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Comparison of analytical methods for measuring proanthocyanidins in wines and their relationship with perceived astringency

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Summary The concentration of proanthocyanidins from twenty red wines from cv. Cabernet Sauvignon, five *rosé* wines from cv. Cabernet Sauvignon and five white wines from cv. Sauvignon Blanc was quantified using four analytical methodologies, and their relationship with the perceived astringency was investigated. Proanthocyanidin concentrations were determined by a methylcellulose precipitation assay, a protein precipitation assay and two colourimetric methods (Bate-Smith and vanillin assay). The four methodologies showed high repeatability but differed widely in proanthocyanidin concentrations. The methylcellulose and protein precipitation assays could not quantify proanthocyanidins in *rosé* and white wines. The protein precipitation assay gave the lowest concentration of proanthocyanidins in all of the red wines. The methylcellulose precipitation assay (r = 0.7725; r^2 = 0.59) and the protein precipitation assay (r = 0.6828; r^2 = 0.47) showed a strong correlation with the perceived astringency compared with the colourimetric methods. The strong correlation of the methylcellulose precipitation method with the perceived astringency could be a useful tool to estimate red wine astringency.

Keywords Astringency, Cabernet Sauvignon, methylcellulose, proanthocyanidins, protein precipitation, Sauvignon Blanc.

Introduction

Proanthocyanidin compounds are important qualitative factors in wine due to their role in astringency, bitterness and colour stability (Boss *et al.*, 1996; Gawel, 1998; Brossaud *et al.*, 2001; Kennedy *et al.*, 2006; Ribéreau-Gayon *et al.*, 2006). Astringency is one of the most important sensory attributes in red wines (Gawel *et al.*, 2001; Vidal *et al.*, 2004; Payne *et al.*, 2009; Villamor *et al.*, 2009) and is caused by the binding of condensed tannins or proanthocyanidins with salivary proteins, which produces a sensation that is described as dryness and puckering in the oral surface of the mouth (Lllaudy *et al.*, 2004). The ability to analyse these compounds is important in various areas of knowledge and especially in the winemaking industry, which needs methods that are reproducible, are inexpensive, require minimal analytical skills and equipment and deliver reliable results (Sarneckis *et al.*, 2006). Furthermore, the use of analytical methods that correlate well with perceived astringency is very important for quality control. Although there are several studies on the quantification of proanthocyanidins in wines (Bate-Smith, 1973; Hagerman & Butler, 1978; Saucier *et al.*, 2001; Harbertson *et al.*, 2002; Kennedy *et al.*, 2006; Sarneckis *et al.*, 2006; Mercurio *et al.*, 2007; Seddon & Downey, 2008), there is a lack of studies that quantify proanthocyanidins in red, *rosé* and white wines from Chile while considering methods that are easy to implement in wine cellars and correlate strongly with the perceived astringency. It is important to consider the relationship between the structure of tannins and the astringency perceived. Although sensory analysis is a powerful tool to keep in mind for winemakers, the cost is high and subjectivity is inherently introduced with the use of a sensory panel that is composed of people (Valentová *et al.*, 2002). For this reason, the aim of this study was to

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compare four analytical methods for the quantification of proanthocyanidins in Chilean red, *rosé* and white wines and their correlation with the perceived astringency.

Materials and methods

Chemicals reagents and equipment

Methylcellulose (1500 cP, viscosity at 2%), bovine serum albumin (BSA, fraction V, lyophilised powder), sodium dodecyl sulphate (SDS), triethanolamine (TEA, 98%), (+)-catechin and ferric chloride hexahydrate ($FeCl_3$, 98%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ammonium sulphate, potassium bitartrate, sodium chloride (NaCl), vanillin 99%, glacial acetic acid, hydrochloric acid, sodium hydroxide (NaOH), ethanol, methanol, ethyl acetate, diethyl ether and sulphuric acid were purchased from Merck (Darmstadt, Germany). All reagents were analytical grade or superior. The Sep-Pak Plus tC_{18} environmental cartridge (900 mg) and Sep-Pak Plus Short tC_{18} cartridge (400 mg) were obtained from Waters (Milford, CA, USA). Ultrapure water was obtained from a Purelab Ultra MK2 purification system (Helga, Hertfordshire, UK). Phosphate buffer, pH 7.0, was acquired from Mallinckrodt Baker (Phillipsburg, NJ, USA). Nitrogen gas was supplied by Indura S.A. (Santiago, Chile). The pH was measured with an 8417N pH meter (Hanna Instrument, Smithfield, RI, USA). Centrifugation was performed in a Labofuge 400 centrifuge (Heraeus, Hanau, Germany). Heating was performed in a WB/OB 7-45 water bath (Memmert GmbH, Büchenbach, Germany). Absorbances were measured using a Shimadzu UV–Vis spectrophotometer model 1700 Pharmaspec (Kyoto, Japan).

Wine samples

Twenty red wines from cv. Cabernet Sauvignon, five *rosé* wines from cv. Cabernet Sauvignon and five white wines from cv. Sauvignon Blanc from different geographical regions of Chile from the 2009 vintage were purchased in supermarkets and specialty wine stores in the Santiago Metropolitan Region.

Wine chemical analyses

The analytical methods recommended by O.I.V. (2011) were used to determine pH, titratable acidity (g H_2SO_4 L^{-1}) and alcohol content (% v/v). The total phenol content was determined by UV absorptiometry at 280 nm and was expressed as g GAE L^{-1} (GAE: gallic acid equivalent) (Glories, 1984). Table S1 presents the basic data for the wines used in this study. All analyses were performed in triplicate.

Determination of proanthocyanidins by methylcellulose precipitation

The proanthocyanidin level was measured in wines using methylcellulose as a precipitant according to the methylcellulose precipitation method published by Sarneckis *et al.* (2006). The precipitation of proanthocyanidins was performed using a 0.04% methylcellulose solution (w/v, in deionised water). The proanthocyanidin concentration was determined by the difference in absorption at 280 nm between the tube without the addition of methylcellulose and the methylcellulose precipitated. The proanthocyanidin content was quantified against a (+)-catechin standard curve and expressed as g (+)-catechin equivalent (CE) per litre of wine sample.

Determination of proanthocyanidins by protein precipitation

The proanthocyanidin concentration was also determined by protein precipitation as previously described (Harbertson *et al.*, 2002). Proanthocyanidins were precipitated from wines using a protein solution prepared by dissolving BSA in a buffer (200 mM acetic acid, 170 mM NaCl, pH 4.9) to give a final protein concentration of 1 g L^{-1}. The samples were then centrifugated at 1400 ***g***, the precipitate was resuspended in an alkaline TEA/SDS buffer, and the absorbance was measured at 510 nm. The proanthocyanidin absorbance was measured after the addition of $FeCl_3$ solution. The quantification was against a (+)-catechin standard curve and was expressed as g CE L^{-1}.

Determination of proanthocyanidins by the Bate-Smith assay

The proanthocyanidin concentration was also determined using the methodology proposed by Bate-Smith (1981). Each wine sample was diluted at a ratio of 1/50 (v/v) with deionised water. In two separate test tubes, 4 mL of the diluted sample, 2 mL of deionised water and 6 mL of hydrochloric acid were added. One test tube (reaction tube) was placed in a water bath at 100 °C for 30 min, and the other test tube (blank tube) was left to stand in the dark for the same time. After 30 min, 1 mL of ethanol was added to each tube, and the tubes were left in the dark until the heated reaction tube was cooled. The absorbance of each test tube was measured in a spectrophotometer at 550 nm using deionised water as the blank. The absorbance difference was multiplied by the factor 19.33, and the concentration of proanthocyanidins was expressed in g CE L^{-1}.

Determination of proanthocyanidins by vanillin assay

The fractionation of proanthocyanidins in wines into monomers (FI), oligomers (FII) and polymers (FIII) of

flavan-3-ols was performed by the separation of flavan-3-ol fraction on C_{18} Sep-Pak cartridges according to the methodology described by Sun *et al.* (1998a). The quantification of proanthocyanidins in each fraction was carried out by the modified vanillin assay (Sun *et al.*, 1998b). A 2.5-mL aliquot of a 1:3 v/v sulphuric acid/methanol solution and 2.5 mL of a 1% (w/v) vanillin in methanol solution were mixed with 1 mL of the sample previously obtained by fractionation. The tubes were incubated at 30 °C for either 15 min (FI fraction) or for period of time long enough to allow maximal reaction (FII and FIII fractions). The absorbance of each tube was measured at 500 nm. A blank was prepared by substituting the vanillin solution in the reaction mix with methanol. The absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample, and the value was compared with standard curves. Quantification was performed by means of standard curves prepared from monomers (for FI), oligomers (for FII) and polymers of flavan-3-ol (for FIII) isolated from grape seeds, as previously described (Sun *et al.*, 1998b). The proanthocyanidin concentration was calculated as the sum of the three fractions and was expressed as g CE L^{-1}.

Sensory evaluation

Red wines were evaluated by a panel of twelve people (five women and seven men aged 23–35 years) who were all students and workers from the Department of Agro-Industry and Oenology. All judges had previous experience with sensory evaluation. A previous training session was conducted to standardise criteria among the judges. The sensory evaluation was performed in two sessions of 90 min for 2 days. In a session, each panellist had to assess two flights consisting of five wines. The wines were evaluated in individual temperature-controlled tasting booths, and water and unsalted crackers were provided for palate cleansing. Aliquots of 20 mL of wine were served at 18–19 °C in dark wine-tasting glasses (R. Cristal, Mendoza, Argentina) labelled with a three-digit code using a completely randomised order. The dark glasses were used to prevent the interaction of visual sensations and encourage focus on the mouthfeel sensation. The judges were forced to have a 1-min break between each wine. During each break, panellists chewed on a cracker and then rinsed their mouth with deionised water. The astringency intensity was scored on a 15-cm unstructured line scale anchored from 'low' to 'high'. All judges rated each wine in duplicate during the 2-day evaluation. The data were collected on a paper ballot.

Statistical analyses

Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were used to compare the proanthocyanidin concentrations among the analytical methods with a significance level of 95% ($P < 0.05$). Linear regression analysis was used to correlate the sensory analysis with the chemical parameters. All the statistical analyses were performed with the Statgraphics Centurion statistical software (version 15.2; Statpoint Technologies, Inc., Warrenton, VA, USA) and Excel 2007 (version 12.0; Microsoft Corp., Redmond, Washington, USA).

Results

The total phenol content ranged from 1.2 to 2.1 g GAE L^{-1} in red wines, from 0.6 to 1.5 g GAE L^{-1} in *rosé* wines and from 1.0 to 1.5 g GAE L^{-1} in white wines. In case of general parameters, in red wines, the pH value ranged from 3.3 to 3.8, the titratable acidity ranged from 3.9 to 5.9 g H_2SO_4 L^{-1}, and the alcohol content varied from 12.0 to 14.8% (v/v). For *rosé* wines, the values were as follows: pH, 2.9–3.2; titratable acidity, 4.4–5.9 g H_2SO_4 L^{-1}; and alcohol content, 12.5–14.0% v/v. For white wines, the ranges were as follows: pH, 2.9–3.4; titratable acidity, 3.8–4.8 g H_2SO_4 L^{-1}; and alcohol content, 12.5–14.5% v/v (Table S1).

Table S2 presents the proanthocyanidin concentrations in wine samples quantified by the four methodologies. In red wines, the ranges were as follows: methylcellulose precipitation, 0.40–1.78 g CE L^{-1}, with an average of 1.00 g CE L^{-1} (% CV = 7.5); protein precipitation, 0.01–0.28 g CE L^{-1}, with an average of 0.17 g CE L^{-1} (% CV = 5.6); Bate-Smith assay, 2.70–4.19 g CE L^{-1}, with an average of 3.28 g CE L^{-1} (% CV = 3.4); and vanillin assay, 0.99–2.18 g CE L^{-1}, with an average of 1.45 g CE L^{-1} (% CV = 6.5).

In this study, only the Bate-Smith and vanillin assay could quantify proanthocyanidin concentration in white and *rosé* wines (Table S2).

The relationship between perceived astringency and proanthocyanidin content was only investigated for red wine samples. Figure S1 shows the relationships among the methods of proanthocyanidins quantification in red wines; there was no significant correlation between the Bate-Smith assay and the methylcellulose precipitation ($r = 0.3404$) or protein precipitation methods ($r = 0.1094$), but there was significant correlation with the vanillin assay ($r = 0.5675$). The correlation between the proanthocyanidin content by the Bate-Smith assay with both precipitation methods was relatively weak compared with the moderately strong correlation with the vanillin assay. There was also a significant correlation ($r = 0.5880$) between methylcellulose precipitation and protein precipitation (Fig. S2).

Figures 1 and 2 show the results of the linear regression analyses between the astringency and the proanthocyanidin concentrations determined by methylcellulose and protein precipitation, respectively. A significant cor-

relation was found between the perceived astringency and methylcellulose precipitation ($r = 0.7725$; $r^2 = 0.59$) and perceived astringency and protein precipitation ($r = 0.6828$; $r^2 = 0.47$).

Figure S3 shows the results of the linear regression analyses between proanthocyanidin concentrations determined by the Bate-Smith and vanillin assays and the perceived astringency. Both methods showed non-significant relationships with the perceived astringency.

Discussion

Comparison among wine proanthocyanidin concentration and analytical methods

A fundamental condition of any proanthocyanidin methodology is that it can be applied to red, *rosé* and white wines. In this study, only the Bate-Smith and vanillin assays could quantify proanthocyanidin concentration in white and *rosé* wines. These results can be explained from the chemical basis of each methodology. The Bate-Smith assay is based on the transformation of proanthocyanidins into anthocyanidins in hot acid solution (Schofield *et al.*, 2001). The vanillin assay is a colourimetric method for the quantification of proanthocyanidins. It is quite specific to a narrow range of flavanols (monomers and polymers) and presents more sensitivity and specificity than other colourimetric methods (Sun *et al.*, 1998b). In contrast, the precipitation-based methods are based on the precipitation of proanthocyanidins with a polysaccharide or protein. Both precipitation methods have a stage of aggregation between the precipitating agent (methylcellulose, bovine serum albumin) and the proanthocyanidins in the wine sample, which leads to the formation of a pellet. Although both colourimetric methods have several factors that may influence the quantification of proanthocyanidins in wine, they could be more sensitive to low concentrations of proanthocyanidins than those methods based on precipitation. This would be the main reason that precipitation-based methods cannot quantify proanthocyanidins in white and *rosé* wines due to the nonformation of the pellet.

Moreover, there was a remarkable difference in the proanthocyanidin concentrations in wines among the different methods. The protein precipitation gave the lowest values of proanthocyanidin concentrations in red wines and differs from the values found by Mercurio & Smith (2008) who reported values from 0.16 to 0.59 g L^{-1}. The methylcellulose precipitation gave higher concentrations than protein precipitation in red wines, although lower values were reported by other authors (Sarneckis *et al.*, 2006; Mercurio & Smith, 2008; Mercurio *et al.*, 2010). The differences in concentration were high between the methods, but the four methodologies presented coefficients of variation (% CV) within normal ranges (< 10%), representing good repeatability. Because the four methodologies had good repeatability, it is necessary to keep in mind that there are other aspects that affect the choice of a methodology such as reagent prices, equipment needs or the steps necessary to measure the concentration of proanthocyanidins in a wine sample.

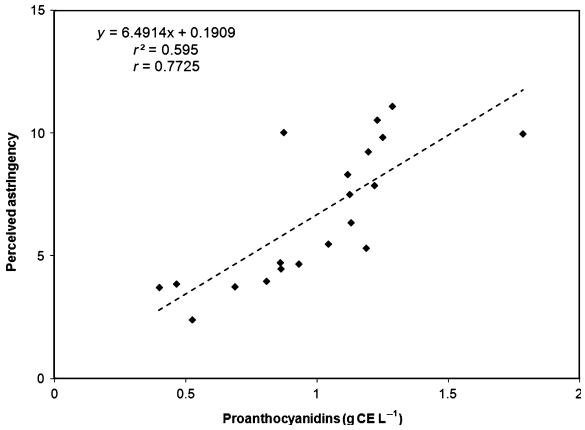

Figure 1 Relationship between the perceived astringency and methylcellulose precipitation in red wines. CE: (+)-catechin equivalent.

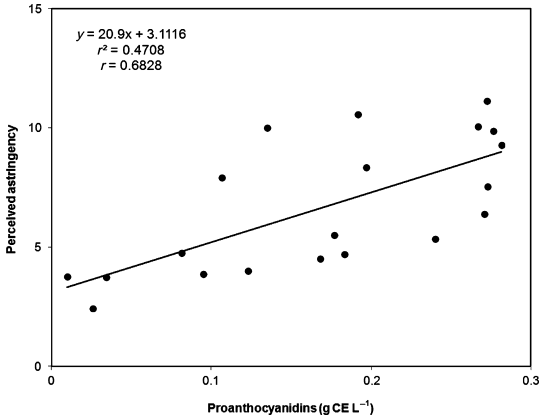

Figure 2 Relationship between the perceived astringency and protein precipitation in red wines. CE: (+)-catechin equivalent.

The Bate-Smith assay is the most widely used proanthocyanidin quantification method in Chilean wineries because it is inexpensive and requires minimal reagents and equipment. In this case, the Bate-Smith assay in red wines gave proanthocyanidin concentrations three times higher than methylcellulose precipitation, twenty times higher than the protein precipitation method and approximately two times higher than the vanillin assay. This methodology could be influenced by several variables that can affect the kinetics of colour formation such as the amount of water in the sample (Waterman & Mole, 1994), the incomplete

transformation of proanthocyanidins into anthocyanidins, reaction yields that depend on both the structure and polymerisation degree of the proanthocyanidins and side reactions that are common during the transformation that leads to the formation of red brown polymers (Schofield *et al.*, 2001). These factors could result in an estimation error by increasing the concentration of proanthocyanidins in the sample because red wines have more proanthocyanidins than white or *rosé* wines (Table S2), which leads to an incorrect concentration value. For these reasons, the application of this method for quantitative analysis of proanthocyanidins is limited in wines. Moreover, the great diversity of factors that affect the colourimetric reaction could explain the low correlation found with the precipitation-based methods.

Figure S1 shows the relationships among the methods of proanthocyanidin quantification in red wines. There was a significant correlation between methylcellulose and protein precipitation with a moderately strong relationship (Fig. S2) although a weaker correlation compared with those found by other authors in red wines (Mercurio & Smith, 2008). The differences in the chemical basis between the Bate-Smith assay and the precipitation-based methods could explain the lack of a relationship between them. The precipitation-based methods have the ability to bind proanthocyanidins with a precipitating agent (either a polysaccharide or protein), and it is therefore expected that a strong relationship would exist between them (Kennedy *et al.*, 2006; Sarneckis *et al.*, 2006) (Fig. S2).

With respect to the vanillin assay, several parameters may affect the accuracy such as reaction time, temperature, water content, vanillin concentration, acid nature and concentration. Sun *et al.* (1998b) studied these critical factors and concluded that the (+)-catechins and proanthocyanidins in the sample must be separated and then quantified separately. C_{18} Sep-Pak cartridges can be used to separate the compounds and obtain the monomers, oligomers and polymers of flavan-3-ol (Sun *et al.*, 1998a). Each fraction can then be quantified by the vanillin assay. Although the vanillin assay is a good methodology for the quantification of proanthocyanidins, the separation into the three fractions of flavan-3-ol, (+)-catechins, oligomers and polymers makes this quantification very difficult due to the large number of steps involved in the separation of flavan-3-ol (Sun *et al.*, 1998a). The increased time required to separate and then quantify the fractions could be a problem in wineries that need to analyse a large number of wine samples in a short period of time.

Hümmer & Schreier (2008) indicated that the problem with the Bate-Smith assay is that proanthocyanidins with high degrees of polymerisation produce more anthocyanidin than the flavan-3-ol dimers due to the existence of more extension units. For this reason, we expected that the samples that had high polymer contents when separated by C_{18} Sep-Pak cartridges and quantified by the vanillin assay would have a positive correlation with the proanthocyanidin quantified by the Bate-Smith assay. Indeed, data collected in this investigation showed a moderately strong correlation ($r = 0.5732$) between the concentration of polymers, that is, approximately 88% of the total content of proanthocyanidins quantified by the vanillin assay and the concentration of proanthocyanidins in red wines measured by the Bate-smith assay. This could be the reason for the high correlation between the Bate-Smith assay and the vanillin assay in red wines.

With regard to methylcellulose and protein precipitation, both methodologies showed a disparity in proanthocyanidin concentrations in red wines, with significant differences among wine samples for each method. The two methods are divided into two main stages: an 'isolation or precipitation stage' in which the precipitating agent interacts with the proanthocyanidins and the 'screening stage' in which the proanthocyanidin concentrations are measured. It is therefore important to identify which stage of the process explains the concentration differences found in this study. The differences may relate to the effect of the various reagents used in each methodology on the absorbances recorded. The slight differences of pH, acidity and alcohol content among the samples (Table S1) could explain these differences. However, other authors showed that these variables had no significant influence on the development of these methodologies due principally to the fact that the influence of pH is neutralised using appropriate buffer solutions (Sarneckis *et al.*, 2006).

On the other hand, the choice of a particular standard for establishing the calibration curve could affect the final results. Mercurio & Smith (2008) showed that the choice of monomer (+)-catechin or (−)-epicatechin has a minimal influence on the slope and intercept of the calibration curve, information that is supported in this work and is detailed in Table S3. The differences between the calibration curves were not significant and did not explain the differences. Therefore, the concentration differences between these two methodologies are not caused by the detection step but rather by differences in the isolation and precipitation stage. Methylcellulose and bovine serum albumin have a different binding and precipitating affinities for proanthocyanidins with different structural features and for each flavanol subclass. Other studies demonstrated by reverse-phase HPLC analysis that methylcellulose removes more of the flavanols from the matrix sample compared with the bovine serum albumin. Moreover, both methods have a different ability to bind and precipitate a subclass of the proanthocyanidins, the polymeric pigments (Harbertson *et al.*, 2003; Mercurio &

Smith, 2008; Seddon & Downey, 2008). These reasons could explain the differences in concentration found in this work. However, further research is necessary to check the actual effects on the results. Therefore, the specificity in binding and precipitating proanthocyanidins in the precipitation-based methods compared with the colourimetric assays could also explain the differences in concentration among the methodologies studied in this investigation in red wines and the lack of precipitation and subsequent detection by the precipitation-based methods in the white and *rosé* wine samples.

Correlation of analytical methods with perceived astringency

Due to the lack of specificity in the methods based on precipitation with white and *rosé* wine samples, the relationship between perceived astringency and proanthocyanidin content was only made for red wine samples.

Several variables in the wine composition such as pH, acidity, alcohol content and concentration and composition of proanthocyanidins can modulate the sensory perception of astringency (Gawel, 1998). Because the astringency is usually measured in wines by tasting, this requires a sensory panel, which adds some subjectivity to the final result of this analysis (Valentová *et al*., 2002). Because astringency is one of the most important factors in wine quality, wineries need a method to quantify proanthocyanidins that also represents perceived astringency. Condelli *et al*. (2006) and Monteleone *et al*. (2004) successfully developed predictive models for perceived astringency ($r^2 = 0.95$) by exploring the ability of wine to develop turbidity when mixed with the protein mucin. Lllaudy *et al*. (2004) developed a predictive model for perceived astringency using ovalbumin as a precipitation agent and tannic acid solutions as standards ($r^2 = 0.7737$). Kennedy *et al*. (2006) found strong correlations between astringency and protein precipitation ($r^2 = 0.82$), gel permeation chromatography ($r^2 = 0.74$) and phloroglucinolysis ($r^2 = 0.73$). In the same field, Mercurio & Smith (2008) found strong correlations between perceived astringency and the protein precipitation ($r^2 = 0.90$) and methylcellulose precipitation assays ($r^2 = 0.83$).

Figures 1 and 2 show the results of the linear regression analyses between the proanthocyanidin concentrations determined by methylcellulose and protein precipitation, respectively. These results are lower than those reported by other authors (Kennedy *et al*., 2006; Mercurio & Smith, 2008). Despite the differences among the studies, the correlations measured in this work are still strong. Figure S3 shows that the results of the linear regression analyses between the proanthocyanidin concentrations determined by the Bate-Smith and vanillin assays did not have a significant relationship with the perceived astringency. This is expected if the analytical principles of the precipitation-based methods, that is, the interaction and precipitation of tannins based on the basic mechanism of astringency, are accounted for (Lllaudy *et al*., 2004). Although a strong relationship between the analytical and sensory evaluations is paramount when choosing the appropriate methodology to predict perceived astringency, there are also several practical considerations that are important for each method, such as simplicity and efficiency. However, even if a methodology is strongly correlated with perceived astringency, the perceived astringency depends not only on the amount of proanthocyanidins in the wine sample but also on several other factors such as pH, acidity, sugar content, alcohol content and density (Kallithraka *et al*., 1997; Prinz & Lucas, 2000; Nurgel & Pickering, 2005; Fontoin *et al*., 2008; Obreque-Slier *et al*., 2010). Thus, the quantification of the proanthocyanidin concentration of a wine sample cannot be expected to accurately quantify the perceived astringency. Therefore, analysing the correlation between the proanthocyanidin content of wine samples and the perceived astringency only provides an estimate of the actual value.

Conclusions

Four methodologies for the quantification of the proanthocyanidin concentration in red, *rosé* and white wines were investigated. The proanthocyanidin concentration differed widely among the methodologies. The methylcellulose and protein precipitations could not quantify proanthocyanidins in *rosé* and white wine samples. The strong correlation between the methylcellulose precipitation method with the perceived astringency and the simplicity of this methodology suggests that it could be used in wine production to reliably estimate red wine astringency.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationship between the proanthocyanidin concentrations in red wines measured by the Bate-Smith assay and the concentrations determined by methylcellulose precipitation, protein precipitation and vanillin assay against Bate-Smith assay. CE: (+)-catechin equivalent.

Figure S2. Relationship between the methylcellulose and protein precipitation measurements of the proanthocyanidin concentrations in red wines. CE: (+)-catechin equivalent.

Figure S3. Relationship between the perceived astringency measured by the Bate-Smith assay and the vanillin assay in red wines. CE: (+)-catechin equivalent.

Table S1. Analytical parameters of wines from different geographical regions of Chile.

Table S2. Proanthocyanidin concentration in wines quantified by four analytical methods.

Table S3. Influence of the (+)-catechin or (_)-epicatechin monomer utilised in the calibration curve for the methylcellulose and protein precipitation methods.