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Chapter

Pathogenesis-Related Proteins in Wine and White Wine Protein Stabilization

Bin Tian and Roland Harrison

Abstract

Protein stabilisation in white wine is of great concern to winemakers as denaturation of wine proteins may cause haze formation, which is usually considered a wine fault. Pathogenesis-related (PR) proteins derived from grapes are the major soluble proteins remaining in the finished wine, which are mainly responsible for haze formation. The development of PR proteins in grapes during ripening and the extraction of PR proteins from grapes into juice can largely affect the concentration of PR proteins in the final wine, which consequently influence wine protein stability. Bentonite fining is the most common method to remove proteins in white wine before bottling, but it can cause the loss of wine volume and the removal of beneficial aromas. Thus, a number of alternative methods have been proposed for their potential to replace bentonite fining.

Keywords: bentonite fining, haze formation, pathogenesis-related proteins, phenolics, stabilisation, wine

1. Introduction

Wines, like many other natural food products, contain varying amounts of different nitrogenous substances, the most important of which are proteins [1]. Proteins found in wine are mostly derived from grape berries. Using immunological methods, the specific polyclonal antibodies raised against the total proteins of a Portuguese Malvasia Fina monovarietal wine have been applied to analyse the origin of the wine proteins [2] which are entirely from the berry pulp. Three years later, a similar immune detection study was conducted [3] and three various polyclonal antibodies raised against must, yeast, and bacteria proteins were applied to analyse the origin of proteins in Chardonnay wine. The results indicated that most of the wine proteins came from grapes and many of them were glycoproteins, but there were also some proteins from the yeast. Yeast may affect the wine protein composition in two ways: by transferring proteins to the wine during the process of yeast autolysis and/or hydrolysing the must proteins via the exocellular protease present in the yeasts [1]. Furthermore, the analysis of a Sauvignon Blanc wine using nano-high performance liquid chromatography (HPLC)/tandem mass spectrometry showed that within the 20 identified proteins there were two proteins from bacteria and one from fungi, which could be attributed to sources in the vineyard including natural infections and improper handling during harvest [4].

Grape variety	Sample type	MW	pI	Reference
Sauvignon Blanc	Wine	14.6–77.1 kDa		[4]
Riesling and Gewürztraminer	Grape	11.2–190 kDa		[5]
	Wine	11.2–65 kDa	4.1–8.0	[5]
Chardonnay, Verdeca and Pinot Noir	Wine	6–200 kDa	3.6–9.0	[6]
Macabeo, Xarel-lo, Parellada and Malvar	Wine	14–94 kDa	3.0–5.6	[7]
Muscadine	Grape	19–100 kDa	5.6–7.6	[8]
	Wine	12–50 kDa	4.6–8.8	[9]

Table 1.
Proteins reported in grapes and wines from different grape varieties: molecular weight (MW) and isoelectric point (pI).

In previous studies, proteins from grapes and wines have been reported with molecular weight (MW) in the range 6–200 kDa and isoelectric points (pI) in a range 3–9 kDa, as shown in **Table 1** [4–9]. However, the majority of wine proteins have MW and pI in a low range (20–30 and 4.1–5.8 kDa, respectively) and possess a net positive charge at the pH of the wine [2, 5, 10]. Studies on fractions of wine proteins using denaturing polyacrylamide gel electrophoresis have shown that the wine protein fraction is mainly composed of only a few polypeptides with MW ranging from 15 to 30 kDa, but a more detailed examination of whole protein fraction indicates a very large number of distinct polypeptides, exhibiting similar MW but subtle differences in electrical charges [11]. In that study, the authors also revealed via highly specific antibodies and N-terminal sequencing analysis that most wine polypeptides were structurally similar, suggesting the existence of a common precursor to most or all of the wine proteins which could generate all of the detected polypeptides by limited proteolysis.

In a study on Muscat of Alexandria wine [12], two major proteins with MW of 24 and 32 kDa, respectively, by SDS-PAGE are found with significant contribution to protein haze, and the 24 kDa protein produced about 50% more haze than the 32 kDa protein. The N-terminal sequence of the protein with MW of 24 kDa showed homology to thaumatin and to a number of plant thaumatin-like proteins, and the N-terminal sequence of enzyme digested peptides of the protein with MW of 32 kDa showed homology to plant chitinases [13]. Another study analysed the two main wine proteins present in sodium dodecyl sulphate capillary gel electrophoresis (SDS-CGE), which were determined with MW at 22 and 26 kDa, respectively, being concluded as corresponding to thaumatin-like proteins (TLPs) and chitinases [14]. Both thaumatin-like proteins and chitinases in wine are pathogenesis-related (PR) proteins derived from grapes.

2. Pathogenesis-related proteins in grapes and juice

Pathogenesis-related proteins are a group of plant proteins induced in pathological or related situations [15]. They were first discovered in tobacco as a result of a hypersensitive reaction to tobacco mosaic virus (TMV) [16]. PR proteins are typically acidic, of low molecular weight and highly resistant to proteolytic degradation and to low pH values. On the basis of similarities in amino acid sequences, serological relationship and/or enzymatic or biological activity, 11 families have been recognised and classified for tobacco and tomato [17]. Some of these PR protein

family members have also been found in grapevine. The two prominent soluble proteins accumulated in grapes during ripening have been identified as chitinases (PR-3 family) and thaumatin-like proteins (PR-5 family) [18, 19]. However, in early studies, the β-1, 3-glucanases (PR-2 family), a potential indicator of pathogen attack, were not found in grape juice and/or berry extracts [19–22]. With the accomplishment of grapevine genome sequencing programs in 2007 [23, 24] and the development of technology in protein analysis, proteomic analysis of grapevine has significantly improved knowledge of grape proteins and produced a better understanding of their characteristics [25]. These have consequently shown that there are more PR protein family members found in grapevines, such as osmotins (PR-5 family), β-1, 3-glucanases (PR-2 family) and the PR-10 proteins [26–28]. The two major PR proteins in wine, thaumatin-like proteins and chitinases, have been found present in both grape skin and pulp but not in grape seed [29].

Protein content in grape berries generally increases during ripening [30–33]. The accumulation of PR proteins in grape berries during ripening has been observed [33, 34], with *véraison* being the trigger for gene expression. The expression of PR genes in grapes can also be modulated by the classical PR protein inducers such as wounding, chemical elicitors, pathogen attack and abiotic stress [18, 35]. Although the level of PR proteins in grape berries increases, the diversity of PR proteins decreases during grape ripening [36]. In addition, the level and proportion of PR proteins in grapes are dependent on the cultivar, region, climate and viticultural practices [36–41]. Therefore, the actual protein composition in ripe grape berries is a result of the interactions between environmental conditions and intrinsic factors.

Sunlight-exposed fruits presented generally higher total soluble solids, anthocyanins and phenolic compounds and lower titratable acidity, malate and berry weight than non-exposed or canopy-shaded fruits [42–46]. One study on Riesling must show that the total amino acid concentration was significantly lower for fruits exposed to ambient UV-B levels than the low UV-B treatment and reduced UV-B affected amino acid composition, causing higher levels of arginine and glutamine, the main sources of amino acid for yeast metabolism [47]. In a later study [48], UV exclusion resulted in a lower concentration of not only phenolic compounds such as tannins but also PR proteins in grape skin. Interestingly, UV exclusion showed no effect on the PR proteins in the grape pulp.

Fungal infection can significantly influence the concentration of PR proteins in grapes. Grey mould caused by *Botrytis cinerea* is one of the main fungal diseases found in grapevines. A study that compared the juice from healthy grapes against *Botrytis* [49] showed that most proteins normally present in the healthy juice, namely, those between 20 and 30 kDa and a major glycoprotein at 62/64 kDa disappeared in the *Botrytis* infected juice. These results suggested that some proteinases secreted by *Botrytis cinerea* could degrade grape proteins. Another study on *Botrytis cinerea* infection on Chardonnay and Semillon grapes has also revealed that the concentrations of both PR proteins and total proteins in botrytised grape juice decreased compared to the juice from healthy grapes [50]. Conversely, powdery mildew infection on grape berries has been documented as increasing levels of PR proteins [48, 50, 51]. The strongly induced expression of some PR genes such as Vv*Chi*3 (coding for an acidic class III chitinase), Vv*Glu*b (coding for a basic class I glucanase) and Vv*Tl*2 (coding for a thaumatin-like protein) has been reported in powdery mildew infected grape berries [35]. A recent study [38] also showed that a number of proteins were induced in leaf tissues of Cabernet Sauvignon in response to powdery mildew infection, suggesting that Cabernet Sauvignon is able to initiate a basal defence but is unable to restrict fungal growth or slow down disease progression.

Extraction of PR proteins from grapes into juice can be greatly influenced by harvesting and grape processing conditions. Studies carried out in Australia [52, 53] showed that the juice obtained from mechanical harvesting coupled with long-distance transport had a higher concentration of PR proteins than juice obtained from hand harvesting fruit, which is likely due to the long skin contact during transport. A more recent study [54] conducted in New Zealand showed that Sauvignon Blanc juices from machine harvesting followed by 3 h skin contact had a significantly lower concentration of proteins, including PR proteins, than those from hand harvesting followed by 3 h skin contact. It was likely due to the greater juice yield in machine harvesting treatment and the interactions between proteins and phenolic compounds. In the following study [55], the authors confirmed that longer skin contact can increase the extraction of PR protein but the final concentration of PR proteins in juice can be modulated by the co-extracted phenolic compounds.

3. Protein haze formation in white wine

Protein haze can appear in bottled white wine as shown in **Figure 1** if unstable proteins are not removed before the wine bottling. Although research studies have investigated the protein stabilisation in white wines [12, 13, 56–59], the precise mechanism of protein haze formation still remains incompletely understood. One hypothesis [60] is that the first step in protein haze formation in wines is protein denaturation, a process accelerated by heating, after which the denatured proteins aggregate into large enough particles to be visually detected as haze, a process that may be affected either positively or negatively by non-protein wine components. In a recent study [61], two different mechanisms were proposed responsible for the heat-induced precipitation of the Arinto wine proteins: (1) at the higher pH values, it appears to result from isoelectric precipitation of proteins; (2) at the lower pH values, it seems to be associated with the presence of the non-protein wine components.

As the main soluble proteins remain in the finished wine, the slow denaturation of PR proteins is thought to lead to protein aggregation, flocculation into a hazy suspension and formation of precipitates. A study using purified thaumatin-like proteins and chitinases from grape juice [62] suggested that chitinases are the primary cause of heat-induced haze formation and their concentration was directly correlated to the turbidity of heat-induced haze formation, but conversely, thaumatin-like proteins seemed to have no measurable impact on turbidity. This result was confirmed by a latter study [54] in which chitinases were found to have a good linear correlation with protein stabilisation in Sauvignon Blanc wine. Different protein haze formation behaviour between thaumatin-like proteins and chitinases could be due to the difference in the protein structure of these two types of proteins. The thaumatin-like proteins start unfolding (or denaturing) at 62°C, but most of the proteins will refold again when the temperature drops down [63]. In contrast, chitinases have a lower unfolding temperature and this denaturation is irreversible. Thus, once chitinases are unfolded, they may aggregate and precipitate out of the solution.

A study used a reconstitution method [64] to investigate the heat-induced aggregation behaviour of purified wine proteins and showed that the chitinases were the protein most prone to aggregate and the one that formed the largest particles. It is important to note that in the reconstitution experiment, four thaumatin-like protein isoforms, chitinases, phenolics and polysaccharides in a Chardonnay wine were isolated individually, and the wine stripped of these compounds was used as a base to reconstitute each of the proteins alone or in combination with the isolated phenolics and/or polysaccharides. Although phenolics and polysaccharides did not show a significant impact on aggregation behaviour of chitinases,

Figure 1.
Protein haze formed in Sauvignon Blanc wine after heating at 80°C for 2 h.

the thaumatin-like protein isoforms varied in susceptibility to haze formation and interactions with phenolics and polysaccharides. These observations obtained in the model system indicated the importance of non-protein factors in affecting protein haze formation.

3.1 Phenolics

Phenolic compounds are important constituents in white wines since they contribute to many characteristics such as appearance, taste, style and quality [65]. A reduction in protein haze observed in commercial wine fined with the addition of polyvinylpolypyrrolidone (PVPP) suggested that phenolics may play a modulating role in haze formation [60]. The interaction between phenolics and proteins has been studied in relation to haze formation [59, 66]. Hydrophobic bonding was suggested as the major model of interaction in tannin-protein complexes [67]. A conceptual model for the protein-phenolics interaction is that a protein molecule has a fixed number of polyphenol binding sites and more sites were exposed when protein hydrogen bonds were broken [68]. Thus, the concentration of various

phenolics in wine could have a great impact on heat stability through the interaction between phenolics and proteins.

3.2 Polysaccharides

Most polysaccharides present in musts are derived from grape cell walls, which include arabinogalactans, galacturonans, arabinans and smaller amounts of xyloglucans, cellulose and mannans. In the musts, type II arabinogalactan proteins are the main polysaccharides released from berries at the initial time of pressing; in the resulting wine, polysaccharides consist essentially of type II arabinogalactan proteins and rhamnogalacturonan-II [69]. Wine polysaccharides can affect the characteristic pattern of haze formation, increasing protein instability under moderately high temperature (40–50°C) [58]. However, mannoproteins, the polysaccharides derived from yeast, have been described as protecting wines from protein haze formation [70]. This polysaccharide is considered a promising prospect for preventing protein haze formation in white wine.

3.3 Wine pH

In a model wine system, maximum haze formed at pH 4.0–4.5 when ethanol was 12%, with less haze at lower or higher pH values [71]. However, a study using six Portuguese varietal wines indicated that wine proteins were increasingly heat stable when the pH increased from wine pH to 7.5 [58]. When the wine pH was adjusted to a typical wine pH value at 2.80, 3.00, 3.34, 3.65 and 3.85, lower bentonite dosages for stability was observed in lower pH wines, which was likely due to the improved efficiency of protein adsorption by bentonite at reduced wine pH [72]. This observation is in agreement with another study which investigated the protein haze formation in an Italian white wine as affected by pH ranging from 3.00 to 3.60, and the increased heat stability of wine was found in wines at lower pH [73].

3.4 Sulphate

It has been found that the sulphate anion in white wine was an essential factor that is required for protein haze formation [60]. In that study, the authors investigated various common wine anions such as sulphate, acetate, chloride, citrate, phosphate and tartrate and wine cations such as iron and copper. When these ions were added into artificial model wine solutions at typical white wine concentrations, only sulphate was found to be essential for protein haze formation. Furthermore, in this model wine system, the thaumatin-like protein (150 mg/L) required approximately 150 mg/L sulphate, and the chitinase (150 mg/L) required approximately 15 mg/L sulphate, for visible haze formation. The range of sulphate in Australian wines between 1994 and 1997 was from 56 to 1780 mg/L, with a mean of 385 mg/L, which exceeds the requirement of both thaumatin-like protein and chitinase for haze formation. A recent study [74] confirmed that sulphate was essential in the aggregation of grape chitinases and thaumatin-like proteins in a model system, and furthermore, the authors pointed out that the aggregation mechanisms of thaumatin-like proteins and chitinases are different and influenced by the ionic content of the model wine.

3.5 Metal ions

There are many ions present in wine, and these ions could play a role in white wine protein haze formation. Metal ions, particularly copper and iron, have been

implicated in the formation of protein hazes in white wines, but as they are also associated with hazes of non-protein origin, their role in protein haze formation is very poorly understood [75]. The copper concentration in wine decreased after protein haze removal, suggesting that copper was part of the protein precipitation [76].

3.6 Ion concentration and electrical conductivity

In a model wine system, increasing the ionic strength and electrical conductivity could increase protein haze formation after heating by reducing electrostatic repulsion of proteins [74, 77]. A study on Chilean Sauvignon Blanc wine reported that more protein haze formation in wine was observed by increasing the electrical conductivity [78]. However, a more recent study on a range of Australian white wines showed a negative correlation between protein haze formation and electrical conductivity [79]. These contradictory results could be related to the differences in other wine components of importance to protein haze formation.

3.7 Sulphur dioxide

A recent study [80] revealed the role of sulphur dioxide in the aggregation of heat unstable wine proteins. In comparison to chitinases, TLPs are more reactive to sulphur dioxide. The aggregation of TLPs could be triggered by sulphur dioxide during cooling after heating, with aggregates held by hydrophobic interactions and intermolecular disulphide bonds.

4. Protein stability tests

To avoid protein haze formation in bottled white wine, a protein stability test is usually conducted before bottling in the winery. If the wine is not protein stabilised, a range of bentonite fining trials will be carried out to determine the minimum required dosage of bentonite addition for protein stabilisation. The most common protein stability test is the heat test, which is a heating procedure to force protein haze formation. Wine samples are normally heated to 80°C for 6 h and then left to cool down to 4°C overnight. The turbidity in heated wine samples is measured by a nephelometer and expressed as nephelometer turbidity units (NTU). Turbidity measurement of less than 2.0 NTU is usually recommended. Different temperatures and durations of heating could have a great impact on the resulting haze formation [81]. A recent study suggests that the less severe condition of heating at 80°C for 2 h is more appropriate to predict bentonite requirement for wine stored in the short term to medium term [82]. The cooling temperature and time are also critical to the accuracy of heat test results. A recent study [83] investigated the influence of heating and cooling conditions on protein heat test results. In this study, white wines were heated at 80°C for a time ranging from 0.5 to 6.0 h and then cooled down for 0.5–18 h at 0, 4 or 20°C, respectively. The results indicated that heating at 80°C for 2 h and then cooled at 20°C for 3 h enabled the repeatable production of haze and bentonite requirement.

As traditional heat test is very time-consuming, near infrared (NIR) spectroscopy has been studied for its potential to predict protein stability with high efficiency [84]. Results from 111 white wines representing multiple regions and varieties in California showed that the turbidity of wine could be predicted from the short-wavelength NIR spectra, but further IR analysis on a large number of wines will be required for the application of NIR in the global wine industry, and the high cost of equipment may limit its widespread use.

There are also commercial reagents, i.e. Bentotest and Proteotest, available for winemakers to check the protein stability in white wine. In general, these commercial reagents are considered to be harsher than the heat test, and as a result, the addition of bentonite is normally more than the actual requirement for protein stabilisation. Other tests including trichloroacetic acid (TCA), tannin and ethanol tests were originally established for protein quantification by precipitation of proteins in wine, but they are rarely used in the winery for checking the protein stability as bentonite requirement can be significantly overestimated using those tests.

5. Protein removal by bentonite and potential alternatives

Removal of the proteins remaining in finished wine before bottling is critical for wine protein stabilisation. In the wine industry, bentonite, a swelling 2:1 aluminosilicate clay, is usually added to wine to remove the proteins [85]. As proteins in wine are positively charged at wine pH and bentonite carries a net negative charge, wine proteins can be absorbed onto bentonite by cationic exchange [1, 85]. Hsu and Heatherbell [86] have shown that wine proteins with higher pI (5.8–8.0) and intermediate MW (32–45 kDa) are preferentially removed by bentonite fining, but a proportion of wine proteins, which have a MW range from 60 to 65 kDa and pI range from 4.1 to 8.0, are highly resistant to removal by bentonite fining. In that study, the authors also pointed out that the removal of proteins with lower pI (4.1–5.8) and lower MW (12.6 and 20–30 kDa) was necessary for protein stabilisation. In contrast to this conclusion, another study [56] showed that the amount of protein depletion correlated linearly with the level of bentonite addition, implying no bentonite selectivity based on isoelectric point. Different conclusions from these two studies might be partly attributed to the different methods used to separate and quantify the proteins [87].

Bentonite fining is effective in removing proteins to stabilise the wine, but the use of bentonite could also remove some important aroma and flavour compounds [88], as well as result in loss of wine as lees [89]. Thus, alternative treatments for protein stabilisation that can reduce or eliminate the use of bentonite are of great interest for winemakers. Potential bentonite alternatives that have been studied are summarised in **Table 2**.

Ultrafiltration is very effective in removing proteins in wine, and up to 90% of wine proteins can be retained using a 10 kDa molecular weight cut-off membrane; as a result, bentonite requirement is greatly reduced [90]. However, in addition to the high setup and running costs, ultrafiltration could also result in the loss of beneficial aroma compounds in wine [91]. Studies on Riesling and Gewürztraminer wines using ultrafiltration with membrane nominal MW cut-off from 10 to 50 kDa showed that ultrafiltration can significantly decrease the overall aroma intensity and fruity, floral, sweet and honey/caramel aromas, but also increase vegetative aroma [92]. Furthermore, a reduction in browning colour (A420 nm) and total phenolics was also observed in filtered Riesling and Gewürztraminer wines [93].

Short-term pasteurisation (90°C for 1 min) can reduce bentonite requirement up to 70% [94], but heating of juice could impart negative sensory implications into resultant wine [95]. A recent study reported that applying heating of juice at 75°C for 1 min in the presence of a heat-tolerant protease, aspergillopepsin (AGP), derived from fungus *Aspergillus niger*, before fermentation showed a significant reduction of PR proteins without damaging wine quality [96]. In general, thaumatin-like proteins and chitinases are highly resistant to proteases, but heating unfolds these proteins and thus they can be degraded by protease. Another promising protease BcAP8 (aspartic acid protease) from *Botrytis cinerea* has been proven to

Bentonite alternatives	Advantages	Disadvantages	Reference
Ultrafiltration			
Ultrafiltration	Efficient in protein removal and reduction of bentonite requirement	High setup and running costs and possible loss of flavour compounds	[90, 91]
Heating			
Short-time pasteurisation	Acceleration of protein denaturation in juice and thus lower bentonite requirement in wine	Impart negative sensory implications into wine	[94, 95]
Enzymes			
Aspergillopepsin	Very active towards wine proteins during flash pasteurisation	Heating required for better efficiency, which may alter sensory properties of wine	[96]
Aspartic acid protease	Capable of degrading chitinases without heat denaturation	Does not remove all PR proteins	[97]
Polysaccharides			
Carrageenan	Effective in heat stabilising white wines at low addition rates without deleterious sensory impacts	Not a good settling agent	[99, 100]
Mannoproteins	Protective effect on protein haze formation in wine	Long-term prevention of protein haze formation is unknown	[101]
Chitin and chitosan	Interact and remove PR proteins and protect wine from browning	Interact with phenolics which may alter wine colour and texture	[103]
Nanomaterials			
Nanoparticles	High surface area and efficiency in removing proteins	Removal of nanoparticles and the costs	[104, 105]

Table 2.
Summary of bentonite alternatives for white wine protein stabilisation.

be effective against grape chitinases during juice fermentation without the need for heating [97]. This protease could potentially reduce the bentonite requirement, but it is less effective on the degradation of thaumatin-like proteins.

Novel fining agents such as polysaccharides could be another potential class of bentonite alternatives [98]. Carrageenan, a food grade polysaccharide extracted from seaweeds, is effective in heat stabilising white wines at low addition rates (125–250 mg/L) without deleterious sensory impacts compared to bentonite treated wines [99, 100]. However, technical issues including frothing, slower filterability and risk of over-fining should be considered when applying carrageenan for protein stabilisation, particularly when it is used prior to or during fermentation. In addition, yeast mannoproteins, the highly glycosylated polypeptides present in yeast cell walls, have also been reported to have a protective effect on protein haze formation in wine [101]. Thus, mannoproteins extracted from purified yeast cell walls could be added into juice/wine to reduce the addition of bentonite, but further research is required to understand whether this protection against protein aggregation is suitable for long-term wine storage. Chitin [102] and chitosan [103], polysaccharides principally from *Aspergillus niger*, also have potential to remove haze-forming

proteins in wine, but the wine colour and texture could be affected as chitosan interacts with phenolics and organic acids in wine.

Nanotechnology is currently a very active research topic in food science. Recently some researchers also become interested in using nanomaterials to remove proteins in wine. Magnetic steel nanoparticles coated with acrylic acid have been tested and are highly efficient in attracting and thus removing haze-forming proteins [104]. Another study using mesoporous nanomaterials to fine Muscat Ottonel and Pedro Ximenez wines also confirmed the high efficiency of nanomaterials in removing haze-forming proteins, and the loss of aroma compounds in wine due to addition of nanomaterials was even less than bentonite [105]. Nanomaterials have shown great potential as bentonite alternative to remove proteins in wine, but the cost of coating the nanoparticles and the removal of nanoparticles from wine after the treatment are the main concerns for their wide application in the wine industry.

6. Conclusions

Pathogenesis-related proteins in grapes play an important role in plant defence mechanisms. The concentration of PR proteins in grapes generally increases during ripening, but a number of factors, e.g. fungal infection and UV radiation, can influence the accumulation of PR proteins in grapes. Extraction of PR proteins from grapes into juice during grape processing, influenced by factors like harvesting (manual or mechanical), skin contact time and grape pressing, could have a great impact on PR protein extraction. The concentration of PR proteins in juice can largely predict their final concentration in wine. If PR proteins were not removed before white wine bottling, they could potentially denature and could form a haze in bottled wine, especially when wines are stored under unfavourable conditions, e.g. high temperatures. Bentonite is commonly used in white wine protein stabilisation, but bentonite is not specific to absorb proteins; it can also remove positive aroma compounds and cause the loss of some wine volume as bentonite lees. Alternatives to bentonite have been widely studied; however, they are not yet enough efficient to replace the cheap bentonite. Carrageenan and some proteolytic enzymes can be in a near future interesting tools to get white protein stabilisation, perhaps more efficient than the classic bentonite.

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