fermentation

MDPI

Article

Polysaccharide Functionality in Wine-like Model Systems with Oat and Egg White Model Proteins

Olivia Burken and Stephan Sommer *

Grape and Wine Institute, University of Missouri, 223 Eckles Hall, Columbia, MO 65211, USA
* Correspondence: ssommer@missouri.edu

Abstract: Interactions between wine proteins and polysaccharides have the capacity to regulate the stability, shelf-life, and turbidity of red wines. Understanding these macromolecular interactions helps with maintaining stability and reproducing high quality wines. Model polysaccharides (carboxymethyl cellulose, mannoproteins, and fruit pectin) and model proteins (egg white and oat protein) were selected to assess protein–polysaccharide interactions within a model wine solution. The wine-like solution was created to simulate the correct pH, ethanol strength, and pigment content. Any interactions with polymeric pigments—anthocyanins and tannins—can also be investigated in this matrix. To analyze the aggregative potential of the macromolecules, particle size and Zeta-potential (ζ-potential) measurements were recorded for the samples with increasingly complex compositions. Carboxymethyl cellulose was found to increase particle sizes, likely binding more than proteins, but also improved the overall stability of the solution. Fruit pectin and mannoprotein were effective at causing precipitation while not removing the color of the model wine. The use of mannoprotein ensued in overall smaller particles for both suspended aggregate and precipitate sizes, indicating higher selectivity. Fruit pectin increased precipitate sizes and decreased suspended aggregate sizes. This study implements model proteins to evaluate complex macromolecular interactions using measurements of ζ-potential and particle size.

Keywords: macromolecule; protein; carboxymethylcellulose; pectin; mannoprotein; fining; precipitate; red wine

Citation: Burken, O.; Sommer, S. Polysaccharide Functionality in Wine-like Model Systems with Oat and Egg White Model Proteins. *Fermentation* **2024**, *10*, 573. https://doi.org/10.3390/fermentation10110573

Academic Editor: Giuseppe Italo Francesco Perretti

Received: 14 September 2024
Revised: 1 November 2024
Accepted: 8 November 2024
Published: 9 November 2024

1. Introduction

Protein presence in wine, even in low concentrations, can significantly impact the stability and turbidity of the product [1]. However low the concentration, protein interaction with other wine constituents, especially phenolic compounds and polysaccharides, is the primary provenance of haze formation [2]. Regardless of the grape variety, the haze-causing proteins left after the winemaking process are pathogenesis-related (PR). PR proteins actually contribute to an elevated disease resistance in grape cultivars, but their presence leads to instabilities in the finished wine [3].

Egg whites are predominately used in red wine to remove harsh precipitable tannins [4], though their use is tedious as the protein denatures and precipitates rather quickly [5]. Egg white proteins are a blend of twelve or more differing proteins; however, in comparison to wine proteins, they are chemically similar. The molecular masses of the egg white proteins range from 14 to 76 kDa, while their isoelectric points range from 3.9 to 10.7 [1]. This is very similar to the molecular masses of wine proteins ranging from 11 to 65 kDa and possessing isoelectric points from 4.1 to 8.0 [1,6], so egg white makes a beneficial model wine protein, in addition to their use as a fining agent. Sommer et al. estimate 85% of egg white proteins to be of the same range as wine proteins. Egg white proteins comprise approximately 66% albumin, 2–8% globulin, 11% ovomucoid, 3.5% ovomucin, 3.4% lysozyme, and several other proteins [1].

Oat is a favorable source of protein as it presents no common allergic characteristics and can function as an antioxidant, among other functions [7]. Oat protein, depending on

the variety, is composed of 70–80% globulin, 4–15% prolamins, 1–12% albumin, and <10% glutenin, which is a type of glutelin [7]. This is markedly similar to the grape endosperm protein content found by Gazzola et al. [8], who reported 58.4% globulin and albumin with the rest being prolamin and glutelin. The molecular masses of oat proteins fall between 10 and 90 kDa, with the majority between 54 and 60 kDa [7], which is also very similar to the wine proteins. Oat proteins also have very comparable isoelectric points, ranging from 4 to 7.5 [9].

Polysaccharides are the primary contributors to the organoleptic properties of wines but also influence stability and clarity [10]. The polysaccharides commonly found in wine, such as pectin from the berry cell wall, are very diverse, and their size, structure, and type all influence the interactions of polysaccharides with other constituents. Certain polysaccharides have been able to reduce or slow haze formation by interfering with particle charge interactions [11], and their interaction with other wine constituents can create "protective colloids" [10], which can be used to prevent precipitation of pigments, tannins, and anthocyanins.

Carboxymethyl cellulose (CMC) has been used within wines to stabilize tartrate salt and control the growth of potassium bitartrate crystals [12]. CMC has been known to interact with the majority of other wine components and is likely to increase protein turbidity [12,13]. Another common issue with CMC is the observed color loss, which has been identified as the consequence of a protein-bridged reaction with anthocyanins [13].

Mannoproteins are polysaccharides derived from *Saccharomyces cerevisiae* yeast cells and have been used to stabilize proteins, tartrates, and wine color [11,14]. Protective and haze-reducing properties, although potentially not long-term [15], have also been observed in mannoproteins [2,16]. They can inhibit the crystallization of tartrate salts, reducing precipitate formation [15], and have been reported to improve thermal stability of the wine [17]. However, various yeast sources do have different compositions, so they can behave differently depending on the mannoprotein's origin [18].

Pectic polysaccharides affect wine clarity and viscosity but have been identified interacting with phenolic components [2]. Studies have reported that pectin fragments can lead to competitive dissociation with tannin–protein interactions [12] and interact with anthocyanins to form colored compounds that can be resistant to polymerization [19]. It should be acknowledged that, like mannoproteins, the method of extraction and source of pectin can lead to significant compositional or structural variation [20].

Tannins, obtained from the skins and seeds of grapes, contribute to the structure, taste, color, and aging process of red wines. They are categorized as either condensed or hydrolysable [10]. Condensed tannins can have an astringent or bitter taste, so they heavily contribute to wines' flavor profiles [21]. Anthocyanins are water-soluble flavonoids that are the source of color in red grapes and wines [22]. They are also known to aid in wine and foam stability, as well as stabilize tannins and improve wine color [10]. Polymeric pigments, consisting of anthocyanins, tannins, and other phenolic compounds, are more resistant to sulfite bleaching than their smaller counterparts, and therefore, provide the stable color of aged red wines [23]. The most recent classification of polymeric pigments differentiates between protein-precipitable (PP) and non-precipitable pigments (NP). Pigments may precipitate with the other wine constituents, specifically polyphenols and polysaccharides [24].

Zeta-potential (ζ-potential) measurements are representative of the electrokinetic potential of colloidal dispersions; therefore, these values can be used to assess the stability of the system [25]. The ζ-potential values are measured at the boundary between the stern and diffuse liquid layers of the particles within the suspension. The charge of the value denotes the overall charge of particles within the sample. Large values, regardless of charge, signal a high repulsion amongst particles, and therefore, a lower probability that the particles would aggregate [26], while low ζ-potential values indicate a greater possibility of precipitation. The isoelectric point (pI) is also the pH level at which the ζ-potential would theoretically equal zero. Since ζ-potential values also represent the charge of suspended

particles, the impact particle charge has on the macromolecular interactions can also be evaluated. Sommer et al. indicates that precipitation is greatly affected by the suspended particle charges [13]. Since pH levels higher than the IEP generate a negative charge and levels lower than the IEP create a positive charge [27], Sommer also concluded that pH can drive precipitation reactions [13]. The same conclusion of charge-driven interactions was reached by Graves and Sommer during their evaluation of protein–polyphenol interactions, specifically in the presence of polysaccharides [28].

Aggregate size measurements are exceedingly important when evaluating aggregation and precipitation tendencies of the macromolecules. This assessment provides insights into the polysaccharide–protein interactions and how each influences haze or precipitation. Describing macromolecular and wine constituent behaviors can provide knowledge for adjustments within the winemaking process to produce and replicate stable, high-quality wines [1]. The most direct, effective method to clarify and stabilize wine would be to remove the macromolecules causing instability and haze formation. This common wine clarification technique uses fining agents, absorptive substances, to remove wine constituents that contribute to haze or precipitate formation. The inorganic fining agent bentonite has an unfavorable influence on wine quality but is currently the most frequently used fining agent [2,10]. Some beneficial wine constituents and positively charged compounds are removed due to bentonite and most other fining agents, which are non-protein specific [2]. This can reduce the organoleptic properties that improve the quality of the wine. This same issue arises with ultrafiltration methods that are used for protein stabilization [2,29]. More investigation is required to determine the success of potential alternative fining agents. Sommer and Tondini examined carboxymethyl cellulose, chitosan, polystyrene, and a *Saccharomyces paradoxus* yeast strain in comparison with bentonite. Chitosan and the yeast strain both proved promising for selective fining. Carboxymethyl cellulose and polystyrene require further examination regarding their interactions with wine proteins [30]. Further research is also being conducted on the potential for mannoproteins as fining agents [10,29]. Egg albumin, milk caseinates, and fish gelatine are other alternatives that are used to bind larger polymeric materials, but knowledge of their selectivity for and interactions with wine proteins is limited [10].

The objective of this study was to demonstrate the use of ζ-potential and particle size measurements in a wine model system to identify interactions on a molecular level. The technique evaluates both the suspended particles and those that have precipitated out of solution. The suspended particles present information as to how the polysaccharides and proteins interact and aggregate, while any focus on the precipitates can illuminate any potential as additives or fining agents. Precipitate size measurements also grant a closer look at whether the polysaccharides could be binding mostly proteins or the other suspended macromolecules that are important to a wine's profile.

2. Materials and Methods

The three model polysaccharides selected for macromolecular interaction analysis were carboxymethyl cellulose (AEB-Group, Brescia, Italy), Sure-Jell Premium Fruit Pectin (KraftHeinz Sure-Jell, Northfield, IL, USA), and MANNOSTAB™ LIQUIDE 200 (Laffort, Petaluma, CA, USA). To simulate the proteins occurring in wine, two model proteins were selected: egg white protein (The Barry Farm, Wapakoneta, OH, USA) and oat protein (Lantmännen, Norrköping, Sweden). The particle sizes of the polysaccharides and proteins were considered as they had to be well suited for measurement by the Malvern Nanoseries Zetasizer (Malvern Panalytical, Worcestershire, UK).

The model wine solution created contained 103 g/L of 200 proof ethanol, 2.7 g/L tartaric acid, 2.0 g/L malic acid, and 0.15 g/L potassium metabilsulfite (all chemicals from Sigma-Aldrich, Burlington, MA, USA). The pH was then adjusted to 3.5 with potassium hydroxide using the HI2209-01 Benchtop pH/mV Meter (HANNA Instruments, Woonsocket, RI, USA). Then, 1.0 g/L anthocyanin extract (non-commercial as described in [13]), and 1.0 g/L grape skin tannins (UvaTan™, Scott Laboratories, Petaluma, CA, USA) were added

to the solution. This solution had slight precipitate formation with minimal-to-no haze, so the solution was centrifuged—using the 5804 R Benchtop Centrifuge (Eppendorf, Hamburg, Germany)—prior to the addition of the model polysaccharides and proteins. The precipitates within the model wine were discarded to ensure that any precipitates formed during the study were the result of polysaccharide or protein addition.

Each macromolecule was dissolved, individually, at 0.25%, 0.5%, and 1.0% concentration in 10 mL of deionized water at neutral pH—and then within 10 mL of the model wine solution. The individual assessments indicate the influence of the concentration on the system's stability and individual particle sizes prior to evaluating any macromolecular interactions. The 0.25% concentration levels resulted in the most accurate, according to sample standard deviation, particle size and ζ-potential measurements. This also promotes better dissolution of the macromolecules within the model. As such, 0.25% concentrations of macromolecules were used for the samples with any combination of macromolecules. Varying concentration measurements were considered to better understand any significant interactions.

The macromolecules were dissolved in 10 mL of the wine-like solution at room temperature, maintaining the individual 0.25% concentrations, in 15 mL tubes. To prevent denaturation, mechanical agitation was used to dissolve the samples, and heat and ultrasounds were avoided. These samples were then stored overnight at 4 °C.

The Malvern Nanoseries Zetasizer recorded particle size and ζ-potential measurements. The 'Protein' Standard Operating Procedure (SOP) was used for the samples with a refractive index of 1.450 and absorption value of 0.001. The temperature was set to 25 °C and the equilibration time to 120 s. The measurements were performed in the same cuvette and capillary cells, supplied by Sarstedt Küvetten (Sarstedt, Nümbrecht, Germany) and Malvern Nanoseries (Malvern Instruments, Worcestershire, UK), respectively. Three measurements were recorded for both particle size and ζ-potential. It should be noted that precipitates were either settled or centrifuged prior to the ζ-potential measurements being recorded. Therefore, the stability evaluation is of the suspended particles post-precipitation, not the stability prior to precipitation.

As the majority of samples contained precipitation, and the Nanoseries Zetasizer only measures particle sizes from 0.3 nm–10 μm, the Malvern Mastersizer 3000 (Malvern Panalytical, Worcestershire, UK) was required to report the size of the precipitates formed as it measures particle sizes from 10 nm to 1000 μm. The hazy samples, precipitates included, were transferred to the Mastersizer. The preset 'Protein' SOP was selected with a refractive index of 1.450, particle density of 1.03, and absorption index of 0.001. The dispersant used was deionized water, and its index was 1.33. A total of five measurements were taken and then averaged.

The data analysis and visualization of suspended aggregates were generated using the Malvern Nanoseries Zetasizer operating software (Malvern Instruments, Zetasizer software v7.12, Worcestershire, UK). The Mastersizer and ζ-potential statistical visualizations were created via SigmaPlot 11.2 (Systat Software Inc., San Jose, CA, USA). Statistical data analysis was also performed with SigmaPlot 11.2.

3. Results and Discussion

3.1. Individual ζ-Potential and Particle Size Baseline Measurements in Neutral pH

The dissolution of egg white proteins (EWPs) in deionized water indicated slightly better stability at lower concentrations. The ζ-potential at 0.25% and 1.0% EWP measured −21.2 mV and −18.3, respectively. There was little to no difference in the particle sizes measured between these two concentrations. EWP aggregate size peaks were measured at 183.4, 6.757, and 4314 nm.

Oat proteins (OP) also had very minimal differences between ζ-potential values: −30.65 mV at 0.25% OP, and −31.5 mV at 1.0% OP. There was also little to no difference between the particle sizes measured at the individual OP concentrations. At 0.25% OP, the particle size peaks were measured at 299.2 and 45.3 nm, which were smaller values than

those for the suspended EWP aggregates. The oat proteins did not completely dissolve within the neutral pH sample, so the insoluble particles were excluded post-centrifugation.

Burken and Sommer (2024) previously determined that, according to ζ-potential measurements, carboxymethyl cellulose (CMC) was more stable in higher concentrations, but did possess larger particle sizes. Both the fruit pectin (FP) and mannoprotein (STAB) had better stability at lower concentrations. The particle size peaks for 0.25% CMC, 0.25% FP, and 0.25% STAB were measured at 291.9, 318.8, and 337.8 nm, respectively [31].

3.2. Individual ζ-Potential and Particle Size Measurements in Model Wine

The macromolecules were individually analyzed within the model wine solution to assess how their varying concentrations might affect the ζ-potential value and particle size measurements. The baseline ζ-potential of the model wine, without any added polysaccharides or protein, was −9.75 mV. The only suspended particle size measurement was averaged at 314.4 nm. This size measurement would correspond to the anthocyanins and grape skin tannins that remained suspended after centrifugation. After centrifugation of the model wine, no precipitates remained in the sample, so any later precipitation is due to the presence of polysaccharides or protein. For this reason, the precipitates in the model wine were not measured.

The dissolution of EWPs in the model wine reliably resulted in precipitate and some haze formation within the samples. Though the model wine solution possesses a negative ζ-potential, the addition of EWPs ensued in a positively charged system. The positive charge is due to the isoelectric point of the majority of egg white proteins being greater than 3.5. The overall stability of the system was reduced by EWP addition. The 0.25% EWP sample possessed a 6.77 mV ζ-potential, less stable compared to the starting −9.75 mV ζ-potential. There was a negligible change between the 0.25% and 1.0% EWP concentrations. The 1.0% EWP ζ-potential only differed 0.45 mV from the 0.25% EWP sample, and the 0.5% EWP sample was 1.5 mV higher for both the 0.25% and 1.0% concentrations, so no clear trend in stability was observed with the change in EWP concentration. The suspended particle sizes increased as the EWP concentration increased. At 0.25% EWP, the particles measured 488.7 nm (Figure 3), while at 1.0% EWP, the particles measured 1151 nm. This demonstrates the increased aggregation of the egg white proteins at higher concentrations. The precipitates were sized at 6720 and 82.35 nm when EWP was present in the model wine (Figure 4). The Mastersizer somewhat negates changes in concentration as the sample is dispersed in the deionized water, so the samples present nearly the same precipitate sizes.

Oat protein also resulted in precipitate formation; however, there was no haze with the OP samples, particularly post-centrifugation. The negative ζ-potential was maintained within the OP samples, which were more stable at the lower concentrations of oat protein: −6.36 mV at 0.25% OP, and −4.35 mV at 1.0% OP (Figure 1). These values indicate that adding oat proteins to the wine solution decreases stability, and therefore, increases the likelihood of precipitation. The oat protein had much smaller suspended particles than the egg white protein, and though few, larger precipitates were also detected. The precipitates were measured at a peak of 81.2 µm (Figure 4). The suspended particle sizes increased with the concentration of oat protein, measuring 336.2 nm at 0.25% OP, 422 nm at 0.5% OP, and 647.9 nm at 1.0% OP. Despite the clear precipitation, there was very minimal haze seen at the varying concentrations of oat protein, which is supported by the smaller suspended particles measured.

CMC was the only polysaccharide that made the suspension more stable, according to the definition of ζ-potential. It was also the only polysaccharide with a larger ζ-potential value, indicative of better stability, at higher concentrations. Given the large negative ζ-potential, it was expected that smaller particles would be detected since larger particles are less stable and more likely to precipitate. However, CMC gave rise to the largest suspended particle sizes out of the evaluated polysaccharides (Figure 2). This does suggest that the interaction between CMC and anthocyanins or tannins is minimal, as previously

suspected [13]. This is favorable for a fining agent since, ideally, only proteins, which are not present in this sample, would be removed.

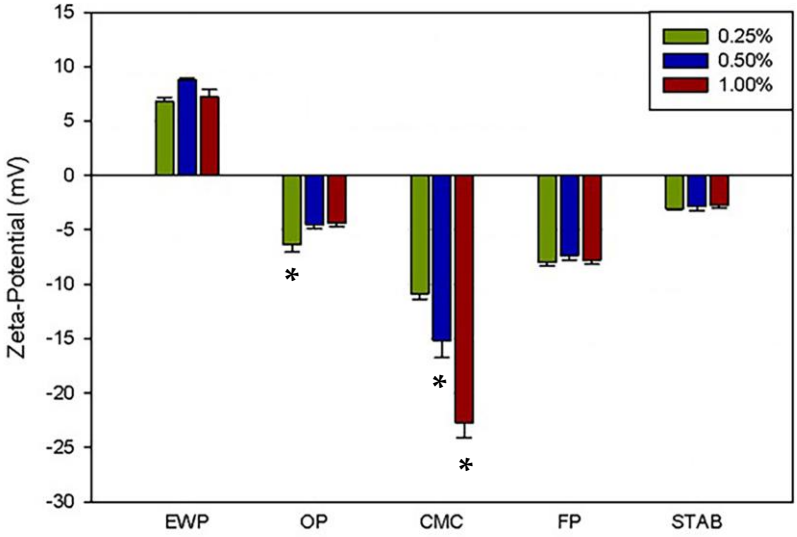

Figure 1. The individual ζ-potential measurements of the proteins and polysaccharides at 0.25%, 0.50%, and 1.00% concentrations in model wine solution. The error bars represent the variability (standard deviation) between the three ζ-potential measurements taken for each sample. (EWP: egg white protein, OP: oat protein, CMC: carboxymethyl cellulose, FP: fruit pectin, STAB: mannoprotein). An asterisk indicates statistically significant differences ($\alpha = 0.05$) between concentrations if applicable.

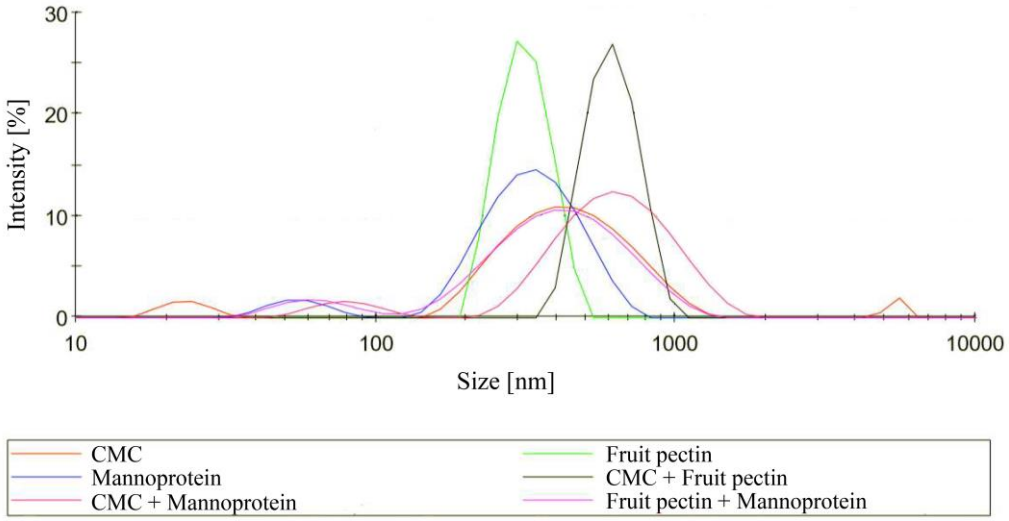

Figure 2. Zetasizer measurements of the suspended particle sizes formed by the interactions of polysaccharides—carboxymethyl cellulose (CMC), fruit pectin, and mannoprotein—in the model wine solution, at pH 3.5, on a logarithmic scale (distributed by intensity).

There was some precipitation, or insoluble particles, observed with fruit pectin (FP) incorporation into the model wine solution; however, the sample was otherwise clear with no haze present. The solids observed were measured at 186 and 9.27 µm. There was a very minimal change in the measured stability. Dissolving fruit pectin in the model wine resulted in a slightly less stable solution, with a decrease in the absolute ζ-potential from −9.75 mV to −7.97 mV. Changes in FP concentration did not appear to affect the ζ-potential values (Figure 1); however, slightly larger suspended particles were observed at lower concentrations. The measured suspended particle size was 354.0 nm at 0.25% and 259.4 nm

at 1.0%. Though the change in concentration was minimal, this was unexpected since lower concentrations would generally lead to less aggregation.

No precipitation was observed for the samples that contained only Mannostab™ (STAB), despite the instability indicated by very small ζ-potential values. The ζ-potential of 0.25% STAB was −3.07, while the value for 1.0% STAB was only slightly lower at −2.74 mV. Based on these values, increasing concentrations of STAB lead to minor and insignificant reductions in stability. The higher STAB concentrations also produced slightly larger suspended particles. Peak particle sizes of 371.1 and 57.17 nm were observed in the 0.25% STAB sample. Increasing the concentration to 1.0% STAB resulted in a peak at 435.3 nm, while the smaller measurement was not observed. It was expected that greater amounts of STAB would increase aggregation, and therefore, the size of aggregates. Given the low ζ-potential, the inclusion of Mannostab™ in the model wine should result in precipitation once other macromolecules are introduced.

3.3. ζ-Potential and Particle Size Measurements of Protein–Polysaccharide Interactions in Model Wine

The egg white protein samples had considerably larger suspended particle sizes than the oat protein samples. This was most pronounced when in the presence of CMC (Figure 3). The only exception to their different suspended particle sizes was their isolated interactions with the fruit pectin or the Mannostab™. The EWP/FP and EWP/STAB suspended particles were 316.6 and 392.6 nm, respectively, while the OP/FP and OP/STAB sizes were 378.0 and 344.9 nm, respectively. Based on the overall larger suspended particle sizes associated with the egg white protein, it appears that precipitating the egg white was more challenging than the oat protein.

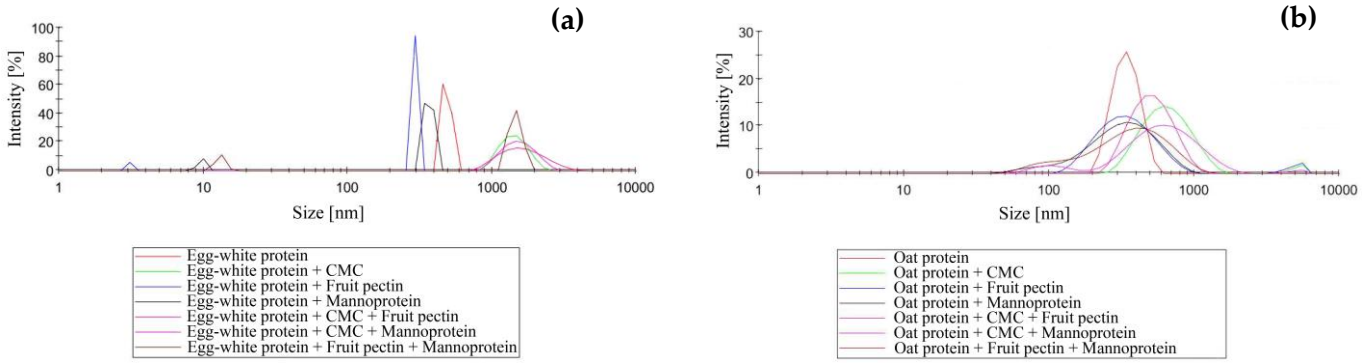

Figure 3. The Zetasizer measurements of the suspended particles developed during the egg white (**a**) and oat protein (**b**) interactions with polysaccharides—carboxymethyl cellulose (CMC), fruit pectin, and mannoprotein—in the model wine solution, at pH 3.5, on a logarithmic scale (distributed by intensity).

The precipitate sizes of EWP and OP were actually most similar in the presence of CMC (Figure 4). The most obvious difference in precipitates was observed with their interactions with fruit pectin and/or Mannostab™. The largest precipitates recorded occurred between EWP and FP, while the smallest were the result of EWP and STAB interaction (Figure 4). There were very little differences in the precipitate sizes formed with the oat protein (Figure 4); OP/FP and OP/STAB had the same peak precipitate measurements—81.2 and 12.7 µm. Overall, the oat protein samples were all very similar, both the precipitate and suspended particle size measurements had little to no change between varying polysaccharide interactions.

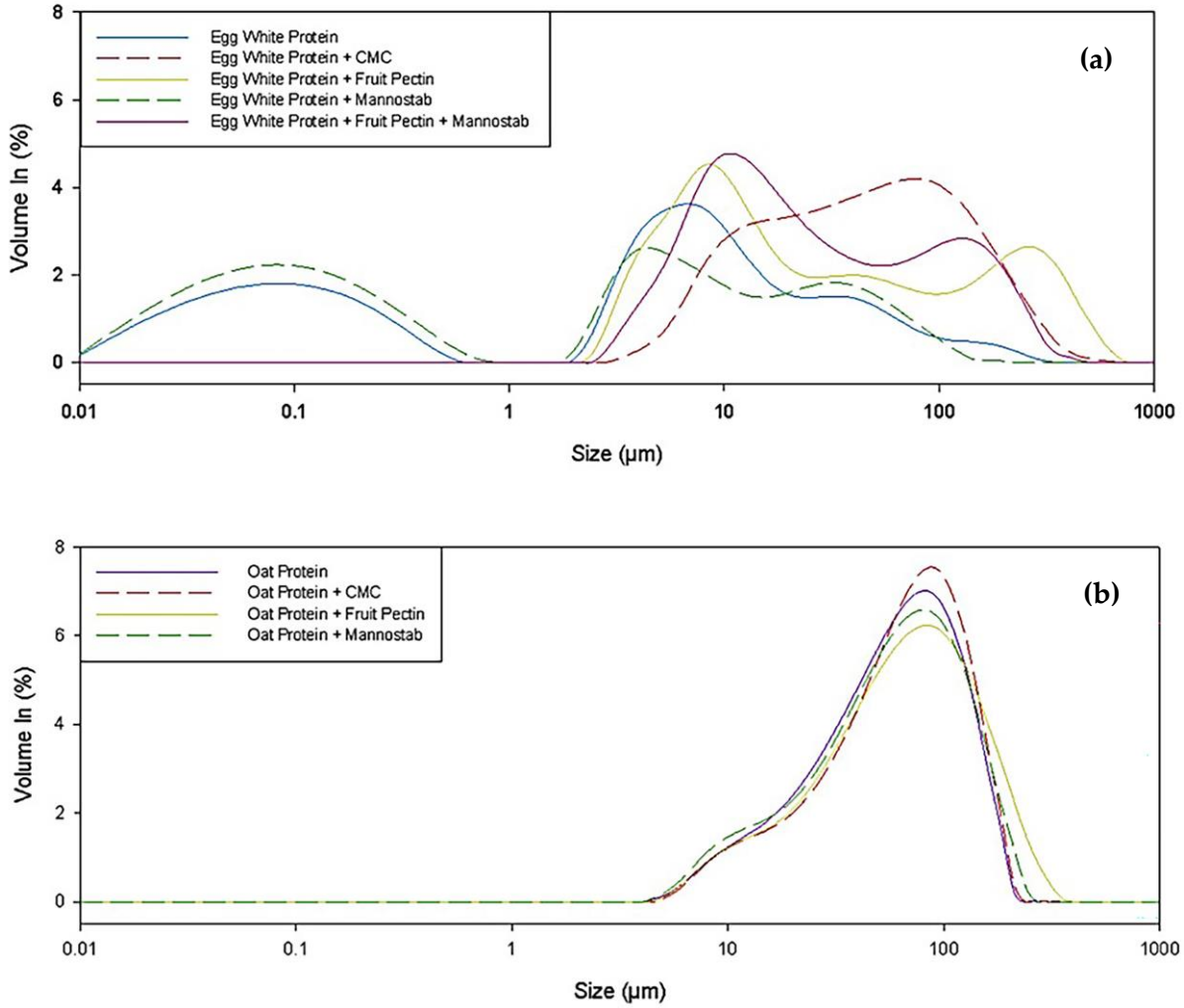

Figure 4. Mastersizer measurements of the precipitates that resulted from the egg white (**a**) and oat protein (**b**) interactions with polysaccharides—CMC, fruit pectin, and mannoprotein—in the model wine solution, at pH 3.5, on a logarithmic scale (distributed by volume).

The egg white and oat protein samples had roughly the same overall stability, but other than in the presence of CMC, the egg white protein systems possessed an overall positive charge, while the oat protein systems were negative (Figure 5). This would indicate that the EWPs, or any particles formed from their subsequent interactions, have a lower isoelectric point than the OP. The oat protein also saw incredibly little variation in size measurements, unlike the egg white protein (Figures 3 and 4), both before and after the inclusion of polysaccharides. This could indicate that the egg white proteins more readily interact with other compounds than the oat proteins.

The majority of CMC interactions produced larger suspended particle sizes compared to the fruit pectin and mannoprotein (Figures 2 and 3), but the precipitate sizes remained consistent across the samples. CMC was the only polysaccharide that maintained the negative ζ-potential despite protein addition and precipitate formation. Since the ζ-potential of CMC is so negative, and wine pH levels are below the pI of most proteins, it is extremely likely to bind the proteins present. This can be observed better through its interactions with FP and STAB, both of which also have a negatively charged system, as they did not result in precipitate formation. This leads us to believe that its affinity towards proteins is very strong. However, regarding the fining ability of CMC, the larger suspended particle sizes are problematic as they are likely to eventually precipitate. The samples containing a protein and CMC presented obvious color loss, meaning the polymeric pigments were

precipitated along with the protein(s), which is unsuitable for fining agents. However, CMC could still be used as a fining agent in white wines.

The samples without the CMC present contained minimal or no haze despite precipitating. This may be partially due to the greater stability associated with the CMC samples. According to the definition of ζ-potential [25], particles are more likely to remain suspended rather than precipitate out of the sample. Given that there are proteins within the solution, this would potentially lead to more haze formation. CMC also had adverse effects regarding color removal from the sample. This was not observed with the fruit pectin and/or mannoprotein samples. Sommer et al. recognized that color loss associated with CMC was the result of a protein-bridged reaction with anthocyanins [13]. This explains why total color loss was not observed with CMC alone but once the proteins were included.

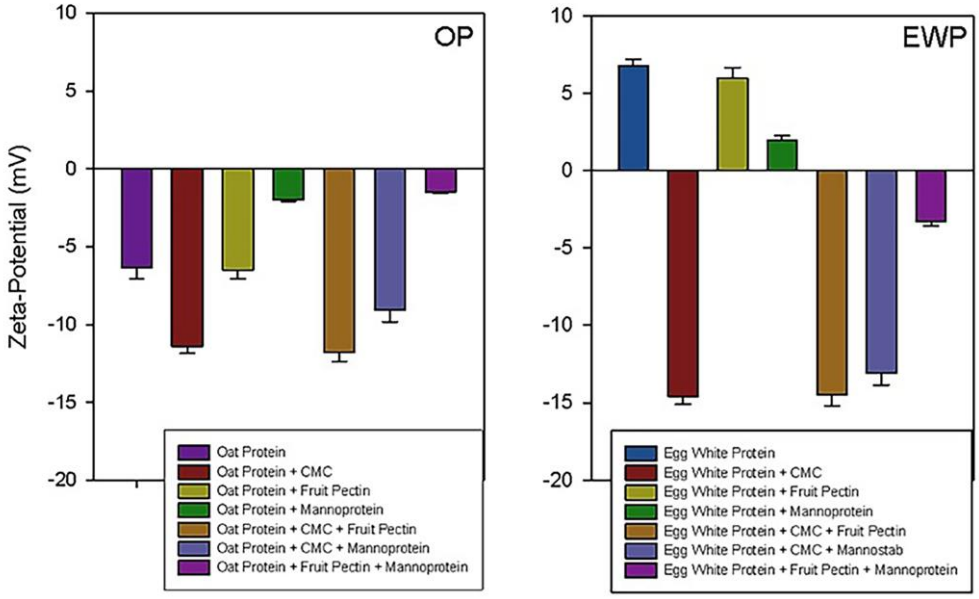

Figure 5. ζ-potential of the oat protein (OP) and egg white protein (EWP) and their subsequent interactions with the polysaccharides—carboxymethyl cellulose (CMC), fruit pectin, and mannoprotein.

Although fruit pectin addition did not produce any haze within the solution, it did result in a small amount of precipitate. And though few, these precipitates were the largest recorded amongst the samples. The particles left suspended after centrifugation were much smaller than those left within the samples containing CMC (Figure 3). Since fining methods aim to leave the wine more stable, smaller suspended particles would be more favorable. Adding fruit pectin also left the sample slightly less stable, but given its potential purpose to precipitate protein, it is reasonable that the stability would be reduced.

STAB reduced the size of suspended and precipitated aggregates in most of the samples, excluding those with FP present (Figures 3 and 4). This indicates that STAB could have better selectivity for proteins, likely binding fewer anthocyanins and/or tannins. The smaller precipitate sizes could suggest that fewer, or smaller, polysaccharide–polyphenol adducts were formed with STAB presence than with the other polysaccharides, indicating greater selectivity. However, this might also mean that the proteins may not be fully removed. The presence of STAB also reduced the stability of each solution, so partial protein precipitation would be much more likely after a mannoprotein is introduced.

Macromolecular charges are also a major contributor or driving force behind interactions [1]. The strong negative charges of CMC and the positive charges of EWP (Figure 5), and therefore, attractive electrostatic forces, may also be important contributors to the large particle sizes observed (Figure 3). The equal but opposite charges between FP and EWP may contribute to such large precipitations (Figure 4), yet smaller observed particle sizes (Figure 3). Inspection of charges can also explain the smaller suspended aggregates. Since

OP is negatively charged in the wine-like solution, like the polysaccharides, this could help explain the suspended aggregates being consistently smaller than the particles found within the EWP samples.

It is important to note that the change from a negative to a positive ζ-potential value consistently resulted in precipitation. As the charge changes from negative to positive, the ζ-potential value intersects 0 mV, which should be at a pH equal to the macromolecules isoelectric point (pI). Considering proteins, they are generally least soluble at their pI, which results in isoelectric precipitation [32]. This is when the change in pH is responsible for precipitate formation in the sample. For two or more macromolecules with similar isoelectric points, especially around the model wine pH, the increased acidity reduces repulsive electrostatic forces [3] and may be the driving force behind the formation of larger aggregates. This would imply that when the isoelectric point of the dry mixture is reached, the macromolecules would precipitate out of the solution. This is not a requirement for precipitation to occur but would be a major contributor.

4. Conclusions

Overall, within the model wine, egg white and oat proteins behaved similarly with only a few differences. The egg white protein samples possessed a positive ζ-potential, while the oat protein systems were negative, though they were around the same stability. The egg white proteins had greater haze formation and variation in particle size measurements than the oat protein. Though not necessarily advantageous for the wine quality, their effectiveness as a model wine protein depends entirely on their closeness to the behavior of grape proteins found within real red wines.

None of the polysaccharide–polysaccharide interactions caused visible haze, which was also supported by the results finding smaller suspended aggregates, so it can be hypothesized that the haze is the result of protein presence in the sample. There was also little to no precipitation observed, so it may be assumed that the polysaccharide affinities for each other are lower than their affinities towards the proteins, both within the model wine and at a neutral pH [31].

The mannoprotein presence had minimal impact on precipitate formation but did remove the haze associated with the egg white. There was very little difference in precipitate sizes from the baseline protein precipitates to the precipitates formed after STAB was introduced to the sample. This would indicate greater selectivity but perhaps less effective fining, depending on the composition of the precipitates. The fruit pectin showed promising fining capability as it affected the protein precipitation. Fruit pectin also left the samples without haze and slightly more stable than the mannoprotein, both beneficial attributes of a fining agent. Though CMC did result in precipitation and possessed the greatest stability post-precipitation, its selectivity is problematic. CMC had adverse effects on the aggregate sizes and the model wine color.

The usefulness of these polysaccharides is heavily contingent on their selectivity towards proteins. Therefore, assessing the composition of precipitates formed would further the applicable knowledge of these macromolecules. Since proteins denature with heat, protein haze is especially common once stored wine is warmed. However, increased temperatures should be evaluated to determine how they affect the stability and precipitation ability of the polysaccharides. Their stability long term and in the presence of heat are extremely important considerations for any potential additive or fining agent. Analyses of ζ-potential and particle size are tools providing unmatched insight into molecular interactions and the resulting overall stability of a complex system like wine.

Author Contributions: Conceptualization, O.B. and S.S.; methodology, S.S.; formal analysis, O.B.; investigation, O.B.; resources, S.S.; data curation, O.B.; writing—original draft preparation, O.B.; writing—review and editing, S.S.; visualization, O.B. and S.S.; project administration, S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sommer, S.; Weber, F.; Harbertson, J.F. Polyphenol–protein–polysaccharide interactions in the presence of carboxymethyl cellulose (CMC) in wine-like model systems. *J. Agric. Food Chem.* **2019**, *67*, 7428–7434. [CrossRef] [PubMed]
2. Ferreira, R.B.; Piçarra-Pereira, M.A.; Monteiro, S.; Loureiro, V.l.B.; Teixeira, A.R. The wine proteins. *Trends Food Sci. Technol.* **2001**, *12*, 230–239. [CrossRef]
3. Van Sluyter, S.C.; McRae, J.M.; Falconer, R.J.; Smith, P.A.; Bacic, A.; Waters, E.J.; Marangon, M. Wine protein haze: Mechanisms of formation and advances in prevention. *J. Agric. Food Chem.* **2015**, *63*, 4020–4030. [CrossRef] [PubMed]
4. Rolland, J.M.; Apostolou, E.; De Leon, M.P.; Stockley, C.S.; O'Hehir, R.E. Specific and sensitive enzyme-linked immunosorbent assays for analysis of residual allergenic food proteins in commercial bottled wine fined with egg white, milk, and nongrape-derived tannins. *J. Agric. Food Chem.* **2008**, *56*, 349–354. [CrossRef]
5. Lacorn, M.; Ristow, R.; Weiss, T.; Immer, U. Collaborative tests of ELISA methods for the determination of egg white protein and caseins used as fining agents in red and white wines. *Food Anal. Methods* **2014**, *7*, 417–429. [CrossRef]
6. Hsu, J.-C.; Heatherbell, D.A. Isolation and characterization of soluble proteins in grapes, grape juice, and wine. *Am. J. Enol. Vitic.* **1987**, *38*, 6–10. [CrossRef]
7. Rafique, H.; Dong, R.; Wang, X.; Alim, A.; Aadil, R.M.; Li, L.; Zou, L.; Hu, X. Dietary-nutraceutical properties of oat protein and peptides. *Front. Nutr.* **2022**, *9*, 950400. [CrossRef]
8. Gazzola, D.; Vincenzi, S.; Gastaldon, L.; Tolin, S.; Pasini, G.; Curioni, A. The proteins of the grape (*Vitis vinifera* L.) seed endosperm: Fractionation and identification of the major components. *Food Chem.* **2014**, *155*, 132–139. [CrossRef]
9. Ma, C.Y.; Harwalkar, V.R. Chemical characterization and functionality assessment of oat protein fractions. *J. Agric. Food Chem.* **1984**, *32*, 144–149. [CrossRef]
10. Jones-Moore, H.R.; Jelley, R.E.; Marangon, M.; Fedrizzi, B. The interactions of wine polysaccharides with aroma compounds, tannins, and proteins, and their importance to winemaking. *Food Hydrocoll.* **2022**, *123*, 107150. [CrossRef]
11. Siebert, K.J.; Carrasco, A.; Lynn, P.Y. Formation of protein−polyphenol haze in beverages. *J. Agric. Food Chem.* **1996**, *44*, 1997–2005. [CrossRef]
12. Claus, H.; Tenzer, S.; Sobe, M.; Schlander, M.; König, H.; Fröhlich, J. Effect of carboxymethyl cellulose on tartrate salt, protein and colour stability of red wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 186–193. [CrossRef]
13. Sommer, S.; Dickescheid, C.; Harbertson, J.F.; Fischer, U.; Cohen, S.D. Rationale for haze formation after carboxymethyl cellulose (CMC) addition to red wine. *J. Agric. Food Chem.* **2016**, *64*, 6879–6887. [CrossRef] [PubMed]
14. Wan, M.; Wang, M.; Zhao, Y.; Deng, H.; Tan, C.; Lin, S.; Kong, Y.; Tong, Y.; Meng, X. Extraction of mannoprotein from Saccharomyces cerevisiae and analysis of its chemical composition and molecular structure. *Int. J. Biol. Macromol.* **2021**, *193*, 2252–2259. [CrossRef] [PubMed]
15. Guise, R.; Filipe-Ribeiro, L.; Nascimento, D.; Bessa, O.; Nunes, F.; Cosme, F. Comparison between different types of carboxyl-methylcellulose and other oenological additives used for white wine tartaric stabilization. *Food Chem.* **2014**, *156*, 250–257. [CrossRef]
16. Dupin, I.V.; McKinnon, B.M.; Ryan, C.; Boulay, M.; Markides, A.J.; Jones, G.P.; Williams, P.J.; Waters, E.J. Saccharomyces c erevisiae mannoproteins that protect wine from protein haze: Their release during fermentation and lees contact and a proposal for their mechanism of action. *J. Agric. Food Chem.* **2000**, *48*, 3098–3105. [CrossRef]
17. Wu, J.; Guan, Y.; Zhong, Q. Yeast mannoproteins improve thermal stability of anthocyanins at pH 7.0. *Food Chem.* **2015**, *172*, 121–128. [CrossRef]
18. Nguyen, T.; Fleet, G.; Rogers, P. Composition of the cell walls of several yeast species. *Appl. Microbiol. Biotechnol.* **1998**, *50*, 206–212. [CrossRef]
19. Larsen, L.R.; Buerschaper, J.; Schieber, A.; Weber, F. Interactions of anthocyanins with pectin and pectin fragments in model solutions. *J. Agric. Food Chem.* **2019**, *67*, 9344–9353. [CrossRef]
20. Lara-Espinoza, C.; Carvajal-Millán, E.; Balandrán-Quintana, R.; López-Franco, Y.; Rascón-Chu, A. Pectin and pectin-based composite materials: Beyond food texture. *Molecules* **2018**, *23*, 942. [CrossRef]
21. Mamet, T.; Ge, Z.-Z.; Zhang, Y.; Li, C.-M. Interactions between highly galloylated persimmon tannins and pectins. *Int. J. Biol. Macromol.* **2018**, *106*, 410–417. [CrossRef] [PubMed]
22. He, F.; Liang, N.-N.; Mu, L.; Pan, Q.-H.; Wang, J.; Reeves, M.J.; Duan, C.-Q. Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules* **2012**, *17*, 1571–1601. [CrossRef] [PubMed]
23. Harbertson, J.F.; Picciotto, E.A.; Adams, D.O. Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. *Am. J. Enol. Vitic.* **2003**, *54*, 301–306. [CrossRef]

24. Weilack, I.; Mehren, L.; Schieber, A.; Weber, F. Grape-derived pectic polysaccharides alter the tannin and pigment composition of Cabernet Sauvignon red wines. *Curr. Res. Food Sci.* **2023**, *6*, 100506. [CrossRef] [PubMed]
25. Larsson, M.; Hill, A.; Duffy, J. Suspension stability; why particle size, zeta potential and rheology are important. *Annu. Trans. Nord. Rheol. Soc.* **2012**, *20*, 209–214.
26. Carneiro-da-Cunha, M.G.; Cerqueira, M.A.; Souza, B.W.; Teixeira, J.A.; Vicente, A.A. Influence of concentration, ionic strength and pH on zeta potential and mean hydrodynamic diameter of edible polysaccharide solutions envisaged for multinanolayered films production. *Carbohydr. Polym.* **2011**, *85*, 522–528. [CrossRef]
27. Vernhet, A.; Pellerin, P.; Prieur, C.; Osmianski, J.; Moutounet, M. Charge properties of some grape and wine polysaccharide and polyphenolic fractions. *Am. J. Enol. Vitic.* **1996**, *47*, 25–30. [CrossRef]
28. Graves, J.; Sommer, S. Polysaccharides influence the results of polymeric pigment analysis in red wines. *ACS Food Sci. Technol.* **2021**, *1*, 1770–1775. [CrossRef]
29. Moriwaki, C.; Matioli, G.; Arévalo-Villena, M.; Barbosa, A.M.; Briones, A. Accelerate and enhance the release of haze-protective polysaccharides after alcoholic fermentation in winemaking. *Eur. Food Res. Technol.* **2015**, *240*, 499–507. [CrossRef]
30. Sommer, S.; Tondini, F. Sustainable replacement strategies for bentonite in wine using alternative protein fining agents. *Sustainability* **2021**, *13*, 1860. [CrossRef]
31. Burken, O.; Sommer, S. Evaluation of protein–polysaccharide interactions through ζ-potential and particle size measurements to assess their functionality in wine. *J. Food Sci.* **2024**, *89*, 6413–6424. [CrossRef] [PubMed]
32. Wang, H.; Sun, H.; Gao, C.; Chen, Q.; Dong, W.; Chang, Y.; Luo, H. A phase separation process induced by pH change for purification of His-tagged protein at low salt concentration. *Biochem. Eng. J.* **2023**, *191*, 108792. [CrossRef]