Impact of winemaking practices on the concentration and composition of tannins in red wine

P.A. SMITH¹,², J.M. MCRAE and K.A. BINDON

¹ The Australian Wine Research Institute, Urrbrae, SA 5064, Australia; ² Flinders Centre for Marine Bioproducts Development (CMBD), and Department of Medical Biotechnology, School of Medicine, Flinders University, Bedford Park, SA 5042, Australia

Corresponding author: Dr Paul Smith, email paul.smith@awri.com.au

Abstract

This review summarises key findings from research on the impact of winemaking practices on the concentration and composition of tannins in red wines. The impact from the point of crushing onwards is summarised from our research and recent literature taking into account the effect of maceration, yeast selection, addition of mannoproteins, addition of oenotannins, fining by animal and plant proteins, filtration, oxygen exposure, barrel treatment, bottling and accelerated-ageing techniques. A sufficient body of evidence has developed in certain areas to make generalisations, but in several other areas tannins continue to show large variability of response to winemaking interventions depending on the grapes and their particular methods of treatment. Progress has been made in determining the underpinning mechanisms for some of the effects, but significant further research is required to understand why many of the effects occur. Knowledge gaps remaining in the area and proposals for future research are identified that will provide the opportunity for improved management of vineyards and winemaking to optimise tannins in grapes and wine, and for increased capacity to meet wine specification, consumer expectations and profitability.

Keywords: colour, enzyme, extraction, filtration, fining, maceration, packaging, tannin, winemaking, yeast

Introduction

There are many points along the wine value chain where knowledge of factors influencing the concentration and composition of tannins can be used to influence the final wine style. Such knowledge can inform decision making concerning harvest, winemaking, ageing and packaging. It has also provided the opportunity for improved management of vineyards and winemaking to optimise tannins in grapes and wine, and for increased capacity to meet wine specification, consumer expectations and profitability.

Tannins, including grape-derived condensed tannins (flavanoids) and oak-derived hydrolysable tannins (non-flavanoids), produce sensations of astringency in food and drink and contribute significantly to the ‘structure’ of wine. The term astringency refers to the drying and puckering sensations in the mouth and is a characteristic of red wine that is considered pleasant when balanced with other factors including alcohol and sugar content (Gawel 1998). Wine acidity also influences astringency with wines of greater acidity being perceived as more astringent (Fontoin et al. 2008). The sensory perception of wine tannins is dependent on the maximum intensity, total duration and time taken to reach maximum intensity, as well as the extent of mouth drying and mouth roughness (Gawel 1998). Knowledge of the structure of the tannins in a wine matrix, the points where they can be manipulated and the impact of these structures on the sensory properties of wine is essential for improving the end product.

Tannin chemistry

Tannins are highly reactive phenolic substances and are categorised as either condensed or hydrolysable. Grape phenolic substances are chemically modified as soon as grapes are crushed, which continues through fermentation and ageing. Many types of reactions happen at different rates at the various stages of wine production, and these reactions determine the final colour and taste properties. Tannins are one of the critical classes of phenolic substances that undergo significant changes during winemaking; in particular the conversion of ‘grape’ tannins (a) into more chemically complex ‘wine’ tannins (b) (Figure 1).

Hydrolysable tannins

Hydrolysable tannins are generally extracted from the oak barrels used in wine ageing. Their structure consists of a glucose molecule acylated with galloyl groups. The quantity of these oak-derived tannins depends on the amount of time the wine is aged in the barrels, whether the barrels were new or had been used previously and the origin of the oak. Relative to condensed tannins, hydrolysable tannins occur only in low concentration in oaked wines, and as such will not be discussed in this review.

Grape tannins

Condensed tannins are readily extracted from the skin, flesh and seeds of grapes during the winemaking process and contribute the majority of tannins to red wine, up to 4 g/L. They consist of repeating flavan-3-ol units, such as catechin, epicatechin, epigallocatechin and epicatechin gallate (Herderich and Smith 2005). The subunits are linked by acid-labile interflavan bonds. They are extracted from the skin, seeds and, to a lesser extent, the flesh of grapes in variable proportion during winemaking. Skin tannins consist of long polymeric chains ranging from an average of 3 to 83 flavonol subunits [mean degree of polymerisation (DP)] and constitute a larger proportion of galloacatechin-derived units because of the greater proportion of prodelphinidins to procyanidins in grape skin. These tri-hydroxylated subunits consist mainly of epigallocatechin, but with trace amounts of gallocatechin and epigallocatechin 3-O-gallate. Seed tannins are much smaller...
Although critical to the outcome of the wine, this review will which influence the concentration and composition of tannins. There are many points during the red wine production process received as bitter (Fontoin et al. 2008, McRae et al. 2013). Flavanol monomers and other non-flavonoid phenolics, are perceived as astringent while smaller molecular mass tannins, such as large molecular mass tannins are perceived as more stability. The structure of tannins influences the sensory properties. Larger molecular mass tannins are perceived as more astringent.

**Wine tannins**

Structurally, wine tannins are less well understood than grape tannins. This is largely because the structure of wine tannins is mostly resistant to methods of analysis, such as acid-catalysed cleavage of the interflavan bonds and subsequent thiolysis or reaction with phloroglucinol (Cheynier et al. 2000, Kennedy and Jones 2001, Herderich and Smith 2005). Once condensed, tannins have been extracted from grapes; they are structurally modified by yeast, enzymes and fermentation by-products (e.g. acetaldehyde). After fermentation, wine tannins continue to undergo chemical changes that gradually alter the purple hue of young wine to a brick red colour and generally the tannins become less astringent.

Structural alterations of tannins involve both direct and indirect condensation reactions and depolymerisation of the tannins. The mechanisms of these processes have been discussed in detail elsewhere (Cheynier et al. 2000). In brief, direct condensation reactions occur with flavanol-3-ol monomers, anthocyanins, anthocyanin-derived pigments and oligomers. Indirect condensation reactions are facilitated by oxidation products, including acetaldehyde from ethanol and also produced by yeast; pyruvic acid from glycerol; and glyoxylic acid from tartaric acid. These can alter the interflavan bonds, susceptibility to acid-catalysed cleavage and sensory characteristics. For example, acetaldehyde-mediated condensation reactions lead to ethyl-linked tannins but these ethyl-linked structures are unstable. The incorporation of anthocyanins and anthocyanin-derived pigments leads to the formation of coloured pigmented tannins that are generally more resistant to bleaching by sulfur dioxide and contribute to long-term colour stability. The structure of tannins influences the sensory properties. Larger molecular mass tannins are perceived as more astringent while smaller molecular mass tannins, such as flavanol monomers and other non-flavonoid phenolics, are perceived as bitter (Fontoin et al. 2008, McRae et al. 2013).

**Maceration**

For the purpose of this review, which has a specific focus on the concentration and composition of tannins, maceration is defined as the period during which the fermenting must or wine is in contact with the grape cap (grape insoluble solids, pomace, or marc). To identify maceration techniques that alter tannin extraction, the authors aim to highlight winemaking methods that influence the transfer of tannins to wine during the period of cap contact. To clarify this definition, Figure 2 shows the structure of a grape cell and the localisation of potential sources of grape tannins for wine. Winemaking intervention can target tannins according to their location within the grape cell, thereby altering the degree to which it is extracted. These effects, however, may also be exerted by changing the degree to which tannins are lost as a precipitate, adsorbed to sedimenting materials (i.e. lees) or by affecting other colloidal properties which underpin its stability in wine. The period of cap contact can affect all of these aspects, thereby altering points at which tannins are extracted, removed or stabilised during the winemaking process.

Winemaking techniques that manipulate the maceration process affect the extent or rate of transfer from the cap to the must (wine). These techniques may either increase the solubility of extractable components, in this instance the focus of the current review, tannins, or decrease the integrity of the cap in some way (Figure 2). This may occur simply through extending the length of cap contact time or by a more strategic intervention that facilitates breakdown of the plant cell wall or intracellular membranes, releasing the cell contents to the wine medium. It should be noted that grape composition may strongly influence this process. For example, it is considered that anthocyanins may be necessary to facilitate the solubilisation and retention of tannins (Figure 2) during fermentation through the formation of polymeric pigments (Kilmister et al. 2014).

Various effects of maceration on grape and wine phenolic substances have been reviewed (Sacchi et al. 2005, Casassa and Harbertson 2014). In terms of the management of tannins specifically, these reviews have highlighted that enzyme addition

---

**Impact of the winemaking process on tannins**

There are many points during the red wine production process which influence the concentration and composition of tannins. Although critical to the outcome of the wine, this review will not discuss the factors that influence tannins during grape production or grape properties at harvest. This review will focus from the point of crushing onwards and include a summary of recent literature reporting on the effect of maceration, yeast selection, addition of mammoproteins, addition of oenotannins, fining (animal and plant proteins), filtration, oxygen exposure, barrel treatment, bottling and accelerated-ageing techniques.

---

**Figure 1.** Tannins are one critical class of phenolic substances that undergo significant changes during winemaking, in particular the conversion of (a) ‘grape’ tannins into more chemically complex (b) ‘wine’ tannins.
and extended maceration (EM) are winemaking techniques that consistently increase tannins in the finished wine. To add to the points raised in these reviews, experimental results are summarised below from a range of studies that aim to understand the way that such winemaking techniques affect the extraction and composition of tannins during the maceration process. Observations from the current literature and reviews highlight that during the early stages of fermentation, extraction of tannins from the skin exceeds that from the seed while in the later stages of maceration, extraction of seed tannins predominates. Reports about the extraction of skin and seed tannins during fermentation have identified a lag phase during which seed tannins are poorly extractable (Peyrot des Gachons and Kennedy 2003, Cerpa-Calderón and Kennedy 2008, Hernández-Jiménez et al. 2012), which appears to be related to the period in which hydration of the seed takes place. In the developing grape berry, accumulation of seed tannins is known to occur up to veraison, after which it declines. The decline of seed tannins during grape ripening may be related to a period of programmed oxidation, whereby tannins may be rendered less extractable, and the seed itself less permeable, in preparation for senescence (Kennedy et al. 2000).

Further to this, work by Peyrot des Gachons and Kennedy (2003) and Cerpa-Calderón and Kennedy (2008) identified an approach by which the extraction of skin tannins could be studied during the winemaking process. Skin tannins appear to follow a Boltzmann sigmoid extraction model, reaching a plateau (Cerpa-Calderón and Kennedy 2008), while the extraction of seed tannins follows a linear model once the initial hydration phase is complete (Hernández-Jiménez et al. 2012). According to these studies, extraction of skin tannins may be more variable depending upon the conditions of maceration, while seed tannins could increase in a linear manner when maceration is extended. It is noted, however, that the studies of Cerpa-Calderón and Kennedy (2008) and Hernández-Jiménez et al. (2012) were undertaken on a limited sample set, hence extrapolation to other conditions is not possible at present. The environmental, genetic and ripeness effects that limit skin and seed hydration and their resulting extractability should be an important component of further research.

Cerpa-Calderón and Kennedy (2008) also showed that the height of the plateau reached in terms of the extraction of skin tannins was dependent upon the conditions of maceration. In that study uncrushed grapes were compared with varying degrees of grape crushing. Generally, a higher level of grape crushing was found to increase the extraction of both skin and seed tannins (maximum at 75% crush) and also decrease the time required to reach 50% extraction. In light of this, it would appear probable that the degree to which the cap (skins, seeds) remains in contact with the must/wine will significantly affect the extraction of tannins. While this may seem logical, the effect of cap management during winemaking has received surprisingly little research attention. During fermentation, the cap can be: punched down by periodically plunging the buoyant solids;
within the gross (early racking) wine lees. Gross wine lees are recently is the potential losses of tannins as an adsorbed fraction activity favour the degradation of seed cell walls, promoting et al. (2013) showed that polygalacturonase and cellulase later confirmed by the results of Busse-Valverde et al. (2011) nents. A role for enzymatic degradation of seed cell walls was enzyme application is exerted on both skin and seed compo-

degradation of pectic material from the grape cell wall (Romero-Cascales et al. 2012, Apolinar-Valiente et al. 2014, Zietsman et al. 2015). Hence their application in maceration may significantly enhance the degradation of pectic material usually contain pectin-degrading enzyme activities, and may contain certain additional enzymes such as hemicellulases (Bautista-Ortín et al. 2007a, Romero-Cascales et al. 2012, Zietsman et al. 2015). Therefore it is observed that the higher concentration of tannins in finished subunits from phloroglucinolysis) for all treatments during degradation of the skin and seed cell walls. The role of oxygen on the extraction of tannins in winemaking will be discussed later in this review. A further aspect of maceration which affects the extraction of tannins is the degree of grape cell wall integrity. Fermentation causes a loss of pectic polysaccharides and associated hemicelluloses from the grape skin cell wall (Doco et al. 2007, Romero-Cascales et al. 2012, Bindon and Smith 2013, Zietsman et al. 2015). Macerating enzymes available for commercial use may contain certain additional enzymes such as hemicellulases (Bautista-Ortín et al. 2007a, Romero-Cascales et al. 2012, Zietsman et al. 2015). Hence their application in maceration may significantly enhance the degradation of pectic material from the grape cell wall (Romero-Cascales et al. 2012, Apolinar-Valiente et al. 2014, Zietsman et al. 2015). There is evidence that the removal of the pectic fraction from cell walls decreases the adsorption properties of the cell wall for tannins (Bindon and Smith 2013, Ruiz-Garcia et al. 2014). By inference, this may render tannins more extractable from the skins during fermentation. Based on this, it is observed that the application of macerating enzymes is generally successful in increasing the extraction of tannins during red winemaking (Ducasse et al. 2010, Busse-Valverde et al. 2010, 2011, Ortega-Heras et al. 2012, Bautista-Ortín et al. 2013, González-Neves et al. 2013, Nel et al. 2014), although there are some instances where the effect is variable or negligible depending on cultivar, environmental conditions or stage of the winemaking process (Bautista-Ortín et al. 2007a, Busse-Valverde et al. 2010, Ortega-Heras et al. 2012, Nel et al. 2014). In terms of the composition of tannins, however, the application of macerating enzymes does not show consistent effects. For example, Ducasse et al. (2010) showed for Merlot that increased wine tannins in response to a variety of enzyme treatments had a variable effect on mean degree of polymerisation (mDP), % galloylation and % epigallocatechin of tannins. This may indicate that the effect of enzyme application is exerted on both skin and seed compo-

ments. A role for enzymatic degradation of seed cell walls was later confirmed by the results of Busse-Valverde et al. (2011) and Bautista-Ortín et al. (2013). In particular, Bautista-Ortín et al. (2013) showed that polygalacturonase and cellulase activity favour the degradation of seed cell walls, promoting release of seed proanthocyanidins. An aspect which has not received significant attention until recently is the potential losses of tannins as an adsorbed fraction within the gross (early racking) wine lees. Gross wine lees are expected to be primarily yeast biomass and some tartrates, and yet the evidence suggests that grape-derived solids (pulp) co-sediment with the wine lees and have the capacity to adsorb tannins (Figure 2) (Bindon et al. 2010a,b, Hanlin et al. 2010). Grape pulp cell walls have been identified as having a high binding capacity for grape-derived tannins (Bindon et al. 2010a,b) and are present in significant proportion in the fermenting must. A hypothesis proposed here is that macerating enzymes may not only affect extraction, that is, facilitate release of tannins from the skin or seed cell wall, but that they might also limit loss of tannins via adsorption and subsequent sedi-
mentation. Degradation of the cell walls in the presence of macerating enzymes, in particular losses of pectic polysaccharides (Le Bourvellec et al. 2012a, Ruiz-Garcia et al. 2014), can reduce the adsorption capacity for tannins. We propose that this may be an additional factor which enhances the retention of tannins in solution in enzyme-treated wines, while recognising that extraction may simply be enhanced via degradation of the skin and seed cell walls. To understand in greater detail how a disruption of cellular integrity during the maceration process can influence extraction of tannins, other winemaking techniques can be discussed which have produced similar results to enzyme application. The impact of variations in temperature profile within the ferment has recently been reported in relation to impact on tannins and colour (Lerno et al. 2015). There was a relatively constant increase in extraction of tannins over time, and it can be seen that increasing fermentation temperature led to increased extraction of tannins and seed tannins (as assessed by galloylated subunits from phloroglucinolysis) for all treatments during active fermentation. Treatments in which the must temperature was the same (regardless of the cap temperature) were not significantly different in the concentration of tannins. The Sacchi et al. (2005) review discussed that the application of thermovinification (heating the must to between 60 and 70°C) can lead to an increase in extraction of anthocyanins when included with a skin contact period after heating. That review concluded, however, that effects on extraction of tannins were unknown or variable. Since that report, more recent studies have focused on the application of alternative heating tech-

niques, such as flash détente or microwave, to facilitate the maceration process. Flash détente is the process of rapidly heating the grapes (to around 95°C) for a set period, then applying a strong vacuum. The process is proposed to degrade the cellular structure. Evidence for this has been provided by Doco et al. (2007) who found that the process, when included with skin contact, increased extraction of the polysaccharides of the grape berry cell wall, namely arabinoxylans and rhamnogalacturonans. The extent to which the process degraded grape cell walls was found to be highly cultivar dependent; therefore limitations may exist in the effectiveness of the technique. In terms of a corresponding effect on the concentra-

tion of wine tannins, Morel-Salmi et al. (2006) showed that for two seasons on the grape cultivars, Grenache, Mourvedre and Carignan, flash détente-treated wines had a higher tannin concentration as well as an increased tannins-to-anthocyanins ratio. When pressed directly after treatment, however, phenolic substances were lost. Cap maceration was therefore a require-

ment of the treatment, which is in strong agreement with the findings of Doco et al. (2007) with respect to polysaccharides. Another method which applies heat to grape must is the use of microwave maceration, which has not yet been applied on a commercial scale as has flash détente. It is, however, noted to be a promising technology for cultivars such as Pinot Noir that are traditionally poor in tannins (Carew et al. 2014a,b). Studies on Pinot Noir have shown that microwave application causes a
release of cellular contents not achievable with standard thermostvinification (Carew et al. 2014a). Similar to the results for flash détente, microwave-treated Pinot Noir resulted in a higher concentration of wine tannins only when fermented with continued cap contact (as opposed to early press off) (Carew et al. 2014a,b). Interestingly, microwave-treated Pinot Noir musts pressed before the onset of fermentation resulted in wines with equivalent phenolic profiles to that of a control treatment fermented with cap contact (Carew et al. 2014a).

A promising technique, which is non-thermal but may facilitate extraction during the maceration process by disruption of cellular integrity, is pulsed electric field (PEF). This is a relatively new technology to winemaking, and is not yet widely in use. It is a processing method that causes permeabilisation of cell membranes with low energy requirement, apparently minimising deterioration of other important food components (Puértolas et al. 2012). Key pilot studies have been conducted by Delsart et al. (2012, 2014) who showed that PEF (500–700 V/cm, 40–100 ms) application on Merlot disrupted the cells located in the hypodermis and epidermis, increasing the diffusion of tannins to the must, and ultimately to the wine. Their results suggested that under their PEF conditions, the cells of the Merlot grapes studied became more permeable. Delsart et al. (2014) and Cholet et al. (2014) undertook further studies on PEF treatment of Cabernet Sauvignon; high energy, long duration PEF produced wine higher in tannins (by 34%) than that of a standard maceration treatment. The treatment appeared to depolymerise high molecular mass tannins into smaller, more extractable tannin components. The high energy PEF treatment appears to modify the tannins associated with the skin cell walls while lower energy PEF affects grape vacuolar tannins (see Figure 2). Delsart et al. (2012, 2014) suggest that the conditions of PEF application produce a highly specific response in terms of the quantity and type of tannins extracted. Therefore, while this technique is promising, it requires considerable optimisation before it becomes more widely applied.

The processes discussed so far focus primarily on disruption of the grape cellular structure for increased extraction of tannins during maceration. The Sacchi et al. (2005) review discussed early work on must freezing that demonstrated increased concentration of wine tannins, and highlighted that this approach may cause both cellular disruption and protection from oxidation in the early stages of maceration. More traditional maceration methods attempt to influence key stages of the maceration process. The process known as cold soak is a pre-fermentation maceration step which can involve holding crushed must at low temperature, or the application of dry ice. Sacchi et al. (2005) did not report a consistent effect of cold soaking on wine phenolic substances. In reviewing the variety of recent reports that have applied these techniques, it is evident that a high level of variability in response is expected, because of grape cultivar, vintage, regional and/or environmental factors which may limit the effectiveness of the method. For example, key studies by Busse-Valverde et al. (2010) and González-Neves et al. (2013) applied low temperature techniques pre-fermentation to three grape cultivars, and found that while cold soaking could effectively increase the concentration of proanthocyanidins in wine, the effect was not consistent across grape cultivars. For both studies, the concentration of tannins in Shiraz wines was not affected by the cold soak treatment. Moreno-Pérez et al. (2013) studied Monastrell and found that the cold soak-treated wines generally contained a higher concentration of proanthocyanidins, but this was region specific, with one of four regional studies having a negligible result. Further studies by De Beer et al. (2006), Ortega-Heras et al. (2012) and Nel et al. (2014) found a variable effect of cold soak on wine tannins, which may demonstrate further examples of the influence of vintage, cultivar and region.

According to studies by Busse-Valverde et al. (2010, 2011), the effect of cold soaking on wine tannins is related to enhanced seed tannin extraction in the wines. Cold soaking is proposed to facilitate the activity of endogenous grape maceration enzymes in degrading cell wall structure, to limit polyphenol oxidase activity, or to delay fermentation; factors which might be expected to enhance either the extraction or preservation of grape-derived phenolic substances in wine. In light of the crucial observations by Hernández-Jiménez et al. (2012), however, a hydration period is required before significant extraction of seed tannins proceeds. Therefore the possibility exists that a cold soak period simply extends the maceration period relative to a standard maceration period. Simply stated, the extension of the maceration period, rather than the temperature of the pre-fermentative cold soak, may facilitate extraction from grape seeds. To explore this hypothesis further, more detailed EM studies are required.

Extended maceration is a technique whereby cap contact is held for longer than the usual short period during which alcoholic fermentation occurs (5–10 days) and instead extends up to 50 days. This technique has been extensively reviewed by Sacchi et al. (2005) as well as by Casassa and Harbertson (2014) and was proposed to be one of the principal methods by which an increase in extraction of tannins can be achieved. A key observation in both reviews is that, generally, EM does not enhance anthocyanins (colour) in wine, but the primary effect is on phenolic substances, polymeric pigments or tannins. This current review has summarised recent literature, and in light of the observations also highlighted by Casassa and Harbertson (2014) some key points emerge:

1. For most studies of EM, an increase in the extraction of seed tannins has been observed as cap contact is continued. Exceptions to this were found where the maceration period was not sufficiently long, or where maceration times were comparatively close.

2. The effect of EM varied with respect to grape cultivar, season, ripeness and region.

There were a few cases in which the application of an EM period produced a limited effect on wine tannins. Álvarez et al. (2006) found that for Monastrell subjected to maceration times of 4 and 8 days, no significant difference in extraction of tannins to wine occurred. Given the observations of Hernández-Jiménez et al. (2012) and Cerpa-Calderón and Kennedy (2008), the time point for the maximum extraction of skin tannins and the initiation of seed hydration with subsequent extraction of seed tannins might be beyond this time period. This might explain why no effect was observed. A further study by Cadot et al. (2012) compared Cabernet Franc wines produced from grapes at two stages of maturity and with two superimposed maceration times of 9 and 15 days. That study found that at these two maceration times, no difference was observed in the concentration or composition of tannins. The observations of Cadot et al. (2012) are perhaps less representative of the general outcomes of EM; longer EM trials exceeding 20 days following crushing have produced more consistent results. In these, the differences in extraction of tannins are clear, and importantly the predominance of the extraction of seed tannins in the later stages of maceration is observed. The study of Cerpa-Calderón and Kennedy (2008) has previously been highlighted as key in the understanding of the transfer of
tannins from grapes to wine during the maceration process. The fundamental point of this research is that for crushed fruit, a plateau in the extraction of skin tannins occurs at approximately day 9 of maceration. Extraction of seed tannins predominates after this point. In light of the points raised above, it is important to consider that the timing of maceration assigned by different researchers for EM studies can significantly impact their results. Some fundamental studies were conducted by Harbertson et al. (2009) and Casassa et al. (2013a,b) where EM was continued for 20 or 30 days, respectively, significantly longer than previously reported work. These studies considered Merlot and Cabernet Sauvignon grapes and observed that the increased tannins from EM were primarily derived from seed tannins. Wines which had not undergone EM (10 days) tended to have a more a balanced proportion of seed and skin tannins. In the more detailed study of Casassa et al. (2013b), EM (30 days) led to increased extraction of tannins during maceration, which at the final sampling point was ~73% seed derived. Similarly, Gil et al. (2012) observed that for Cabernet Sauvignon and Tempranillo, wines produced from riper grapes contained a higher proportion of skin tannins, and tannins increased with EM time independent of grape ripeness. Accordingly, tannin mDP and the proportion of epigallocatechin as a function of the composition of tannins decreased with EM, a strong indication that extraction of tannins from seeds was increased with EM. In a detailed study Busse-Valverde et al. (2012) macerated wine up to 20 days, yet compared tannins extracted at 5, 10 and 20 days. Tannins in wines increased significantly from 5 to 10 days, but only a small increase occurred from 10 to 20 days. In this study, the proportion of skin-derived proanthocyanidins was consistently higher at all the maceration stages analysed, but it is noted here also that the time of analysis was important. The largest increase in wine tannins was from 5 to 10 days, and thereafter only a small increase occurred to 20 days which was mainly seed tannins. This example is one where skin tannins appeared to plateau by 10 days of maceration for all the samples fermented, yet a small increase in tannins was derived from seeds with additional maceration time. The Busse-Valverde et al. (2012) study is an example of a winemaking where seed tannins were poorly extractable, and the extraction of skin tannins predominated irrespective of the maceration time. Irrespective of grape composition, the only increase in wine tannins from 10 to 20 days maceration was derived from seeds. This indicates that grape composition may significantly impact on the extractability of skin or seed tannins, and as highlighted previously, this warrants further study.

**Yeast**

The wide-ranging impact of different yeast on wine aroma and colour has been well established over many years; the effect of yeast on tannins, however, has been less thoroughly investigated. With recent advances in analytical methods with improved specificity and sensitivity for determining the concentration and composition of tannins, research has demonstrated that choice of yeast can also have a major effect on tannins in wine.

Early research (Bautista-Ortín et al. 2007b) that specifically included measurement of tannins reported that in 2002 and 2003 Monastrell grapes that were fermented with INRA 7303 and Rhone 2323 yeast strains showed effects in 2003 only for a different concentration of tannins in wine. Specifically, Rhone 2323 led to a 43% increase in polymeric tannins post-ferment, and at 8 months of age the increase was maintained at 28%. *Saccharomyces cerevisiae* and *S. bayanus* have well established colour impacts in red wine, and early research reported similar impact of tannins in red wine (Hayasaka et al. 2007); several *Saccharomyces* interspecific hybrids are also able to ferment grape juice to completion. Eleven commercially available *Saccharomyces* strains were used in one study (Blazquez-Rojas et al. 2012) with Australian Cabernet Sauvignon – *S. cerevisiae* (7), *S. bayanus* (2) and interspecific *Saccharomyces* hybrids (2). Yeast selection increased the final concentration of tannins by up to 33%. Specifically, wines prepared with *S. bayanus* AWRI 1176 and *S. cerevisiae* AWRI 1486 had the lowest and highest concentration of tannins (1.51 and 2.06 g/L epicatechin equivalents, respectively), and those prepared with *S. cerevisiae* AWRI 1555 and AWRI 1486 had the lowest and highest concentration of pigmented polymers (15.4 and 29.5 mg/L, malvidin-3-glucoside equivalents, respectively). Both *S. bayanus* wines had a high concentration of pigmented polymers. *Saccharomyces cerevisiae* strains produced wines with lower colour density (13.2–15.5 absorbance units (a.u.)), and *S. bayanus* strains and *Saccharomyces* hybrid (AWRI 1501) produced wines with the highest colour density (15.7–16.0 a.u.). *Saccharomyces bayanus* wines had the highest concentration of SO₂-resistant pigments and lowest anthocyanins. A range of intermediate values for tannins and colour were reported for the strains AWRI 796, 838, 1493, 1553, 1554, 1501 and 1503.

A further study (Holt et al. 2013) which evaluated the impact of fermenting Shiraz must with different yeast strains also showed that choice of strain had a strong influence on several aspects of wine composition, including up to a 37% increase in the concentration of tannins for the same fruit. Across three parcels of Shiraz over two vintages (2009, 2010) wine fermented with *S. cerevisiae* AWRI 1631 had consistently the highest concentration of tannins (an average of 1165 mg/L epicatechin equivalents over 2009 and 2010) and, as observed by Blazquez-Rojas et al. (2012), wine fermented with *S. bayanus* AWRI 1375 was consistently the lowest in tannins (an average of 850 mg/L epicatechin equivalents over 2009 and 2010), but highest in non-bleachable pigments. The authors additionally showed that the size of tannins as measured by mDP was lowest for AWRI 1375 across these wines. Wine prepared with *S. cerevisiae* AWRI 1537 had also consistently a low concentration of tannins (887 mg/L epicatechin equivalents average over 2009 and 2010) but was different from that fermented with *S. bayanus* AWRI 1375 in that it was low in non-bleachable colour. A range of intermediate values of tannins and colour were reported for the strains AWRI 1575, 1375, 1493, 796, 1483 and 1620, many of which are commercially available.

While Cabernet Sauvignon and Shiraz traditionally have a moderate level of tannins available in the fruit, Pinot Noir can be limited both in tannins and colour. The low skin to seed tannin ratio and the less diverse anthocyanin profile can make it difficult to achieve higher concentration of wine tannins and stable wine colour. This has led to a range of investigations into ways to improve the colour stability and concentration and composition of tannins in Pinot Noir wines. Yeast selection has been shown to be an effective tool in diversifying these characteristics (Carew et al. 2013). The strain *S. cerevisiae* EC1118 (AWRI 838) was compared as a control treatment against: inoculation at day 0 with strain RC212 (common in Pinot Noir winemaking); a ‘wild’ ferment that was subsequently inoculated at day 3 with EC1118; a day 0 inoculation with *S. bayanus* AWRI 1176; and a day 0 inoculation with *Torulopsis delbrueckii* subsequently inoculated at day 3 with *S. cerevisiae* EC1118 (AWRI 838). Importantly, this study also assessed the persistence of the post-ferment effects at 6 months of age. The wines from RC212 showed significantly higher concentration of tannins post-ferment and at 6 months relative to that of the wild-initiated ferment and *S. bayanus* AWRI 1176 treatments (which had 70%
and 60% less tannins, respectively, at 6 months). Although lower in tannin, the two sequential inoculations had a higher proportion of trihydroxylated tannin subunits suggesting they may have a higher proportion of skin tannins. Wines fermented with RC212 had a relatively higher component of galloylated subunits indicating a higher relative proportion of seed tannin subunits, highlighting the impact that yeast selection can have on composition of the tannins, not just concentration.

The variability observed in the concentration and composition of tannins for these studies may stem from several mechanisms. Different rates of fermentation and of ethanolic extraction may account for some of the observations, although in the Shiraz, Cabernet Sauvignon and Pinot Noir studies discussed, all fermentations had the same length of skin contact so this does not account for the differences reported. Enzyme activity of wine yeast has also been linked to the extraction of compounds from grape skins, and may play a role. The variable increase in pigmented polymer formation has been ascribed to the evolution of reactive intermediates such as aldehydes which can directly link phenolic substances and can also create polymeric tannins that undergo subsequent rearrangement and modification during ageing. Acetaldehyde-mediated linkages that result in ethyl-linked bridges are one such example, where these intermediate polymers subsequently undergo degradation and rearrangement to new polymeric forms. As discussed previously, a growing body of literature demonstrates that the relationship between the concentration of tannins, ethanol concentration and pomace contact time is complex. Extraction of tannins is influenced by: the physical breakdown of grape solids which affects its adsorption; differential yeast adsorption of tannins from the liquid phase of wine; variable expression by yeast of enzymes contributing to the release of tannins from the grape matrix (e.g. β-glucosidase, pectinase, proteolytic enzymes); and the colloidal stability of soluble macromolecules that bind to tannins. What is clear is that the phenolic style of red wines can influence by the formation of colloids of relatively unknown stability and, as such, there is interest in the effect of mannoprotein addition on colour effects and tannins. Low to medium molecular mass mannoproteins have been shown to be more effective than high molecular mass mannoproteins at limiting particle growth of seed tannins in model wine conditions (pH 3.4 buffers containing 2 g/L tartaric acid, 12% ethanol) (Pontet-LeGrand et al. 2007), but knowledge gaps remain on their effects on colloids in red wine. Mannosylated yeast invertase was shown to have tenfold lower binding affinity for tannins than bovine serum albumin (BSA), but in model wines they may have greater solution stability than that of other yeast proteins (Rowe et al. 2010).

Mannoproteins
Mannoproteins, naturally present in wines and derived from the cell walls of S. cerevisiae, are increasingly being added in oenological products to wines with the intention of preventing tartrate instability or modulating mouthfeel. In red wines they have the potential to interact with tannins and other phenolic substances through the formation of colloids of relatively unknown stability and, as such, there is interest in the effect of mannoprotein addition on colour effects and tannins. Low to medium molecular mass mannoproteins have been shown to be more effective than high molecular mass mannoproteins at limiting particle growth of seed tannins in model wine conditions (pH 3.4 buffers containing 2 g/L tartaric acid, 12% ethanol) (Pontet-LeGrand et al. 2007), but knowledge gaps remain on their effects on colloids in red wine. Mannosylated yeast invertase was shown to have tenfold lower binding affinity for tannins than bovine serum albumin (BSA), but in model wines they may have greater solution stability than that of other yeast proteins (Rowe et al. 2010).

Early mannoprotein research focused on colour effects, but more recent reports have investigated in more detail the impact on the concentration and composition of tannins. Two commercial preparations containing yeast mannoproteins were added to three Portuguese wines after malolactic fermentation (MLF) at 0.2 g/L and 0.4 g/L (Rodrigues et al. 2012) and aged for 21 months. The wines were also subjected to an ‘accelerated’ ageing test at 35°C. The commercial mannoprotein products did not have an effect on colour stabilisation in these wines and, in general, the overall evolution of the composition of tannins did not differ significantly relative to that of the control with no addition. One of the commercial mannoproteins limited the change of grape tannins with mDP between 8 and 14. Many of the tannins present after fermentation chemically rearrange during ageing into new forms of ‘wine’ tannins that are not directly accounted for through acid-catalysed depolymerisation, except through the ‘mass conversion yield’ parameter. This parameter was not reported, but the authors of this review advocate that it should be as it allows an estimate of the rearrangements of tannins. Nonetheless, the mass conversion yield is relatively unchanged in this study, likely as a result of the low variance in the proportion of subunits reported for the tannins in the ‘accelerated’ ageing test.

By contrast, addition of mannoproteins at the start of winemaking has resulted in a decrease in the concentration of wine proanthocyanidins and in wine stable colour (Guadalupe and Ayestarán 2008) but with no effect on monomeric phenolic substances and anthocyanins. The concomitant decrease in mannoproteins led the authors to propose a destabilisation of polysaccharide–tannin colloids for the loss of tannins, and while this appears likely, no direct evidence is provided for such colloidal instability in that work. The composition of tannins was not assessed nor was the impact on sensory properties. Previous research (Guadalupe et al. 2007) with a similar experimental design (mannoprotein addition at the start of winemaking) also showed a decrease in non-bleachable colour, colour intensity and in the concentration of wine tannins, but sensory evaluation showed a decrease in astringency and an increase of the wine sweetness and roundness. A recent study (Rinaldi et al. 2012) on the impact of mannoproteins on sensory and saliva precipitation has reinforced earlier findings that mannoproteins decrease the perception of astringency in red wines.

Oenotannins
The impact of oenotannin addition on wine has been recently reviewed (Versari et al. 2013), with limited literature published in the interim. One key observation is that, in general, oenotannins have little impact on colour stabilisation regardless of timing, dose, oenotannin type, grape cultivar or maturity. Occasionally colour impacts are observed soon after completion of fermentation, but they are often not maintained through ageing (and in some cases the experimental wines were not assessed after ageing). In the few reports where the sensory impact of addition of tannins was evaluated, their impact on astringency was often observed, leading to the conclusion that oenotannin addition can affect mouthfeel properties. The nature of these mouthfeel properties needs to be assessed individually, however, as the composition of the oenotannins is highly variable and in some wines with the addition of a high dose, the sensory impact was considered as negative (Harbertson et al. 2012).

Recent research (Bautista-Ortín et al. 2015) has provided further insight into the dynamics of what may happen to oenotannins when they are added. The study analysed the impact of cell walls on the concentration and composition of commercial oenotannins, reinforcing the earlier discussion about interactions between tannins and cell-wall material from grape skin and pulp that could eliminate tannins (particularly larger tannins) from must through adsorption and phase separation. They propose that oenotannins should be added to wine when the cell-wall material suspended in the must is low (e.g. at
the end of alcoholic fermentation or after pressing) especially for cultivars with high binding capacity cell walls and that oenotannins with a smaller proportion of high molecular mass tannins should be used to reduce the impact on loss of tannins. This reinforces other observations made using purified grape tannins (Bindon et al. 2010a,b, Bindon and Smith 2013).

**Fining**

The mechanisms of interaction between proteins and phenolic substances, including tannins, have been thoroughly reviewed (De Freitas and Mateus 2012). The authors noted that the drawback of some of the earlier literature investigating phenolic substance protein interactions was the use of methods that lacked specificity and sensitivity. Some recent wine-focused applications have been reported that do use improved methods and as such merit discussion.

**Animal proteins**

Gelatin is widely used for fining wines, and several key references report the effect on concentration and composition of tannins. The general observation from early research was that higher molecular mass tannins are removed preferentially, and the tannins removed tend to be more highly galloylated (Sarmi-Manchado et al. 1999, Maury et al. 2001). Different types of gelatin removed different amounts of tannin (9-16%) depending on the wine and the gelatin composition. One knowledge gap left from these two reports is that they measured only tannin that was able to depolymerise under acid-catalysed thiolysis conditions and because there is often a large proportion of tannins that cannot be depolymerised, these tannins are left unaccounted for when using only this method. Gel permeation chromatography of wine tannins allows a more accurate assessment of size and complements the information available from acid-catalysed depolymerisation. The combination of both methods in addition to quantitation by precipitation with BSA and RP-HPLC has been used to assess the effect of gelatin and egg albumin in a Pinotage wine (Oberholster et al. 2013). This research showed that both fining agents decreased the concentration of tannins, although to varying degrees depending on the analytical method selected to measure the tannins, emphasising the need to understand the interrelation of analytical methods. Again, preferential removal of galloylated tannins was shown and removal of larger molecular mass (MM) species (~25% decrease in mDP) was demonstrated by both gel permeation chromatography and acid-catalysed depolymerisation methods. A wider range of fining agents (potassium caseinate, isinglass, egg albumin and three gelatins) and of dosage was assessed using similar methods, with the additional reporting of the mass conversion yield effect on the tannins (Bindon and Smith 2013). Removal of tannins at the maximal dose ranged between 9 and 19% and, again, higher MM tannins were preferentially removed. The effect of these proteins was also compared against plant fibres, as discussed subsequently. A similar selection of proteins (egg albumin, isinglass, gelatin, casein, potassium caseinate) was assessed by fractionating monomeric, oligomeric and polymeric phenolic substances that were quantified using the vanillin assay and by performing acid-catalysed depolymerisation (Cosme et al. 2009). This showed a decrease in mDP between 6 and 14% for the polymeric fraction for all fining agents and, similarly to the work in Pinotage, ~25% decrease in mDP for egg albumin. Swim bladder isinglass was found to be more effective than fish skin isinglass. Casein removed the most monomers, Treatment of four wines with four fining agents (bentonite, gelatin, gluten protein, egg albumin) decreased tannins by ~10% as assessed using two methods with lower specificity, but no compositional information was determined (González-Neves et al. 2014).

**Plant-based fining agents**

There is increased interest in finding alternatives to animal proteins for use as fining agents in wine. Initial observations of the binding affinity of apple cell wall material for tannins (Renard et al. 2001) have led researchers to report on the effectiveness of plant cell wall materials from apples and grapes as wine fining agents [Bindon and Smith (2013), Guerrero et al. (2013), Bautista-Ortín et al. (2015) and references therein]. Such plant cell walls have proved to be effective fining agents, with apple cell walls achieving reduction in tannins as high as 42% and grape fibres as high as 38% (Bindon and Smith 2013). Apple and grape fibres generally show the characteristic preference for higher MM tannins but grape fibres sometimes remove both high and low MM tannins. The number of reports in this area is large and comprehensive review is beyond the scope of the current review. The impact of cell walls on the extractability of tannins in wine has been reviewed (Hanlin et al. 2010) as has, more broadly, the interactions of phenolic substances with macromolecules (Le Bourvellec and Renard 2012b).

A range of plant proteins has also been assessed. Patatin, a glycoprotein from potato, performed effectively with ~10% reduction in tannin concentration, decreased astringency and effectiveness similar to gelatin but better than egg albumin and casein and with little effect on colour (Gambuti et al. 2012). Gluten and yeast extract protein have been claimed as effective but despite measuring tannins by colorimetry in hot acid, the authors did not report the values; however, the total phenolic index was reduced (Iturmendi et al. 2010). Corn proteins have also showed effectiveness at removing tannins at a level between 6 and 24% depending on the dose or protein composition (Simonato et al. 2009). Proteins from soybean, pea, lentil flour and gluten have also been applied to wines and the impact on tannins from dimer to decamer was assessed using Matrix Assisted Laser Desorption Ionisation Mass Spectrometry MS. In a model solution of oligomeric procyanidins, lentil flour was the most effective (16% decrease) followed by gluten, soy and pea proteins (Granato et al. 2010).

**Impact of wine filtration**

Filtering red wines prior to bottling is considered essential by many winemakers for maintaining clarity and stability in the finished wine (El Rayess et al. 2011) as well as removing residual fining agents (Deckwart et al. 2014). Wines are generally first treated with more coarse filtration membranes such as pad filtration with hydrophilic polyvinylidene fluoride (Bohanan et al. 2012) or cross-flow filtration (El Rayess et al. 2012a,b) and can then be filtered through a series of membranes with decreasing pore size (Ulbricht et al. 2009). Filtering red wines can be a source of concern for winemakers for two main reasons. First, filtering red wines in particular can foul the membranes and this brings extra costs associated with the time required for changing membranes as well as the cost of the membranes themselves. Second, filtering is perceived to alter the texture and mouthfeel of wine and can lead to the phenomenon of ‘bottle-shock’ – a period of altered sensory characters in the product. Research into filtration effects has focused on the sensory impact of wine filtration as well as the cause of membrane fouling and thus how fouling can be predicted and mitigated either in the wine or in the membrane manufacture.

The extent to which a wine will foul a membrane depends on the wine colloidal properties and the type of membrane used in the filtration. Red wine colloids include tannins,
polysaccharides and tannin–polysaccharide complexes (El Rayess et al. 2012a,b). The concentration of tannins has been found to decrease significantly after fining with cross-flow membranes (El Rayess et al. 2012a, Oberholster et al. 2013), suggesting that tannins are involved in membrane fouling. A higher concentration of tannins in wine also significantly decreased the rate of flux in wines passing through a ceramic cross-flow membrane which further implied that high concentration of tannins in wine (greater than 1.25 g/L) could potentially foul membranes (El Rayess et al. 2012a,b). The contribution of tannins to membrane fouling is not considered as significant as that of polysaccharides, particularly pectins, which have been shown to form a gel-like coating over cross-flow membranes, and mannoproteins (Vernhet and Moutounet 2002, El Rayess et al. 2012a,b). Cross-flow filtration can also alter tannin composition, with filtered wines showing a decrease in size of tannins (mDp) by up to 25% (Oberholster et al. 2013).

Different types of membranes used in wine filtration have different surface properties that can also influence the extent to which tannins bind to filtration membranes (Vernhet and Moutounet 2002, Ulbricht et al. 2009). Model wines sterile filtered with membranes made of polyethersulfone (PES) demonstrated lower fluxes and a greater proportion of adsorbed tannins and polysaccharides than the same wine filtered with polypropylene membranes (Ulbricht et al. 2009). Other studies have demonstrated that PES membranes bind significantly fewer tannins than membranes made from polyvinylpyrrolidone (Schroën et al. 2010). The reason for the different tannin-binding capacity of filtration membranes relates to differences in the surface properties. Membranes with greater polarity have greater interaction with phenolic substances because of greater hydrogen bonding (Vernhet and Moutounet 2002, Schroën et al. 2010). Research into ways of modifying the surface properties of membranes may therefore reduce the extent of membrane fouling by tannins.

The impact of filtration on the concentration and composition of tannins has prompted much research into the sensory impacts of red wine filtration. Several studies have noted that, despite the decrease in the concentration and in size distribution of tannins of filtered wines, there is negligible difference in texture and mouthfeel between unfiltered control wines and either cross-flow filtered or sterile-filtered red wines, regardless of the membrane type used (Schoibinger et al. 1992, Bohanan et al. 2012, Buffon et al. 2014). This suggests that when considering the wine production chain, the inconvenience of membrane fouling may be a greater issue than any loss of mouthfeel of red wines.

Methods for reducing the potential of red wines to foul membranes include treating wines with pectinolytic enzymes prior to filtration (El Rayess et al. 2012a,b) and changing the membrane surface to minimise tannin interaction (Vernhet and Moutounet 2002, Schroën et al. 2010). Further research in this area is required to understand better the role of tannins and wine colloids in membrane fouling in order to develop methods that can predict the membrane-fouling capacity of a red wine prior to filtration. More research is also required to understand the long-term effects of filtration on wine colloid structure and mouthfeel.

**Oxygen exposure, barrel treatment, bottling and accelerated-ageing techniques**

Ageing red wines has long been used to allow flavours to develop and astringency to mellow (Chira et al. 2012). Greater astringency intensity is directly associated with higher concentration and larger molecular size of tannins (Vidal et al. 2003), so the reasons for the change in wine astringency with ageing have been suggested to involve a decrease in the concentration of tannins because of precipitation and a decrease in molecular size of tannins because of subunit cleavage (Cheynier et al. 2006, Chira et al. 2012). Aged red wines, up to 50 years old, however, can have concentration and average molecular size of tannins similar to that of tannins of younger wines (McRae et al. 2012, Bindon et al. 2014) and yet have lower astringency. This suggests that other changes have occurred to the structure of the tannin molecules that cause the decrease in wine astringency (McRae et al. 2013). The cost of maintaining barrels and long-term storage of wine has prompted much research into new technologies to accelerate the ageing process. This has involved different methods of oxygen exposure, the use of oak staves, and ageing on wine lees (Tao et al. 2014). The impact of oxygen, barrel-ageing and bottle-ageing as well as the impact of the more common accelerated ageing techniques on the structure and development of tannins are discussed below.

The most significant change in the structure of tannins with ageing involves oxidation and depolymerisation reactions. Oxygen reacts readily with phenolic substances as reviewed in Oliveira et al. (2011). The presence of oxygen produces highly reactive quinones from flavonoids and acetaldehyde from ethanol, which promote polymerisation of tannins because of direct and indirect condensation reactions between tannins and flavan-3-ols or anthocyanins (He et al. 2008). These reactions increase tannin size by incorporating more polymer subunits as well as reducing the water solubility of the tannins because of the cross-linking of subunits through hydroxyl groups (Cheynier 2006, He et al. 2008, Poncet-Legrand et al. 2010). Structural rearrangement reactions also occur with wine ageing as a result of the low pH of wine. The chemical bonds between tannin subunits—flavan-3-ols—are continually broken and reformed, which also changes the tannins structure in such a way as to reduce the water solubility (Haslam 1980). This can lead to precipitation of tannins and thus sediment forming in the bottle (Waters et al. 1994). The extent of the precipitation of tannins from solution can potentially be mediated by interactions with polysaccharides (Maury et al. 2001, Cheynier et al. 2006) which can keep in solution the tannins that would have otherwise precipitated. The structural modifications to tannins occur faster at lower pH (Kontoudakis et al. 2011, McAra et al. 2013). As a result of these reactions, wine tannins are structurally different from the original grape tannins, and aged wine tannins are different even from young wine tannins. The main difference is that the number of bonds between subunits that can be broken by exposure to acid is significantly reduced as a result of subunit cross-linking and the incorporation of anthocyanins (He et al. 2008). In terms of analysis, these changes manifest as a decrease in the mDp, which is a way of determining molecular size of tannins (Vernhet et al. 2011). This may have contributed to the assumption that tannins become smaller with age and yet other techniques for size measurements of tannins, including gel permeation chromatography and small-angle x-ray scattering, have indicated that tannins remain of similar (McRae et al. 2012) or slightly larger size with wine ageing (McRae et al. 2014a) while others show decreased size with age (Bindon et al. 2014) and as such further research work is required to understand size and composition changes with age.

Wine astringency is positively associated with the concentration and molecular size of tannins (Vidal et al. 2003). Given that the changes in tannins that occur during red wine ageing have limited impact on either of these parameters, it must be
concluded that the changes that occur in wine astringency with ageing are because of other structural characteristics of tannins. Astringency is a tactile sensation that occurs largely as a result of wine tannins interacting with salivary proteins and oral epithelial cells (Payne et al. 2009, De Freitas and Mateus 2012) and is measured in vitro by assessing the extent of the interaction between tannins and a model protein (Obreque-Siler et al. 2010). Tannins isolated from wines that were about 10 years old have been shown to interact less strongly with proteins than tannins isolated from younger wines (McRae et al. 2010). Astringency studies have also indicated that pigmented polymers, those tannins that contain anthocyanins, are less astringent than pigmented tannins (Vidal et al. 2004a). Thus the combined effects of the structural changes that occur in tannins during wine ageing all contribute to the softening of astringency during red wine ageing.

Red wines traditionally undergo barrel ageing in French or American oak prior to bottling. Ageing wines in barrels not only imparts favourable flavour compounds, particularly oak lactones and phenolic aldehydes, but the long-term exposure to oxygen through the pores in the oak induces changes in the tannins and thus in wine astringency (Cano-Lopez et al. 2008, Oberholster et al. 2015). The impact of barrel ageing on red wine will vary depending on the type of oak used. French oak is more porous than American oak, allowing greater oxygen ingress (Tao et al. 2014). Generally, the amount of oxygen ingress through barrels has been estimated to be between 1.7 and 2.5 mL/(L · month) (Cano-Lopez et al. 2008). Wine also extracts hydrolysable tannins from the oak, such as ellagittannins, which are characterised as a glucose moiety bound to multiple gallic acid subunits via ester linkages (McManus et al. 1985). The extraction of phenolic substances from oak staves is low, with a concentration up to 250 mg/L (Quinn and Singleton 1985) compared to around 2000–4000 mg/L of tannin in red wines regardless of oak treatment (Herderich and Smith 2005). The extraction into white wines is also reportedly below sensory threshold (Pocock et al. 1994), suggesting that the astringency and mouthfeel of red wines is predominately because of the condensed tannins from grapes.

The reduction in astringency intensity of red wine with barrel ageing is therefore more likely to involve changes to the structure of tannins because of gradual oxidation and ageing processes. Hydrolysable tannins have also been shown to be oxidised in preference to catechins, and produce a significantly larger concentration of peroxides in wine, which promote the polymerisation of condensed tannins (Vivas and Glories 1996). This suggests that although hydrolysable tannins play a limited direct role in wine astringency, their presence can influence wine astringency by promoting structural changes in wine tannins. Oak cellulose can also adsorb phenolics (Barrera-Garcia et al. 2007), and the extent of this interaction depends upon the age of the oak barrel as well as the type of oak (Sánchez-Iglesias et al. 2009). Adsorption of condensed tannins to oak can lower the concentration of tannins in the wine and this also contributes to the reduction in overall astringency during barrel ageing.

Wine tannins undergo further changes during bottle ageing. Oxidation can occur gradually during bottle ageing as a result of the oxygen ingress through the bottle closure as well as the consumption of the total package oxygen (TPO) (Ugliano et al. 2012, McRae et al. 2013). Total package oxygen refers to the oxygen present in both in the bottle headspace and dissolved in the wine during transfer and bottling and the greater the TPO, the greater the extent of oxidation reactions and the more rapid the changes in the structure of tannins. The type of closure used in the bottle will impact the amount of oxygen that the wine is exposed to over time (Ugliano et al. 2012, Gambuti et al. 2013) with cork generally allowing greater oxygen ingress than screw caps. Wines bottled under closures that allowed for greater oxygen ingress have been shown to have a reduced interaction with protein than the same wines bottled under wines with low oxygen permeation (Gambuti et al. 2013).

Common techniques to reduce the time involved in wine ageing involve the controlled gradual application of oxygen to wine using macro-oxygenation during alcoholic fermentation or micro-oxygenation (MOX) before or after MLF. Macro-oxygenation normally occurs during primary fermentation during pump-overs although the amount of oxygen ingress can vary greatly depending on how the process is carried out (Moene et al. 2014). In a more controlled environment, bubbling large volumes of air through active fermentations in rotary fermenters has been shown to induce changes in tannins and astringency that are consistent with characteristics of aged wines (McRae et al. 2014b). These oxygen-treated wines demonstrated similar tannin characteristics after 2 months of ageing to those of the control wines after 2 years of ageing, and the differences between the control and oxygen-treated wines were still significant after 2 years of bottle ageing (McRae et al. 2014b). The oxygen-treated ferments contained a lower concentration of tannins, and the composition of tannins showed lower molecular mass and mass conversion yield. Further research into controlled macro-oxygenation is warranted.

Exposing wines to small amounts of oxygen with MOX is increasingly a popular technique for developing wine colour and texture, and much research has been conducted in this area and reviewed recently (Gomez-Plaza and Cano-Lopez 2011, Schmidtke et al. 2011, Anli and Cavuldak 2012). Micro-oxygenation involves the controlled addition of small doses of oxygen to wines in tanks, usually either pre-MLF [10–30 mL/(L · month)] or post-MLF [1–5 mL/(L · month)] (Gomez-Plaza and Cano-Lopez 2011). The effect of MOX reportedly increases colour stability through the formation of pigmented polymers and decreases astringency; however, results vary greatly depending on wine composition, timing of MOX application, dosage and temperature (Gomez-Plaza and Cano-Lopez 2011, Oberholster et al. 2015). Micro-oxygenation has been shown to be most effective on high phenolic wines and when additions were made pre-MLF (Cano-Lopez et al. 2008). Combination treatments of MOX with oak staves are also more effective than MOX alone in accelerating wine ageing as well as producing favourable sensory attributes (Cano-Lopez et al. 2008, Sánchez-Iglesias et al. 2009, Oberholster et al. 2015). One of the effects of adding oxygen to wine is the production of acetaldehyde from ethanol, which promotes polymerisation of tannins and the formation of pigmented polymers through ethyl linkages. A different approach to accelerating wine ageing is the direct addition of acetaldehyde as a tannin cross-linking agent (Sheridan and Elias 2015). This technique resulted in a decrease in the concentration of tannins and an increase in colour stability, as expected with wine ageing, although the impact of acetaldehyde addition on flavour and mouthfeel are yet to be determined.

Ageing on lees is another method for improving the developed characteristics of a wine. As well as the aroma and flavour impacts [summarised in Tao et al. (2014)], ageing on lees can change the phenolic composition of wine. This is mostly because of the adsorption of phenolic substances to yeast cell walls and sterol in the cell membranes, resulting in the removal of some tannins and an overall decrease in the concentration of tannins leading to lower astringency. Storing wines on lees also results in yeast autolysis, releasing polysaccharides which can reduce

© 2015 Australian Society of Viticulture and Oenology Inc.
wine astringency (Vidal et al. 2004b). Wines aged on lees in combination with MOX have been shown to have greater colour stability and more pigmented polymers than either method alone (Sartini et al. 2007, Tao et al. 2014). Other processes that have been investigated include the application of electric fields, gamma radiation and ultrasonic waves, which have been thoroughly reviewed by Tao et al. (2014). More research into combination treatments for accelerated ageing of red wines may reduce the need for extended wine ageing. Overall, wines of lower pH stored under a closure that allows for greater oxygen ingress will develop more rapidly than wines of higher pH and limited oxygen exposure. The application of oxygen to wines either as macro-oxygenation during primary fermentation or as MOX post-fermentation can induce characteristics of wine ageing more quickly. Understanding how red wine tannins change with ageing and how these changes impact the astringency of wine is enabling new technologies to be developed that shorten the ageing period of young red wines.

**Conclusions**

Research undertaken to improve the understanding of the impacts of winemaking practices on the concentration and composition of tannins in red wines shows that significant impacts occur at many points of the wine production process, including through maceration, yeast selection, addition of mannoproteins, addition of oenotannins, fining by animal and plant proteins, filtration, oxygen exposure, barrel treatment, packaging/bottling and accelerated ageing techniques. A sufficient body of evidence has developed in certain areas to make generalisations, but several other areas continue to show large variability of tannins in response to winemaking interventions depending on the grapes and their particular methods of treatment. Progress has been made in determining the underpinning mechanisms for some of the effects, but significant further research is required to understand why many of the effects occur. Future research will provide the opportunity for improved management of vineyards and winemaking to optimise tannins in grape and wine, and for increased capacity to meet wine specification, consumer expectations and profitability.

**Acknowledgements**

This work was supported by Australian grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The Australian Wine Research Institute is a member of the Wine Innovation Cluster in Adelaide.

**References**


© 2015 Australian Society of Viticulture and Oenology Inc.
Impact of winemaking practices on tannins


© 2015 Australian Society of Viticulture and Oenology Inc.
Smith et al.
Impact of winemaking practices on tannins 613


Manuscript received: 12 August 2015

Revised manuscript received: 15 September 2015

Accepted: 16 September 2015

© 2015 Australian Society of Viticulture and Oenology Inc.