The Great Hundredths Hoax

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Analytical methods for the analysis of grape juice, must, and wine have developed significantly over the past forty years. Early wet chemical methods have improved through control or elimination of the interfering components in these biological samples, and many new procedures have been described. Enzymes have been incorporated into tests to selectively react with desired components; and instrumentation such as HPLC (High Performance Liquid Chromatography), Capillary Electrophoresis and FTIR Spectroscopy (Fourier Transform Infra Red) have been applied to solving complex sample matrix problems.

Despite these improvements, there is still a great deal of confusion and frustration among winemakers at all levels of winemaking proficiency as to what comprises a satisfactory analysis. The reason for this situation is what I call **"the great hundredths hoax."**

The hundredths hoax has not been perpetrated, but rather has evolved as a result of the large amount of research conducted on improving the quality of grapes and the quality of the resultant wine. The hoax has two major components: ***need*** and ***delivery***.

Let's talk first about delivery. I'll define delivery as the accuracy and precision you can actually expect when you perform an analysis for a component in a wine sample.

Let's look first at one popular method, the enzymatic analysis of malic acid. From the directional insert for one malic acid kit offered by a large commercial firm, we can learn that the precision,[a] or repeatability, of their method is ± 1.8% when testing a wine sample with an average result of 2.97 g of malic acid/Liter. That's really quite good, but we should note that the directional insert does not provide any information on the accuracy of this kit method, i.e., how close the 2.97 g/L is to the actual amount of malic acid in the sample.

How does this kit capability of ± 1.8% compare to actual results? In 1999 the California Enology Research Association sent out some samples of wines to a dozen winery laboratories for analysis.[19] When analyzing a blush wine with an average malic acid level of 2.98 g/L the laboratories achieved an overall precision (Coefficient of Variation) of 6.7%. That's pretty good considering the number of people and the number of pieces of equipment involved. How did these labs do when measuring another wine sample with an average malic acid level of 90 mg/L? Not so well! The CV was a whopping 54.8%. That means two thirds of the labs reported results from 43 mg/L up to 137 mg/L, but another third of the laboratories reported results from about 0 mg/L up to 174 mg/L. That's not very precise when you're trying to determine if malolactic fermentation is complete at around 30 mg/L, and are operating below the sensitivity limit for paper chromatography and thin layer chromatography.

Has performance gotten any better since this first study was conducted? For at least the past five years a committee of The American Society for Enology and Viticulture (ASEV) has been sending out specific wine samples to winery laboratories to check on the performance of their laboratories. About fifty laboratories participate in these trials, but these laboratories represent wineries with about 90% of the wine production in the United States. Presumably these are the larger wineries with full time laboratory staff and equipment suitable to the task and properly maintained. During the first six sample rounds, which took place in 1999 and 2000, samples with malic acid levels above 1.0 g/L were analyzed with precision levels ranging from 3.9% to 12.7%, about the same level of reasonable precision observed in the earlier study. It should be

added that laboratories reporting results significantly different form the consensus average were eliminated from the calculation of precision. If we look at the results for wine samples with malic acid levels below 400 mg/L, the reported precision ranged from 19% to 34%. This means that for a typical sample with an average value of 160 mg/L, laboratories reported results that ranged from 80 to 240 mg/L. And remember, all the labs knew which samples were part of the evaluation program, and all labs performed their analyses in duplicate.[20]

As part of the continuing ASEV program, during 2003 and 2004 eight samples were sent out with malic acid levels above 1.0 g/L. These samples were analyzed with precision levels ranging from 6.1% to 16.3%, a slightly poorer performance than with the earlier sample set, and with data from an average of four laboratories per set excluded from the precision calculation because of "extreme" variability in their data. For four samples with malic acid levels below 400 mg/L, the precision ranged from 26% to 35%. A typical set of data from 39 laboratories on a sample of Zinfandel wine with an average value of 165 mg/L, the reported results ranged from 67 mg/L all the way up to 277 mg/L. (Two of the excluded laboratories from this set reported results of just 24 mg/L and 25 mg/L. Wow!)[20]

[It's now another ten years later. Has laboratory performance improved? According to the recent results provided by the Collaborative Testing Services, the results are actually worse:

- ***A 2013 sample (44A) with a malic acid content of 163 mg/L had a coefficient of variability (CV) of 47%, with results as low as 20 and 40, and as high as 330 and 390;***
- ***A 2013 sample (44B) with a malic acid content of 187 mg/L had a CV of 44%, with results as low as 10, 10, and 55, and as high as 370 and 423;***
- ***A 2014 sample (47A)with a malic acid content of 152 mg/L had a CV of 41%, with results as low as 10, 20, 20, and 25, and as high as 270 and 323;***
- ***A 2014 sample (47B)with a malic acid content of 192 mg/L had a CV of 38%, with result as low as 10, 20, and 25, and results as high as 340 and 388;***
- ***A 2015 sample (50A)with a malic acid content of 165 mg/L had a CV of 46%, with results as low as 20, 60. And 75, and results as high as 268, 270, and 280;***
- ***A 2015 sample (50B)with a malic acid content of 146 mg/L had a CV of 37%, with results as low as 10, 40, and 55, and as high as 220, 225, and 263.***
- ***(The results reported above were AFTER 10% of the laboratories had their results excluded because the results were so erratic!)***

These results, still provided by the larger, more capable winery labs and run in duplicate, very often would lead to incorrect winemaking decisions by the winemaker, even though they were obtained using modern instrumentation and reported to two decimal places. Instrumentation can easily lead to precise, but precisely wrong, answers! Often simpler, but more reliable methods are better.] [23]

What are some of the causes for the variation in reported results versus kit "capability"? While a study would have to be done to identify these with certainty, a review of the directions and precautions in the directional insert for some enzymatic kits provides some clues.[22] Six different pipettings, some involving quantities as small as 10 microliters, have to be done for each assay. Samples with malic acid levels above 150 mg/L need to be diluted. Samples containing carbon dioxide should be degassed, for example, by filtration or by addition of sodium bicarbonate. Acidic samples, like wine, should be adjusted to pH 8 to 10. Colored samples, like wine, should be measured against a sample blank and/or treated with PVPP. Some samples may exhibit a creep reaction, so extrapolate the absorbance back to the time of initial addition of all the reagents. No guidance is given as to how colored a "colored sample" has to be before the listed adjustment(s) are made. No specific directions are given regarding whether or not to use solid chemicals for the adjustments or whether to use solutions. No instruction is given on whether to correct the assay answers for dilutions caused by sample pretreatments. Each lab has to determine the appropriate steps for its own samples and ensure that these adjustments are made reproducibly and consistently.

What about some other analytes? For volatile acidity, normal primary fermentation produces 200 – 400 mg/L of acetic acid. Bacterial contamination can produce more acetic acid, and we would like to know if the amount present is growing to a level which would make a wine unpalatable. The taste/odor threshold of acetic acid is about 800 mg/L in wine, so we'd like to know if a wine has a volatile acidity level in the 400 – 650 mg/L range in order for us to take corrective action before we can taste the acetic acid and have a very

expensive problem to correct. Looking at the ASEV program data, during 2003 and 2004 seven wine samples had volatile acidity levels in the 400 – 650 mg/L range. The data are listed below:[21]

Sample	Average	Range Reported
SA25	580	505 - 720
SA26	421	325 - 550
SA27	501	335 - 630
SA28	606	460 - 710
SA30	595	490 - 750
SA31	583	395 - 720
SA32	642	505 - 705

For those samples with an average value near the middle of our range of concern, 400 – 650 mg/L, the assays confirmed that the samples were above baseline levels, but did not provide information with confidence as to where the actual value was. For the two samples with average values of 421 and 501 mg/L, about half the results suggested no action was required, yet the average value suggested a corrective action was needed. The results obtained on these seven samples from the laboratories in the study suggest that the main method used, the Cash still method is useful for deciding if a wine is in trouble, but is not helpful for providing information as to whether a wine is just starting to show the effects of contamination by lactic acid bacteria, or if it is about to become a disaster.

Turning to Free SO_2, the most recent survey, report #018, described the analysis of two Chardonnay samples, which had average assay values of 17.9 ppm and 20.8 ppm, and precision values of 16.9% and 16.7%. The ranges for the results reported by the 50 participating laboratories were 13.0 – 25.5 ppm and 14.5 – 27.5 ppm, respectively.[21] The prior survey, report #017, described the analysis of two Zinfandel samples, with average assay values of 19.4 ppm and 8.5 ppm and precision values of 20.3% and 48.9%. The respective ranges were 8.0 – 28.0 ppm and 3.0 – 19.0 ppm.[21] These results are spread across a broad range, with the results for the red wines somewhat more variable than the results for the white wines. Technically these are not very good analytical results, but practically they generally give the right message. For the white wines, at the reported pH of 3.46 the Free SO_2 should be 23 – 37 ppm. For the red wines, at the reported pH of 3.52, the Free SO_2 level should be 26 – 42 ppm. Most of the individual assays suggest increasing the Free SO_2 levels measured in these four wine samples by 50 – 100%, and that would produce a wine with adequate microbial control. Thus, even with the wide variation reported, the correct conclusion can be drawn from the analytical results.

Let's look at the survey performance regarding pH measurement. Measurements were taken by each lab, in duplicate, using digital pH meters presumably calibrated against standard buffers. In the two most recent surveys, the ranges reported for four wine samples were 3.32 – 3.57, 3.32 – 3.62, 3.42 – 3.70, and 3.41 – 3.65.[21] The standard deviation averaged 0.034 pH units. The variation in the reported results suggests that just because a digital readout flashes a two or three decimal number, the answer may not be accurate to those two or three decimals. Temperature control, electrode cleanliness and conditioning, and buffer accuracy are all important in arriving at a pH result. From the reported data, this is a challenge for the working winery lab, but once again the requirements for winemaking decisions, ± 0.1 pH unit, can be routinely achieved with the pH meter, and with the ACCUVIN AV-pH test kit.

The other aspect of the hundredths hoax is need. Need has to do with relevance and with significance, with a significant number being defined as the number when reached that requires action. Are these numbers in the hundredths?

In the case of "acids" in wine, there are two key macro parameters: pH and Titratable Acidity. The pH, a measure of free hydrogen ions, is important for ensuring optimal fruit character at harvest, for optimizing both primary and malolactic fermentations, for optimizing color and color stability, and to a minor extent controlling astringency. It is also important to know the pH value in order to make the correct addition of sulfur dioxide, SO_2. In numerous publications on winemaking the optimum pH range for still white wines is given as 3.1 or 3.2– 3.4, and for still red wines as 3.3 – 3.5 or 3.6.[12-16] Notice that all these numbers are given to the nearest tenth of a pH unit. This matches the information provided to ACCUVIN in a survey of commercial winemakers from around the world prior to development of the AV-pH test kit. The participating ***winemakers***

reported that they made their winemaking decisions based on pH results reported to the nearest tenth of a pH unit as well. But what about the need for knowing the pH when calculating SO_2 additions? It's true that a 0.1 pH difference can result in a 20% difference in the calculated amount of free SO_2 needed, but remember that in the pH ranges normally encountered in wine this amounts to an actual difference of only about 6 - 8 ppm, an absolutely minimal difference with respect to microbial inhibition and with respect to taste.

Titratable Acidity levels are important for maintaining optimal varietal character and for achieving flavor balance. Winemaking resources describe a range of 6 – 8 g/L (as tartaric acid) for red table wines, and 7 – 9 g/L for white table wines.[12-15] To achieve these targets, a method must deliver results to ± 0.5 g/L.

There are other parameters in wine which also fall into similar control ranges or threshold management levels. For example, fermentation produces up to 0.4 g/L of volatile acidity, but this is not a worry until bacterial contamination or other adverse situation increases the VA level to the taste threshold of 0.6 g/L. [13, 16-18]

O.K. The information presented above seems straightforward, but don't many studies show assay values with a lot more decimal places? Let's look at the purposes of some of these studies. Typically these studies have a primary purpose other than providing guidance on how to make a quality wine. These include method comparison studies, where precise measurement is needed in order to evaluate the benefits of one method versus another.[1-5] Another type of study is designed to identify the impact variations in vineyard practices has on wine quality.[6-8] And a third major type of study is conducted for measuring the outcome of different winemaking practices.[9-11] In all of these studies precise measurement is required because the variations in outcome are usually small, and the improvements incremental. That's how wine science improves, but it's not how quality wine is routinely produced. Quality wine is produced by **measuring** key parameters, **monitoring** these parameters over the course of the winemaking process, and keeping them within broad ranges or above or below thresholds.

What does all this mean? Each laboratory generally agrees reasonably well with itself when running duplicate assays by a given method, and when running different samples with the same method. Obviously, there is a lot less agreement between laboratories using the same method, and even more variability when different methods are employed. In none of these data sets is a report of, for example, the true malic acid level in the submitted samples. My observations are:

A. Wine is a pretty nasty sample and test methods for wine are subject to a lot of technique and measurement variables, so we should consider oenological analyses as estimates rather than absolute measurements. Some methods in a controlled environment have better capability than others, but the bottom line to consider is what each method actually delivers in a working winery setting with the personnel available.
B. When considering a method, consider the range you will be operating in and what the significant decision level for that analyte is. For example, from the malic acid test data discussed above, the spectrophotometric method did fairly well with malic acid levels above 1 g/L, but the application of the method suffered at lower malic levels such as those found at or near the end of malolactic fermentation. A better choice for testing in the lower range might be the ACCUVIN Quick Tests, which are optimized for samples with lower levels.
C. Just because your method readout gives you an answer with a lot of decimal places, it doesn't mean that your answer is correct to those decimal places. Be aware of the variations reported in the interlaboratory studies, and assume that your method has just as much variation unless you have specifically done something to make your performance significantly better than that of the laboratories in the study. And if you have done something like that, publish an article and help out everyone else.
D. Check your units! An answer in *mg/L* is the same as the answer in *ppm*. To get *g/L*, divide *mg/L by 1000*. Be aware that sometimes results are reported in *g/100 mL*, which is equivalent to *mg/L divided by 100*. An answer in g*/100 mL* is numerically the same as the answer in *%*.
E. Winemaking is <u>not</u> an exact science. It requires information to help with winemaking decisions in order to achieve the winemaking objective of each winemaker. Most decisions in winemaking are based on ranges, not on specific assay values. For example, consider adjusting the pH of a red wine if it is not with the range of 3.3 – 3.6. It is not necessary to know if a wine is at a pH of 3.56 vs. 3.63. Similarly,

in the medical field, “normal” individuals have blood sugar levels of 70 – 109 mg/100 mL. A blood sugar above 126 mg/100 mL suggests diabetes. From 110 – 125 mg/L you have overlap. Similarly, below 60 mg/100 mL you have low blood sugar. The region 61 – 69 mg/100 mL is overlap.

F. Wine is not sold as a chemical solution. True, it is made of chemicals, but it is a food. It is important that this food be prepared within certain ranges according to the variety and style of wine desired, but it is not necessary, and may be undesirable, to produce a wine to an exact chemical formula. Many people take sugar in their coffee or tea. I know of no one who weighs the sugar so that there is no variation from cup to cup, yet almost all of these coffee drinkers are satisfied with their results.

G. Method to method comparisons outside of a research activity should be taken with a grain of salt. There are methods with known biases, and with known range and sensitivity limits. After these method characteristics are eliminated or corrected for, the issue to decide is whether or not a particular method reliably provides you with the information you need to manage your winemaking process the way you want to manage it.

H. Even with (some of) the variation in results, measurement and monitoring are good and allow us to make better the wines we want to make compared to if all we did was taste, smell, and guess.

Definitions:

a. Precision in this article is reported as CV, the coefficient of variation. The CV is a statistical term that covers the range within which 69% of measurements fall in a particular trial. For example, if the average is 100, and the CV is 8%, then 69% of the results will fall within the range from 92 to 108. Another 26% of the results, or 95% of all the results, will fall within the range 84 to 116. The last 5% of the results are spread out a little more, but statistically will fall within the range 76 to 124.

References:

1. B. H. Gump, S. Saguaneekul, G. Murray, J. Teresita Villar, “Determination of Malic Acid in Wines by Gas Chromatography,” *Am. J. Enol. Vitic.* 36: 248 – 251 **1985.**
2. A. Schneider, V. Gerbi, M. Redoglia, “A Rapid HPLC Method for Separation and Determination of Major Organic Acids in Grape Musts and Wines,” *Am. J. Enol. Vitic.* 36: 151 – 155 **1987.**
3. T. Kandl, S. Kupina, “An Improved Capillary Electrophoresis Procedure for the Determination of Organic Acids in Grape Juice and Wine,” *Am. J. Enol. Vitic.* 50: 155 – 161 **1999.**
4. L. Moio, A. Gambuti, L. di Marzio, P. Piombino, “Differential pH Meter Determination of Residual Sugars in Wine,” *Am. J. Enol. Vitic.* 53: 271 – 274 **2001.**
5. B. H. Gump, B. W. Zoecklein, K. C. Fugelsang, R. S Whiton, “Comparison of Analytical Methods for Prediction of Prefermentation Nutritional Status of Grape Juice,” *Am. J. Enol. Vitic.* 53: 325 – 329 **2002.**
6. J. R. Morris, C. A. Sims, D. L. Cawthon, “Effects of Excessive Potassium Levels on pH, Acidity, and Color of Fresh and Stored Grape Juice,” *Am. J. Enol. Vitic.* 34: 35 – 39 **1983.**
7. T. K. Wolf, B. W. Zoecklein, Performance of Alternative Winegrape Varieties in Virginia,” *Vineyard & Winery Mgmnt.,* 22: 49 – 53 **1996.**
8. A. G. Reynolds, D. A. Wardle, M. A. Cliff, M. King, “Impact of Training System and Vine Spacing on Vine Performance, Berry Composition, and Wine Sensory Attributes of Seyval and Chancellor,” *Am. J. Enol. Vitic.* 55: 84 – 95 **2004.**
9. C. S. Ough, V. L. Singleton, “Wine Quality Prediction form Juice Brix/Acid Ration and Associated Compositional Changes for ‘White Riesling’ and ‘Cabernet Sauvignon’,” *Am. J. Enol. Vitic.* 19: 129 - 138 **1968.**
10. M. Kudo, P. Vagnoli, L. F. Bisson, “Imbalance of pH and Potassium Concentration as a Cause of Stuck Fermentations,” *Am. J. Enol. Vitic.* 49: 295 - 301 **2004.**
11. G. Nicolini, R. Larcher, G. Versini, “Status of Yeast Assimilable Nitrogen in Italian Grape Musts and Effects of Variety, Ripening and Vintage,” *Vitis,* 43: 89 – 96 **2004.**
12. M. Dharmadhikari, “White Wine Production,” *Vineyard and Vintage View,* 14: **1999,** and “Red Wine Production,” *Vineyard and Vintage View,* 15: **2000.**
13. Y. Margalit, *Wine Chemistry,* Wine Appreciation Guild, South San Francisco, CA **1997**, pp. 298,
14. R. B. Boulton, V. L. Singleton, L. F. Bisson, R. E. Kunkee, *Principles and Practices of Winemaking,* Chapman & Hall, New York **1996**, pp. 54
15. P. Jackisch, *Modern Winemaking,* Cornell University Press, Ithaca, NY **1985**, pp. 103
16. C. S. Ough, M. A. Amerine, *Methods for Analysis of Musts and Wines,* John Wiley & Sons, New York, **1988**, pp.52

17. B. W. Zoecklein, K. C. Fugelsang, B. H. Gump, F. S. Nury, *Wine Analysis and Production*, Chapman and Hall, New York, **1995**. pp. 192 - 196.
18. E. Peynaud, *Knowing and Making Wine,* John Wiley and Sons, New York, **1984**. pp. 42 - 43.
19. C. E. Butzke, "2000/2001 Survey of Winery Laboratory Proficiency," *Am. J. Enol. Vitic.* 55: 163 - 169 2002.
20. Collaborative Testing Services, Inc., Sterling Virginia, 2003, available at http://www.collaborativetesting.com/wine/current_reports.html:
    Wine Industry Interlaboratory Survey Report #013, Spring 2003;
    Wine Industry Interlaboratory Survey Report #014, Summer 2003;
    Wine Industry Interlaboratory Survey Report #015, Fall 2003
21. Collaborative Testing Services, Inc., Sterling Virginia, 2004, available at http://www.collaborativetesting.com/wine/current_reports.html:
    Wine Industry Interlaboratory Survey Report #016, Spring 2004;
    Wine Industry Interlaboratory Survey Report #017, Summer 2004;
    Wine Industry Interlaboratory Survey Report #018, Fall 2004
22. Directional Inserts:
    i. L-Malic Acid, Kit 10 139 068 035, Boehringer Mannheim/R-Biopharm, Darmstadt, Germany
    ii. Enzytec™ L-Malic Acid, Scil Diagnostics GmbH, Martinsried, Germany.
    iii. L-Malic Acid, Kit K-LMALR, Megazyme International, Bray, Ireland
23. Collaborative Testing Services, Inc., Sterling Virginia, 2013, 2014, 2015, available at http://www.collaborativetesting.com/wine/current_reports.html:
    Wine Industry Interlaboratory Survey Report #044, Summer 2013;
    Wine Industry Interlaboratory Survey Report #047, Summer 2014;
    Wine Industry Interlaboratory Survey Report #050, Summer 2015