LWT - Food Science and Technology 241 (2026) 119055

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Use of proteases combined with heat treatment for protein stabilisation of white wines improves their foaming properties

Nadia Gregori [a], Jordi Gombau [a], Arnau Just-Borràs [a], Dolores Pérez [b], Jose Maria Heras [b], Fernando Tarin [c], Francesca Fort [a], Joan Miquel Canals [a], Fernando Zamora [a,*]

[a] Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, C/Marcel.li Domingo 1, 43007, Tarragona, Spain
[b] Lallemand Bio S.L., Barcelona, Spain
[c] Cheste Agraria Coop. V., Calle Estación 5 y Calle La Bodega n°2, Cheste, 46380, Valencia, Spain

ARTICLE INFO

Keywords:
White wine
Proteins
Protein haze
Bentonite
Proteases
Foaming properties

ABSTRACT

In this study we evaluate the effectiveness of combining protease treatment with heat treatment as an alternative method to bentonite fining for achieving protein stability in white wines. Our results demonstrate that protease treatment can achieve complete protein stability or, at least, substantially reduce the amount of bentonite needed in highly unstable varieties such as Muscat of Alexandria. This reduction or even elimination of the amount of bentonite required mitigates its adverse effects. In addition to its role in achieving protein stability, protease treatment significantly enhances both the foamability (HM) and foam stability (HS) of wines. Notably, these improvements persist even when protease treatment is followed by bentonite fining. These enhancements offer a considerable advantage in the production of sparkling wines, especially in regions or with grape varieties that inherently produce base wines with limited foaming potential. Protease treatment could therefore play a valuable role in optimising the sensory and textural qualities of sparkling wines, thereby expanding production possibilities particularly in regions or with grape varieties that typically yield base wines with limited foaming capacity.

1. Introduction

Although wine proteins are generally found in very low concentrations, their study is of great interest for oenological science for several reasons. Unstable proteins may aggregate into light-scattering particles, resulting in wine turbidity and, in some cases, visible precipitation phenomena collectively known as protein haze (Esteruelas et al., 2009a; Van Sluyter et al., 2015; Waters et al., 1991). The main proteins responsible for protein haze formation are thaumatin-like proteins and chitinases, which belong to the group of pathogenesis-related proteins derived from grapes (Marangon et al., 2011; Waters et al., 1996). In addition, proteins such as β-glucanase, grape ripening-related proteins such as GRIP22 and GRIP32, invertase, and lipid transfer proteins are recognised as minor contributors to haze formation (Esteruelas et al., 2009a; Falconer et al., 2010; Sauvage et al., 2010).

The appearance of haze in a bottle of wine can seriously harm both the image of the wine and its acceptance by consumers (Carrasco & Siebert, 1999; Siebert, 2009). Winemakers therefore apply treatments to prevent protein haze formation (Cosme et al., 2020; Waters et al., 2005). However, treatments applied to prevent this problem (mainly bentonite fining) can seriously affect wine quality (Moine-Ledoux & Dubourdieu, 1999; Pocock et al., 2011; Ubeda et al., 2021). For example, some authors have reported that treatment with very high levels of bentonite can negatively affect both wine aroma (Lambri et al., 2010; Lubbers et al., 1996; Vincenzi et al., 2015) and mouthfeel (Guillou et al., 1998; He et al., 2020; Ribeiro et al., 2014). Using bentonite can also lead to volume loss and waste generation in the wine due to its swelling and settling properties (Horvat, Radeka, Plavsa, & Lukic, 2019). Other authors have also found a close relationship between protein concentration and foam quality in sparkling wines (Brissonet & Maujean, 1993; Medina-Trujillo et al., 2017; Vanrell et al., 2007). Proteins act as surfactant substances, which enhance foam stability and appear to contribute to film elasticity and film strength (Aguié-Béghin et al., 2009; Malvy et al., 1994; Senée et al., 1999). For this reason, bentonite treatment negatively impacts the foaming properties of sparkling wines (Andrés-Lacueva et al., 1996; Puig-Deu et al., 1999). Winemakers,

* Corresponding author.
E-mail address: fernando.zamora@urv.cat (F. Zamora).

https://doi.org/10.1016/j.lwt.2026.119055
Received 18 September 2025; Received in revised form 11 January 2026; Accepted 16 January 2026
Available online 19 January 2026

especially those who produce sparkling wines, are therefore greatly interested in alternative methods to bentonite for achieving protein stabilisation in white wine.

In recent years various alternative methods to bentonite treatment for achieving protein stabilisation in white wines have been investigated (Silva-Barbieri et al., 2022). These methods have been designed to minimise or prevent the undesirable impacts of bentonite, such as aroma loss and foam stability reduction in sparkling wines.

The most promising methods for stabilising wines against protein haze are ultrafiltration (Hsu et al., 1987), flash pasteurisation (Pocock et al., 2003), use of zirconium (Salazar et al., 2010), treatment with heat and proteases (Marangon et al., 2012), high hydrostatic pressure (Tabilo-Munizaga et al., 2014), use of zeolites (Mierczynska-Vasilev et al., 2019), treatment with high-power ultrasound (Celotti et al., 2021), and treatment with functionalised mesoporous silica (Marangon et al., 2023). Although all these approaches have shown potential in reducing or eliminating protein instability in wine, they vary significantly in terms of practicality, cost, and scalability. Despite growing interest in these innovative techniques, the only method that has been significantly implemented in commercial winemaking is treatment with heat and proteases (Robinson et al., 2012, pp. 24–30; Seidel et al., 2025). Combining the use of proteases with heat treatment is reported to be better than applying treatment alone on account of protein unfolding due to heat treatment since unfolded proteins are more susceptible to proteolysis (Comuzzo et al., 2020). In this context, Marangon et al. (2012) reported that using aspergillopepsins in combination with the flash pasteurisation of grape must is a promising alternative to bentonite fining. The proteases remained active against wine proteins, and the heat treatment did not negatively affect the wine's sensory properties. This technique is therefore considered the most feasible and effective alternative to traditional bentonite fining.

However, since proteins play a key role in the formation and stability of foam in sparkling wines, we need to consider whether their partial degradation by proteolytic enzymes influences the wine's foaming properties and compare this impact with that of conventional bentonite treatment. In this study, therefore, we analyse how protease treatment at the winery scale affects the foaming properties of base wines for sparkling wine production. The results indicate that protease treatment is not only effective in eliminating the risk of protein haze, but also improves the foaming properties of base wines, suggesting that it may be a very promising tool for improving sparkling wine production.

2. Materials and methods

2.1. Chemicals and equipment

Absolute ethanol (96 % v/v) and hydrochloric acid (≥37.0 %) were purchased from Panreac (Barcelona, Spain). Water was ultrapure Milli-Q quality (Millipore, Bedford, MA, USA). Ammonium acetate (≥99.0 %), ammonium formate (≥98.0 %), and bovine serum albumin (≥98.0 %) were purchased from Sigma-Aldrich (Madrid, Spain). A pullulan molecular weight calibration kit Shodex P-82 was obtained from Waters (Barcelona, Spain), while a pullulan 1.3 kDa and four dextrans Bio-Chemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, MO, USA). The polysaccharides used as external standards for quantification were pectins from citrus fruit (≥90 %) and dextrans from *Leuconostoc mesenteroides* (≥99.9 %) purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Wines

This study was conducted at full winery scale, thereby providing greater validity to the results. Two wines from AOC Valencia (Spain) made from grape varieties Macabeo (Variety number VIVC, n.d.: 13127) and Muscat of Alexandria (Variety number VIVC, n.d.: 8241) were used in this experiment. These varieties were selected due to their low protein instability potential (Macabeo) and high protein instability potential (Muscat of Alexandria). The wines were produced and processed at the *Cheste Agraria Cooperativa Valenciana* winery following standard winemaking protocols and without the addition of bentonite. The general analytical parameters of each wine are provided in Table 1, which shows that both wines exhibit highly typical and common analytical data for these types of wines. The wines were kept during all the process protected from air and at a temperature of 16 ± 2 °C.

The bentonite doses required for protein stabilisation of the wines were determined using the rapid heat test (Sarmento et al., 2000) according to the protocol described by Esteruelas et al. (2009b).

A total volume of 50,000 L of each wine was used for stabilisation treatment following the scheme shown in Fig. 1. For both wines, 5000 L were allocated for stabilisation with bentonite, while the remaining 45,000 L were subjected to protease treatment.

To clarify the wines with bentonite, two types of calcium-sodium bentonites supplied by Erbslöh Geisenheim GmbH (Geisenheim, Germany) were used: BlancoBent (powder form) and Aktivit (granular form). Macabeu wine was treated with 200 mg/L of BlancoBent and 200 mg/L of Aktivit, which represents a total of 400 mg/L of bentonite. Muscat of Alexandria wine, on the other hand, was treated with 2000 mg/L of BlancoBent and 2000 mg/L of Aktivit, which represents a total of 4000 mg/L of bentonite.

Protein stabilisation with proteases was performed by adding 50 μL/L of the commercial pectolytic enzyme Lallzyme Stabpro™ (Lallemand Inc, Montreal, Canada). This enzyme is a liquid preparation of aspergillopepsin I (EC 3.4.23.18). The wine was subsequently subjected to a controlled heat treatment consisting of an initial pre-heating phase of 2 min at 60 °C, followed by flash pasteurisation at 80 °C for 90 s. This two-step process was implemented to ensure partial denaturation of the wine proteins, as unfolded protein structures are more accessible to enzymatic cleavage. The rationale behind this approach lies in the fact that proteases exhibit greater efficiency when acting on denatured substrates, thereby facilitating the hydrolysis of proteins responsible for haze formation and improving the overall clarification efficiency of the treatment (Comuzzo et al., 2020). According to EFSA (2025), the activity of this enzyme declines above 30 °C and is completely lost at 70 °C; consequently, virtually no enzymatic activity is anticipated to persist under such conditions. The wines were then immediately cooled to 6–7 °C.

Macabeo wine was fully stable after protease treatment, whereas Muscat of Alexandria wine remained unstable despite a significant reduction in protein instability. Muscat of Alexandria wine was therefore then treated with 1000 mg/L of BlancoBent and 1500 mg/L of Aktivit, which represents a total of 2500 mg/L of bentonite. After this treatment the wine was fully stable.

2.3. General parameters of the wines

Ethanol content, titratable acidity, pH, total sulphur dioxide and volatile acidity were determined according to the analytical method recommended by the International Organisation of Vine and Wine (OIV, 2020).

Table 1
General parameters.

Parameter	Macabeo	Muscat of Alexandria
Ethanol content (% v/v)	11.5	12.0
Titratable acidity (g tartaric acid/L)	4.9	4.6
pH	3.70	3.50
Volatile acidity (g acetic acid/L)	0.2	0.25
Total sulphur dioxide (mg/L)	125	80

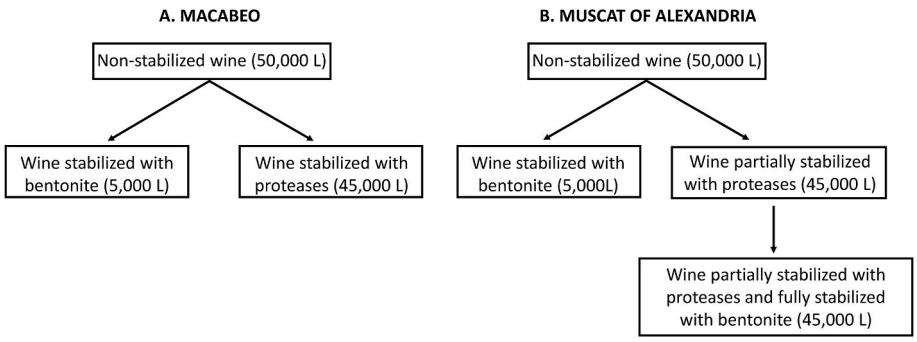

Fig. 1. Experimental protocol.

2.4. Protein stability test

The wines were centrifuged (10 min at 12,000 g) and filtered (0.45 μm) before use for the stability tests. All stability tests were performed in triplicate in accordance with the fast heat test (Sarmento et al., 2000). Briefly, 40 mL of wine was subjected to 90 °C for 1 h in a thermostatic water bath, followed by a temperature of 4 °C for 6 h in a refrigerator. The wines were considered protein stable when the increase in turbidity between a heated sample and an unheated control was less than 2 NTUs. All samples were equilibrated at room temperature (25 °C) prior to turbidity measurements. The samples were stirred well and turbidity was measured against a blank using a Hach 2100N Turbidimeter (Hach Company, Loveland, CO, USA).

2.5. Protein extraction and determination by HRSEC-DAD

The samples were processed and analysed by HRSEC-DAD using the method described by Canals et al. (1998). Aliquots (15 mL) of the white wine were concentrated following two-step dialysis in tubes with a MM cut-off of 3.5 kDa (Membrane Filtration Products, San Antonio, TX, USA). The first step was completed over 48 h with distilled water following the original protocol (water dialysed, or WD) or with 0.3 mol/L ammonium acetate solution (ammonium acetate dialysed, or AAD). In both cases a sample-to-solution ratio of 1:10 was applied with constant agitation. A new dialysis step was then immediately completed for all samples with distilled water for a further 48 h using the same sample-to-solution ratio and with constant agitation. The dialysed samples (WD and AAD) were then lyophilised and frozen at −20 °C. The lyophilised samples were re-suspended in 0.6 μL of ammonium acetate solution (300 mmol/L) and centrifuged at 12,000 g for 5 min. The supernatant was filtered through 0.22 μm acetate cellulose filters (Merck Millipore) and 100 μL of supernatant was then injected into the chromatographic system. Analyses were carried out in an HPLC Agilent 1200 Series system (Agilent Technologies, Barcelona, Spain) with a DAD to monitor output at 230, 280, 320 and 420 nm. Separation was achieved at 20 °C using an S 165 Shodex gel permeation HPLC column 210 (OHpak 166 SB-803 HQ, 300 mm × 8 mm i.d.; Showa Denko). The mobile phase comprised an aqueous solution of 300 mmol/L ammonium acetate applied at a constant flow rate of 0.6 mL/min for 70 min. The molecular weight (MW) distribution of the protein wine fractions was determined by calibration with a Gel Filtration Calibration Kit LMW (Healthcare, Uppsala, Sweden). The eluting proteins were quantified according to the peak area for each fraction using the external standard method with a bovine serum albumin standard (Sigma-Aldrich, Madrid, Spain) ranging from 0 to 1 mg/mL ($r^2 > 0.99$). All assays were performed in triplicate.

2.6. Polysaccharide extraction and determination by HRSEC-RID

The samples were processed using the method described by Ayestarán et al. (2004). Briefly, 10 mL of sample in triplicate were concentrated to a final volume of 2 mL using a vacuum evaporator (Univap 148 100ECH; Progen Scientific, London, UK). Total soluble polysaccharides were precipitated by adding 10 mL of cold acidified ethanol (hydrochloric acid 0.3 M in absolute ethanol) and kept for 24 h at 4 °C. The samples were then centrifuged (10,000×*g* for 15 min) and the supernatants discarded. Finally, the precipitates were dissolved in 1 mL of ultra-pure water, frozen to −20 °C and freeze-dried using a lyophiliser (Telstar LyoQuest HT40, Barcelona, Spain). The soluble fractions were analysed by high-resolution size-exclusion chromatography (HRSEC) to determine the molecular distribution and quantify the polysaccharides obtained from the samples. The lyophilised samples were resuspended in 1 mL of 50 mM ammonium formate and filtered through 0.22 μm acetate cellulose filters (Merck Millipore, Darmstadt, Germany). Then 100 μL were injected into the chromatographic system. The analyses were performed in an HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, USA) with a refractive index detector. Separation was conducted at 20 °C using two Shodex gel permeation HPLC columns (OHpak SB-186,803 HQ and SB-804 HQ, 300 mm × 8 mm I.D.; Showa Denko, Japan). The mobile phase consisted of an aqueous solution of 50 mM ammonium formate applied at a constant flow rate of 0.6 mL/min for 60 min, and the cell RID temperature was 35 °C. The molecular weight distribution of the wine fractions was monitored by calibration with a Shodex P-82 pullulan calibration kit (P-5, MW = 5.9 kDa; P-10, MW = 11.8 kDa; P-20, MW = 22.8 kDa; P-50, MW = 47.5 kDa; P-100, MW = 112 kDa; P-200, MW = 212 kDa; P-400, MW = 404 kDa; and P-800, MW = 788 kDa) purchased from Waters (Barcelona, Spain) and four dextrans (BioChemika; 12, 25, 50 and 80 kDa) purchased from Fluka (St. Louis, MO, USA). The polysaccharides were quantified according to the peak area for each fraction using the external standard method with pectin and dextran commercial standards (Sigma–Aldrich, Saint Louis, MO, USA) in a range of 0–2 g/L ($r2 > 0.99$).

2.7. Measuring the foam properties

The samples were degassed using a magnetic stirrer for 15 min, followed by centrifugation at 4000×*g* for 5 min at 4 °C. The foam properties were then measured using the Mosalux method (Station Oenotechnique de Champagne, Epernay, France) following the procedure described by Maujean et al. (1990) and adapted by Just-Borras et al. (2022). Each sample was analysed in triplicate. Parameters assessed in this study included maximum foam height (HM) and stable foam height (HS). HM reflects foamability, while HS indicates foam stability.

2.8. Statistics

All analytical data are expressed as the arithmetic average ± the standard deviation of three replicates. One-factor analysis of variance (ANOVA) was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL). The existence of statistically significant differences among means was evaluated using Tukey's post hoc test, and differences were

considered significant at $p < 0.05$.

3. Results and discussion

3.1. Influence of the treatments on protein stability

Fig. 2 shows how the treatments affected the protein stability of the two wine samples. Macabeo wine, for example, exhibited slight protein instability, as is evidenced by a turbidity increase of approximately 3 NTUs following the rapid heat test. In contrast, Muscat of Alexandria wine displayed a markedly higher degree of instability, with a turbidity increase of over 150 NTUs, which indicates severe susceptibility to protein haze formation. This figure also shows that bentonite treatment was effective in ensuring protein stability in both wine samples. This result is evidenced by the turbidity increases observed following the fast heat test, which remained well below two NTUs in both cases. Treatment with proteases also led to protein stability in Macabeo wine. However, despite a notable improvement, this treatment did not achieve full stability in Muscat of Alexandria wine. Specifically, the increase in turbidity following the stability test was significantly lower than in the control sample, which indicates a partial but insufficient enhancement in protein stabilisation for this wine. Muscat of Alexandria wine was therefore also treated with bentonite in order to achieve protein stability. However, in this case a lower bentonite dose was needed (2500 mg/L rather than 4000 mg/L), which indicates that protease treatment helped to reduce the amount of this fining agent required to achieve protein stability in the wine.

3.2. Influence of the treatments on protein fraction

Fig. 3 shows how the treatments affected the protein fraction of the two wine samples. The initial Macabeo wine, for example, had a relatively low protein content (roughly 55 mg/L), whereas the initial Muscat of Alexandria wine was considerably richer in proteins (roughly 200 mg/L). These results are consistent with the differences observed in protein stability, since Macabeo wine exhibited only slight instability while Muscat of Alexandria wine showed a high degree of instability.

Bentonite treatment of Macabeo wine (Fig. 3A) had little effect on total protein concentration as no significant reduction was observed following treatment. Similarly, no significant changes were detected in any of the molecular weight fractions. This finding is rather unexpected as bentonite functions by removing proteins (Vanrell et al., 2005, 2007), and applying it successfully achieved protein stabilisation in the wine. A plausible explanation for this result is that the initial protein content of Macabeo wine was already very low. Therefore, although full stabilisation was attained, the changes in protein concentration were too small to reach statistical significance.

In contrast, bentonite treatment of Muscat of Alexandria wine (Fig. 3B) led to a significant reduction in total protein concentration, which was also evident across all molecular weight fractions. This effect was particularly noticeable in the low molecular weight fraction (F3), where the main unstable proteins are found (Esteruelas et al., 2009a; Waters et al., 1996).

Protease treatment of Macabeo wine did not lead to a significant reduction in either total protein concentration or any of its molecular weight fractions. These results are similar to those observed with bentonite treatment. However, protein stabilisation was also successfully achieved. These findings are thus consistent and suggest that the initial protein concentration of Macabeo wine was already very low and that the minor reductions generated by both treatments were insufficient to reach statistical significance.

In contrast, protease treatment of Muscat of Alexandria wine led to a highly significant reduction in total protein concentration that was reflected across all molecular weight fractions. This effect was particularly evident in the fraction corresponding to low molecular weight proteins (F3), which, as previously noted, contains the main unstable protein fractions (Esteruelas et al., 2009a; Waters et al., 1996). However, the reduction in protein content was less pronounced than that achieved with bentonite treatment. This may explain why, despite the substantial decrease in protein instability observed with protease application, complete stabilisation was not achieved. For this reason, and as previously discussed, Muscat of Alexandria wine was treated with a lower dose of bentonite following protease treatment. This combined approach led to a further decrease in total protein concentration as well as across all molecular weight fractions, reaching levels almost comparable to those achieved with the initial bentonite treatment, and successfully led to complete protein stabilisation.

3.3. Influence of the treatments on polysaccharide fraction

Fig. 4 shows how the treatments affected the polysaccharide fraction of the two wine samples. For example, both wines exhibited total polysaccharide concentrations in the 300–400 mg/L range. These concentrations are consistent with the typical values reported in the literature for white wines (Martínez-Lapuente et al., 2024; Pons-Mercadé et al., 2022).

Bentonite treatment of Macabeo wine did not significantly affect the concentration of total polysaccharides or any of their molecular weight fractions. In contrast, bentonite treatment of Muscat of Alexandria wine led to a small but significant decrease in total polysaccharide content that was reflected in both the high molecular weight fraction (F1) and the low molecular weight fraction (F3). Bentonite treatment did not appear to substantially affect the polysaccharide content of the wine, while the slight decrease observed in Muscat of Alexandria wine is

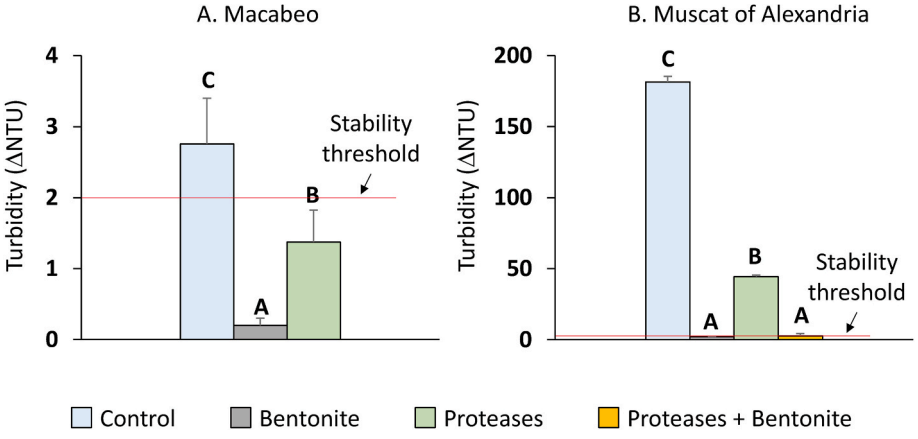

Fig. 2. Influence of the treatments on protein stability test.

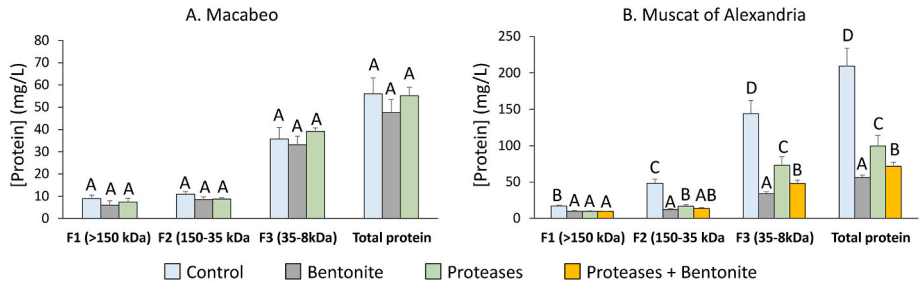

Fig. 3. Influence of the treatments on protein fraction.

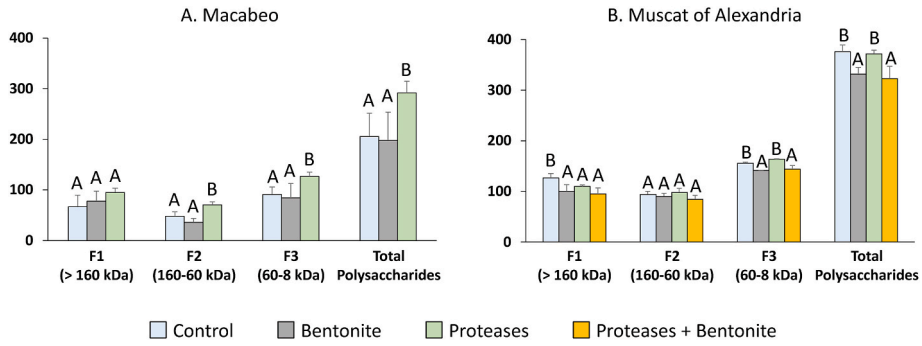

Fig. 4. Influence of the treatments on polysaccharide fraction.

probably attributable to nonspecific adsorption of these macromolecules. Another explanation may be that some polysaccharides bonded to proteins through intermolecular interactions were eliminated from the wine along with the proteins removed by bentonite.

As occurred with bentonite treatment, protease treatment of Macabeo wine did not affect polysaccharide content. In contrast, a slight but significant decrease was observed in Muscat of Alexandria wine that affected only the high molecular weight fraction (F1). In any case, we can conclude that protease treatment had a minimal impact on the polysaccharide fraction of the wine.

Finally, bentonite treatment following protease application did not affect polysaccharide concentration in Macabeo wine but led to a slight reduction in Muscat of Alexandria wine that specifically impacted the low molecular weight fraction (F3). We can therefore conclude that neither bentonite nor protease treatment significantly affected the polysaccharide fraction of the wine.

3.4. *Influence of the treatments on foam properties*

Fig. 5 shows how the treatments affected the foam properties of the two wine samples. As expected, bentonite treatment resulted in a significant reduction in maximum foam height (HM) in both wines. Although this reduction was relatively low in both cases, it was statistically significant. No changes were observed in stable foam height (HS). This detrimental effect of bentonite on the foaming properties of wine is well documented in the literature (Andrés-Lacueva et al., 1996; Puig-Deu et al., 1999; Vanrell et al., 2005, 2007) and, as previously noted, explains why winemakers, especially those who work with sparkling wines, are greatly interested in alternative methods to bentonite fining for protein stabilisation (Salazar et al., 2010; Silva-Barbieri et al., 2022).

In contrast, protease treatment produced a substantial increase in both maximum foam height (HM) and stable foam height (HS) in both wines, which indicates a marked improvement in their foaming properties. A possible explanation for this enhancing effect is that, through the proteolysis of wine proteins, proteases generate smaller peptides with higher surfactant activity. These peptides could reduce the surface tension of the wine, which in turn may positively influence both foamability and foam stability, facilitating the formation of more stable and persistent foam. Subsequent application of bentonite to Muscat of Alexandria wine (previously treated with proteases to ensure complete protein stabilisation) led to a decrease in maximum foam height (HM) due to the decrease in the protein concentration required to achieve stability. Despite this reduction, however, the HM remained significantly higher than in the untreated wine. This additional bentonite treatment did not affect stable foam height (HS). These results suggest

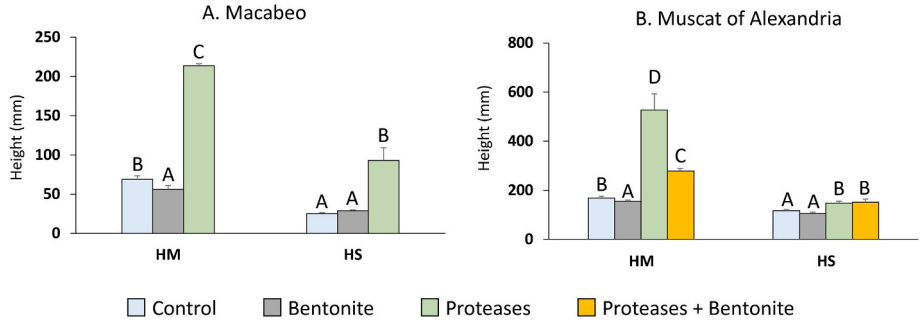

Fig. 5. Influence of the treatments on the foam parameters.

that the beneficial effect of protease treatment on the foaming properties of the wine is largely retained, although somewhat reduced, following bentonite treatment when such fining is required to achieve complete stabilisation of the wine against protein haze.

4. Conclusions

The results of this study demonstrate that protease treatment is an effective alternative to bentonite fining for achieving protein stability in white wines. In some cases, however, especially in varieties with high protein instability (such as Muscat of Alexandria), subsequent bentonite treatment may be needed to achieve complete protein stability. Nevertheless, protease treatment significantly reduces the amount of bentonite required, which can have a positive impact on the final quality of the wine. Moreover, even when followed by bentonite fining to ensure complete protein stability, protease treatment significantly enhances both the foamability (HM) and foam stability (HS) of the treated wines. This represents a considerable advantage for sparkling wine production, especially in regions or with grape varieties that typically yield base wines with limited foaming capacity. Further research is required to elucidate the underlying mechanisms through which protease treatments enhance the foaming properties of wines, as well as to investigate how the characteristics of the sparkling wines produced from these base wines using the traditional method evolve throughout the aging period, including their aromatic profile and sensory analysis. It would also be worthwhile to extend this investigation to wines from other grape varieties in order to determine whether the observed effects are consistent across different varietal matrices.

CRediT authorship contribution statement

Nadia Gregori: Investigation, Formal analysis. **Jordi Gombau:** Supervision, Methodology, Data curation, Conceptualization. **Arnau Just-Borràs:** Supervision, Investigation. **Dolores Pérez:** Supervision, Conceptualization. **Jose Maria Heras:** Supervision, Conceptualization. **Fernando Tarin:** Supervision, Methodology, Conceptualization. **Francesca Fort:** Supervision, Conceptualization. **Joan Miquel Canals:** Supervision, Conceptualization. **Fernando Zamora:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Lallemand Inc. for their financial support. We also express our gratitude to *Cheste Agraria Cooperativa Valenciana* winery for conducting the stabilisation trials and providing the wine samples used in this study.

Data availability

Data will be made available on request.

References

Aguié-Béghin, V., Adriaensen, Y., Péron, N., Valade, M., Rouxhet, P., & Douillard, R. (2009). Structure and chemical composition of layers adsorbed at interfaces with Champagne. *Journal of Agricultural and Food Chemistry, 57*, 10399–10407. https://doi.org/10.1021/jf9016948

Andrés-Lacueva, C., López-Tamames, E., Lamuela-Raventós, R. M., Buxaderas, S., & de la Torre-Boronat, M. C. (1996). Characteristics of sparkling base wines affecting foam behavior. *Journal of Agricultural and Food Chemistry, 44*, 989–995. https://doi.org/10.1021/jf9504879

Ayestarán, B., Guadalupe, Z., & León, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta, 513*, 29–39. https://doi.org/10.1016/j.aca.2003.12.012

Brissonet, F., & Maujean, A. (1993). Characterization of foaming proteins in a champagne base wine. *American Journal of Enology and Viticulture, 44*, 297–301. https://doi.org/10.5344/ajev.1993.44.3.297

Canals, J. M., Arolam, L., & Zamora, F. (1998). Protein fraction analysis of wine by FPLC. *American Journal of Enology and Viticulture, 49*, 383–388. https://doi.org/10.5344/ajev.1998.49.4.383

Carrasco, A., & Siebert, K. J. (1999). Human visual perception of haze and relationships with instrumental measurements of turbidity. Thresholds, magnitude estimation and sensory descriptive analysis of haze in model systems. *Food Quality and Preference, 10*, 421–436. https://doi.org/10.1016/S0950-3293(99)00029-4

Celotti, E., Osorio-Barahona, M. S., Bellantuono, E., Cardona, J., Roman, T., Nicolini, G., & Natolino, A. (2021). High-power ultrasound on the protein stability of white wines: Preliminary study of amplitude and sonication time. *LWT - Food Science and Technology, 147*, Article 111602. https://doi.org/10.1016/j.lwt.2021.111602

Comuzzo, P., Voce, S., Fabris, J., Cavallaro, A., Zanella, G., Karpusas, M., & Kallithraka, S. (2020). Effect of the combined application of heat treatment and proteases on protein stability and volatile composition of Greek white wines. *Oeno One, 54*, 175–188. https://doi.org/10.20870/oeno-one.2020.54.1.2952

Cosme, F., Fernandes, C., Ribeiro, T., Filipe-Ribeiro, L., & Nunes, F. M. (2020). White wine protein instability: Mechanism, quality control and technological alternatives for wine stabilisation—An overview. *Beverages 2020, 6*, 19. https://doi.org/10.3390/beverages6010019

EFSA. (2025). Safety evaluation of the food enzyme aspergillopepsin I from the non-genetically modified Aspergillus sp. strain AE-PRHF and does not contain pectolytic activity. *EFSA Journal, 23*, Article e9561. https://doi.org/10.2903/j.efsa.2025.9561, 2025.

Esteruelas, M., Poinsaut, P., Sieczkowski, N., Manteau, S., Fort, F., Canals, J. M., & Zamora, F. (2009a). Characterization of natural haze protein in Sauvignon white wine. *Food Chemistry, 113*, 28–35. https://doi.org/10.1016/j.foodchem.2008.07.031

Esteruelas, M., Poinsaut, P., Sieczkowski, N., Manteau, S., Fort, F., Canals, J. M., & Zamora, F. (2009b). Comparison of methods for estimating protein stability in white wines. *American Journal of Enology and Viticulture, 60*, 302–311. https://doi.org/10.5344/ajev.2009.60.3.302

Falconer, R. J., Marangon, M., Van Sluyter, S. C., Neilson, K. A., Chan, C., & Waters, E. J. (2010). Thermal stability of thaumatin-like protein, chitinase, and invertase isolated from sauvignon blanc and semillon juice and their role in haze formation in wine. *Journal of Agricultural and Food Chemistry, 58*, 975–980.

Guillou, C., Aleixandre, J. L., Garcia, M. J., & Lizama, V. (1998). Clarification influence upon sensorial and analytical characteristics of Muscat dry wine. *Journal International des Sciences de la Vigne et du Vin, 32*, 111–119. https://doi.org/10.20870/oeno-one.1998.32.2.1053

He, S., Hider, R., Zhao, J., & Tian, B. (2020). Effect of bentonite fining on proteins and phenolic composition of Chardonnay and Sauvignon blanc wines. *South African Journal of Enology and Viticulture, 41*, 113–120. https://doi.org/10.21548/41-1-3814

Horvat, I., Radeka, S., Plavsa, T., & Lukic, I. (2019). Bentonite fining during fermentation reduces the dosage required and exhibits significant side-effects on phenols, free and bound aroma, and sensory quality of white wine. *Food Chemistry, 285*, 305–315. https://doi.org/10.1016/j.foodchem.2019.01.172

Hsu, J., Heatherbell, D. A., Flores, J. H., & Watson, B. T. (1987). Heat-unstable proteins in grape juice and wine. II Characterization and removal by ultrafiltration. *American Journal of Enology and Viticulture, 38*, 17–22. https://doi.org/10.5344/ajev.1987.38.1.17

Just-Borras, A., Pons-Mercadé, P., Gombau, J., Giménez, P., Vilomara, G., Conde, M., Cantos, A., Canals, J. M., & Zamora, F. (2022). Effects of using cationic exchange for reducing pH on the quality and composition of sparkling wine (Cava). *Oeno One, 56*, 179–192. https://doi.org/10.20870/oeno-one.2022.56.2.5399

Lambri, M., Dordoni, R., Silva, A., & De Faveri, D. M. (2010). Effect of bentonite fining on odor-active compounds in two different white wine styles. *American Journal of Enology and Viticulture, 61*, 225–233. https://doi.org/10.5344/ajev.2010.61.2.225

Lubbers, S., Charpentier, C., & Feuillat, M. (1996). Study of the binding of aroma compounds by bentonites in must, wine and model systems. *Vitis, 35*, 59–62.

Malvy, J., Robillard, B., & Duteurtre, B. (1994). Influence des protéines sur le comportement de la mousse des vins de Champagne. *Science des Aliments, 14*, 87–98.

Marangon, M., Bernardi, L., Brearley-Smith, E., Marangon, C. M., Angiuli, F., Caramori, S., Argazzi, R., Triulzi, G., & Basana, A. (2023). Functionalized mesoporous silica is a viable alternative to bentonite for wine protein stabilization. *IVES Conference Series, OENO Macrowine*, Article 2023. https://ives-openscience.eu/41879/.

Marangon, M., Van Sluyter, S. C., Neilson, K. A., Chan, C., Haynes, P. A., Waters, E. J., & Falconer, R. J. (2011). Roles of grape thaumatin-like protein and chitinase in white wine haze formation. *Journal of Agricultural and Food Chemistry, 59*, 733–740. https://doi.org/10.1021/jf1038234

Marangon, M., Van Sluyter, S. C., Robinson, E. M. C., Muhlack, R. A., Holt, H. E., Haynes, P. A., Godden, P. W., Smith, P. A., & Waters, E. J. (2012). Degradation of white wine haze proteins by Aspergillopepsin I and II during juice flash pasteurization. *Food Chemistry, 135*, 1157–1165. https://doi.org/10.1016/j.foodchem.2012.05.042

Martínez-Lapuente, L., Guadalupe, Z., Higueras, M., Ayestarán, B., Pérez-Porras, P., Bautista-Ortín, A. B., & Gómez-Plaza, E. (2024). Effect of pre-fermentative treatments on polysaccharide composition of white and rosé musts and wines.

Journal of Agricultural and Food Chemistry, 72, 1928–1937. https://doi.org/10.1021/acs.jafc.2c08976

Maujean, A., Poinsaut, P., Dantan, H., Brissonet, F., & Cossiez, E. (1990). Étude de la tenue et de la qualité de mousse des vins. II. Mise au point d'une technique de mesure de la moussabilité, de la tenue et de la stabilité de la mousse des vins effervescents. *Bulletin de l'OIV, 711–712*, 405–426.

Medina-Trujillo, L., Matias-Guiu, P., López-Bonillo, F., Canals, J. M., & Zamora, F. (2017). Physicochemical characterization of the foam of white and rosé base wines for sparkling wine production (AOC Cava). *American Journal of Enology and Viticulture, 68*, 485–495. https://doi.org/10.5344/ajev.2017.17008

Mierczynska-Vasilev, A. M., Wahono, S., Smith, P. A., Bindon, K. A., & Vasilev, K. (2019). Using zeolites to protein stabilize white wines. *ACS Sustainable Chemistry & Engineering, 7*, 12240–12247. https://doi.org/10.1021/acssuschemeng.9b01583

Moine-Ledoux, V., & Dubourdieu, D. (1999). An invertase fragment responsible for improving the protein stability of dry white wines. *Journal of the Science of Food and Agriculture, 79*, 537–543. https://doi.org/10.1002/(SICI)1097-0010(19990315)79:4<537::AID-JSFA214>3.0.CO;2-B

OIV. (2020). *Compendium of international methods of analysis of wines and musts*. Paris, France: International Organization of Vine and Wine.

Pocock, K. F., Hoj, P. B., Adams, K. S., Kwiatkowski, M. J., & Waters, E. (2003). Combined heat and proteolytic enzyme treatment of white wines reduce haze forming protein content without detrimental effect. *Australian Journal of Grape and Wine Research, 9*, 56–63. https://doi.org/10.1111/j.1755-0238.2003.tb00232.x

Pocock, K. F., Salazar, F. N., & Waters, E. J. (2011). The effect of bentonite fining at different stages of white winemaking on protein stability. *Australian Journal of Grape and Wine Research, 17*, 280–284. https://doi.org/10.1111/j.1755-0238.2011.00123.x

Pons-Mercadé, P., Giménez, P., Vilomara, G., Conde, M., Cantos, A., Rozès, N., Ferrer, S., Canals, J. M., & Zamora, F. (2022). Monitoring yeast autolysis in sparkling wines of nine consecutive vintages produced by the traditional method. *Australian Journal of Grape and Wine Research, 28*, 347–357. https://doi.org/10.1111/ajgw.12534

Puig-Deu, M., López-Tamames, E., Buxaderas, S., & de la Torre-Boronat, M. C. (1999). Quality of base and sparkling wines as influenced by the type of fining agent added prefermentation. *Food Chemistry, 66*, 35–42. https://doi.org/10.1016/S0308-8146(98)00093-4

Ribeiro, T., Fernandes, C., Nunes, F. M., Filipe-Ribeiro, L., & Cosme, F. (2014). Influence of the structural features of commercial mannoproteins in white wine protein stabilization and chemical and sensory properties. *Food Chemistry, 159*, 47–54. https://doi.org/10.1016/j.foodchem.2014.02.149

Robinson, E., Scrimgeour, N., Marangon, M., Muhlack, R., Smith, P., Godden, P., & Johnson, D. (2012). *Beyond bentonite*. Wine & Viticulture Journal. November/December 2012.

Salazar, F., Zamora, F., Canals, J. M., & López, F. (2010). Protein stabilization in sparkling base wine using zirconia and bentonite: Influence on the foam parameters and protein fractions. *Journal International des Sciences de la Vigne et du Vin, special issue Macrowine*, 51–58. June 2010.

Sarmento, M. R., Oliveira, J. C., Slatner, M., & Boulton, R. B. (2000). Influence of intrinsic factors on conventional wine protein stability tests. *Food Control, 11*, 423–432. https://doi.org/10.1016/S0956-7135(00)00004-9

Sauvage, F. X., Bach, B., Moutounet, M., & Vernhet, A. (2010). Proteins in white wines: Thermo-sensitivity and differential adsorption by bentonite. *Food Chemistry, 118*, 26–34. https://doi.org/10.1016/j.foodchem.2009.02.080

Seidel, L., Albuquerque, W., Gand, M., Zorn, H., Lee, K. Z., Will, F., & Schweiggert, R. (2025). Investigations into protein reduction in grape must and wine: Screening the efficacy of 21 peptidases and the effects of thermal treatments, ultrasound, and reducing agents. *European Food Research and Technology, 251*, 1935–1950. https://doi.org/10.1007/s00217-025-04751-9

Senée, J., Robillard, B., & Vignes-Adler, M. (1999). Films and foams of Champagne wines. *Food Hydrocolloids, 13*, 15–26. https://doi.org/10.1016/S0268-005X(98)00061-7

Siebert, K. J. (2009). Haze in beverages. *Advances in Food & Nutrition Research, 57*, 53–86. https://doi.org/10.1016/S1043-4526(09)57002-7

Silva-Barbieri, D., Salazar, F. N., López, F., Brossard, N., Escalona, N., & Pérez-Correa, J. R. (2022). Advances in white wine protein stabilization technologies. *Molecules 2022, 27*, 1251. https://doi.org/10.3390/molecules27041251

Tabilo-Munizaga, G., Gordon, T. A., Villalobos-Carvajal, R., Moreno-Osorio, L., Salazar, F. N., Pérez-Won, M., & Acuña, S. (2014). Effects of high hydrostatic pressure (HHP) on the protein structure and thermal stability of Sauvignon blanc wine. *Food Chemistry, 155*, 214–220. https://doi.org/10.1016/j.foodchem.2014.01.051

Ubeda, C., Lambert-Royo, M. I., Gil-Cortiella, M., Del Barrio-Galán, R., & Peña-Neira, A. (2021). Chemical, physical, and sensory effects of the use of bentonite at different stages of the production of traditional sparkling wines. *Foods, 10*, 390. https://doi.org/10.3390/foods10020390

Van Sluyter, S. C., McRae, J. M., Falconer, R. J., Smith, P. A., Bacic, A., Waters, E. J., & Marangon, M. (2015). Wine protein haze: Mechanisms of formation and advances in prevention. *Journal of Agricultural and Food Chemistry, 63*, 4020–4030. https://doi.org/10.1021/acs.jafc.5b00047

Vanrell, G., Canals, R., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2007). Influence of the use of bentonite as a riddling agent on foam quality and protein fraction of sparkling wines (Cava). *Food Chemistry, 104*, 148–155. https://doi.org/10.1016/j.foodchem.2006.11.014

Vanrell, G., Esteruelas, M., Canals, J. M., Zamora, F., Poinsaut, P., Sieczkowski, N., & Lebouef, D. (2005). Influence du type de clarification du vin de base et des adjuvants de tirage sur la qualité de la mousse des vins effervescents. *Revue des Oenologues et des techniques vitivnicoles et oenologiques, 114*, 28–30.

Vincenzi, S., Panighel, A., Gazzola, D., Flamini, R., & Curioni, A. (2015). Study of combined effect of proteins and bentonite fining on the wine aroma loss. *Journal of Agricultural and Food Chemistry, 63*, 2314–2320. https://doi.org/10.1021/jf505657h

VIVC. Vitis international variety catalogue. https://www.vivc.de/. (Accessed 22 August 2025).

Waters, E. J., Alexander, G., Muhlack, R., Pocock, K. F., Colby, C., O'neill, B. K., Høj, P. B., & Jones, P. (2005). Preventing protein haze in bottled white wine. *Australian Journal of Grape and Wine Research, 11*, 215–225. https://doi.org/10.1111/j.1755-0238.2005.tb00289.x

Waters, E. J., Shirley, N. J., & Williams, P. J. (1996). Nuisance proteins of wine are grape pathogenesis-related proteins. *Journal of Agricultural and Food Chemistry, 44*, 3–5. https://doi.org/10.1021/jf9505584

Waters, E. J., Wallace, W., & Williams, P. J. (1991). Heat haze characteristics of fractionated wine proteins. *American Journal of Enology and Viticulture, 42*, 123–127. https://doi.org/10.5344/ajev.1991.42.2.123