Getting proactive about protein

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Heat tests and bentonite fining are common practices across the winemaking world. Recent research at the AWRI has identified a more convenient and reliable format for the heat test, which is being adopted across winery labs. Promising results are also being seen for a number of possible alternatives to bentonite for protein stabilisation.

INTRODUCTION

Grapes contain proteins that persist through winemaking and, if not removed, can cause hazes in white, rosé and sparkling wines. Most winemakers use bentonite fining to remove protein and prevent haze formation, and use a heat test to determine the required bentonite dose. Recent research on wine proteins at the AWRI has closely investigated the chemistry behind the heat test, and developed new recommendations to maximise both convenience and reliability. In addition, possible alternatives to bentonite for protein stabilisation continue to be investigated, with some interesting results.

REVISITING THE HEAT TEST

Heat tests are widely used to determine the amount of bentonite required to remove enough protein from a wine to prevent a haze from developing. The test is performed by heating a wine sample, cooling it down, and measuring the turbidity (cloudiness) before and after heating. The original heat test method (Pocock *et al*. 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 of heating and cooling times and temperatures have been used by wine laboratories around Australia and the world, with reports received of variable results.

This prompted AWRI researchers to revisit the heat test in some depth, looking at the effect of different heat test conditions on the amount of haze formed, the predicted bentonite dose and the relationship between the haze formed in the heat test and the haze formed after longer-term storage.

COOLING IS AS IMPORTANT AS HEATING

The first key finding from this work was that cooling time and temperature were very important in achieving consistent heat test results. This is because protein haze production following heating is a three-stage process (Van Sluyter 2015):

- Proteins in the wines are unfolded from their normal configuration due to the high temperature.
- The unfolded proteins start to interact with each other and with other wine components to form aggregates.
- As the wine is cooled, the aggregates grow larger and interact with other aggregates to form a visible haze.

For the heat test to work, the wine samples must be heated long enough for the proteins to unfold and start to aggregate and then be cooled fast enough and long enough for the aggregates to form a visible haze. Because of this, wine samples that are not cooled immediately from 80°C to ≤ 20°C (or are not cooled for a long enough time) 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.

Heating time was also found to be important, with different amounts of haze formed when wines were heated for different times. Experiments comparing the amount of haze formed following different periods of heating suggested that heat test samples should be heated for a minimum of two hours for consistent results.

Predicted bentonite dose was usually greater after longer heating and cooling times (e.g. after the original 24-hour test compared to a five-hour test) although the cooling temperature did not change the test outcome, providing the cooling time was at least three hours.

TESTING LONGER-TERM STABILITY

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. Given that different combinations of heating and cooling time resulted in different predicted bentonite doses, this meant either that the lower predicted

AT A GLANCE

- Proteins in wine have potential to cause haze if not removed. This might be considered desirable or undesirable, depending on wine style.
- Bentonite fining is the most common treatment used to prevent protein haze, with a heat test used to determine the bentonite dose.
- AWRI researchers have confirmed the reliability of a shorter heat test, requiring only two hours of heating and three hours of cooling.
- Enzymes, pasteurisation and magnetic nanoparticles are all showing favourable results as potential alternatives to bentonite for ensuring haze-free wines.

doses were under-fining the wine or the higher doses were over-fining. It was therefore important to conduct longer-term storage trials to test which version of the heat test best predicted the minimum dose of bentonite required for long-term stability.

Selected wines were fined at bentonite doses predicted by two heat tests, one with six hours' heating and 18 hours' cooling and the other with two hours' heating and three hours' cooling. The wines were then stored for 12 months at 17°C and 28°C. Wines fined using both versions of the test were clear and bright after 12 months of storage, suggesting that the shorter (and therefore more convenient) version of the text could be recommended to industry.

This shorter version (two hours' heating at 80°C, three hours' cooling at 20°C) has now been promoted widely to industry via technical publications and a well-attended webinar. It will be included in the next issue of the Australian wine industry's key analysis text book and will be discussed at the upcoming Australian Wine Industry Technical Conference in July 2019.

BENTONITE ALTERNATIVES

While bentonite is an effective way to remove haze-forming proteins from wine, it has some drawbacks. It is not selective in its action, as it removes all proteins, not just those that contribute to a haze. It also increases the time wines spend in tank, can lead to loss of volume and quality and creates waste disposal challenges. These issues and associated costs have led researchers around the world, including at the AWRI, to conduct research on possible alternatives.

Enzymes

The most promising bentonite alternative to date is flash pasteurisation of grape juice in the presence of aspergillopepsin (AGP) enzymes. In general, the structures of wine haze-forming proteins make them inherently resistant to enzymes, but once they are heated to 75°C, these structures break down, leaving them vulnerable. Such high temperatures also tend to break down most enzymes; however, unusually, AGP enzymes are active at the same temperatures that make haze-forming proteins vulnerable. Adding AGP enzymes to juice just prior to flash pasteurisation and then fermentation can produce heat-stable wine without needing to add bentonite (Marangon 2012). AGP enzymes have been approved by FSANZ for use in Australian wine and are also legal to be used in wines destined for major export markets. Cost analyses indicate that this protein removal treatment costs less per batch of wine than bentonite. Several companies are now developing AGP enzymes for commercial application.

Pasteurisation

While AGP enzymes are not yet commercially available, juice flash pasteurisation alone can still substantially reduce protein concentration and the amount of bentonite needed to heat stabilise white wines. Heating juice changes the structures of the proteins and makes them more likely to stick together to form aggregates, much like the effect of the heating step in the heat test. This means that after fermentation, the aggregated proteins are more likely to drop out of solution along with the yeast lees.

Heating juice in a controlled environment like a flash pasteuriser or heat exchanger for one to two minutes has been shown not to have adverse effects on the sensory properties of wine (Marangon 2012). In that trial, the concentration of protein in a Sauvignon Blanc wine was reduced by half after juice was heated for one minute, substantially reducing the concentration of bentonite required to stabilise the wine. In smaller-scale trials, heating juice for two minutes removed almost all the protein in a Semillon wine and a Sauvignon Blanc wine. Further research is under way to investigate the impact of two minutes of heating juice on the sensory properties of wine.

Magnetic nanoparticles

Plasma polymer coated magnetic nanoparticles (MNPs) – nanometre-scale particles that can be moved around using external magnets – are the basis of another novel method for removing haze proteins from wines (Mierczynska-Vasilev *et al*. 2017, 2019). Proteins are adsorbed onto the magnetic particles and can then be removed from the wines, along with the particles, when an external magnet is applied. Testing of wines following a trial of the MNP treatment found that haze-forming proteins were removed, even from wines with very high protein content, while other components in the wines, such as phenolics and metal content, were unaffected. In addition, since the adsorption of proteins onto the nanoparticles is a reversible process, there is potential for the particles to be regenerated and reused, saving on waste.

WHERE NEXT WITH PROTEIN RESEARCH?

Recent developments in understanding haze formation have enabled better predictions of haze and development of new strategies to prevent it. Now that the work on the heat test has concluded, protein research efforts at the AWRI will continue to explore and refine alternatives to bentonite, to provide winemakers with sustainable, economical and efficient protein stabilisation options.

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