AUSTRALIAN SOCIETY OF VITICULTURE AND OENOLOGY

ASVO

Australian Government

Australian Grape and Wine Authority

Incoming Scholars Program: Associate Prof. Harbertson and Professor Dr Durner

FINAL REPORT to
The Australian Grape and Wine Authority

Project Number : GWT 1410
Principal Investigator : Dr P. Petrie.

Research Organisation:
Australian Society of Viticulture and Oenology (ASVO)

Date: Friday, 11 March 2016

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Abstract

The Australian Society of Viticulture and Oenology (ASVO) organises and conducts seminars on specialised topics to promote education in viticulture and oenology and help ensure and maintain the highest standards of quality for such educational objectives. The 2014 ASVO Seminar was held in Adelaide and two international scholars – Prof. Harbertson and Prof. Durner – were invited to attend.

The aim of the Adelaide seminar was to present some of the latest viticultural and winemaking research findings, as well as some new and existing techniques and management options that could help growers to improve fruit quality and vineyard profitability at all production price points.

The purpose of the invitation was to allow two scholars of renown to interact with researchers and industry and to present at the seminar. Their visit was intended to give an international perspective and complement a local programme of information regarding science and application in process efficiency and vineyard profitability.

This report summarises the activities and key outcomes arising from the visit by the two scholars. It also summarises the conference presentations given to the Australian wine industry.

Executive summary

- 114 delegates in attendance at the 2014 ASVO seminar: 'Seminar: Inputs and outputs – is less more?' - November 19 - 20.
- AGWA supported the ASVO to fund travel costs for two international speakers; Associate Prof. Harbertson, Extension Enologist, Washington State University, Prosser USA & Prof. Dr. Dominik Durner (Fachgebiet Oenologie und Lebensmitteltechnologie Weincampus Neustadt.
- A diverse group of conference speakers presented mostly published work about methods and strategies to improve fruit quality and profitability.

Background

The ASVO serves the interests of practising winemakers and viticulturists by encouraging the exchange of technical information. One of the ways the ASVO achieves this is by holding seminars. These seminars are open to both members and non-members and cover areas that are topical to the Australian industry and include both domestic and international speakers. They are one of the few events to regularly bring international speakers to present to the Australian industry. The inclusion of an international perspective is critical to the profile of these seminars and their ability to attract a viable audience. It is also an important opportunity for the Australian industry to access topical information from overseas. **Project objectives and performance targets**

Output	Performance Targets	Date
A summary paper for industry, translated for practical application, from Prof. Harbertson & Prof Dr Durner seminar presentations	ASVO to provide a summary paper for industry, translated for practical application, from Prof. Harbertson & Prof Dr Durner seminar presentations	20/11/2014
ASVO to provide responses from Prof. Harbertson & Prof Dr Durner. to questions from industry. The questions and	ASVO to provide responses from Prof. Harbertson & Prof Dr Durner. to questions from industry. The questions	20/11/2014

responses to be made available to industry in video format.	and responses to be made available to industry in video format.	

Papers from Prof. Harbertson & Prof Dr Durner are attached to this report.

Activities associated with this objective were carried out however the video was subsequently deleted. As there was no way of retrieving the data Anne Duncan (AGWA) and Chris Waters (ASVO) agreed that the ASVO is no longer required to provide a video of the questions and answers to industry.

Conclusion

The ASVO's annual two-day Adelaide seminar was a successful and effective means of information dissemination. The seminar provided an ideal opportunity for scientists and practitioners to exchange the latest information on research, to promote adoption of reliable and consistent techniques and ideas.

In addition to the required outputs, ASVO collaboratively worked with AWRI and supported a seminar on the WIC campus at which Prof Dr Durner presented.

Acknowledgements

The activities detailed in this report were supported financially by Australia's grape growers and winemakers through their investment body the Australian Grape and Wine Association with matching funds from the Australian Government.

Visiting Scholar abstracts

Understanding the impact of cultural practices on fruit quality in California.

Extension Enologist, Washington State University, Prosser
Martin Mendez-Costabel, Manager Wine and Grape Supply / GIS E&J Gallo Winery, Modesto, CA, USA

Cultural practices are widely known to affect grape and wine yield and quality. Although many studies have looked at the effects of these practices in primary and secondary metabolites, as well as their influence in vine yield components, growers usually have few options to manage them in the vineyard. Among these are irrigation and canopy management, both proven to affect fruit quality and yield.
The development of new technologies involving remote sensing and geographical information systems (GIS) represents a new opportunity to implement management practices at a large(r) scale, bringing at the same time a new meaning to the term 'precision' viticulture. The application of them to specific situations will be discussed in order to gain a broader understanding on how cultural practices are impacting fruit quality in commercial vineyards across California. The challenges associated with these new technologies will be covered as well, especially focusing in the links between research and practical outcomes that are directly relevant to industry.

Tannin Addition Use in the Production of Red Wines
James Harbertson1, Giuseppina P. Parpinello2, Hildegarde Heymann3 and Mark O. Downey4

Tannins are a heterogeneous class of phenolic compounds that have numerous chemical and sensorial properties, which are important for wine quality. Although tannins can be extracted from grape skins and seeds during alcoholic fermentation and/or from oak barrels during aging, they can also be added to wine as a powder referred to as oenological tannins. Oenological tannins are extracts derived from oak, grapes and exotic trees. Although the use of oenological tannins is widespread in the global wine industry there are a limited number of studies that evaluate their efficacy. In this work we characterized several commercially available tannins using iron and protein precipitation. Oenological tannins (one condensed and hydrolysable) were added at two rates (manufacturer's recommended and excessive) to a Washington State (USA) Cabernet Sauvignon wine after it had been pressed. Phenolic analysis and sensory evaluation by descriptive analysis was carried out on the wines. It was found that the recommended addition rate had little impact the wine tannin concentration for either oenological tannin added. Although the excessive addition rate had a significant impact on wine tannin concentration it was also found to have multiple sensory impacts considered to be negative (brown coloration, bitterness and earthy aroma). The results in this study call into question the use of such additions to modify the tannin concentration of wine as neither result observed here were justifiable.

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Financial Statement

Australian Government
Australian Grape and Wine Authority

END OF PROJECT
FINANCIAL STATEMENT
Statement of Receipts and Expenditure

At the conclusion of each Project, AGWA requires a Statement of Receipts and Expenditure of AGWA funds received in relation to the Project.

Project Title:	ASVO Adelaide Seminar: Inputs and outputs – is less more?

AGWA/GWRDC Project Number	Project Start Date	Project End Date
GWT 1410	**1 July 2014**	**1 August 2014**

Financial Year (e.g. 2010-11)	(a) *Funds brought forward from previous year	(b) #Approved GWRDC Budget (full year)	(c) Total budget available (a)+(b)	(d) Actual expenditure ($)	(e) Difference ($) (c)-(d)
Year 1: 20__/__	**n/a**		0.00		0.00
Year 2: 2014/15	0.00	3,065.00	3,065.00	4,838.64	-1,773.64
TOTAL		3,065.00		4,838.64	**-1,773.64**

* "Funds brought forward" refers to any unspent AGWA funds from the previous year and approved by AGWA/GWRDC to be spent the following year (i.e. column *(e)* from previous year).
# "Approved GWRDC Budget" (now administered by AGWA) is the funding amount specified in the Project Agreement, including any approved Variations after Project commencement.

Was funding expended as approved in the Project Agreement? Yes ✓ No ☐

*If **NO**, please provide reasons:*

Has the cash and/or in-kind funding from Contributing Agencies been received and applied to the Project as approved in the Project Agreement? Yes ✓ No ☐

*If **NO,** please provide reasons:*

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative) ……

…… …… ……
Name: *Title:* *Date:*

Attachment 1 DOMINIK DURNER PAPER

Micro-oxygenation and its impact on polyphenols and sensory characteristics of Pinot noir

Dominik Durner[1,2], Patrick Nickolaus[1], and Hai-Linh Trieu[1]

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Abstract

The continuous addition of measured amounts of oxygen, referred to as micro-oxygenation (MOX), is a widely applied technique to promote greater colour and balanced tannicity of red wines. This study focused the evolution of phenolic and other oxygen-induced compounds during MOX of a 2013 Pinot noir and examined the sensory impact of different oxygen dosages. Before alcoholic fermentation (AF) the Pinot noir grapes were split into two equal portions. One part was fortified with grape tannins yielding higher tannin-to-anthocyanin ratio. After completion of malolactic fermentation (MLF) two different oxygen dosages were applied to the wines over a 12 week period. Photometric analysis showed a continuous increase in colour intensity, which was amplified by higher oxygen dosages. At the same time MOX caused an accumulation of acetaldehyde, which was sensorially not critical until concentrations of 50 mg/L. Acetaldehyde concentrations > 50 mg/L were reached for a oxygen dosage of 20 mg O_2/L/month after one month. These acetaldehyde levels concomitantly increased the SO_2 demand of the wines rendering them as being over-oxygenated. The application of MOX triggered the formation of red/purple-coloured anthocyanin-flavanol oligomers (up to 25 mg/L) and orange-coloured vitisin B (up to 7 mg/L). Higher tannin-to-anthocyanin ratio favoured the formation of anthocyanin-flavanol oligomers verifying that tannins are responsible for the initiation of polymerisation reactions. The concentration of the orange-coloured vitisin B was not dependent on the tannin-to-anthocyanin ratio of the wine. Descriptive analysis confirmed higher colour intensity and revealed enhanced strawberry flavour in the wine subjected to a moderate oxygen supply (10 mg O_2/L/month no longer than three months after MLF). In case of over-oxygenation, the sensory panel observed a higher intensity in dry tannins and a decrease in fruitiness. For best MOX results according to this study moderate oxygen supply with 10 mg/L/month for three months is recommended as early as possible after AF. Besides high anthocyanin concentrations, a high proportion of tannins is recommended for a satisfactory formation of polymeric pigments, which do not only improve the colour, but also soften the astringency perception.

Evolution of colour and anthocyanins during winemaking and elaboration

The evolution of red wine colour is subject to dramatic changes throughout the vinification process. During AF a high proportion of anthocyanins gets lost due to adsorption to yeast cells (Morata et al. 2005; Mazauric and Salmon 2006), ß-Glucosidase-catalyzed cleavage (Wenzel 1989), or polymerisation/derivatisation reactions (Fulcrand et al. 2004). This loss in anthocyanins is usually accompanied by a colour decrease. The phenomenon becomes particularly obvious during AF of a thermovinified wine (Fig. 1) since the extraction process of anthocyanins has been completed before AF. After AF, the decrease in colour density slowed down. However, colour evolution is still well correlated to the loss in anthocyanins until the end of MLF. During the three months MOX period, colour and anthocyanin curves developed differently; while the wine colour increased, the anthocyanin concentration decreased nearly 30 % within this period indicating the formation of deeply coloured polymeric pigments.

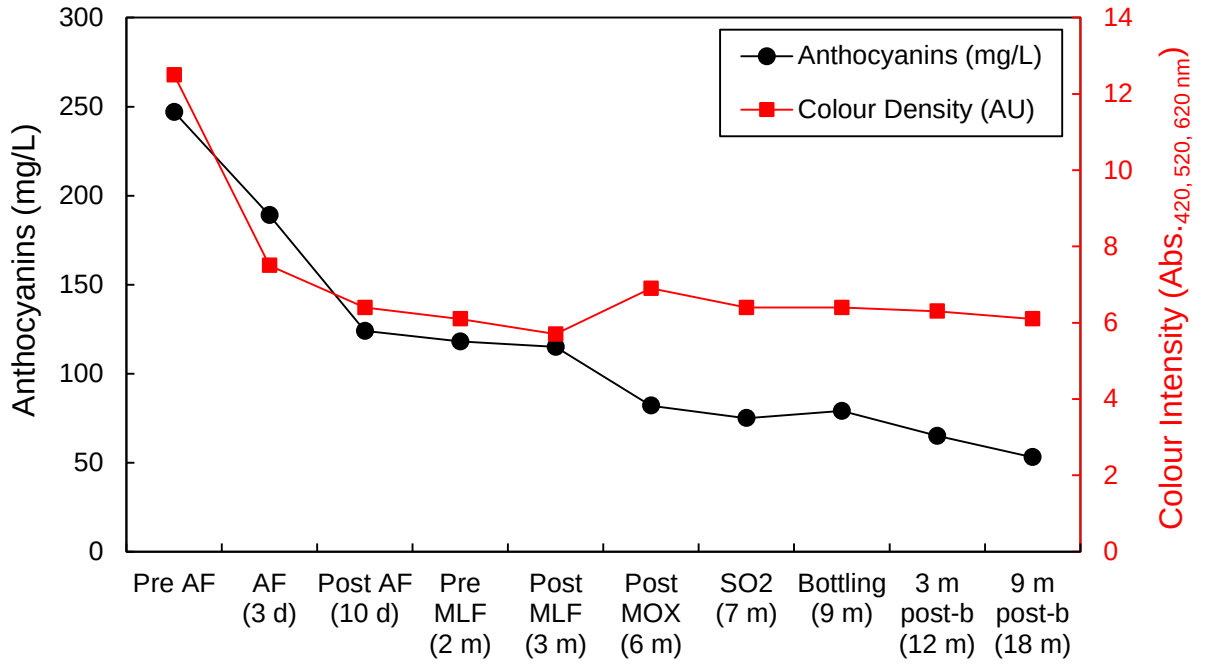


Fig. 1: Evolution of colour during winemaking and elaboration of a thermovinified 2012 Pinot noir; thermo-extraction at 85°C for 2 min; 3 hL juice fermentation at 20°C for 10 days; MLF at 18°C for 35 days; MOX with10 mg O_2/L/month at 15°C for 12 weeks; 90/35 mg/L total/free SO_2; bottle storage at 15°C for 9 months.

Beneficial and detrimental MOX effects

As shown in Fig. 1, MOX improved the colour of the wine. Oxygen-induced polymeric pigments, particularly the ethylidene-bridged anthocyanin-flavanol oligomers (Timberlake and Bridle 1976;

Escribano-Bailon et al. 1996; Es-Safi et al. 1999), are thought to be responsible for the higher colour stability in MOXed wines. At the same time, the formation of polymeric pigments seems to soften astringency perception of red wines (Weber et al. 2013). This effect has been shown in MOX trials before claiming that small amounts of oxygen promote balanced tannicity (Del Carmen Llaudy et al. 2006; De Beer et al. 2008; Durner et al. 2010). Alongside these advantages, however, other repercussions of MOX have to be considered. Acetaldehyde, a compound that causes off-flavour at high concentrations, is produced in the course of oxygen-triggered reactions of polyphenols (Wildenradt and Singleton 1974). An accumulation of acetaldehyde could therefore be detrimental for wine. Another issue is the occurrence of dry tannins due to excessive oxygen addition (Lemaire 1995; Durner et al. 2010). Accordingly, there is a demand to monitor beneficial and detrimental effects during MOX in order to identify optimum oxygen dosages for different phenolic preconditions.

Winemaking protocol and MOX experiment

The MOX experiment was carried out on a Pinot noir, clone Mariafeld, which was grown in Oberderdingen (Württemberg) and harvested on October, 12th, 2013. Must analysis revealed 20.9°Bx, pH 3.2, 240 mg/L YAN, and negligible volatile acidity and gluconic acid concentrations. Prior to alcoholic fermentation de-stemmed and crushed grapes were heated to 82°C for 2 min and cooled down to 40°C for depectinization using 30 µL/L pectinase (Trenolin Frio DF, Erbsloeh, Geisenheim, Germany). After 13,5 h, grapes were pressed and the juice was clarified using a separator. An aliquot of the clarified juice was supplemented with 200 mg/L condensed tannins (UVATANN ST, Ever Intec, Pramaggiore, Italy). Both, fortified and regular juice were inoculated with 200 mg/L of the hydrated Mycoferm Cru 05 yeast strain according to manufacturer's instructions (Ever Intec, Pramaggiore, Italy). After two days, the two batches were inoculated with the Extremo IT14 strain (Ever Intec, Pramaggiore, Italy) to initiate malolactic fermentation (MLF). During alcoholic fermentation and simultaneous MLF both wines were kept at 20°C. After completion of MLF (36 days), the wines were racked off, divided into six equal portions per fortified and regular wine, and transferred to 100 L stainless steel tanks with a diameter of 200 mm and a height of 3200 mm. The wine analysis at this point revealed an anthocyanin concentration of 102 mg/L. Depending on the grape tannin supplementation before AF, the tannin concentrations after MLF were 131 mg/L and 174 mg/L, respectively. These measures resulted in two different molar tannin-to-anthocyanin ratios: 2.2 for the regular wine and 2.9 for the tannin-supplemented Pinot noir.

After racking off, two different MOX treatments with oxygen dosages of 10, or 20 mg O_2/L/month were started for a period of 12 weeks at a constant temperature of 15°C in duplicate. Oxygen delivery was conducted using food grade oxygen (BIOGON O E948, Linde Gas, Pullach, Germany), a VinO2 MOX system (Ever Intec, Pramaggiore, Italy), and ceramic diffusers with a pore size of 0.4 µm (Thonhauser, Perchtolsdorf, Austria). The diffusers were suspended 50 mm above the bottom of the tanks. Control batches of the same two wines were stored without oxygen addition for the same time period and at the same temperature in duplicate. Upon completion of MOX, the wines were sulphited by consecutive additions of SO_2 in order to achieve a stable level of 35 mg/L free SO_2. After bottling, wines were stored horizontally at room temperature for two months until sensory sessions started.

Evolution of colour and acetaldehyde during MOX

As stated by many other authors, oxygen triggers the formation of stable pigments with higher colour intensity and a purple colour hue (Cano-Lopez et al. 2006; Del Carmen Llaudy et al. 2006; De Beer et al. 2008; Cano-Lopez et al. 2008). Monitoring the colour intensity revealed a steady rate of increase throughout the 12 weeks MOX process for both treated wines (Fig. 2). While the colour intensity in the control wine remained unchanged during the first six weeks, both MOX wines immediately inclined in colour. At completion of MOX, the colour intensity was increased by 10 % in the control wine, by 23 % in the wine which was treated with 10 mg O_2/L/month, and by 36 % in the wine which received twice the amount of oxygen. Accordingly, the colour gain was 13 % per 10 mg O_2/L/month for three months. The increase in colour of the control wine resulted probably due to polymeric pigments triggered by residual oxygen in the wine.

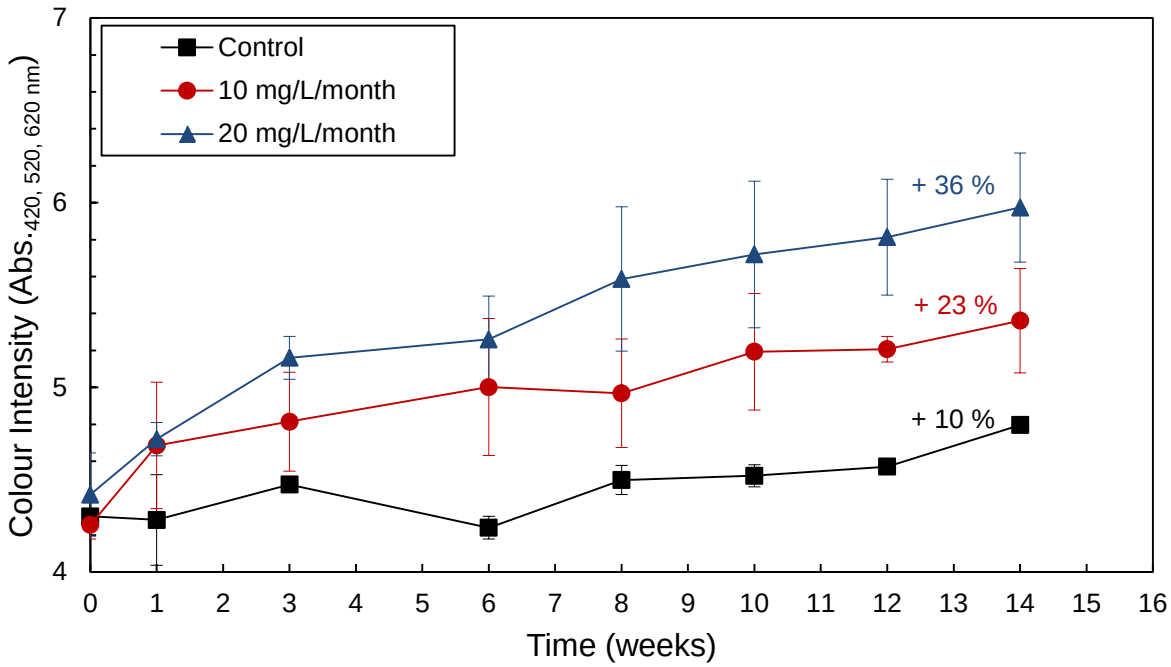


Fig. 2: Evolution of colour during 12 weeks MOX with different oxygen dosages in Pinot noir (error bars indicate 2SD).

The increase in colour has continued for two weeks after completion of MOX suggesting a time delay in the process of forming polymeric pigments. Considering the reaction paths of oxygen in red wine, three consecutive and interdependent steps take place: First, the oxidation of catechol-containing phenolics yielding highly reactive hydrogen peroxide (Wildenradt and Singleton 1974); second, the hydrogen peroxide-induced oxidation of ethanol to acetaldehyde (Fenton 1894); and third, the formation of anthocyanin derivatives, polymeric pigments, and other polymerized polyphenols utilising the formed acetaldehyde (Cheynier et al. 2002; Vernhet et al. 2011). As an intermediate product, acetaldehyde plays a major role during MOX and was suggested as a control parameter for the MOX process (Carlton et al. 2007). On average, red wines contain 30 mg/L acetaldehyde (Liu and Pilone 2000). It can contribute fruity aromas to red wine at low levels. At higher concentrations the wine aroma is considered a defect and is reminiscent of rotten apples. The threshold of acetaldehyde in wine ranges between 100-125 mg/L (Liu and Pilone 2000). An accumulation of acetaldehyde is supposed to determine the endpoint of MOX (Carlton et al. 2007). Monitoring the acetaldehyde by the use of enzymatic testing (R-Biopharm Test-Combination No. 10668613035, Darmstadt, Germany) revealed fast-rising concentration during 12 weeks MOX for the wine which was treated with 20 mg O_2/L/month (Fig. 3). After 10 weeks, the concentration levelled off at 75 mg/L suggesting a state of equilibrium between formation and consumption of acetaldehyde. The low MOX dosage showed a slower and later increase

in acetaldehyde, finally reaching a concentration of 45 mg/L. The control wine remained at stable acetaldehyde levels during the 12 weeks.

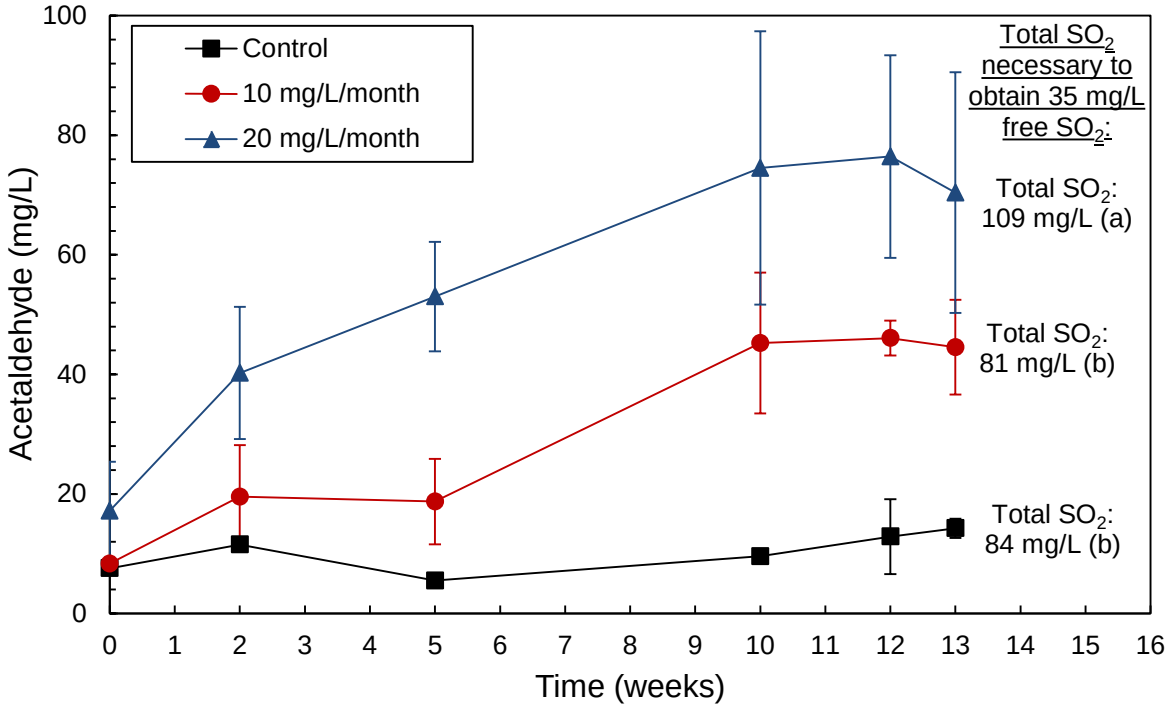


Fig. 3: Acetaldehyde during 12 weeks MOX with different oxygen dosages in Pinot noir (error bars indicate 2SD; Total SO_2 data with different letters indicate significant differences for $p \leq 0{,}05$).

The acetaldehyde curves (Fig. 3) show good correlation with the evolution of wine colour (Fig. 2). However, adverse effects of acetaldehyde may also appear when its concentrations are too high. These could be either a deterioration of organoleptic characteristics or a higher demand in SO_2. In order to assess the negative impact of the acetaldehyde, the concentration of total SO_2 necessary to obtain 35 mg/L free SO_2 was determined (Fig. 3). Despite its higher acetaldehyde level, the wine which was treated with 10 mg O_2/L/month retained the same total SO_2 as the control, but with a considerable portion (57 %) in the bound form. In contrast, significant elevation of total SO_2 was observable in the wine treated with 20 mg O_2/L/month which could be attributed to the high concentration of acetaldehyde. Since too high levels in SO_2 should be avoided with regard to legal limits, inhibition of lactic acid bacteria, and negative sensory aspects, such an increase in SO_2 demand should be regarded as over-oxygenation.

MOX induced formation of polymeric pigments

As mentioned earlier, MOX-induced polymeric pigments are thought to be responsible for the observed colour intensification. Vitisin B, four isomers of the ethylidene-bridged malvidin-3-glucoside-flavanol dimer, and 16 isomers of the double ethylidene-bridged flavanol-malvidin-3-glucoside-flavanol trimer were analysed using LC-Q-ToF-MS analyses. Exact masses of molecular ions and fragmentation patterns of the molecules were used for identification. Vitisin B was quantified using malvidin-3-glucoside as an external standard. The ethylidene-bridged dimer was quantified using the ethylidene-bridged dimer cyanidin-3-glucoside-catechin. The ethylidene-bridged trimer was quantified using the the double ethylidene-bridged trimer catechin-cyanidin-3-glucoside-catechin. The standards were synthesised by and obtained from Weber and Winterhalter (2014). Fig. 4 shows the concentrations of vitsin B and ethylidene-bridged anthocyanin-flavanol dimers and trimers after completion of MOX with different oxygen dosages in regular and tannin-supplemented Pinot noir. As expected, the highest concentrations were found in wines treated with 20 mg O_2/L/month. Regarding the different oxygen dosages, the red/purple-coloured anthocyanin-flavanol oligomers showed a faster increase in concentration than the orange-coloured vitisin B. In relation to the progressive colour intensification shown in Fig. 2, the disproportionately low increase in vitisin B concentration seems to be sensorially irrelevant. Considering the fast increase in anthocyanin-flavanol oligomers, the anthocyanin-flavanol dimer was predominantly formed upon 10, the trimer upon 20mg O_2/L/month, suggesting that not only the concentration but also the size of MOX-induced polymeric pigments is dependent on the oxygen dosage applied.

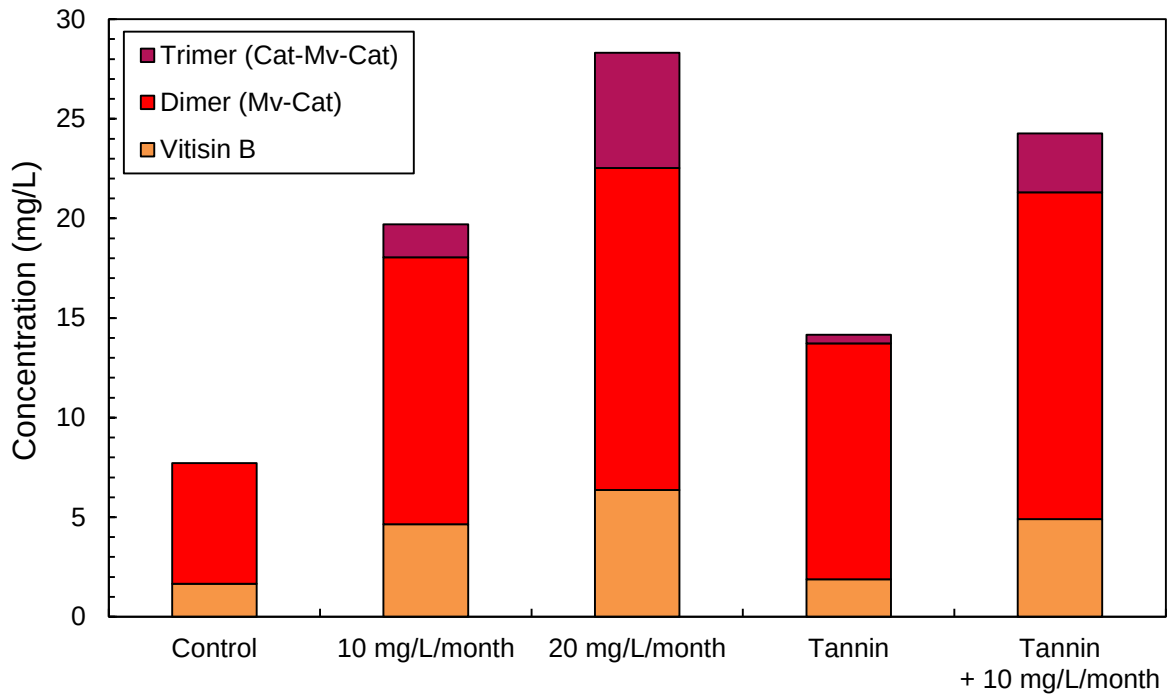


Fig. 4: Concentrations of vitsin B and ethylidene-bridged anthocyanin-flavanol dimers and trimers after completion of MOX with different oxygen dosages in regular and tannin-supplemented Pinot noir (Abbreviations: Cat: Catechin; Mv: Malvidin-3 glucoside).

The comparison of regular and tannin-supplemented Pinot noir in Fig. 4 revealed that higher tannin-to-anthocyanin ratio favoured the formation of anthocyanin-flavanol oligomers. At the same time the MOX-induced production of orange-coloured vitisin B was not dependent on the tannin-to-anthocyanin ratio of the wine. This observation confirms that tannins are responsible for the initiation of polymerisation reactions of anthocyanins and tannins.

Sensory impact of MOX

The descriptive sensory analysis was conducted with 18 trained judges according to the method described by Durner et al. (2010). According to statistically processed sensory data, MOX treatment yielded significant changes in color intensity, colour hue, strawberry aroma, and dry tannin impression (Fig. 5). The wine benefited from low dosage MOX, as colour increased and fruit intensity improved confirming the hypothesis that a moderate acetaldehyde concentration can contribute fruity aromas to red wine (Liu and Pilone 2000). With a purple tint reminding more of Cabernet Sauvignon, the 20 mg O_2/L/month dosage did not result in a visual colour improvement of the Pinot noir. Furthermore, there was an unpleasant increase in dry tannin perception which may be also regarded as an indicator for over-oxygenation (Lemaire 1995).

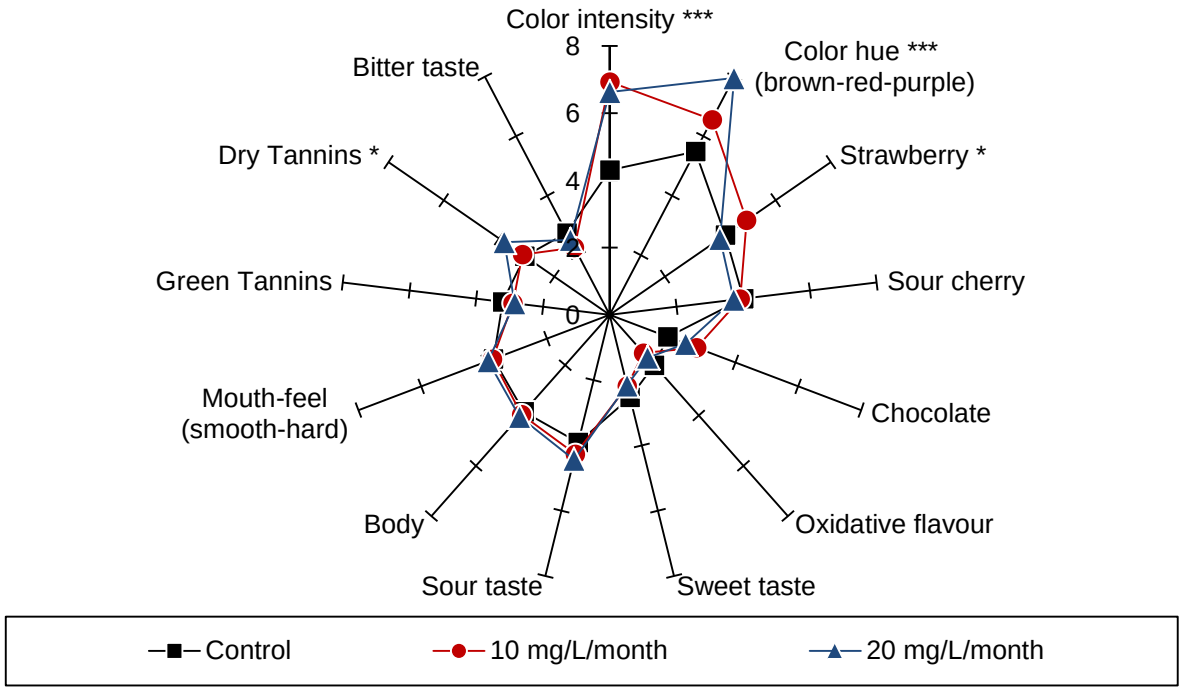


Fig. 5: Sensory impact of MOX with different oxygen dosages in Pinot noir three months after bottling (n=18×2; Scale: 0-10; Significance levels: * $p \leq 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$).

Examining the sensory profile for the tannin-supplemented Pinot noir, the addition of 200 mg/L grape tannins before AF had only little impact on the descriptors (Fig. 6). However, the MOX-induced colour intensification was more pronounced in the tannin-supplemented wine confirming that a higher tannin-to-anthocyanin ratio accelerates the formation of red coloured polymeric pigments. On the other hand, the strawberry aroma, which was increased upon the 10 mg O_2/L/month dosage, remained unchanged when tannins were added to the wine. The dry tannin intensity was significantly increased in the tannin-supplemented Pinot noir at 10 mg O_2/L/month dosage. In comparison to the regular wine (Fig. 5), the unpleasant increase in dry tannin perception was only observed at the 20 mg O_2/L/month dosage suggesting that MOX-induced effects are reached faster with a higher tannin-to-anthocyanin ratio.

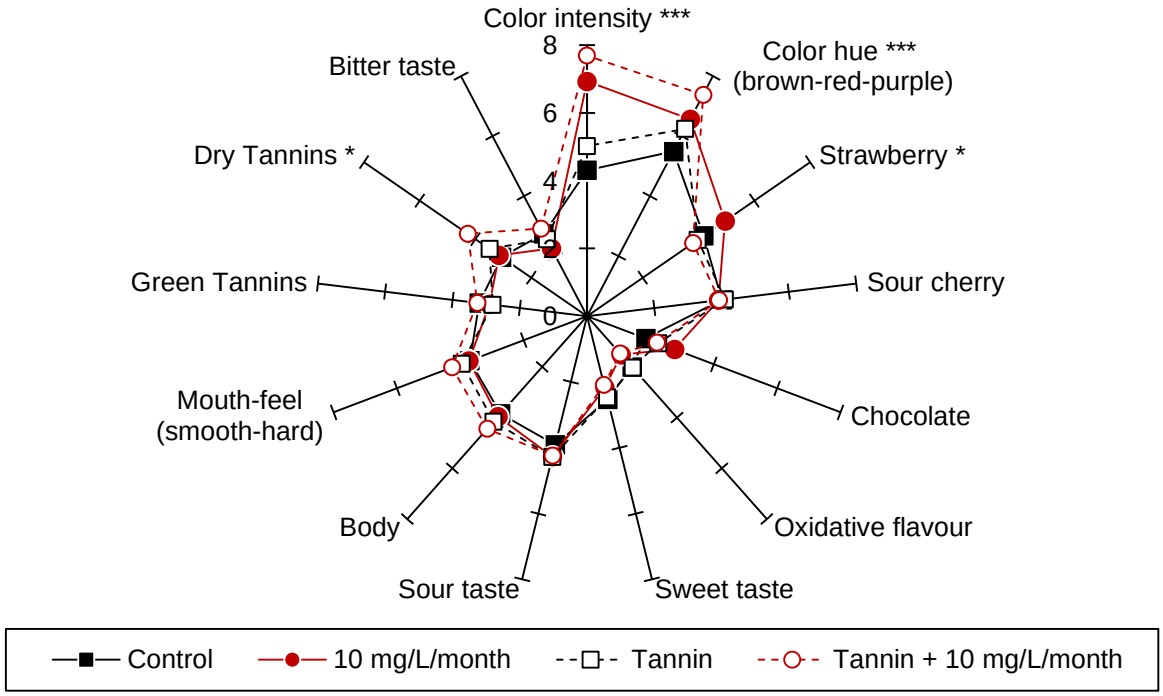


Fig. 6: Sensory impact of grape tannin addition with and without MOX in Pinot noir three months after bottling (n=18×2; Scale: 0-10; Significance levels: * $p \leq 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$).

Conclusions

The experiment demonstrated that MOX is a useful tool to intensify and stabilise the colour of a red wine with medium anthocyanin concentration. To achieve the desired results it is necessary that laccase and other PPOs are completely inactive. The MOX-induced colour increase is always accompanied with an accumulation of acetaldehyde. Higher oxygen dosages are linked to a higher rise in acetaldehyde concentration and accordingly to a larger increase in colour. However, acetaldehyde concentrations > 50 mg/L cause higher SO_2 demand and concentrations > 100 mg/L are considered as an off-flavour.

Acetaldehyde is necessary for the formation of the red/purple-coloured ethylidene-bridged anthocyanin-flavanol oligomers. These oligomers are most likely responsible for the MOX-induced colour intensification. They increase in concentration as the oxygen dosage increases. Besides these compounds, the orange-coloured vitisin B is also growing in concentration with a higher oxygen dosage. Due to the disproportionately low increase in vitisin B concentration, it can be assumed that vitisin B is irrelevant for the wine colour at this stage. Tannin addition before AF favoured the MOX-induced formation of anthocyanin-flavanol oligomers. Vitisin B concentration was not dependent on the tannin-to-anthocyanin ratio of the wines. These observations suggest that tannins are essential for the initiation of polymerisation reactions responsible for higher colour stability.

Sensory analysis confirmed higher colour intensity due to 10 mg O_2/L/month treatment and revealed an increase in strawberry aroma which is attributed to moderate acetaldehyde concentrations in the wine. A high oxygen dosage of 20 mg O_2/L/month resulted in an atypical purple hue and an unpleasant increase in dry tannin perception. These descriptions indicate over-oxygenation. The supplementation of grape tannins without MOX showed only minor changes in the sensory profile. However, the increase in colour intensity was even larger in association with MOX treatment confirming the hypothesis of an accelerated formation of red coloured polymeric pigments at higher tannin-to-anthocyanin ratio. Besides colour, the tannin-fortified and MOX treated wine has been described with higher dry tannin intensity already after low oxygen dosage. These results suggest that the oxygen demand of a red wine decreases with an increasing tannin-to-anthocyanin ratio.

Acknowledgements

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Attachment 2 JAMES HARBERTSON PAPER

Tannin Addition Use in the Production of Red and White Wines

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Abstract

Tannins are a heterogeneous class of phenolic compounds that have numerous chemical and sensorial properties, which are important for wine quality. Although tannins can be extracted from grape skins and seeds during alcoholic fermentation and/or from oak barrels during aging, they can also be added to wine as a powder referred to as oenological tannins. Oenological tannins are extracts derived from oak, grapes and exotic trees. Although the use of oenological tannins is widespread in the global wine industry there are a limited number of studies that evaluate their efficacy. In this work we characterized several commercially available tannins using iron and protein precipitation. Oenological tannins (condensed and hydrolysable) were added at two rates (manufacturer's recommended and excessive) to a Washington State (USA) Cabernet Sauvignon wine after it had been pressed. Phenolic analysis and sensory evaluation by descriptive analysis was carried out on the wines. It was found that the recommended addition rate had little impact the wine tannin concentration for either oenological tannin added. Although the excessive addition rate had a significant impact on wine tannin concentration it was also found to have multiple sensory impacts considered to be negative (brown coloration, bitterness and earthy aroma). The results in this study call into question the use of such additions to modify the tannin concentration of wine as neither result observed here were justifiable.

Introduction

Tannins are primarily important as a result of sensory attributes, particularly astringency (Gawel 1998). Tannins as with many phenolic compounds have antioxidant properties and will also react with anthocyanins to form pigmented tannins that are part of the collective group of compounds known as polymeric pigments (Somers 1971). Tannins are extracted from the skins and seeds during alcoholic fermentation and maceration. The amount of tannins extracted is dependent on the winemaking technique employed (Sachi et al. 2004) and there is evidence that different winemaking techniques alter the amount of tannin extracted from the skins and seeds differentially (Cerpa Calderón and Kennedy 2008, Harbertson et al. 2009). Grapes are quite rich in tannins containing ~ 4 mg/g fresh weight of skin and seed tannins though their concentration varies by cultivar, viticultural management practices (Downey et al. 2006).

Although it has been understood that only a portion of the tannins available in grapes are extracted into the wine (Boulton et al. 1996) it has only recently been discovered that there are problems with the extraction of tannins (Hazak et al. 2005, Adams and Scholz 2008). Briefly, grape cell wall materials behave like fining agents during the fermentation and larger tannins are removed during the fermentation by binding to the cell wall materials (Bindon et al. 2010ab). To counteract, enological tannins, which are purified from different sources can be added as a powder during the winemaking process (Kovac et al. 1992, Kovac et al. 1995, Main and Morris 2007, Parker et al. 2007, Bautista‐Ortín

et al. 2014) Although this is not the only reason oenological tannins are added to wine. It also possible to vary the winemaking technique to extract more phenolics during the winemaking process and has been reviewed here (Sacchi et al. 2005, Casassa and Harbertson 2014).

Tannins Defined

Tannins are chemically defined by their ability to precipitate protein (Hagerman 2002) but can also be subdivided into categories based on their behavior under acidic conditions. Under acidic conditions, condensed tannins also known as proathocyanidins will break down into the red colored anthocyandins (Porter et al. 1986), while hydrolysable tannins will break down to ellagic or gallic acids (Hagerman 2002). The type of tannin found in a particular oenological preparation is highly dependent on the source material (Versari et al. 2013) although in some cases products contain impurities (Obreque-Slíer et al. 2009). Grape skins, seeds and stems only contain condensed tannins while most wood derived tannins contain primarily hydrolysable tannins and in some cases a small amount of condensed tannins (Puech et al. 1999).

Oenological Tannins

Oenological tannins are extracts derived from oak, grapes and exotic trees, and are made into powder so they can be easily weighed out and added to wine. Oenological tannins are regulated in different winemaking areas of the world according to the country's particular allowances (Versari et al 2013). The Organisation Internationale de la Vigne et du Vin (OIV) provides a codex that contains guidelines on oenological tannins (Codex Alimentarius Commission
2009, International Oenological Codex 2012). The codex also provides information for the chemical evaluation of various oenological tannins (for regulatory usage) and the amount of tannin required (by the provided methodology) to be defined as a particular type of tannin. The listed purity of tannins for grapes is worryingly low with the requirements of 50 mg/g catechin equivalents and proanthocyanidin tannin 0.5 mg/g catechin equivalents which corresponds to 5 and 0.05% by mass respectively. As many of the companies that produce oenological tannins are based in Europe this might be observed to be somewhat problematic. This can be partially explained by the fact that in Europe, oenological tannins are only allowable as processing aids, which essentially means they cannot impact the color or olfactory properties of the wine (Codex Alimentarius Commission 2009) and as such purity may be less of an issue. However in other countries (United States, Australia, New Zealand, South Africa) where tannins are allowed can be added for adjusting tannin content, purity may be more important.
Some research has been carried out in an attempt to evaluate the contents of various different oenological tannins. Neither study utilized the methodologies provided by the OIV for the evaluation of tannins (Obreque-Slier et al. 2009, Harbertson et al. 2012). Obreque-Slier et al. (2009) utilized numerous methodologies including high performance liquid chromatography (HPLC) precipitation with gelatin, and heated acid treatments to release anthocyanidins from proanthocyanidins. The range of procyanidins measured in 10 commercial products were from 1.2 mg/g to 72 mg/g of powder which are more pure than standards set by the OIV, however it is difficult to know whether the values are comparable given the difference in methodologies. Similarly Harbertson et al. (2012) using BSA protein precipitation found protein precipitable tannins ranged between 193 mg/g to 425 mg/g of powder in 8 products that originated in Europe. A recent study compared the purity of 4 commercial grape skin oenological tannin products to 4 different purified skin tannin samples by means of phloroglucinolysis, and gel permeation found the purity ranged from 39.9% to 61.7% for the oenological tannins and 44%-92.4% for the purified skin samples (Bautista‐Ortín et al. 2014). They also found the size distribution for the oenological skin tannins was quite different than skin tannin samples which is likely due to fact that oenological tannins

are water extracts whereas the fractionation done in research laboratories utilizes solvents (methanol, acetone).
As there are numerous oenological tannins available, clearly more research is required to understand the variance in concentration and purity in the products. Further, as there are numerous methodologies available for the measurement of tannins (nicely summarized here Versari et al. 2013) it would be important for the tannins to be assessed by several methods similar to the approach of Obreque-Slier et al. (2009) and to include the methods provided in the OIV codex for comparison.

Uses of Oenological Tannins in Wine Production

Removal of Proteins and Enzymatic Activity

Protein hazes are typically problematic in white wine production with grape parthogenesis-related proteins (Waters et al. 1996), berry chitinases (Waters et al. 1998) and thaumatin proteins (Claus et al. 2014) all being cited as potential causes of hazes in wine. Bentonite, a phyllosilicate clay is primarily used to prevent protein hazes by interacting with positively charged proteins or amino acids in the juice or must and can lead to more prolonged fermentations as a result of depleting arginine and lysine (Bach and Hoffman 1978). Bentonite is also known to strip some aroma compounds from wine (Armanda and Falqué 2006). Bentonite lees disposal and the loss of wine due to occlusion into bentonite lees are economically problematic (Muhlack et al. 2006). In-line dosing of bentonite combined with centrifugal clarification have been demonstrated to be effective (Nordestgaard et al. 2007). Unfortunately, smaller wine producers will not have access to centrifuges although in-line dosing is certainly within their means. Tannins have been suggested as a means of removing proteins and unwanted enzymatic activity such as laccase in white wines (Versari et al. 2013) although no published data suggesting the feasibility of this has been published. The ability of tannins to denature enzymes has long been recognized (Goldstein and Swain 1965). Anecdotal evidence suggests that the addition of hydrolysable and condensed tannin mixtures added at 500 mg/L was able to reduce laccase activity, however made the wine bitter. The bitterness is likely due to low molecular weight phenolics that are present in most enological tannin preparations which will not interact with proteins when larger tannins are present (Harbertson et al. 2014). Research has been conducted that shows proteins and metals can be bound by immobilized hydrolysable tannins that have been covalently bound into a water-insoluble matrix (Chibata et al. 1986). Although this research has not been tested with wine or juice, there is potential for removing proteins or metals without the danger of leaving residual low molecular weight phenolics that would cause unwanted bitterness. The removal of metals that have been demonstrated to play a significant role in the oxidation of wine may also be beneficial (Danilewicz 2007).

Color Stabilization

As discussed earlier, anthocyanins, the primary pigment in wine will react different classes of compounds to form polymeric pigments (Somers 1971). The importance of polymeric pigments is their bisulfite bleaching resistance and the ability to maintain their coloration across the wine pH range (Somers 1971). There are several proposed reaction mechanisms for pigmented tannin formation for both condensed and hydrolysable tannins (Haslam 1998, Fulcrand et al. 2006, Quideau et al. 2005, Chassaing et al. 2010). Some of the pigmented tannin adducts have been recovered in wine (Remy et al. 2000, Bakker and Timberlake 1997, Schwarz and Winterhalter 2004, de Frietas and Mateus 2004) and some have been created in model solutions where the starting concentration of the reactants may not be observed in vivo (Quideau et al. 2005, Chassaing et al. 2010).
In any case, numerous experiments have been carried out to evaluate the effect of tannin addition on color stabilization in wine. The results have shown varied impacts likely due to differences in the chemistry of the wine or must, the oenological tannins that were added, and the timing of the addition.

For the most part, studies that have been carried out where the enological tannins have been added at the manufacturers recommended rate (<300 mg/L) have seen very small differences in polymeric pigment formation (Parker et al. 2007, Main and Morris 2007, Harbertson et al. 2012). When additions have been made in excess of the manufacturers recommended rate (>300 mg/L) increases in polymeric pigments have been observed when both condensed (Bautista-Ortin et al. 2007, Harbertson et al. 2012) and hydrolysable tannins have been added (Harbertson et al. 2012).

Astringency Modification

Several studies on the sensory aspect of oenological tannins have been carried out. Model solutions of various oenological tannins from different origins including exotic wood, and grape skins and seeds were assessed both by sensory and an electronic tongue (Puech et al. 2007). It was found that the tannins from tara, galls, quebracho, chestnut were more astringent than those from grape skins, seeds and oak. It was also found that grape skins and seeds as well as the oak tannins were found to be dissimilar to wine tannins. The authors suggested the lack of recognition was as a result of the purification process that removed hints that tannins were grape derived. In a study where enological tannins were added to a Shiraz wine, Parker et al. (2007) found only enhancements in astringency despite the fact that tannins were added to wine at different times (pre- and post fermentation). However, the increases in astringency detected by sensory evaluation were small. In other work an addition of 200 mg/L was made to a Monastrell wine and a ~ 50 mg/L difference in tannins was found (Diaz-Plaza et al. 2002). However the wine with oenological tannins added was considered the least preferred of the various treatments in the experiment by a sensory panel. Similarly, Bautista-Ortin et al. (2005) found among the numerous treatments applied to Monastrell that a 200 mg/L addition of oenological tannins (two different types) made the wines unbalanced with an increased herbaceous character and dryness.

In another study condensed and hydrolysable tannins were added to a Cabernet Sauvignon wine at excessive and the manufacturer's recommended rate (Harbertson et al. 2012). Sensory evaluation found that the wines with excessive amounts of hydrolysable and condensed tannin (600 and 800 mg/L respectively) had a significant increase in astringency and negative characteristics attributed to them (bitterness, brown color and earthy aroma). The treatments where the tannins were added at the manufacturers recommended rate did not exhibit the same negative characteristics but did have an enhancement in astringency. Unfortunately the trends observed here suggest that enological tannins whether they are hydrolysable or condensed, don't enhance astringency in all cases and frequently are deemed unsuitable for the style of wine, or give the wine negative aromatic and flavors. In the cases where the manufacturers guidelines are followed, at least the negative aromatic aspects are not observed

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