Chitin, chitinase, chitosan ...

GO BACK

by Karien O'Kennedy | 1 Mar, 2019 | Oenology research, Winetech Technical

PHOTO: Pixabay.

In the past 10 years various studies were published that highlighted these three entities and their roles in winemaking. It is therefore worthwhile to take a closer look at what they are, what they do and why winemakers should be excited.

What they are

Chitin is the second most abundant polysaccharide in nature after cellulose.[1] It is a structural polysaccharide and is the main component of the exoskeletons of crustaceans (shrimps, prawns, crabs and lobsters) and insects.[2] It is also found in the cell walls of fungi and yeasts.

Chitinases are enzymes found in grapes and the resulting wine.[3,4,5] It forms part of the family of pathogeneses-related proteins. Their role in grapes is to protect grapes against fungal infection by binding to and breaking down the chitin in the fungal mycelial cell walls. Even though chitinases only comprise a small percentage of the total protein content in grape juice and wine, it is the biggest culprit in terms of protein instability. The reason for that is because chitinase denatures at a fairly low temperature compared to the other grape proteins. It is therefore really only necessary to remove chitinases from wine to render the wine fairly protein stable and not all the proteins in wine. It should be mentioned that another group of pathogeneses-related proteins (thaumitin-like proteins) can also contribute to protein instability, however, to a lesser extent than chitinases.[5]

Chitosan is produced commercially by partial deacetylation of chitin.[2] However, chitosan also occurs naturally in the cell walls of certain fungi. Most chitosan in the world is produced from crab, shrimp and prawn wastes. The production of chitosan from seafood is much more cost-effective than production from fungal sources. Chitosan, being a "natural" product, has various applications in the food, pharmaceutical and agricultural industries.[6] It can bind certain metals, is anti-microbial and an antioxidant, to name a few relevant specific to winemaking. Chitosan (and chitin) can also bind to chitinases in juice and wine, thereby rendering wines more protein stable.[4]

What the laws permit

Commission Regulation (EU) No. 53/2011 (January 2011) permits the use of chitosan and chitin-glucan of fungal (*Aspergillus niger*) origin in winemaking for the purposes of the: "reduction in the heavy metal content, particularly iron, lead, cadmium and copper – 100 g/hL; prevention of ferric casse and copper casse – 100 g/hL; reduction of possible contaminants, especially ochratoxin A – 500 g/hL; and reduction in the populations of undesirable microorganisms, in particular *Brettanomyces*, solely by means of treatment with chitosan – 10 g/hL".

South Africa has adopted the exact same resolution in our wine laws.

Chitosan as anti-microbial agent

Chitosan is effective against various wine microorganisms, such as acetic acid bacteria, some lactic acid bacteria and *Brettanomyces.*[7] In the case of Brett, its mode of action is to disrupt cell walls and membranes, causing leakage of cell constituents. It also causes Brett cells to aggregate to the bottom of a tank/barrel. It is important to know that chitosan is mainly fungistatic and not fungicidal, meaning that the Brett population can increase again after a certain amount of time, if the wine is not racked off the chitosan-Brett lees.

Interestingly chitosan is also used as an anti-microbial agent in natural textiles, such as sportswear, as well as in the medical field in wound dressings.[8]

Chitosan and protein stabilisation

Currently EU legislation does not stipulate the use of chitosan for protein stabilisation. However, a recent study demonstrated the possibility thereof.[4] The study was conducted on model wines, as well as Moscato commercial wines. 1 g/L Chitosan was added to the model wine and real wine samples and unfined controls were kept. Chitosan reduced both tartaric acid and malic acid in the model wines. The highest reduction for tartaric acid was 0.65 g/L and 0.46 g/L for malic acid. The total protein content of wines fined with chitosan was on average 14% lower than the control. Chitosan-fined wines were almost completely deprived of chitinases, but there was no significant effect on the TL-proteins. After a 60°C heat test, the control NTU was 11.07 and the chitosan-treated wine 1.95. After a 62°C heat test, the control NTU was 8.96 and the chitosan-treated wine 2.10. Chitosan treatment also reduced the calcium, potassium, iron and sodium content of the wines. A possible negative effect is that it did reduce some of the free terpenols, such as nerol, geraniol and linalool, which are important aroma compounds for a wine such as Moscato. The glycosylated precursors were mostly unaffected. No other classes of aromatic compounds were affected by chitosan treatment.

Due to the reduction in protein haze, chitosan is a potential alternative to bentonite treatment. Unfortunately compared to bentonite addition of the same dosage, chitosan addition is not at all economically viable at this stage since only, more expensive to produce, fungal-derived chitosan (*Aspergillus niger*) is approved for use in winemaking.

In addition to rendering wines protein stable, the removal of potassium and calcium ions can also have a positive effect on the tartrate stability of wines. The removal of iron can reduce a wine's oxidative capacity. No sensory analysis was done in this study and chitosan's effect on free terpenols should be further investigated.

Chitin, yeast and protein stabilisation

A study conducted by Dr. Thuli Ndlovu at the Institute for Wine Biotechnology, Stellenbosch University, under the leadership of Prof. Florian Bauer, revealed that fermenting Chardonnay and Sauvignon blanc musts with *Saccharomyces paradoxus* rendered wines more protein stable than wines fermented with normal *Saccharomyces cerevisiae* wine yeasts.[3] This can be attributed to the higher cell wall chitin content of the former compared to the latter. It was also found that inactivated versions of the *S. paradoxus* yeasts can remove chitinases, but to a lesser extent than the live cells. The significance of the study is quite profound since it finally presents a viable possibility of being bentonite-free one day. The solution might not lie with the chitin content of *Saccharomyces cerevisiae* cell walls, but other species of wine yeasts might be able to provide us with a no more bentonite future. Investigations are ongoing.

Currently no commercial *Saccharomyces paradoxus* exists, but a *Saccharomyces cerevisiae/paradoxus* hybrid is commercially produced and sold by Anchor Yeast. In the mentioned study, it was not as effective as the native *S. paradoxus* strains in improving protein stability, but performed slightly better than the *S. cerevisiae* strains. The use of yeast strains to reduce protein haze in white wines has been patented by Stellenbosch University. The research was funded by Winetech.

Conclusions

There are various commercial products available to winemakers that contain chitosan, chitosan in combination with other products and chitin-glucan. Most of the applications are for microbiological control. Winemakers are advised to contact suppliers directly to determine which products will best suit their needs. Examples of products commercially available are: OenoBrett, BactiControl and MicroControl (Laffort); Stab Micro and Stab Micro M (Enartis); and No Brett Inside and Bactiless (Lallemand). With the exception of Bactiless, all products are chitosan-based. Bactiless contains chitin-glucan and chitosan.

Chitosan and chitin-glucan are bio-degradable, non-allergenic and of plant origin.

References

1. Pillet, O., 2013. Chitosan and *Brettanomyces*: Origin, impact and mode of action. No. , December, 1 – 10.
2. Nunes, C., Maricato, É., Cunha, Â., Rocha, M.A.M., Santos, S., Ferreira, P., Silva, M.A., Rodrigues, A., Amado, O., Coimbra, J., Silva, D., Moreira, A., Mendo, S., Lopes Da Silva, J.A., Pereira, E., Rocha, S.M. & Coimbra, M.A., September 2016. Chitosan-genipin film, a sustainable methodology for wine preservation. *Green Chemistry* 18(19): 5331 – 5341.
3. Ndlovu, T., Divol, B. & Bauer, F.F., July 2018. Yeast cell wall chitin reduces wine haze formation. *Applied and Environmental Microbiology* 84(13): e00668 – 18.
4. Colangelo, D., Torchio, F., De Faveri, D.M. & Lambri, M., October 2018. The use of chitosan as alternative to bentonite for wine fining: Effects on heat-stability, proteins, organic acids, colour and volatile compounds in an aromatic white wine. *Food Chemistry* 264, 301 – 309.
5. Ndlovu, T., Buica, A. & Bauer, F.F., April 2019. Chitinases and thaumatin-like proteins in Sauvignon blanc and Chardonnay musts during alcoholic fermentation. *Journal of Food Microbiology* 78(11): 201 – 210.
6. Castro-Marín, A., Buglia, A.G., Riponi, C. & Chinnici, F., July 2018. Volatile and fixed composition of sulphite-free white wines obtained after fermentation in the presence of chitosan. *LWT – Food Science and Technology* 93, 174 – 180.
7. Petrova, B., Cartwright, Z.M. & Edwards, C.G., March 2016. Effectiveness of chitosan preparations against *Brettanomyces bruxellensis* grown in culture media and red wines. *Oeno One* 50(1): 49 – 56.

8. Kong, M., Chen, X.G., Xing, K. & Park, H.J., November 2010. Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology* 144(1): 51 – 63.

– For more information, contact Karien O'Kennedy at karien@winetech.co.za.

You may like to read these:

Management and re-use of treated winery wastewater (Part 1): New Zealand wineries

Carolyn went to New Zealand to participate in a technical tour focussing on the management and re-use of treated… Read More

Vinpro Production Plan survey (Part 1): The 2018 vintage

A watershed year for the South African wine industry?   In 2018 the Vinpro Production Plan survey was conducted… Read More

The role of leaf and soil analyses in vineyard management (Part 1)

Is it worth the effort for producers to take regular soil and leaf samples, or does this only generate… Read More