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Title: **"Phenolics: A comparison of diverse analytical methods"**

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Russian, South African, New Zealand and California researchers come together in this article to clarify which phenolic compounds is it exactly that we are measuring when using the different analytical methods available today. A rather technical paper –so you might want to skip this one-, but a very valuable comparison to have as a reference.

• In the first part of the paper, the authors present a very useful overview on how the different types of phenolics tend to be measured and how the different methods work:

1) **Total phenols:** the most common method is the *Folic-Ciocalteau assay* (Singleton, 1999). Recently, a method called *cyclic voltammetry* has been used to measure wine **total phenols** based on their redox potential. Two other modern methods able to measure total phenols based on their antioxidant activity are: *Free radical scavenging*, and *Lipid peroxidation*.

2) **Monomeric phenols:** generally measured using *reversed-phase high performance liquid chromatography* (RP-HPLC).

3) **Flavanols:** normally involve reactions with aldehydic reagents, followed by a spectrophotometric reading. (Because of the way the most-common used reagent reacts with the A-ring of phenols, proanthocyanidins are included in this measurement, but anthocyanins and flav**o**nols, with slightly different structures, are not.)

4) **Proanthocyanidins:** can be measured by cleavage with a specific reagent into their subunits; then using the method for monomeric phenols above.

5) **Tannins:** can be measured by protein precipitation. Later, this method was coupled to the use of bisulfite bleaching, giving rise to the *UC Davis protein precipitation assay*, also known as the Harbertson-Adams assay, which measures **anthocyanins, tannins, small polymeric pigments and large polymeric pigments** (Harbertson, 2002).

6) **Polymeric phenols:** can be measured, besides with protein precipitation, using *normal-phase high pressure liquid chromatography* (NP-HPLC). A later improvement of this method is able to divide polymeric phenols into **low-** and **high- molecular-weight polymers** (LMWP and HMWP, respectively). When this method is coupled to 520 nm absorbance, a total of 4 fractions can be resolved: **low-** and **high- molecular-weight colored polymers** (LMWCP and HMWCP), and **low-** and **high-molecular-weight non-colored polymers** (LMWP and HMWP).

• In case you were wondering, yes, there is some overlapping in the fractions above and in what each method measures; this is unavoidable.

• In the second part of the paper, the authors concentrate in their goal: to study the phenolic content of a variety of California white and red wines using these different methods, and to determine the relationships between them. The established methods (Folin-Ciocalteau, RP-HPLC, NP-HPLC, and DAC) were compared to the new methods (protein precipitation, cyclic voltammetry, and antioxidant assays).

• To keep this summary brief, please refer to the original paper to learn about how each assay was conducted, or the nature of the wines used for each. In fact, I will also skip part of the results, and jump right into the comparisons between methods.

• **Comparison of assays measuring total phenols.** Even though the *absolute numbers* differed, a reasonably good correlation was obtained when comparing total phenols by Folin-Ciocalteau, the most widely used method, with the RP-HPLC method and the cyclic voltammetry method. The correlation was better for the RP-HPLC than for the cyclic voltammetry, and stronger in reds than in whites. The Folin-Ciocalteau method has the advantages of being highly reproducible, and its basis is well understood. The drawback is that it is difficult to compare samples with very different phenolic composition. The same problem exists when using UV absorption to measure total phenols, as individual phenols differ greatly in absorbance maxima. As for cyclic voltammetry, the main disadvantage is that it does not normally measure anthocyanins (in red wines), nor most of the phenols present in white wines (that would require using very high potentials, which is complicated).

• **Comparison of assays measuring monomeric phenols.** The *flavanol* content in red wines measured using DAC (white wines had very little) correlated well with the values obtained using RP-HPLC or with those using NP-HPLC. Each of the three methods had their pro's and con's. The major disadvantage with DAC and NP-HPLC is that flavanols are not being measured exclusively: DAC measures terminal flavanols in proathocyanidins, whereas the "monomer" peak in NP-HPLC includes also monomers that are not flavanols. As for RP-HPLC, it has a hard time measuring catechin and epicatechin due to coelution with other "stuff".

• As for *anthocyanins*, the authors compare measurements using RP-HPLC and the "colored monomers" by NP-HPLC. The correlation between both was very good. Thus, the authors were able to confirm the assumption that most of the colored monomer content in wines is anthocyanins. Additionally, the very high correlation between total anthocyanins and malvidin-3-glucoside demonstrates that malvidin-3 glucoside is an excellent marker of relative anthocyanin content in wines. The advantage of RP-HPLC over NP-HPLC is that individual anthocyanins can be quantified, whereas with NP-HPLC the individual anthocyanin "peaks" are not separated (which often times is enough for our purposes).

• **Comparison of assays measuring polymeric phenols.** *Tannins* were measured in red wines (white wines had very little) using the protein precipitation assay and NP-HPLC. Tannin measured with the protein precipitation assay correlated well with both the HMWP (high molecular-weight polymer) and the total polymer using NP-HPLC. Given that tannin content by protein precipitation correlated better with HMWP than with total polymers, the authors concluded that LMWP (the low molecular-weight pigment portion of total polymers) is probably not measured using the standard protein precipitation tannin assay (as previously suggested by Dr. Adams).

• As for *polymeric pigments*, both SPP (small polymeric pigment) and LPP (large polymeric pigment), as well as their sum (total polymeric pigment by the tannin protein precipitation assay), all correlated well with HMWCP, but not with LMWCP. Thus, it seems that both SPP and LPP in the protein precipitation assay are measured as HMWCP in the NP-HPLC method. NP-HPLC is the most common method for polymeric quantification, as polymers of different sizes can be separated. But the protein

precipitation tannin assay is rapid and inexpensive (and preferable, according to the authors, if only an estimation of total polymer is needed).

In conclusion, because phenolic substances are so complex, the complete characterization of the phenolic content of a wine is still not possible, and many compounds -particularly those in the polymeric fraction- are still unidentified. Considering this complexity, the authors believe it is not practical to seek a single best assay. Choosing an appropriate assay depends on what information is required and how much the user is willing to invest to generate the data . Depending on the needs of an experiment, a combination of assays is often the best approach.

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