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"Evaluation of a comprehensive red wine phenolics assay using a microplate reader"

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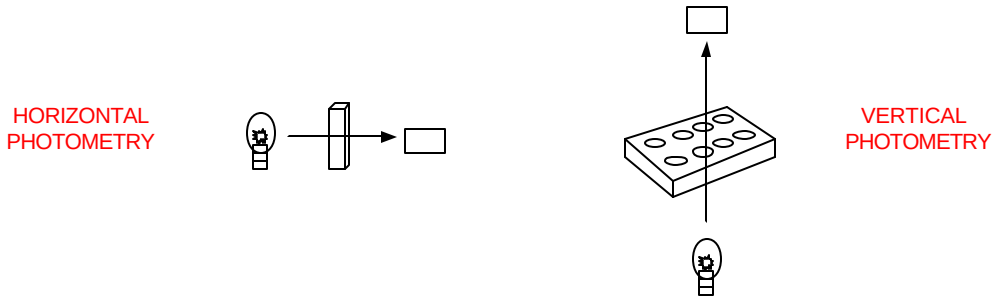
In this article the authors introduce and validate a method that considerably speeds up the analysis of complex phenolic compounds in a winery laboratory.

• The featured winery, Joseph Phelps Vineyards, uses the UC Davis protein precipitation assay (developed by Drs Harbertson and Adams) to measure tannin, iron-reactive phenolics, anthocyanins, and polymeric pigments in their red wines. But, although they very pleased with the assay's strong results, they found it to be somewhat time-consuming (2-3 hours for 12 samples) for use with many samples. So, in 2004 they introduced a microplate reader to speed up the process. A 96-well microplate reader uses the same spectrophotometric principle as the traditional method, but allows for a three-fold increase in throughput (2-3 hours for 36 samples). As a bonus, labor, reagent costs, and the numbers of disposable tubes and cuvettes needed are all significantly reduced. In this article, the authors analyze 40 wines representing a wide range of phenolic composition to evaluate how well the readings obtained with the microplate reader compare with the traditional readings using a spectrophotometer.

• **Transitioning to microplates.** Because calculating a concentration from an absorbance reading requires that the pathlength of the absorbing material be known, it was critical to calibrate the pathlength in the microplate so that it would mimic that in the traditional method. In horizontal photometry, as performed in spectrophotometers, this is determined by the physical dimensions of the cuvette, normally 1cm. In vertical photometry, as performed by microplate readers, the pathlength is dependent on the volume in the well, so adjustments need to be made.



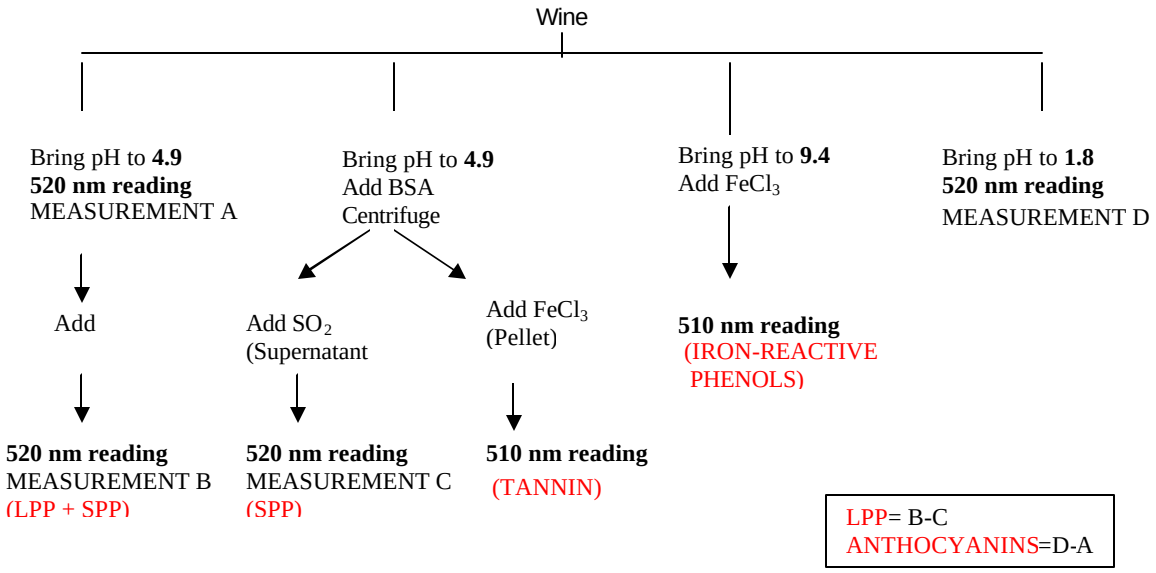
Even though, with the microplate method, most of the incubations have to be performed in a tube and later transferred to the microplate for reading (the wells being too small for adequate mixing), the rest of the protocol is identical to that of the original assay, simply scaled down to fit in the well.

• For a review of the determination of the 4 classes of phenols (polymeric pigments, tannin, iron-reactive phenolics, and anthocyanins), see *Summary 4*. Briefly, there are 4 major absorbance measurements in the whole assay: A, B, C, D. "B" measures polymeric pigments. "C" measures **small polymeric pigments. "**B minus C" measures **large polymeric pigments.** And "D minus A" measures **anthocyanins**. **Tannin** and **iron-reactive phenols** are read directly after subtracting the background absorbance.

• **Validation of the microplate reader**. Validation was established by calculating the instrumental difference between the microplate and the spectrophotometer for each class of phenol and for each wine. The average difference ranged from 0.5 to 6.7%, which is within acceptable range. The least discrepancy was for anthocyanins (1-4% error), and the greatest discrepancy was for small and large polymeric pigments (6.7%). Incidentally, the authors found that elimination of the small and large polymeric pigment steps of the assay is a choice that yields significant time savings and allows a greater number of samples to be assayed.

• **Reproducibility of the microplate reader.** The authors tested the reproducibility of the assay for each instrument by calculating range, standard deviation, and percent coefficient of variation for each phenolic class on a Merlot sample replicated 10 times. The values collected with the microplate reader were at least as precise as those collected using the spectrophotometer.

In conclusion, the authors validated the use of a 96-well microplate reader for its positive correlation to the U C Davis tannin assay. The efficiency of the assay is such (36 samples in 2-3 hours) that all fermentations at their winery could be monitored for phenolic development during the critical, hectic maceration period. As the authors point out, this tool will allow winemakers to make quick and reliable decisions about fermentations, pressing, and blending on the same day the samples are submitted to the lab. For more information on the actual assay and the solutions and reagents required, see Table 1 in the original text or at Dr Doug Adams' website: http://wineserver.ucdavis.edu/adams/tannin/totalassay.pdf



From Dr. Doug Adams, modified

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