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"Protein haze in bottled white wines: How well do stability tests and bentonite fining trials predict haze formation during storage and transport?"

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• Measuring the protein stability –or haze potential- of a white wine is a key tool in the winemaker's toolbox. This allows adjustment of the wine's protein content before bottling, thus ensuring the wine will not develop a haze during transport or storage. In this study, the authors compare different methods of evaluating protein stability.

• Stability predictive assays usually involve 1) *inducing a haze*, by heat or other methods, followed by 2) *measuring the induced haze* through a variety of methods available. In the event that the wine were to be declared "protein unstable", the winemaker would "clean it up", or reduce its protein content, normally through bentonite fining.

• Predictive assays tend to use those conditions that would induce the most haze (the idea is to get all bases covered later by going overboard now). The authors compared 3 **methods of *inducing* a haze**: 1) Heating the wine samples at 80°C for 2 hours, 2) heating the samples at 80°C for 6 hours, and 3) the 'Bentotest', which consists of adding increasing amounts of protein to the wine, then evaluating which amount triggers the development of a haze. Method 2) is the current method used in Australia to predict protein stability. Additionally, the authors also studied the impact of substituting the 80°C above by heating at 60°C or 70°C.

• The authors also compared 3 **methods of *measuring* the induced haze**: 1) Nephelometry: samples were considered protein-unstable when the difference between heated and unheated controls was >2NTU as measured by a nephelometer. 2) Spectrophotometry: samples were considered protein-unstable when the difference between heated and unheated controls was >0.02 absorbance units measured at 520nm. 3) Visually: samples were considered protein-unstable if any haze could be observed when shining a flashlight beam through the wine, preferably in a dark room. (A 1998 Australian survey showed that 29 out of the 34 responding wineries relied on visual estimation, rather than instrumentation, to predict protein stability).

• **Effect of temperature and time on estimated bentonite dosage**. As expected, heating at 80°C predicted higher dosage rates of bentonite required for stability than heating at 60°C or 70°C. Similarly, heating for a longer period predicted higher bentonite dosage rates required for stability.

• **Heat tests *versus* 'Bentotest'.** Bentotest always indicated higher bentonite dosage rates than the two heat tests (as assessed by nephelometry). That is, it tends to overfine. For this reason, a previous author suggested that, when winemakers use Bentotest, they make their decision on the final dose of bentonite to use based on the sample with a very slight haze, instead of the sample completely free of haze.

• **Comparison of methods for reading haze**. As expected, visual inspection was difficult and varied with the observer. As for measurements using a spectrophotometer, they were unreliable and subjected to additional error due to the fact that wine color changes after heating (and a spectrophotometer picks that up and adds it to the reading). A nephelometer, on the other hand, is rather blind to these color changes, and is the instrument of choice for this purpose.

• Next, to evaluate the suitability of current bentonite fining trials in avoiding haze development after storage, the authors chose 8 commercial dry white wines and either *fined* them at differing bentonite doses or left them *un-fined*. Then they subjected the wines to the following "storage extremes": 1) bottles in cartons were held at 35°C for 1 month (transport conditions test); 2) bottles were held one day at 35°C followed by one day at 20°C, then back to 35°C and so on for eight days (fluctuating temperature test); 3) the remaining bottles were held in closed cartons under controlled temperature and humidity (13- 17°C) (best practice test). See results in next bullet point.

• **Effectiveness of bentonite fining in avoiding haze after wine storage**.
- *Best practice conditions (13-17°C).* All the bentonite-fined wines –except Riesling- were bright (considered protein-stable). Two of the 8 unfined wines were also bright (6 developed a haze).
- *Commercial transport conditions (35°C).* All the bentonite-fined wines –except Riesling- were bright. One of the 8 unfined wines (Sultana) was also bright (7 developed a haze).
- *Fluctuating temperature conditions (20°C/30°C back and forth)*. All the bentonite-fined wines –except Riesling- were bright. All the unfined wines developed a haze.

A few take-home messages based on these results:

_ most of the wines fined with bentonite at dosages determined by the three predictive methods remained bright after storage with the harshest conditions. Therefore, the least severe stability test, 80°C for 2 hours, is adequate to accurately predict short-to-medium-term stability.
_ even 80°C for 2 hours may lead to overfining (70°C for 30 min, even 80°C for 5 min, would have adequately predicted the right dosage). In the authors' opinion, the wine industry is biased towards overfining. Still, they believe that continuing to use use 80°C for a 2-hour period is the prudent thing to do.
_ we need to be aware that the temperature of heat tests given in the literature is usually referring to the temperature of the sample itself, not the temperature to set the apparatus to maintain the prescribed sample temperature. Laboratory staff should always determine the "set point" for their particular water bath that results in the wine –not the water bath- reaching the stipulated temperature. For instance, in order to bring the samples to 80°C for 2 hours, as prescribed in the literature, the authors needed to set their heating block to 94°C and have the tubes in the block for 2.5 hours. This is very important!
_ a spectrophotometer is not designed to measure hazes. And even a nephelometer should be given some time to stabilize before recording the reading (bubbles rise, large particles sink). This is particularly important when dealing with low nephelometric values.
_ finally, non-proteinaceous factors are also involved in haze formation, and these are not fully understood. So, different wines with the same protein content may require different amounts of bentonite to reach stability.

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