A comparison between two tannin precipitation methods – BSA and MCP (Part 4)

Keywords: BSA, MCP.



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Introduction

Tannins are classified as flavonoids and non-flavonoids. Non-flavonoids consist of gallic acid, ellagic acid, hydroxycinnamic acid and hydroxybenzoic acid and their derivatives. Flavonoids consist of flavonols, flavan-3-ols, flavan-3,4-ols and anthocyanins. Tannins are also known as the phenolic composition of the wine, total polyphenols or wine phenols.

A wide variety of methods may be used to analyse wine for phenols. These methods range from simple to complex. There are three principal methods to analyse for phenols or tannins. These methods are the colorimetric, gravimetric and precipitation assays. As early as 1912, Otto Folin and W. Denis started developing a method to analyse the total phenols of wine. In 1927, Otto Folin and Vintila Ciocaltue adapted the original Folin-Denis to improve analysis of total phenols. To this day it is known as the Folin-Ciocaltue method. It is a long method that takes almost three hours and is read at 765 nm on a spectrophotometer.

In 1978, Californians Ann Hagerman and Larry Butler developed a precipitation method, whereby a protein is used to bind to tannins. Hagerman and Butler used bovine serum albumin as a protein and the method is therefore known as the BSA method. James Harbertson adapted this method in 2003, incorporating a bleaching with bisulphite. The reason for this adaptation was that mono pigments (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) could also be analysed. BSA is a more indirect method of establishing tannin, seeing that it is read at 510 nm on a spectrophotometer. Three years later, in 2006, Sarneckis and his colleagues developed a precipitation method in Australia. They used a polysaccharide, methyl cellulose, to bind to tannins. This method, a more direct option since it reads at 280 nm, is known as the methyl cellulose precipitable (MCP) method.

Material and methods

Three criteria were used to compare the two methods with each other, namely ease of practice, repeatability and time efficiency. The evaluation was done on six wine samples that were analysed by hand and each wine was tested in triplicate. The evaluation made use of the complete BSA method. Two cultivars, Cabernet Sauvignon and Shiraz, were harvested on two farms (Plaisir de Merle and Morgenster).

Results

In the course of this study it was found that the tannin extraction, as measured by the BSA and MCP methods, followed the same trend (data not shown). The big difference between these two methods is the concentration, measured as g/L epicatechin (MCP) or catechin (BSA) equivalents. The BSA method was found to be approximately 4.5 times lower than the MCP method.

The correlation between the BSA and MCP was also found to be very good (R^2 = 0.88), whereas Seddon and Downey (2008) found their correlation to be very poor (R^2 = 0.41). Seddon and Downey found that the two methods did not precipitate the same amount and type of tannins (Seddon & Downey, 2008 and Harbertson & Downey, 2009). The correlation obtained by Seddon and Downey (2008) between the BSA and HPLC (R^2 = 0.28) and the MCP and HPLC (R^2 = 0.32) was very poor. A possible reason for this poor correlation is that the standards for the HPLC do not include dimers and poly-mers. These types of tannins are consequently not quantified and may result in a misconception about tannin concentrations. Sarneckis *et al.* (2006) found the contrary and obtained a good correlation between MCP and the HPLC (R^2 = 0.74). On the other hand Seddon and Downey (2008) found a poor correlation between MCP and HPLC (R^2 = 0.25), but a good correlation between BSA and HPLC (R^2 = 0.91).

Ease of practice regarding the execution and time required for analysis

The MCP method is a relatively simple method to use as there are only four steps with a total waiting time of 15 minutes. The MCP takes approximately 45 minutes to analyse six wine samples. The BSA is a more complicated method. There are approximately 15 steps, with a total waiting period of approximately 60 minutes. The entire BSA method takes approximately 90 minutes to conduct.

The reason why the BSA takes so long is that the method analyses not only total tannins, but also mono pigments (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP). If an analysis of MP, SPP and LPP is not required, these steps may be eliminated. The process will thus be shortened. Because the BSA method entails so many steps, different reagents are required and different amounts of these reagents have to be added. The more steps in a process, the bigger is the risk that something can go wrong.

Repeatability

As mentioned above, each wine sample was analysed in triplicate.

TABLE 1. Table showing the repeatability of the two precipitation methods.

	BSA	MCP
Stdev	3 mg/L	86 mg/L
CV%	1.5 - 3.6%	1.9 - 2.1%

An average for each of the wine samples was taken and a standard error obtained.

According to Table 1 the average standard deviation (stdev) is 3 mg/L for the BSA and 86 mg/L for the MCP. Furthermore it is clear that the average coefficient of variation, expressed as a percentage of the average (CV%), is 1.5 - 3.6% for the BSA and 1.9 - 2.1% for the MCP. The two methods are therefor equally repeatable.

As in each study there will be contradictions. This is indeed the case with regard to the two precipitation methods. Harbertson *et al.* (2008) analysed 1 325 red wines using the BSA assay. The cultivars being used were Cabernet Sauvignon, Merlot, Shiraz and Zinfandel, obtained from countries such as the United Kingdom, Australia and France. Harbertson found the standard deviation to be at least half of the average tannin concentration. In another study Brooks *et al.* (2008) found large variations between the tannin concentrations of the same wine sample analysed by different laboratories. Each wine sample was sent to five different laboratories and the tannin concentrations determined according to the BSA assay. The CV% among the laboratories was 27% and Brooks and his collaborators found that the BSA assay was not very repeatable.

Conclusion

There is ample scope for further research. The correlation between the methods and the HPLC may be further investigated. If the standardisation of the HPLC does result in a misrepresentation of tannin concentration, the use of polymeric standards should be investigated. The repeatability of a method also depends on the laboratory and the person doing the analysis. In view of the inconsistency between the two precipitation methods, it would be better to standardise using one method.

Acknowledgement

Winetech for funding the study and Distell for the infrastructure. Thanks also to the personnel at the Distell experiment cellar and the respective farms who sacrificed their grapes.

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Summary

The two most use methods for tannin analyses in the wine industry are the BSA (bovine serum albumin) and the MCP (methyl cellulose precipitable) assays. It was found that there was a 4.5 times difference in concentration levels between these two methods. These methods were compared for ease of practice, time efficiency and repeatability. This study proves that the MCP is easier to do (45 minutes) than the BSA (90 minutes). BSA can analyse not only for tannins but also for monopigments (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP). It was also found that the repeatability of the two methods was very good, but there are discrepancies in other studies.

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