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# Tannin quantification in red grapes and wine: Comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency.

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• The use of tannin-precipitation assays has increased greatly among wine industry researchers and practitioners due to their rapid, simple and robust natures. These researchers wanted to compare the two most common methods: the methyl cellulose precipitable (MCP) tannin assay and the Adams-Harbertson (A-H) protein precipitation tannin assay (using bovine serum albumin or BSA). They compared them for repeatability, time efficiency, throughput (both these assays have been adapted to high throughput formats by use of a microplate reader), and ease of practice. They also assessed the relationships between tannin quantification using both analytical techniques (how well the numbers correlated). And, importantly, the correlation of tannin concentration, as determined by the two methods, to red wine astringency was also investigated in this study. Forty-one commercially-available dry red wines from multiple varieties and vintages with a broad range of tannin concentrations were selected for the analytical portion of this trial. Following rigorous training and selection, the consumer preference portion of the trial commenced. The 12 judges who were selected rated 10 Cabernet and 10 Shiraz wines for 14 appearance attributes, 25 aroma attributes and 12 palate attributes.

• To determine repeatability, the authors used both assays to test the same 6 wines, representing a wide range of tannin concentrations, in triplicate. The results were fairly startling, with the MCP yielding concentrations from 1450 to 2300 mg/L as epicatechin equivalents and the A-H yielding concentrations ranging from 162 to 590 mg/L as catechin equivalents. Although both assays had similar repeatability (as the coefficient of variation between replicates was below 7% for both), it was obvious that their results were significantly different; this will be elaborated on in greater detail. As for the other areas of comparison, these authors felt that because the entire MCP assay can be conducted in 96 well plates, it was more time-efficient (they were able to do 48 samples in less than an hour) and the throughput was better than the A-H assay. The A-H assay, which requires that the first step (tannin isolation) be done in microfuge tubes, followed by several inoculation and incubation steps in the microplate reader, yielded them only 10-15 samples in 90 minutes.

• A comparative study was performed to determine how several variables influenced the final tannin concentrations for both assays. This involved the 40 grape samples and 41 wine samples noted above, performed in duplicate. To remain consistent with reported methods, tannin concentrations were expressed as catechin equivalents for the A-H assay and epicatechin equivalents for the MCP assay. Using linear regression analysis, the authors found a strong correlations between the methods when measuring grape tannin ($r^2$=0.96), and good correlations between the wine samples ($r^2$=0.80). The authors thought this lower correlation for wine samples could possibly stem from the chemical structure differences between grape tannins and wine tannins and the way the two assays function. Grape tannins have less structural diversity, so they probably interact more similarly with methyl cellulose and BSA. Wine tannins, however, are far more heterogeneous, and they also have anthocyanins incorporated as pigmented polymers, forming a whole new subset of tannins. The two assays have different approaches to the quantification of pigmented polymers when testing for total tannin (see article for details), and this may contribute to the reduced

correlation between the assays in wine samples and also the significant differences reported in the actual tannin concentrations in wine.

- The authors found that structural diversity did not completely explain the differences in reported tannin concentrations: the MCP assay was actually removing more tannin material from the samples than the A-H assay. Methyl cellulose complexes and precipitates all pigmented polymers, but BSA does not. The A-H assay differentiates pigmented polymers into those that precipitate with BSA (large polymeric pigment or LPP) and those smaller polymers which don't (small polymeric pigment or SPP). They state that it remains unclear whether the ability to precipitate with BSA is determined by size alone, or if other physicochemical properties of these pigments are important.

- Another experiment was designed to determine if the MCP and the A-H assays were capable of isolating the same amount of tannin from the sample. To remove the issue of pigmented polymers, they performed the both analyses on the same grape homogenate, using three extracts each and comparing the results. Then to confirm that both assays were capable of isolating and detecting the same amount of tannin, they changed the MCP protocol: after isolating the tannin using the methyl cellulose, they redissolved the MC-tannin complexes in the A-H buffer C, added ferric chloride and analyzed them for iron-reactive phenolics at 510nm by A-H detection protocol. In this way, they found that the numerical values were very similar to those using the A-H assay. This shows that although the assays use different compounds and methodology to isolate tannin, the amount of tannin they isolate is very similar. These results imply that the almost-three-fold difference in numerical values is a function of the different detection steps used for each assay and doesn't reflect the tannin isolation step.

- To investigate the impact of the monomer standard chosen (MCP uses epicatechin equivalents and A-H used catechin), the authors established calibration curves using both standards. The results indicate that there is minimal difference in the slope and intercept of the curves, so it can be concluded that the choice of standard does not account for the significant differences in tannin concentration found in the two assays. The authors then examined the possibility that there may be complex interactions between the chemicals used in the assays that might change the spectral properties of the phenolics. An increase in absorbance at 280nm was observed with the addition of ammonium sulfate and methyl cellulose, but it was not sufficient to explain the large differences shown. It was also shown that BSA had no effect on the absorbance reading of the ferric-tannin complex and, so, did not influence the tannin quantification. So this did not explain the difference either.

- These observations led the researchers to conclude that the difference in reported tannin concentration between the MCP and the A-H assays is not primarily caused by a difference in isolation of tannin material, but rather is a function of the different detection methods used. This work gives guidance to anyone who might want to know how the values between these two assays relate, but a conclusive explanation remains unclear, so further work in this area is required.

- **Correlation of analytical techniques with astringency perception:** The authors finally investigated the correlation of both assays with wine astringency ratings. In another study, the A-H assay was shown to have the highest correlation with wine astringency ($r^2$=0.82) of the methods tested. In this study, they focused on MCP, since it hadn't been studied before. The descriptors were quantified in triplicate. Because the three astringency descriptors used (surface texture, adhesiveness and drying) were so highly correlated, only results for "drying" attribute were reported. Linear regression analyses show a strong correlation between MCP tannin assay values and the "drying" character ($r^2$=0.83). Interestingly, with these wines an even stronger correlation with astringency was observed using the A-H assay ($r^2$=0.90) than had been reported in earlier publications. Thus, these authors concluded that the MCP assay "models wine astringency with reasonable confidence. The simplicity and efficiency of this assay, coupled with its ability to objectively predict wine astringency, could prove very useful for both researchers and wine industry practitioners."

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