



## “Influence of intrinsic factors on conventional wine protein stability tests”

By: M. Sarmiento, J. Oliveira, M. Slatner, and R. Boulton

In: Food Control, 11:423-432, 2000

These authors study how well different protein stability tests work. And when they don't work, what factors in the wine might be interfering with the results.

- Protein precipitation may be due to internal or external changes, such as pH, ethanol content, storage temperature, and phenolic content. These changes may arise during storage or when different wines are blended together, changing the equilibrium. To avoid protein precipitation when it's least desired, such as in the bottled product, wineries perform stability tests to assess whether a wine may become protein-unstable.
- Most stability tests function by forcing the precipitation of proteins in some manner. This allows the user to estimate the amount of bentonite needed to avoid protein instability. The amount of bentonite required could be none, if the wine is already stable, or up to 2.5 g/l for the most unstable wines. Bentonite works by adsorbing to proteins, thus causing them to precipitate out of solution.
- Protein stability tests can be classified as: 1) total protein assays (Coomassie blue staining), 2) chemical denaturation (trichloroacetic acid precipitation), 3) heat denaturation (heat tests), and 4) decrease of solubility (ethanol precipitation and tannin precipitation).
- *Heat tests* are the most commonly used in winery laboratories. They do have several inherent problems, however. For instance, it is not known whether some proteins are more sensitive than others to heat, necessitating more than one heating regime on the same sample to establish stability. And another questionable issue is whether the result is the same when you precipitate the proteins quickly at high temperature (as happens in the standard heat test), or very slowly at low temperature (as happens in normal storage).
- *Ethanol precipitation tests* are based on the fact that increasing amounts of ethanol will decrease protein solubility, causing the proteins to precipitate out of solution as a haze. The least soluble protein fractions are expected to precipitate first.
- *Tannin precipitation tests* are based on the assumption that proteins precipitate in wine by linking to high molecular weight compounds, like tannins. Oversimplifying a bit, the more protein, the more precipitation, if there is not a shortage of tannin.
- The authors applied all three tests to 23 commercial Portuguese and Austrian white wines involving almost as many varieties. (You are referred to the original text for specifics on how the tests were carried out, so we can jump right into the results).

• **Comparison among heat test, ethanol test, and tannin test.** The turbidity developed in the *heat test*, both with a fast (90°C, 1 hr) and a slow (60°C, 4 days) precipitation method, was proportional to the protein content. Therefore, it is a good stability test to determine the amount of fining agent required. In the remaining methods (ethanol precipitation test, tannin precipitation test), the haze that developed was not proportional to the protein content, but instead, was influenced by other factors in the wine. The *ethanol test* showed interference by calcium in the wine and by the pH. Briefly, the *tannin test* was sensitive to even more wine factors (pH, iron, potassium, copper), beside the problem of tannin saturation effect, mentioned above.

• **Sensitivity to precipitation of different protein fractions.** The authors allowed the wines to age and precipitate naturally for over one year. Then they compared the HPLC protein profiles for 5 of the wines before and after storage. Which protein fractions were missing? Which appeared for the first time? The short answer is that **natural precipitation does not change the protein profiles greatly** – no given protein fraction was particularly sensitive to precipitation in normal storage conditions. As the authors state, the best way to ensure protein stability is to remove all fractions.

• **Influence of tannin, ethanol, pH, and temperature on wine protein stability.** To study the impact of the interfering factors, the authors adjusted wine samples to *two levels* of each of the factors examined (for example, low and high pH, more tannins or less, more ethanol or less, store at low or high temperature). Then they compared the haze that developed in each of the two samples. Ethanol content was the only factor studied that did not affect turbidity. **The most important factor affecting haze formation was the change in tannin concentration.** Other factors, including pH and temperature, also affected turbidity, but only when tannins had also been added. Therefore, when determining the required amount of fining agent to use, variations in tannin content that might have resulted from blending or storage changes should be taken into careful consideration.

So the method the industry relies on most often, the heat test, was shown to be the best of the three compared by these authors. No particular protein fraction was shown to be unusually sensitive to precipitation –so knowing the type of protein in our wines cannot predict the likelihood of precipitation yet. Finally, protein haze formation appears to be unaffected by the amount of ethanol, but it was strongly affected by the amount of tannin in these tests. If tannin concentration changes significantly in a wine, then changes in pH and temperature may impact haze formation as well.

*Author: Bibiana Guerra, Editor: Kay Bogart. This summary series funded by J. Lohr Vineyards & Wines.*