Beyond bentonite

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Until now, bentonite treatment has been the winemaker's best answer to troublesome haze-causing proteins. Breakthroughs in understanding the structure and properties of those proteins at the AWRI have led to the discovery of a potentially viable and practical alternative. Laboratory, pilot and industry-scale trials of proctase have now been successfully completed.

SEEING THROUGH THE HAZE

When wines develop a haze, the culprit is usually protein, particularly when the wines are exposed to high temperatures or after a long time in storage. Winegrapes contain proteins that persist throughout the winemaking process and, if not removed, they can produce an unsightly haze in white, rosé and sparkling wines.

To remove the protein and prevent haze formation, most winemakers use bentonite fining. While bentonite itself is effective, this step in the winemaking process is not selective, as it removes all proteins, not just those that contribute to a haze. It also increases the time wines spend in tank; it can lead to loss of volume and quality; and it creates waste disposal challenges and costs.

A recent study estimated the hidden cost of bentonite fining to be around $1 billion worldwide (AWRI publication #1307). These issues and costs have led researchers around the world to try to find an alternative. Until recently, however, their efforts have been hampered by a lack of understanding: only now do we know why grape proteins form a haze in wines.

NEW TECHNIQUES, NEW KNOWLEDGE

The first breakthrough came with the novel application of two laboratory techniques – strong cation exchange (SCX) and hydrophobic interaction chromatography (HIC) – to isolate the haze-causing proteins (AWRI publication #1180). These techniques allowed scientists to collect better quality samples of the proteins in larger amounts, paving the way for new discoveries.

Samples were then analysed using differential scanning calorimetry (DSC) to assess, for the first time, their unfolding temperatures and behaviour (AWRI publication #1187). Unfolding is key to haze formation. Scientists then identified which proteins were causing haze, and how. They discovered that of the pathogenesis-related (PR) proteins, chitinases were the main culprits, since they unfolded irreversibly and aggregated, or clumped together. When other PR proteins unfolded – namely, thaumatin-like proteins (TLPs) that are also responsible for haze formation – the process was reversible; they are much less likely to aggregate over short periods of time.

Researchers discovered the link between protein unfolding, induced by heat, and aggregation by using another technique called dynamic light scattering (DLS) (AWRI publication #1272). They also found that sulfate concentration and the overall ionic strength of wines played a part, as the presence of sulfates and other ions in sufficient quantities can favour protein aggregation.

Today, as a result, wine researchers have reached a much deeper understanding of wine proteins and why they unfold and aggregate. The AWRI has used this knowledge to identify new ways to break proteins down, which could eliminate the need for bentonite.

FINDING AN ENZYME IN A HAYSTACK

For some time, wine scientists have wondered whether the alternative to bentonite could be enzymes that break down proteins. Proteolytic enzymes have been tried before, but with limited success. This is because grape PR proteins are particularly resistant to enzyme attack and the proteolytic enzymes are not sufficiently active under normal winemaking pH and temperature conditions.

Now, wine scientists have adopted a new approach. They have taken an enzyme and put it to work on the proteins after they have unfolded, when they are much more susceptible to enzyme attack. They have also identified proctase as a lead candidate, due to its activity at wine pH and at temperatures close to the unfolding temperatures of the PR proteins.

For the process to work, the juice must be heated so that the proteins unfold. While some winemakers may be wary of this, trials suggest that short-term heating does not cause a negative sensory effect in the resulting wine. Some studies even suggest that heating can lead to the release of important

At a glance

- Proctase treatment has been identified as a viable alternative to bentonite fining in reducing haze in white wines
- The treatment targets those specific proteins responsible for haze formation. It builds on research that has revealed new information about how haze-causing proteins behave when exposed to heat
- Sensory evaluation has revealed no difference between wines treated with proctase and those treated with bentonite.
- Economically, in-line bentonite dosing may be more cost-effective, but proctase treatment may be cheaper for smaller wineries that cannot afford to invest in the required infrastructure. The economic benefit of proctase, in relation to batch dosing of bentonite, is significant
- The AWRI is working with regulatory bodies to ensure that proctase-treated wines do not encounter regulatory hurdles – in Australia and overseas.

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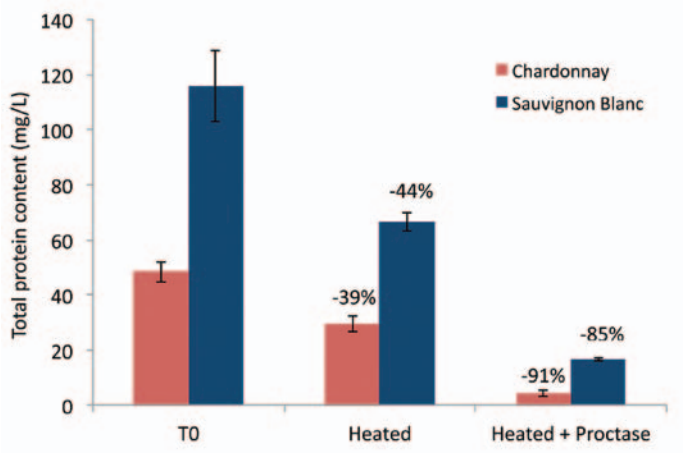

Figure 1. Total protein content of Chardonnay and Sauvignon Blanc juice samples: T0 (unheated juice (control)); heated, 75°C for one minute; heated + proctase (15mg/L), 75°C for one minute. Unheated juice + proctase not shown.

flavour compounds. In contrast, however, heating wine can produce negative sensory effects, so proctase treatment should only be applied to juice, not wine.

FROM LAB-SCALE TO PILOT-SCALE

The next step was laboratory testing using different concentrations of proctase and juice at different temperatures. Researchers found the best combination to be 15mg/L proctase concentration in juice heated to a nominal temperature of 70-75°C for one minute. This combination was then tested during the 2011 vintage (AWRI publication # 1444).

The pilot-scale experiment took two juices (one Chardonnay and one Sauvignon Blanc, both from the Barossa Valley) and applied four initial treatments to each juice:

- an unheated control
- unheated juice + proctase
- heated juice + proctase
- heated without proctase.

Protein analysis immediately after treatment showed that heating alone reduced protein content by around 40 percent for both juices. When proctase was used, protein was reduced by 85% in the Sauvignon Blanc and 91% in the Chardonnay juice (Figure 1).

The three different juice treatments and the control (untreated) juice were then fermented in triplicate 80L volumes. The wines made from the control juice were divided into two after fermentation, with one half left untreated and the other half fined with bentonite to represent normal industry practice. This resulted in five different treatments for each variety available for further analysis. Protein content results for the wines echoed the juice results closely, with the proctase + heat treatment leading to a 84% and 81% reduction in total protein content in Sauvignon Blanc and Chardonnay, respectively (Figure 2).

Further analysis showed that the majority of proteins removed by the proctase treatment are those known to contribute most significantly to haze formation (chitinases, in particular).

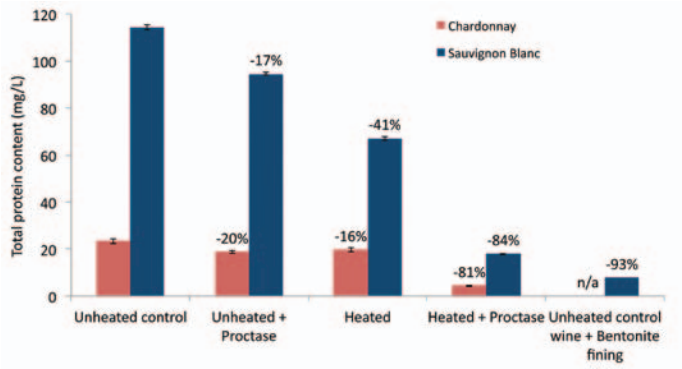

Figure 2. Average protein content of wines (three replicates): unheated control, no bentonite; unheated + proctase; heated; heated + proctase; unheated (control) + bentonite (Sauvignon Blanc only).

SENSORY EFFECT

With protein removal successfully confirmed, the next step was to check for sensory effect. A triangle test was used to assess sensory differences among treatments, with 47 experienced panellists involved. Wines made from the proctase-treated juices, with and without heating, were not found to be significantly different to the bentonite-fined control wine. This showed that proctase treatment does not produce a sensory effect when compared with bentonite treatment.

Figure 3. AWRI staff providing onsite support for proctase treatment.

OUT OF THE LAB AND INTO THE TANK FARM

With such positive results from the 2011 pilot-scale trial, the AWRI was ready to scale-up again in 2012. The researchers knew that proctase treatment worked well, but they wanted to assess how well it could work in a commercial winery using existing, rather than specialised, equipment.

Two industry partners came onboard to try out the new treatment for protein removal. With the support of AWRI engineers on site, a total of three juice varieties (Riesling, Sauvignon Blanc and Chardonnay) were treated across the two wineries at a 5000L-scale.

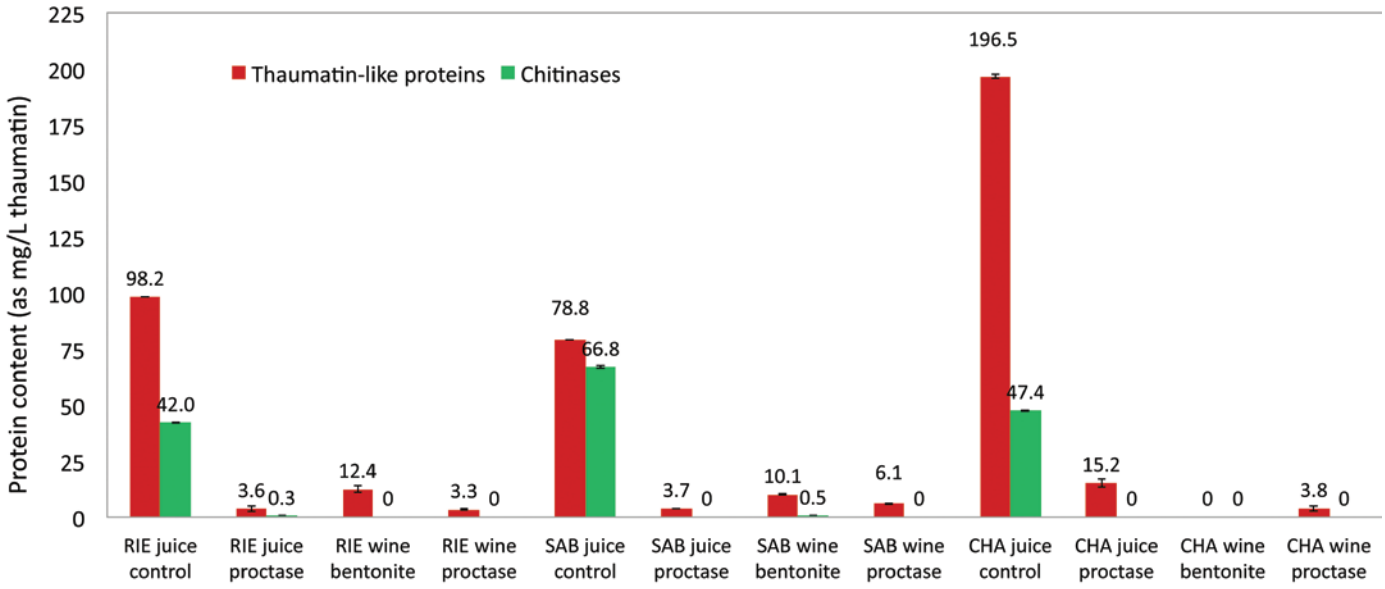

Figure 5. Average levels of chitinases and thaumatin-like proteins in treated Riesling (RIE), Sauvignon Blanc (SAB) and Chardonnay (CHA) juice and wine samples. Error bars indicate standard deviation across three replicates.

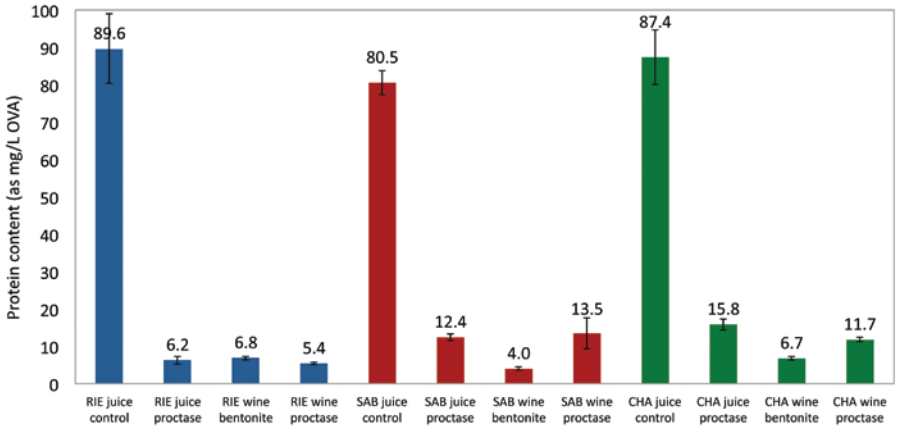

Figure 4. Average protein content of treated Riesling (RIE), Sauvignon Blanc (SAB) and Chardonnay (CHA) juice (j) and wine (w) samples. Error bars indicate standard deviation across three replicates.

This time, the experiment was simplified. For each juice variety, the proctase + heat treatment was compared against the industry standard bentonite treatment. Each juice was split into two parcels, with one parcel heat treated with proctase, while the other parcel acted as the control. The two parcels were then fermented under identical conditions, with the control subsequently fined with bentonite post-fermentation as per typical industry procedures.

After cold stabilisation, sub-samples of each wine were brought to the AWRI for packaging under typical commercial conditions. Juices (pre- and post-proctase treatment) and wines (treated and bentonite fined) were analysed for protein content and composition. The total protein content of the samples (analysed in triplicate) is summarised in Figure 4.

In all three juices, proctase treatment caused a reduction in protein content from more than 80mg/L to less than 16mg/L, similar to the results achieved following bentonite fining. The AWRI then used high performance liquid chromotography (HPLC) analysis to provide more information about the types of proteins that remained in the juice and wine samples.

The HPLC results (Figure 5) show that while chitinases were present in all of the control (untreated) juices, the proctase treatment successfully removed them and also reduced the concentration of TLPs dramatically. In two out of three varieties, the wine made from the Proctase-treated juice contained lower levels of TLPs than the equivalent bentonite-treated control wine. This shows that proctase is as effective as bentonite in removing proteins.

Formal sensory evaluation has only been carried out on one of the wines (Riesling) to date. An expert panel of eight experienced tasters assessed the wines for colour and condition, aroma and palate attributes, and provided ratings for acceptability of the wines and the presence of any taints or faults. The average ratings of the proctase and bentonite-treated wines were identical, with no taints or faults identified. Formal sensory evaluation of the Sauvignon Blanc and Chardonnay wines is expected to be carried out in the near future.

THE HEAT TEST CONUNDRUM

To check for protein stability, winemakers currently use a heat test, where a sample of filtered wine is heated at 80°C for six hours and its turbidity is compared with an unheated sample. This stringent test brings out of solution all proteins from the wine, including those known not to contribute to haze formation. This can lead to a false positive result when proctase treatment has been used and can incorrectly suggest that a wine is protein unstable.

Since proctase is a selective treatment, targetting haze-forming proteins and not other proteins, a new approach is required. In 2011 and 2012, researchers used a modified version of the protein stability test, which involved heating for two hours at 80°C followed by two hours of chilling and subsequent measurement at room temperature (AWRI publication #943). Both the Riesling and Sauvignon Blanc wines made from proctase-treated juice passed this test. However, the Chardonnay wine appeared to be a borderline fail.

To support the adoption of proctase as an alternative to bentonite treatment, the

AWRI recognised the need for a better, more reliable test. Currently, HPLC can characterise the proteins in a wine sample and find out if any haze-forming culprits are still present. This is not a quick or easy test, however, and cannot be carried out by most winery labs. An alternative test is now in development.

The new test will likely involve a lower test temperature, to preserve proteins that do not contribute readily to haze formation. It is hoped that this could become the new industry 'standard' test and provide a more rapid analysis of protein stability in all white wines, irrespective of the method used for protein stabilisation.

WHAT ABOUT LONG-TERM PERFORMANCE?

The AWRI is still gathering data about the long-term performance of wines treated with proctase. For example, the 2011 trial Sauvignon Blanc wines were revisited after one year in bottle, to see if any changes in protein content or composition had occurred during storage.

Turbidity tests showed that the wine produced from the proctase-treated juice was still haze-free after one year of storage, whereas the unfined control had thrown a light haze. Protein content measurement and heat stability tests showed similar results to those obtained a year earlier, with the only exception being the unheated + proctase treatment, which showed a slight decrease in protein concentration over the year period. This was not entirely surprising, given that this is the treatment most likely to have residual enzyme activity.

The results to date suggest that proctase is an effective, long-term treatment for achieving protein stability in white, wines and might ultimately prove to be a viable alternative to bentonite.

HOW DO THE COSTS COMPARE?

To be economically viable, any alternative to bentonite must deliver cost savings. Therefore, detailed economic analysis was conducted to compare operating costs between proctase and bentonite treatments. For completeness, in-line bentonite addition was also included – this method is used by several large Australian wineries.

The study took processing conditions into account (flow rates, temperatures, heat exchanger specifications, etc). It also analysed heating and refrigeration energy, heat exchanger losses, pumping requirements and proctase purchase costs. To compare batch and in-line bentonite addition, wine volume and downgrade losses were included, together with filtration and centrifuge performance, as well as energy and labour requirements. Results are shown in Figure 6 (see page 30).

Further analysis revealed that operating costs are more sensitive to bentonite requirements and heat exchanger performance than to fluctuations in operating temperature and process flow rate. Increasing the cost of the proctase enzyme by a full 100% resulted in an operating cost increase of approximately 12-25% under commercial conditions, suggesting that the process is relatively insensitive to proctase cost variability.

The analysis also highlights that juices with high protein levels benefit most from proctase treatment in terms of process efficiency and cost. This makes sense, considering that juices

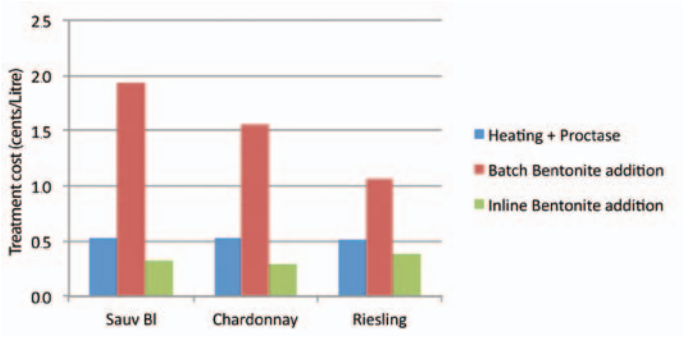

Figure 6. Economic analysis of heating + proctase addition, compared with batch and in-line bentonite addition for Sauvignon Blanc, Chardonnay and Riesling juices (treatment cost in cents per litre).

or wines with higher protein levels require higher bentonite doses, which carry higher associated costs. This means that the cost differential is more pronounced in high protein juices than it is for low protein juices where a smaller bentonite dose is needed.

In-line bentonite treatment costs were lower when compared with the combination of heat and proctase treatment. This suggests that if suitable equipment is available for in-line bentonite dosing, this option offers some advantages when processing juices or wines with lower protein concentrations. Considerable capital investment is associated with in-line bentonite dosing, however. Consequently, this method is cost prohibitive for all but the largest commercial wineries.

WHAT IS THE REGULATORY LANDSCAPE FOR PROCTASE?

The AWRI is currently seeking clarification on the regulatory status of the enzymes present in proctase from Food Standards Australia New Zealand (FSANZ). A review of the Food Standards Code indicates that enzymes of the same class and origin (Aspergillus niger var. macrosporus) as those present in proctase are listed (as Carboxyl proteinases) as permitted enzymes under clause 17 of the Food Standards Code 1.3.3. The AWRI is pursuing formal recognition of proctase with FSANZ, but until such formal registration has been obtained, proctase cannot be used in commercial winemaking for the upcoming vintage.

Once that formal approval has been obtained, proctase will be permitted for wine production in Australia, provided that the finished wine is destined for the domestic market. However, wines treated with proctase are not currently permitted for export to the EU. The AWRI is anticipating working with the OIV (International Organisation of Vine and Wine) to gain approval for proctase-treated wines to be permitted for export to the EU in the coming months.

Regardless of these regulatory hurdles, a number of Australian wineries are set to trial this alternative protein stabilisation technique during the 2013 vintage, now that the efficacy of proctase has been proven on a number of wines at a commercial-scale. Bottled samples of the 2012 trial wines will soon be available for independent assessment by any wine producers who are interested in this alternative approach to protein stabilisation.

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