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Effect of SO_2 Concentration on Polyphenol Development during Red Wine Micro-oxygenation

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A Merlot wine in 15 L research tanks was subjected to micro-oxygenation at 10 mL O_2 per liter of wine per month over a 16 week period with additions of 0, 50, 100, and 200 mg/L SO_2. A large decrease in monomeric anthocyanins and flavan-3-ols was seen in wines with a lower concentration of SO_2, coupled with an increase in nonbleachable pigments; an increase in tannin, measured using precipitation with methyl cellulose; and a greater size and red coloration of a proanthocyanidin extract obtained using Sephadex LH-20. These changes were largely suppressed in wines initially treated with 200 mg/L SO_2 and occurred more slowly in wines stored in bottles in the absence of O_2. The concentration of SO_2 is shown to regulate the polyphenol chemistry involved in the formation of polymeric pigments and changes in tannin structure affecting wine astringency.

KEYWORDS: Micro-oxygenation; red wine; polyphenols; sulfur dioxide

INTRODUCTION

Micro-oxygenation (MOX) is a winemaking process used to introduce small controllable amounts of oxygen into red wines stored in stainless steel tanks and other vessels (*1*). MOX aims to mimic the oxygen input experienced by wines during barrel aging, in contrast to the large doses of oxygen received during periodic racking operations. The benefits claimed during red wine MOX include a softening of tannins, stabilization of wine color, and a lessening of vegetative aromas (*2*). Reactions involving wine polyphenols are seen as key to these processes, including changes in proanthocyanidin chain length and its influence on wine astringency, and the linking of anthocyanins with wine tannins to form more stable colored structures such as pyranoanthocyanins, mediated by the acetaldehyde generated from ethanol oxidation (itself coupled to initial polyphenol oxidation processes) (*3*, *4*).

A distinction has been drawn between the influence of MOX before or after malolactic fermentation, related particularly to differences in the concentration of sulfur dioxide and its reaction with peroxide and aldehyde, thus limiting the condensation of anthocyanins with tannins (*5*, *6*). This observation can be related to a lower rate of pigmented polymer formation from model solutions of malvidin-3-glucoside and tannin in the presence of a higher concentration of SO_2 (*7*). However, the role of SO_2 during MOX has not been closely evaluated. While SO_2 is not thought to react directly with O_2 under wine conditions (*8*), it has the ability to reduce oxidized polyphenols back to their reduced forms (*9*) and to remove peroxide (*10*). Sulfonation of tannins has also been shown to occur in experiments involving much higher concentrations of bisulfite than normally used in winemaking (*11*) or at elevated temperature and pH (*12*), but the formation of such adducts of a lower molecular weight may still occur more slowly under regular wine conditions.

In this study, MOX at a rate of 10 mL O_2 per liter of wine per month was applied to a Merlot wine in 15 L research vessels with different SO_2 additions up to 200 mg/L. Changes in a range of polyphenol measures were recorded, which showed that the concentration of SO_2 had a marked effect on wine development.

MATERIALS AND METHODS

A 292 kg amount of Merlot grapes was handpicked on April 22, 2005, from the Waikoukou Valley vineyard of Matua Valley Wines (Waimauku, Auckland, New Zealand). At this point, the grapes were at 22.2 °Brix, with a titratable acidity of 8.1 g/L tartaric acid equivalents and a pH of 3.50. After destemming and crushing, 7.8 g of pectolytic enzyme (Rohapect VR-C, AB Enzymes, Germany) was added into the 260 L of must, along with 40 mg/L of potassium metabisulfite, to suppress polyphenol oxidase enzymes and wild yeast activity. The pomace was then stirred and cold-soaked for 72 h at a low temperature of 12 °C to minimize the onset of spontaneous fermentation. After 3 days, the must was inoculated with 90 g of ICVD 254 yeast and the fermentation proceeded in a stainless steel tank at a temperature of 19−24 °C for 2 weeks. A 52 g amount of diammonium phosphate (200 mg/L) was added on day two. Manual cap plunging was applied every 12 h from the time of crushing until the end of fermentation. The wine was left on skins for 15 days following fermentation to achieve maximum tannin extraction, after which the wine coming from a pneumatic press was transferred to a 225 L Hungarian oak barrel at 16−19 °C. The barrel was used previously but, prior to use, was cleaned and flushed with 90 °C water and stored for a period of 2 months with a 25 L solution of potassium metabisulfite (20% v/v) and citric acid (4 g/L). The barrel was flushed four times with fresh water prior to refilling. One gram of lactic acid bacteria was added into the barrel to facilitate malolactic fermentation, and the barrel was topped up regularly

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using Merlot wine from the same vintage stored in a stainless steel vessel (with a floating lid). Six months later, the wine was pumped into a 200 L stainless steel tank, which was sealed and stored in a 12 °C temperature-controlled room. During storage, argon was added regularly, but no SO_2 additions were made so that a low SO_2 control wine could be obtained to which SO_2 additions were made.

MOX Trial. For the MOX trial, 12 sealable 15 L stainless steel research vessels were used, which were purpose-built at the University of Auckland to include a range of lid fittings and entry points for the 0.188 m loop of 6 mm diameter, 1 mm thick, fluorinated ethylene−propylene tubing, from Zeus Industrial Products (South Carolina), used to introduce oxygen into the wine. After 5 months of storage in the 200 L tank, the wine was racked and filtered through a 0.45 μm membrane filter to remove residual yeast lees and used to fill the tanks, which had been purged with argon immediately prior to filling via a Swagelok connection and pressure relief valve in the tank lid. A blanket of argon was maintained over the wine as it was pumped into the tanks by again applying a very low flow rate (approximately 20 mL/min) through the Swagelok fittings. The tanks were then sealed, the headspace volume (approximately 180 mL) was displaced with argon, and the wines were allowed to sit for 24 h for temperature equilibration. The wines were kept in a temperature-controlled room at 15 °C and stirred continuously using magnetic stirrers, with an insulating plate placed between the stirrers and the tank to eliminate heat transfer from the stirrer unit to the fluid. O_2 was introduced at a rate of 10 mL per liter of wine per month, using a previous determination that a pressure of 200 kPa of Food Grade O_2 controlled by a gas regulator (Victor SGL 500) in the plastic tubing would supply oxygen to the tanks at this rate. For this purpose, a series of replicated trials were undertaken to monitor the rise in dissolved oxygen in sealed 15 L tanks containing previously deareated deionized water or mixed solutions with up to 15% ethanol. A slow steady increase in dissolved oxygen was monitored continuously for 300 h, which allowed the conversion between gas pressure and oxygen delivery to be established for wine conditions, details of which will be the subject of a separate communication. A series of four MOX treatments were set up, including wine with no additional SO_2 and wines to which 50, 100, and 200 mg/L of SO_2 were added. The headspace of each tank was displaced with argon twice weekly to minimize any contamination with O_2 from the atmosphere. At the beginning of the trial, 12 wines were also bottled with the same SO_2 additions but not treated with MOX and were only opened and analyzed after 114 days. The initial pH of the wine was 3.47, and the pH remained within 0.1 units of this value in all treatments throughout the trial.

Wine Sampling. The wines were sampled 7, 14, 28, 42, 62, 85, and 112 days after MOX commenced. Sampling without contamination of O_2 from the atmosphere was considered critical, and to achieve this, a flow of argon was maintained over the headspace during sampling. With argon flowing, the blanking plate on the tank was removed and replaced with a specially designed sampling tube with lid. The argon pressure applied to the headspace was used to force wine through the tube and into a 50 mL Schott bottle, previously evacuated with argon, after which the original blanking plate was refitted.

Sulfur Dioxide. The concentration of free and total SO_2 was determined using the aspiration method (*13*).

Spectrophotometric Analyses. A number of spectrophotometric measures of wine anthocyanins and polyphenols were made at a Cary 50 UV-spectophotometer (Varian Inc., Palo Alto, CA), using established procedures (*13*). These included diluting the wine 50-fold in 1 M HCl to obtain a measure of total phenols given by the absorbance at 280 nm (A_{280}^{HCl}), recording the red wine color at 520 nm (A_{520}), and mixing 2 mL of wine with 30 μL of 25% (w/v) potassium metabisulfite in distilled water for 45 min, to give a value at 520 nm for pigments resistant to SO_2 bleaching ($A_{520}^{SO_2}$). A measure of monomeric anthocyanins was given by total red pigments (A_{520}^{HCl}) minus SO_2 resistant pigments ($A_{520}^{SO_2}$).

Tannin Analyses. The total tannins were measured throughout the trial using the methyl cellulose (MC) precipitable tannin assay (*14*), using the difference in 280 nm absorbance obtained before and after the addition of MC (Sigma, St. Louis, MO) and converting to epicatechin equivalents (EEs) (mg/L). At the end of the 16 week trial, the samples were also analyzed for tannins using the Adams−Habertson assay based upon precipitation with bovine serum albumin (BSA) (*15*) and converting to catechin equivalents (CEs) (mg/L). Both analyses were run in triplicate on each sample.

High-Performance Liquid Chromatography (HPLC) Analyses. Monomeric wine polyphenols were determined using an HPLC method previously outlined (*16*). In brief, about 1 mL of wine was filtered through a 0.45 μm cellulose filter (Ministart RC-4), of which 20 μL was injected onto a Phenomenex Luna C18 column (4.6 mm × 250 mm, 5 μm particle size) (Torrence, CA) on an Agilent 1100 series instrument (Waldbronn, Germany). Chromatograms were obtained with the diode array detector set at 280 (for flavan-3-ols), 320 (for hydroxycinnamic acids), 365 (for flavonols), and 520 nm (for anthocyanins). A ternary solvent was run over 2 h employing water, 5% aqueous acetic acid, and acetonitrile; between 30 and 105 min in the run, the concentration of acetonitrile went from 0 to 55%, and further details are provided in ref *16*. The following standards were obtained from Sigma, gallic acid, catechin, caffeic acid, and quercetin, while malvidin-3-glucoside was obtained from Extrasynthèse (Genay, France).

A proanthocyanidin fraction was obtained by introducing 5 mL of wine onto a Sephadex LH-20 column (Amersham Biosciences, Sweden) and eluting with 50% acetone (with 0.2% acetic acid) in Milli-Q water, according to a published procedure (*17*). Following solvent removal using a rotary vacuum evaporator at 37 °C, the proanthocyanidin fractions were made up to a volume of 5 mL using 50% methanol in Milli-Q water. Spectrophotometric measures of total phenols (A_{280}^{HCl}) and red color (A_{520}) were taken at this point. The fractions were then subjected to thiolysis using 5% benzyl mercaptan in 0.2 M HCl in methanol in a process adapted from past publications (*3*, *18*), sealing them in small glass bulbs for 5 h at 60 °C. Stability trials showed that most of the terminal and extension (thioether) units peaked in concentration after 2−3 h of thiolysis, with a gradual degradation for thiolysis times longer than 5 h. The mean degree of polymerization (MDP) was determined by HPLC (*19*), using the column and detector (at 280 nm) described above with aqueous acetic acid and acetonitrile at a flow rate of 1.0 mL/min. Calibration curves were established using catechin, epicatechin, and epicatechin gallate (Sigma).

The various chemical analyses are reported plus or minus the standard deviations of the results. Statistical analyses of the chemical data were also undertaken using analysis of variance (ANOVA) single factor (Microsoft Excel, 2002) and Fisher's least significant difference ($LSD_{0.05}$).

RESULTS AND DISCUSSION

At the beginning of the MOX trial, the Merlot wine had a very low level of total SO_2, with a measured value of 2.0 (±0.6) mg/L, and fortunately had not been affected by microbial spoilage to this point. The concentration of SO_2, both free and total, was monitored throughout the trial (**Figure 1A**,**B**), and a steady decline was seen over the 16 week trial in the wines to which SO_2 was added. The wines that originally received a 200 mg/L addition of SO_2 dropped to 40 mg/L total SO_2 by the end of the trial, with free SO_2 below 10 mg/L. In this case, the bound SO_2 stayed in the 80−90 mg/L range for the first 40 days before declining to 30 mg/L by day 114. This SO_2 would be bound to both anthocyanins and to various aldehydes and ketones, which could be released to react with polyphenols as the trial progressed. The concentration of free SO_2 dropped to values below 10 mg/L after 14 and 62 days for the wines initially treated with 50 and 100 mg/L SO_2, respectively. In the second half of the trial, a free SO_2 of less than 2 mg/L was recorded in these two wines, although the bound SO_2 component remained steady at around 20 mg/L. The decline in SO_2 was likely due not only to the influence of oxygen coming into the wine at 10 mL per liter of wine per month but also to the effect of sparging the headspace with argon during wine sampling, which could displace some of the SO_2 in its volatile molecular form. By contrast, the wines bottled at the beginning of the trial and only

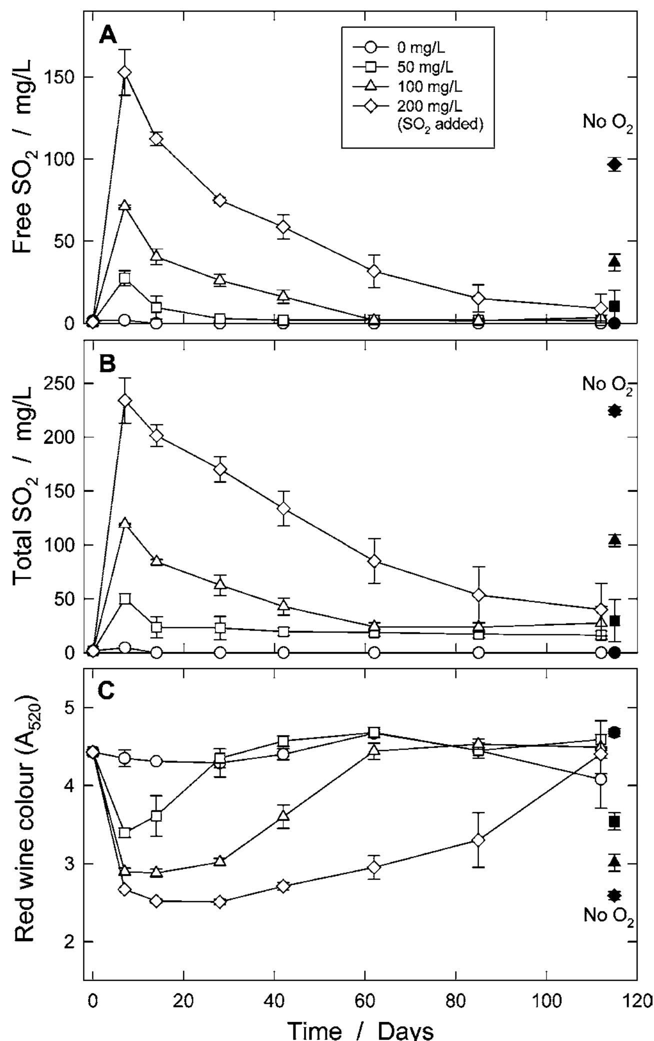

Figure 1. Concentration of (**A**) free SO_2, (**B**) total SO_2, and (**C**) red wine color (A_{520}) during the 16 week MOX trial for wines with different initial additions of SO_2. Values for wines stored in bottles until the end of the trial with "no O_2" are shown on the right. Error bars are given for the standard deviation in each value ($n = 3$).

opened and sampled after 16 weeks showed a similar total SO_2 to the amount originally added (the individual points at 114 days labeled as "no O_2" in **Figure 1B** and on the other figures).

The effect of the SO_2 additions on wine color was a consistent drop in the 520 nm absorbance, and the wines, which received 200 mg/L SO_2 initially, lost nearly half of their red wine color (**Figure 1C**). The extent of bleaching was closely related to the amount of SO_2 in the wines, and as the concentration of SO_2 fell throughout the trial, the 520 nm absorbance progressively rose, returning (once the total SO_2 fell below 40 mg/L) to values within 0.2 units of the starting level, indicating that the bleaching effect was largely reversible. The drop in the absorbance of the control wine by 0.4 units for the final reading at day 112 can be related to the occurrence of microbial spoilage noted in two of the three control tanks in the final weeks of the trial, given by a smell of vinegar and white surface growth in these two tanks, which was not apparent in any of the other wines. Some precipitation of wine solids also occurred in most of the wines toward the end of the trial, except those that initially received 200 mg/L SO_2 (see below). On the other hand, the wines bottled for 16 weeks maintained both higher SO_2 and the same initial level of bleaching (**Figure 1C**).

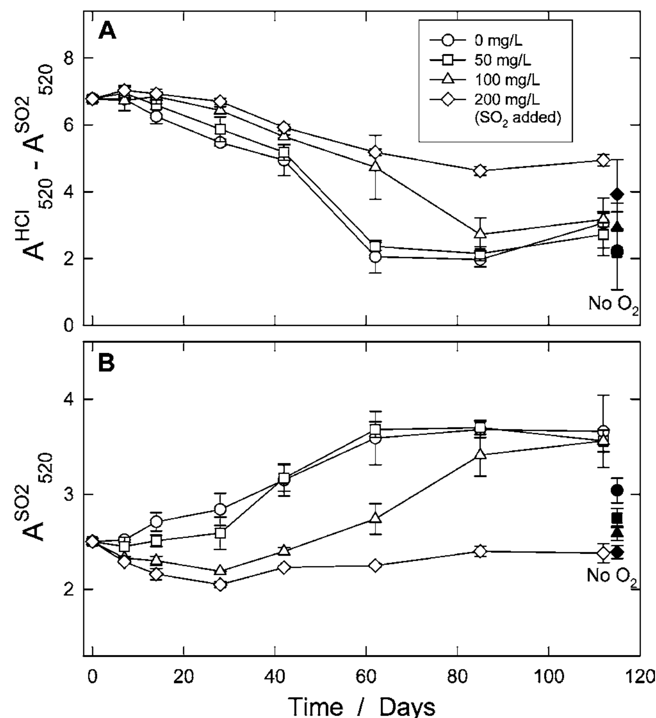

Figure 2. Levels of (**A**) monomeric anthocyanins (A_{520}^{HCl}–$A_{520}^{SO_2}$) and (**B**) nonbleachable pigments ($A_{520}^{SO_2}$) given by spectrophotometric measures for the 16 week MOX trial for wines with different initial additions of SO_2. Values for wines stored in bottles until the end of the trial with "no O_2" are shown on the right. Error bars are given for the standard deviation in each value ($n = 3$).

The effect of the MOX treatment on various pigment classes, monitored using spectrophotometric measures, included an expected decline in monomeric anthocyanins, as given by the subtractive measure A_{520}^{HCl} minus $A_{520}^{SO_2}$ (**Figure 2A**). This decline was greater in the lower SO_2 wines and was more gradual in the wines originally treated with 200 mg/L SO_2. Coupled to these changes was an increase in nonbleachable pigments ($A_{520}^{SO_2}$), expected to be due largely to polymeric pigments, with increases seen in the 0 and 50 mg/L initial SO_2 wines from the beginning of the trial and with the 100 mg/L initial SO_2 wines once the total SO_2 had declined below 40 mg/L from 62 days (**Figure 2B**). By contrast, there was no increase in nonbleachable pigments for the wines originally treated with 200 mg/L SO_2. The bottled wines also showed a larger increase in nonbleachable pigments at lower SO_2 concentrations, but the extent of increase was much less than in the MOX wines (**Figure 2B**). The formation of nonbleachable pigments was thus most rapid with oxygenated wines in a low SO_2 environment. By contrast, the processes by which tannins combine with anthocyanins to form the nonbleachable pigments that develop as a wine ages appear to be significantly retarded at higher SO_2 concentrations.

The level of total phenols, given by the 280 nm absorbance, was not greatly altered during the MOX trial (**Figure 3A**), until the final weeks when a decline was seen, particularly for the lower SO_2 wines, which can be related to the precipitation of solids, which occurred in many of the wines. Wines to which 200 mg/L of SO_2 was originally added did not form a precipitate, and in this case, the addition of sulfonate groups onto wine tannins, through a chemical bonding of SO_2 onto the polyphenol structure, may have made them more soluble and less prone to precipitation (*12*). The presence of sulfonated compounds of this sort needs to be confirmed in further experiments, including the use of LC-MS identification.

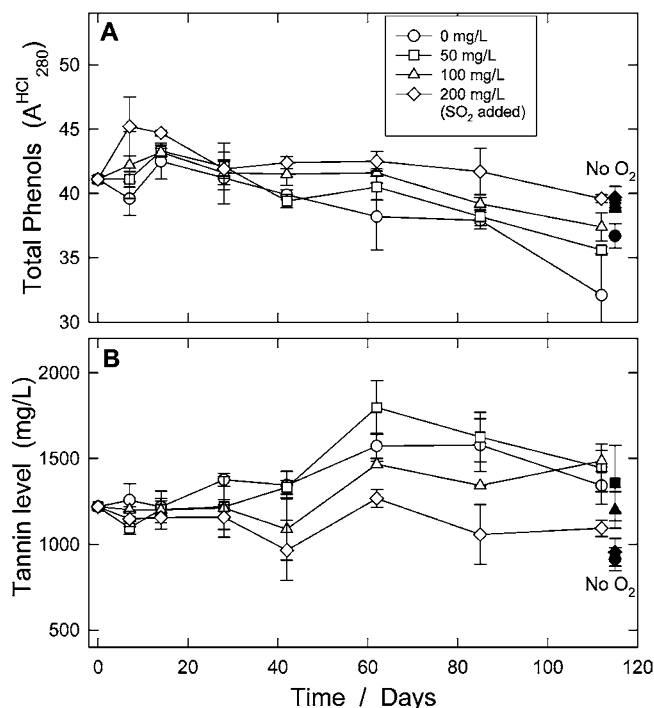

Figure 3. Levels of (**A**) total phenols (A^{HCl}_{280}) and (**B**) tannin given by binding with MC for the 16 week MOX trial for wines with different initial additions of SO_2. Values for wines stored in bottles until the end of the trial with "no O_2" are shown on the right. Error bars are given for the standard deviation in each value ($n = 3$).

The level of tannins, given by the amount bound by the MC polymer, was steady in the early stages of the trial (**Figure 3B**). However, from 42 days on, an increase in tannin was seen in all of the wines, somewhat in contrast to the total phenols result, except for those wines originally treated with 200 mg/L SO_2, which again remained largely unchanged. These trends were followed in part in the bottled wines, except for a lower tannin value obtained in the wines with no added SO_2. This separation in values at the end of the trial was also seen in the tannin measure obtained through binding with BSA (**Table 1**), where a lower value was recorded for the 200 mg/L SO_2 wines. An increase in the value obtained from the Harbertson−Adams assay, alongside a steady total phenols value, has been reported in a previous MOX trial (*2*), while in a further trial a lower tannin value was obtained where binding with ovoalbumin was measured (*1*). Changes in the size and distribution of polyphenols in the lower SO_2 wines are likely to have occurred as a result of MOX, leading to more compounds large enough to bind effectively with either MC or BSA, processes that were largely suppressed under the influence of the highest SO_2 addition employed in this trial. Any sulfonation of the tannin structure with the higher SO_2 treatments may also alter the interaction of the tannins with MC or BSA. The increase in tannin in the lower SO_2 wines may be large enough to produce an increase in astringency intensity (*20*).

To examine the tannin structure in more detail, a proanthocyanidin extract was obtained using Sephadex LH-20. Using this fraction, the MDP was found to be in the 13−15 range 7 days into the trial (**Figure 4C**). Throughout the trial, there was relatively little change in the proanthocyanidin extract for the wines originally treated with 200 mg/L SO_2. In this case, the total phenols of the extract (A^{HCl}_{280}) stayed around 3 units and even declined a little for samples taken later in the trial (**Figure 4A**)−A decline in the size of a proanthocyanidin extract has been reported for a previous MOX trial (*3*). Likewise, the red color of the extract remained quite low with a small decline across the trial (**Figure 4B**). By contrast, for the lower SO_2 wines, including the 0 and 50 mg/L SO_2 wines from 42 days and the 100 mg/L SO_2 wines from 85 days (when the total SO_2 was below 30 mg/L), the proanthocyanidin extract contained more phenolic material as the trial progressed (**Figure 4A**) and over twice the red coloration (**Figure 4B**). These increases are consistent with the development of more polymeric material, and in particular more pigmented polymers, of a sufficient size to be retained on the Sephadex column and eluted with acidified acetone. At the same time, the MDP of the lower SO_2 wines declined to values below 10 (**Figure 4C**).

Given the increase in the amount of tannin present in the proanthocyanidin fraction, it is not possible to conclude that the tannins present are getting smaller in size, as it is likely that smaller polyphenol units were linked together to create new polymers large enough to join this fraction. It has been previously recognized that, as a result of tannin−tannin condensation reactions, the production of new proanthocyanidin molecules can lead to an increase or decrease in MDP depending upon the level of monomeric flavanols present (*21*), which will vary with different wines. Indeed, in a preliminary trial to this report, a different red wine was stored at 15 °C with and without weekly saturation with O_2 and with and without treatment with 100 mg/L SO_2 (creating four treatments, all run in triplicate). In this case, no significant change in the total phenols of the proanthocyanidin extract was observed. Instead, a decline in MDP was seen in the wines treated with additional SO_2 from a value of 8.7 ($\pm$0.1) to values within 0.2 units of 7.5 after 7 weeks. This decline occurred regardless of the addition or absence of dissolved oxygen. By contrast, the low SO_2 wines (with or without O_2) retained an MDP value within 0.3 units of 8.8 for 4 weeks, then increased to values of 10.5 ($\pm$0.1) (no O_2) and 10.65 ($\pm$0.05) (with O_2) by the end of the trial. Despite this difference in MDP shift from the larger trial described in this paper, an increase in wine nonbleachable pigments was seen for wines stored without SO_2 in the preliminary experiment but little change when SO_2 was present (regardless of O_2 content), consistent with the larger trial.

The inclusion of a greater amount of red-colored material in the proanthocyanidin extract is also consistent with the increase in nonbleachable pigments given by the $A^{SO_2}_{520}$ measure (**Figure 2B**). This increase in the extract was again much reduced for the wines stored in bottles (**Figure 4B**), while the extract total phenols stayed low in all of the bottled wines (**Figure 4A**), pointing to the role of oxygen, alongside low SO_2 levels, in the tannin chemistry occurring here. The combination of anthocyanins and tannins has been postulated to lead to a decrease in tannin astringency (*20*, *22*), an effect that needs to be considered alongside changes in other tannin measures, such as the increase in binding with MC seen in **Figure 3B**.

Changes in the concentration of individual polyphenols at the end of the trial determined by HPLC were consistent with the observations noted above (**Table 1**). There was a large decline in monomeric anthocyanins in the MOX wines in a low SO_2 environment. The concentration of malvidin-3-glucoside was 27−29 mg/L in the wines bottled with 100−200 mg/L SO_2, while lower values were seen in the MOX wines initially containing 200 mg/L SO_2 (23 mg/L) and in the wines bottled with 50 mg/L SO_2 (25 mg/L) or with no added SO_2 (14 mg/L). By contrast, the MOX wines with 0, 50, and 100 mg/L initial SO_2 showed a near complete absence of malvidin-3-glucoside by the end of the trial (**Table 1**) and of other monomeric anthocyanins. The concentration of the flavan-3-ols catechin and

Table 1. Concentration of Tannins and Monomeric Polyphenols by HPLC at the End of the 16 Week MOX Trial ($n = 3$)[a]

initial SO_2 addition (mg/L)	tannin level with MC (mg/L EE)	tannin level with BSA (mg/L CE)	malvidin-3-glucoside (mg/L)	catechin (mg/L)	epicatechin (mg/L CE)	caftaric acid (mg/L CAE)	caffeic acid (mg/L)	quercetin-3-glucoside (mg/L QE)	quercetin (mg/L)
				MOX wines					
0	1342 (±108) ab[b]	469 (±66) ab	ND[c] e	48 (±12) c	19 (±11) c	6 (±3) c	28 (±5) a	1.29 (±0.25) c	3.9 (±2.5) b
50	1446 (±139) a	581 (±121) a	ND e	47 (±8) c	24 (±5) c	20 (±2) a	19 (±2) b	1.18 (±0.17) c	2.4 (±0.5) bc
100	1486 (±62) a	465 (±18) ab	0.9 (±0.7) e	71 (±12) b	37 (±4) b	21 (±1) a	20 (±1) b	1.36 (±0.12) c	0.8 (±0.1) c
200	1093 (±47) b	335 (±32) b	22.8 (±3.2) c	114 (±2) a	65 (±4) a	22 (±0.5) a	22 (±0.3) b	1.72 (±0.06) b	0.27 (±0.03) c
				bottled wines					
0	913 (±70) bc		13.5 (±0.3) d	108 (±2) a	64 (±2) a	15 (±1) b	27 (±0.4) a	1.96 (±0.07) ab	11.8 (±0.7) a
50	1357 (±220) ab		24.7 (±0.2) bc	119 (±5) a	72 (±2) a	21 (±1) a	23 (±0.4) b	2.10 (±0.04) a	12.2 (±0.6) a
100	1200 (±107) b		27.1 (±0.8) ab	121 (±9) a	73 (±4) a	20 (±2) a	22 (±1) b	2.04 (±0.10) a	11.8 (±1.1) a
200	953 (±81) bc		28.8 (±0.5) a	122 (±1) a	75 (±1) a	20 (±1) a	23 (±0.4) b	2.04 (±0.06) a	11.3 (±1.2) a

[a] Standard deviations are given in parentheses after each value. [b] Significantly different based on a single factor ANOVA and multiple comparison using Fisher's $LSD_{0.05}$. [c] ND, not detected.

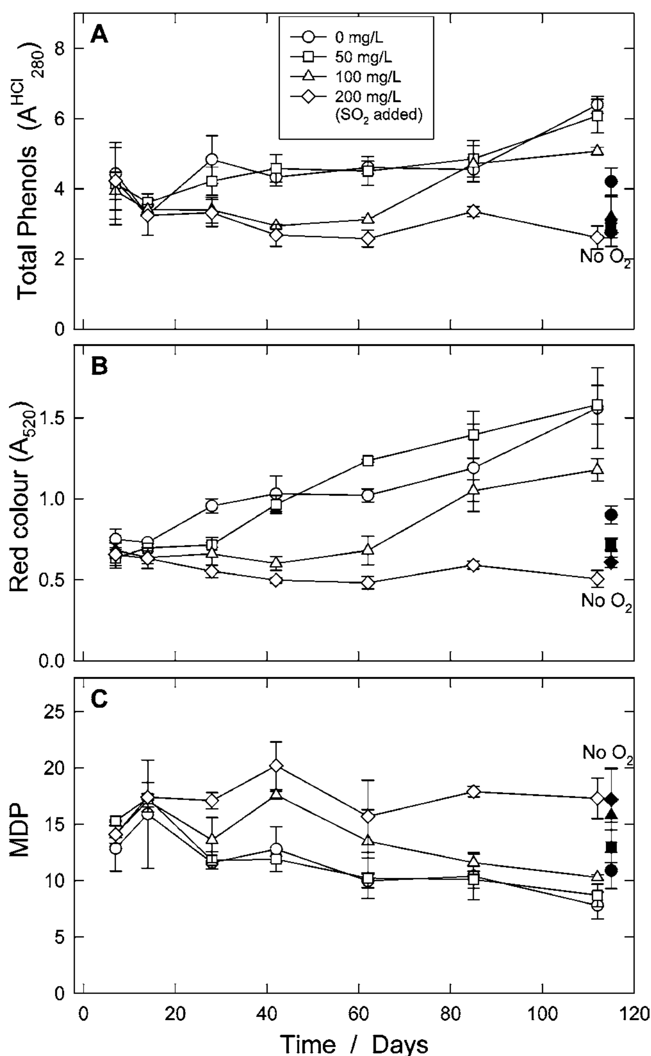

Figure 4. Properties of a proanthocyanidin extract obtained using Sephadex LH-20: (**A**) total phenols (A_{280}^{HCl}), (**B**) extract red color (A_{520}), and (**C**) MDP for the 16 week MOX trial for wines with different initial additions of SO_2. Values for wines stored in bottles until the end of the trial with "no O_2" are shown on the right. Error bars are given for the standard deviation in each value ($n = 3$).

epicatechin also declined in the MOX wines, with more extreme losses seen for wines with a lower SO_2 content to below half of the values seen in wines bottled with added SO_2 (**Table 1**). Lower levels of anthocyanins and flavan-3-ols have been reported in previous MOX studies (*2*, *3*, *23*). The inclusion of both anthocyanins and flavan-3-ols into larger polymeric structures would serve to raise the amount of tannin (**Figure 3B**) and polymeric pigments (**Figures 2B** and **4B**).

The concentrations of the hydroxycinnamates caftaric acid and caffeic acid were largely unaffected by the MOX treatment, except for the MOX wines without added SO_2 where the concentration of caftaric acid dropped from around 20 mg/L in the other wines to 6 (±3) mg/L (**Table 1**). This was accompanied by an increase in free caffeic acid, suggesting that some hydrolysis had occurred in these wines. Regarding the flavonol content, the concentration of quercetin-3-glucoside declined by a small amount in the MOX wines, but the concentration of free quercetin was much lower in the MOX wines as compared to levels of 11−12 mg/L in the bottled wines (**Table 1**) and, somewhat counter to the previous polyphenol trends, was lower in the higher SO_2 wines, pointing to an interaction of SO_2 with quercetin or an oxidized form, which differs from processes that occur with other polyphenols.

The results of this trial clearly show that sulfur dioxide has a moderating effect on the interaction of oxygen with wine polyphenols. Alongside the reversal of the oxidation of B-ring catechol groups, a reduction process can occur with a range of polyphenols, or removal of peroxide within the wine, and SO_2 is likely to affect pathways involving the formation of carbocations at the C4 position of proanthocyanidins (*12*) and the way in which these will combine with other polyphenols, including anthocyanins, to create new tannin and polymeric pigment compounds. The extent of SO_2 addition to a red wine undergoing MOX will thus affect the rate of development of wine polyphenol chemistry, including the stabilization of color in polymeric pigment forms and changes in tannin structure affecting wine astringency.

ABBREVIATIONS USED

MOX, micro-oxygenation; MDP, mean degree of polymerization; A_{520}, red wine color at 520 nm; $A_{520}^{SO_2}$, SO_2 resistant pigments; A_{520}^{HCl}, absorbance at 520 nm after dilution in HCl; A_{280}^{HCl}, total phenols given by the absorbance at 280 nm; MC, methyl cellulose; BSA, bovine serum albumin; EE, epicatechin equivalents; CE, catechin equivalents; CAE, caffeic acid equivalents; QE, quercetin equivalents.

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