

Application of Toasted Oak and Micro-oxygenation to Ageing of Cabernet Sauvignon Wines

Dr. Jeff McCord
StaVin Inc., California, USA

Introduction

For many years, toasted oak in the form of barrels has been used for wine storage and to influence the ageing of white and red wines. Alternatives to barrels, most notably oak chips, have also been utilised to enhance the character of bulk wines fermented and aged in stainless steel (SS) tanks. However, these wines have rarely matched the complex tastes and aromas imparted by barrels. The use of toasted staves in SS tanks for ageing of wines has provided a great improvement, yielding a more barrel-like flavour profile. This approach, however, is still not able to match the ageing characteristics that barrels impart through a slow leakage of air into wines.

In the mid 1980's, Dr. Michel Moutounet began research on a technique now known as micro-oxygenation. This technique involves the addition of very small amounts of pure oxygen into wine over time. This process, while thought to be new and revolutionary, is in reality a refinement of the barrel ageing process. There is a slow, very slow, permeation of air through oak staves and joints of the barrel; however, most air contact with wine in barrels arises through the removal of the bung. The process of bung removal to top up barrels, additions of SO₂ and racking, all allow oxygen to be absorbed into wines. How these processes are performed gives the winemaker some control over the amount of air the wine absorbs, and therefore the wine style.

The use of micro-oxygenation devices has given the winemaker a level of control not previously available in the production of wine. Furthermore, the use of these devices, in combination with toasted oak in SS tanks, can encourage maturation and complexity in wines similar to those made using barrels.

There has been considerable controversy surrounding the use of micro-oxygenation since its introduction as a new winemaking tool. Most of this controversy is a result of the subjective nature of trials conducted to date; this highlights a need for large-scale objective trials.

The aim of this study was to determine the effect of toasted oak products with and without micro-oxygenation on the ageing of Cabernet sauvignon wine. A second aim was to evaluate various analytical methods for their ability to provide an effective and useful tool to monitor the maturation of wine undergoing micro-oxygenation. At present, the most effective tool winemakers have is their own experience in the production of their wines, and their judgement of their wine's ability to age.

Materials and Methods

Cabernet sauvignon fruit was harvested from the Edna Valley region of San Luis Obispo County, California in 2001. Approximately 500 tons was harvested at an average of 22.5° Brix in early November. Grapes were divided into fermentation vessels varying from 10 ton to 20 ton in size with an addition of 50 ppm SO₂ at the crusher. Fermentation trials were conducted to determine the effects of enzymes, toasted oak

addition and type of fermentation, comparing punch down with pump over techniques. All fermentations had enough Chardonnay concentrate added to bring the final alcohol concentration to 14%. The must was inoculated with yeast CY3079 and was pressed off at 0° Brix.

After pressing, all the wine was combined together and then divided into duplicate lots for the ageing trials: no oak / no oxygen (control) to two 3,000 gallon (11,360 L) tanks; staves only / no oxygen to two 3,000 gallon (11,360 L) tanks; segments only / no oxygen to two 3,000 gallon (11,360 L) tanks; oxygen only / no oak to two 12,000 gallon (45,420 L) tanks; staves and oxygen to one 6,000 gallon (22,710 L) tank and one 12,000 gallon (45,420 L) tank; and lastly, segments and oxygen to one 6,000 gallon (22,710 L) tank and one 12,000 gallon (45,420 L) tank. Toasted oak was added at 3 g/L in the form of StaVin French Oak Medium Plus staves or StaVin French Oak Medium Plus segments.

The combined wine, before division into tanks for individual treatments, was inoculated with Oeno strain of malolactic bacteria. At completion of malolactic fermentation, all tanks were clean racked and SO₂ was added at 30 ppm. Micro-oxygenation treatments were dosed with oxygen for one month at 10 mL O₂/L/month then reduced to 5 mL O₂/L/month for the remainder of the trial, using two 4-channel Ox Boxes from StaVin Inc. Dosing of oxygen began 15 January 2002 and was halted on 15 June 2002. Wines were bottled from their respective tanks in August of 2002.

Analysis

Wines were analysed by ETS Laboratories in St. Helena, California for 48 chemical attributes: ethanol, protein reactive tannins, *trans*-oak lactone, *cis*-oak lactone, vanillin, furfural, 5-methylfurfural, guaiacol, 4-methylguaiacol, eugenol, isoeugenol, gallic acid, catechin, epicatechin, polymeric phenols, caftaric acid, caffeic acid, quercetin glycosides, quercetin aglycone, malvidin glucoside, polymeric anthocyanins, total anthocyanins, monomeric anthocyanins, hydrogen sulfide, ethyl mercaptan, methyl mercaptan, diethyl sulfide, dimethyl sulfide, diethyl disulfide, dimethyl disulfide, other sulfides, 4-ethylguaiacol, 4-ethylphenol, free sulfur dioxide, pH, titratable acidity, total sulfur dioxide, volatile acidity, absorbance at 420 nm, absorbance at 520 nm, the sum of absorbances at 420 nm and 520 nm, and the ratio of absorbances 420 nm/520 nm; tristimulus L, a, b, hue angle, and chroma values.

Further analysis was performed by Felipe Laurie, under the direction of Dr. Andrew Waterhouse, Department of Viticulture and Enology, University of California, Davis. This analysis measured the Adams assay, tannin, total colour, large polymeric pigments, small polymeric pigments and monomeric pigments. Normal phase HPLC was used for analysis of monomers, oligomers and polymers absorbing at 280 nm, and for monomers, oligomers and polymers absorbing at 520 nm. Reverse phase HPLC was used for analysis of catechin, epicatechin, gallic acid, caffeic acid and quercetin.

Analysis of variance was performed on the data using a MiniTab statistical package, Minitab Inc., State College, PA, USA.

Results and Discussion

Of all the chemical attributes measured by ETS and at UC Davis, only those found to differ between treatments at the 95% confidence level will be discussed.

Total and free SO₂

Figure 1 illustrates the difference between oxygenated and non-oxygenated wines for free and total SO₂ at eight months. Although Figure 1 only gives the 8 month snapshot of the wine, the measurement of free and total SO₂ has been found to be an effective tool for monitoring micro-oxygenation of wines if done on a weekly basis and charted. The rate of decrease of free SO₂ can be indicative of how the wine is responding to micro-oxygenation. Too rapid a rate of decrease indicates too high a rate of oxygenation. Too low or no decrease would indicate that the rate of oxygenation could be increased. Important points to be made with monitoring free and total SO₂ concentrations are the following: free SO₂ levels should not fall below 10 ppm, to help ensure proper protection of the wine; a decrease in total SO₂ appears to indicate that the free SO₂ has dropped below 10 ppm leaving the wine prone to severe oxidation; and total SO₂ measurements will not be additive after doing SO₂ additions to maintain free SO₂ levels above 10 ppm. For example with this study there were three 30 ppm additions of SO₂.

Free mercaptans and sulfides

Figure 2 demonstrates that all of the wines with micro-oxygenation had a significantly lower level of both methyl and ethyl mercaptan. The effect was sufficient to lower the concentration of these two compounds below their aroma thresholds of 1 ppb. Much concern has been raised over the possible production of disulfides during the oxygenation of wines containing above aroma threshold levels of mercaptans. This study demonstrated no such formation of disulfides; measured levels of disulfides were not significantly different in all treatments. Dimethyl sulfide (DMS) is usually associated with bottle ageing, especially in white wines, generating a characteristic aroma of creamed corn. Normal levels in red wines approach 50 ppm and are associated with aroma complexity. Non-biological production of DMS is thought to occur as a slow oxidative process. Therefore, we expected an increase of DMS concentration in all oxygenated treatments. However, the results clearly show there was not an increase of DMS concentration with the addition of oxygen. Interestingly, a significant decrease in DMS level is seen in all treatments with added toasted oak. The cause of this decrease is not understood.

Spectral effects

Figures 3 & 4 demonstrate differences in spectral characteristics between treatments. All micro-oxygenated treatments increased in absorbance, with an absorbance maximum at 528 nm and an absorbance minimum about 420 nm. No shift in absorbance wavelength was detected at the maximum. There was a slight shift of absorbance minimum from about 428 nm to 421 nm between treatments. This shift may help to explain why certain wines do not appear to increase in brown colour as much as expected with micro-oxygenation. Figure 5 is a simpler illustration of the effect of oxygen and oak on red colour (measured at 520 nm) and brown colour (measured at 420 nm). In all cases micro-oxygenation increased absorbance at 520 and 420 nm, increasing the apparent colour in the wines.

Reverse Phase HPLC Analysis of Phenolics

The wines were analysed for numerous types and classes of phenolic compounds. Figure 6 demonstrates the results using the Price HPLC method to determine protein reactive tannin, polymeric phenols, total anthocyanins and malvidin glucoside. Except for protein reactive tannin, only those compounds or classes of compounds that provided significant differences are shown. The Price HPLC method identifies polymeric material as the area under the last eluting peak that is detected at 280 nm. The area under that same peak, when detected at 520 nm, is referred to as the polymeric anthocyanin peak, as shown in Figure 7. Protein reactive tannin is measured as the difference between the polymeric peak of a wine before and after addition of gelatin. The idea being that the polymeric compounds that react with the gelatin and precipitate may be correlated with astringency or a particular type of astringency, eg. harsh, coarse, or soft. This measure was thought to be a potentially useful indicator of mouth feel. However, across the treatments we saw no significant difference in protein reactive tannin at the 95% confidence level. However, there did appear to be a trend towards a higher level of protein reactive tannin in the micro-oxygenated treatments; thus this analysis may warrant further investigation.

The polymeric phenol levels, as shown in Figure 6, were significantly different. The amount increased with micro-oxygenation and also with oak added in the form of segments. This increase may correlate with a decrease in monomeric procyanidins, and it will be discussed later. This may indicate that both oxygen and toasted oak encourage formation of tannin polymers. However, the type and size of these polymers is very much in question as none of the methods used in this study were able to yield this information.

Also shown in Figure 6 are the levels of total anthocyanin and malvidin glucoside, as measured by HPLC. It is clearly demonstrated that micro-oxygenation decreases both malvidin glucoside and total anthocyanin. Malvidin glucoside also demonstrated a larger decrease in the presence of toasted oak and oxygen than with either toasted oak alone or oxygen alone. This may indicate the occurrence of an interaction between oxygenation of the wine and compounds extracted from toasted oak. At this point we can hypothesise that anthocyanins are more effectively linked to oligomeric and polymeric procyanidins in the presence of toasted oak and oxygen. To provide further support for this process, Figure 7 demonstrates clearly an inverse relationship between monomeric anthocyanins and polymeric anthocyanins. Again, oxygen plus toasted oak, especially the segment form, showed the most significant polymerisation of anthocyanins.

Figure 8 compares two analytical methods that measure the overall tannin concentration of wines, the Folin-Ciocalteu method and the newer Adams tannin assay. Here, the Folin method did not demonstrate any clear difference between treatments. However, the Adams method showed a clear trend between treatments. In the absence of oxygen, toasted oak staves slightly increased the tannin level, compared to the control; it was further increased with toasted segments. Higher tannin levels occurred with all oxygenated samples, with or without toasted oak. Thus, the Adams method does appear to be capable of discerning differences between the treated wines. Unfortunately, sufficient work has not been conducted to correlate these higher Adams tannin values to the sensory perception of the wines.

Normal Phase HPLC Analysis of Phenolics

Normal phase analysis of wine phenolics can better distinguish and quantify molecular weights of these classes of compounds. The detected compounds were grouped into three classes: monomers, oligomers (up to 7 catechin units) and polymers (greater than 7 catechin units). Using two absorbance wavelengths, 280 nm and 520 nm, differences between polymers with and without a covalently bonded anthocyanin can be distinguished. Figure 9 depicts the results between treatments at 520 nm. Figure 10 depicts similar trends at 280 nm. While error bars are shown in Figures 9 and 10, the significance of the data was not calculated. So only general observations can be made for these results. The results agree with those shown in figures 6 & 7, derived by reverse phase HPLC analysis, in that monomers with 520 nm absorbance tend to decrease with oxygenation. It was hoped that a significant difference in quantity of oligomers and polymers might be seen between oxygenated and non-oxygenated wines. Unfortunately, there was no such trend, and normal phase HPLC analysis did not yield usable winemaking information.

Other Phenolic based compounds

Three other compounds analysed by the Steve Price reverse phase HPLC method were: caftaric acid, a useful compound for indicating the impact of oxidation on white wines; quercetin aglycone, a potentially bitter compound; and epicatechin, a building block of tannins. Figure 11 shows that the treatment using toasted oak segments without oxygenation maintained the highest concentration of caftaric acid, indicating a better ability of this treatment to protect the highly oxidizable compound. Quercetin aglycone showed a significant decrease in concentration in all treatments with micro-oxygenation. This may be a partial explanation for the perceived smoothness of oxygenated wines, although this has not yet been proven with quantitative sensory analysis. Also seen is a decrease in epicatechin across all treatments with added oxygen. This decrease in concentration agrees with work (Vidal et al. 2002) using a wine-like model solution, that has shown that flavanol monomers, such as epicatechin, react with products of the breakdown of polymeric proanthocyanidins. This leads to a reduction in the average size of the proanthocyanidin polymers, and that change of polymer size may influence the perceived astringency. This may help to explain a frequent decrease of perceived harshness in wines with micro-oxygenation.

Figure 12, confirms the findings of the Price reverse phase HPLC method. These results, from the Waterhouse Laboratory, used normal phase HPLC and show very similar trends for these small molecular weight phenolic compounds.

Effects on Oak Extractives

Figure 13 shows the abundance of six compounds known to be extracted from oak and found in all treatments at less than 100 ppb. All treatments had detectable amounts of these six compounds, even though some treatments had no added toasted oak, because the ageing trial was performed on a combination of wines that received various treatments during fermentation, including some toasted oak addition. No significant treatment effect was found for *trans*- and *cis*-oak lactone, guaiacol, 4 methylguaiacol or eugenol. A slight effect was noted for isoeugenol. Both eugenol and isoeugenol have

clove like aroma, and isoeugenol may be slightly more prone to oxidation than eugenol. The treatments with added toasted oak showed a significant increase in all compounds over treatments with no oak addition. Toasted oak segment treatments, with or without micro-oxygenation, were very similar. The toasted oak stave treatment showed lower concentrations for most of these six compounds, indicating lower or slower extraction rates. Figure 14 shows the abundance of oak-derived compounds detected above 100 ppb in some treatments: 5-methylfurfural, vanillin and furfural. As expected, both treatments without added toasted oak showed low concentrations of these three compounds. However, the treatment with toasted oak staves also showed low concentrations of the three compounds. Interestingly, the oxygenated treatment with oak staves showed similar concentrations to the toasted oak segment treatments. Knowing that exactly the same amount of toasted staves was added to both the non-oxygenated and oxygenated treatment led us to speculate that there may be an interaction between staves and oxygen. One possible explanation is that oxygenation of the wine produces a stronger driving force to extract these compounds from the staves. Why this is not seen with the segments may be due to the higher exposure of end grain, which is known to increase extraction in toasted oak products, equalizing extraction rates for this product. Further work needs to be done to clarify the phenomenon and its effect on the resulting wine.

Conclusions

This study has provided conclusive evidence that micro-oxygenation of wine during ageing leads to an increase of colour intensity and a concurrent decrease in free mercaptan concentrations without an increase of disulfide concentrations. We have also demonstrated an interaction between the form of toasted oak and micro-oxygenation. Further work is being conducted to relate the effects seen in this study with the sensory impact of the resulting wines. We have also shown a direct relationship between the decrease in monomeric anthocyanins and the increase in polymeric anthocyanins with the addition of toasted oak, with micro-oxygenation, or with the combination of the two. This may lead to a softening of the wine when monomeric anthocyanins are bound to polymers, as demonstrated in the talk earlier by Dr. Leigh Francis. This study has also shown that micro-oxygenation led to a decrease in monomeric procyanidins, epicatechin, and an increase in polymeric phenolics. Both of these observations tend to support the perceived softening of wine that has been micro-oxygenated.

The quest for straightforward analytical methods to monitor the effects of micro-oxygenation and to aid the winemaker in the use of micro-oxygenation has still to be met. This study did show that many complex analytical methods are not adequate alone to monitor oxygenation. However, certain simpler methods, such as the Adams tannin method do show promise as a simple tool. Much more work should be done with the Adams method to relate results to treatment effects on wine and, most of all, to sensory implications.

Acknowledgments

Christian Roguenant, Orcutt Road Cellars

Gordon Burns, Dr. Steve Price and Eric Herve, ETS Laboratories

Felipe Lurie and Dr. Andrew Waterhouse, Dept. Viticulture and Enology, UC Davis

Leslie Norris, Flavorsense

Rebecca Bleibaum, Tragon Corporation

References

Vidal, S.; Cartalade, D.; Souquet, J.-M.; Fulcrand, H. and Cheynier, V. (2002). Changes in proanthocyanidin chain length in winelike model solutions. *J. Agric. Food Chem* 50(8): 2261-2266.

Adams, D.O. and J.F. Harbertson. Use of alkaline phosphatase for the analysis of tannins in grapes and wine. *AJEV*. 1999, 50, 247-252.

Harbertson, J.F., J.A. Kennedy and D.O. Adams. Tannins in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot Noir berries during ripening. *AJEV*, 2002, 53, 54-59.

Waterhouse, A.L., S. Ignelzi, and J. R. Shirley. A comparison of methods for quantifying oligomeric proanthocyanidins from grape seed extracts. *AJEV*, 2000, 383-389.

Waterhouse, A.L., S.F. Price, J.D. McCord. Reversed-Phase High-Performance Liquid Chromatography Methods for Analysis of Wine Polyphenols. *Methods in Enzymology*, 199, 8 299: 113-121.

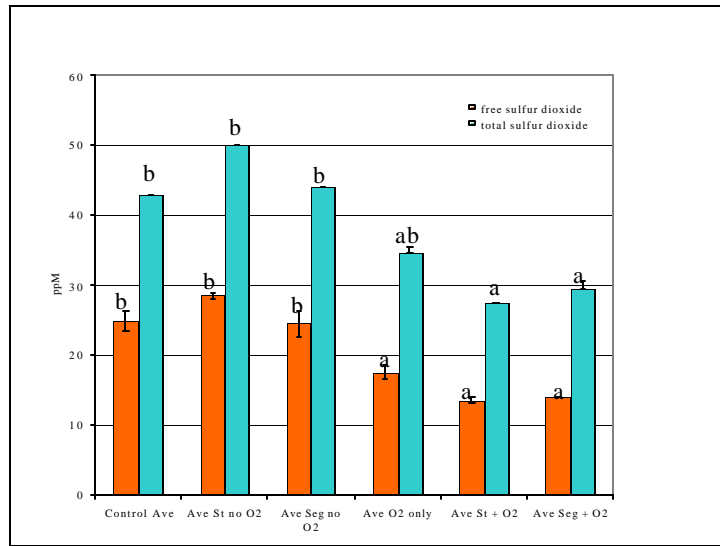


Figure 1. Analysis of free and total SO₂ at 8 months of ageing.

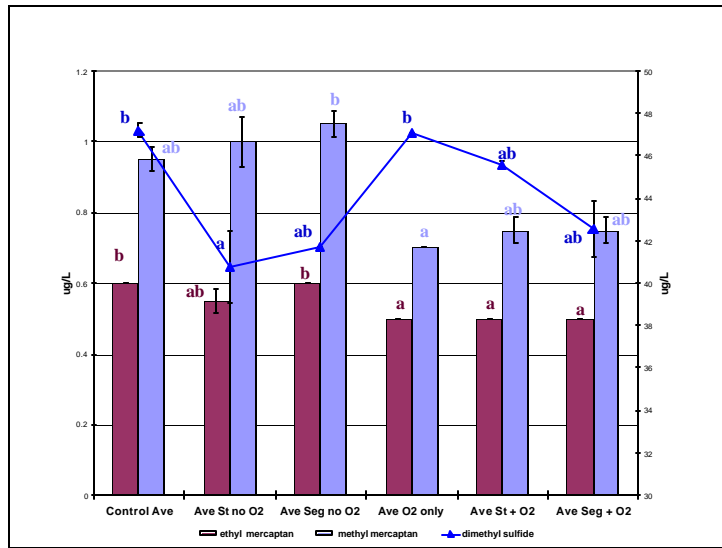


Figure 2. Analysis of thiols and sulfides.

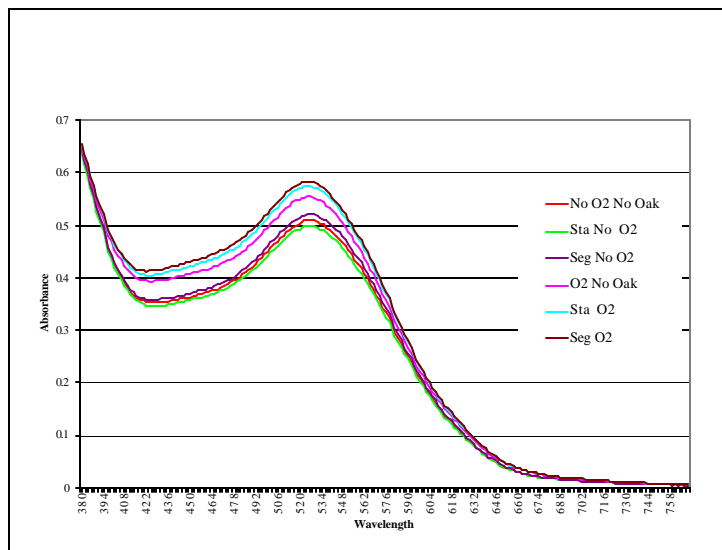


Figure 3. Spectral comparisons between treatments.

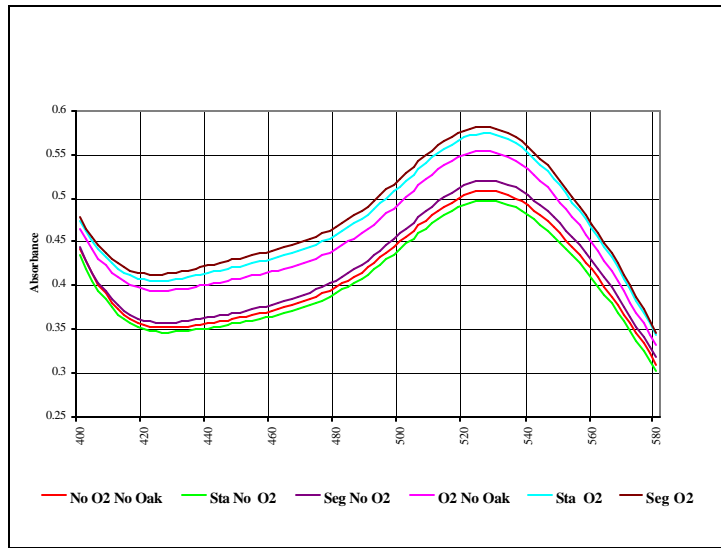


Figure 4. Enlarged 400 – 580 nm region of spectral comparisons between treatments.

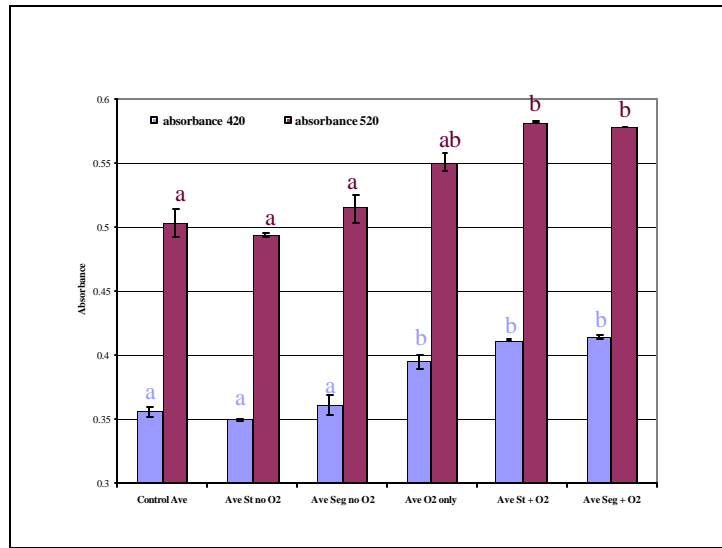


Figure 5. Absorbance at 420 and 520 nm of all treatments.

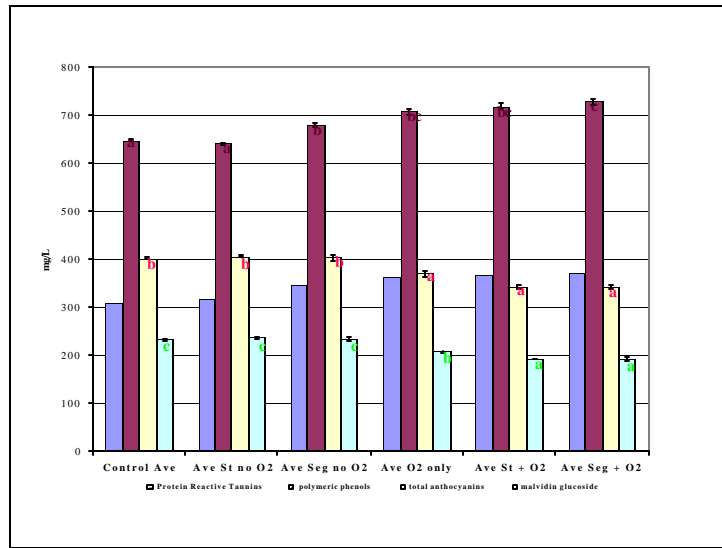


Figure 6. Reverse phase HPLC results for major phenolic classes.

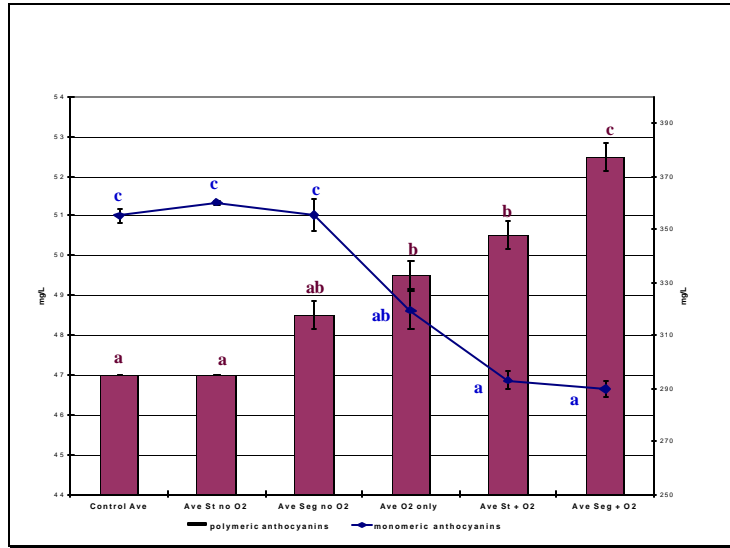


Figure 7. Comparison of polymeric anthocyanins vs monomeric anthocyanins.

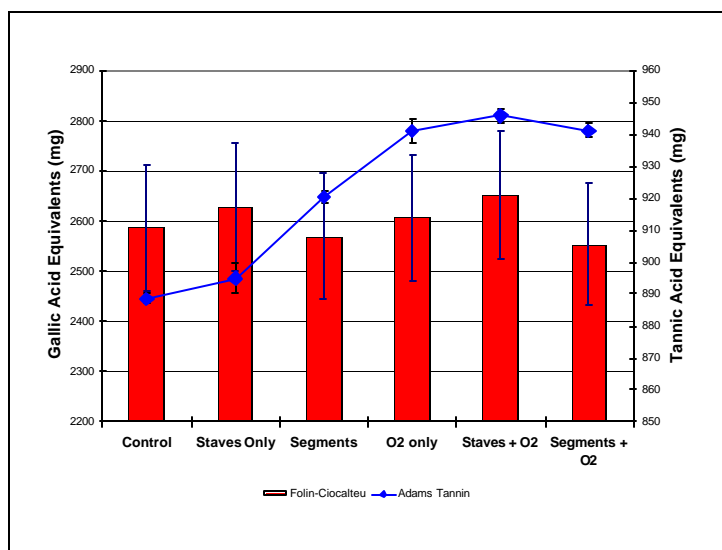


Figure 8. Standard tannin analysis comparison for treatments, Folin Ciocalteu assay and the Adams tannin assay

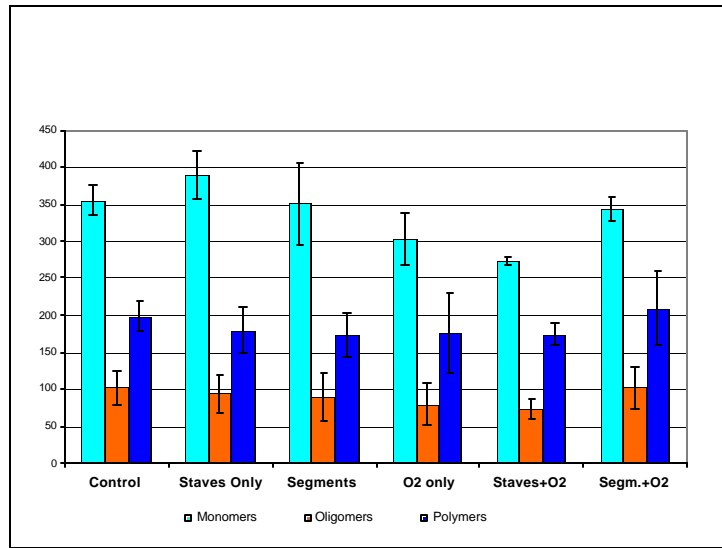


Figure 9. Normal phase HPLC of monomers, oligomers and polymers absorbing at 520 nm.

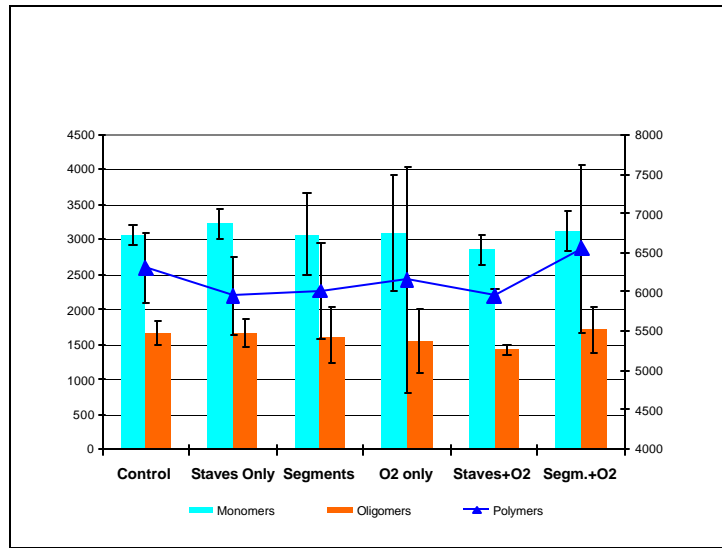


Figure 10. Normal phase HPLC of monomers, oligomers and polymers absorbing at 280 nm.

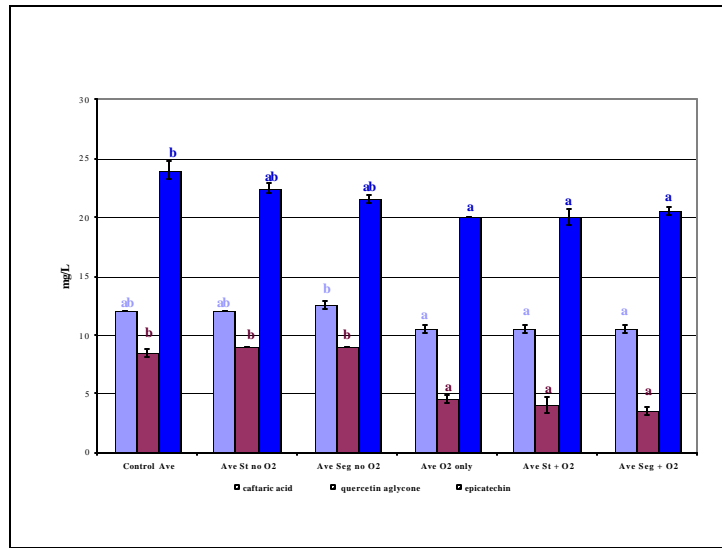


Figure 11. HPLC analysis of caffeic acid, quercetin aglycone, and epicatechin.

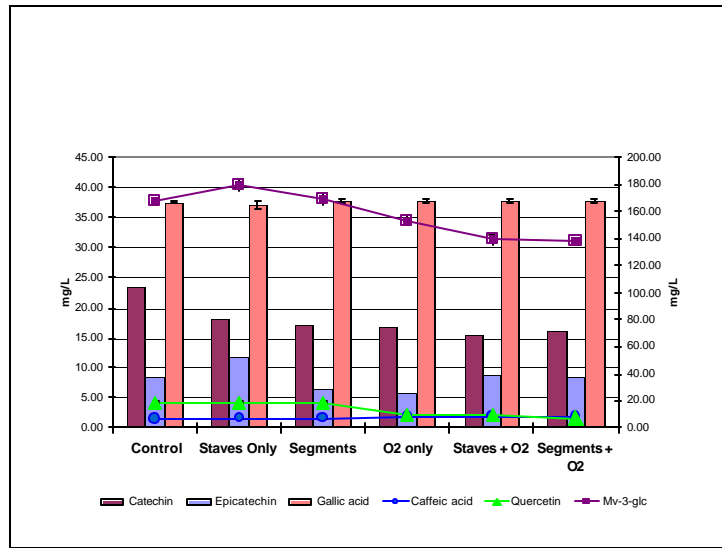


Figure 12. Normal phase HPLC analysis of catechin, epicatechin, gallic acid, caffeic acid, quercetin, and malvidin-3-glucoside

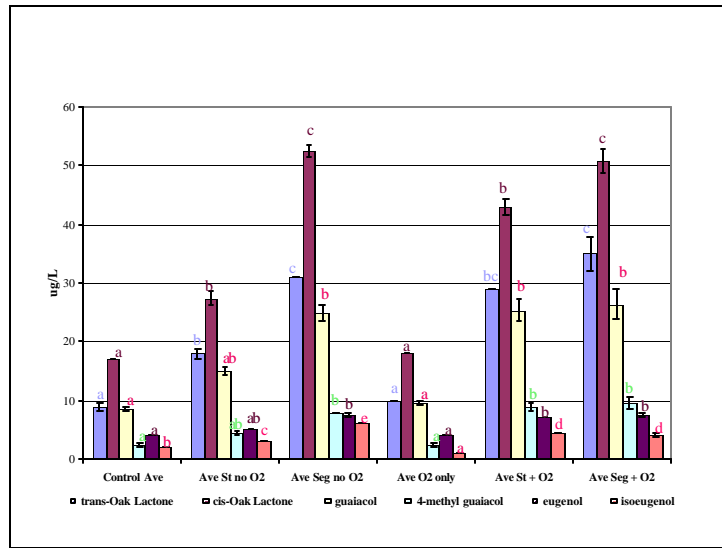


Figure 13. Analysis of minor oak extracted compounds.

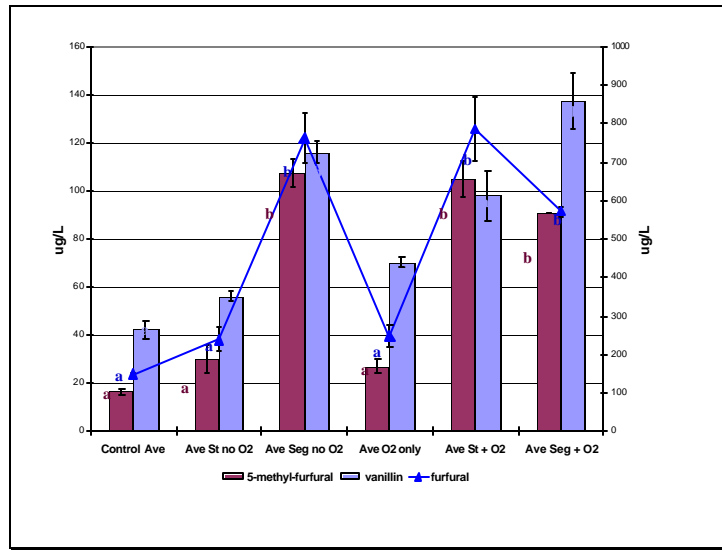


Figure 14. Analysis of major oak extracted compounds.