Sustainable Replacement Strategies for Bentonite in Wine using Alternative Fining Agents

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Introduction

Polymeric compounds like proteins, polyphenols, and polysaccharides can interact with one another or other smaller molecules in wine, causing a haze and in severe cases a precipitation in the bottle. Currently, the main solution is the use of bentonite, an aluminium phyllosilicate, which removes proteins based on charge interactions and physical adsorption. While the use of bentonite in wine is fairly well understood, the application has disadvantages associated with handling and disposal, especially if it is used on a larger scale. Efforts to find a safer, less expensive, or more sustainable alternative have been ongoing for several years. A promising and renewable approach is *Saccharomyces paradoxus*, a yeast strain that has high concentrations of chitin in its cell wall and was shown to adsorb protein onto the cell surface. Chitosan is a purified alternative material derived from chitin and was shown to remove chitinases, which can, among others, lead to haze formation in wine. Another option could be carboxymethyl cellulose which has been shown to bind proteins and precipitate them independent of the chemical composition of the wine. Polystyrene surfaces have been studied as a matrix to adsorb proteins for medicinal purposes and to study binding mechanisms. Although other synthetic polymers are also known to bind proteins, the use in wine as a bentonite alternative has not been reported yet.

The objective of this study was to evaluate a selection of different more sustainable protein fining agents in comparison to bentonite in a broad range of wine styles in order to identify an alternative solution for achieving protein stability. While the main experiments were done in real wine, concentration effects and removal rates were verified in model systems as well.

Materials and Methods

Grape cultivars for this experiment were selected based on their expected tendency to develop a higher level of protein instability, with focus on cultivars that usually exhibit low polyphenolic content and high pH values. All grapes were sourced from the California State University Fresno Farm in 2019. Muscat Canelli, Cabernet Sauvignon, and Zinfandel grapes were destemmed and pressed right after hand-harvesting, thus minimizing skin contact and phenolic extraction. The three resulting wines were white or slightly pinkish. A Barbera rose and a Touriga Nacional red wine were partially or fully fermented on the skins to extract phenolic material. All wines were fermented with a *Saccharomyces cerevisiae* yeast (Ionys WF, Lallemand Inc., Montreal, Canada) at 20°C, clarified and stabilized with the addition of 100 ppm of sulfur dioxide. All wines were racked twice and were visually clear before the fining trials.

Table 1 Experimental setup performed on all five wines.

Experiment	Treatment	Dosage rate
Control	/	/
S. paradoxus yeast hulls	Strain P01-161	1 g/L
S. paradoxus yeast hulls	Strain P01-167	1 g/L
Polystyrene	Polystyrene foam beads 2-2.5 mm	0.3 g/L
Chitosan	Chitocel© (AEB)	1 g/L
Bentonite	Na-Ca bentonite	1 g/L

Pure fining agents and additives were added directly or, in the case of bentonite, after swelling in water with 1% citric acid for 12 hours. Fining trials were performed in 375 mL wine bottles. The bottles were placed on a shaker table and agitated for 10 hours overnight. Two sets of samples were taken out of each bottle, centrifuged at 6000 RPM (3750 RCF) for 10 minutes, filtered through a 0.45 µm syringe filter, and analyzed for protein stability.

White and light rosé wines (Muscat Canelli and Cabernet Sauvignon Blanc de Noir) were tested with Bentotest® solution (Erbslöh Geisenheim AG, Geisenheim, Germany) according to the manufacturer's instructions. All wines were also tested with a heat-test method at 80°C for 6 hours. Turbidity in each sample was analyzed by spectrophotometry at 860 nm.

In order to evaluate the concentration effect of selected fining methods, additional trials were performed in duplicate in 50 mL centrifuge tubes. Instead of using wine with an unknown protein concentration, a model system containing 1 g/L egg-white protein was chosen. This variation in methodology allows for a more accurate calculation of removal rates and concentration dependent efficiencies. Fining efficiency was evaluated using a photometric Bradford assay to quantify the remaining protein.

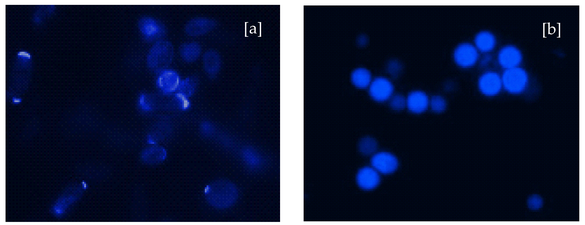

Figure 1 Fluorescence microscopic picture using a Calcufluor White chitin stain on *Saccharomyces cerevisiae* (Ionys WF) [a] and *Saccharomyces paradoxus* (P01-167) [b].

Results and Discussion

The fining trials reveal large differences in efficiency between the additives but also among the range of wine styles that were tested. It was reported before that Bentotest®-treatment leads to higher turbidity readings than a heat-test (84% difference on average in these experiments), most likely because the acids in the testing solution denature most of the proteins, not just the heat labile fraction. The same can be seen for the two white wines that were tested with both methods.

Table 2 Spectrophotometric turbidity readings of treated wine samples at 860 nm.

Treatment	Muscat Canelli		White Zinfandel	Barbera Rosé	Touriga Nacional	Cabernet Sauvignon Blanc de Noir	
	Heat test	Bentotest	Heat test	Heat test	Heat test	Heat test	Bentotest
Control	0.015 ± 0.005a	0.058 ± 0.007a	0.010 ± 0.001a	0.025 ± 0.001a	0.038 ± 0.013a	0.028 ± 0.003a	0.097 ± 0.001a
P01-161	0.015 ± 0.004a	0.058 ± 0.004a	0.007 ± 0.001b	0.017 ± 0.002b	0.035 ± 0.007a	0.001 ± 0.001b	0.033 ± 0.001b
P01-167	0.012 ± 0.001a	0.057 ± 0.002a	0.007 ± 0.001b	0.017 ± 0.001b	0.024 ± 0.007b	0.002 ± 0.001b	0.039 ±0.004b
Polystyrene	0.011 ± 0.001a	0.052 ± 0.003a	0.008 ± 0.001ab	0.013 ± 0.001b	0.014 ± 0.004c	0.001 ± 0.001b	0.027 ± 0.005b
Chitosan	0.011 ± 0.001a	0.039 ± 0.001b	0.013 ± 0.001a	0.019 ± 0.002b	0.018 ± 0.011bc	0.001 ± 0.001b	0.033 ± 0.004b
Bentonite	0.003 ± 0.001b	0.007 ± 0.002c	0.001 ± 0.001c	0.002 ± 0.001c	0.012 ± 0.001c	0.001 ± 0.001b	0.018 ± 0.004c

The protein removal efficiency is higher in white wines (40.5% on average), which could be attributed to phenolic material that competes with proteins for potential binding sites on the surface of the additive. If the data for the *S. paradoxus* strains are combined into one dataset, the difference between white and red wines actually becomes significant (p=0.022). Although the binding mechanism between chitin and haze-forming proteins like chitinases is documented and is not expected to be disrupted by phenolics, yeast cells have a wide variety of surface properties and charges that can bind pigments, tannins, and polysaccharides. This surface cover might essentially disrupt the efficient fining performance in red wines more than in whites.

Chitosan showed a slightly higher efficiency than the chitin in white wines, which might be due to the purified form that was added. In red wines, however, the protein removal rate was the lowest of all fining agents tested. The reasons for this can be speculated to be due to stronger interactions and higher affinities between tannins, polysaccharides, and the chitosan, making the binding sites unavailable for proteins. The difference between white and red wine efficiency is less pronounced with bentonite and polystyrene, which can be attributed to the mechanism with which these fining agents bind to protein. It is more likely that a changing degree of polarity, hydrophobicity, and surface charges remove different fractions of proteins from wine. A combination of both fining agents could potentially improve the efficiency by lowering the required addition, and as a result, making the use more sustainable.

In model wine, the addition of bentonite reduces the protein concentration stepwise by about 60% of the initial concentration (R^2=0.998). A similar behavior can be observed with *Saccharomyces paradoxus*, however, the reduction rate is lower with around 40% at the highest addition and confirms the range that was observed in real wine. The chitosan results prove the selective nature of the fining mechanism, since it only binds to chitinases that are not present in egg-white. Polystyrene displays a fining efficiency that is independent from the concentration that was added to the model wine, most likely due to the hydrophobic nature of the foam spheres and the mixing problems that are associated with that. CMC shows promising results but the application needs to be optimized to determine dosage rates and recommendations .

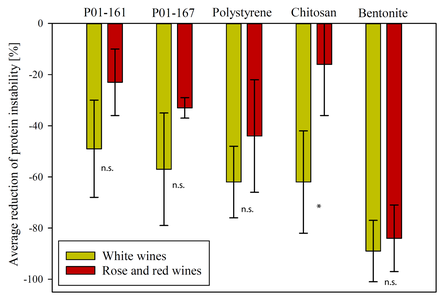

Figure 2 Average reduction in protein haze in the experimental wines arranged by fining agent and wine style. Error bars represent all variability among grape cultivars and experimental replicates. Statistical analysis by t-test shows significant differences between white and red wines for each treatment (*: significant, n.s.: not significant).

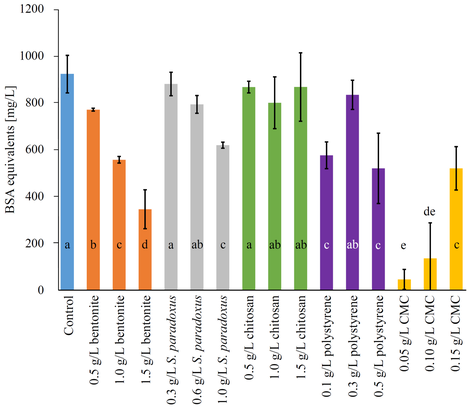

Figure 3 Fining efficiency in model wine analyzed via Bradford assay. Error bars represent experimental duplicates. Statistical analysis shows differences from the control ($p < 0.05$).

Conclusion

For wines that have a high level of protein instability, bentonite remains the only fining agent that is able to remove haze proteins reliably. Alternative more sustainable treatments like *Saccharomyces paradoxus*, polystyrene, chitosan, or CMC might be an alternative for products with lower protein instabilities, however, there is a tendency for higher efficiency in white wines compared to red wines.

Literature

[1] Ndlovu, T. et al. (**2018**). Applied and Environmental Microbiology, 84, e00668–18.
[2] Sommer, S. et al. (**2021**). Sustainability 13, 1860.

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