A Review of Tannin Determination Methods Using Spectrophotometric Detection in Red Wines and Their Ability to Predict Astringency

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Astringency is an important sensory attribute that influences red wine quality. The astringent sensation inside the mouth is caused by a group of molecules called tannins. These molecules in wine can be determined and analysed by spectrophotometric, analytical and recently electrochemical methodologies. This article focuses on the three methods most frequently used by the wine industry: Bate Smith or acid hydrolysis method, Adams Harbertson assay or BSA tannin assay, and methylcellulose precipitation (MCP) method. These methods differ on the principle upon which they are based, as well as on the kind of tannin that they can determine. The purpose of this article is to present the main advantages and disadvantages of the three spectrophotometric methods acid hydrolysis, BSA assay and MCP methods for tannin determination in red wine, in order to review their efficacy, group of tannins each method can determine, and their suitability for astringency prediction.

INTRODUCTION

Within the multiple parameters that are used to characterize red wines, astringency is one of the factors that is important in assessing their quality (Brossard *et al.*, 2016; García-Estévez *et al.*, 2017). Tannins, which are known to be the main contributors to astringency in red wine, are secondary metabolites of plants and are usually classified as condensed tannins or proanthocyanidins, and hydrolysable tannins (Sarneckis *et al.*, 2006).

Condensed tannins are flavan-3-ol polymers composed by different isomeric forms of catechin and gallocatechin (Aron & Kennedy, 2008; Kuhnle, 2018) and they are usually linked by C4-C8 and C4-C6 bonds giving rise to varying degrees of polymerization (Pascal *et al*., 2007; Vidal *et al*., 2004), expressed as mean degree of polymerization (mdP). Condensed tannins originate in the grape berry, where they are found both in skin and seeds (Harbertson *et al.*, 2002). On the other hand, hydrolysable tannins are molecules that are easily decomposed in the presence of a weak acid and/or high temperatures to yield pyrogallol. Hydrolysable tannins are divided into gallotannins and ellagitannins, which yield different products under acid hydrolysis. Gallotannins produce a sugar molecule and gallic acid, whilst ellagitannins yield the same reaction products and additionally a molecule of ellagic acid (Smeriglio *et al.*, 2017). Hydrolysable tannins can react among themselves to form large polymeric complexes. Hydrolysable tannins may also be present in red wine as a result of extraction from oak barrels or through the addition of commercial tannins.

Both condensed and hydrolysable tannins exist in a wide range of molecular weights, and can be found bound to other compounds present in red wines, such as sugars (Werner, 2011) or gallic acid (Mercurio *et al.*, 2007). The associations between tannins and other red wine compounds, add more complexity to the chemical structure of tannins (Jensen *et al.*, 2008; Mercurio & Smith, 2008), making the development of reliable methods for wine tannin determination and quantitation a challenging task.

From the sensory point of view, one of the most important characteristics of tannins is their ability to interact with salivary proteins present inside the mouth and produce their precipitation (Bajec & Pickering, 2008). The astringency mouthfeel is the result of interaction between tannins and a class of salivary proteins called proline-rich proteins (PRPs) (Mcrae & Kennedy, 2011). The subsequent aggregation and precipitation of the resulting complexes have been shown to reduce the lubricity of saliva, increasing friction between oral surfaces (Quijada-Morín *et al.,* 2012). The precipitation reaction inside the mouth leads to the formation of tannin-protein aggregates, producing the loss of oral lubrication, which causes the astringent sensation (Upadhyay *et al.*,

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2016). Astringency perception associated with red wine consumption has been commonly described as a tactile sensation of drying, puckering, roughening and shrinking in the oral cavity (Gawel *et al.*, 2000; Soares *et al.*, 2016).

The astringent quality of some foods has been a topic of debate in the literature, with some authors defining astringency as a flavour (Bajec & Pickering 2008), and others stating that astringency is a tactile sensation associated to food texture (Bajec & Pickering, 2008; Ployon *et al.*, 2018). The vocabulary used to describe the astringent sensation has also been a matter of study. Gawel *et al.(*2000) developed a series of terms to describe the astringent sensation of red wines organized in a mouth feel wheel (MFW). The MFW describes 13 different sub-qualities of astringency, with each sub-quality being divided into smaller tactile groups to better describe it. Despite the MFW being useful as a guide, the complexity of the terminology is hard to fully understand for tasters, and wine consumers. Additionally, extensive training is needed for applying the MFW to wine sensory analysis (Vidal *et al.*, 2015), as astringency is a multifactorial sensation, which makes its sensory evaluation more complex (Ferrer-Gallego *et al.*, 2014).

Regarding the impact of tannin content on wine quality, a proportional relationship between the amount of tannins and the intensity of the astringency perceived during wine consumption has been observed (Gawel *et al.*, 2007). However, other factors are also important to consider: for example, astringency resulting from skin tannins is described as pleasant and, thus, favourable for wine quality (Cortell *et al.,* 2005). On the other hand, astringency resulting from seed tannins is associated with undesired sensory "green" qualities in wine, associated to a greater proportion of galloyl groups that increase the affinity towards proteins due to hydrogen bonds which stabilize the tannin-protein interaction (Smith *et al.*, 2015; Ferrer-Gallego *et al.*, 2016; García-Estévez *et al.*, 2017). Furthermore, it is important to consider the different cellar techniques which may enhance tannin extraction from grape berries into the must and therefore increase astringency, for instance the use of pectolytic enzymes, pre and post fermentative maceration, and *saignée* (Harbertson *et al.*, 2009).

In the literature, different methods for wine tannin analysis, such as those based on tannin precipitation and colourimetric detection have been reported. The present study will focus on a review of the most used tannin analysis methods; Bate Smith or acid hydrolysis method, Adams Harbertson or BSA tannin assay, and methyl cellulose precipitation (MCP). In order to understand what each method is measuring, the chemical basis of the method is reviewed, and later, tannin concentrations in different red wines, as measured by each method, are compared, in order to assess variability of tannin measured by each one of the three methods herein reviewed. Differences in the values reported by each method could be explained by the chemical nature of each method. Also, on the basis of the chemical basis of the method, advantages and disadvantages of each method are discussed. Then, correlations between tannin content as evaluated by each method, with sensory astringency, are also reviewed. Likewise, differences in how each method correlates with sensory astringency could also be explained by the chemical nature of the methods, which could thus have an impact on each method reliability for the purpose of astringency prediction.

METHODS USED FOR TANNIN ANALYSIS

Wine tannin can be determined by means of colourimetric, gravimetric, chromatographic, or precipitation methods (Sarneckis *et al.*, 2006). Additionally, indirect methods for astringency prediction in wine have been reported, such as the gelatine index (Sartini *et al.*, 2011). Colourimetric assays for tannin determination are based on the reaction of the tannin with another chemical molecule to yield a coloured compound. The resulting concentration of the coloured compound is quantified by spectrophotometry using a calibration curve, and the tannin content in the wine analysed can thus be easily determined (Herderich & Smith, 2005). An example of this kind of method is the Bate Smith method, based on the Bate Smith reaction of condensed tannins (Ribéreau-Gayon & Stonestreet, 1966).

Precipitation methods for tannin determination rely on selective tannin precipitation with particular chemical reagents (Herderich & Smith, 2005). Hagerman and Butler (1978) pioneered the protein precipitation method for tannin determination using bovine serum albumin (BSA) to precipitate and quantify tannins. The method was later modified by Adams and Harbertson (Harbertson *et al.*, 2003) and is nowadays known as the BSA assay. Likewise, the MCP method developed by Sarneckis *et.al*. (2006) is also a precipitation method, where the polysaccharide methyl cellulose is used to precipitate and quantify tannins. Currently, these two last methods are the most used for tannin determination in wine samples (Scollary, 2010).

Bate Smith Method or acid hydrolysis

The Bate Smith method is a colourimetric method based on the hydrolysis reaction of proanthocyanins in a heated acidic medium to give coloured anthocyanin pigments. The analysis must be performed in parallel using two glass tubes, where only one tube is submitted to the acidic hydrolysis. This is necessary to correctly assess the colour change after the hydrolysis reaction. For the analysis, the wine sample is diluted in a 1:50 ratio with distilled water. Afterwards, 6 mL of hydrochloric acid (37% fuming) are added to 4 mL of the diluted wine samples. The reaction tube is heated at 100°C for 30 minutes to perform the acidic hydrolysis. Meanwhile, the tube that was not submitted to the acidic hydrolysis is saved for the next step. Before the spectrophotometric measurement, 1 mL of ethanol (95% v/v) must be added to both tubes, with and without hydrolysis (Ribéreau-Gayon & Stonestreet, 1966). The consequent colour formation in the reaction tube is directly proportional to the absorbance measured at 550 nm by the spectrophotometer and represents the tannin concentration expressed as g/L of (+)-catechin equivalents (Cáceres-Mella *et al.*, 2013). An adaptation of this methods was reported (Vignault *et al.*, 2018). In this procedure (Figure 1) the red wine sample (20 µL), water (1.48 mL) and hydrochloric acid (1.5 mL, 37%) are placed in two tubes. Tube A is placed in an ice bath (0ºC) while tube B is placed in a warm bath (100ºC). After 30 min, 600 µL of ethanol are added to both tubes to stop the reaction. The

proanthocyanidin concentration in g/L is obtained multiplying the difference in absorbance at 550 nm between tube B and tube A by 19.33, which is the absorptivity coefficient of cyanidin after the acidic cleavage of the condensed tannins (Bate-Smith reaction), as summarised in equation 1.

$$\text{Tannins(g/L)} = (\text{Tube B}_{\text{Hydrolysed}} - \text{Tube A}_{\text{Control}}) \times 19.33 \quad \text{(Eq.1)}$$

The main advantage of this method is its easy implementation and reliability (Cáceres-Mella *et al.*, 2013). However, some limitations regarding this method have been reported. First, the acid hydrolysis reaction only gives an estimation of the total tannin content without considering the chemical structure of tannins present in the wine sample analysed (Ribéreau-Gayon *et al.*, 2006). Moreover, tannin concentration in wine is often overestimated, and it is possible to observe an increase over time, which does not correspond to an increase in tannin content (Aleixandre-Tudo *et al.*, 2017). Also, the hydrolysis reaction performed at high temperature (100ºC) for a long period, can lead to caramelization of sugars with a consequent browning, known as Maillard reaction. This reaction is particularly important in sweet wines because it can lead to an overestimation of tannin measurement (Oliveira *et al.*, 2011).

Adams Harbertson assay

The Adams Harbertson or BSA assay (Harbertson *et al.*, 2003) is a modification of the precipitation method published by Hagerman and Butler in 1978. The method was first adapted for wine analysis by Adams & Harbertson (1999), and then modified by Harbertson *et al.* (2002) and Harbertson *et al.* (2003). First, the wine sample has to be diluted in a model wine (12 % v/v of ethanol; pH 3.3). The diluted sample reacts with the globular bovine serum albumin (BSA) protein derived from cow's blood, giving as a result a precipitate after the centrifugation of the reaction tube. After discarding the supernatant, the tannin-protein complex is resuspended in a sodium dodecyl sulphate (SDS) and 5% triethanolamine (TEA) resuspension buffer and the absorbance at 510 nm (background tannin) is obtained. After an incubation period with the resuspension buffer, ferric chloride (0.27 g $FeCl_3$/100 mL solution) is added and, finally, the absorbance of the solution is measured at 510 nm (total tannin). Tannin concentration in the sample is given by the subtraction between total tannin and background tannin. Quantitation of tannin in wine samples is performed by interpolation of this value in a standard calibration curve. The results obtained are expressed in g/L of (+)-catechin equivalents (Harbertson *et al.*, 2003). The modification incorporated by Adams and Harbertson to the original method described by Hagerman and Butler, includes anthocyanins bleaching with bisulphite after the protein-tannin pellet precipitation. This modification allows to distinguish between large and small polymeric pigments. Recently, a modification on the composition of the resuspension buffer was reported (Harbertson *et al.*, 2015), where the original resuspension buffer of SDS at pH 9.4 was replaced by urea (8.3 mol/L, pH 7.8). Results obtained after the analysis of 44 red wine samples using this modification showed that the urea buffer significantly increases the amount of tannin recovered and reduces the background absorbance shift. The new conditions assayed at neutral pH (Figure 2) may contribute to the stability of the protein-tannin complex and, therefore, improve tannin yield.

An experiment with isolated condensed tannins from cacao, from monomer to octamer, determined that the BSA assay has the ability to precipitate condensed tannins of different size, from trimers to octamers. However, this method is not able to determine monomers and dimers because BSA does not cause their precipitation (Harbertson *et al.*, 2014). The reliability of tannin determination in wine samples using BSA assay is strongly influenced by very high tannin concentrations present in wines and also by the existence of a threshold tannin level necessary to produce protein precipitation (Jensen *et al.*, 2008). Both factors can cause an underestimation of tannin concentration of the

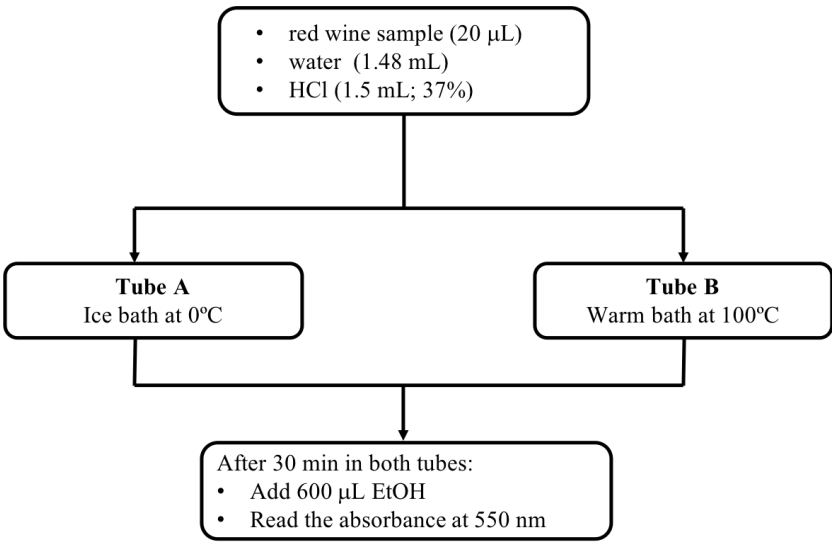

FIGURE 1
Diagram of acid hydrolysis reaction method for tannin determination in red wine samples (adapted from Vignault *et al.*, 2018)

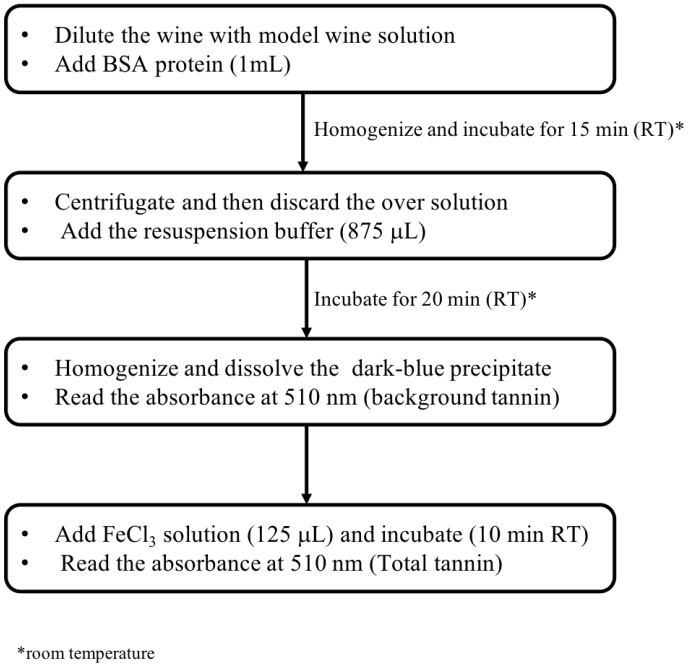

FIGURE 2
Diagram of BSA assay for tannin determination in red wine samples for a total volume of analysis of 2 mL (adapted from Hartbertson *et al*., 2015)

samples analysed using the BSA assay. In his study, Jensen *et al*. (2008) recommends to carefully dilute wine samples in order to obtain absorbance responses between 0.3 to 0.75 units. Additionally, a tannin-protein ratio between 17 to 45 (mole of tannin CE/mole BSA) was calculated, so as to ensure a proficient precipitation of tannins according to the saturation stoichiometry of BSA.

Results obtained by Kemp *et al*. (2011), showed that tannin precipitation using the BSA assay exhibited a linear behaviour related to tannin concentration since protein is in excess (for the range 0.40 – 1.80 mg BSA/mL). Additionally, this study supports the suggestion made by Jensen *et al*. (2008) about the existence of a tannin threshold concentration for precipitation with BSA to occur. However, they suggest a minimum tannin concentration of 100 mg CE/L, instead of ~140 mg CE/L proposed before. In addition to this, it has been reported that the results of the BSA assay can vary on the basis of variables such as tannin and protein solution homogenization and contact time between tannins and BSA protein (Ropiak *et al.*, 2017).

Methyl cellulose precipitation method

The MCP method was published in 2006 by Sarneckis *et al.* This method is based on the ability of tannin to precipitate with the polysaccharide methyl cellulose, and it can be used for tannin analysis of wine, 50% ethanol grape extract and aqueous tannin solutions. The determination is performed spectrophotometrically at 280 nm by the difference in absorbance of the supernatant obtained before and after tannin precipitation with a 0.04% methyl cellulose solution in the presence of a saturated ammonium sulphate solution (Figure 3). Quantitation of tannin in the wine or grape extract samples is performed by fitting a standard calibration curve. Tannin results are expressed in g/L of (-) - epicatechin equivalents. In order to validate the method, Sarneckis *et al*. (2006) compared the tannin measured by the MCP precipitation with tannin determination by reverse-phase high performance liquid chromatography (HPLC). For this purpose, 121 red wines and 54 grape extracts samples were used. All samples were analysed by both MCP and HPLC methods. Validation results exhibited a good correlation for the determination of total tannin content between 0.1 and 3.0 g/L of epicatechin equivalents ($r = 0.74$) and a coefficient of variation of 3.0% (Sarneckis *et al.*, 2006).

According to Sarneckis *et al*. (2006) the MCP method is selective for condensed tannins and does not suffer interference from other 280 nm absorbing phenolics such as anthocyanins or catechin monomers. The spectrophotometric determination of tannins at 280 nm avoids any need for indirect quantitation methods such as radioactive tracers (Henson *et al.*, 2004) or non-specific colourimetric assays (Harbertson *et al.*, 2003). Furthermore, a high throughput version using a 96-well plate reader has been proposed (Mercurio *et al.*, 2007). This modification of the original method leads to a reduction of sample and reagents to a miniaturised format of 1mL. Validation studies conducted by Mercurio *et al*. (2007) showed that this format is equally efficient and reproducible as the analogous original version. The main advantage of the MCP method is that it is simple, fast, easy to implement and robust (Sarneckis *et al.*, 2006; Mercurio & Smith, 2008). This versatility makes the MCP method especially useful for quality control in the wine industry.

It is important to mention that significant differences in tannin concentration have been reported for the same wine samples using the MCP and BSA assays (Mercurio & Smith,

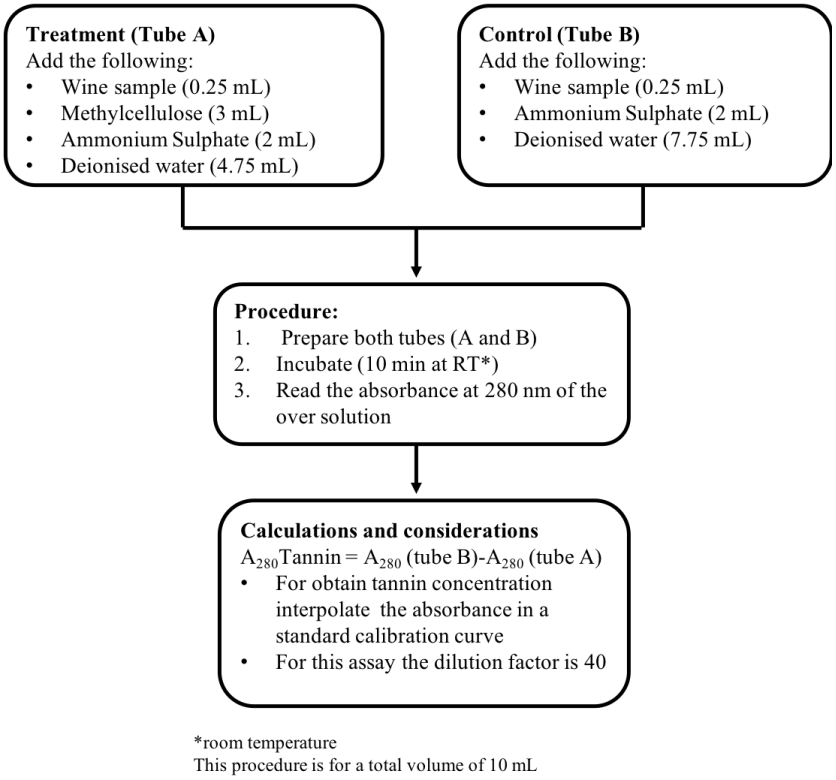

FIGURE 3
Diagram of MCP method for tannin determination in red wine samples (adapted from Mercurio & Smith, 2008)

2008; Seddon & Downey, 2008; Harbertson & Downey, 2009; Cáceres-Mella *et al.*, 2013). The main explanation for this behaviour is that MCP tannin assay quantifies as tannin the contribution from anthocyanin subunits incorporated into the tannin. On the other hand, the BSA assay would not include the anthocyanin compounds incorporated into the tannins because the ferric chloride step to produce the final colour change does not react with anthocyanins (Mercurio & Smith, 2008). Additionally, the BSA assay is not able to cause the precipitation of dimer and monomeric molecules of tannins (Harbertson *et al.*, 2014). Apparently, tannin size is the most important factor for the formation of the protein-tannin complex, although other factors related to tannin structure could be equally important (Koerner *et al.*, 2009; Ropiak *et al.*, 2017). Additionally, it has been reported that the polysaccharide methylcellulose has a higher binding affinity for condensed tannin than the globular protein BSA (Kemp *et al.*, 2011), which could affect tannin concentration results obtained using both MCP and BSA assays. Finally, Table 1 summarises the information of the three methods reviewed.

Other methods available for tannin analysis

It is important to mention that the methods reviewed in this study are not the only options available to determine and quantify tannins in red wines and grape extracts. A modification of the acid hydrolysis method has been reported, which uses the same chemical principle of the Bate Smith reaction but employing a mixture of n-butanol/HCl (95:5) to perform the tannin hydrolysis (Porter *et al.*, 1985).

A gravimetric method based on tannin precipitation by a copper-acetate complex has been reported as an alternative to the Folin-Ciocalteu analysis (Yebra *et al.*, 1995). Tannin measurement is performed indirectly by the quantitation of the unprecipitated copper using atomic absorption spectrometry. Results are expressed in μg/mL of tannic acid with a detection limit of 0.7 μg/mL. Recoveries of tannic acid added to three wine samples and one tea sample ranged between 96.1 and 104.3%, which shows the reliability of the method.

Another method reported is based on the reduction of Fe (III) to Fe (II) by tannins, producing a coloured dark blue solution. The Fe (II) obtained reacts with phenanthroline to from a coloured orange-red complex. The obtained complex exhibits UV-vis absorption at 335 and 540 nm (Tinkiliç & Uyanik, 2001). However, this method reports unreliable tannin concentration, especially for beer and wine matrices.

A predictive method which combines Fourier transform mid-infrared (FT-MIR) spectroscopy and chemometric techniques has also been developed using protein precipitation and phloroglucinolysis as analytical reference methods (Fernández & Agosin, 2007). This method was able to determine tannin concentration as well as the mDP of tannins by means of the analysis of FT-MIR spectrum, by multivariate analysis using Partial Least Squares (PLS). High correlation and accuracy for the prediction of tannin concentration ($r = 0.995$; RMSEP = 9.4%) and mDP ($r = 0.958$; RMSEP = 10.3%) was obtained, with protein precipitation and phloroglucinolysis used as analytical reference methods. Similarly, using near-infrared spectroscopy (NIR) combined

TABLE 1
Summary of the tannin determination methods reviewed

Method	Detection	Molecules determined	Advantages	Disadvantages
Acid hydrolysis	Colourimetric	Proanthocyanidins and flavanol monomers	Fast Easy implementation Reliable Sensitive for low concentrations	Overestimation problems affected by sugar (Maillard reaction)
BSA	Precipitation	Proanthocyanidins (trimers to octamers)	Protein as precipitating agent, similar to astringency mechanism	Wine dilution problems Saturation point of BSA Precipitation threshold Only for red wines
MCP	Precipitation	Proanthocyanidins	Fast Easy implementation Robust	Precipitation threshold Only for red wines

with chemometrics, a large number of methods have been reported, especially for the determination of the total polyphenolic content in wines. However, among all the articles published, only three of them (Cozzolino *et al.*, 2008; Ferrer-Gallego *et al.*, 2011; Zhang *et al.*, 2017) paid special attention to tannin quantitation with good results.

TANNIN ANALYSIS AND ASTRINGENCY PERCEPTION IN RED WINES

The main challenge for tannin analysis has been to correlate tannin content with perceived astringency, as evaluated by trained panels, and also to be able to predict astringency in terms of wine quality (Kennedy *et al.*, 2006; Gawel *et al.*, 2007). So far, the only direct way of determining sensory astringency has been using a trained sensory panel, but even when these panels are comprised of expert panellists, they are difficult to train with an objective vocabulary, are time consuming and expensive (Ren *et al.*, 2017).

Table 2 presents the results of studies that have analysed the relationship between tannin concentration in red wine using at least one of the three methods reviewed in the present article, with perceived sensory astringency. In spite of colourimetric methods being usually described as having low specificity to tannins (Herderich & Smith, 2005), tannin determination of 57 red wine samples using acid hydrolysis showed good correlation results (R^2=0.8) with the sensory astringency perceived by a trained panel (Rinaldi *et al.*, 2012).

Currently, in the winemaking industry, both MCP and BSA assay are the most used methods for astringency prediction, based on the tannin content of red wine, with good linear correlation coefficient (R^2) with sensory astringency (Scollary *et al.*, 2012). In particular, 4 studies which compare the tannin content with the BSA method and sensory astringency perceived showed correlation coefficients (R^2) from 0.47 to 0.90 (Kennedy *et al.*, 2006; Mercurio & Smith, 2008; Cáceres-Mella *et al.*, 2013; Boulet *et al.*, 2016). For the MCP method 3 different studies showed correlation coefficients (R^2) from 0.59 to 0.86 (Mercurio & Smith, 2008; Cáceres-Mella *et al.*, 2013; Brossard *et al.*, 2016). It is important to mention that the study with lowest correlation coefficients, for both BSA (R^2 = 0.47) and MCP (R^2 = 0.59), included five *rosé* and five white wine (Sauvignon Blanc) samples, demonstrating that both the MCP and the BSA assay are not proficient for tannin determination for these kind of wines, as both methods consider aggregation between the precipitating agent and proanthocyanidins, which leads to pellet formation. The low concentration of procyanidins in *rosé* and white wines would not be high enough to reach the threshold for pellet formation (Cáceres-Mella *et al.*, 2013)

Among all the tannin determination methods reviewed in this article, precipitation methods for tannin determination, such as the BSA and the MCP assay are specially interesting for astringency prediction because the interaction between tannins and the precipitant agent is similar to the astringency mechanism (Soares *et al.*, 2018). Furthermore, the results enounced in Table 2 show that both of these methods are equally proficient for astringency prediction in red wines samples.

Last, even while precipitation methods give only relative information about wine tannin, they are useful from a viticultural or winemaking perspective because they are easy to use and implement in a winery, unlike HPLC tannin determinations, which are appropriate for analytical studies where the absolute amount of tannin, and tannin subunit composition are important.

CONCLUSIONS

Tannin quantitation is an important issue for the wine industry because, from a sensory point of view, tannins are one of the main molecules responsible for astringency and mouthfeel perception in red wines. Therefore, having a reliable, fast, proficient, and easy-to-implement method for tannin determination in red wines is a useful tool for astringency management during winemaking

Among all tannin determination methods reviewed in the present article, the acid hydrolysis method has been used in several studies, mostly in Europe. The latter is mainly because this method is easy to implement and cost effective. However, acid hydrolysis may overestimate

TABLE 2
Comparison between the linear regression coefficient (R^2) obtained for the tannin determination methods reviewed with sensory astringency perceived in different red wine samples

Method	(R^2) *	References	Sample
Acid hydrolysis	0.80	Rinaldi *et al.*, 2012	57 commercial red wines including Aglianico, Merlot, Cabernet Sauvignon, Syrah and Sangiovese
BSA assay	0.90	Mercurio & Smith, 2008	20 Australian commercial red wines, 10 Cabernet Sauvignon and 10 Shiraz
	0.83	Boulet *et al.*, 2016	21 red wines mainly from the South of France
	0.47	Cáceres-Mella *et al.*, 2013	20 Chilean Cabernet Sauvignon wines
	0.82	Kennedy *et al.*, 2006	6 US red wines
MCP	0.83	Mercurio & Smith, 2008	20 Australian commercial red wines, 10 Cabernet Sauvignon and 10 Shiraz
	0.59	Cáceres-Mella *et al.*, 2013	20 Chilean Cabernet Sauvignon wines
	0.86	Brossard *et al.,* 2016	4 red wines

*Linear regression coefficient obtained from the comparison of tannin concentration obtained and the perceived astringency using a trained panel.

tannin concentration because it exhibits interaction with other wine components like sugars and additionally gives only an estimation of the total tannin content and does not differentiate between the different tannin structures that may be present in wine.

Both MCP and BSA assays are methods based on the precipitation of tannins, which is similar to the astringency mechanism inside the mouth. The two methods are specific for tannins derived from flavan-3-ol family or proanthocyadins. Results obtained for wine samples using both methods have shown that the MCP method usually exhibits higher values for total tannin content, than the BSA assay. This behaviour has been mainly attributed to the fact that BSA is only able to precipitate tannin of a specific size (from trimers to octamers) and does not precipitate polymeric pigment associated to tannin, which induces an underestimation in the total tannin content compared with the values obtained using MCP analysis.

Comparisons of MCP and BSA assay with instrumental methods, such as HPLC, have shown that instrumental methods are generally more precise and give more information about tannin composition and chemical structure. However, neither instrumental nor spectrophotometric tannin determination methods can give the exact tannin composition of wine, because currently there are few commercial standards available. It is thus only possible to estimate a degree of polymerization based on the identity of the monomeric terminal units.

Finally, the best method to determine the tannin content in wine samples is dependent on the information required by the researcher. For accurate research about chemical structure, the best option are instrumental methods, for instance HPLC. On the other hand, for daily routine analysis in a wine cellar the acid hydrolysis, MCP or BSA assay, are good options. However, if the aim of the analysis is to predict the intensity of astringency it is more suitable to use either the MCP or BSA assays. Finally, it would be interesting to test tannin determination methods with different enological practices, grape varieties, and with the use of hydrolysable tannins, so as to establish their performance and, thus, practical application for the wine industry.

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