sensor spot inside a sight-glass to measure liquid flowing through a pump. It is also possible to use a 'dipping probe' for this device in which the chemiluminescent material is bonded inside a narrow stainless steel probe and linked to the measuring device with a fibre optic cable. Other manufacturers produce this type of equipment and the AWRI selected the Firesting from PyroScience (Germany) as a comparison and also for work in the laboratory where multiple channels are required. A comparison (del Alamo-Sanza et al. 2014) of the above-mentioned equipment has recently been published and the Firesting trace was assessed to provide the most accurate data with the least noise error. The Firesting and the Presens are more suited to the laboratory environment, so more appropriate adaptations of the chemiluminescent technology were also assessed during the 2014 vintage. Handheld DO meters have been available for a number of years and the Hach HQ30d was taken as an example of this type of meter found in many wineries. It is battery operated, comes with a 5-metre lead and a weighted probe cowl allowing easy deployment into deep tanks – a rugged stainless steel version is also available. This particular model has a several reading modes: continuous, interval and press-to-read; the data generated can be saved and downloaded or reviewed on the handheld meter. The active part of the unit is the LDO sensor cap containing the chemiluminescent material and a separate memory chip 'iButton' which holds the calibration data. The cap has a fixed lifespan of 365 days and replacement is very inexpensive. Regular calibration checks are recommended using a two-point calibration (see below).

Most of the above devices are portable and do not integrate into a winery environment or on a fixed platform. The Firesting and the Presens have separate thermocouple wire and a fibre-optic cable which is inconvenient (the Hach has the thermocouple integrated into the probe housing). The process-style probes, represented in this study by the Mettler-Toledo InPro 6000 LDO probes, (Hach equivalent is the 410 Orbisphere with a M1100 or K1100 probe) present as much more robust. The probe housings are stainless steel and the controllers carry an IP6x rating which can also be mounted into power control unit on a pump/filtration/skid.

Measurement of DO is important in the waste-water sector and several devices which differ from those typically used in the wine industry may have potential for a technology transfer. Many of those devices are integrated data loggers that are deployed in the field for considerable periods of time. They are relatively inexpensive and small in format. The miniDOT from Precision Measurements Engineering (USA) was selected. This has the ability to store up to 60 days data, depending on the data measurement frequency which can be up to one reading per minute. The unit is autonomous and can be constructed from food grade plastics. The advantage of this device is that it can be placed inside equipment without the need to run cables back to a control unit. One example that will benefit oenology research is the ability to monitor DO inside a membrane press. The device can also be deployed in fermenters or other vessels that do not have access ports for

classical DO probes. Frequently the measurement of DO is compromised by the sampling methods or even the potential of introducing oxygen into a vessel in which a probe is being deployed or removed. Therefore, another added advantage of this type of device lies in the 'deploy and forget' nature of a watertight data logger.

More information on this work can be found in Day and Wilkes (2014) and a fact sheet entitled 'Ways to introduce oxygen into an active red ferment' available from the AWRI website at: https://www.awri.com.au/wp-content/uploads/2015/02/introducing_oxygen.pdf

Industry adoption

Case study from The Oxford Landing Winery

Research of this type has most value when trialled by, and ideally adopted by, industry. Following a workshop held at the 16th Australian Wine Industry Technical Conference in 2013, the AWRI was approached by The Yalumba Wine Company about trialling oxygen additions to production-scale ferments at The Oxford Landing Winery (OLW) during the 2014 vintage. The following summary highlights some of the practical experiences of working with industry partners to support adoption of this research.

2014 vintage trials

The trials were conducted in four 100-tonne sweeping arm Potter (SWAP) fermenters using Cabernet Sauvignon fruit from the Oxford Landing vineyards. The project team decided to use a more conservative dose of 1.6 g/L oxygen compared to the doses added during the pilot-scale trials (2.8 g/L and 5.5 g/L), based on the site equipment and an achievable cost/benefit return.

Three different types of oxygen introduction device were tested:

- 3-inch venturi injector (Mazzei) placed at bottom or top of pump-over line
- Pulsair tanks in normal operation
- Air sparger at bottom of tank.

Results from the trial were very encouraging, with all of the wines ending up in their intended blends, a decision which was a small leap of faith for the winemakers! No sulfidic aromas were detected, although this is not normally a significant problem for Cabernet Sauvignon wines. With the venturi configuration, the DO measured before and during the aerated pump-over (probe placed just before irrigator) rose to 19.9% air saturation which from the given total soluble sugars (TSS) at the time of aeration gave a DO of 1.43 mg/L. When the venturi injector was at the top of the SWAP fermenter, the DO rose to 42.2% air sat or 2.92 mg/L. From a practical point of view, the venturi injectors – placed directly after the pumps at the bottom of the tanks – did not work well in the set-up at OLW because the in-place pump-over pumps are a high flow, low pressure design. This meant that the flow rate was dramatically reduced and changed the dynamic of the pump-over system, creating another variable in the trial. It also would not be feasible to retrofit the venturi injector into the existing pump system. With the right pump design a venturi system might be suitable for a new installation. Because of the passive nature of the venturi device and the fact that the inlet pressure was not measured, which meant the actual volume of gas delivered could not be calculated, the project team reluctantly decided not to continue with this device.

2015 vintage trials

Following the positive outcomes from the 2014 vintage, the OLW project team decided to continue and expand their trials in 2015 to include rotary fermenters as well as SWAPs. The necessary compressed air lines were attached to the existing Pulsair fittings and connected to the SCADA system for solenoid control and logging. Compressed air was only supplied to the fermenter while it was being pumped over to ensure mixing. The air flow rate was set at 200 L/min and 130 kg O_2 were delivered over five days to 100 t of Cabernet and Shiraz grapes from the Riverland. Treated wines were compared with similar untreated batches.

The rotary fermenters had a capacity of 30 t and were fitted with a manifold containing several stainless steel sinters. The air supply was connected to a gas turret fitted with a safety switch to prevent rotation with the gas line attached, and they were also connected to the SCADA system. The air flow rate in this case was 100 L/min delivering 33 kg of O_2 over the five days of fermentation. Because of the smaller head height in the rotary fermenters compared to the SWAPs, the bubble residence time was much lower. However, with the mixing that occurred during rotation, it was hoped that the headspace oxygen would be incorporated back into the fermenting must. Three batches of Cabernet Sauvignon and one each of Merlot and Shiraz (all from the Riverland) were used in the trial. Control wines were made with the same fruit without addition of air. After the first day or so, the sinters in the rotary fermenters became blocked and were replaced with 1-2 mm holes drilled along the length of the gas supply line in the fermenter. Fortunately, these did not block and were well suited to purpose. No other major engineering problems occurred. One key observation during the trial was that none of the ferments treated with oxygen required the addition of DAP to stop 'stinky' sulfur odours, compared to three out of the five control experiments.

The trial wines were tasted at OLW and Yalumba three months after bottling. For the Shiraz wines made in the rotary fermenters, six of the nine tasters preferred the oxygen-treated wines over the control and one had no preference. No negative impacts on colour were noted and the tannins were considered to be 'smoother'. A slight 'sour' and 'aldehydic' note was picked up by the two tasters that preferred the control Shiraz. All tasters preferred the oxygen-treated Cabernet wines made in the rotary fermenters. For the wine fermented in SWAPs there were unfortunately no control wines available at the time of tasting; however, the oxygen-treated samples looked very good, in particular the Shiraz wines, which had smoother tannins than other wines of similar age.

Analysis of the wines was undertaken by the AWRI at the time of tasting. Volatile sulfur compounds were analysed by GC-SCD (gas chromatography with sulfur-chemiluminescence detection) which detected the presence of hydrogen sulfide (H_2S), methanethiol (MeSH), dimethylsulfide (DMS) and carbon disulfide (CS_2). As with the AWRI trials, the concentration of MeSH was lower in the air-treated wines; however, there was little difference for the other compounds. The trends in tannin composition were also similar to the AWRI pilot-scale trial. Free anthocyanins and total tannin concentrations were lower in oxygen-treated wines as they had been combined into more complex and evolved tannins in which the colour is stabilised. Such tannins tend to be less astringent and are associated with wine ageing.

Further contact with industry

During preparation and execution of the 2017 vintage, several industry players contacted the AWRI for advice in using oxygen during red ferments. These wineries ranged from premium boutique wineries in the Western Australia and Limestone Coast, as well as two large-scale wineries in Victoria and Barossa.

Some quotes from email with one happy winemaker:

Sent: Friday, 7 April 2017 4:02 PM

Hi Martin
Working well, We are running about 20 litres minute of filtered compressed air on a 10 Tonne ferment. Normal pumpover would have been 30 minutes, air for 15minutes is working well during the pumpover. I'm basically looking for the milkshake vision.
cheers

Sent: Tuesday, 11 April 2017 8:51 AM

Hi Martin

Basically I've been rotating it on all shiraz and merlot ferments, about a dozen so far

Each one at 9-10 Baume is getting 15-30 minute intervals, then again at 6-7 Baume.

Seen no foaming issues, its appears frothy but that subsides very quickly.

The effects have been excellent, when compared to my traditional aerative pumpovers into bins, then back over the top.

The team prefer it as well! Only because its less work!

Sent: Wednesday, 9 August 2017 11:35 AM

Hi Martin

All went really well.

We removed the necessity to do any traditional rack and returns on the static fermenters. The oxygen sparger lead the charge and was utilised on all Cabernet, Merlot and shiraz static ferments at least twice through fermentation.

Our rates were 15-30 minutes of air, set at approximately 20 litres/minute, so half the actual pumpover time allocated depending on batch sizes; which were between 6 and 14 tonnes.

The ferments appeared aromatically brighter, with no reductive issues in the ferments and fermentation timing/temperature etc saw no difference to normal practices.

I think we will push parcels harder next year, we really were quite cautious I think.

cheers

Outcome and conclusion

Greater oxygen exposure during fermentation trials in vintage 2012 produced wines with more 'aged' characteristics with respect to greater hue, fewer anthocyanins, lower tannin concentrations, and smaller tannins with more modified structure. These changes were similar to those induced by 12 months of bottle ageing in wines deprived of oxygen during fermentation. The 40% O_2 and Air treatments scored lowest for 'bitter' and for 'astringency', while the N_2 treatment scored highest for 'astringency'. This suggests that increased oxygen exposure during winemaking may reduce the need for extended wine ageing, saving winemakers the cost of tannin fining and extended storage, and possibly increasing consumer preferences. Recent research on white wine phenolics adds to the body of evidence that oxygen is likely to affect texture; the research established that two of the major phenolics in wine (GRP and caftaric acid) that are influenced by oxygen exposure also modulate astringency and increased oiliness (Gawel et al. 2014).

In addressing these questions this research also improved the understanding of how oxygen management and use during processing and fermentation affects areas other than fermentation efficiency and continued to explore how oxygenation during fermentation can be used to remediate or prevent reductive aromas and enhance attributes generally considered to have a positive impact on wine style. In addition, decreased metal concentration in a wine post-ferment may benefit a wine's shelf life and evolution. Finally, the significantly faster rates of malolactic fermentation might provide a practical tool to assist in the reliable completion of malolactic fermentation. As such, investigations into the MLF implications of oxygen exposure are continuing.

The different oxygen levels that occurred in the vintage 2014 trial arose simply through pressing and handling operations – no active oxygen additions were conducted. The choice of pressing mode and the extent to which juice or wine was protected from oxygen during handling were both shown to

affect a wine's final chemical composition and sensory characteristics; in this particular case affecting 'floral' and 'citrus' characters. For juices prepared through normal (i.e. aerobic) pressing, no significant differences were introduced through the choice of handling method. This seems to suggest that, at least for Chardonnay, there is little need to invest too much time and money protecting juice and fermenting wine from oxygen, if it has been produced through aerobic pressing. Other white varieties may behave differently so caution should be used before dispensing with inert gas blanketing altogether! On the other hand, if a juice is produced by inert pressing then sufficient phenolics remain which can be affected by further oxygen exposure during normal handling. Inertly pressed juices therefore need continued protection through reductive handling, if oxidation is to be avoided.

Having observed and quantified the chemical and sensory differences that occur through passive oxygen exposure in the 2014 study, trials during the 2015 vintage focused on making deliberate but controlled oxygen additions during fermentation which were expected to have a greater impact on wine style. The addition of oxygen during white wine fermentation had a positive effect on the kinetics of fermentation, rather than style of wine. This observation could have major impact on the efficiency of fermentation by allowing a wine to finish several days earlier than normal, while maintaining style through unaltered fermentation temperatures. This is a particularly valuable outcome considering the growing need to manage fermentations in compressed vintages. The sensory effects of adding oxygen were minimal. The effect of the timing of oxygen additions was also assessed and the preferred timing of oxygen addition appeared to be in the first half of fermentation when sugars had dropped by 20% of the starting concentration. It was still beneficial, however, to make a late addition, even once the sugar concentration had dropped by 80%, as this ensured that the ferment achieved dryness safely. Sensory analysis confirmed that there were no negatives issues associated with using a reasonable amount of oxygen.

During 2016 vintage trials, type of fermenter used and the way the aeration was carried out were modified to demonstrate how oxygen addition could be achieved in wineries not equipped with rotary fermenters and with minimal capital outlay. The timings used in the 2015 trial were replicated in 2016 with additional treatments of a daily dose and a post-press addition. In order to achieve the positive benefits of enhancing the 'bright red fruit' attributes and suppressing low-level reductive aromas, it was important to use an early aeration during the first few days of active fermentation.

Recommendations

Five pilot-scale vintage trials and numerous controlled laboratory experiments were carried out during this funding period. In parallel, several industry partners trialled the use of air additions at small, medium and large-scale wineries across the country.

In white winemaking oxygen additions can lead to increases in fermentation efficiency without having negative effects on sensory outcomes. Modulating the extent of oxygen exposure at the very earliest stages of juice preparation was an important tool in understanding the effect of oxygen in white winemaking. Although the project did not set out to assess the merits of inert pressing, results have highlighted some subtle effects that can be achieved from pressing in low, if not totally inert

environments. This is an area that should receive some further investigation, particularly looking at sensory variations and fermentation efficiency gains in a range of grape varieties.

There is still a need to establish the appropriate dose of oxygen for any given must, red or white, and to monitor this in large-scale fermentation tanks. The observed DO during active ferment will most probably be a strong indication of the amount of oxygen consumed by the ferment and accurate oxygen measurement is the 'missing link' in defining a universal approach to controlling dosage. Some engineering solutions may be required to ensure correct DO measurement on large-scale fermenters, which is where the greatest benefits could be achieved. Work on redox potential probes to complement DO measures may also contribute to improved dosage control. The ultimate aim would be to create a 'calculator' to decide the amount of oxygen to add from easily measured juice parameters.

Many winemakers have already attended workshops on the use of oxygen in the winery, and further extension activities would be highly desirable to support adoption and regional trials.

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