Technical Brief

Assessment of Potential Effects of Common Fining Agents Used for White Wine Protein Stabilization

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Abstract: A variety of fining agents are commercially available to the wine industry, including proteins and inorganic ion exchangers. These fining agents are essentially used to control the levels of phenolics in wine, but they also have the potential to interact with other wine components, most often as a side effect. They are therefore expected to influence, at least in part, the potential for wine protein haze formation. Six common fining agents—casein, egg albumin, isinglass, chitosan, chitin, and polyvinylpolypyrrolidone (PVPP)—were analyzed to assess their effects on wine protein haze-forming potential and on the levels of proteins and phenolic compounds in a Muscat of Alexandria wine. Bentonite was selected as the positive control, whereas nonfined wine was used as the negative control. Differential results were detected among the selected fining agents when compared to the controls. Egg albumin and chitosan, although incapable of stabilizing the wine, originated a small but significant decrease in the protein haze formed, whereas chitosan and PVPP were second to bentonite in removing the most polyphenols from the wine. Thus, while chitosan fining removes a fraction of polyphenols from the wine and seems to induce a small decrease in its haze-forming potential, PVPP eliminates more polyphenols while leaving its haze-forming potential unaltered. The fining agents analyzed did not significantly affect wine protein content but did remove considerable levels of polyphenols and presented no apparent effect on protein stabilization of the fined wines. Results show that these fining agents do not contribute significantly to protein stabilization in white wines, confirming that bentonite was the most effective agent in wine protein stabilization.

Key words: fining agent, haze, polyphenol, protein, white wine, wine

Enological fining is a typical practice to clarify and, depending on the fining agent, stabilize wines. Fining is responsible for elimination of some phenolic compounds of a colloidal nature, implicated in oxidation phenomena and excess astringency, thus contributing to the improvement of some sensory characteristics of wines (Sims et al. 1995, Castillo-Sánchez et al. 2008). When used during fermentation, the fining agents act as insoluble solids which promote yeast growth, helping fermentation to proceed faster and to completion (Groat and Ough 1978). Fining agents are diverse and are usually prepared from animal proteins (Ribéreau-Gayon et al. 2006), vegetable proteins (Maury et al. 2003, Tschiersch et al. 2010), and inorganic compounds (Puig-Deu et al. 1999). With the emergence of bovine spongiform encephalopathy in the 1980s, that has been considerable interest in the replacement of animal-derived protein (in particular gelatin) in food processing (Karim and Bhat 2008) and the alternative use of plant-derived proteins (Puig-Deu et al. 1999).

Proteins commonly used as fining agents exhibit a wide diversity of molecular masses, isoelectric points, and/or surface charge densities. These characteristics have been shown to influence differentially the properties of the fining agents under the conditions prevailing in each wine and their interactions with specific wine components (Versari 1998, Tschiersch et al. 2010). Protein fining agents can form complexes with wine tannins, resulting in negatively charged hydrophobic colloids that precipitate in the presence of metal cations. This reaction will induce particle association which will precipitate after flocculation. Proteins that do not react with tannins may combine with particles in suspension or in colloidal solution, most of which are negatively charged. Other proteins (such as casein) will flocculate exclusively due to the low wine pH, but the presence of tannins is necessary for precipitation and clarification (Ribéreau-Gayon et al. 2006). Nonprotein fining agents are now being used, such as polyvinylpolypyrrolidone (PVPP), activated charcoal, chitosan, and chitin (Bornet and Teissedre 2008, Tschiersch et al. 2008, Sanborn et al. 2010). Chitosan and chitin have also been described to reduce wine heavy metals content (Pb and Cd), Fe, and ochratoxin A, thus improving wine safety (Bornet and Teissedre 2008). Bentonite, a cation exchanger clay, is an inorganic fining agent that removes wine proteins by electrostatic adsorption (Pocock et al. 2011). Additional bentonite effects include electrostatic interactions with other nitrogen compounds (such as peptides;

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Gorinstein et al. 1984) and, theoretically, with all compounds bearing/carrying a positive net charge at wine pH. The objectives of fining wines are achieved essentially by removing several wine components, including phenolics, which are involved not only in the phenomena described above but also in wine antioxidant activity (Ghiselli et al. 1998), eventually interacting with wine proteins to form haze (Pocock et al. 2007).

White wine protein haze formation is associated with the presence of residual proteins that can aggregate into light-dispersing particles under elevated temperatures during storage or transportation (Waters et al. 1992). Protein haze formation is now recognized as a multifactorial process, in which there is an absolute requirement for one or more as yet unknown nonproteinaceous wine components, generally termed the X factor, in addition to the wine proteins themselves (Batista et al. 2010). It has been suggested that phenolic compounds are probably involved in the mechanism of haze appearance in white wines (Esteruelas et al. 2011), corroborating another study that reported PVPP fining of commercial wines resulted in a reduction of protein haze formation after heat stability testing (Pocock et al. 2007).

The current use of numerous fining agents by the wine industry deserves an evaluation of their potential side effects, that is, interactions with wine components other than those for which the agent is best suited. In this study, six fining agents were selected (casein, egg albumin, isinglass, chitosan, chitin, and PVPP), all known to remove polyphenols, improve clarification processes, and clarify wines with different haze problems (Spagna et al. 1996, OIV 2012a). They are all widely used by the wine industry except for chitin. Chitosan has also been reported to prevent protein haze by the partial precipitation of excess proteinaceous matter (OIV 2012a). Therefore, the main goal of this study was to study the effect of these six fining agents on both total phenolic and total protein contents of a wine, thus evaluating their impact in the prevention of protein haze formation. Bentonite fining was used as the positive control.

Materials and Methods

Wine. The wine used in all experiments was a varietal Moscatel of Alexandria white wine, 2009 vintage, from the Terras do Sado region, Portugal. The grapes were mechanically harvested in September 2009, pressed, and the free running juice was fermented at controlled temperature. No malolactic fermentation took place. The resulting wine had an alcohol content of 14.3% (v/v), 5.6 g/L total acidity (expressed as tartaric acid), 0.33 g/L volatile acidity (expressed as acetic acid), pH 3.2, 25 mg/L free SO_2, and 144 mg/L total SO_2. The wine was divided into 100 mL aliquots and stored frozen at -20°C until used.

Protein instability test. Evaluation of the wine susceptibility to form protein haze was according to a published protein instability test (Bruijn et al. 2009) with minor modifications. Five mL samples of each wine were saturated with nitrogen and sealed in test tubes with screwcaps. The samples were heated at 80°C for 2 hr in a water bath, followed by cooling at 6°C for 14 hr. After allowing the samples to warm to 25°C, the increase in turbidity was detected spectrophotometrically at 540 nm in 1 mL plastic cuvettes. Differences in wine turbidity (before and after the heat treatment) have been reported to correlate directly to wine protein instability (Pachova et al. 2002). All measurements were performed in triplicate for posterior statistical analysis.

Total polyphenol index. The total polyphenol index was calculated as described in method OIV-MA-AS2-10 (OIV 2012a). This index derives from the oxidation of the wine phenolic compounds by the Folin-Ciocalteu reagent, originating a blue-colored product with an absorption maximum at 750 nm, and is proportional to the total quantity of phenolic compounds originally present in the wine analyzed (OIV 2012a).

Fining agents and experiments. The fining agents analyzed in this work were casein (C7078, Sigma-Aldrich, St. Louis, MO), egg albumin (A5503, Sigma-Aldrich), chitosan (C3646, Sigma-Aldrich), chitin (C9753, Sigma-Aldrich), isinglass (Cristalline Supra, IOC, Epernay, France), polyvinylpyrrolidone (77627, Fluka, Buchs, Switzerland), and sodium bentonite (Enartis, Trecarte, Italy) (Table 1).

Fining experiments involved the addition of standard concentrations of the selected fining agents, prepared as recommended by the corresponding manufacturers (Table 1). The trials were performed at a laboratory scale using 50 mL aliquots of wine. Unfined wine was used as the negative control. Wine previously clarified by centrifugation at 4300 *g* for 15 min and fined with 1 g bentonite/L was used as the positive control. The fining agents were added to wine, previously clarified by centrifugation at 4300 *g* for 15 min, and incubated for 72 hr at 25°C. The samples were then centrifuged at 4300 *g* for 15 min and the pellet was discarded before analysis. All experiments were performed in triplicate.

Statistical analysis. All data are expressed as the mean ± standard deviation (n = 3). Statistical comparisons between values were established with one-way ANOVA and Tukey's post-hoc test ($p = 0.05$) using Statistica software (ver. 8; StatSoft, Tulsa, OK).

Results and Discussion

Effect on protein haze formation. Samples of the Moscatel of Alexandria white wine were individually treated with each of the six fining agents, with unfined wine and bentonite-fined wine as the negative and positive controls, respectively. All samples were subsequently subjected to the heat

Table 1 Commercial fining agents used for fining Moscatel of Alexandria white wine.

Agent	Concn	Manufacturer
Casein	40 g/hL	Sigma-Aldrich
Egg albumin	10 g/hL	Sigma-Aldrich
Isinglass	4 g/hL	Cristalline
Chitosan[a]	100 g/hL	Sigma-Aldrich
Chitin[b]	100 g/hL	Sigma-Aldrich
PVPP	80 g/hL	Fluka
Bentonite	100 g/hL	Enartis

[a]Minimum 85% deacetylated.
[b]From crab shells.

stability test to assess the possible effects of the fining agents on the wine haze-forming potential (Figure 1). As expected, fining a wine sample with 100 g bentonite/hL (positive control) warrants the wine protein stability. Fining with bentonite is still the most widely used treatment to prevent protein haze formation in white wines (Pocock et al. 2011). The mechanism underlying bentonite fining differs from those displayed by all other fining agents analyzed in this study, as it removes wine proteins (carrying a net positive charge at the wine pH) by electrostatic adsorption because of its net negative charge. Bentonite fining was therefore selected as the positive control in the present study by comparing the effectiveness of this treatment to those displayed by the other fining agents.

Both positive and negative controls behaved as expected (Figure 1). Among the six different fining agents, four (casein, isinglass, chitin, and PVPP) did not produce any statistically significant effect when compared to the negative control. However, two fining agents (egg albumin and chitosan) originated a small but significant decrease in the haze formed after the heat stability test. Nevertheless, the wines fined by egg albumin and chitosan were still considered susceptible to forming protein haze, presenting an absorbance value higher than 0.02, the pass-fail point in protein stability tests suggested by Pocock and Waters (2006).

PVPP fining produced no significant difference when compared to the negative control. This particular result does not agree with Pocock et al. (2007), who reported that PVPP-fined wines formed less haze after heating than the unfined controls. However, these results are in accordance with the conclusion drawn by the same authors that wine protein haze formation cannot be eliminated by removing polyphenolic compounds by PVPP. In an attempt to understand if the effect of PVPP on wine protein haze formation is somehow related or dependent upon incubation time, wine samples were incubated in the presence of PVPP (80 g/hL) for 0 (control), 1, 5, and 72 hr. A 1 hour-long incubation was assumed as a reasonable time for the interaction of PVPP with the wine components. The results obtained after fining suggest that the contact time of PVPP with the wine sample does not influence removal of the compounds which interact with wine proteins to form haze (Table 2). In contrast, after PVPP addition to tea, catechin adsorption attained equilibrium after 5 hr (Dong et al. 2011). PVPP exhibits a high affinity toward wine total phenols and flavonoids. It has been reported to remove these compounds from white wines (Sims et al. 1995) and to affect the particle size of denatured, aggregated proteins, possibly through cross-linking. Results here indicate that PVPP treatment does not affect the haze protein potential of the wine and is independent of the duration of PVPP treatment (Table 2). Moreover, trials performed in our laboratory using higher doses of PVPP on sample wines (above the maximum established by the OIV) presented no significant difference after heat stability test when compared to the untreated wine (data not shown).

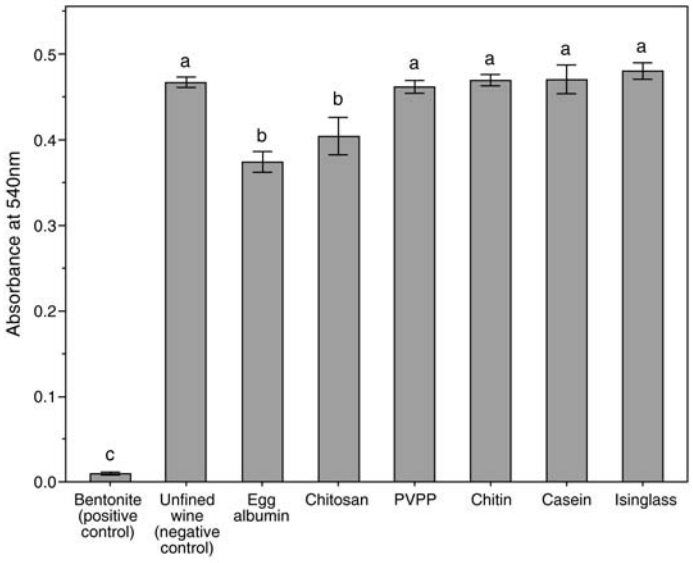

Figure 1 Changes in turbidity detected by the difference in the absorbance at 540 nm in samples of fined wine and unfined (negative control) wine measured after heat stability test. Bars indicate mean ± SD (n = 3). Different letters represent different homogeneous subsets for $p = 0.05$.

Chitosan fining is described as a treatment to prevent protein haze (OIV 2012b). The wine fined with chitosan (100 g chitosan/hL wine; the maximum recommended by OIV) presented a minor but significant decrease in haze formed when compared with the unfined control, which was not strong enough to stabilize the wine (Figure 1). This result seems to indicate that chitosan is not a good option to guarantee protein haze stability or, in other words, the maximum chitosan concentration recommended is not sufficient to ensure removal of the compounds that interact with the wine proteins to form haze.

Effect on the levels of phenolic compounds. According to the Folin-Ciocalteu index, the positive control, bentonite fining, removed the greatest proportion of the wine total phenolic fraction (Figure 2). These results are in accordance with other reports that wines fined with bentonite showed a significant decrease in total phenolics (Main and Morris 1994). In addition to adsorbing proteins (such as polyphenoloxidase; PPO), bentonite is described to remove other positively charged molecules as well as phenols (Main and Morris 1991).

As expected, all six fining agents removed significant amounts of phenolics from the wine, with chitosan and PVPP the most efficient. Chitosan fining does not apparently significantly affect wine protein (Figure 3), does remove a significant fraction of polyphenols from the wine (Figure 2), and seems to induce a small decrease in its haze-forming potential (Figure 1). PVPP also does not seem to significantly affect wine protein (Figure 3), does eliminate a higher fraction of polyphenols

Table 2 Wine protein haze formed after the heat stability test performed on a wine sample previously incubated with 80 g PVPP/hL for different periods of time. Values are mean ± SD (n = 3).

	Wine protein haze (Δ540 nm)			
	Control	1 hr	5 hr	72 hr
Sample wine	0.467 ± 0.006	0.457 ± 0.006[a]	0.460 ± 0.007[a]	0.462 ± 0.007[a]

[a]Not significant compared to the control ($p = 0.05$).

from the wine compared to the other fining agents, but leaves the haze-forming potential unaltered (Figure 1). The most logical explanation for these different effects relies on different classes of wine phenols removed by each of the fining agents.

It has been suggested that haze formation during storage of white wines is a result of temperature fluctuations and of a decrease in the wine redox potential, which may facilitate the exposure of protein hydrophobic binding sites suitable for tannin complexation (Marangon et al. 2010). Possibly, the mechanism described above is not the main factor responsible for protein haze formation, since the PVPP-fined wine, containing less polymeric polyphenols (especially the high molecular mass polymeric tannins), presented no significant difference in the formed haze when compared to the control wine after heat stability test.

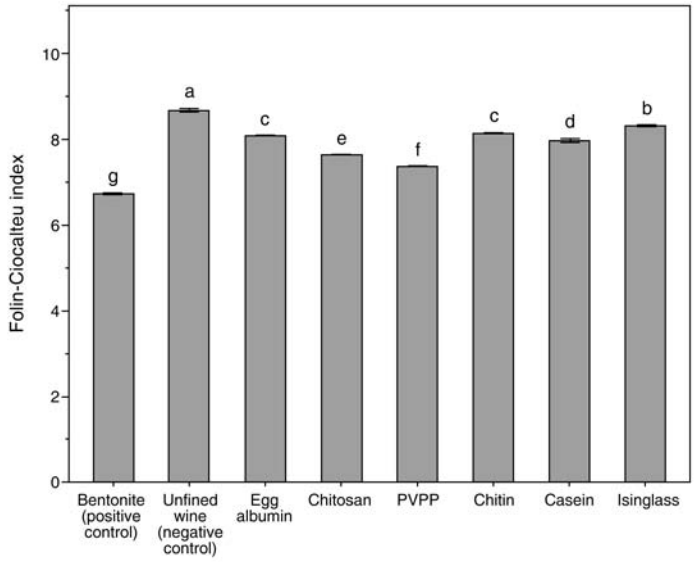

Figure 2 Folin-Ciocalteu index of the wine samples after fining with the different agents under analysis. Bars indicate mean ± SD (n = 3). Different letters represent different homogeneous subsets for $p = 0.05$.

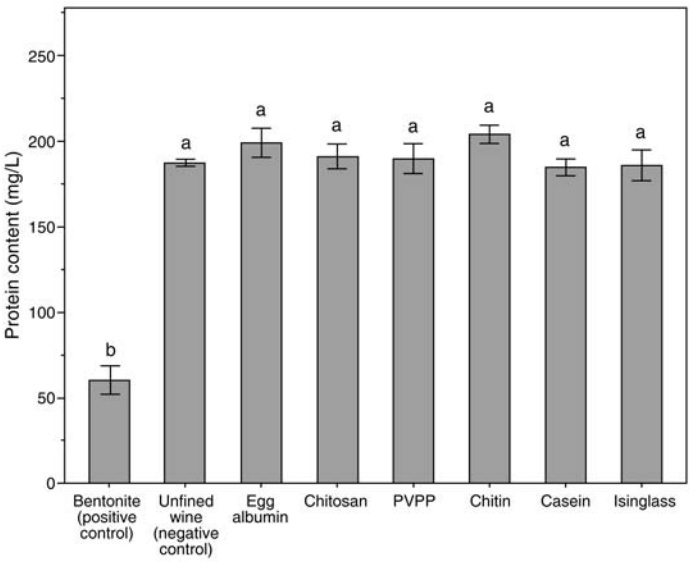

Figure 3 Protein content (in mg/L) of the wine samples after fining with different fining agents. Bars indicate mean ± SD (n = 3). Different letters represent different homogeneous subsets for $p = 0.05$.

Effect on protein levels. After analyzing the effect of the six fining agents on wine protein haze formation potential and wine phenolic content, the next logic parameter was to determine whether any of the agents removed wine proteins to any significant extent and whether any exogenous proteins (those comprising some of the fining agents themselves) remained after fining. After protein quantification, no significant differences were detected when the negative control was compared the fined samples (Figure 3). Slightly higher protein content was detected in the wine fined with chitin and egg albumin compared to the unfined wine. For egg albumin fining, this observation may indicate that some exogenous protein may have been left behind, remaining dissolved in the wine after centrifugation but with no detectable negative impact on the wine protein haze formation potential, as assessed by the heat stability test. In contrast, egg albumin fining did slightly reduce the haze-forming potential of the wine (Figure 1). The importance of this discussion on possible incremental wine protein content derives from the potential health risks posed by the proteins themselves, in particular by the animal-derived proteins known to elicit clinical reactions in food-allergic patients (such as egg albumin). However, Kirschner et al. (2009) reported that wines treated with fining agents, including egg albumin applied at commercial concentrations, do not appear to present health risks to allergic individuals when filtered after fining.

As expected, the positive bentonite control had a residual protein content of 60 mg/L, but presented no visible haze after heat stability test. This result may indicate that protein haze formation in white wines is triggered only above a certain protein concentration threshold. Dawes et al. (1994) showed that haze formation and protein levels in wine decreased in parallel upon bentonite fining, fully stabilizing the fined wine to 23.3 mg protein/L. It would be of great interest to identify the residual proteins left after full bentonite-induced wine stabilization and to determine the lower and upper limits of protein concentration which trigger white wine protein haze formation, although that will most certainly also depend on factors of nonproteinaceous origin, such as wine pH and the X factor, meaning that they will likely vary from wine to wine.

Conclusion

The fining agents casein, egg albumin, isinglass, chitosan, chitin, and PVPP did not significantly affect the tendency of the wine to form protein haze. Comparisons were established among the six fined wine samples, together with an unfined wine sample (negative control) and a bentonite-fined wine sample (positive control). The fining agents here are known to remove essentially phenolic compounds, considered as capable of interacting with wine proteins to form haze, which they did to varying extents. PVPP removed the highest amount of phenolic compounds followed by chitosan, casein, egg albumin, chitin, and isinglass. These results clearly support the view that phenolic compounds, or at least those removed by the fining agents, albeit possibly interacting with the wine

proteins, are not the main cause/factor involved in white wine protein haze formation.

Bentonite fining removed significantly both protein and phenolic compounds from the wine, stabilizing it after heat stability test. With the single exception of the positive control (wine fined with bentonite), the protein content of all fined samples remained essentially constant, indicating that there was no protein removal or addition during the fining trials.

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