



# Proactive Protein Stabilization

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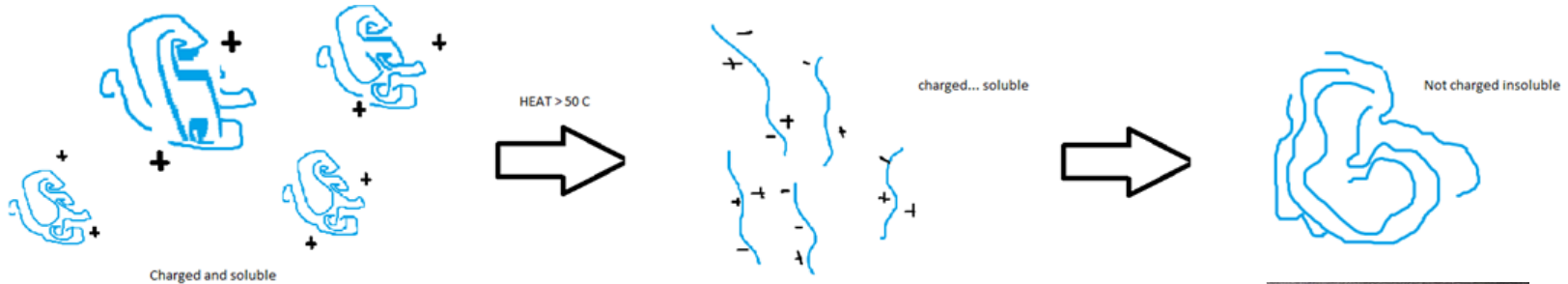
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- Background/review
- Trends for vintages
- Factors for vintage variability
- Bentonite additions pre/post fermentation
- Some enological tools
- New things on the horizon

## Overview

- Formation of protein haze in wine, either by denaturation from heat, or induced by ethanol over time (Pocock et al., 2003)



### Why focus on protein stabilization?

- Several winemakers have reported unusually high amounts of bentonite required for stabilization of proteins for the 2018 vintage

Protein Stability vs. Vintage

Actual:



- *What affects wine protein instability levels?*
  - **Variety** : Sauv. blanc, Semillon, Pinot Gris (Grigio), Grüner Veltliner
  - **Vintage**:
    - PR proteins (pathogenesis related proteins) sub group called Thaumatin and Chitinase proteins, related to vine stress (Marangon et al., 2010c; Marangon et al., 2011b).
    - Warmer climate = more protein instability (Salazar 2012)
    - Related to wind, water, and salts stress = more unstable
    - Related to botrytis and powdery mildew or other pathogen pressures
  - **Winemaking**:
    - Fermentation temperature, higher temp = less PR proteins (Ndlovu et al. 2019)
    - Use of enzymes, tannins, mannoproteins etc.

Bentonite effects on wine:

- Removes beneficial mannoproteins (Rodriguez 2012)
- Decreases terpenes, C13-norisoprenoids, C6 alcohols, ethyl esters, acetates, and thiols (Moio et al. 2004, Armada and Falque 2007, Baiano et al. 2012, Vela et al. 2012).
- Lose wine volume
- Addition of metals Na, Ca

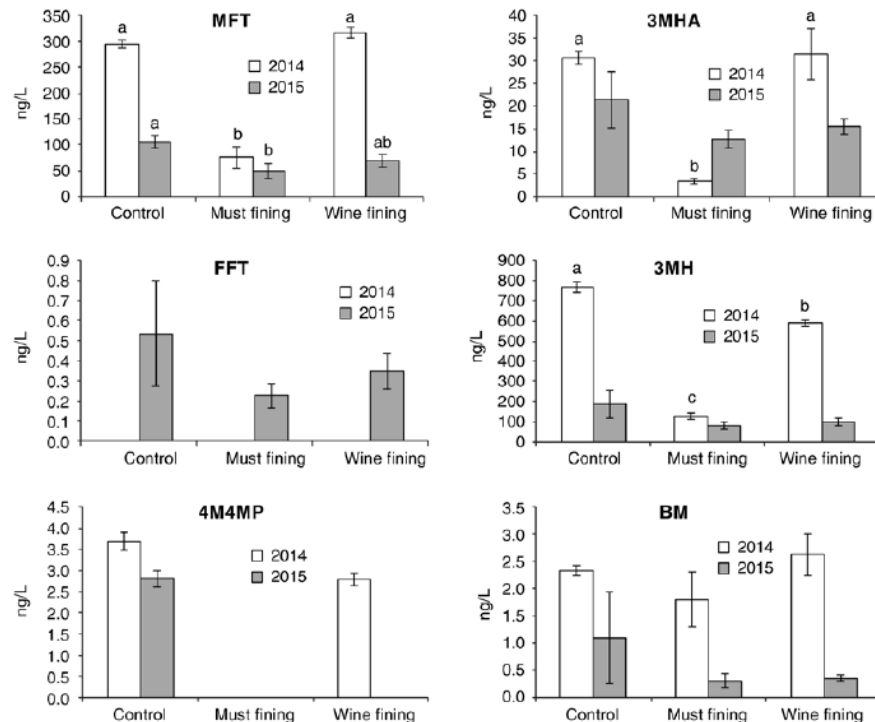


Which is best from a total bentonite addition perspective?

- Some authors have reported that the use of bentonite in juice/must is more efficient and reduces loss of aromatic compounds (Lambri et al. 2012).
- Others have reported that the best time to add bentonite is during fermentation, because a minimal amount is required and the concomitant removal of aromatic compounds is apparently lower (Miller et al. 1985, Pocock et al. 2011, Lira et al. 2015).
- Some authors bentonite additions are more efficient in the finished wine (Somers and Ziemelis 1973, Puig-Deu et al. 1999).

For thiols content:

- Changes thiolic varieties like SB
- Should supplement musts with precursors or preservative treatments



**Figure 1** Contents of 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA), 4-mercapto-4-methylpentan-2-one (4M4M2P), 2-furfurylthiol (FFT), benzyl mercaptan (BM), and 2-methyl-3-furanthiol (MFT) (expressed as ng/L of FFT) for wine control, treated with bentonite during fermentation (Must fining) and after fermentation (Wine fining) from vintages 2014 and 2015. Values are averages of independent vinifications (n = 2), error bars are two standard deviations. a, b, c: Different letters indicate mean is significantly different among samples at  $p < 0.05$  by Duncan's test after a statistically significant one-way ANOVA.

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Effect of Bentonite on Wine Aroma

Am. J. Enol. Vitic. 68:1 (2017)



# Tan Skin for recovery of thiolic aromas

Selected for its high content of 3-S-glutathionyl mercaptohexan-1-ol and 3-S-cysteinyl mercaptohexan-1-ol precursors when compared among other commercial grape tannins

Table 1: Thiols content of wines split by cultivar vs treatment (tannin addition at juice stage)

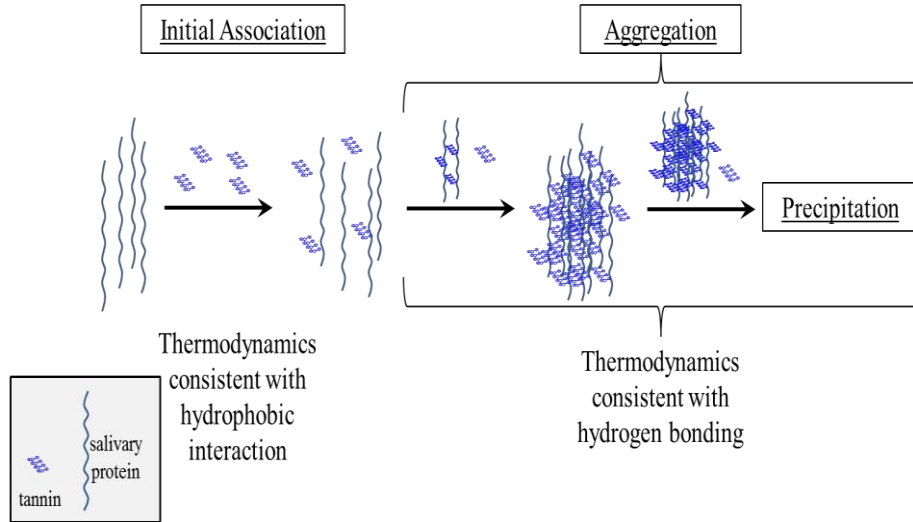
	Control	Tannin low precursor content	Enartis Tan Skin high precursor content
Gewürztraminer	Mean (n = 6)	Mean (n = 6)	Mean (n = 6)
3MH (ng L <sup>-1</sup> )	195	175	558
3MHA (ng L <sup>-1</sup> )	5	5	20
Sauvignon Blanc	Mean (n = 6)	Mean (n = 6)	Mean (n = 6)
3MH (ng L <sup>-1</sup> )	642	536	1168
3MHA (ng L <sup>-1</sup> )	67	45	114

Results extracted from “Importance of polyfunctional thiols on semi industrial Gewürztraminer wines and the correlation to technological treatments”, T. Roman et al., *Eur Food Res Technol* (2017)



High MW grape skin tannin

## Protein binding and removal





### Dosages and timing of application

- Applied to juice in tank at 5-10 g/hL dissolved in 10x water.
- Be sure not to add this adjacent to enzyme addition in concentrated form...

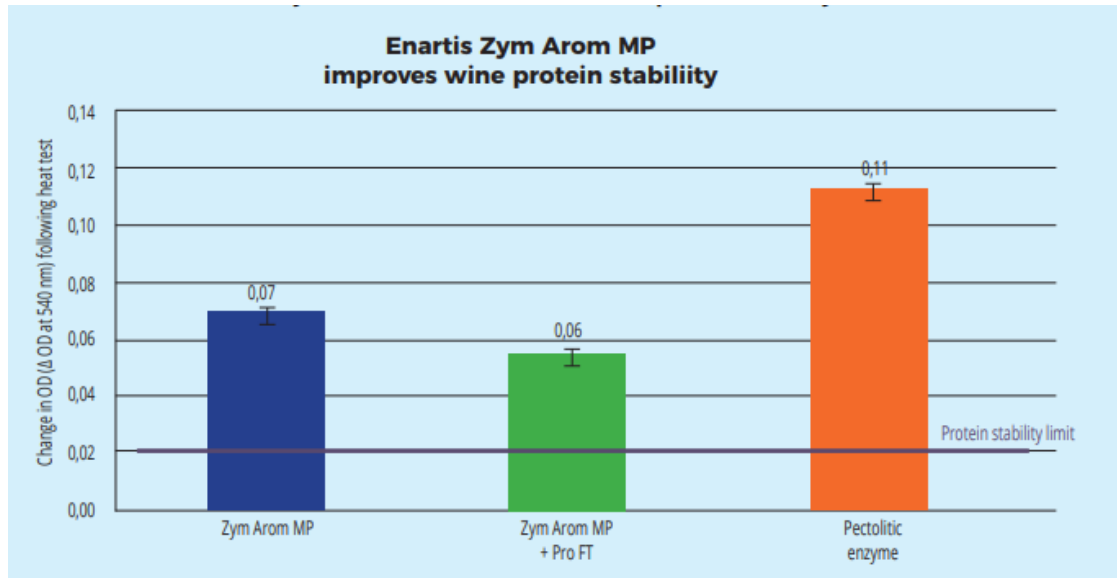
Pectinase, Cellulase and Hemicellulase, Protease

- Easier to clarify musts
- More aromas released
- Cuts up proteins into smaller pieces



## Effectiveness of Arom MP

- Reduces bentonite requirements up to 40%





### Dosages and timing of application

- Applied to grapes at 20 – 40 g/ton dissolved in 10x water or juice at 2 – 4 g/hL dissolved in 10x water
- Use higher dosage if low pH (<3.2) and low temp (<10 C)
- Allow 2 - 3 hours (minimum) for enzyme to work

Yeast hulls with immediately available mannoproteins

- Mannoproteins have been shown to have a stabilizing effect on heat unstable proteins (Ribeiro 2016, Dupin *et al.*, 2000a, Gonzalez-Ramos *et al.* 2009)
- Other Benefits: Pro Uno increases wine length and mid palate



## How it's applied and Dosages

- Pro Uno is dissolved in 10x water at 20 g/hL and added to the tank and mixed well into the juice. It can be added just at the onset of fermentation if there is sufficient natural mixing.



## Proactive Protein Stabilization

- Reducing unstable proteins from the start of the process
- Improving quality along the way
- Three products which will reduce bentonite additions:





Just how much money will it cost a winemaker to do this protocol?

☛ At retail cost of the product:

- Zym Arom MP = @ 54\$ for 250 g and 4 g/ hL = 4 c/Gallon
- Tan Skin = @ 425\$ for 1 kg and 10 g/hL = 16 c/Gallon
- Pro Uno = @ 154\$ for 1 kg and 20 g/hL = 11 c/Gallon

Total cost for protocol : 0.31 \$ /Gallon

Compare to treatment with bentolit super: @ 3.25\$ for 1 kg

Dosage is 50 g/hL = .006 \$ / Gallon

☪ Non-Sacc. Yeast derivatives for protein stabilization

**Protection of wine from protein haze using *Schizosaccharomyces japonicus* glycoproteins**

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Thank you for  
your  
participation!  
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