CHAPTER 4

Microoxidation in Wine Production

Paul A. Kilmartin

Contents

Abstract

Microoxygenation (MOX) is now widely applied for the maturation of red wines as an alternative to barrel aging. The proposed improvements in wine quality arising from MOX include color stabilization, removal of unwanted off-odors, and improvements

Wine Science Programme, The University of Auckland, Auckland, New Zealand

Advances in Food and Nutrition Research, Volume 61
ISSN 1043-4526, DOI: 10.1016/S1043-4526(10)61004-2

in wine mouthfeel. In this review, an outline is provided of oxygenation systems, particularly microbullage and polymer membrane delivery, and of the current understanding of wine oxidation processes. A summary of the results from published studies into red wine MOX is then provided, beginning with observations on O_2 and acetaldehyde accumulation, and the moderating effect of added sulfur dioxide. Effects upon red wine color, particularly the more rapid formation of polymeric pigments and higher color retention, have been consistently demonstrated in MOX studies, along with further effects on specific polyphenol compounds. A few reports have recently examined the effect of MOX on red wine aromas, but these have yet to identify compounds that consistently change in a manner that would explain sensory observations regarding a lowering of herbaceous and reductive odors. Likewise, tannin analyses have been undertaken in several studies, but explanations of the decline in wine astringency remain to be developed. The accelerated growth of unwanted microorganisms has also been examined in a limited number of studies, but no major problems have been identified in this area.

I. INTRODUCTION

Microoxygenation (MOX) has been widely applied as a technique for the aging of red wines since its introduction in the mid-1990s (Dykes and Kilmartin, 2007; Oenodev, 2009; Parish *et al*., 2000; Paul, 2000). The aims of MOX match the benefits expected for red wine exposed to oxygen during barrel aging, including improvements in mouthfeel characteristics, stabilization of wine color, and the removal of unwanted reductive or herbaceous odors. These aspects of red wine maturation can be linked to changes in polyphenol content, particularly involving the anthocyanins that give red wine its color, and flavanol oligomers and polymers associated with astringency. Changes in the structures and concentrations of these compounds during red wine aging have been the subject of recent research developments (Alcalde-Eon *et al*., 2006; Garcia-Puente Rivas *et al*., 2006).

The number of scientific studies published on red wine MOX was very limited prior to 2006, but over the last 3 years, several research groups have published results that confirm a number of observations made from practical winery experience. In this review, MOX systems currently available to industry will be briefly described, centered upon oxygen delivery methods and oxygen spatial considerations within large tanks. An overview of the role of oxygen in red wine maturation will then be presented, prior to a review of published studies on MOX. These studies will be grouped according to the generation of aldehydes and the loss of sulfur

dioxide from the wine, effects on red wine color, aromas, and mouthfeel, and finally microbiological considerations.

II. MICROOXYGENATION IN INDUSTRY

A. MOX technology: Microbullage delivery

The addition of tiny bubbles to introduce O_2 into wine at a controlled rate, as an alternative to barrel aging, was developed initially in France in the early 1990s (Lemaire, 2000). It was the slow, metered additions of oxygen that differentiated the technique of MOX from traditional approaches such as periodic racking or more intense oxygen sparging in stainless steel tanks, while providing cost savings versus the purchase of oak barrels. The generation of microbubbles from a porous diffuser (microbullage) is the most widely used technique in commercial applications, with several systems and units currently available (KauriWine, 2009; Oenodev, 2009; Vinovation, 2009). Porous ceramic diffusers with 2–4-μm pore diameters are known to generate microbubbles with diameters around 400 μm (Devatine and Mietton-Peuchot, 2009). The oxygen is then supplied to the wine at dosages of several milliliter of O_2 per liter of wine per month (mL/L/month), with some systems calibrated to deliver a certain volume of oxygen, and others based upon the mass of O_2 delivered (note 10 mL/L/month = 14.3 mg/L/month at 25 °C). A minimum tank height of about 2 m is required to ensure that bubbles coming from the diffuser are fully dissolved into the wine and do not escape the wine surface and strip out wine volatile components.

MOX can be undertaken at a number of stages in a wine's development, from directly after alcoholic fermentation through to post-malolactic fermentation (post-MLF). The choice of O_2 dosage rate and the length of time to apply MOX are still very much determined by experience and ongoing wine tastings. Decisions relating to MOX depend upon the initial tannin and anthocyanin content of the wine, aroma profiles (such as the presence of reductive odors), along with SO_2 content, pH, and temperature, which can be controlled. A wine that is lower in polyphenol content, or whose existing astringency may want to be retained in the final wine, might not be a good candidate for MOX.

Some of the early descriptions of the effect of MOX on red wine included an interesting cycling effect where certain organoleptic aspects of wine quality appear to get worse before the continued application of MOX leads to the desired improvements (Dykes and Kilmartin, 2007; Parish *et al.*, 2000; Vinovation, 2009). In the first ''structuring'' phase (for several days to weeks), the wine tannins are said to become more aggressive and the varietal aromas decrease, after which the tannins soften and

aroma complexity develops in the ''harmonization'' phase (for several weeks to months). The optimum in wine quality can be exceeded should MOX continue for too long, in which case an ''over-oxygenation'' phase is reached with an increase in astringency and oxidized aromas, and is to be avoided.

B. Alternative oxygenation procedures using polymer membranes

An alternative means of introducing oxygen into a fluid, besides the supply of O_2 bubbles, is through a permeable membrane, such as plastic tube or vessel (Kelly and Wollan, 2003; Paul, 2000). In this case, the oxygen will dissolve directly into the wine in a ''bubbleless'' procedure. An example of this approach is the ''O2mate'' technology developed in Australia, which makes use of gas diffusion through a permeable membrane in the form of a silicone rubber tube for oxygen delivery (O2mate, 2009). Units have been designed for both small- (e.g., barrels) and large-sized vessels, and allow quite small volumes of wine to be oxygenated.

Oxygen delivery by diffusion through a dense polymer membrane made out of fluorinated ethylene–propylene has been investigated in detail for use with specially designed research scale (15 L) tanks (Dykes, 2007). The tanks were fitted with a 0.188-m sealed-end polymer tube that was calibrated by measuring O_2 accumulation in water/ethanol solutions over a period of 10 days. The rise in dissolved oxygen (DO) was found to be quite linear during this time, and was used to establish the diffuser feed pressure required to deliver O_2 at 10, 17, 23, 30, and 36 mg/L/month (namely 200, 300, 400, 500, and 600 kPa). In this process, oxygen is absorbed into the polymer on the gas side and transported by diffusion to the liquid side where desorption of oxygen into the wine occurs. The O_2 mass transfer resistance is much larger on the liquid side, due to the presence of a large boundary layer, than on the gas side, which is an important consideration in modeling the system. This method of O_2 delivery has been applied to a number of MOX trials at the University of Auckland (Dykes and Kilmartin, 2007; Tao *et al.*, 2007), and allows for replicated trials on small volumes of wine, without concern over the loss of volatile components in the gas bubbles that can escape from small tanks when microbullage delivery is used.

A related approach has been the introduction of maturation vessels constructed out of high density polyethylene (HDPE), in which the tank walls, of a certain thickness (typically 4 mm), are themselves used as the membrane to introduce O_2 into the wine (Flecknoe-Brown, 2006). The term ''permeation'' is used in this context, rather than ''diffusion,'' when the movement is controlled by a pressure difference and described by Darcy's law. ''Flextank'' maturation vessels have been designed to

allow oxygen permeation at rates around 20 mg/L O_2 per year, similar to those achieved in wooden barrels. The vessels are claimed to lead to very low aroma losses (through either adsorption or permeation), and not to suffer from the clogging of pores that occurs over time with oak barrels.

C. Oxygen spatial considerations

A limited amount of research has been undertaken on issues to do with oxygen transfer rates from bubbles, and the spatial gradients that can develop when O_2 is supplied from a fixed bubbler at the bottom of a large tank. Initial modeling of the oxygen distribution has shown how the DO is present at a high concentration around the bubble plume with little initial mixing due to the small fluid velocities involved (Fig. 4.1C). Simulations of this sort can show how certain volumes of the wine could be exposed to higher O_2 concentrations during the MOX process, affecting subsequent oxidation processes, and well above the much lower average DO levels in the remainder of a large commercial wine tank.

A further consideration that has been highlighted in recent research is the effect of dissolved CO_2 on lowering the efficiency of O_2 transfer (Devatine *et al.*, 2007). Using engineering principles governing oxygen mass transfer kinetics and tests undertaken in 3-L tanks, an increase in bubble diameter with CO_2 desorption has been cited as a reason to explain the decrease in O_2 transfer fluxes in wines with a higher CO_2 content. Through modeling of oxygen transfer from a rising bubble, the

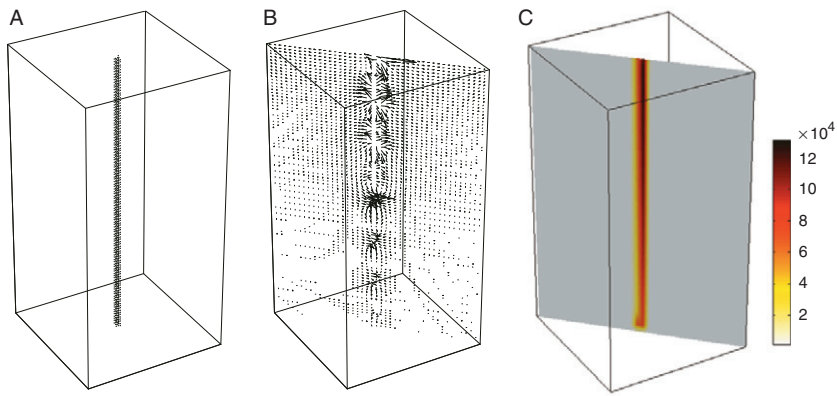

FIGURE 4.1 Application of computational fluid dynamics to simulate: (A) bubble position after 120 s during O_2 dosage at 26 mg/L/month and 670-μm bubble size; (B) represents a diagonal slice of the wine phase velocity field, and (C) the dissolved oxygen concentration in milligram per liter. Reprinted with permission from Dykes and Kilmartin (2007). Copyright 2007 Winetitles Pty Ltd.

influence of CO_2 was further confirmed, along with a recognition of the importance of column height relative to diffuser pore diameter (Devatine and Mietton-Peuchot, 2009).

These studies illustrate how a number of physical factors such as diffuser position and size, and additional wine gases, can influence the exposure that the wine will receive from added O_2, which cannot be assumed to be uniform or equal for all wines.

III. OXIDATION PROCESSES IN WINE

A. Oxygen in wine

There are several stages in red winemaking in which the wine is exposed to oxygen as a part of regular wine aging and development. These include quite large aerations during pump-overs, in which case the concentration of DO can reach a saturation value of 6 mL/L (8.6 mg/L) at room temperature, and higher values at lower wine temperatures. Much slower rates of O_2 ingress occur in the barrel, but this small rate of oxygenation is considered an essential aspect of barrel maturation. A wine saturated with O_2 will typically take 1–2 weeks to consume the oxygen, which is taken up by reactions involving polyphenols (Singleton, 1987) and by the yeast lees, when present. The later process occurs through a mild oxidation of yeast membrane lipids (Salmon *et al.*, 2000), which can provide a rapid initial O_2 consumption (Salmon *et al.*, 2002). In a comparison of oxygen uptake by a Cabernet Sauvignon wine, a saturation level of 9 mg/L of O_2 was removed within 2 days for the wine still on yeast lees, but after filtering and removal of the lees, it took 6 days for the oxygen to be depleted (Dykes, 2007; Dykes and Kilmartin, 2007). Among the impacts of periodic saturations of a wine with oxygen is the loss of protective SO_2, important for the microbial stability of the wine (Castellari *et al.*, 2000).

However, the concentration of DO in the wine is typically well below the saturation level and can be monitored at a Clark electrode (Laurie *et al.*, 2008; Vidal *et al.*, 2004a), or using alternatives such as photoluminescence-based systems (Nevares and del Alamo, 2008). During several winemaking operations, the following mean DO values were recorded (Castellari *et al.*, 2004): 0.37 mg/L for racking at 15–20 °C (but around 1 mg/L for racking at 10 °C); 1.75 mg/L for mixing wines from different casks; less than 0.3 mg/L during filtration; up to 1.2 mg/L for centrifugation; 1.27 mg/L for cold stabilization at −5 °C; but little increase as a result of batonnage during barrel aging. Historical estimates of the amount of oxygen that enters a wine during barrel aging are of the order of 20–30 mL/L/year (Nevares and del Alamo, 2008; Paul, 2000; Vivas *et al.*, 2004). The case of controlled MOX will be considered separately below.

There is also considerable interest in the amount of O_2 that enters a wine during bottling, which can vary considerably depending upon the technology applied, with concentrations of around 0.8 mg/L typically seen (Castellari *et al.*, 2004). The oxygen that remains in the headspace above the wine (ullage) can pass into the wine over a period of some months and sustain wine oxidation processes at a slow rate (Vidal and Moutounet, 2006). This can add a few milligram per liter of O_2 to the wine and match the expected oxygen addition of several months of mass transfer across the wine closure (Kontoudakis *et al.*, 2008), which has been determined to be less than 1 μL of O_2 a day for storage under good quality cork and screw-cap closures (Lopes *et al.*, 2006, 2007). The additional O_2 supplied for a large ullage under screw cap (64 vs. 4 mL) in a Cabernet Sauvignon wine trial, was found to lead to greater losses of SO_2 after bottling, more oxidized and less flint/rubber aromas, and a greater change in the colored anthocyanins over a 3-year period (Kwiatkowski *et al.*, 2007). When a wine is stored under synthetic plastic closures, the rate of O_2 ingress and wine oxidation processes are greater. With the use of polyethyleneterephtalate (PET) bottles for wine storage, a slow oxygenation will occur unless an oxygen scavenger is incorporated into the PET bottle, in which case rates of SO_2 and anthocyanin loss can be less than for storage in glass containers, due to the removal of O_2 already present in the wine at bottling (Giovanelli and Brenna, 2007).

B. Polyphenol-mediated oxidation processes

The current understanding of wine oxidation processes centers around polyphenols as the main initial substrate of wine oxidation, with crucial roles for catalytic metals in facilitating the reactions (Danilewicz, 2003). The amount of oxygen that can be taken up by a particular wine has been found to be proportional to the polyphenol content, an uptake that proceeds more rapidly at a higher pH where the phenolate anion forms of the polyphenols are more abundant (Singleton, 1987).

Oxygen in its initial triplet state first needs to be activated, and can react with catechol-containing polyphenols via cycling with Fe^{2+} (Fig. 4.2), which is typically present in wine at a few milligram per liter. Copper ions will further increase the rate of polyphenol oxidation through the redox cycling of iron (Danilewicz, 2007). As O_2 is reduced (by up to four electrons before water is produced), it creates a range of reactive species that progressively include the hydroperoxyl radical ($HO_2^{\cdot}$), hydrogen peroxide (H_2O_2), and the very reactive hydroxyl radical ($OH^{\cdot}$) through the Fenton reaction. The hydroxyl radical will react quickly with most organic molecules and will oxidize alcohols to aldehydes (Waterhouse and Laurie, 2006). Alongside acetaldehyde (CH_3CHO) production from ethanol, further aldehydes such as

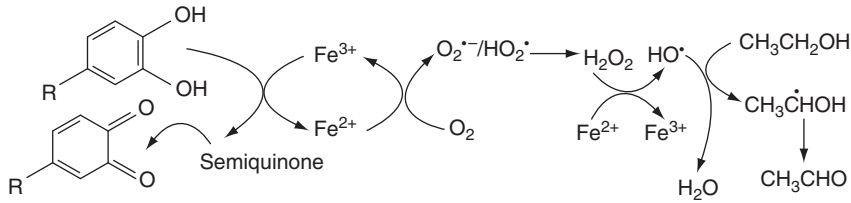

FIGURE 4.2 Wine oxidation processes; adapted from Danilewicz (2003); R = further organic groups such as the three-ring structure in the flavonoids, and further groups in nonflavonoid polyphenols.

glyceraldehyde can be produced through the action of the hydroxyl radical on the likes of glycerol (Laurie and Waterhouse, 2006b). In the absence of polyphenols, ethanol and tartaric acid are quite stable against oxidation (Wildenradt and Singleton, 1974).

Two of the major products of the polyphenol oxidation process important for further wine reactions are thus polyphenol quinones and aldehydes. The quinones can react with various sulfur-containing species, including glutathione naturally present in wine, and sulfur-containing aroma compounds (see below). The quinones can also combine with additional polyphenols to create new products that can oxidize further, and through their more extended conjugation, take on a brown color (Monagas *et al.*, 2005a). The reactivity of polyphenol quinones can be inhibited by the presence of free SO_2 in the wine, which has been shown to rapidly reduce certain quinones back to their original polyphenol forms (Makhotkina and Kilmartin, 2009).

Aldehydes such as acetaldehyde can play important roles in wine-aging reactions and can create covalent links between polyphenols, including anthocyanins and wine tannins (Fig. 4.3). Glyceraldehyde additions to (epi)catechin and malvidin-3-glucoside illustrate the sorts of condensed products that can arise (Laurie and Waterhouse, 2006a). These processes occur alongside other linkage reactions involving small molecules such as pyruvate generated from yeast activity to form the colored visitins, or with direct anthocyanin–tannin linkages (Fig. 4.3; Fulcrand *et al.*, 2006). These processes create a range of pigment classes that form and degrade during wine aging with impacts on red wine color (Alcalde-Eon *et al.*, 2006; Garcia-Puente Rivas *et al.*, 2006; Monagas *et al.*, 2005a,b). Many of the new compounds are resistant to sulfite bleaching, resulting in an increase in the measured ''sulfite-resistant pigments'' at the expense of the monomeric anthocyanins. An increase in red polymeric pigments was observed to be a consequence of periodic saturations of red

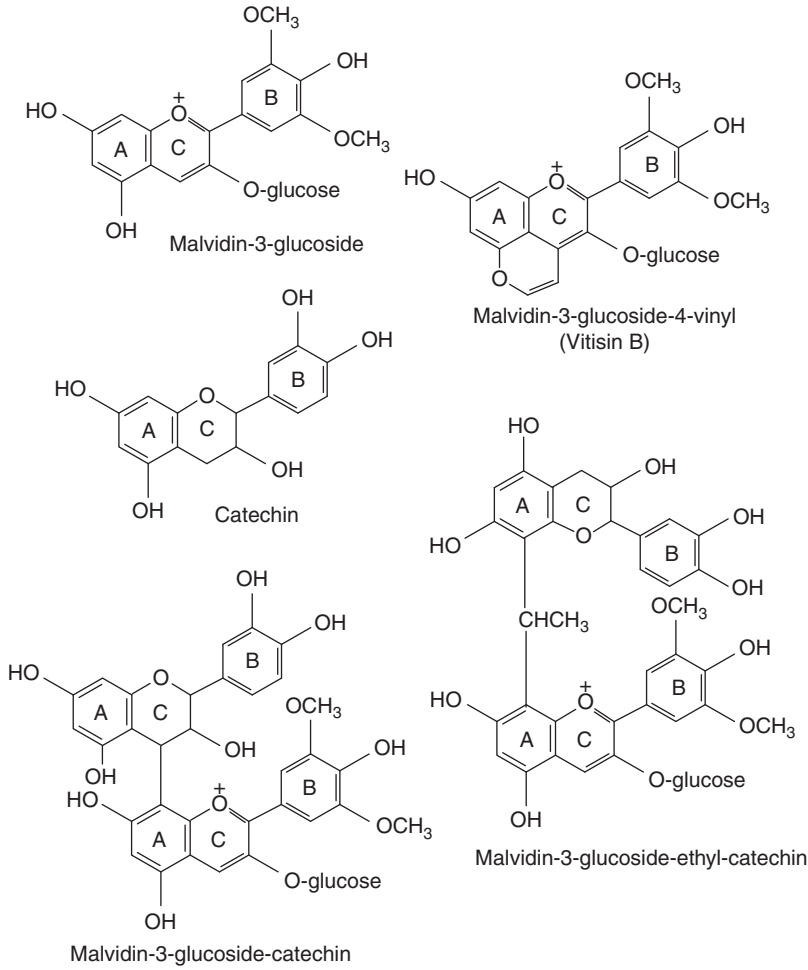

FIGURE 4.3 Representative wine polyphenol structures.

wine with oxygen (Castellari *et al.*, 2000), and to be a general trend for the aging of red wines in the bottle (Saenz-Lopez *et al.*, 2004).

As a result of these condensation processes, concentrations of methoxy-containing malvidin anthocyanins decline more quickly in red wines than hydroxycinnamic acids such as caffeic acid with easily oxidizable catechol groups (De Beer *et al.*, 2008). This is particularly the case when higher levels of SO_2 are maintained (Tao *et al.*, 2007), an observation that can also be linked to the ability of free SO_2 to readily regenerate the original caffeic acid form (Makhotkina and Kilmartin, 2009).

C. Oxidation of wine aromas

The impact of oxygen on wine aroma is likely to involve several oxidation mechanisms. One pathway involves polyphenol quinones, particularly in the case of the removal of unwanted sulfur-containing off-odors (RSH; Mestres *et al.*, 2000), as illustrated in Fig. 4.4A.

In addition to reactions of quinones with unwanted ''reductive'' or rubbery sulfur-containing compounds (e.g., mercaptans), there has been some indication that quinones can react with desirable varietal aromas such as 3-mercaptohexanol (3MH), which imparts passion fruit or berry-type aromas in wines (Blanchard *et al.*, 2004; Danilewicz *et al.*, 2008). On the other hand, the aroma loss might also proceed via reaction with H_2O_2 or other reactive oxygen species produced during wine oxidation, affecting a wide range of aroma classes. Sulfur-containing compounds acting as nucleophiles can also combine with the carbocation formed as proanthocyanidin polyphenols are hydrolyzed, which has been the basis of a measure of the mean degree of polymerization (MDP) of tannin fractions (Prieur *et al.*, 1994).

There is also an expectation that thiols can be directly oxidized through to disulfides (RSSR in Fig. 4.4B) (Mestres *et al.*, 2000; Rauhut *et al.*, 1996), a mechanism also suggested for the case of 3MH (Murat *et al.*, 2003) where a protective effect from anthocyanins present in the wine was noted. In one study, the concentrations of both ethanethiol and the related oxidized form of diethyl disulfide in a red wine were found to decrease over a 60-day period, and at a greater rate under aeration (Majcenovic *et al.*, 2002). However, in a survey of wines over five vintages, the older wines were shown to contain higher concentrations of diethyl disulfide, and lower concentrations of ethanethiol (Fedrizzi *et al.*, 2007).

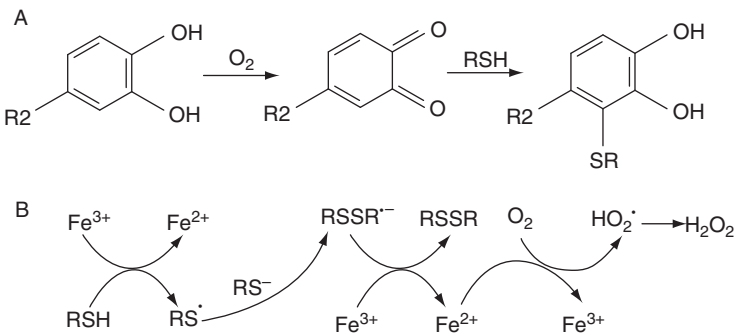

FIGURE 4.4 (A) Polyphenol-mediated oxidation; and (B) metal-catalyzed thiol oxidation mechanisms by which sulfur-containing compounds in wine can be removed; adapted from Danilewicz *et al.* (2008).

It has been suggested that the disulfides may subsequently be reduced back to the volatile thiols (with lower perception thresholds) by SO_2 present in the wine during bottle storage under low oxygen concentrations (Bobet *et al.*, 1990), or be released by hydrolysis from thioacetate esters (Rauhut *et al.*, 1996).

Wine oxidation has also been associated with the formation of unwanted aromas (du Toit *et al.*, 2006b), including 3-(methylthio)propionaldehyde (methional) with a ''farm-feed'' descriptor, phenylacetaldehyde described as ''honey-like,'' 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) with a ''kerosene'' odor, and 4,5-dimethyl-3-hydroxy-2(5-H)-furanone (sotolon) (Silva Ferreira *et al.*, 2003). Increases in the concentrations of methional and TDN were observed in accelerated aging studies of wines at 45 °C and saturated with O_2, in which case varietal compounds with floral aromas, the terpenes, and norisoprenoids, decreased in concentration (Silva Ferreira *et al.*, 2002). A number of these aldehydes were found at higher concentrations in aged versus younger red wines and above their odor activity values, particularly methional and phenylacetaldehyde (Cullere *et al.*, 2007). Further aldehydes produced during wine oxidation are also expected to contribute to the oxidized aroma, although many of these compounds have yet to be identified (Escudero *et al.*, 2002).

IV. MICROOXYGENATION RESEARCH FINDINGS

A. Generation of acetaldehyde

The increase in DO in wines undergoing MOX is meant to remain low, with the aim of ensuring that the wine will take up the oxygen without O_2 reaching higher levels where negative impacts may result, such as microbial spoilage (see below). Early descriptions of the operation indicated that with an oxygen dosage rate of up to 3 mL/L/month, the concentration of DO did not exceed 0.05 mg/L (Moutounet *et al.*, 1996). In one study, an O_2 concentration of 0.04 mg/L was observed for MOX at a rate of 5 mL/L/month, similar to wines in the barrel, while higher values were seen during the cooler months of the year (Castellari *et al.*, 2004). A further report also for red wine MOX at 5 mL/L/month for several months determined values of DO at 0.1–0.25 mg/L, with no significant effect of having oak present (Laurie *et al.*, 2008; Waterhouse and Laurie, 2006). Higher rates of MOX at 30–60 mL/L/month for a few days led to higher O_2 concentrations that could exceed 2 mg/L. These levels dropped back to around 0.025 mg/L when the MOX rate was just 1 mL/L/month post-MLF, whereas the control wines without O_2 additions remained at about 0.01 mg/L or less. These results indicated that even when very small MOX rates are applied that an increase in DO content can be

detected, meaning that the rate of oxygen consumption is slower than the rate of oxygen dissolution (Laurie *et al.*, 2008). Similar low O_2 concentrations largely in the range of 0.01–0.06 mg/L were recorded for wines undergoing MOX at 1.5–2.5 mL/L/month, with similar levels seen for wines stored in barrels (Nevares and del Alamo, 2008).

Measurements of acetaldehyde accumulation were reported in some of the earliest descriptions of the MOX process, where 5 months of MOX at 3 mL/L/month was found to raise the acetaldehyde concentration to 33 mg/L, compared to a control wine at 13 mg/L (Moutounet *et al.*, 1996). Further trials at Oenodev for a Syrah wine in 300-L tanks, and subject to elevated O_2 delivery rates of 30, 60, or 90 mL/L/month for 3 weeks, have shown that acetaldehyde will progressively accumulate to be perceived by a tasting panel from an early stage. Increased concentrations of acetaldehyde by GC were confirmed for all treatments over the control by the end of the trial, with very high concentrations (50 mg/L) seen in the 90-mL/L/month MOX treatment (Oenodev, 2009).

In the presence of free SO_2, it can be expected that acetaldehyde will bind with the SO_2, and thus the acetaldehyde build up might be seen through an increase in bound SO_2. An increase in bound SO_2 was seen in one study on Pinot noir wine subject to MOX at 3 mL/L/month for 2 months, at 6 versus 2 mg/L bound SO_2 in the control wine (Lesica and Kosmerl, 2006). A buildup of bound SO_2 was observed in another study using an alternative microoxidation approach at glassy carbon rods, where the acetaldehyde production was quite marked due to the electrochemical oxidation of ethanol, but it was only toward the end of the 12-week trial that the measured acetaldehyde increased significantly (Fig. 4.5; Fell *et al.*, 2007). However, a decrease in both free and bound SO_2 might instead be observed due to oxidative losses of SO_2 outweighing the binding of SO_2 with acetaldehyde, as was seen in related periodic oxygenation experiments in the same trial.

Throughout the course of MOX trials with commercial scale Monastrell wines, the concentration of acetaldehyde was not found to increase over a 5-month period (Cano-Lopez *et al.*, 2006), even though more colored compounds involving acetaldehyde were found to form in the MOX wines (see below). However, in similar trials, the acetaldehyde concentration was higher with the MOX treatments after a final MOX phase at 3 mL/L/month post-MLF by up to an additional 13 mg/L; both free and total SO_2 were depleted in the MOX wines by the end of the trial (Cano-Lopez *et al.*, 2008). In these trials, the MOX operation was discontinued during MLF for a period of 1–2 months, on the expectation that the bacteria will consume the acetaldehyde produced during this period.

In a further trial involving a high O_2 dosage rate (76 mg/L/month in a pilot trial with 141-L tanks), binding of acetaldehyde with anthocyanins and flavanols was suggested as a reason for low concentrations of

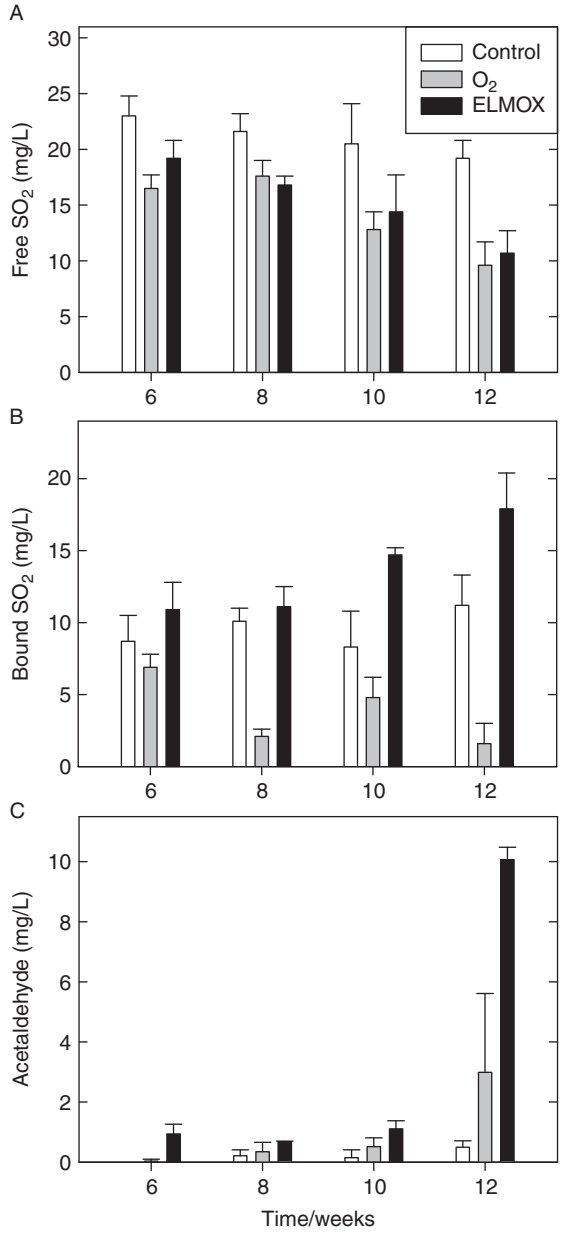

FIGURE 4.5 Changes in the concentrations of (A) free SO_2, (B) bound SO_2, and (C) acetaldehyde during the final 6 weeks of a trial using periodic oxygen additions (O_2) and electrochemical microoxidation (ELMOX). Reprinted with permission from Fell *et al.* (2007). Copyright 2007 American Society for Enology and Viticulture.

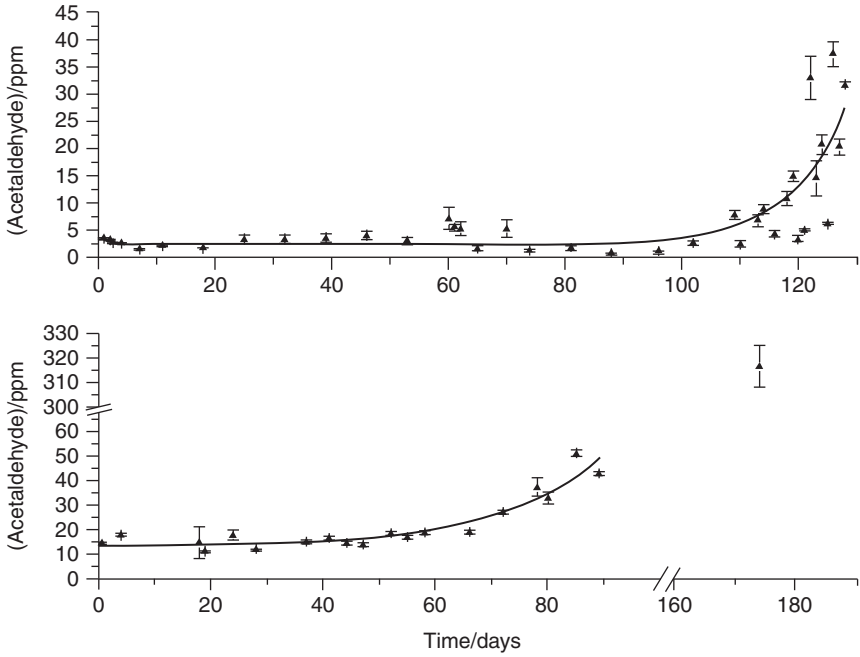

FIGURE 4.6 Development of acetaldehyde concentrations during the microoxygenation of a Merlot wine for (A) a 141-L pilot plant study and (B) a 2400-L study. Reprinted with permission from Carlton *et al.* (2007). Copyright 2007 American Chemical Society.

acetaldehyde being observed for several weeks prior to a marked increase in measured acetaldehyde by about 1 mg/L/day (Fig. 4.6; Carlton *et al.*, 2007). The point at which excess free acetaldehyde begins to be observed (also seen in commercial scale 2400-L tanks at the lower O_2 rate of 9.3 mg/L/month) could then be used as a marker for monitoring the progress of the MOX, and may indicate when oxygenation needs to be decreased to avoid elevated levels of aldehydes leading to overoxidized characters in the wine.

B. Influence of SO_2 and wine antioxidants

It has been widely recognized in the practical application of MOX, that the concentration of free SO_2 in the wine has a major impact upon the oxygenation process (Paul, 2000; Vinovation, 2009). Rather than reacting directly with DO, free SO_2 has been found to have an antioxidant effect through a fast scavenging of hydrogen peroxide (Danilewicz, 2003), and to play a role in binding up the acetaldehyde produced in wine oxidation processes (Danilewicz *et al.*, 2008). SO_2 has a further influence on

polyphenol oxidation processes through a rapid reduction and regeneration of certain oxidized polyphenols (Cheynier *et al.*, 1989, 1993; Saucier and Waterhouse, 1999). In a recent examination of the interaction of polyphenol quinones with wine antioxidants, the rate of reduction was most rapid in the case of caffeic acid, a representative hydroxycinnamic acid, and also with the flavan-3-ol catechin, but very little reduction was evident with the flavonol quercetin (Makhotkina and Kilmartin, 2009). The removal of hydrogen peroxide, acetaldehyde, and quinones by free SO_2 will all alter chemical processes when a wine is exposed to oxygen.

Similar quinone reduction processes have been described for the natural grape and wine antioxidant glutathione, of well-known importance in limiting the effects of enzymatic oxidation upon caftaric acid in grape musts (Singleton *et al.*, 1985). Glutathione has been ascribed a similar protective role in wines due to its ability to react with quinones in preference to varietal aroma compounds such as 3MH (Dubourdieu *et al.*, 2000). The rapid reaction of glutathione with polyphenol quinones, in a similar manner to free SO_2, has also been recently confirmed (Makhotkina and Kilmartin, 2009). While a similar role in reducing quinones has been ascribed to ascorbic acid (Danilewicz, 2003; Peng *et al.*, 1998; Singleton, 1987), this reaction has been more difficult to confirm in model solution studies with typical wine polyphenols (Makhotkina and Kilmartin, 2009). However, the direct scavenging of oxygen, catalyzed by iron species, remains of interest in the case of ascorbic acid (Danilewicz, 2003), although the ascorbic acid present naturally in grapes can be lost quite quickly in the must upon exposure to oxygen (Singleton, 1987).

The influence of SO_2 on a Merlot wine undergoing MOX for 16 weeks was examined in 15-L research vessels with O_2 supplied through fluorinated ethylene–propylene tubing at 10 mg/L/month (Tao *et al.*, 2007). The use of a polymer membrane for the oxygen supply allows low flow rates and multiple small-scale research tanks to be employed to address effects in a reproducible manner. The control wine had very low levels of measured SO_2, and was compared to further treatments with additions of 50, 100, and 200 mg/L of SO_2. These additions had an immediate bleaching effect on the wine pigments leading to a decrease in the 520-nm absorbance, by up to half in the case of the 200 mg/L SO_2 additions (Fig. 4.7). Throughout the course of the trial, the SO_2 concentrations declined progressively, and a corresponding reversal of the bleaching effect was also seen; wines stored in bottles from the beginning of the trial showed only a small decline in SO_2 during the 16-week trial. The SO_2 content had a significant moderating effect on wine-aging processes. The content of monomeric anthocyanins declined and sulfite-resistant pigments increased quite rapidly over the first 10 weeks of the trial in the lower SO_2 wines (and after 7 weeks in the wine that received an initial 100 mg/L SO_2 addition, when the free SO_2 content dropped below

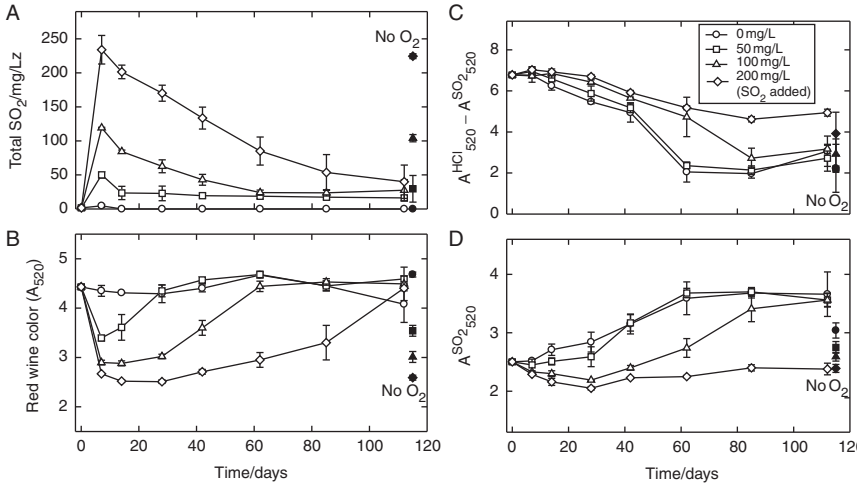

FIGURE 4.7 Concentration of (A) total SO_2, (B) red wine color given by 520-nm absorbance, (C) monomeric anthocyanins given by the subtractive spectrophotometric measure ($A_{520}^{HCl} - A_{520}^{SO_2}$), and (D) sulfite-resistant pigments ($A_{520}^{SO_2}$); for a Merlot wine undergoing MOX with different initial SO_2 additions ($n = 3$). Values for wines stored in bottles until the end of the trial with "no O_2" are shown on the right. Reprinted with permission from Tao *et al.* (2007). Copyright 2007 American Chemical Society.

25 mg/L). The 200 mg/L SO_2 wine, on the other hand, showed little increase in sulfite-resistant pigments over the course of the trial.

Further effects on the polyphenol content of the wines were seen in this trial (Tao *et al.*, 2007), including greater losses of catechin and malvidin-3-glucoside under low SO_2 conditions; by contrast, more quercetin was lost in the MOX wines under a higher SO_2 content, which can be potentially related to the limited ability of SO_2 to reduce quercetin quinones (Makhotkina and Kilmartin, 2009), along with further reaction products arising from the interaction of quercetin with SO_2. By contrast, hydroxycinnamic acids such as caftaric acid and caffeic acid were largely unaffected by MOX while adequate concentrations of free SO_2 were maintained in the wines.

C. Effects on red wine color and polyphenol development

The trends in wine pigment development seen above for the SO_2 study have been consistently reported in MOX trials. These include a greater formation of sulfite-resistant pigments, a more rapid loss of monomeric anthocyanins, and a greater retention of wine color. Higher color intensity was one of the findings reported in early research work on the MOX

technology for wines subject to O_2 additions at 1 and 3 mL/L/month for 5 months (Moutounet *et al.*, 1996). Further research at Oenodev using higher O_2 rates of 30–90 mL/L/month for 3 weeks also produced color intensities up to 50% higher under MOX (Oenodev, 2009). The application of MOX to a Barbera wine for 45 days at 1.7–2.5 mL/L/month in 50-L tanks led to higher color intensity and the quicker development of sulfite-resistant pigments (Bosso *et al.*, 2000). An early trial on Pinot noir wine subject to MOX at 2 mL/L/month for 7 months in a 1000-L tank also showed that MOX accelerated reactions in which anthocyanins combine with tannins (Castel *et al.*, 2001).

In a detailed survey of the pigment composition of a Cabernet Sauvignon wine subject to MOX at 5 mL/L/month for 7 months, several spectroscopic and LC measures were compared (Table 4.1; Atanasova *et al.*, 2002). The MOX wine showed a larger loss of free anthocyanins (determined by LC), and greater development of sulfite-resistant pigments (A_{520}^{SO2}), than the control wine, which also showed pigment development in this direction. The loss of wine color density ($A_{620} + A_{520} + A_{420}$) that was evident in the control wine was also lessened as a result of the MOX operation, despite the greater loss of free anthocyanins. On the other hand, the increase in 420-nm absorbance associated with aged wines was no greater in the case of the MOX wine than the control. Importantly, among the individual pigment compounds quantified using LC, MOX led to a greater formation of compounds involving acetaldehyde, including the pyranoanthocyanins and ethyl-bridged compounds.

In a commercial scale trial using MOX at 5–10 mL/L/month on a Cabernet Sauvignon wine in 11,000–45,000-L tanks, the 420-nm and 520-nm absorbances were greater in wines subject to MOX, including tanks in which toasted oak staves or segments were added at 3 g/L (McCord, 2003). HPLC analyses also showed a larger polymeric anthocyanin peak in the MOX wines (and more so with added oak segments), and lower concentrations of the monomer malvidin-3-glucoside. Some further differences were seen in the concentrations of individual polyphenols, with lower epicatechin and quercetin levels in the final MOX wines.

TABLE 4.1 Changes in wine color parameters for a Cabernet Sauvignon wine subject to MOX for 7 months at 5 mL/L/month; data from Atanasova *et al.* (2002)

Color parameter	Initial	Control	MOX
Free anthocyanins (abs. units)	23.20 ± 0.70	14.92 ± 0.27	12.62 ± 0.27
$A_{620} + A_{520} + A_{420}$	21.8 ± 0.2	18.5 ± 0.1	21.1 ± 0.1
$A_{520}^{SO_2}$	4.03 ± 0.06	4.73 ± 0.12	5.33 ± 0.06
A_{420}/A_{520}	0.2 ± 0	0.6 ± 0	0.6 ± 0

In a further trial involving large commercial wine tanks, and a control tank of similar size, MOX rates of 1.5–4 mg/L/month were applied to various red wines post-MLF with free SO_2 maintained at 25–35 mg/L throughout (du Toit *et al.*, 2006a). MOX was more effective at increasing the color density of younger red wines, where more sulfite-resistant pigments developed alongside a greater loss of catechin monomers. These trends also translated into a greater proportion of polymeric pigments in some of the MOX wines at the expense of free anthocyanins (Fig. 4.8).

It was also recognized that difficulties in conducting replicate experiments on a commercial scale were a problem that might be overcome through new systems to accurately deliver lower rates of O_2 into smaller tanks (du Toit *et al.*, 2006a).

An extended series of studies on Monastrell wines was undertaken using 17,500-L tanks, and two oxygenation levels set initially at 5 mL/L/month (T1) and 10 mL/L/month (T2) for 3 weeks prior to MLF, during which the MOX operation was stopped, before being restarted at lower rates post-MLF for further 2 months, finishing at rates of 1.5 and 2.5 mL/L/month, respectively (Cano-Lopez *et al.*, 2006). A number of color properties were examined in detail, and these confirmed that monomeric anthocyanins such as malvidin-3-glucoside (malv-3-gluc) decreased more rapidly, and more sulfite-resistant pigments were formed at higher O_2 exposures (Table 4.2). In addition, the concentrations of various ethyl-linked compounds (e.g., malv-3-gluc-ethyl-cat) and pyranoanthocyanins

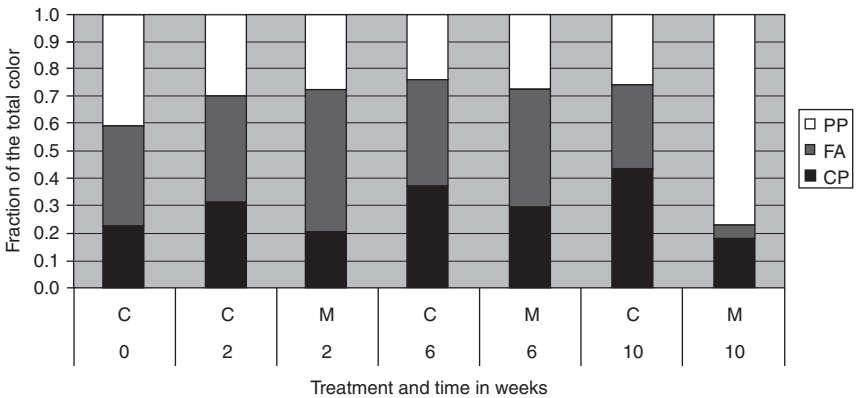

FIGURE 4.8 Development of wine color properties for wines undergoing MOX at 3 mg/L/month (M), versus control wines (C); with the fraction of color due to the polymeric fraction (PP), free anthocyanins (FA), and copigmentation (CP) reported. Reprinted with permission from du Toit *et al.* (2006a). Copyright 2006 South African Society for Viticulture and Enology.

TABLE 4.2 Changes in selected wine color parameters for a Monastrell wine subject to MOX, initially at 5 mL/L/month (T1) and 10 mL/L/month (T2), at the end of MOX and after 6 months in the bottle; data from Cano-Lopez *et al.* (2006, 2007)

	Initial	Control	T1	T2
Malv-3-gluc (mg/L)	157.9 ± 1.5	109.6 ± 1.0	105.6 ± 3.3	94.1 ± 8.0
Malv-3-gluc-ethyl-cat (μg/L)	1082 ± 33	1215 ± 99	1271 ± 52	1446 ± 108
Malv-3-glu-4-vinyl (μg/L)	1343 ± 110	978 ± 29	1281 ± 110	1331 ± 268
Malv-3-glu-cat (μg/L)	800 ± 108	900 ± 110	900 ± 125	800 ± 148
HPLC polymeric peak (mg/L)	19.7 ± 0.2	19.2 ± 0.7	21.6 ± 2.3	23.4 ± 1.1
$A_{620} + A_{520} + A_{420}$	12.7 ± 0.1	11.6 ± 0.1	13.7 ± 0.1	14.0 ± 0.2
$A_{520}^{SO_2}$	1.9 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.9 ± 0.1
A_{420}/A_{520}	0.59 ± 0.01	0.61 ± 0.01	0.60 ± 0.01	0.61 ± 0.01
After 6 months in the bottle				
$A_{620} + A_{520} + A_{420}$		10.8 ± 0.7	12.4 ± 0.3	12.4 ± 0.5
$A_{520}^{SO_2}$		2.5 ± 0.2	3.4 ± 0.1	3.5 ± 0.1
Σ Ethyl-linked compounds (mg/L)		6.69 ± 0.23	6.65 ± 0.55	7.04 ± 0.39
Σ Pyranoanthocyanins (mg/L)		6.03 ± 0.67	9.23 ± 1.05	9.40 ± 0.72

(e.g., malv-3-glu-4-vinyl; Fig. 4.3) were higher in the MOX wines versus the control, whereas concentrations of direct adducts (e.g., malv-3-glu-cat) were not different. Further, there was no extra increase in the hue value (A_{420}/A_{520}) in the MOX wines.

The wines were tested again after 6 months in the bottle (Cano-Lopez *et al.*, 2007), and a number of the color characteristics which differentiated the MOX from the control wines continued to hold. These included more sulfite-resistant pigments, which continued to increase in all of the wines (alongside a decrease in monomeric anthocyanins) (Table 4.2). There were also higher wine color density, ethyl-linked compounds, and pyranoanthocyanins in the MOX wines, indicating that the benefits of the MOX step had persisted, despite overall decreases in these measures across the wine samples (contrasted by an increase in color density for wines stored in oak barrels rather than in bottles for 6 months, to a value of $A_{620} + A_{520} + A_{420} = 14.2$ for previously MOXed wines). Many of these trends were confirmed for trials undertaken on three additional Monastrell wines from a later vintage, starting initially at 10 mL/L/month O_2 delivery (Cano-Lopez *et al.*, 2008). In particular, monomeric

anthocyanins were lost and ethyl-linked compounds were formed more rapidly in the MOX wines, along with more sulfite-resistant pigments.

Among further MOX trials, it has been shown that wine color density and polymeric pigments increased, leading to more stable color resistant to sulfite bleaching, for a Pinot noir wine subject to MOX at 3 mL/L/month for 2 months in 30-L tanks (Lesica and Kosmerl, 2006). The issue of the combined effect of MOX and added oak chips at 2 g/L was examined in a trial on a Sangiovese wine with MOX applied at 3 mL/L/month for 90 days in 50-L tanks post-MLF with 50 mg/L total sulfites, or at 9 mL/L/month when 90 mg/L yeast lees were also included (Sartini *et al.*, 2007). The color intensity ($A_{520} + A_{420}$) was again higher in the MOX wines (even against a slight loss of color suggested for the absorbing capacity of the chips), linked to the greater formation of red polymeric color and associated loss of anthocyanins (Table 4.3). Further results included a greater loss of small polyphenols such as quercetin and catechin in the MOX wines (but some protection for caftaric acid afforded with added lees), but somewhat surprisingly a decline in wine hue (A_{420}/A_{520}), which may have reflected the color of the particular polymeric pigment species formed in this trial. The effect of added lees in addition to MOX was difficult to evaluate given the different MOX rates employed. The subsequent storage of these wines in

TABLE 4.3 Changes in selected wine color parameters for a Sangiovese wine subject to MOX with and without added oak chips and yeast lees; data from Sartini *et al.* (2007)

	Control	Chips	Chips + MOX (3 mL/L/month)	Chips + Lees + MOX (9 mL/L/month)
$A_{520} + A_{420}$	7.7 ± 0.010	7.645 ± 0.006	7.788 ± 0.009	7.84 ± 0.003
Total anthocyanins (mg/L) by HPLC	315± 0.8	316 ± 0.2	305 ± 1.8	301 ± 1.6
Red polymeric color	2.51± 0.005	2.548 ± 0.007	2.613 ± 0.007	2.665 ± 0.013
A_{420}/A_{520}	0.54 ± 0.002	0.541 ± 0.003	0.506 ± 0.002	0.507 ± 0.004
Quercetin (mg/L)	8.36 ± 0.173	5.61 ± 0.074	4.16 ± 0.293	5.1 ± 0.030
Catechin (mg/L)	45.3 ± 0.36	44.5 ± 0.16	39.5 ± 0.18	38.9 ± 0.13
Caftaric acid (mg/L)	45.4 ± 0.82	45.7 ± 0.07	43.3 ± 0.32	45.6 ± 0.05

the bottle for 5 months showed that the MOX wines were more stable in terms of many of the measures employed.

The development of a Cabernet Sauvignon wine, undergoing MOX at different oxygen dosage rates, was undertaken at the University of Auckland in 15-L research tanks with O_2 supplied through a previously calibrated dense polymer membrane (Dykes, 2007; Dykes and Kilmartin, 2007). Using a PCA projection of the complete HPLC output for chromatograms at 280, 320, 265, and 520 nm (targeting polyphenols generally, hydroxycinnamic acids, flavonols, and anthocyanins, respectively, and using peak alignment software), the wines were seen to follow a similar trajectory across the 15-week trial, but the wines subject to MOX at the highest rates advanced most rapidly (Fig. 4.9). The slowest development was seen in the control wine without added O_2. These results illustrate how the oxygen dosage can affect the rate of wine-aging processes, which are accelerated as a result of MOX.

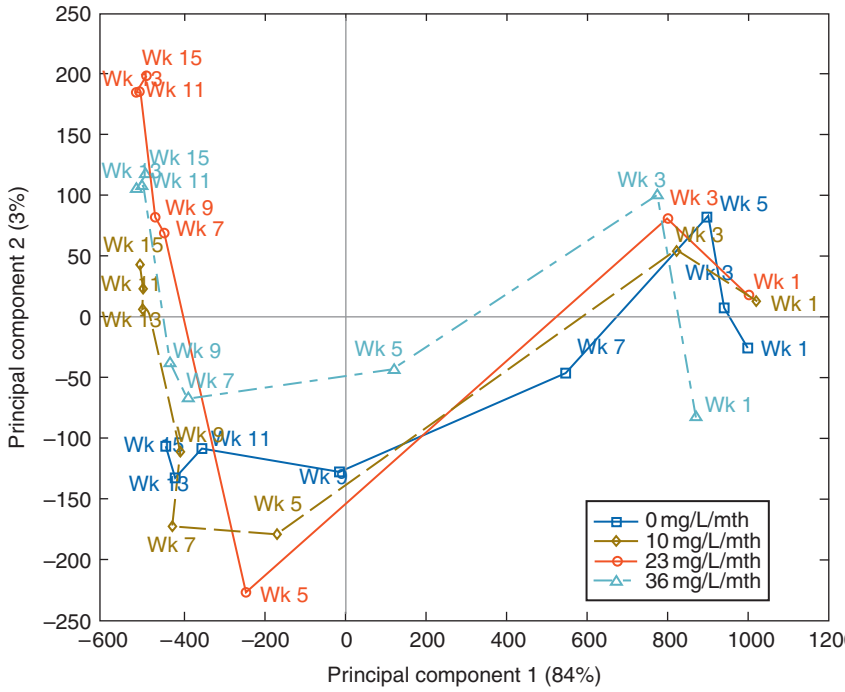

FIGURE 4.9 PCA projection of the combined results for HPLC chromatograms taken at 280, 320, 365, and 520 nm, for a Cabernet Sauvignon wine undergoing MOX at various oxygen dosage rates during 15-week trial, with sampling every 2 weeks (Wk1, Wk3, etc.). Reprinted with permission from Dykes and Kilmartin (2007). Copyright 2007 Winetitles Pty Ltd.

A series of MOX studies were undertaken on Mencia, Tinta de Toro, Tinta del Pais, and Tempranillo wines, in which effects on colored compounds and polyphenol antioxidant measures were examined (Perez-Magarino *et al.*, 2007, 2009; Rivero-Perez *et al.*, 2008). Trials on each of the four wines were conducted in 2000-L tanks across 3 consecutive years, during which 23–42 mL of O_2 was added over a 3-week period pre-MLF. The rate and duration of MOX was determined by individual factors for each of the 12 wines, such as the presence of any reductive characters, and MOX was stopped when tasters considered that vegetal characters had been lost and green tannins had evolved into hard tannins (Perez-Magarino *et al.*, 2007). Across the 12 trials, a higher percentage of polymeric anthocyanins and greater loss of monomeric anthocyanins were seen in the MOX wines. A higher color intensity was seen in several cases and an increase in blue tonalities. In a survey of 162 wines made from these four Spanish varieties, the changes in phenolic composition after MOX did not lead to differences in the results obtained for antioxidant scavenging-based assays (Fig. 4.10), which was related to the maintenance of a similar number of hydroxyl groups after condensation reactions had occurred (Rivero-Perez *et al.*, 2008). The assays where some differences were observed were one in which DNA damage was monitored (where MOX wines were more effective), and a lipid peroxide assay (ABAP-LP) (where a lower activity was observed with the MOX wines). It was suggested that a greater proportion of polymeric phenols in the MOX wines could have been more effective in protecting DNA against damage, but were less able to be incorporated into microsomal membranes as required for the lipid peroxidation assay. As well as providing some

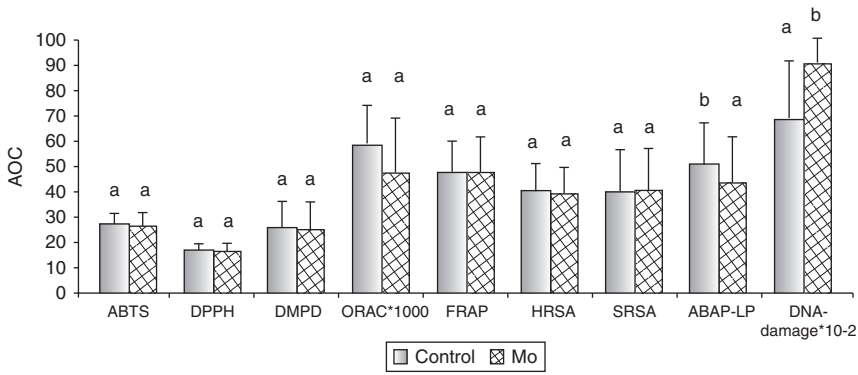

FIGURE 4.10 Average responses in antioxidant assays for microoxygenated (mo) and control wines, for 4 replicates of 162 Spanish wines ($n = 648$). Values that are significantly different (LSD test, $p = 0.05$) are given different letters. Reprinted with permission from Rivero-Perez *et al.* (2008). Copyright 2008 Elsevier.

interesting insights into the methodology of the different antioxidant assays, this extensive study also showed how a number of polyphenol properties can remain largely unchanged as a result of the MOX process.

In further trials on Mencia and Tinta del Paris wines, the presence of French or American oak chips at 4 g/L during MOX led to very few observable differences in terms of polyphenol and color parameters (Perez-Magarino *et al.*, 2009). Some influence of oak toasting degree was related to the ability of compounds released from the chips to combine with anthocyanins to form new pigments, and to a different extent with the two wines examined, but these effects were outweighed by the impact of MOX itself on phenolic composition and wine color.

A range of wine parameters were also investigated for discrete oxygen doses at monthly intervals to supply 2.5 and 5.0 mg/L/month for a Pinotage wine in 20-L tanks for up to 6 months with free SO_2 maintained at 25 mg/L, and a second trial with O_2 applied every 2 weeks for 2 months at a target addition of 1.0 mg/L/month (De Beer *et al.*, 2008); air was supplied through a gas diffuser until the DO reached the required concentration. In the first trial, a decline was observed in the free radical scavenging capacity of the oxygenated wines as measured using the ABTS assay, which was seen as a negative outcome. These changes were matched by a decrease in total phenol content (using the Folin-Ciocalteu reagent) and total monomer content (by HPLC) in the oxygenated wines, with more pronounced decreases in individual flavonols, flavan-3-ols, and monomeric anthocyanins and an associated increase in polymeric anthocyanins and changes in L*a*b* color parameters (to lower L* and a* values); the hydroxycinnamic acids such as caftaric acid, on the other hand, remained largely unchanged. The phenolic composition was not significantly altered during the second trial by the lower 1.0 mg/L/month O_2 additions. While this study was not an MOX procedure as such, but rather a study in periodic oxygen additions chosen for the smaller research scale vessels, the results did show polyphenol and color trends consistent with previous MOX studies, and provided useful information on sensory aroma and mouthfeel measures (see below).

Changes in the polyphenols present in a diethyl ether extract of wines subject to MOX at 4 mL/L/month for 5 weeks have been observed, alongside molecular changes revealed through the application of ^1H NMR spectroscopy, which pointed to an increase in oxygen-containing compounds and an increase in acetylated sugars (Conte, 2008; Piccolo *et al.*, 2009). A further analytical methodology that has been recently applied to characterize microoxygenated wines is the electronic tongue based upon potentiometric chemical sensors (Rudnitskaya *et al.*, 2009). Shiraz wines from three successive vintages were treated with MOX post-MLF in 300-L tanks at a rate of 2 mL/L/month, with and without oak chips added at 14 g/L, and with free SO_2 maintained at 30–35 mg/L.

The wines were then bottled and stored for 8–32 months before a wide range of chemical and instrumental analyses were undertaken at the same time. Using a total of 28 physicochemical parameters, the wines were clearly separated by vintage, and according to the treatments applied in some cases. Separation by vintage was also largely achieved using the electronic tongue data. The MOX wines were found to be lower in ionized anthocyanins, but higher in CIE-Lab coordinates (except a*). These two studies show the potential for new instrumental methodologies to be applied in the study of red wine aging and the effects of MOX treatments.

D. Effects on aromas

The increase in aldehydes leading to oxidized characters in wines subject to high O_2 dosages or prolonged MOX was noted above, and their appearance has been associated with excessive oxygenation and a detrimental effect on the wine. Prior to this point, benefits of lowering vegetative or reductive odors, and increasing fruity or varietal aromas, have been ascribed to MOX (Moutounet *et al.*, 1996; Parish *et al.*, 2000). The chemical changes affected by wine oxygenation that are responsible for these sensory observations have yet to be determined, although recent studies have measured aroma concentrations and/or undertaken sensory trials and have shown that certain compounds are not greatly affected by the addition of O_2, or provided an indication of the aroma compounds that can change in MOX wines.

The impact of MOX upon reductive odors was included in the study of McCord (2003) for MOX at 5–10 mL/L/month over 5 months on a Cabernet Sauvignon wine in commercial scale tanks. Lower concentrations of methyl mercaptan and ethyl mercaptan were observed in the oxygenated wines, but no impact was seen upon disulfides, in spite of the suggestion that concentrations of the disulfides could increase due to direct oxidation of sulfides. Dimethyl sulfide concentrations were not affected, except that lower concentrations were seen in wines with added toasted oak staves or segments, with or without MOX. The concentrations of various oak extracted compounds were also measured in this study, with similar levels seen with and without MOX alongside appreciable increases due to the presence of the oak staves or segments; in some cases (e.g., lactones and vanillin), oxygenation appeared to enhance aroma extraction.

In an initial survey at the University of Auckland in 2004 on Cabernet Sauvignon, Merlot and Malbec wines, subject to MOX in 2000-L tanks at 4–8 mg/L/month for 12 weeks post-MLF, no changes were seen in the aroma profiles of the wines (vs. controls) for a wide range of aroma compounds, including herbaceous methoxypyrazines and C6 alcohols, floral terpenes and β-ionone, or for fruity esters and higher alcohols (Rowdon, 2005). Likewise, the concentrations of the varietal thiol 3MH,

in the range of 300–600 mg/L, were the same in the MOX and control wines providing no obvious candidates for diminished vegetal characters and enhanced fruitiness in wines treated by MOX.

For the MOX trials undertaken with four South African red wines on a commercial scale at 1.5–4 mg/L/month, sensory evaluations were undertaken to provide data on aroma and tasting intensities (du Toit *et al.*, 2006a). Triangle tests confirmed that a sensory panel could distinguish MOX from control wines after a suitable treatment period, although mean scores for several sensory attributes, such as fruitiness, spiciness, vanilla/butterscotch, and oak/coconut (the last two were higher for wines stored in barrels), were not significantly different between MOX and control wines. The oxidized/aged character was scored higher in one of the 3 mg/L/month MOX wines after 6 months compared to the control or the lower 1.5 mg/L/month dosage wine, which was seen as an indication that the wine had become overaged, reflected in a lower quality rating for this wine. In the monthly oxygenation of Pinotage wines in 20-L tanks (at 2.5 and 5.0 mg/L/month via periodic O_2 additions), scores for the berry/plum intensity and for overall wine quality both fell in the oxygenated wines (De Beer *et al.*, 2008). On the other hand, in a second trial undertaken using 1.0 mg/L/month O_2 additions, a decline in the berry/plum intensity was still observed, but without a loss in the overall quality score, suggesting that care needs to be taken with the level of O_2 input so as not to overoxidize the wine. At the same time, we can note that periodic O_2 additions need to be monitored carefully to ensure that volatile compounds are not stripped along with air bubbles that escape from the wine during this type of oxygenation.

Surveys of several classes of aroma compounds were also included in MOX studies undertaken on Mencia and Tinta de Toro Spanish wines, with the inclusion of the impact of oak barrels or wood chips (Ortega-Heras *et al.*, 2008; Rodriguez-Bencomo *et al.*, 2008). The wines in one of the studies received O_2 doses of 50–60 mL/L/month for 10 days pre-MLF, said to be necessary to eliminate the reductive compounds that develop after fermentation and allow improved fruit expression through the removal of vegetal properties, prior to a lower dose of 20–30 mL/L/month applied for up to 10 days (Ortega-Heras *et al.*, 2008). At the end of MOX, all of the wines were racked off and aged for 12 months in oak barrels. Aroma analyses were undertaken at the end of alcoholic fermentation (point A), at the end of the MOX treatment (point B), at the end of MLF (point C), upon transfer to barrels (0MB), and after 4, 8, and 12 months in barrel (4MB, 8MB, 12MB), included in the profile of esters presented in Fig. 4.11.

In the case of the esters, considered responsible for the fruity aromas of young wines, there was no consistent difference brought about by the oxygenation procedure between the MOX (dotted lines) and control (solid

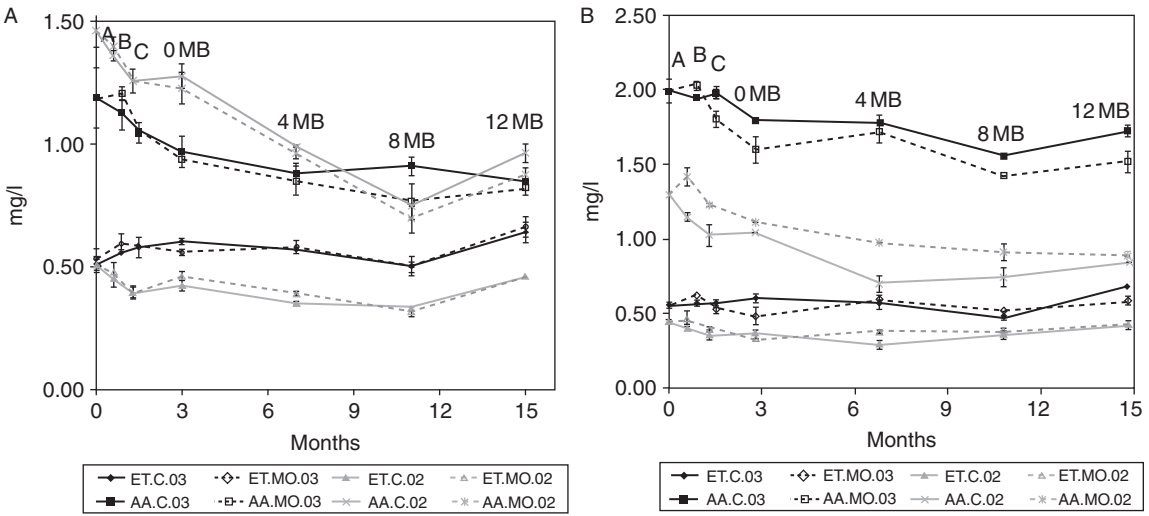

FIGURE 4.11 Evolution of ethyl esters (ET) and alcohol acetate esters (AA) for (A) Mencia and (B) Tinta de Toro wines, and for microoxygenated (MO) and control (C) wines from 2002 (02) to 2003 (03). Reprinted with permission from Ortega-Heras *et al*. (2008). Copyright 2008 Springer-Verlag.

lines) wines, against a background of hydrolysis losses, especially for the acetate esters. Little influence of MOX was found with the concentrations of C6 alcohols and terpene compounds, and only small increases in short chain fatty acids and higher alcohols in some of the MOX wines. Once again, the perception of a lowering of vegetal aromas could not be linked to changes in the concentrations of the aroma compounds examined in this study. On the other hand, there was a clear barrel influence upon certain volatile compounds, with increases seen in furfural, eugenol, and vanillin, for example, during barrel aging. What was observed was fewer aldehydes in the MOX wines, linked to aldehydes extracted from wood being taken up in polyphenol-linking reactions. In a second study, MOX was undertaken on a Mencia wine both before (44 mL/L/month for 17 days in 1500-L tanks) and after MLF in the presence of different oak chips (2 mL/L/month for 28 days in 300-L tanks) (Rodriguez-Bencomo *et al.*, 2008). Again, the evolution of oak-derived aroma compounds with time was clearly seen, and the highest concentrations were typically reached after about 21 days, including furfural, whiskey lactone, eugenol, and vanillin, among other compounds, with differences seen according to the type of oak chip applied. On the other hand, there was practically no effect of MOX upon the extraction of the volatile compounds, with some minor differences noted in the concentrations of vanillin and syringaldehyde at a few time points.

A further extensive aroma survey was undertaken with Cabernet Sauvignon and Tempranillo wines subject to MOX at 60 mL/L/month for 15 days pre-MLF, and for subsequent storage in oak barrels or stainless steel tanks, with and without MLF, and with and without additional MOX (Hernandez-Orte *et al.*, 2009). Samples were taken pre-MLF, after MLF (when a free SO_2 concentration of 32 mg/L was established), and after 4 and 8 months of maturation. In this study, a number of differences, usually small, between MOX and control wines were noted, but the two wines responded differently, and more compounds were found to be affected by the use of MOX in the Cabernet Sauvignon wine. For example, a lower concentration of acetoin was found in the Tempranillo wines subject to MOX, but a higher concentration in the MOX Cabernet Sauvignon wines (pre-MLF) (Table 4.4). Further differences included higher concentrations of methyl vanillate in the MOX wines, while after MLF, the vanillins were generally at higher concentrations in the non-MOX wines. Volatile phenols such as guaiacol were found at higher concentrations in the non-MOX wines.

On the other hand, the terpene content was not consistently affected by MOX, the only differences being a higher initial level of citronellol in the Tempranillo wine, which disappeared after MLF, and more geraniol in the MOX Cabernet Sauvignon wine after 4 months, but this became lower after 8 months of aging. Some higher initial concentrations of ethyl

TABLE 4.4 Concentrations of selected volatile compounds prior to malo-lactic fermentation in Cabernet Sauvignon and Tempranillo wines; data from Hernandez-Orte *et al.* (2009)

	Cabernet Sauvignon		Tempranillo	
	No MOX	MOX	No MOX	MOX
Acetoin (mg/L)	13.7 ± 9.4	12.8 ± 9.5	9.76 ± 10	37.9 ± 7.55
Methyl vanillate (μg/L)	4.38 ± 0.34	3.82 ± 0.20	20.0 ± 0.8	16.8 ± 2.83
Guaiacol (μg/L)	2.78 ± 0.77	1.87 ± 0.14	1.96 ± 0.94	0.94 ± 0.24
Ethyl decanoate (μg/L)	13.2 ± 0.73	33.6 ± 23.3		
Isobutyl acetate (μg/L)			52.9 ± 6.55	70.0 ± 13.7

esters in the MOX Cabernet Sauvignon wine (e.g., ethyl decanoate) and of acetate esters in the MOX Tempranillo wine (e.g., isobutyl acetate), also disappeared post-MLF, again indicating that effects seen immediately after MOX were often undetectable after MLF and aging. Importantly, no differences in the short chain fatty acids or C6 alcohols (hexanol and Z-3-hexenol) were seen between MOX and control wines, again failing to provide support for the hypothesis that a lowering of the herbaceous character as a result of MOX could be linked to a decrease in the concentrations of the C6 alcohols. Further, the intensity ratings for descriptive analyses undertaken by a sensory panel showed no significant differences between MOX and control wines for Tempranillo pre-MLF, while immediately after MLF, the panel scored the wood note higher in the non-MOX wines. Likewise, ''fresh fruit'' 4 and 8 months after MLF was higher in the non-MOX wines, whereas the currant note was dominant in the MOX wines. In the case of the Cabernet Sauvignon wines, the MOX wines were more herbaceous than the control wines (also seen in the Tempranillo wines before MLF). However, due to a possible association of herbaceousness with green pepper notes (typical of Cabernet Sauvignon), it was suggested that MOX reinforces varietality rather than increasing the herbaceous note (Hernandez-Orte *et al.*, 2009).

The impact of MOX upon reductive aromas is being examined in a further research project at the University of Auckland, using SPME GC–MS (Nguyen *et al.*, 2010). The wines subject to MOX, or stored in an O_2-permeable Flextank, recorded lower concentrations of compounds such as methanethiol (aroma of cooked cabbage, with a perception threshold of 0.3 μg/L; Mestres *et al.*, 2000), and 3-(methylthio)-1-propanol

TABLE 4.5 Concentrations of reductive sulfur compounds and the varietal thiol 3MH (μg/L) in a Cabernet Sauvignon wine in 300-L tanks after 16 weeks of MOX or storage in Flextanks ($n = 3$); data from Nguyen *et al.* (2010)

	Control	MOX (5 mg/L/month)	MOX (20 mg/L/month)	Flextank
Methanethiol	0.87 ± 0.09	0.69 ± 0.09	0.55 ± 0.15	0.60 ± 0.15
Dimethyl disulfide	0.39 ± 0.08	0.31 ± 0.11	0.21 ± 0.03	0.19 ± 0.02
Dimethyl sulfide	11.2 ± 0.5	11.0 ± 1.6	9.6 ± 0.3	9.2 ± 0.2
Methyl thioacetate	3.0 ± 0.3	3.0 ± 0.4	2.9 ± 0.2	2.9 ± 0.1
Ethyl thioacetate	3.2 ± 0.3	3.0 ± 0.2	2.8 ± 0.3	3.1 ± 0.2
2-(Methylthio)-1-ethanol	56 ± 8	42 ± 12	29 ± 4	28 ± 1
3-(Methylthio)-1-propanol	2057 ± 147	1815 ± 343	1335 ± 89	1324 ± 84
3-Mercaptohexanol (3MH)	0.45 ± 0.02	0.47 ± 0.03	0.45 ± 0.04	0.41 ± 0.02

(cauliflower, with a perception threshold of 1200 μg/L; Mestres *et al.*, 2000), compounds that exceeded their perception thresholds in this study (Table 4.5). A decline was also observed with dimethyl disulfide (although this compound was present at concentrations well below the perception threshold of 20–45 μg/L; Mestres *et al.*, 2000), despite suggestions that oxidation processes could lead to increases in disulfide formation (Mestres *et al.*, 2000; Rauhut *et al.*, 1996). On the other hand, oxygenation was again found to have little impact on the varietal thiol 3MH. The concentrations of the S-thioacetates were also similar in MOX and control wines.

E. Effects on mouthfeel

The further benefit ascribed to MOX, of a decrease in astringency and greater smoothness in oxygenated wines (Moutounet *et al.*, 1996), has also been difficult to relate to measurable changes in tannin content and structure. Longer tannin molecules are known to be more astringent (Vidal *et al.*, 2003), and an increase in the total amount of tannin present size is also expected to lead to a more astringent wine, unless other factors intervene such as the formation of bridged structures or capping of tannin chains with anthocyanins (du Toit *et al.*, 2006b; Vidal *et al.*, 2004b). Various observations and experimental results pertaining to wine astringency and related polyphenol content from reported MOX trials are summarized below.

In a trial on a Barbera wine subject to MOX at 1.7–2.5 mL/L/month for 45 days in 50-L tanks, the wines were found to be higher in smoothness 4–5 months after the end of the MOX process (Bosso *et al.*, 2000). In the trial on Cabernet Sauvignon wines subject to MOX at 5 mL/L/month for 7 months (Atanasova *et al.*, 2002), a polymeric fraction from a Toyopearl column was analyzed by thiolysis to determine the MDP. After 7 months, the MDP values were similar for the MOX (12.2 ± 0.9) and control (12.6 ± 0.3) wines, and both greater than the initial wine value of 10.1 ± 0.4. On the other hand, it was noted that the total amount of tannins (by LC), originally 1434 ± 50 mg/L, declined further in the MOX wines (1214 ± 39 mg/L) compared to the control (1340 ± 44 mg/L) after 7 months.

In the commercial scale trial using MOX at 5–10 mL/L/month on a Cabernet Sauvignon wine (McCord, 2003), the MOX wines showed higher tannin levels according to the Adams tannin assay, while no difference was seen in the total phenols measure provided by the Folin-Ciocalteu assay. However, the increase by around 10% in tannin values over the control wines by the Adams assay might not be sufficient to have an appreciable sensory impact. An attempt was made to monitor wine astringency during commercial scale trials during which MOX rates of 1.5–4 mg/L/month were applied post-MLF (du Toit *et al.*, 2006a). In this case, differences in gelatine index values (sometimes higher, sometimes lower in MOX wines vs. the control) did not correlate with sensory ratings (similar intensity scores were given for astringency and bitterness), pointing to the need for more research in this area.

In the trial on a Cabernet Sauvignon wine in 15-L tanks at the University of Auckland using oxygen delivered at 10, 23, and 36 mg/L/month through with a dense polymer membrane for 15 weeks, sensory evaluations were undertaken for various mouthfeel measures (Dykes, 2007; Dykes and Kilmartin, 2007). Using principal response curves to collect together the sensory data, some cycling in the response was seen for each treatment compared to the control, with higher points related to higher mouthfeel and astringency scores being seen earlier with the higher MOX rates prior to a decrease in the measure. Among further chemical analyses undertaken in this trial, the MDP values for a proanthocyanidin extract obtained using Sephadex LH-20 were found to increase from around 11 to 18 over the first 11 weeks of the trial, with little difference between the various O_2 dosage rates, followed by a decline in MDP once SO_2 was added post-MLF, which appeared to have a greater impact than the oxygen supplied. The impact of SO_2 was confirmed in a further experiment using wine stored in 1-L airtight containers (with and without weekly oxygen saturations, and with and without SO_2 added at 100 mg/L; Dykes and Kilmartin, 2007). With added SO_2, the MDP values were seen to decline from days 7 to 21 irrespective of the presence or

absence of added O_2; without SO_2, the MDP values remained steady until day 30, when an increase was seen (and to the same extent with or without added O_2), which was not observed in the wines with added SO_2. In the subsequent trial described above for the impact SO_2 levels have on wine color development using 15-L tanks (Tao *et al.*, 2007), the MDP values remained relatively unchanged for the highest initial SO_2 addition of 200 mg/L, while some decline was observed in the wines with less SO_2 present (Fig. 4.12C), in this case, in the opposite direction to the smaller 1-L trial.

It can be noted that the amount and type of tannins which are collected using various extraction procedures can vary, and in these trials, changes in MDP need to be considered alongside the total amount of tannin extracted, and how well these represent the tannins responsible for mouthfeel properties. In the case of the above trial, the lower SO_2 wines were in fact marked by a greater polyphenol content in the proanthocyanidin extract (Fig. 4.12A; Tao *et al.*, 2007). The tannin level, determined using both the methyl cellulose and BSA-based assays, was also lower in the high 200 mg/L initial SO_2 wine at the end of the trial. Smaller chain lengths can be associated with a lowering of astringency, whereas more total tannin would likely increase astringency; which will dominate with a particular wine is a further question.

In more recent studies, the tannin content measured in terms of BSA protein precipitation remained a little higher in MOX wines from commercial scale trials undertaken on Monastrell wines, starting initially at 10 mL/L/month O_2 delivery (Cano-Lopez *et al.*, 2008). At the same time, changes in tannin MDP values were variable across the trials (sometimes higher, sometimes lower in the MOX wines). No change was recorded in the sensory ''astringency'' measure for the Pinotage wines subject to monthly oxygenation in 20-L tanks (at 2.5 and 5.0 mg/L/month using periodic O_2 additions), although ''fullness'' scores were higher in the oxygenated wines after 6 months (De Beer *et al.*, 2008). It was noted that the optimal oxygenation rate and the time required for sensory quality will vary depending upon the initial tannin and anthocyanin content of the particular wine.

F. Microbiological considerations

Oxygen additions also have the potential to promote the growth of unwanted aerobic microorganisms, particularly if DO concentrations become too high (du Toit *et al.*, 2006b). Problem areas could include growth of *Brettanomyces* yeast, avoided by watching out for residual sugar and maintaining good pH and SO_2 control, and increased levels of volatile acidity (VA) due to acetic acid bacteria, although decreases in VA were noted for one trial on South Australian wines (Paul, 2000).

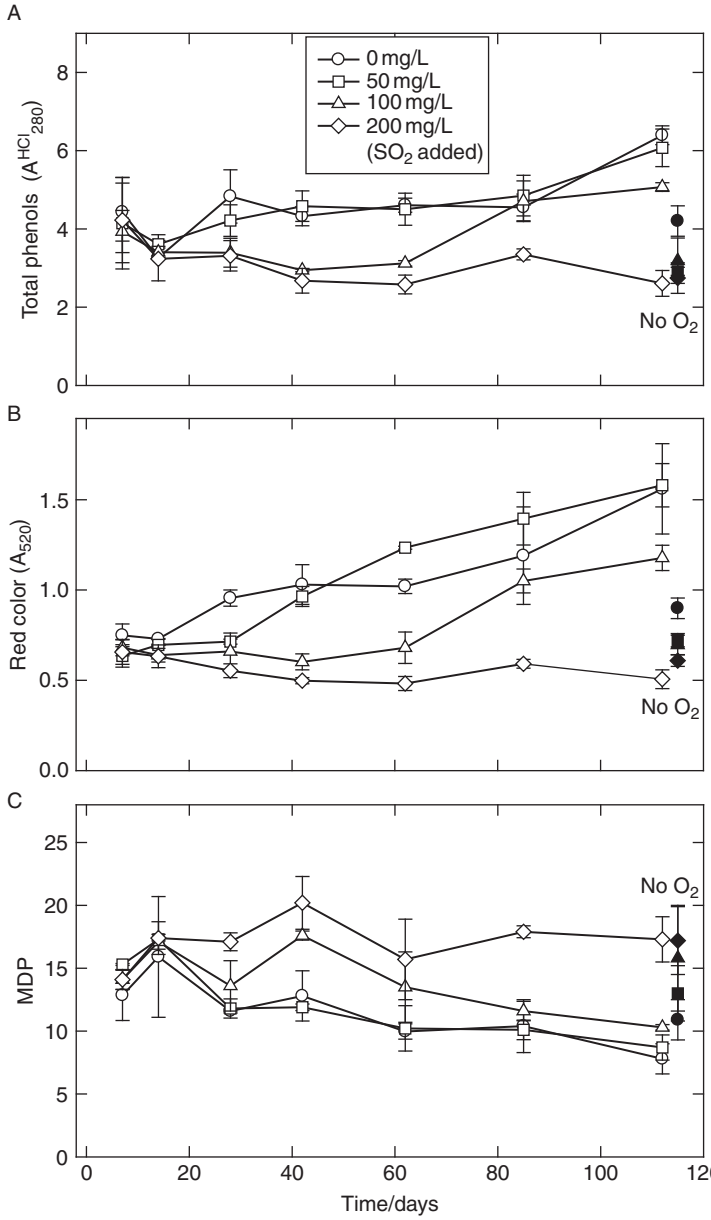

FIGURE 4.12 Concentrations of (A) total phenols given by 280-nm absorbance, (B) red wine color given by 520-nm absorbance, and (C) mean degree of polymerization (MDP) in a proanthocyanidin extract; for a Merlot wine undergoing MOX at 10 mg/L/month with different initial SO_2 additions ($n = 3$). Values for wines stored in bottles until the end of the trial with "no O_2" are shown on the right. Reprinted with permission from Tao *et al.* (2007). Copyright 2007 American Chemical Society.

The growth of acetic acid bacteria and *Brettanomyces* was monitored, by plating and enumerating, during commercial scale MOX trials at 1.5–4 mg/L/month on various South African red wines (du Toit *et al.*, 2006a). Acetic acid bacteria numbers declined in the control tanks, but remained higher in the MOX wines, although an increase in VA was not noted. When the free SO_2 concentration fell to 18 mg/L during MOX, cell counts of *Brettanomyces* increased (along with the sensory barnyard/medicinal character), but dropped off again after addition of SO_2 to 35 mg/L free SO_2. While SO_2 addition can be used to control *Brettanomyces*, it was noted that excessive SO_2 concentrations could inhibit favorable polymerization reactions (du Toit *et al.*, 2006a), thus negating some of the benefits of applying MOX to the wine. An increase in acetic acid concentration in MOX wines, associated with the activity of acetic bacteria, was also seen in Shiraz wines treated with MOX post-MLF in 300-L tanks at 2 mL/L/month (Rudnitskaya *et al.*, 2009).

V. FINAL COMMENTS

The technology of MOX provides winemakers with a lower cost alternative to barrel aging for the development of red wines, while avoiding the effects of higher DO concentrations generated during racking procedures. MOX also provides the researcher with an approach to examine the influence of small doses of O_2, in the absence of oak barrels, to gain more insight into the role that oxygen specifically plays in red wine maturation.

As summarized above, recent MOX studies have consistently confirmed the more rapid development of polymeric pigments associated with more stable red wine color, seen through a decline in monomeric anthocyanins and an increase in sulfite-resistant pigments. The involvement of acetaldehyde in pigment development, itself generated as a product of polyphenol-mediated oxidation processes, has also been demonstrated, along with the moderating influence of SO_2. On the other hand, chemical changes in aroma compounds and in tannin content, relevant to sensory changes in wine aromatic and mouthfeel properties, remain to be determined. Further research avenues that may shed more light on the widely recognized benefits of MOX, namely, a lowering of vegetative and reductive aromas and of astringency, include more studies on the effect of O_2 on sulfur-containing compounds, and more in-depth studies of tannin structure as a wine ages. Likewise, the inclusion of synergistic effects, for example, the masking effect of sulfur-containing compounds and newly formed aldehydes require more systematic study, along with the effects of polyphenols on the volatility of aroma

compounds (Aronson and Ebeler, 2004; Lund *et al*., 2009), and how this may change as the tannin structure develops as a result of oxygenation.

There is also a growing recognition of the need to better understand the spatial gradients of DO within large tanks, and include these effects in modeling wine maturation processes (Devatine and Mietton-Peuchot, 2009; Dykes, 2007; Dykes and Kilmartin, 2007). Considerations of this sort, alongside O_2 dosage rate and SO_2 adjustment, will allow winemakers to make the most effective use of MOX to improve the quality of specific red wines prior to bottling.

REFERENCES

Alcalde-Eon, C., Escribano-Bailon, M. T., Santos-Buelga, C., and Rivas-Gonzalo, J. C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing. *Anal. Chim. Acta* **563**, 238–254.

Aronson, J. and Ebeler, S. E. (2004). Effect of polyphenol compounds on the headspace volatility of flavors. *Am. J. Enol. Vitic.* **55**, 13–21.

Atanasova, V., Fulcrand, H., Cheynier, V., and Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of winemaking. *Anal. Chim. Acta* **458**, 15–27.

Blanchard, L., Darriet, P., and Dubourdieu, D. (2004). Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. *Am. J. Enol. Vitic.* **55**, 115–120.

Bobet, R. A., Noble, A. C., and Boulton, R. B. (1990). Kinetics of the ethanethiol and diethyl disulfide interconversion in wine-like solutions. *J. Agric. Food Chem.* **38**, 449–452.

Bosso, A., Guaita, M., Vaudano, E., and Di Stefano, R. (2000). Influence of oxygen on the evolution of phenolic compounds during red wine aging. *Ind. Bevande* **29**, 630–640.

Cano-Lopez, M., Pardo-Minguez, F., Lopez-Roca, J. M., and Gomez-Plaza, E. (2006). Effect of microoxygenation on anthocyanin and derived pigment content and chromatic characteristics of red wines. *Am. J. Enol. Vitic.* **57**, 325–331.

Cano-Lopez, M., Pardo-Minguez, F., Lopez-Roca, J. M., and Gomez-Plaza, E. (2007). Chromatic characteristics and anthocyanin profile of a micro-oxygenated red wine after oak or bottle maturation. *Eur. Food Res. Technol.* **225**, 127–132.

Cano-Lopez, M., Pardo-Minguez, F., Schmauch, G., Saucier, C., Teissedre, P.-L., Lopez-Roca, J. M., and Gomez-Plaza, E. (2008). Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents. *J. Agric. Food Chem.* **56**, 5932–5941.

Carlton, W. K., Gump, B., Fugelsang, K., and Hasson, A. S. (2007). Monitoring acetaldehyde concentrations during micro-oxygenation of red wine by headspace solid-phase microextraction with on-fiber derivatization. *J. Agric. Food Chem.* **55**, 5620–5625.

Castel, C., Morand, A., Pujol, G., Peyron, D., and Naudin, R. (2001). Influence on phenolic composition and sensory characteristics of microoxygenation on grape pomaces and during aging of red wines in Burgundy. *Ind. Bevande* **30**, 271–276.

Castellari, M., Matricardi, L., Arfelli, G., Galassi, S., and Amati, A. (2000). Level of single bioactive phenolics in red wine as a function of the oxygen supplied during storage. *Food Chem.* **69**, 61–67.

Castellari, M., Simonato, B., Tornielli, G. B., Spinelli, P., and Ferrarini, R. (2004). Effects of different enological treatments on dissolved oxygen in wines. *Ital. J. Food Sci.* **16**, 387–396.

Cheynier, V., Basire, N., and Rigaud, J. (1989). Mechanism of trans-caffeoyltartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *J. Agric. Food Chem.* **37**, 1069–1071.

Cheynier, V., Masson, G., Rigaud, J., and Moutounet, M. (1993). Estimation of must oxidation during pressing in Champagne. *Am. J. Enol. Vitic.* **44**, 393–399.

Conte, P. (2008). 1H NMR spectroscopy with multivariate statistical analysis as a tool for a rapid screening of the molecular changes occurring during micro-oxygenation of an Italian red wine. *Open Magn. Reson. J.* **1**, 77–80.

Cullere, L., Cacho, J., and Ferreira, V. (2007). An assessment of the role played by some oxidation-related aldehydes in wine aroma. *J. Agric. Food Chem.* **55**, 876–881.

Danilewicz, J. C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *Am. J. Enol. Vitic.* **54**, 73–85.

Danilewicz, J. C. (2007). Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. *Am. J. Enol. Vitic.* **58**, 53–60.

Danilewicz, J. C., Seccombe, J. T., and Whelan, J. (2008). Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. *Am. J. Enol. Vitic.* **59**, 128–136.

De Beer, D., Joubert, E., Marais, J., and Manley, M. (2008). Effect of oxygenation during maturation on phenolic composition, total antioxidant capacity, colour and sensory quality of Pinotage wine. *S. Afr. J. Enol. Vitic.* **29**, 13–25.

Devatine, A. and Mietton-Peuchot, M. (2009). A mathematical approach for oxygenation using micro bubbles. *Chem. Eng. Sci.* **64**, 1909–1917.

Devatine, A., Chiciuc, I., Poupot, C., and Mietton-Peuchot, M. (2007). Micro-oxygenation of wine in presence of dissolved carbon dioxide. *Chem. Eng. Sci.* **62**, 4579–4588.

du Toit, W. J., Lisjak, K., Marais, J., and du Toit, M. (2006a). The effect of micro-oxygenation on the phenolic composition, quality and aerobic wine-spoilage microorganisms of different South African red wines. *S. Afr. J. Enol. Vitic.* **27**, 57–67.

du Toit, W. J., Marais, J., Pretorius, I. S., and du Toit, M. (2006b). Oxygen in must and wine: A review. *S. Afr. J. Enol. Vitic.* **27**, 76–94.

Dubourdieu, D., Moine-Ledoux, V., Lavigne-Cruege, V., Blanchard, L., and Tominaga, T. (2000). Recent advances in White Wine Aging: The Key Role of the Lees. *In* ''ASEV 50th Anniversary Meeting''. pp. 345–352. American Society for Enology and Viticulture, Seattle, Washington.

Dykes, S. I. (2007). The Effect of Oxygen Dosage Rate on the Chemical and Sensory Changes Occurring During Micro-Oxygenation of New Zealand Red Wine. PhD thesis, The University of Auckland, Auckland.

Dykes, S. I. and Kilmartin, P. A. (2007). Micro-oxygenation: Optimising the maturation process. *Aust. NZ Wine Ind. J.* **22**, 31–45.

Escudero, A., Asensio, E., Cacho, J., and Ferreira, V. (2002). Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* **77**, 325–331.

Fedrizzi, B., Magno, F., Badocco, D., Nicolini, G., and Versini, G. (2007). Aging effects and grape variety dependence on the content of sulfur volatiles in wine. *J. Agric. Food Chem.* **55**, 10880–10887.

Fell, A. J., Dykes, S. I., Nicolau, L., and Kilmartin, P. A. (2007). Electrochemical microoxidation of red wine. *Am. J. Enol. Vitic.* **58**, 443–450.

Flecknoe-Brown, A. (2006). 'Diffusive' methods of oxygenating wine: Simpler, better, lower-cost barrel replacement. *Aust. NZ Grapegrow. Winemak.* August, 64–69.

Fulcrand, H., Duenas, M., Salas, E., and Cheynier, V. (2006). Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.* **57**, 289–297.

Garcia-Puente Rivas, E., Alcalde-Eon, C., Santos-Buelga, C., Rivas-Gonzalo, J. C., and Escribano-Bailon, M. T. (2006). Behaviour and characterization of the color during red wine making and maturation. *Anal. Chim. Acta* **563**, 215–222.

Giovanelli, G. and Brenna, O. V. (2007). Oxidative stability of red wine stored in packages with different oxygen permeability. *Eur. Food Res. Technol.* **226**, 169–179.

Hernandez-Orte, P., Lapena, A. C., Escudero, A., Astrain, J., Baron, C., Pardo, I., Polo, L., Ferrer, S., Cacho, J., and Ferreira, V. (2009). Effect of micro-oxygenation on the evolution of aromatic compounds in wines: Malolactic fermentation and ageing in wood. *LWT—Food Sci. Technol.* **42**, 391–401.

KauriWine (2009). http://www.kauriwine.com/parsec_mox.html.

Kelly, M. and Wollan, D. (2003). Micro-oxygenation of wine in barrels. *Aust. NZ Grapegrow. Winemak. Annu. Tech. Issue* **473a**, 29–32.

Kontoudakis, N., Biosca, P., Canals, R., Fort, F., Canals, J., and Zamora, F. (2008). Impact of stopper type on oxygen ingress during wine bottling when using an inert gas cover. *Aust. J. Grape Wine Res.* **14**, 116–122.

Kwiatkowski, M. J., Skouroumounis, G. K., Lattey, K. A., and Waters, E. J. (2007). The impact of closures, including screw cap with three different headspace volumes, on the composition, colour and sensory properties of a Cabernet Sauvignon wine during two years' storage. *Aust. J. Grape Wine Res.* **13**, 81–94.

Laurie, V. F. and Waterhouse, A. L. (2006a). Glyceraldehyde bridging between flavanols and malvidin-3-glucoside in model solutions. *J. Agric. Food Chem.* **54**, 9105–9111.

Laurie, V. F. and Waterhouse, A. L. (2006b). Oxidation of glycerol in the presence of hydrogen peroxide and iron in model solutions and wine. Potential effects on wine color. *J. Agric. Food Chem.* **54**, 4668–4673.

Laurie, V. F., Law, R., Joslin, W. S., and Waterhouse, A. L. (2008). In situ measurements of dissolved oxygen during low-level oxygenation in red wines. *Am. J. Enol. Vitic.* **59**, 215–219.

Lemaire, T. (2000). Managing micro-oxygenation and other maturation techniques on a large scale: Fine tuning, away from the recipe. *In* ''ASVO Use of Gases in Winemaking'', (M. Allen, S. Bell, N. Row, and G. Wall, Eds), pp. 54–59. ASVL, Adelaide, SA.

Lesica, M. and Kosmerl, T. (2006). Microoxidation of Pinot Noir wine. *Acta Agric. Slov.* **87**, 461–475.

Lopes, P., Saucier, C., Teissedre, P.-L., and Glories, Y. (2006). Impact of storage position on oxygen ingress through different closures into wine bottles. *J. Agric. Food Chem.* **54**, 6741–6746.

Lopes, P., Saucier, C., Teissedre, P.-L., and Glories, Y. (2007). Main routes of oxygen ingress through different closures into wine bottles. *J. Agric. Food Chem.* **55**, 5167–5170.

Lund, C. M., Nicolau, L., Gardner, R. C., and Kilmartin, P. A. (2009). Effect of polyphenols on the perception of key aroma compounds from Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **15**, 18–26.

Majcenovic, A. B., Schneider, R., Lepoutre, J.-P., Lempereur, V., and Baumes, R. (2002). Synthesis and stable isotope dilution assay of ethanethiol and diethyl disulfide in wine using solid phase microextraction. Effect of aging on their levels in wine. *J. Agric. Food Chem.* **50**, 6653–6658.

Makhotkina, O. and Kilmartin, P. A. (2009). Uncovering the influence of antioxidants on polyphenol oxidation in wines using an electrochemical method: Cyclic voltammetry. *J. Electroanal. Chem.* **633**, 165–174.

McCord, J. (2003). Application of toasted oak and micro-oxygenation to ageing of Cabernet Sauvignon wines. *Aust. NZ Grapegrow. Winemak.* July, 43–53.

Mestres, M., Busto, O., and Guasch, J. (2000). Analysis of organic sulfur compounds in wine aroma. *J. Chromatogr. A* **881**, 569–581.

Monagas, M., Bartolome, B., and Gomez-Cordoves, C. (2005a). Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci. Nutr.* **45**, 85–118.

Monagas, M., Gomez-Cordoves, C., and Bartolome, B. (2005b). Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in the bottle. *Eur. Food Res. Technol.* **220**, 607–614.

Moutounet, M., Ducournau, P., Chassin, M., and Lemaire, T. (1996). Apparatus for supplying oxygen to wines and its technological significance. Oenol. 95, Symp. Int. Oenol., 5th, Bordeaux, June, 1995, pp. 411–414.

Murat, M.-L., Tominaga, T., Saucier, C., Glories, Y., and Dubourdieu, D. (2003). Effect of anthocyanins on stability of a key odorous compound, 3-mercaptohexan-1-ol, in Bordeaux rose wines. *Am. J. Enol. Vitic.* **54**, 135–138.

Nevares, I. and del Alamo, M. (2008). Measurement of dissolved oxygen during red wines tank aging with chips and micro-oxygenation. *Anal. Chim. Acta* **621**, 68–78.

Nguyen, D. D., Dykes, S., Nicolau, L., and Kilmartin, P. A. (2010). Micro-oxygenation influence on reductive sulfur off-odors and colour development in a Cabernet Sauvignon wine. *Am. J. Enol. Vitic.* **61** in press.

O2mate (2009). www.o2mate.com.

Oenodev (2009). www.oenodev.com.

Ortega-Heras, M., Rivero-Perez, M. D., Perez-Magarino, S., Gonzalez-Huerta, C., and Gonzalez-Sanjose, M. L. (2008). Changes in the volatile composition of red wines during aging in oak barrels due to microoxygenation treatment applied before malolactic fermentation. *Eur. Food Res. Technol.* **226**, 1485–1493.

Parish, M., Wollan, D., and Paul, R. (2000). Micro-oxygenation: A review. *Aust. NZ Grapegrow. Winemak. Annu. Tech. Issue* **438a**, 47–50.

Paul, R. (2000). Micro-oxygenation: Where now? *In* ''ASVO Use of Gases in Winemaking'', (M. Allen, S. Bell, N. Row, and G. Wall, Eds), pp. 18–22. ASVO, Adelaide, SA.

Peng, Z., Duncan, B., Pocock, K. F., and Sefton, M. A. (1998). The effect of ascorbic acid on oxidative browning of white wines and model wines. *Aust. J. Grape Wine Res.* **4**, 127–135.

Perez-Magarino, S., Sanchez-Iglesias, M., Ortega-Heras, M., Gonzalez-Huerta, C., and Gonzalez-Sanjose, M. L. (2007). Color stabilization of red wines by microoxygenation treatment before malolactic fermentation. *Food Chem.* **101**, 881–893.

Perez-Magarino, S., Ortega-Heras, M., Cano-Mozo, E., and Gonzalez-Sanjose, M. L. (2009). The influence of oak wood chips, micro-oxygenation treatment, and grape variety on colour, and anthocyanin and phenolic composition of red wines. *J. Food Compos. Anal.* **22**, 204–211.

Piccolo, A., Spaccini, R., and Smejkalova, D. (2009). Molecular changes occurring during micro-oxygenation of an aglianico red wine as observed by NMR spectroscopy. *Adv. Food Sci.* **31**, 27–33.

Prieur, C., Rigaud, J., Cheynier, V., and Moutounet, M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **36**, 781–784.

Rauhut, D., Kurbel, H., Dittrich, H. H., and Grossmann, M. (1996). Properties and differences of commercial yeast strains with respect to their formation of sulfur compounds. *Wein-Wissenschaft, Wiesbaden* **51**, 187–192.

Rivero-Perez, M. D., Gonzalez-Sanjose, M. L., Muniz, P., and Perez-Magarino, S. (2008). Antioxidant profile of red-single variety wines microoxygenated before malolactic fermentation. *Food Chem.* **111**, 1004–1011.

Rodriguez-Bencomo, J. J., Ortega-Heras, M., Perez-Magarino, S., Gonzalez-Huerta, C., and Gonzalez-San Jose, M. L. (2008). Importance of chip selection and elaboration process on the aromatic composition of finished wines. *J. Agric. Food Chem.* **56**, 5102–5111.

Rowdon, G. (2005). The Characterization of Volatile Compounds During Micro-Oxygenation of Wine. MSc thesis, The University of Auckland, Auckland.

Rudnitskaya, A., Schmidtke, L. M., Delgadillo, I., Legin, A., and Scollary, G. (2009). Study of the influence of micro-oxygenation and oak chip maceration on wine composition using an electronic tongue and chemical analysis. *Anal. Chim. Acta* **642**, 235–245.

Saenz-Lopez, R., Fernandez-Zurbano, P., and Tena Maria, T. (2004). Analysis of aged red wine pigments by capillary zone electrophoresis. *J. Chromatogr. A* **1052**, 191–197.

Salmon, J. M., Fornairon-Bonnefond, C., Mazauric, J. P., and Moutounet, M. (2000). Oxygen consumption by wine lees: Impact on lees integrity during wine ageing. *Food Chem.* **71**, 519–528.

Salmon, J. M., Fornairon-Bonnefond, C., and Mazauric, J. P. (2002). Interactions between wine lees and polyphenols: Influence on oxygen consumption capacity during simulation of wine aging. *J. Food Sci.* **67**, 1604–1609.

Sartini, E., Arfelli, G., Fabiani, A., and Piva, A. (2007). Influence of chips, lees, and micro-oxygenation during aging on the phenolic composition of a red Sangiovese wine. *Food Chem.* **104**, 1599–1604.

Saucier, C. T. and Waterhouse, A. L. (1999). Synergetic activity of catechin and other anti-oxidants. *J. Agric. Food Chem.* **47**, 4491–4494.

Silva Ferreira, A. C., de Pinho Paula, G., Rodrigues, P., and Hogg, T. (2002). Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. *J. Agric. Food Chem.* **50**, 5919–5924.

Silva Ferreira, A. C., Hogg, T., and Guedes de Pinho, P. (2003). Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **51**, 1377–1381.

Singleton, V. L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *Am. J. Enol. Vitic.* **38**, 69–77.

Singleton, V. L., Salgues, M., Zaya, J., and Trousdale, E. (1985). Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *Am. J. Enol. Vitic.* **36**, 50–56.

Tao, J., Dykes, S. I., and Kilmartin, P. A. (2007). Effect of SO_2 concentration on polyphenol development during red wine micro-oxygenation. *J. Agric. Food Chem.* **55**, 6104–6109.

Vidal, J. C. and Moutounet, M. (2006). Monitoring of oxygen in the gas and liquid phases of bottles of wine at bottling and during storage. *J. Int. Sci. Vigne Vin* **40**, 35–45.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., and Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food Agric.* **83**, 564–573.

Vidal, J. C., Toitot, C., Boulet, J. C., and Moutounet, M. (2004a). Comparison of methods for measuring oxygen in the headspace of a bottle of wine. *J. Int. Sci. Vigne Vin* **38**, 191–200.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., and Waters, E. (2004b). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta* **513**, 57–65.

Vinovation (2009). http://www.vinovation.com/MOxmain.htm.

Vivas, N., Debeda, H., Menil, F., De Gaulejac, N. V., and Nonier, M. F. (2004). Demonstration of oxygen transfer through barrel staves by using a novel system to measure wood porosity. Preliminary results. *Sci. Aliments* **23**, 655–678.

Waterhouse, A. L. and Laurie, V. F. (2006). Oxidation of wine phenolics: A critical evaluation and hypotheses. *Am. J. Enol. Vitic.* **57**, 306–313.

Wildenradt, H. L. and Singleton, V. L. (1974). Production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Viticult.* **25**, 119–126.