

## FOOD COMPOSITION AND ADDITIVES

# Adams-Harbertson Protein Precipitation-Based Wine Tannin Method Found Invalid

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**The poor precision of the Adams-Harbertson wine tannin assay which was proposed for commercial winemaking, thereby creating the real possibility of quality control problems, is documented. The method is a version of the Hagerman and Butler protein precipitation-based tannin method. An extensive invalidation of the assay results with luxury wine data shows that the assay cannot distinguish bottled wine with reasonable accuracy. Five laboratories used Adams-Harbertson to assay 9 replicates each, of 3 bottled wines ( $n = 135$ ) found in California supermarkets, with tannin concentrations of nominally 500 and 1000 ppm by high-performance liquid chromatography (HPLC). Reliability exceeded the  $\pm 5\%$  industry requirement by nominally 5 times (z-score based on 5% distribution). Coefficient of variation was  $\pm 27\%$ , making the standard deviation range 54% for Pinot Noir, 34% for Merlot, and 44% for Cabernet Sauvignon. Validity exceeded the 100% requirement. Intralaboratory validity recovery was 55–63%. Interwinery validity was 71–178% of the mean for Pinot Noir, 81–144% for Merlot, and 83–164% for Cabernet Sauvignon. Range as a function of the mean was 89% for Pinot Noir, 55% for Merlot, and 67% for Cabernet Sauvignon. Expect intermethod validity to be nominally 50%, i.e., percent recovery to HPLC. These statistically significant errors were predicted by the literature. First-order error is related to the tannin-protein equilibrium constant ( $K_a$ ), as suggested by the original author, Hagerman, and the protein equivalence point error as suggested by Silber. This does not obviate second-order errors for tannin-protein analytical chemistry. Winemakers using the measurements risk making wines that are relatively more tannic than the measurements report.**

There are widespread problems with the Hagerman and Butler (1–3) tannin assay, which has been repeatedly questioned (4), including by Hagerman (2, 3). There is a danger of using protein precipitation-based assays for commercial winemaking without validation, because they can give incorrect tannin measurements that affect taste quality scores (5). Winemakers are motivated to include tannin in quality control because economists have noted that taste quality scores are the primary marketing information for luxury wine, the most profitable sector of the California wine market (6). Tannin concentrations have also been correlated with perceived astringency using previously published analytical methods by Kennedy and Jones (7), who correlated astringency with protein precipitation ( $R^2 = 0.82$ ) and high-performance liquid chromatographic (HPLC;  $R^2 = 0.74$ ) measurements. Moreover, the reported results showed protein precipitation measurements were 18–38% lower than HPLC measurements (7).

Winemakers see a tremendous competition between commercial and public laboratories to develop an industry standard method, since tannin concentration was first used in quality control by a commercial laboratory (8). An industry standard can be created by using either proprietary or freely available analytical technologies. For an analytical method to function as a standard, whether open source or proprietary, the single most important functional requirement is precision. The precision of a method is collectively defined by 2 statistics: reliability, percent relative standard deviation (RSD), and validity (% recovery) against a widely used benchmark.

The Hagerman and Butler protein precipitation-based tannin assay and variants have not been validated in the literature. Analytical errors exist in the original method adapted to wine (4). Martin and Martin first noted that there were errors related to the equilibrium constant ( $K_a$ ) of protein-tannin reactions; tannin measurements were a function of both protein and tannin concentrations (9). Because the protein-tannin precipitation reaction is not stoichiometric, the amount of protein necessary to precipitate 100% of the tannin from each wine sample is not linear. Hagerman and Klucher (10) confirmed Martin and Martin's results. They improved the original method by defining the

**Table 1. Adams-Harbertson interwinery validity for bottled Pinot Noir**

Pinot Noir	Commercial	Winery S	Winery J	Winery T	Winery R	Consortium
<i>n</i>	9	9	9	9	9	45
Mean	262	412	352	362	202	318
Minimum	218	353	308	298	186	186
Maximum	326	468	384	413	218	468
Range	108	115	76	115	32	282
Standard deviation	37	39	29	45	11	84
Coefficient of variation, %	14.1	9.6	8.3	12.5	5.4	27
Validity (recovery, %)	100	157	134	138	77	121
Low, %	83	134	118	113	71	71
High, %	124	178	146	157	83	178

protein equivalence point (PEP; 4, 10). Hagerman and Robbins (11) warned that the original Hagerman and Butler method failed to detect tannin in some plant samples altogether. Silber et al. (12) proposed a mathematical solution for tannin-protein precipitation assays for wine, including the Adams-Harbertson assay. In 2006, Silber and Fellman (4) exposed the analytical chemistry problems inherent in the original Hagerman and Butler assay for wine, saying “the accuracy and precision of these protocols have been repeatedly questioned over the years.” J.K. Fellman (personal communication, 2007) confirmed this result for wine: “Importantly, the method of Martin and Martin provided a high frequency of negligible results (35.9%) in white and diluted wines.” Despite these concerns, a variant of the original Hagerman and Butler precipitation assay protocol was recently promoted by Adams and Harbertson as a potential industry standard technique to compete with commercial HPLC measurements at the Recent Advances in Enology and Viticulture (RAVE) conference on March 22, 2007, at the University of California, Davis (13).

Enologists will attempt to use the precipitation-based techniques, most of which are derived from Hagerman and Butler’s procedure published in the *Journal of Agricultural Food Chemistry*, with the assumption that they are precise. The negative results noted by Silber and Fellman have not been emphasized by Adams and Harbertson in wine industry periodicals. This may explain why Adams and colleagues have received significant attention in the California wine industry (13).

The question among analytical chemists is whether the Adams-Harbertson (14–17) or Silber et al. (4, 12) methods can be independently validated for wine. The question among winemakers is, Which tannin assay can safely be used to manage bottled wine tannin concentrations? Analytical methods are typically validated by the regulatory agencies using AOAC guidelines. The Adams-Harbertson assay has not been validated by any independent organization using procedures approved by AOAC. Further, the American Society for Enology and Viticulture Technical Projects Committee did not validate this method before it was

**Table 2. Adams-Harbertson interwinery validity for bottled Merlot**

Merlot	Commercial	Winery S	Winery J	Winery T	Winery R	Consortium
<i>n</i>	9	9	9	9	9	45
Mean	479	649	616	602	427	554
Minimum	429	607	586	526	386	386
Maximum	533	689	670	677	452	689
Range	104	82	84	151	66	302
Standard deviation	40	27	26	49	19	96
Coefficient of variation, %	8.4	4.2	4.3	8.1	4.5	17
Validity (recovery, %)	100	136	129	126	89	116
Low, %	90	127	122	110	81	81
High, %	111	144	140	141	94	144

**Table 3. Adams-Harbertson interwinery validity for bottled Cabernet Sauvignon**

Cabernet Sauvignon	Commercial	Winery S	Winery J	Winery T	Winery R	Consortium
<i>n</i>	9	9	9	9	9	45
Mean	453	697	578	614	395	547
Minimum	403	652	488	600	377	377
Maximum	527	745	601	680	421	745
Range	124	93	112	81	44	368
Standard deviation	50	25	34	26	14	122
Coefficient of variation, %	11.1	3.6	5.9	4.2	3.6	22.3
Validity (recovery, %)	100	154	127	136	87	121
Low, %	89	144	108	132	83	83
High, %	116	164	132	150	93	164

presented to RAVE attendees (Sarita Cardozo, Alcohol and Tobacco Tax and Trade Bureau, personal communication, December 2007). Winemakers attending RAVE 2007 embarked on a collaborative study of the Adams-Harbertson tannin assay.

## METHOD

In order to determine precision of the Adams-Harbertson tannin assay, an official protocol was adopted. The organization of the study followed AOAC recommendations for method validation. The procedure for validating analytical methods can be found at the official Web site, <http://www.aoac.org>.

### Samples

A General Referee organized testing of 9 replicate samples of 3 bottled wines. Identical test kits were created for each laboratory involved to ensure that samples were assayed blind. The test kits comprised 9 replicate samples each of a 2005 Sonoma County Pinot Noir, a 2004 Sonoma County Merlot, and a 2004 Sonoma Valley Cabernet Sauvignon for a total of 27 samples. Bottled wines chosen were purchased from a local grocery store and are widely available in the marketplace. Samples were sent to each laboratory blind and were labeled with a unique alphanumeric code that could be discerned only by the Referee. Sample analysis was conducted by trained analysts from 4 independent enological laboratories within wineries and one commercial testing laboratory offering the Adams-Harbertson tannin assay for sale. The commercial laboratory served to measure intralaboratory precision by performing the analysis on the same wines on 2 different occasions.

### Analytical Protocol

Participants received clearly and unambiguously written instructions detailing the design of the study, the testing protocol, and the reporting forms (MS Excel format). Test kits,

bottled wine samples, and documentation developed by the General Referee were forwarded to the independent testing laboratory. Each participant assayed the blind commercial bottled wines to determine the statistical precision.

Briefly, the Adams-Harbertson assay was performed as directed by the method and was obtained from the Douglas Adams page on the University of California, Davis Web site (18). Tannins were precipitated from wines by adding 1 g/L bovine serum albumin (BSA) solution, followed by centrifugation. The precipitate was collected and resuspended in an alkaline triethanolamine/sodium dodecyl sulfate buffer, measured for background absorbance at 510 nm, and measured for tannin absorbance after the addition of FeCl<sub>3</sub> solution. Each winery ran 9 blind replicate samples of each wine and submitted results to the Referee. The Referee then reported the values in Tables 1–4. For quantitation, the results were standardized to catechin and reported as mg/L catechin equivalents.

### Statistical Procedures

Precision of the method is defined by both reliability (defined as percent RSD) and validity (% recovery) against a benchmark over time. Reliability was determined by calculating the coefficient of variation (CV) as shown in Equation 1. Validity was calculated by (1) Normalizing individual winery results to commercial laboratory results, and (2) normalizing commercial laboratory measurements to a second analysis by the same commercial laboratory. Validity is expressed as % recovery, as shown in Equation 2a and b.

$$CV = (SD/mean) \times 100 \quad (1)$$

$$\text{Recovery, \%} = \frac{\text{winery laboratory result}}{\text{commercial laboratory result}} \times 100 \quad (2a)$$

$$\text{Recovery, \%} = \frac{\text{first commercial laboratory result}}{\text{second commercial laboratory result}} \times 100 \quad (2b)$$

**Table 4. Adams-Harbertson validity for commercial analytical laboratory**

Cabernet Sauvignon	Pinot Noir No. 1	Pinot Noir No. 2	Merlot No. 1	Merlot No. 2	Cabernet No. 1	Cabernet No. 2
<i>n</i>	9	4	9	4	9	4
Mean	262	414	479	854	453	828
Minimum	218	404	429	791	403	810
Maximum	326	429	533	887	527	851
Range	108	25	104	96	124	41
Standard deviation	37	12	40	43	50	19
Coefficient of variation, %	14.1	2.8	8.4	5.1	11.1	2.3
Validity (recovery, %)	63	100	56	100	55	100
Low, %	83	98	90	93	89	98
High, %	124	104	111	104	116	105

## Results and Discussion

The California wine industry commercial laboratories' generally accepted standard for precision is  $100 \pm 5\%$ , largely to create interoperability between analysts, and to support future label approval by regulatory agencies such as the Alcohol and Tobacco Tax and Trade Bureau (Sarita Cardozo, personal communication, 2007). The Adams-Harbertson protein precipitation-based tannin assay, as performed in this interlaboratory comparison did not meet these criteria, as it produced measurements with a nominal interchangeability of  $55 \pm 27\%$  between commercial and winery laboratories. Reliance upon this measurement for commercial winemaking risks production of defective wines. The results were nominally one-half of HPLC measurements, just as previously reported (5, 7).

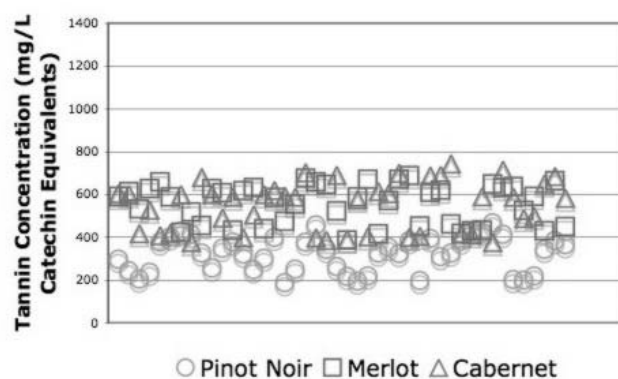
The measurements of low and high tannin concentration in bottled wine samples overlapped each other (Figure 1). Three wines with a nominal 2-fold difference in tannin concentration (500–1000 ppm by HPLC) could not be distinguished from each other by a single measurement within 5% of the mean. No one has demonstrated that the Adams-Harbertson method meets this precision requirement in any systematic study reported in the literature.

A mathematical model exists for errors in protein precipitation-based methods (12), which clearly explains quantitatively the dependence of tannin–protein complex precipitation upon the protein concentration. When tannin–BSA complexes fall below the level necessary for large aggregate formation, i.e., 100% precipitation, further addition of protein molecules to the solution reduces the average number of tannin bonds to each protein, causing the precipitate to redissolve. This model predicts that tannin concentration affects the tannin measurements when the protein-to-tannin ratio is  $>1$ . The problem is that protein-tannin precipitation reactions are not stoichiometric. This study confirms the model, showing poor recovery for the range of tannin found in bottled California wines.

The mean interlaboratory measurements ranged from 71 to 178% of those produced by the leading commercial provider of Adams-Harbertson measurements, a difference of over 2-fold between analysts (Tables 1–3). Table 4 shows that intralaboratory measurements ranged from 53 to 64%, a difference of 2-fold within one company.

*Wine quality control.*—Measurements did not distinguish between grape varieties, rendering the method unacceptable to winemakers. Pinot Noir minimum 186 and maximum 468 ppm overlapped with Merlot minimum 386 and maximum 689 ppm, and with Cabernet Sauvignon minimum 377 and maximum 745 ppm.

*Recommended analytical chemistry.*—Measurements can be improved by including assessment of the protein-precipitating capacity of each sample with simultaneous determination of the PEP to improve sensitivity, accuracy, and consistency of measurements (4; J.K. Fellman, personal communication, 2007). First, determine PEP with a trial to measure protein precipitated by each wine sample. Next, produce the measurement at the PEP value of 1.0, i.e., 100% precipitation. Produce the measurements by including



**Figure 1. Scatter plot for Adams-Harbertson tannin assay of replicate bottled wine samples (3 varieties).**

the model determined by Silber et al. (12) with the Beer-Lambert equation. In our opinion, the fastest solution is to use the methods recommended by Silber and Fellman (4).

*Caveat.*—There may be other problems with the method beyond the primary order physical chemistry reaction, Ka-based PEP problem found by Silber et al. (12), that does not obviate second-order thermodynamics, e.g., heat, which may be masked by the larger problems.

## Conclusions

It is premature to use Adams-Harbertson as a tool to track tannin concentration in commercial winemaking. Precision is the central issue in the growing debate over the use of the Adams-Harbertson protein precipitation-based methods for winery quality control. Winemakers' ability to track changes in measurements in wine manufacturing is determined by reliability. The ability to characterize relationships between wines and the bottled wine markets is a function of validity. This study demonstrates that Adams-Harbertson measurements do not meet acceptable levels of precision and recovery. There is a real danger of making wines that are more tannic than the measurements report, thereby possibly reducing the market value of wines that will then exceed the benchmarks by which consumer critics judge wines.

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