A new heat test for more accurate prediction of bentonite additions to avoid protein haze

Introduction

Grapes contain proteins which can persist through winemaking to be present in wine, particularly white wines. These proteins can come out of solution during later wine storage, causing an unsightly haze that may be off-putting to consumers. Winemakers prevent protein hazes by treating juices or wines with bentonite clay, which binds to the proteins and precipitates them so that they can be removed by racking or filtration. To determine how much bentonite is needed to prevent haze formation in a specific wine, a predictive test is required.

Heat tests are widely used to predict the likelihood of a protein haze forming in a wine and to determine the amount of bentonite required to prevent that haze from developing. Heat tests are a deceptively simple way of assessing protein stability – a sample of the wine is heated and then cooled, with the turbidity (cloudiness) measured before and after heating. Heating causes any unstable proteins to form a haze, and the resultant increase in turbidity is used to assess the amount of bentonite required to remove those proteins. However, there is significant variability in how the test is performed, and how the results are interpreted across industry, which affects the reliability of the results.

The original heat test method (Pocock and Rankine 1973) was designed to produce the greatest amount of haze in a wine sample in the shortest amount of time, and included six hours of heating at 80°C and 16 hours of cooling at 4°C, followed by a further two hours at room temperature. This test was reliable, but it involved a 24-hour turnaround time and may have over-predicted the amount of bentonite required to stabilise a wine. Since this test was developed, many variations have been used by wine laboratories around Australia and the world, most commonly heating for two hours and cooling for unspecified times or temperatures.

Reports from industry of the variable nature of protein stability testing prompted research into the effect of different heat test conditions on the amount of haze formed, the predicted bentonite addition required to achieve stability and the relationship between the haze formed in the heat test and the haze formed after longer-term storage. This work has culminated in the development of new recommendations for conducting heat tests, which are being adopted by industry.

Why cooling time matters

The heat test is generally thought of in terms of the time and temperature of heating, with the cooling step often overlooked. However, this work found that the cooling step has a critical impact on the overall heat test result. Experiments were conducted to assess the impact of cooling times and cooling conditions on haze formation. Selected wines were heated in 20 mL tubes for two hours and the amount of haze formed was measured after cooling at 20°C for one to five hours (Figure 1). Note that wines were still warm to touch after being allowed to cool for 30 minutes after heating for two hours at 80°C, and so the turbidity was not measured at that point.

These trials demonstrated that longer cooling time leads to greater haze formation in some wines. The reason for this is that protein haze production is a three-stage process (Van Sluyter et al. 2015):

- First, the proteins in the wines are unfolded from their normal configuration due to the high temperature.
- Second, the unfolded proteins start to interact with other components of the wine matrix (including phenolics and small particles) to form aggregates.
- Finally, as the wine is cooled, these aggregates grow larger and interact with each other to form a visible haze.

For the heat test to work, a wine sample must be heated for long enough for the proteins to unfold and start to aggregate, and then cooled fast enough and for long enough for the aggregates to form the visible haze. Because of this, wine samples that are not cooled immediately from 80°C to ≤ 20°C, or are not cooled for long enough, will produce less haze than samples that are removed from heat and cooled for longer. The results suggest that the cooling period in the heat test should be a minimum of three hours for consistent results.

Figure 1 also shows that heating for two hours with three hours cooling produced more haze in all three wines than a common industry method of heating for six hours and cooling for

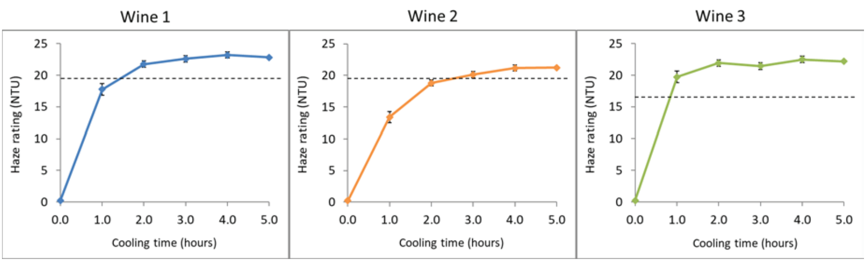

Figure 1. Haze produced in three wines after heating for 2 hours at 80°C and cooling for 1 to 5 hours at 20°C immediately after the allocated heating time. Results are shown as the average of triplicate analyses +/- one standard deviation. Dashed lines indicate the haze produced after 6 hours of heating at 80°C and cooling for 30 minutes at 20°C.

30 minutes (dashed lines). This further demonstrates the importance of sufficient cooling time in driving haze formation.

What about heating time?

Experiments were also conducted to examine the effects of different heating times, with selected wines heated for different periods from 30 minutes to six hours, followed by a consistent cooling period of three hours at 20°C (Figure 2). Wines heated for two hours and cooled for three hours produced four times more haze than wines heated for 30 minutes, and twice as much haze as the wines heated for one hour. Comparatively, wines heated for six hours produced approximately 20% more haze than wines heated for two hours.

These results suggest that haze formation is very dynamic in the first two hours of heating, with large increases in the amount of haze formed. After two hours of heating, the rate of haze formation is much slower. This suggests that heat test samples should be heated for a minimum of two hours for consistent results.

Different heat tests predict different bentonite doses

The main reason for performing the heat test is to determine the amount of bentonite to add to a wine to achieve protein stability. Different forms of the heat test were compared across a range of wines by examining the different bentonite doses they predicted. The tests included:

- 6 hours' heating with 18 hours' cooling (16 hours at 4°C and 2 hours at 20°C)
- 2 hours' heating with 18 hours' cooling (16 hours at 4°C and 2 hours at 20°C)
- 2 hours' heating with three hours' cooling (1 hour at 0°C and 2 hours at 20°C)
- 2 hours' heating with three hours' cooling at 20°C

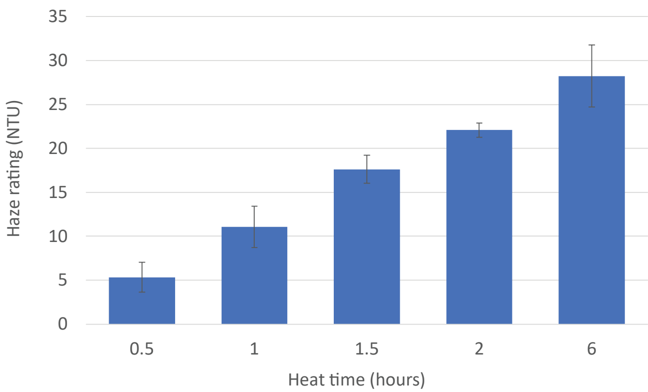

Figure 2. Example of the amount of haze produced in a wine after heating for 30 minutes to 6 hours at 80°C and cooling for 3 hours at 20°C. Results are shown as the average of triplicate analyses +/- one standard deviation.

Wine samples were fined with a series of bentonite doses and each heat test was conducted on the bentonite-fined samples. The lowest bentonite dose that produced a change in turbidity after the heat test of less than or equal to 2.0 NTU (nephelometric turbidity units) was considered the predicted dose for that test. Table 1 shows the bentonite dose predicted by each heat test for the selected wines.

Table 1 shows that predicted bentonite dose was not affected by the cooling temperature in the heat test, even though it did influence the amount of haze formed during the test. This means that more haze does not necessarily mean more bentonite is required to stabilise the wine.

Longer heating time (six hours) with longer cooling time (18 hours) generally predicted a higher bentonite dose, up to 0.3 g/L higher in the Sauvignon Blanc wine, but this was not the case with all wines. For the Semillon/Sauvignon Blanc and the Verdejo wines, the same amount of bentonite was predicted by all four heat tests.

Table 1 also shows that bentonite dose generally increases with protein concentration but there is not a strong correlation between the two variables. This is because there are many other components in wine that can enhance the amount of protein haze formed. Wine with a small amount of protein (such as 10 mg/L) can be unstable and produce a haze whereas another wine with more protein (such as 50 mg/L) can be stable and not produce haze, depending on the other wine components (McRae et al. 2018b).

For example, the protein concentration of the Sauvignon Blanc wine was lower than that of the Pinot Gris (Table 1) but the predicted bentonite dose was around twice as high. Other

Table 1. Protein concentration and comparison of the different bentonite doses (g/L) predicted by different versions of the heat test for selected white wines. Note: the cooling times for the 4°C and 0°C treatments include 2 hours at 20°C

Wine	Protein (mg/L)	Predicted bentonite dose (g/L)			
		6 h heat 18 h cool (4°C)	2 h heat 18 h cool (4°C)	2 h heat 3 h cool (0°C)	2 h heat 3 h cool (20°C)
Chardonnay	11	0.2	0.0	0.0	0.0
Semillon/Sauvignon Blanc	63	0.4	0.4	0.4	0.4
Riesling	87	0.4	0.2	0.2	0.2
Vermentino	112	0.4	0.2	0.2	0.2
Sauvignon Blanc	133	0.8	0.5	0.5	0.5
Pinot Gris	143	0.4	0.2	0.2	0.2
Verdejo	392	1.2	1.2	1.2	1.2

components of the Sauvignon Blanc wine, including higher pH, lower alcohol and more phenolics, made this wine more susceptible to haze formation.

The question from the results shown in Table 1 is whether the test with six hours heating and 18 hours cooling over-predicted the bentonite dose, or if the test with two hours heating and three hours cooling under-predicted the bentonite dose. The answer was investigated with longer-term storage trials.

Heat test haze compared to long-term storage haze

One of the unknown factors of the heat test is how well it relates to real-world haze formation. To assess this, selected wines with and without bentonite fining were stored for 12 months at cellar temperature (17°C) and at elevated temperature (28°C), to better assess the correlation between heat test results and haze formation during longer-term storage.

Several of the un-fined wines became hazy after 12 months storage, particularly at 28°C (Figure 3). One exception was the Riesling, where the results are suspected to be due to factors other than protein haze. The amount of haze formed after 12 months storage was much less than that formed in all the measured heat tests (Figure 4).

The reason for this lack of correlation is that the cause of protein unfolding is different under the conditions of the heat test compared to real world storage. In the heat test, proteins unfold because of the high temperature, which is above the ~65°C necessary to cause them to unfold. When they unfold in this way, wines at higher pH produce more haze.

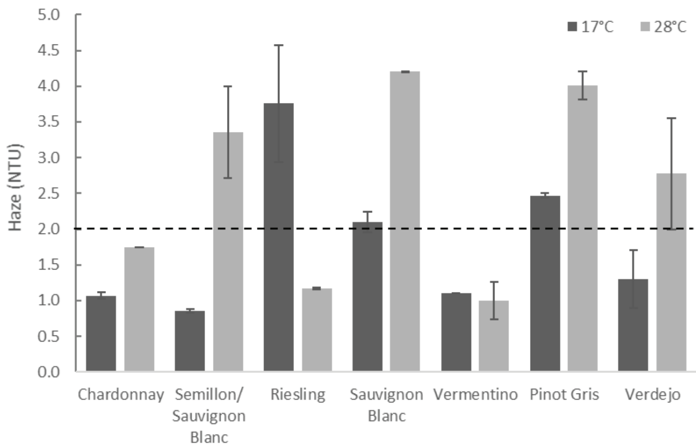

Figure 3. Haze formed in the un-fined control wines after 12 months storage at 17°C and 28°C. Dashed line shows maximum haze for a wine considered heat stable (2 NTU).

In the real world, wines (hopefully!) don't reach such high temperatures but the proteins will still unfold because they are unstable at wine pH. At normal temperatures, wines with a lower pH are more likely to develop haze. This process of haze formation can happen at any temperature – even if the wine is stored in a fridge – but will happen faster at higher temperatures.

The different processes driving haze formation in the heat test and in the real world mean that the heat test can never accurately represent real-world conditions. However, the heat test does show the potential of a wine to form a protein haze and is therefore useful as a rapid measure for predicting wine haze potential and the amount of bentonite needed to prevent that haze from forming during later storage.

How well does the bentonite dose predicted by different heat tests prevent haze formation during storage?

Preventing haze formation is always a balance between over-fining wine with bentonite, which can strip colour and aroma, and under-fining wine, which increases the risk of the wine becoming hazy. The predicted amounts of bentonite required to prevent haze were different for different heat tests (Table 1), which either meant that the lower dose was under-fining the wine or the higher dose was over-fining.

To assess the effect of different bentonite doses on wine stability, selected wines were fined at bentonite doses predicted by two heat tests, one with six hours heating and 18 hours cooling

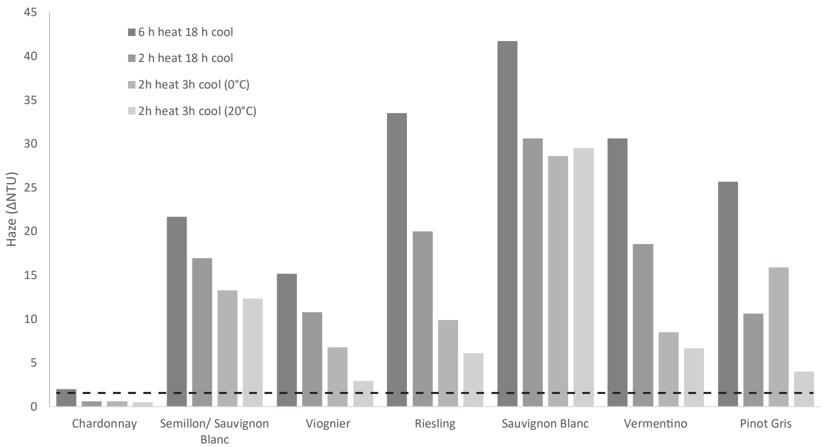

Figure 4. Haze formed in the un-fined control wines after each heat test, expressed as the turbidity difference between samples heated during the test and unheated control samples (ΔNTU). The amount of haze produced by Verdejo wine in the 2 h heat 18 h cool method was 329 ΔNTU (data not shown). Dashed line shows maximum haze for a wine considered heat stable (2 ΔNTU).

and the other with 2 hours heating and 3 hours cooling (20°C). The wines were then stored for 12 months at 17°C and 28°C.

Wines fined at bentonite doses predicted by the test with 6 hours heating and 18 hours cooling were generally clear and bright (<2.0 NTU) after 12 months storage (Figure 5). The Riesling was again an exception, likely due to factors other than protein haze.

Wines fined based on the test with two hours heating and three hours cooling at 20°C were also clear and bright after 12 months' storage at both temperatures (Figure 6). One exception

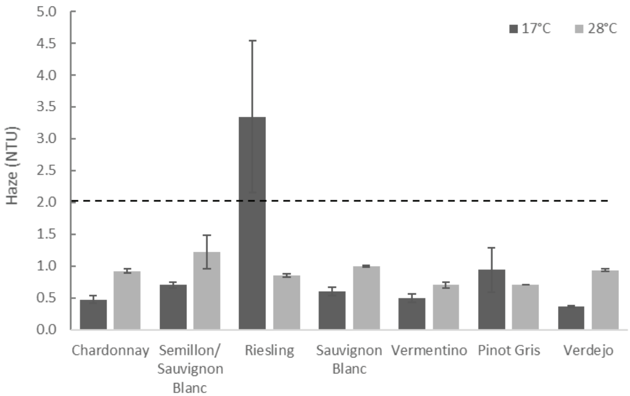

Figure 5. Haze formed in wines fined at a bentonite dose predicted by the 6-hour heating with 18 hours cooling test after 12 months storage at 17°C and 28°C. Dashed line shows maximum haze for a wine considered heat stable (2.0 NTU).

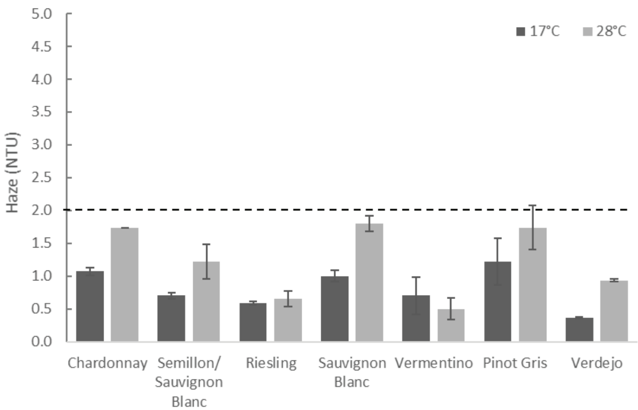

Figure 6. Haze formed in wines fined at a bentonite dose predicted by the two- hour heating with three hours cooling (20°C) test after 12 months' storage at 17°C and 28°C. Dashed line shows maximum haze for a wine considered heat stable (2.0 NTU).

was a single replicate of the Pinot Gris wine stored at 28°C, which produced a slight haze. The reason for this may be that the predicted dose was taken as the bentonite concentration that produced a change in turbidity of exactly 2.0 NTU in the heat test. This highlights the importance of selecting a difference in turbidity that is less than (not equal to) 2.0 NTU in the heat test when determining bentonite addition rates.

Conclusions

Conditions for both heating and cooling influence the amount of haze formed in the heat test. Comparative trials indicate that a test with two hours heating and three hours cooling produces similar or greater haze than the commonly used industry heat test (six hours heating, 30 minutes cooling). Longer-term stability trials showed that wines fined with the amount of bentonite predicted by either a test with two hours heating and three hours cooling, or a test with six hours heating and 18 hours cooling, were generally clear and bright after 12 months of storage.

Heat test recommendations

- Be consistent in the cooling time and temperature as well as the heating time and temperature.
- Results can be achieved in five hours total turnaround time: two hours at 80°C, then three hours at 20°C.
- If considering changing the heat test method currently used, consider conducting side-by-side analyses using both the current test and the new five-hour hour test (two hours heating and three hours cooling), particularly for bentonite fining trials.
- With bentonite fining, always select a predicted dose that produces a change in turbidity less than (not equal to) 2.0 NTU.
- Heat and cool samples in a water bath. Cooling can also be achieved by placing samples in a water vessel large enough to cool the sample without substantially heating the water. If this isn't feasible, cool samples on the bench.
- Remove particles from wine using a 0.45 µm filter before heating to avoid 'seeding' protein aggregation and haze formation.
- Note that any additions made to a wine after it is heat stable can de-stabilise it and therefore wines should be re-checked after any additions.

Acknowledgements

This work is supported by Australian grapegrowers and winemakers through their investment body, Wine Australia, with matching funding from the Australian government. The AWRI is a member of the Wine Innovation Cluster in Adelaide. Ella Robinson and Peter Godden are thanked for their editorial assistance.

References and further reading

McRae, J.M., Barricklow, V., Pocock, K., Smith, P.A. 2018a. Predicting protein haze formation in white wines. Aust. J. Grape Wine Res. DOI: 10.1111/ajgw.12354.

McRae, J.M., Schulkin, A., Dambergs, R.G., Smith, P.A. 2018b. Effect of white wine composition on protein haze potential. Aust. J. Grape Wine Res. DOI: 10.1111/ajgw.12346.

Pocock, K.F., Rankine, B.C. 1973. Heat test for detecting protein instability in wine. Aust. Wine Brew. Spirit Rev. 91: 42–43.

Van Sluyter, S.C., McRae, J.M., Falconer, R.J., Smith, P.A., Bacic, A., Waters, E.J., Marangon, M. 2015. Wine Protein Haze: Mechanisms of Formation and Advances in Prevention. J. Agric. Food Chem. 63(16): 4020–4030.

Jacqui McRae, Senior Research Scientist, jacqui.mcrae@awri.com.au

Viktor Barricklow, Freelance scientist, formerly Miami University student

Ken Pocock, formerly AWRI

Paul Smith, Wine Australia (formerly AWRI).