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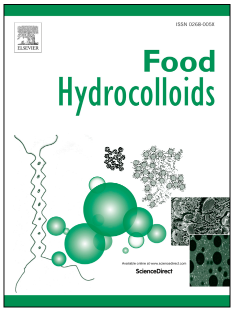

The interactions of wine polysaccharides with aroma compounds, tannins, and proteins, and their importance to winemaking

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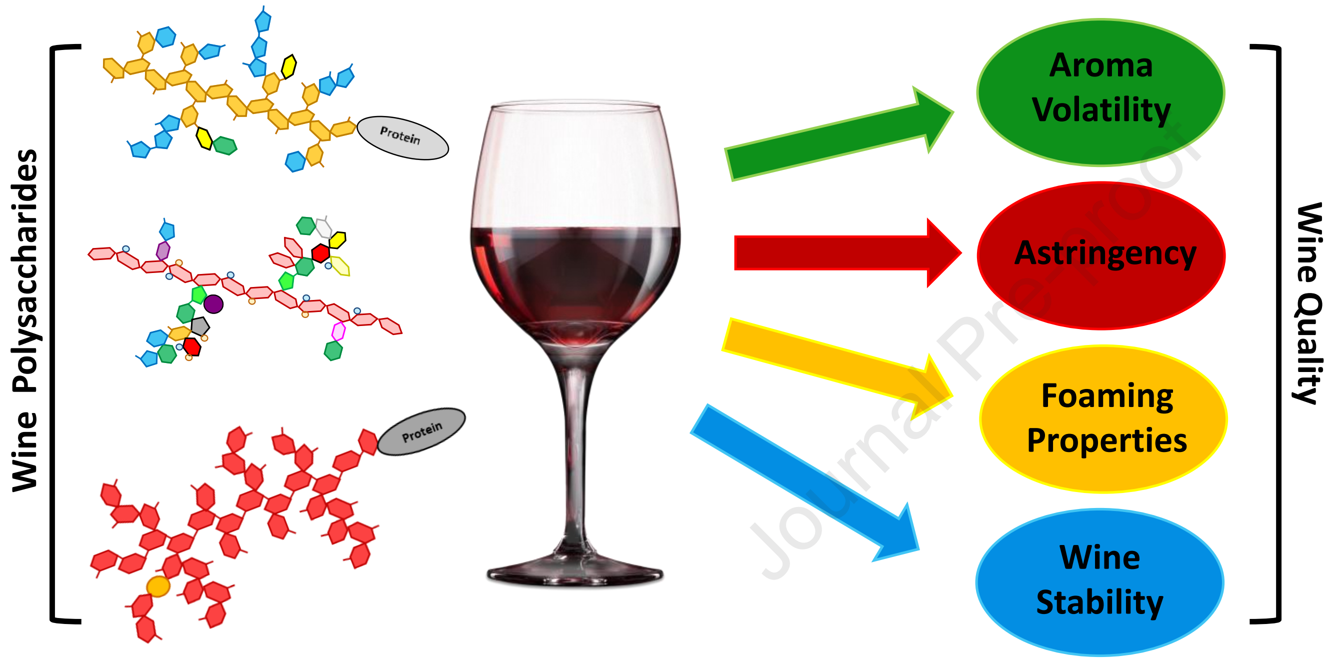

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The interactions of wine polysaccharides with aroma compounds, tannins, and proteins, and their importance to winemaking.

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1.0 Abstract

A current research interest of the wine industry is the improvement of wine quality by producing wines to meet the consumers' demands and desires. However, this requires an understanding of the complex chemical matrix and the nature of interactions between molecular components of the wine. Wine polysaccharides are macromolecules whose presence and interactions with other wine components can lead to the modulation of technological and organoleptic wine quality attributes. Indeed, grape and yeast derived polysaccharides play a major role in modulating wine astringency through interactions with exogenous salivary protein-tannin complexes formed within the oral cavity. Polysaccharides participate in the formation of colloidal particles through their interactions with wine tannins and proteins, with crucial implications on the clarity and stability of finished wines. Additionally, polysaccharides modulate wine aroma volatility and foaming. The extent to which they influence these attributes is dependent on the concentration and physico-chemical properties of all the species involved in these interactions. Overall, the structure, size and type of the polysaccharides are key components governing the success and intensity of their interactions with other species. Therefore, to better understand the relevance of polysaccharides in wine, this review discusses the molecular interactions facilitated by these species and details their potential roles within the wine matrix.

Highlights

1. Elevated glycoprotein concentrations can cause aroma retention.
2. Polysaccharides can improve foam stability in sparkling wines.
3. Physico-chemical parameters of cell wall polysaccharides and tannins can influence their extractability during winemaking.
4. Interactions with endogenous tannin and protein complexes can influence the haze potential of wine.
5. Polysaccharides can play an important role in modulating the astringency of wine.

Keywords

Polysaccharides, wine, stability and organoleptic properties, aroma compounds, tannins and proteins.

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2.0 Introduction

Wine polysaccharides are macromolecules that originate from several sources. It is widely acknowledged that wine polysaccharides can typically be categorised into two classes, they are either grape or yeast derived and are further classified into three families. These include (i) polysaccharides rich in arabinose and galactose (PRAG) including arabinogalactans (AG-I and AG-II) and arabinogalactan proteins (AGP), (ii) rhamnogalacturonans (RG-I and RG-II), both of which are derived from the pectocellulosic cell walls of grape berries, and (iii) mannoproteins (MP) which are released from yeast cells during fermentation and ageing on lees (Guadalupe, Ayestarán, Williams, & Doco, 2014; Jones-Moore, Jelley, Marangon, & Fedrizzi, 2021; Martínez-Lapuente, Guadalupe, & Ayestarán, 2019; Unterkofler, Muhlack, & Jeffery, 2020; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). There are many polysaccharides present in the grape berry, however, many do not survive maceration and/or alcoholic fermentation processes during winemaking and are enzymatically degraded or precipitated; thus the most abundant grape derived polysaccharides found in wine are AGP and RG-II (and sometimes RG-I) (González-Royo, et al., 2013; Jones-Moore, et al., 2021; Vidal, et al., 2003). AGP is a hydroxyproline-rich glycoprotein whose protein moiety is attached to the saccharide backbone *via* a (1 → 4)-ß-D-galactose linkage. The backbone is composed of β-D-galactose (1→3) residues with branched substitutions of β-D-galactose (1→6) residues. Further substitutions can be observed on these branches including (1 → 3) and (1 → 5) linked α-L-arabinose, (1 → 4) linked α-L-rhamnose and (1 → 6) linked β-D-glucuronic acid. (Guadalupe, et al., 2014; Jones-Moore, et al., 2021) RG-II is a highly conserved polysaccharide containing a backbone of (1 → 6) linked β-D-glucuronic acid residues and four different, branched side chains. These side chains contain glycosidic linkages and glycosyl residues unique to plant polysaccharides, allowing for easy identification during compositional analysis (Guadalupe, et al., 2014). Interestingly, RG-II can form complexes with metals such as boron, through its apiosyl-residues to yield a borate-diol ester linkage allowing RG-II to exist as a dimer (dRG-II) (Guadalupe, et al., 2014; Jones-Moore, et al., 2021). Yeast and yeast derivatives can release significant amounts of polysaccharides in the form of mannoproteins, glucans and mannans, mainly originating from cell wall material (Ayestarán, Guadalupe, & León, 2004; Escot, Feuillat, Dulau, & Charpentier, 2001). Mannoproteins are glycoproteins with a backbone of (1→6) linked α-D-mannose, which is often highly branched with other mannose residues connected through α-D-mannose (1→2) or α-D-(1→3)-mannose linkages. Further background information detailing wine polysaccharide structure and profiles are discussed in reviews published by Guadalupe *et al*. and Jones-Moore *et al*. (Guadalupe, et al., 2014; Jones-Moore, et al., 2021).

Other wine polysaccharides can originate from *Botrytis cinerea*, which may be desirable in some cases (König H., 2017), or from exogenous additions including gum Arabic (Apolinar-Valiente, Salmon, et al., 2021; Apolinar-Valiente, et al., 2020; Nigen, et al., 2019) and carboxymethylcellulose (CMC) (Sommer, Weber, & Harbertson, 2019). Wine polysaccharides can exist between the molecular weights of 5–800 kDa (Guadalupe, et al., 2014; Martínez-Lapuente, et al., 2019), and are present in wine at concentrations between 0 and 2 g L^{-1} but these values are highly dependent on factors including vintage, variety, climate, the winemaking stage and winemaking techniques employed (Doco, Brillouet, & Moutounet, 1996; Guadalupe & Ayestarán, 2007; Guadalupe, et al., 2014; Martínez-Lapuente, et al., 2019). Several authors have measured the proportion of each polysaccharide class within certain wines. Vidal *et al.* have reported that Carignan noir wines included in their study were composed of 42% AGP, 35% MP, 19% RG-II and 4% RG-I (Vidal, et al., 2003) and Ayestarán *et al.* identified that red Tempranillo wines were composed of 45% MP, 37% AGP and 15% RG-II (Ayestarán, et al., 2004). Figure 1 below summarises some of the key details discussed above.

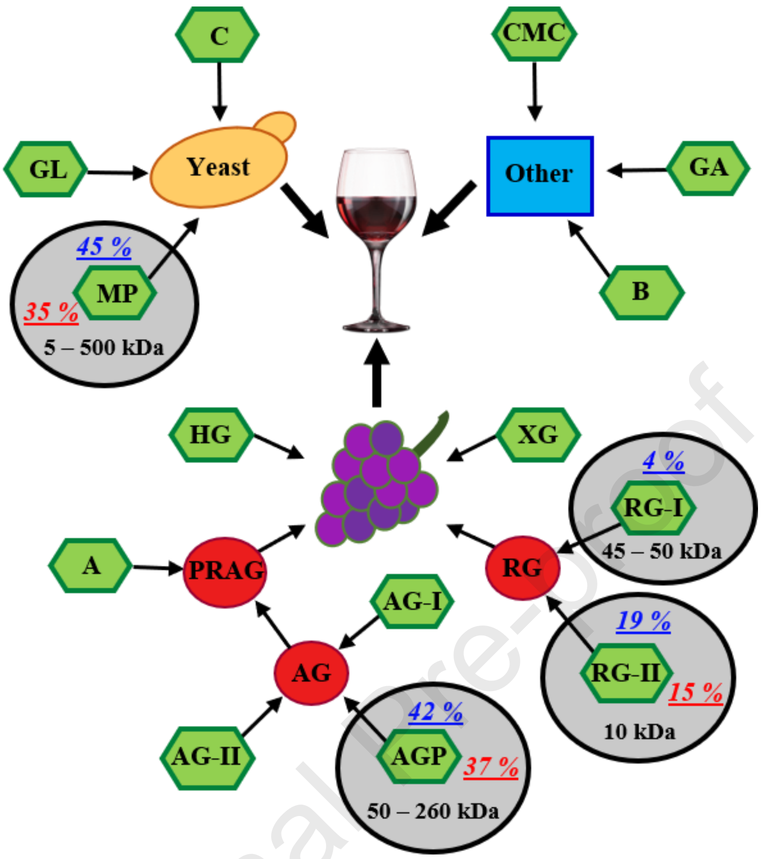

Figure 1. A summary illustration of wine polysaccharides and their respective origins.
The green hexagons represent the polysaccharides, and the red circles show the families that these polysaccharides belong to. The polysaccharides highlighted by the grey circles are the most abundant in wine. The percentages represent their reported compositions in Carignan noir wine (Vidal, et al., 2003) in blue and in Tempranillo in red (Ayestarán, et al., 2004). The respective molecular weights of these polysaccharides are also given in kDa (Guadalupe, et al., 2014; Martínez-Lapuente, et al., 2019).
A: Arabin, AG: Arabinogalactan, AG-I: Arabinogalactan-I, AG-II: Arabinogalactan-II, AGP: Arabinogalactan Protein, B: Bacteria, C: Chitin, CMC: Carboxymethylcellulose, GA: Gum Arabic, GL: Glucans, HG: Homogalacturonan, MP: Mannoprotein, PRAG: Polysaccharides Rich in Arabinose and Galactose, RG: Rhamnogalacturonan, RG-I: Rhamnogalacturonan-I, RG-II: Rhamnogalacturonan-II, XG: Xyloglucan.

The recent review article by Jones-Moore *et al*. discussed the many factors influencing polysaccharide profile of grape and wine, including their evolution and the most crucial steps affecting them throughout vinification (Jones-Moore, et al., 2021).

Due to their colloidal nature, wine polysaccharides are known interact with many other important wine components, including aroma compounds, polyphenols, and proteins. However, as wine polysaccharides are incredibly complex structures, not all polysaccharides exhibit the same behaviour with respect to the examined wine; in particular their influence on wine processes, stability and organoleptic attributes is dependent on the type and concentration of polysaccharide present (Guadalupe, et al., 2014).

Polysaccharides in wine have been reported to play important roles in the stabilisation of other molecules within the medium and in the perceived organoleptic properties of the beverage including astringency (Boulet, et al., 2016; Brandão, et al., 2017; Brandão, Silva, et al., 2020; Susana Soares, et al., 2020), aroma (Dufour & Bayonove, 1999; Jouquand, Ducruet, & Giampaoli, 2004; Villamor, Evans, & Ross, 2013; Villamor & Ross, 2013) and clarity (De Iseppi, et al., 2021; Gazzola, Van Sluyter, Curioni, Waters, & Marangon, 2012; Waters, Pellerin, & Brillouet, 1994a, 1994b). However, their influence on the medium during the winemaking process and towards the organoleptic properties depends not only on the concentration and type of polysaccharide, but also on the presence and quantity of other wine components known to interact with them such as proteins, phenolics and volatiles (Guadalupe, et al., 2014).

A fundamental understanding of the interactions of polysaccharides within the wine matrix and the consequent implications on the quality parameters of the beverage is crucial for oenologists. Such an understanding can assist with the stylisation of their wines, improving quality, and expand the range of successful wines to satisfy consumer desires. This review examines and discusses the important interactions of wine polysaccharides with other wine components, with a focus on their potential roles and implications on the stability and organoleptic properties of wine.

3.0 Molecular interactions of polysaccharides in wine and their implications

3.1 Interaction of polysaccharides with aroma compounds

The aromatic quality of any given wine is an essential organoleptic characteristic governing its overall success and appeal. There are a variety of identifiable aroma compounds within wine, of which four compounds of several of the major aroma groups (methoxypyrazines, sulfur compounds, terpenes and isoprenoids) are highlighted in Figure 2, but a more comprehensive list of aroma compounds are examined in a review published by Villamor *et al.* (Villamor & Ross, 2013). In model systems, polysaccharides generally supress aroma release, either indirectly through a modification of the viscosity of the medium, or by direct molecular interaction with aroma compounds (Jouquand, et al., 2004). Polysaccharides have been reported to individually interact with aroma compounds (Villamor, Evans, Mattinson, & Ross, 2013). Zhu *et al*. (Fengmei Zhu, Du, & Li, 2016) have discussed many factors that can influence the profile of organic volatile compounds within wines, but the scope of their review does not encompass the influence of polysaccharides on aroma substances.

The impact native wine polysaccharides have on the aroma compounds of wine has been investigated by several research groups. Will *et al.* examined the potential interactions of yeast derived MP with aroma compounds in Riesling wine but reported no significant differences in the aroma profile of wines enriched with MP at concentrations levels ranging between 600–1500 mg L^{-1} (Will, Pfeifer, & Dietrich, 1991). However, Lubbers *et al.* (Lubbers, Charpentier, Feuillat, & Voilley, 1994; Lubbers, Voilley, Feuillat, & Charpentier, 1994) investigated MP, yeast cell walls and volatile compounds in model wines and came to a different conclusion, observing that MP and yeast walls did have an impact on the volatility of aroma compounds, reporting that these interactions were dependent on the physico-chemical nature of the volatile compound.

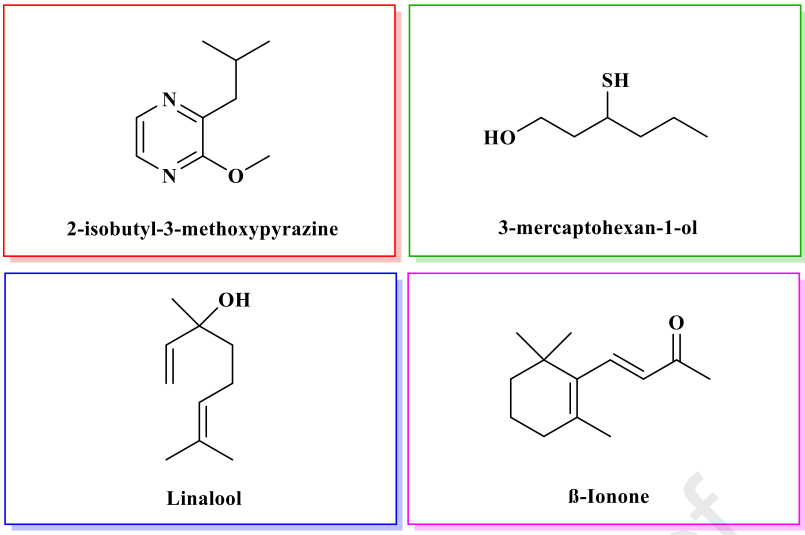

Figure 2. An example of four chemical classes of aroma compounds present in wines, illustrating the chemical structures of a methoxypyrazine, a sulfur compound, a terpene and an isoprenoid (Villamor & Ross, 2013).

The retention of hexanoate and β-ionone by MP was identified by Lubbers *et al.* and this was attributed to an increased protein content of the polysaccharide. The protein portion of a polysaccharide of the MP was therefore thought to be an important factor in governing interactions with volatile aroma compounds of wine (Lubbers, Charpentier, et al., 1994; Lubbers, Voilley, et al., 1994). During further research Lubbers *et al.* observed that the hydrophobic nature of the volatile compound seemed to be an important factor governing their binding affinity for MP. It was thought that increasing the hydrophobicity of both the aroma compound and protein portion of the MP could enhance the interactions and binding of these two species, thus increasing retention and decreasing volatility of aroma compounds (Lubbers, Charpentier, et al., 1994).

Dufour *et al.* (Dufour, et al., 1999) examined the influence of pectic polysaccharides on aromatic volatility, testing the interactions of MP, AGP, monomer RG-II (mRG-II) and dimer RG-II (dRG-II) with a selection of volatile compounds (ethyl hexanoate, isoamyl acetate, hexanol and diacetyl). Corroborating earlier research, increased MP concentrations (1000 mg L^{-1}) reduced the volatility of aroma compounds (e.g. a -12% decrease for ethyl hexanoate), with Dufour *et al*. noting that uronic acid-rich polysaccharides would often "salt out" or precipitate the two volatile esters (isoamyl acetate and ethyl hexanoate) in model wines. Overall, the volatility of these esters was not affected by the addition of any polysaccharides at concentrations between 5 and 20 mg L^{-1}, which is not surprising or significant considering how low these concentrations values are, being much lower than those normally recorded in wine. However, at increased concentration (1000 mg L^{-1}) these two compounds were somewhat supressed in the presence of the protein-containing polysaccharides AGP and MP (Dufour, et al., 1999). This observation is in agreement with the hypotheses proposed by Lubbers *et al.* (Lubbers, Charpentier, et al., 1994; Lubbers, Voilley, et al., 1994), further supporting the idea that it is the protein portion of the MP and AGP that is responsible for the retention of volatile aroma species in wine.

To complicate things further, wine polysaccharides are known to interact with endogenous wine proteins and tannins to form different colloidal species (Marassi, et al., 2021; Mateus, Carvalho, Luís, & de Freitas, 2004), so colloids are a possible candidate responsible for the binding and retention of aroma compounds. Given the involvement of proteins in colloid formation and given that colloids are generally stabilised by the hydrophilic

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portions of the polysaccharide molecules, the interactions between aroma compounds and the protein portion of the colloids must also be governed by the differences in accessibility of the volatiles to the hydrophobic binding sites of the protein portion of the colloids. The involvement of polysaccharides in the colloidal stability of a wine will be discussed in following sections (Sections 3.3 and 3.4).

Mitropoulou *et al.* corroborated findings by Lubbers *et al.* and Dufour *et al.* suggesting that the structure and physico-chemical properties of the polysaccharide and aroma compound are important regarding their interactions (Mitropoulou, Hatzidimitriou, & Paraskevopoulou, 2011). Jouquand *et al.* also reported that the retention of aroma compounds in the presence of polysaccharides seemed to be linked to their hydrophobic nature (Jouquand, et al., 2004). These conclusions suggest that increasing the polysaccharide content could induce preferential binding of the most hydrophobic aroma compounds, and also suggests that a saturation effect may exist within the wine regarding the interaction of these two species (Mitropoulou, et al., 2011).

3.2 Polysaccharide involvement in foam formation and stability for sparkling wines

The foaming properties of sparkling wines are considered key quality attributes that can govern its success, attractiveness and overall appeal (Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015). The foam properties have been linked to the chemical composition of the beverage; with proteins being the first component identified to have an impact due to their intrinsic surfactant properties. This has been supported by several research groups (Blasco, Viñas, & Villa, 2011; Martínez-Rodríguez & Pueyo, 2009; Vincenzi, Crapisi, & Curioni, 2014), but it has been reported that certain proteins have varying effects on the foam. Some proteins are reported to be good foam formers, yet poor stabilisers, and others poor foam formers but good stabilisers (Andrés-Lacueva, Lamuela-Raventós, Buxaderas, & de la Torre-Boronat, 1997; Andrés-Lacueva, López-Tamames, Lamuela-Raventós, Buxaderas, & de la Torre-Boronat, 1996; Cilindre, Liger-Belair, Villaume, Jeandet, & Marchal, 2010; Richard Marchal & Jeandet, 2009; Martínez-Lapuente, et al., 2015). Andrés-Lacueva *et al.* concluded that the presence of proteins and acids in wine resulted in the production of a larger foam volume; however, increasing their content resulted in poorer foam stability (Andrés-Lacueva, et al., 1996). A review by Blasco *et al.* discussed the role of yeast, yeast mannoproteins and other species in foam formation, also noting that foam production and stability depend on other endogenous grape proteins such as the highly glycosylated vacuolar invertase (Blasco, et al., 2011). Dufour *et al.* concluded that the foaming extent of a wine appeared to be correlated to the protein content of the polysaccharides present (Dufour, et al., 1999).

Furthermore, Andrés-Lacueva *et al.* and Cilindre *et al.* discussed how wine variety and ageing can affect foaming properties (Andrés-Lacueva, et al., 1997; Cilindre, et al., 2010). The CO_2 content of a sparkling wine is a crucial parameter influencing effervescence, and Cilindre *et al.* discovered a significant loss of the CO_2 content during ageing, supporting the observation of poorer foaming properties in older sparkling wines. Cilindre *et al.* also suggested that the presence of colloidal material (namely grape derived proteins) is essential in producing satisfactory foam formation and stability. Andrés-Lacueva *et al.* noted that the best Chardonnay foams contain higher quantities of total and neutral polysaccharides, as well as soluble proteins and polyphenols. This finding was attributed to the presence of yeast glycoproteins (MP) and other components deriving from yeast autolysis, a fundamental process in the elaboration of bottle-fermented sparkling wines. Vincenzi *et al.* supported these findings reporting that high molecular weight yeast glycoproteins (MP) gave the highest “foamability”, yet grape derived glycoproteins and proteins did not have an impact on the foam (Kemp, Alexandre, Robillard, & Marchal, 2015; Vincenzi, et al., 2014). However, Vincenzi *et al.* did claim that there were cooperation effects present between the grape and yeast derived glycoproteins with regards to the "foamability" when combined in the model systems. Martínez-Lapuente *et al*. reported that anthocyanins and amino acids contributed positively towards foams height and stability parameters, while tannins contributed in a negative manner (Martínez-Lapuente, et al., 2015). MP and grape derived polysaccharides were identified as poor foam formers but possessed good foam stabilising properties. Furthermore, all polysaccharide fragments had positive influences on foam stabilisation, but grape derived polysaccharides gave a greater correlation coefficient than yeast derived polysaccharides (Martínez-Lapuente, et al., 2015), contradicting findings from Vincenzi *et al*. (Vincenzi, et al., 2014). Overall Martínez-Lapuente *et al.* claimed that the role of polysaccharides in foam stabilisation was attributable to the protein moieties of the glycoproteins, which when present at the gas/liquid interface could interact with other species. It was argued that these interactions (a mix of electrostatic, hydrogen and covalent bonding) could lead to the formation of a strong visoelastic film highly resistant to tension, thus preventing the coalescence of bubbles and improving foam stability (Blasco, et al., 2011; Martínez-Lapuente, et al., 2015). However, more research needs to be conducted to further support these observations.

Recently, Apolinar-Valiente *et al.* demonstrated that *Acacia* gum additions improved the foamability of sparkling base wines treated with bentonite (Apolinar-Valiente, Salmon, et al., 2021; Apolinar-Valiente, et al., 2020). Bentonite, a wine fining agent used to prevent protein haze formation, flocculates wine proteins via electrostatic interactions, consequently causing diminished foaming properties of a wine (Cosme, Fernandes, Ribeiro, Filipe-Ribeiro, & Nunes, 2020; Dambrouck, Marchal, Cilindre, Parmentier, & Jeandet, 2005; R. Marchal, Chaboche, Douillard, & Jeandet, 2002; Van Sluyter, et al., 2015). However, additions of *Acacia* gum can achieve partial foam recovery from this process and, unsurprisingly the foamability recovery was dependent not only on the gum fractions, but also the wine composition. The increased foamability reported by Apolinar-Valiente *et al.* was suggested to be facilitated by possible hydrophobic interactions between PRAG and *Acacia* gum fractions, as the foaming parameters of a wine are highly dependent on the charge characteristics and structural properties of the macromolecules involved (Martínez-Lapuente, et al., 2015). Additional information on sparkling wines is covered in comprehensive reviews published by Kemp *et al.* which identifies the many factors involved at the different stages of bottle-fermented sparkling wines, the chemical components and mechanisms behind foam formation and stabilisation, discussing how these factors and components can influence overall quality (Kemp, et al., 2015; Kemp, et al., 2019).

3.3 Interactions of polysaccharides with proanthocyanidins

Tannins, or proanthocyanidins, are highly reactive, amphipathic polyphenol compounds characterised as either condensed or hydrolysable. Tannins can be considered quality indicators among red wines, critical for establishing several sensory attributes. They play a vital role in pigmentation and are present in the vegetative tissue of the grapevine and many other plant species (Smith, McRae, & Bindon, 2015). Anthocyanins, a sub-group of flavonoids, are phytochemicals present in grape berries responsible for the colour of grapes and thus wines. Tannins are known to interact with anthocyanins to stabilise and enhance the colour of a finished wine (Bautista-Ortín, Fernández-Fernández, López-Roca, & Gómez-Plaza, 2007; Cheynier, et al., 2006; Springer, Sherwood, & Sacks, 2016), a topic discussed in detail by Freitas *et al*. (Freitas, Fernandes, Oliveira, Teixeira, & Mateus, 2017). Tannins are located in the skin and seeds, (in particular in the vacuoles of the cells) of grape berries and during vinification, like polysaccharides, these polyphenols can be extracted and are present in the final wine (Bindon, Li, Kassara, & Smith, 2016; Bourvellec & Renard, 2012; Le Bourvellec, Guyot, & Renard, 2009; Renard, Watrelot, & Le Bourvellec, 2017; Ruiz-Garcia, Smith, & Bindon, 2014; Smith, et al., 2015; Watrelot, Le Bourvellec, Imberty, & Renard, 2014). Tannins are almost-always present in higher concentrations in red wines than in white wines, a consequence of the extensive maceration step during red winemaking (Ducasse, et al., 2010; Smith, et al., 2015; Unterkofler, et al., 2020). Many reactions occur throughout different stages of vinification that contribute to the evolution of tannins and increased complexity of the finished wine (Smith, et al., 2015). Tannins are key contributors to the ageing potential and structure of wine, taste, colour, and mouthfeel (e.g. astringency, hotness) (Gawel, Smith, Cicerale, & Keast, 2018) of the beverage, all important organoleptic characteristic of red wines (García-Estévez, et al., 2017; Riou, Vernhet, Doco, & Moutounet, 2002; Smith, et al., 2015; Vidal, et al., 2004; Watrelot, Schulz, & Kennedy, 2017). An important intrinsic property of tannins is their ability to self-aggregate to form a polymeric structure, which in grapes is often more uniform, however in wine, these polymeric tannin structures possess greater chemical complexity, as highlighted in Figure 3. Monomers such as (-)-epicatechin and (+)-catechin are linked by C4-C8 or C4-C6 interflavan linkages, with mean degrees of polymerisation (mDP) reported to be 18 in grape seeds and 28 in skins (Pinelo, Arnous, & Meyer, 2006; Riou, et al., 2002). Tannins have been reported to exhibit several health benefits in humans (Rauf, et al., 2019), acting as antioxidants (Koleckar, et al., 2008) and anticarcinogens (Cai, et al., 2017). Further information and discussion focussed on red wine tannins and the analytical techniques used to characterise and quantify them can be found in the comprehensive reviews published by Smith *et al*. (Smith, et al., 2015), Zhu (Fan Zhu, 2018), Hanlin *et al.* (Hanlin, Hrmova, Harbertson, & Downey, 2010) and Le Bourvellec *et al.* (Bourvellec, et al., 2012).

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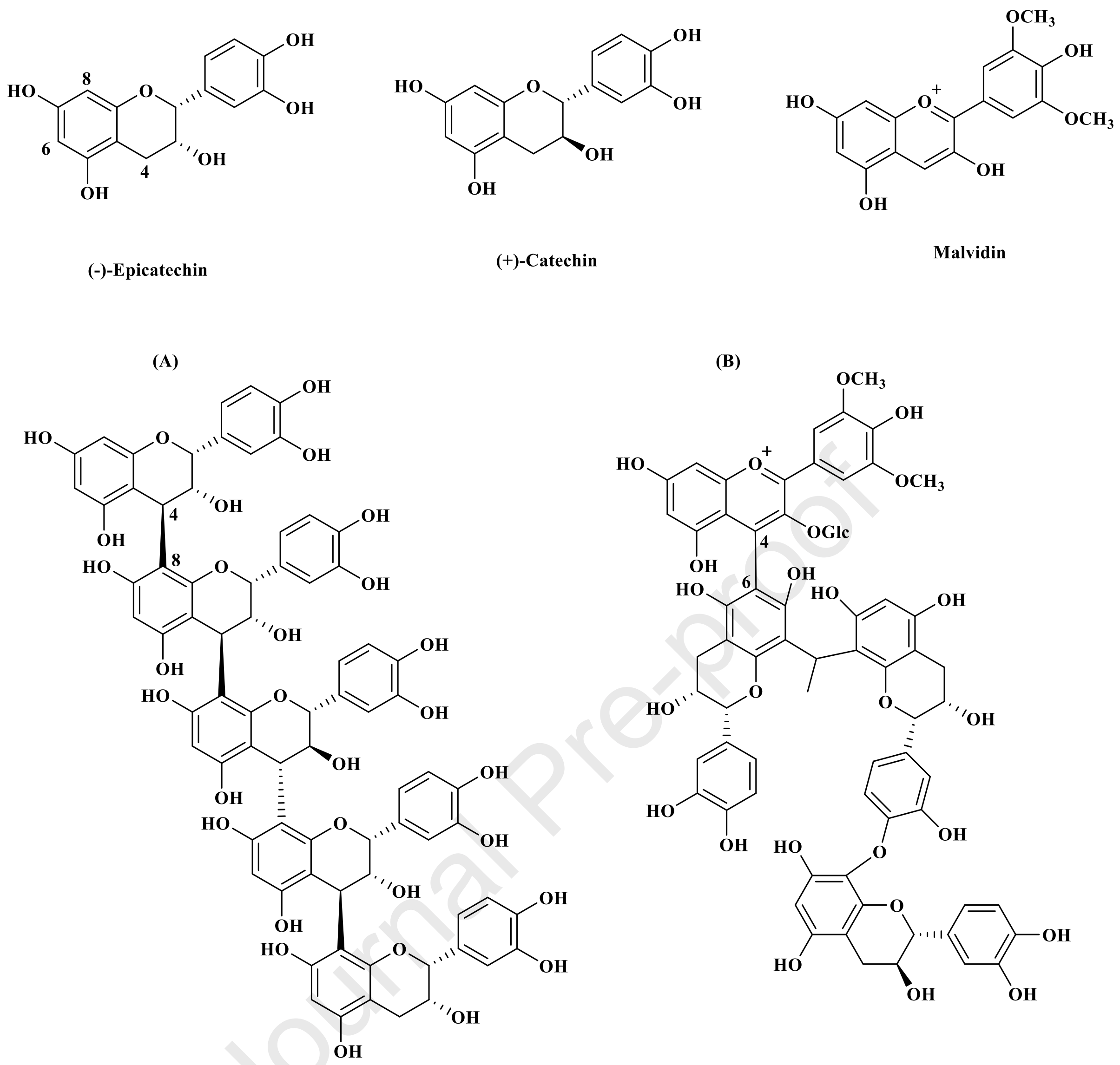

Figure 3. Grape seed and skin tannins undergo structural evolution during the winemaking and ageing processes. This figure illustrates an example of a more uniform, <u>grape derived tannin (A)</u> structure showing C4-C8 linkages, in comparison to a more complex <u>wine tannin</u> (B) structure showing the presence of C4-C6 linkages, with an example of some respective monomers above (Hanlin, et al., 2010; Smith, et al., 2015).

The colloidal behaviour of wine components is an important concept for oenology and studying wine colloids is a challenging task often due to their innate instability (Marassi, et al., 2021). Polysaccharides have been reported to interact with tannins and can act as "protective colloids" (Pellerin & Cabanis, 1998; Waters, et al., 1994a, 1994b). Riou *et al.* performed a study to explore this by investigating the influence of wine polysaccharides on tannin aggregation using several polysaccharides fractions (AGP acidic and neutral, mRG-II, dRG-II and MP) purified from red wines (Riou, et al., 2002). Dynamic light scattering (DLS) analysis showed that procyanidins (condensed tannins) do spontaneously aggregate into colloidal-sized particulates in the presence of tannins at concentrations consistent with those commonly found in red wines. This finding was in agreement with Saucier *et al.* who identified polymeric tannin colloids of 400 nm diameter in model systems (Saucier, Bourgeois, Vitry, Roux, & Glories, 1997). Following the addition of the purified wine polysaccharides, Riou *et al.* discovered that none of the fractions (AGP acidic and neutral, mRG-II, dRG-II or MP) prevented initial tannin aggregation, but some did influence the particle size evolution. It was identified that mRG-II and neutral AGP fractions had no impact on tannin aggregation, even at increased concentrations of tannins; however, acidic AGP and MP notably inhibited particle size growth. Interestingly, Riou *et al.* concluded that dRG-II strongly enhanced tannin aggregation, suggesting a co-aggregation between the dimer of RG-II and tannins. An example illustration of tannin interactions (only hydrogen bonding is shown) with an acidic polysaccharide fraction is presented in Figure 4. As mentioned earlier, acidic AGP and MP inhibited the further growth of colloidal tannin aggregates, whereby two hypotheses are proposed to explain this phenomenon. The first was the competition of polysaccharides with other polyphenol compounds interfering with further tannin aggregation, and the second was the adsorption of polysaccharides onto particles formed by tannins, which would prevent further particle evolution (Marassi, et al., 2021; Riou, et al., 2002). Similar hypotheses have also been used to explain the potential interactions of polysaccharides in tannin-protein association (Brandão, Fernandes, et al., 2020; Brandão, et al., 2017; Brandão, Silva, et al., 2020; Ruiz-Garcia, et al., 2014; Watrelot, et al., 2017).

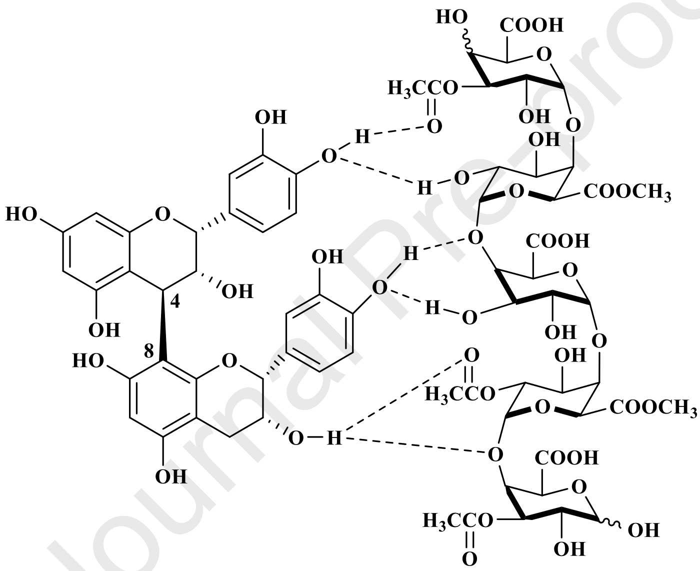

Figure 4. An example of the proposed hydrogen bonding that exists between the galacturonic acid backbone of pectic polysaccharides and monomeric units of the tannin structure (Hanlin, et al., 2010).

Work by Mateus *et al.* supported this hypothesis through highlighting the potential colloidal formation system in model wine that was assessed by nephelometry using BSA, grape seed tannins and carbohydrates (Mateus, et al., 2004). Recently, Marassi *et al.* confirmed this hypothesis by analysing, for the first time, colloids in their native state in real red wines using a specifically developed asymmetrical flow-field flow fractionation (AF4)-multidetection and fractionation method. In this way, the authors showed that polysaccharides are always present in the tannin colloidal particles found in real wine samples and demonstrated that polysaccharides bind to tannin and protein aggregates, “coating” these particles to form a stable colloid that would otherwise precipitate (Marassi, et al., 2021).

Poncet-Legrand *et al.* investigated how the molecular weight of MP could influence their interactions with tannin aggregates (Poncet-Legrand, Doco, Williams, & Vernhet, 2007). It was shown that at typical wine conditions the low to medium molecular weight species (50 kDa) acted as more efficient stabilising agents through steric stabilisation, with high molecular weight species imparting no influence. These polysaccharides were said to act

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as stabilising agents by preventing flocculation of tannin aggregates.

Investigations by Watrelot *et al.* utilising alternative techniques such as isothermal titration calorimetry (ITC) and absorption analysis, examined the interactions between pectic polysaccharides and procyanidins at two different degrees of polymerisation (DP), DP9 and DP30 (Watrelot, Le Bourvellec, Imberty, & Renard, 2013; Watrelot, et al., 2014). The associations between pectic polysaccharides and procyanidins are known to be a combination of hydrophobic interactions and hydrogen bonding (Smith et al., 2015; Watrelot et al., 2014), with the hairy regions, a term used to describe the highly branched polysaccharides of pectin (RG-II and AGP), interacting preferentially with highly polymerised tannins (DP30). No differences were observed between association constants for lower degrees of polymerisation (DP9) (Watrelot, et al., 2014). Watrelot *et al.* observed no interactions between mRG-II or dRG-II at DP9 with only very weak interactions confirmed at DP30, concluding that RG-II does not bind tannins efficiently. Furthermore, Watrelot *et al.* observed obvious aggregation of protein-rich neutral AGP with procyanidins at DP30, contradicting findings outlined by Riou *et al.* (Riou, et al., 2002). The discrepancy between these results could be attributed to the medium in which Riou *et al.* conducted their experiments. The ionic strength of the medium could drastically influence the results obtained as high ionic strength is known to induce the self-aggregation of tannins and also limit the aggregation potential of neutral AGP (Watrelot, et al., 2014). Overall Watrelot *et al.* concluded that interactions between pectic polysaccharides and tannins do exist and increase with greater degrees of polymerisation. These interactions are also dependent on the physico-chemical properties of the neutral sugars side chains of pectins, with a greater association observed between arabinans and arabinogalactans than with rhamnogalacturonans (Watrelot, et al., 2013, 2014). Corroborating these findings, Thongkaew *et al*. reported that affinity of tannins for polysaccharides increased as the tannin DP increased, identifying that polysaccharides must have the appropriate properties to efficiently undergo complexation with tannins (Thongkaew, Gibis, Hinrichs, & Weiss, 2014). Using transmission electron microscopy techniques, Mamet *et al*. noticed that the addition of pectic polysaccharides to solutions of tannin aggregates (DP5 and DP26) decreased the average particle size of the aggregate, indicating that pectic polysaccharides can hinder the self-aggregation of tannins. This highlighted that the strength of these interactions was dependent on the structural characteristics of the polysaccharides, but the complex formation between the two species was governed by hydrophobic interactions and hydrogen bonding (Mamet, Ge, Zhang, & Li, 2018).

3.3.1 Tannin interactions with grape cell walls and cell wall polysaccharides.

The interaction of tannins with cell wall components has important oenological implications regarding extractability of grape derived compounds. Le Bourvellec *et al*. discovered that the presence of tannins in the cell wall limited the extractability of polysaccharides and that the presence of methylated galacturonans within the pectic polysaccharide network further enhanced the binding of tannins (Le Bourvellec, et al., 2009). Furthermore, Le Bourvellec *et al.* claimed that modifications to the protein content of the cell wall had no impact on the adsorption of tannins, thus suggesting that it is the polysaccharides that play a crucial role in the mediation of tannin-cell wall interactions (Bourvellec, et al., 2012). Ethanol has been shown to interfere with tannin-cell wall interactions through a decrease in solvent polarity in an aqueous environment and thus, leading to disruptions in hydrophobic interactions (Hanlin, et al., 2010; Le Bourvellec, Guyot, & Renard, 2004). This suggests that interactions between tannins and cell walls are less pronounced in a wine medium than they are in an aqueous medium.

Ruiz-Garcia *et al.* studied the influence of selective polysaccharide extraction on the binding properties of tannins with the cell wall, identifying that the bulk of cell wall-tannins are bound to a relatively minor component of the cell wall (Ruiz-Garcia, et al., 2014). Hemicellulose fractions were found to have a high intrinsic binding capacity for tannins, with cellulose and lignin by weight having the lowest, which could be explained by the difference in composition and structures of the polysaccharides, as alluded to earlier with findings from Watrelot *et al.* (Ruiz-Garcia, et al., 2014; Watrelot, et al., 2013, 2014). The selective removal of galacturonan-rich pectic polysaccharides from the cell wall resulted in the greatest reduction in tannin-cell wall binding. This demonstrated a higher propensity of tannins to associate with galacturonic acid-rich components of the cell wall, suggesting a strong relationship between the pectic cell wall composition and tannin-cell wall affinity, which is supported by other research (Bindon, et al., 2016; Bourvellec, et al., 2012; Le Bourvellec, et al., 2009; Watrelot, et al., 2014). It has also been identified that grape skin ripeness can influence the adsorption of tannins to cell wall components (Renard, et al., 2017). Ruiz-Garcia *et al.* proposed that all cell wall fractions displayed a preference for the highest molecular weight tannins, suggesting there is selectivity with regards to tannin binding; polysaccharides exhibit greater affinities for tannins that are of a greater degree of polymerisation (Renard, et al., 2017; Ruiz-Garcia, et al., 2014; Watrelot, et al., 2013, 2014). These findings are important as they identify a possible limiting step for the solubility and extraction of polysaccharides and tannins, thus implying that the cell wall structure could be modified to alter the extractability of these compounds with techniques that manipulate pectin and hemicellulose having the biggest influence on extractions. This could allow for strategic implementations during the commercial operations of winemaking to optimise tannin extraction based on the desired outcome of the final wine (Ruiz-Garcia, et al., 2014). Interestingly, the use of insoluble cell wall polysaccharides as endogenous fining agent as a potential replacement for animal and plant based fining agents are also being investigated (Marangon, Vincenzi, & Curioni, 2019).

3.3.2 Inactive yeast additions as a source of polysaccharides; their interactions, and their implications.

In recent years, a large variety of commercial inactivated, dry yeast products have been gaining popularity in enology, primarily as an additional source of polysaccharides, in the form of mannoproteins. These polysaccharides are normally released from active yeast material during fermentation or during ageing on lees (Jones-Moore, et al., 2021). However, their release from lees is often slow, thus alternatives are being studied with the intention of producing wines with similar characteristics to those aged on lees (Del Barrio-Galán, Pérez-Magariño, Ortega-Heras, Guadalupe, & Ayestarán, 2012).

These additives can increase the polysaccharide content of wine, improve mouthfeel and have positive implications on wine colour, and foamability (Del Barrio-Galán, Pérez-Magariño, Ortega-Heras, Williams, & Doco, 2011; González-Royo, et al., 2013; González-Royo, et al., 2017; Mekoue Nguela, Vernhet, Sieczkowski, & Brillouet, 2015). González-Royo *et al.* trialled the use of yeast strains with a greater capacity for releasing polysaccharides and supplementation with inactive yeast derivatives to favour the release of polysaccharides. They identified a 32% increase in polysaccharides within wines fermented using a ‘high polysaccharide-releasing’ yeast strain and up to a 20% increase in polysaccharides in wines supplemented with inactive yeasts. The treated wines were also less bitter than the controls (González-Royo, et al., 2013).

Del Barrio-Galán *et al*. investigated the polysaccharide content of some commercial, inactive yeast derivatives and their influence on Verdejo and Tempranillo wines, noting that their content and composition was dependent on the manufacturing process and the purity of the product (Del Barrio-Galán, et al., 2011). Supplemented red wines had reduced 'green' tannins, increased 'palate softness', and stabilised colour, more notably when additives with a higher release of neutral polysaccharides were employed (Del Barrio-Galán, et al., 2012).

To corroborate findings from González-Royo *et al*., all wines with yeast additions exhibited a statistically significant increase in the concentration of total and neutral polysaccharides (Del Barrio-Galán, et al., 2012; Del Barrio-Galán, et al., 2011). However, during ageing, all treated wines exhibited a notable decrease in the abundance of these polysaccharides; a decrease that could be attributed to their interaction and complexation with other compounds to form unstable, colloidal species that could precipitate (Del Barrio-Galán, et al., 2011; Guadalupe, et al., 2007; Guadalupe & Ayestarán, 2008). Furthermore, Del Barrio-Galán *et al.* observed that yeast lees and other yeast derivatives can also adsorb phenolic compounds, consequently reducing their concentration in the final wine in comparison to controls. This reduction was dependent on the treatment, the phenolic compound, and the stage of vinification or ageing process. Moreover, their results suggested that interaction and adsorption does not occur immediately after supplementation but is time dependent (Del Barrio-Galán, et al., 2011).

Interestingly, Pérez-Magariño *et al.* did not observe any differences in the content of phenolic compounds in sparkling Verdejo and Godello wines treated with inactive yeast derivatives (Pérez-Magariño, et al., 2015). However, the addition of yeast derivatives with the highest mannoprotein content and greatest purity significantly modified the aroma composition, maintaining higher concentrations of terpenes, whilst enhancing the fruity aromas of both wines.

As mentioned in section 3.3.1, grape tannins have a very strong affinity for grape cell wall polysaccharides. Research from Mekoue Nguela *et al.* identified a very strong affinity and high adsorption potential for grape seed and skin tannins towards yeast cells (Mekoue Nguela, Sieczkowski, Roi, & Vernhet, 2015). Yet interestingly, they identified that the chemical evolution of grape tannins to wine tannins during winemaking and ageing (Figure 3) influenced the affinity and adsorption of these compounds to yeast products, with grape tannins having a higher affinity towards yeast derivatives than wine tannins (Mekoue Nguela, Sieczkowski, et al., 2015). More specifically, yeast 'whole cell' products had a greater capacity for irreversible adsorptions of grape and wine tannins in comparison to yeast 'cell wall' products only (Mekoue Nguela, Vernhet, et al., 2015). This reiterates that the type of yeast derivative treatment is important for determining the release of polysaccharides and the respective adsorption properties of the yeast derivative.

3.4 Protein interactions with polysaccharides & tannins

Proteins are generally present in relatively low concentrations in wine and provide little nutritional value in the finished beverage. However, they have crucial technological and economic importance in winemaking, influencing aroma, foam properties and the overall the stability and clarity of a wine. Previous sections established that grape and yeast derived glycoproteins play an important role in the aroma volatility, and the foaming properties of sparkling wine beverages. This section will detail the role of endogenous and exogenous proteins and their interactions and implications during winemaking procedures.

3.4.1 Endogenous proteins role in haze, precipitation, and sediment formation: Colloidal importance

Tannins, by definition are protein binding agents, can act as multi-dentate ligands to bridge proteins or complexes to create aggregates (Carvalho, et al., 2006) and can be responsible for unwanted haze formation. Tannins have been identified to interact with endogenous wine proteins. Bindon *et al.* highlighted a potential mechanism for the loss of grape-extracted tannins from wine during winemaking, concluding that up to 50% of grape-extracted tannins could be lost as a result of complexation and precipitation with grape soluble proteins (Bindon, et al., 2016). The clarity of a finished wine is an important property that contributes to its overall success (Waters, et al., 1994a, 1994b), however, naturally occurring grape derived proteins, in particular pathogenesis-related (PR) proteins such as thaumatin-like proteins (TLP) and chitinases (Gazzola, et al., 2012), can become unstable over time and begin to aggregate and precipitate to form unwanted sediments or haze (Van Sluyter, et al., 2015; Waters, et al., 1994a). PR protein concentrations are generally low in healthy plants, however along with other hydroxyproline-rich glycoproteins (e.g. extensin and AGP), these molecules are involved in the plants primary defence mechanism and can spike in concentration in response to stress-related events such as wounding or pathogenic attack (Ferreira, Piçarra-Pereira, Monteiro, Loureiro, & Teixeira, 2001; Nunan, Sims, Bacic, Robinson, & Fincher, 1998; Ribeiro, et al., 2006; Van Sluyter, et al., 2015; Waters, et al., 2005). Therefore, harvesting and maceration treatments during early winemaking steps could increase the release of these proteins, increasing their presence in grapes. This causes complications as these proteins are resistant to the low pH of wine and proteolysis, and consequently are able to survive winemaking processes (Ferreira, et al., 2001).

Protein-induced tannin precipitation can be detrimental to the success of a finished wine as red wines with low tannin concentrations, due to precipitation, are correlated with lower bottle prices and poorer consumer ratings due to impaired mouthfeel associated with low astringency (Springer & Sacks, 2014). Springer *et al.* discovered that an American interspecific hybrid (*Vitis* spp.) yielded a wine with lower tannin concentrations than European wine varieties (*Vitis vinifera*) (Springer, et al., 2014) and later, Springer *et al.* observed that these hybrids contained a high content of PR proteins (Springer, et al., 2016). It was concluded that the elevated contents of PR proteins react with tannins causing aggregation, leading to precipitation and thus poor tannin retention in the final wine. Springer *et al.* suggested that exogenous additions of condensed tannins (CT) could remedy this issue and during their investigations, it was demonstrated that retention of exogenous CT added to a finished wine was inversely correlated with the concentration of wine protein (Springer, et al., 2016).

During the 1990s, Waters *et al.* reported the identification and characterisation of a wine AGP (Waters, et al., 1994b) and a yeast MP (Waters, et al., 1994a) that were able to alter the colloidal state of a wine. Waters *et al.* provided evidence suggesting that the AGP reduced heat-induced protein haze and the yeast MP acted as a "protective colloid", thereby reducing protein haze spoilage by decreasing haze particle size. These glycoproteins offered a potential alternative to some fining techniques and provided a role for yeast MP in the wine medium (Waters, et al., 1994a). However, more recently there have been discrepancies and contradictions in the literature involving the role of polysaccharides as protective colloids. Research from Moine-Ledoux *et al.* and Brown *et al.* supported the role of MP as protective colloids, reporting improvements in protein stability in the presence of MP (Moine-Ledoux & Dubourdieu, 1999). Brown *et al.* concluded that the overexpression of specific genes coding for haze protective MP from different yeast strains resulted in greater haze protective activity (Brown & Stockdale, 2007). However, research by Mesquita *et al.* (Mesquita, et al., 2001) reported that wine polysaccharides adversely affected haze formation through increasing protein instability. More recently, Gazzola *et al.* compared several types of wine proteins and their ability to form hazes and concluded that chitinases, a class of wine protein identified to be the most susceptible to aggregation, formed the largest haze particulates as determined by scanning ion occlusion sensing (SIOS). Colloidal properties were not significantly altered by the presence of wine polysaccharides or polyphenols (Gazzola, et al., 2012). Furthermore, TLP showed large variability in aggregative and colloidal behaviour, with some isoforms of TLP increasing in particle size in the presence of polysaccharides and polyphenols, while others reduced in size in their presence (Gazzola, et al., 2012). Additionally, Gazzola *et al.* argued that isoforms of the same protein can have different intrinsic haze potentials, a finding later corroborated by Marangon *et al.* (Marangon, Sluyter, Waters, & Menz, 2014). This potentially explains why some wines such as Sauvignon blanc, are reported to be more susceptible to protein haze than others like Chardonnay, resulting in conflicting ideas between haze formations and the ability of other compounds to modulation their aggregation and haze potential (Gazzola, et al., 2012). Overall, the type of protein was a more important factor governing the haze potential than the presence of other components in the medium, such as polysaccharides.

Addition of exogenous AGP from Acacia *senegal* gum can been utilised as a protective colloid, primarily to prevent the precipitation of pigmented compounds in red wine. Nigen *et al.* investigated the role of AGP in this colloidal stability, discovering that the protective activity was dependent on the protein content of AGP and the accessibility of polypeptide backbone; the higher the protein content and the more accessible the polypeptide backbone, the more efficient the colloidal stability (Nigen, et al., 2019).

With the reported benefits of polysaccharides in wine, in particular MP, Guadalupe *et al.* performed a study which examined the potential compositional effects of the addition of an exogenous, commercial MP on Tempranillo wines (Guadalupe, et al., 2008). They observed that the added MP had no influence on the content of grape derived polysaccharides, but more importantly reported a decrease in high molecular weight MP during vinification, coinciding with a decreased tannin content. This suggested a co-aggregation and precipitation of MP with tannins; Guadalupe *et al*. concluded that MP did not act as stabilising colloids under the conditions studied. The colloidal properties and stability of wine is currently a popular research topic, knowledge involving the underlying mechanisms is scarce, however it is widely agreed that polysaccharides have a major role in this process. More research is required to understand the extent to which they are involved.

3.4.2 Interactions involving exogenous proteins: Fining agents

The sediment and haze associated with wine protein and tannin aggregations can be removed by the addition of exogenous fining agents during the clarification processes of winemaking. These techniques provide clarity, and stability to a wine, modifying its organoleptic characteristics, however, some of these techniques can be costly, and prove detrimental to wine quality (Ferreira, et al., 2001).

Fining agents are generally proteinaceous products, utilising the advantageous, intrinsic protein-binding properties of tannins for these procedures. Egg albumin, milk caseinates and fish gelatine are common organic fining agents, but some inorganic agents such as bentonite, carbon and polyvinylpolypyrrolidone (PVPP) are also widely used. Peñas *et al.* discusses common wine fining agents and the implications arising from their use in their review (Peñas, Di Lorenzo, Uberti, & Restani, 2015). Allergenic repercussions associated with the use of animal proteins as fining agents for some consumers are a concern even if trace amounts of material are carried through vinification. The use plant proteins during wine fining has gained attention in recent years as a more allergenic and vegan-friendly option for winemakers. Thus, wine fining using plant proteins has become more popular and its adoption and implications are discussed extensively by Marangon *et al.* (Marangon, et al., 2019). Other literature exploring wine haze and clarification has been published by Mierczynska-Vasilev *et al*. (Mierczynska-Vasilev & Smith, 2015), Van Sluyter *et al*. (Van Sluyter, et al., 2015) and Cosme *et al.* (Cosme, et al., 2020). In their recent publications, Sommer *et al*. and Marassi *et al.* investigated wine colloidal stability confirming that wine polysaccharides do indeed play a major role in this aspect (Marassi, et al., 2021; Sommer, et al., 2019). Wine

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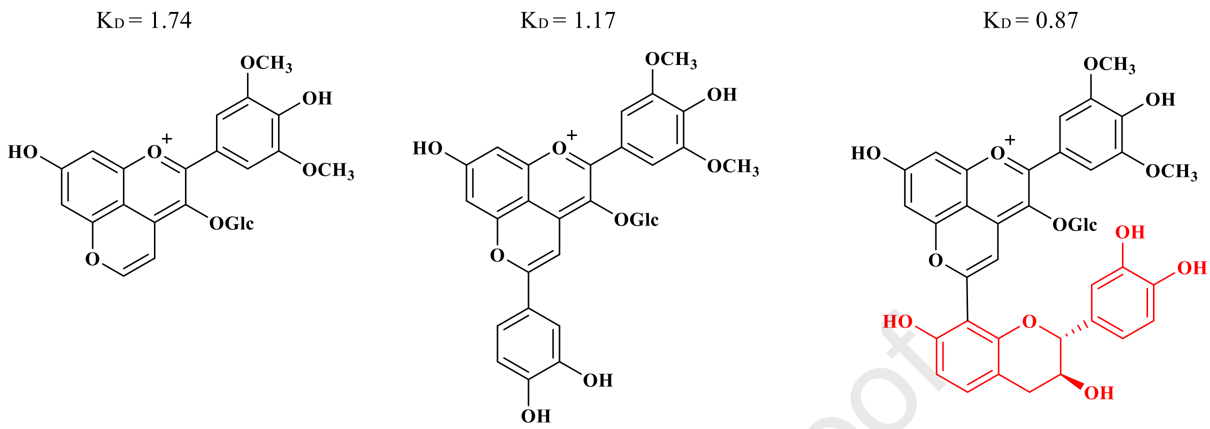
polysaccharides could have negative implications for the effectiveness of wine fining treatments, as one could question whether fining agents can function effectively when the tannins they target are buried in a colloid in wine polysaccharides (Marassi, et al., 2021; Van Sluyter, et al., 2015); an important concept that should be explored further.

3.4.3 Interactions involving exogenous proteins: Salivary proteins

It has been identified that tannins can interact and form complexes with salivary proteins (McRae & Kennedy, 2011), which has important implications involving the organoleptic properties of the beverage (García-Estévez, et al., 2017; Gombau, et al., 2019; Gyémánt, et al., 2009; Ployon, et al., 2018; Alba María Ramos-Pineda, et al., 2017; A. M. Ramos-Pineda, et al., 2019; Sarni-Manchado, Cheynier, & Moutounet, 1999; Susana Soares, et al., 2011; Vidal, et al., 2004; Watrelot, et al., 2017). Much research has been performed to highlight and understand the influence of wine polysaccharides on this tannin-protein complex (Brandão, Fernandes, et al., 2020; Brandão, et al., 2017; Brandão, Silva, et al., 2020; Chong, Cleary, Dokoozlian, Ford, & Fincher, 2019; García-Estévez, et al., 2017; Lei, et al., 2019; Mateus, et al., 2004; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014; Vidal, et al., 2004; Watrelot, et al., 2017). Soares *et al.* summarises the techniques used in the literature to analyse these interactions (Susana Soares, et al., 2020). The ability of wine polysaccharides to influence tannin-protein interactions was recently examined by Watrelot *et al.* using high performance liquid chromatography (HPLC) and ultraviolet-visible spectroscopy (UV-Vis) techniques to quantify the degree of protein precipitation, which they termed "tannin-activity" (Watrelot, et al., 2017). The chemical profiles of several Cabernet Sauvignon (CS) and Pinot noir (PN) wines from different regions were analysed, noting that CS wine tannins had a larger DP than the PN wines. CS wines also had a higher tannin-activity than PN wines, which follows the trend reported in earlier literature whereby tannins with increasing DP resulted in greater tannin affinity towards polysaccharides (Watrelot, et al., 2014; Watrelot, et al., 2017). Overall, the interactive ability (tannin-activity) of tannins towards a hydrophobic surface was not altered significantly following the addition of wine polysaccharides (Watrelot, et al., 2017). This statement supported their hypothesis that tannin-polysaccharide interaction was of non-covalent nature and could be disrupted by HPLC conditions, a finding also corroborated by Marassi *et al.* (Marassi, et al., 2021). However, despite reporting no significant differences, most interactions that did occur, did so when the polysaccharide concentrations were double that of the tannins, and further concluded that tannins had a greater affinity to bind with proteins compared to self-aggregation (Watrelot, et al., 2017).

Recently, more advanced techniques such as synchrotron radiation circular dichroism (SCRD) (Di Gaspero, et al., 2020), saturation transfer difference-NMR (STD-NMR) (Brandão, et al., 2017; García-Estévez, et al., 2017) and MALDI-TOF (García-Estévez, et al., 2017) have been utilised to examine protein-polyphenol interactions and any influences polysaccharides have on these interactions. Anthocyanins are a key family of polyphenols present in red wine and García-Estévez *et-al.* hypothesised whether pyranoanthocyanins, the pigment compounds responsible for the colours in red wine, could influence other organoleptic characteristics such as flavour or astringency (García-Estévez, et al., 2017). García-Estévez *et al.* investigated the interactions between the red wine pyranoanthocyanins, pyranomalvidin-3-glucoside (vitisin B), pyranomalvidin-3-glucoside-catechol, and pyranomalvidin-3-glucoside-epicatechin (Figure 5), and a family of acidic proline-rich salivary proteins (aPRP). The aPRP are the most abundant PRP in human saliva and have been identified to be the most reactive towards tannins (García-Estévez, et al., 2017), thus investigations were performed to understand the nature of the interactions, the potential mechanisms with additional compounds and any important implications as result of these events.



Pymv3glc (Vitisin B) **Pymv3glc-catechol** **Pymv3glc-epicatechin**

Figure 5. An illustration of the three polyphenols studied by Garcia Estevez *et al.* with their respective dissociation constants (K_D). The lower the K_D, the greater the binding affinity for the given protein, thus Pymv3glc-epicatechin, containing a "tannin structural unit" (shown in red) bound with the greatest affinity. Pymv3glc = Pyranomalvidin-3-*O*-Glucoside (García-Estévez, et al., 2017).

The interactions and complexes formed between aPRP and polyphenols were analysed using MALDI-TOF and STD-NMR to determine the dissociation constants (K_D) based on the molecular weights and proton signals of the complexes obtained. The higher the K_D value, the lower the binding affinity for aPRP. García-Estévez *et al.* concluded that different polyphenol compounds form a different number of aggregates with aPRP, stating that the presence of other moieties, such as the tannin "structural unit" monomer epicatechin (highlighted in red in Figure 5), resulted in a greater binding affinity with proteins, reflected by their K_D values. Pymv3glc-epicatechin showed the greatest interaction with aPRP, concluding that the presence of "structural units" of tannins in other polyphenol compounds, such as pyranoanthocyanins, could induce interactions with proteins, thus influencing the organoleptic properties of the wine (García-Estévez, et al., 2017). These findings corroborated research from Mamet *et al.* who identified that the presence of gallate moieties in highly polymerised tannins could enhance the affinity of highly methylated pectic polysaccharides. Using UV-Vis techniques, Mamet *et al.* noticed that increasing the degree of esterification (DE) of the polysaccharide and the DP of the tannin increased absorbance, correlating to increased interactions between the two moieties (Mamet, et al., 2018).

Soares *et al.* highlighted that polysaccharides can have varying propensity for tannin-protein binding and interact through different mechanisms of actions depending on their physico-chemical properties (Susana Soares, Mateus, & de Freitas, 2012). The deeper implications of these findings were investigated by Brandão *et al.* where possible interactions of wine polysaccharides AGP and RG-II on salivary protein-tannin complexes were examined using HPLC and STD-NMR (Brandão, et al., 2017). The two salivary proteins, aPRP and P-B peptide and the tannins studied are depicted in Figure 6. Brandão *et al.* concluded that both polysaccharide fractions were successful at inhibiting or reducing the aggregation or interaction of protein-polyphenol complexation, operating by two different mechanisms dependent on the structural components of the complex (Brandão, et al., 2017). These mechanisms can be competitive in nature (Riou, et al., 2002) or through the formation of a ternary complex

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(protein-polyphenol-polysaccharide (Brandão, et al., 2017; Mateus, et al., 2004; Susana Soares, et al., 2012), Figure 7 serves to illustrate these proposed mechanisms.

Polysaccharide interactions with the P-B peptide complex were thought to occur through a ternary mechanism, whereby polysaccharides surround the complex, enhancing its solubility (Brandão, et al., 2017). With respect to the aPRP complex, polysaccharide interactions were a combination of ternary and competitive interactions. Furthermore, their chromatographic data confirmed that that RG-II was the most effective polysaccharide at preventing aPRP precipitation for both tannins studied. However, for the P-B peptide, the ability of the polysaccharide to interact with the complex was dependent on the tannin present, suggesting, as in agreement with prior conclusions, that the structural components of both the tannin and the protein govern the interactive ability the polysaccharides have towards the complex (Brandão, et al., 2017; García-Estévez, et al., 2017). It would be safe to conclude that the physico-chemical components of the polysaccharide would further influence these interactions (Riou, et al., 2002; Watrelot, et al., 2014).

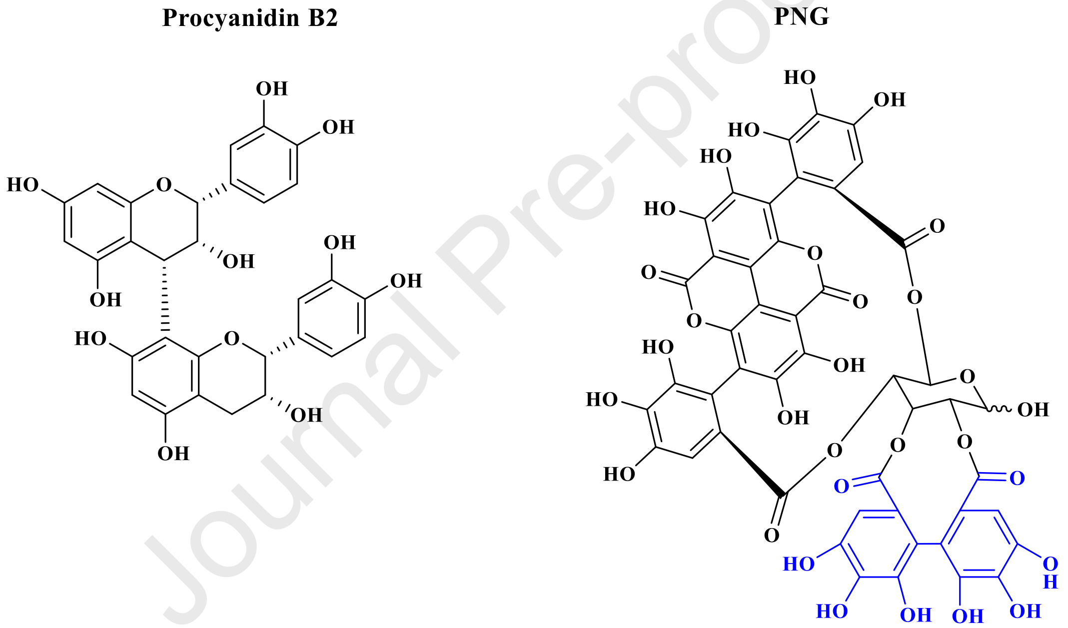

Figure 6. The structures of the two polyphenol compounds studied by Brandão *et al.* (Brandão, et al., 2017). The blue highlights the hexahydroxydiphenoyl (HHDP) moiety, a constituent of some ellagitannins, a diverse type of hydrolysable tannins.

Further work by Brandão *et al.* (Brandão, Fernandes, et al., 2020; Brandão, Silva, et al., 2020) directed towards the specific interaction mechanism of wine polysaccharides confirmed that RG-II fractions exhibit inhibitory effects of protein-tannin complexation by the competitive mechanism. However, they also discovered that this RG-II interaction was absent in the presence of sodium ions in the wine matrix, suggesting that mineral ion content or even ionic strength of the matrix could be a factor in governing polysaccharide interactions (Watrelot, et al., 2014).

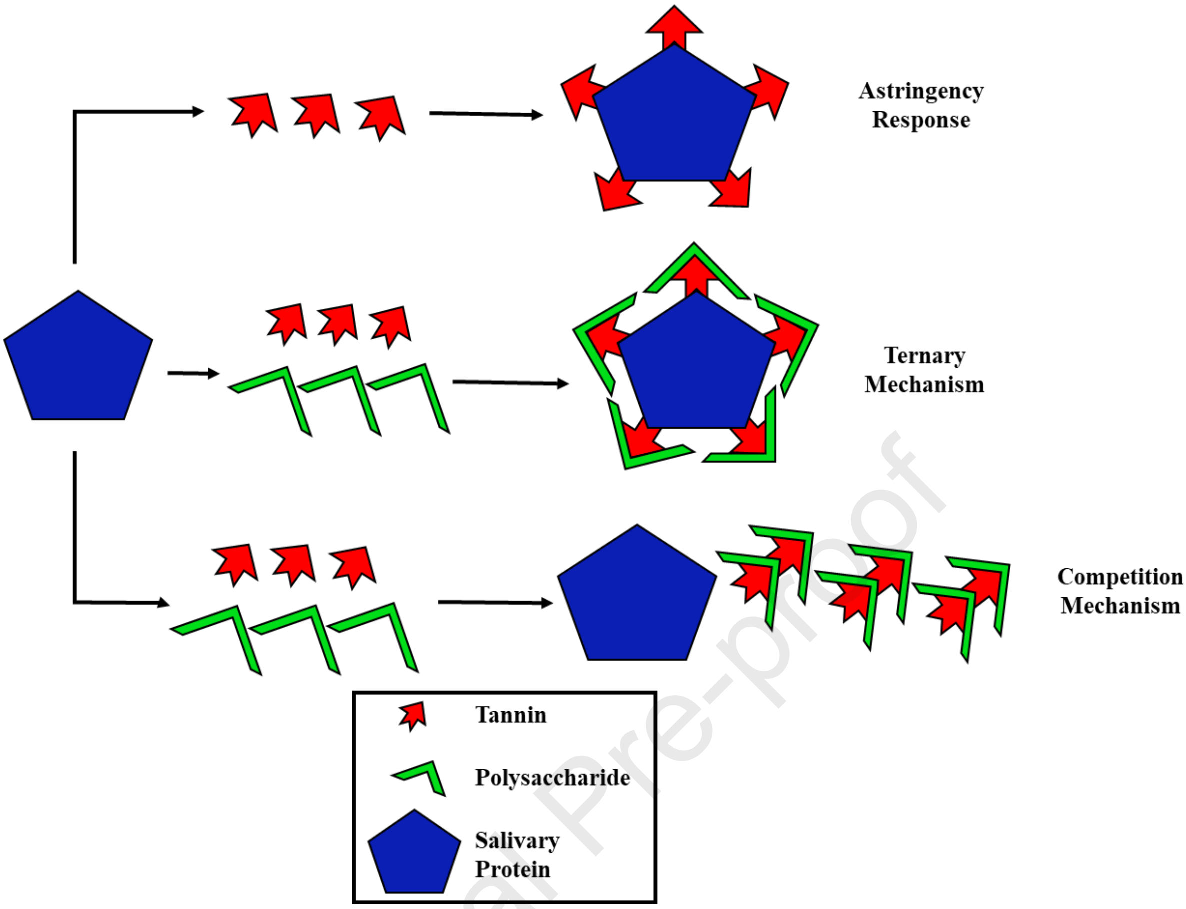

Figure 7. The proposed mechanisms for how polysaccharides interact with the protein-tannin complex to modulate the astringency response and perception. Adapted from Mateus *et al.* (Mateus, et al., 2004), de Freitas *et al.* (de Freitas & Mateus, 2012).

Brandão *et al.* concluded that AGP fractions interacted with protein complexes through both aforementioned mechanisms. However, these interactions were further dependent on the saliva sample, suggesting even further variability for possible interactions due to the intrinsic variations of genetic composition in humans (Brandão, Silva, et al., 2020). Arguably, this factor could result in discrepancies in the perceived intensities of organoleptic characteristic, such as taste or astringency between people. Overall, Brandão *et al.* concluded that the interactions between polysaccharides and tannin-proteins are predominately hydrophobic in nature (Brandão, Fernandes, et al., 2020).

Following on from this research, Lei *et al.* (Lei, et al., 2019) utilised an array of analytical techniques to investigate the influence of wine polysaccharides on flavan-3-ols-BSA protein complexes. Lei *et al.* also wanted to understand any potential structural and conformational changes in protein structure that occur because of polysaccharide interaction. MP and RG-II were the major polysaccharides to alter the interactions of flavan-3-ols with BSA and the interaction of ternary complexed structures was enhanced with increasing concentration of the respective polysaccharide. When the DP of flavan-3-ols ranged between 5 and 7, the secondary structure of the protein was changed from a predominantly α-helical structure to a less uniform, curled structure. Consequently, protein precipitation increased (Lei, et al., 2019). These results are interesting and perhaps counter-intuitive, as mentioned earlier, polysaccharides have been shown to preferentially bind and interact with polyphenols with a

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high DP (Renard, et al., 2017; Ruiz-Garcia, et al., 2014; Watrelot, et al., 2017), thus a DP between 5 and 7 is relatively low compared to other literature. However, the literature in question only focused on the binding of polysaccharides with polyphenols and perhaps the protein moiety does facilitate polysaccharide binding. Considering the results from Lei *et al.* were obtained using bovine serum albumin, it would be interesting to discover if the chemical structure of human salivary proteins followed similar behaviour.

4.0 The role of polysaccharides in the modulation of astringency

The interaction between salivary PRP and plant tannins within the oral cavity and their subsequent precipitation influences the organoleptic perception of many food and beverages (Murray, Williamson, Lilley, & Haslam, 1994). Astringency is a tactile, trigeminal sensation defined as a drying, puckering and shrinking of the mouth epithelia within the oral cavity (Riou, et al., 2002; Vidal, et al., 2004), involving the activation of G-protein coupled signalling of trigeminal ganglion neurons (Schöbel, et al., 2014), and is an important organoleptic characteristic of alcoholic beverages such as wine and cider. Some classes of astringent agents include the salts of multivalent metallic cations, dehydrating agents, and acids; however, the major classes include tannins and other smaller polyphenolic compounds. The mechanism behind this sensation is not fully understood, and is currently under investigation (Canon, et al., 2021), yet it is said to be attributed to the precipitation of salivary proteins elicited by astringent agents, which reduce the lubrication and increase the friction within the oral cavity, with many claiming to experience a "chalky" or rough sensation (Vidal, et al., 2004). However, it has been noted that not all astringent agents interact and precipitate salivary proteins (Schwarz & Hofmann, 2008), as this event is often related to the structure and physico-chemical nature of both the astringent and the salivary protein. Scollary *et al.* (Scollary, Pásti, Kállay, Blackman, & Clark, 2012), de Freitas *et al.* (de Freitas, et al., 2012) and more recently, García-Estévez *et al. (García-Estévez, María Ramos-Pineda, & Teresa Escribano-Bailón, 2018)* have completed comprehensive reviews on the astringency response explaining the event in more detail. Figure 8 was adapted from this work to illustrate an example of the molecular interactions between polysaccharides and protein-tannin complexes.

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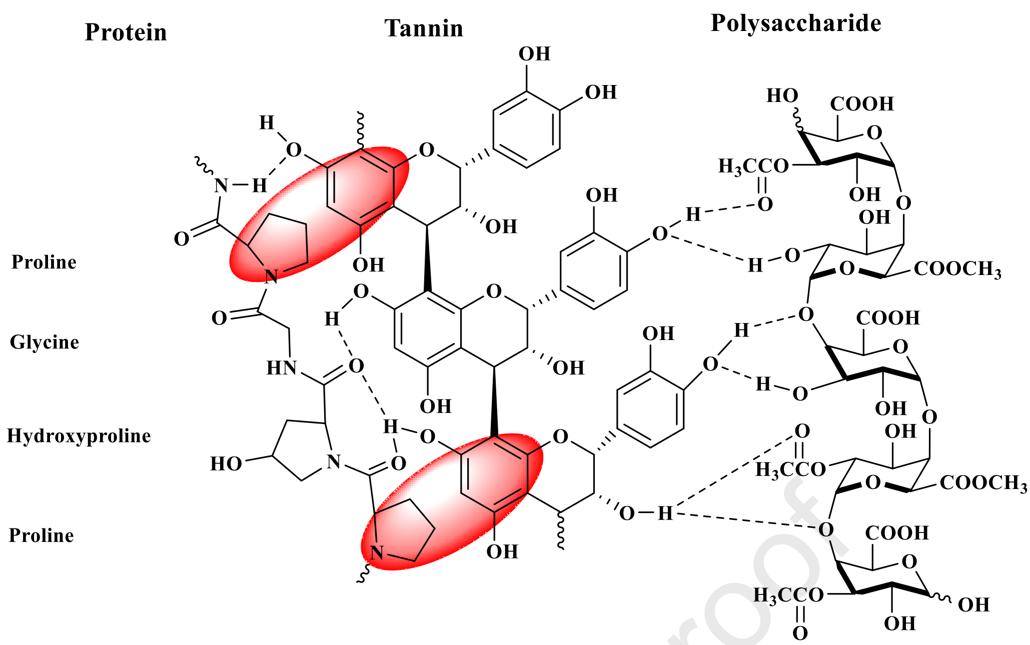

Figure 8. An example illustration of the proposed interactions between the polysaccharide-tannin-protein complex. Hydrogen bonding is represented by the dashed lines and the red circles highlight the potential areas for hydrophobic interactions. Adapted from Scollary *et al.* (Scollary, et al., 2012) and de Freitas *et al.* (de Freitas, et al., 2012). Note that hydrophobic interactions between tannins and polysaccharides are hypothesised to exist but are not included in the diagram due to insufficient information.

Vidal *et al.* compared the astringent characteristics of unripe fruit to a young wine (Vidal, et al., 2004). During maturation both entities experience a decrease in astringent characteristic (Ozawa, Lilley, & Haslam, 1987), with older wines showing decreases in their astringency properties during ageing due to the transformation and evolution of tannins to less bitter and less astringent species (Singleton & Noble, 1976; Vidal, et al., 2004). Vidal *et al*. also investigated the role of certain components of a model wine and their impacts on the "mouth-feel". The results from a panel of fifteen trained judges suggested that an increased tannin concentration correlated to an increased astringency perception; however, this perception was reduced in the presence of RG-II. It was noted that MP, AGP and RG-II significantly increased "mouth-fullness" and RG-II reduced the astringent perception. Further observations included a decrease in the perception of dryness and chalkiness attributed to RG-II in the absence of MP and AGP, yet this was not observed in its presence. Additionally, RG-II had no effect on bitterness in the absence of MP, but enhanced it in its presence, suggesting that RG-II inhibited the reduction of bitterness caused by MP (Vidal, et al., 2004).

Several authors have highlighted rapidly growing interest in the development of instrument or cellular-based analysis alternatives to the traditional assessment of astringency in food and beverage by trained, experienced sensory panels (Mo, Chen, & Wang, 2019; Simoes Costa, Costa Sobral, Delgadillo, Cerdeira, & Rudnitskaya, 2015; Sónia Soares, et al., 2019). The main drawbacks of sensory panels are that they can be time-consuming, expensive and subjective (Boulet, et al., 2016; Simoes Costa, et al., 2015). Several authors have recently attempted to examine correlations between the sensory attributes of different grape varieties and their chemical composition (Arapitsas, et al., 2020; Parpinello, et al., 2019; Piombino, et al., 2020). Piombino *et al.* investigated the diversity of astringency of eleven different Italian cultivar wines to discover any correlations between 'in-mouth' sensory properties and the chemical composition of the grapes. Patterns of astringent features were found to exist; however it was noted that their correlations could not predict the perception of all astringency distinctions based on the total phenols or tannins present in the wine (Piombino, et al., 2020).

Simoes Costa *et al.* investigated the astringency response using an electronic tongue, based on potentiometric and voltammetric sensors, chemical parameters and Fourier transform infrared spectroscopy (FTIR) to quantify phenolics in red, white, and rosé wine, and to measure the astringency perception. Simoes Costa *et al.* concluded that astringency is a complicated phenomenon that cannot be defined only by the concentration of polyphenols present in the wine, also identifying that different compounds are responsible for the astringency perception in red, rosé and white wines (Simoes Costa, et al., 2015).

Boulet *et al.* built prediction models based on multilinear regression using UV-Vis and chemical analysis to propose faster methods of identifying correlations between wine composition and astringency perception. Their model illustrated that astringency was strongly correlated with tannin precipitation with BSA. The models indicated a negative relationship between polysaccharides and astringency, and interestingly, a positive relationship between oligosaccharides and astringency perception. Perhaps the smaller size of these entities could be the reason behind these oligosaccharide results, essentially, they are not large enough to modulate astringency by acting through any of the mechanisms highlighted in Figure 7. Boulet *et al*. also identified that RG-II reduced astringency more efficiently in comparison to the total polysaccharides and PRAG groups, also noting that polysaccharides can directly interact with proteins, which could further influence astringency perception (Boulet, et al., 2016; de Kruif, Weinbreck, & de Vries, 2004).

Quijada-Morin *et al.* examined the relationship of the perceived astringency of red Tempranillo wines and their polysaccharide content, attempting to identify any trends in their composition associated with this phenomenon (Quijada-Morín, et al., 2014). Quijada-Morin *et al.* highlighted that all families (PRAG, RG-II and MP) positively influenced astringency perception, with RG-II and MP having the strongest influence. This suggested that the branched structures of these complex polysaccharides and the presence of unusual glycosidic linkages could be related to the decreased astringency perception. Overall however, there was no clear trend associated with glycoysl residues and astringency, with the role of oligosaccharide fractions in this perception remaining unclear (Apolinar-Valiente, Williams, & Doco, 2021). The glycosyl residues and oligosaccharide fractions are all found within the polysaccharide structures present in the wine, yet they do not elicit a response, primarily because these fractions are too small to encapsulate the colloidal protein-tannin complex associated with reduced astringency. Thus, the ability of polysaccharides to soothe astringency primarily comes down to their size and tridimensionality, which is especially true for MP and RG-II (Quijada-Morín, et al., 2014).

Manjón *et al.* studied the molecular mechanisms by which MP may modulate the astringency perception elicited by tannins. Manjón *et al.* experimented with three different commercial yeast MP, with varying protein and saccharide composition, to identify possible links between MP composition and the mechanism of action modulating astringency; however, no definitive correlation could be established. All three MP reduced astringency but to different extents and through different mechanisms (Manjón, Brás, García-Estévez, & Escribano-Bailón, 2020).

RG-II may act as a modulating agent for the perceived astringency of the beverage (Boulet, et al., 2016; Vidal, et al., 2004). This suggests that polysaccharides could have a role as fining or stabilising agents to help to improve wine stability and organoleptical properties through doping or enriching techniques, potentially modifying astringent wines (Hanlin, et al., 2010), assisting in the production of stylistic wines with tailored properties or traits. However, despite the attractive potential for oenologists, the observations and evaluations discussed prior indicate more research is required to completely understand the intricate and complicated nature of the wine matrix and its components, suggesting there is a crucial, yet delicate balance between the composition and quantity of molecular components with respect to overall sensory properties of the final wine. Overall, there is compelling evidence that in theory polysaccharides from wine can modulate astringency perception, but through making use of recent advances in knowledge and technology in the fields of oenology and chemistry, more research should be conducted into this relationship.

Conclusion

Polysaccharides are an important macromolecule within the wine medium and originate from either grape or yeast derived matter. Polysaccharides can interact with many endogenous wine species such as aroma compounds, tannins and proteins, but also with exogenous species such as salivary protein complexes. This review has highlighted the fundamental importance of these interactions regarding the proposed roles of polysaccharides towards influencing the organoleptic properties of the beverage. Knowledge gained from these observations could affect decision making during the vinification process. Polysaccharides have potential in astringency modulation, protein haze control, foam stability and modulating aroma volatility. However, it is crucial that a complete understanding of the intrinsic properties and the reactive nature of wine polysaccharides, including an understanding of the delicate balance between wine components, exists as an enrichment of a wine with a certain species to enhance or elicit a particular beneficial trait could in fact have detrimental effects on other aspects of the wine. Advances in instrumentation and techniques are paving the way for deeper investigations into the molecular interactions and subsequent understanding of the roles of wine components.

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References

Andrés-Lacueva, C., Lamuela-Raventós, R. M., Buxaderas, S., & de la Torre-Boronat, M. d. C. (1997). Influence of Variety and Aging on Foaming Properties of Cava (Sparkling Wine). 2. *Journal of Agricultural and Food Chemistry*, *45*(7), 2520-2525.

Andrés-Lacueva, C., López-Tamames, E., Lamuela-Raventós, R. M., Buxaderas, S., & de la Torre-Boronat, M. d. C. (1996). Characteristics of Sparkling Base Wines Affecting Foam Behavior. *Journal of Agricultural and Food Chemistry, 44*(4), 989-995.

Apolinar-Valiente, R., Salmon, T., Williams, P., Nigen, M., Sanchez, C., Doco, T., & Marchal, R. (2021). Acacia gums new fractions and sparkling base wines: How their biochemical and structural properties impact foamability? *Food Chemistry, 354*, 129477.

Apolinar-Valiente, R., Salmon, T., Williams, P., Nigen, M., Sanchez, C., Marchal, R., & Doco, T. (2020). Improvement of the foamability of sparkling base wines by the addition of Acacia gums. *Food Chemistry, 313*, 126062.

Apolinar-Valiente, R., Williams, P., & Doco, T. (2021). Recent advances in the knowledge of wine oligosaccharides. *Food Chemistry, 342*, 128330.

Journal Pre-proof

Arapitsas, P., Ugliano, M., Marangon, M., Piombino, P., Rolle, L., Gerbi, V., Versari, A., & Mattivi, F. (2020). Use of Untargeted Liquid Chromatography–Mass Spectrometry Metabolome To Discriminate Italian Monovarietal Red Wines, Produced in Their Different Terroirs. *Journal of Agricultural and Food Chemistry*.

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*(1), 29-39.

Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M., & Gómez-Plaza, E. (2007). The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. *Journal of Food Composition and Analysis, 20*(7), 546-552.

Bindon, K. A., Li, S., Kassara, S., & Smith, P. A. (2016). Retention of Proanthocyanidin in Wine-like Solution Is Conferred by a Dynamic Interaction between Soluble and Insoluble Grape Cell Wall Components. *Journal of Agricultural and Food Chemistry, 64*(44), 8406-8419.

Blasco, L., Viñas, M., & Villa, T. G. (2011). Proteins influencing foam formation in wine and beer: the role of yeast.

Boulet, J.-C., Trarieux, C., Souquet, J.-M., Ducasse, M.-A., Caillé, S., Samson, A., Williams, P., Doco, T., & Cheynier, V. (2016). Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency. *Food Chemistry, 190*, 357-363.

Bourvellec, C. L., & Renard, C. M. G. C. (2012). Interactions between Polyphenols and Macromolecules: Quantification Methods and Mechanisms. *Critical Reviews in Food Science and Nutrition, 52*(3), 213-248.

Brandão, E., Fernandes, A., Guerreiro, C., Coimbra, M. A., Mateus, N., de Freitas, V., & Soares, S. (2020). The effect of pectic polysaccharides from grape skins on salivary protein – procyanidin interactions. *Carbohydrate Polymers, 236*, 116044.

Brandão, E., Silva, M. S., García-Estévez, I., Williams, P., Mateus, N., Doco, T., de Freitas, V., & Soares, S. (2017). The role of wine polysaccharides on salivary protein-tannin interaction: A molecular approach. *Carbohydrate Polymers, 177*, 77-85.

Brandão, E., Silva, M. S., García-Estévez, I., Williams, P., Mateus, N., Doco, T., de Freitas, V., & Soares, S. (2020). Inhibition Mechanisms of Wine Polysaccharides on Salivary Protein Precipitation. *Journal of Agricultural and Food Chemistry, 68*(10), 2955-2963.

Brown, S., & Stockdale, V. (2007). Reducing haziness in white wine by overexpression of Saccharomyces cerevisiae genes YOL155c and YDR055w. *APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY, 73*, 1363-1376.

Cai, Y., Zhang, J., Chen, N. G., Shi, Z., Qiu, J., He, C., & Chen, M. (2017). Recent Advances in Anticancer Activities and Drug Delivery Systems of Tannins. *Medicinal Research Reviews, 37*(4), 665-701.

Canon, F., Belloir, C., Bourillot, E., Brignot, H., Briand, L., Feron, G., Lesniewska, E., Nivet, C., Septier, C., Schwartz, M., Tournier, C., Vargiolu, R., Wang, M., Zahouani, H., & Neiers, F. (2021). Perspectives on Astringency Sensation: An Alternative Hypothesis on the Molecular Origin of Astringency. *Journal of Agricultural and Food Chemistry, 69*(13), 3822-3826.

Carvalho, E., Mateus, N., Plet, B., Pianet, I., Dufourc, E., & De Freitas, V. (2006). Influence of Wine Pectic Polysaccharides on the Interactions between Condensed Tannins and Salivary Proteins. *Journal of Agricultural and Food Chemistry, 54*(23), 8936-8944.

Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J.-M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and Properties of Wine Pigments and Tannins. *American Journal of Enology and Viticulture, 57*(3), 298-305.

Chong, H. H., Cleary, M. T., Dokoozlian, N., Ford, C. M., & Fincher, G. B. (2019). Soluble cell wall carbohydrates and their relationship with sensory attributes in Cabernet Sauvignon wine. *Food Chemistry, 298*, 124745.

Cilindre, C., Liger-Belair, G., Villaume, S., Jeandet, P., & Marchal, R. (2010). Foaming properties of various Champagne wines depending on several parameters: Grape variety, aging, protein and CO2 content. *Analytica Chimica Acta, 660*(1), 164-170.

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, 6*(1).

Dambrouck, T., Marchal, R., Cilindre, C., Parmentier, M., & Jeandet, P. (2005). Determination of the Grape Invertase Content (Using PTA−ELISA) following Various Fining Treatments versus Changes in the Total Protein Content of Wine. Relationships with Wine Foamability. *Journal of Agricultural and Food Chemistry, 53*(22), 8782-8789.

de Freitas, V., & Mateus, N. (2012). Protein/Polyphenol Interactions: Past and Present Contributions. Mechanisms of Astringency Perception. *Current Organic Chemistry, 16*(6), 724-746.

De Iseppi, A., Marangon, M., Vincenzi, S., Lomolino, G., Curioni, A., & Divol, B. (2021). A novel approach for the valorization of wine lees as a source of compounds able to modify wine properties. *LWT, 136*, 110274.

de Kruif, C. G., Weinbreck, F., & de Vries, R. (2004). Complex coacervation of proteins and anionic polysaccharides. *Current Opinion in Colloid & Interface Science, 9*(5), 340-349.

Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Guadalupe, Z., & Ayestarán, B. (2012). Polysaccharide characterization of commercial dry yeast preparations and their effect on white and red wine composition. *LWT - Food Science and Technology, 48*(2), 215-223.

Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Williams, P., & Doco, T. (2011). Effect of Aging on Lees and of Three Different Dry Yeast Derivative Products on Verdejo White Wine Composition and Sensorial Characteristics. *Journal of Agricultural and Food Chemistry, 59*(23), 12433-12442.

Di Gaspero, M., Ruzza, P., Hussain, R., Honisch, C., Biondi, B., Siligardi, G., Marangon, M., Curioni, A., & Vincenzi, S. (2020). The Secondary Structure of a Major Wine Protein is Modified upon Interaction with Polyphenols. *Molecules, 25*(7), 1646.

Doco, T., Brillouet, J. M., & Moutounet, M. (1996). Evolution of Grape (Carignan noir cv.) and Yeast Polysaccharides During Fermentation and Post-Maceration. *American Journal of Enology and Viticulture, 47*(1), 108-110.

Ducasse, M.-A., Canal-Llauberes, R.-M., de Lumley, M., Williams, P., Souquet, J.-M., Fulcrand, H., Doco, T., & Cheynier, V. (2010). Effect of macerating enzyme treatment on the polyphenol and polysaccharide composition of red wines. *Food Chemistry, 118*(2), 369-376.

Dufour, C., & Bayonove, C. L. (1999). Influence of Wine Structurally Different Polysaccharides on the Volatility of Aroma Substances in a Model System. *Journal of Agricultural and Food Chemistry, 47*(2), 671-677.

Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeasts and the influence of released polysaccharides on colour stability and wine astringency. *Australian Journal of Grape and Wine Research, 7*(3), 153-159.

Ferreira, R. B., Piçarra-Pereira, M. A., Monteiro, S., Loureiro, V. l. B., & Teixeira, A. R. (2001). The wine proteins. *Trends in Food Science & Technology, 12*(7), 230-239.

Freitas, V. A. P. d., Fernandes, A., Oliveira, J., Teixeira, N., & Mateus, N. (2017). A review of the current knowledge of red wine colour. *OENO One, 51*(1).

García-Estévez, I., Cruz, L., Oliveira, J., Mateus, N., de Freitas, V., & Soares, S. (2017). First evidences of interaction between pyranoanthocyanins and salivary proline-rich proteins. *Food Chemistry, 228*, 574-581.

García-Estévez, I., María Ramos-Pineda, A., & Teresa Escribano-Bailón, M. (2018). Interactions between wine phenolic compounds and human saliva in astringency perception. *Food & Function, 9*(3), 1294-1309.

Gawel, R., Smith, P. A., Cicerale, S., & Keast, R. (2018). The mouthfeel of white wine. *Critical Reviews in Food Science and Nutrition, 58*(17), 2939-2956.

Gazzola, D., Van Sluyter, S. C., Curioni, A., Waters, E. J., & Marangon, M. (2012). Roles of Proteins, Polysaccharides, and Phenolics in Haze Formation in White Wine via Reconstitution Experiments. *Journal of Agricultural and Food Chemistry, 60*(42), 10666-10673.

Journal Pre-proof

Journal Pre-proof

Gombau, J., Nadal, P., Canela, N., Gómez-Alonso, S., García-Romero, E., Smith, P., Hermosín-Gutiérrez, I., Canals, J. M., & Zamora, F. (2019). Measurement of the interaction between mucin and oenological tannins by Surface Plasmon Resonance (SPR); relationship with astringency. *Food Chemistry, 275*, 397-406.

González-Royo, E., Urtasun, A., Gil, M., Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2013). Effect of Yeast Strain and Supplementation with Inactive Yeast during Alcoholic Fermentation on Wine Polysaccharides. *American Journal of Enology and Viticulture, 64*(2), 268.

González-Royo, E., Esteruelas, M., Kontoudakis, N., Fort, F., Canals, J. M., & Zamora, F. (2017). The effect of supplementation with three commercial inactive dry yeasts on the colour, phenolic compounds, polysaccharides and astringency of a model wine solution and red wine. *Journal of the Science of Food and Agriculture, 97*(1), 172-181.

Guadalupe, Z., & Ayestarán, B. (2007). Polysaccharide Profile and Content during the Vinification and Aging of Tempranillo Red Wines. *Journal of Agricultural and Food Chemistry, 55*(26), 10720-10728.

Guadalupe, Z., & Ayestarán, B. (2008). Effect of Commercial Mannoprotein Addition on Polysaccharide, Polyphenolic, and Color Composition in Red Wines. *Journal of Agricultural and Food Chemistry, 56*(19), 9022-9029.

Guadalupe, Z., Ayestarán, B., Williams, P., & Doco, T. (2014). Determination of Must and Wine Polysaccharides by Gas Chromatography-Mass Spectrometry (GC-MS) and Size-Exclusion Chromatography (SEC). In *Polysaccharides* (pp. np): Editions Springer.

Gyémánt, G., Zajácz, Á., Bécsi, B., Ragunath, C., Ramasubbu, N., Erdődi, F., Batta, G., & Kandra, L. (2009). Evidence for pentagalloyl glucose binding to human salivary α-amylase through aromatic amino acid residues. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 1794*(2), 291-296.

Hanlin, R. L., Hrmova, M., Harbertson, J. F., & Downey, M. O. (2010). Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. *Australian Journal of Grape and Wine Research, 16*(1), 173-188.

Jones-Moore, H. R., Jelley, R. E., Marangon, M., & Fedrizzi, B. (2021). The polysaccharides of winemaking: From grape to wine. *Trends in Food Science & Technology, 111*, 731-740.

Jouquand, C., Ducruet, V., & Giampaoli, P. (2004). Partition coefficients of aroma compounds in polysaccharide solutions by the phase ratio variation method. *Food Chemistry, 85*(3), 467-474.

Kemp, B., Alexandre, H., Robillard, B., & Marchal, R. (2015). Effect of Production Phase on Bottle-Fermented Sparkling Wine Quality. *Journal of Agricultural and Food Chemistry, 63*(1), 19-38.

Kemp, B., Condé, B., Jégou, S., Howell, K., Vasserot, Y., & Marchal, R. (2019). Chemical compounds and mechanisms involved in the formation and stabilization of foam in sparkling wines. *Critical Reviews in Food Science and Nutrition, 59*(13), 2072-2094.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L., & Opletal, L. (2008). Condensed and Hydrolysable Tannins as Antioxidants Influencing the Health. *Mini Reviews in Medicinal Chemistry, 8*(5), 436-447.

König H., U. G., Fröhlich, J. (2017). *Biology of Microorganismson Grapes, in Must and in Wine* (Second ed.). Switzerland: Springer.

Le Bourvellec, C., Guyot, S., & Renard, C. M. G. C. (2004). Non-covalent interaction between procyanidins and apple cell wall material: Part I. Effect of some environmental parameters. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1672*(3), 192-202.

Le Bourvellec, C., Guyot, S., & Renard, C. M. G. C. (2009). Interactions between apple (Malus x domestica Borkh.) polyphenols and cell walls modulate the extractability of polysaccharides. *Carbohydrate Polymers, 75*(2), 251-261.

Lei, X., Zhu, Y., Wang, X., Zhao, P., Liu, P., Zhang, Q., Chen, T., Yuan, H., & Guo, Y. (2019). Wine polysaccharides modulating astringency through the interference on interaction of flavan-3-ols and BSA in model wine. *International Journal of Biological Macromolecules, 139*, 896-903.

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994). Influence of Yeast Walls on the Behavior of Aroma Compounds in a Model Wine. *American Journal of Enology and Viticulture, 45*(1), 29-33.

Journal Pre-proof

Lubbers, S., Voilley, A., Feuillat, M., & Charpentier, C. (1994). Influence of Mannaproteins from Yeast on the Aroma Intensity of a Model Wine. *LWT - Food Science and Technology, 27*(2), 108-114.

Mamet, T., Ge, Z.-z., Zhang, Y., & Li, C.-m. (2018). Interactions between highly galloylated persimmon tannins and pectins. *International Journal of Biological Macromolecules, 106*, 410-417.

Manjón, E., Brás, N. F., García-Estévez, I., & Escribano-Bailón, M. T. (2020). Cell Wall Mannoproteins from Yeast Affect Salivary Protein–Flavanol Interactions through Different Molecular Mechanisms. *Journal of Agricultural and Food Chemistry, 68*(47), 13459-13468.

Marangon, M., Sluyter, S. C. V., Waters, E. J., & Menz, R. I. (2014). Structure of Haze Forming Proteins in White Wines: Vitis vinifera Thaumatin-Like Proteins. *PLOS ONE, 9*(12), e113757.

Marangon, M., Vincenzi, S., & Curioni, A. (2019). Wine Fining with Plant Proteins. *Molecules, 24*(11), 2186.

Marassi, V., Marangon, M., Zattoni, A., Vincenzi, S., Versari, A., Reschiglian, P., Roda, B., & Curioni, A. (2021). Characterization of red wine native colloids by asymmetrical flow field-flow fractionation with online multidetection. *Food Hydrocolloids, 110*, 106204.

Marchal, R., Chaboche, D., Douillard, R., & Jeandet, P. (2002). Influence of Lysozyme Treatments on Champagne Base Wine Foaming Properties. *Journal of Agricultural and Food Chemistry, 50*(6), 1420-1428.

Marchal, R., & Jeandet, P. (2009). Use of Enological Additives for Colloid and Tartrate Salt Stabilization in White Wines and for Improvement of Sparkling Wine Foaming Properties. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 127-158). New York, NY: Springer.

Martínez-Lapuente, L., Guadalupe, Z., & Ayestarán, B. (2019). Properties of Wine Polysaccharides. *Pectins - Extraction, Purification, Characterization and Applications*.

Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., & Pérez-Magariño, S. (2015). Role of major wine constituents in the foam properties of white and rosé sparkling wines. *Food Chemistry, 174*, 330-338.

Martínez-Rodríguez, A. J., & Pueyo, E. (2009). SparklingWines and Yeast Autolysis. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 61-80). New York, NY: Springer.

Mateus, N., Carvalho, E., Luís, C., & de Freitas, V. (2004). Influence of the tannin structure on the disruption effect of carbohydrates on protein–tannin aggregates. *Analytica Chimica Acta, 513*(1), 135-140.

McRae, J. M., & Kennedy, J. A. (2011). Wine and Grape Tannin Interactions with Salivary Proteins and Their Impact on Astringency: A Review of Current Research. *Molecules, 16*(3).

Mekoue Nguela, J., Sieczkowski, N., Roi, S., & Vernhet, A. (2015). Sorption of Grape Proanthocyanidins and Wine Polyphenols by Yeasts, Inactivated Yeasts, and Yeast Cell Walls. *Journal of Agricultural and Food Chemistry, 63*(2), 660-670.

Mekoue Nguela, J., Vernhet, A., Sieczkowski, N., & Brillouet, J.-M. (2015). Interactions of Condensed Tannins with Saccharomyces cerevisiae Yeast Cells and Cell Walls: Tannin Location by Microscopy. *Journal of Agricultural and Food Chemistry, 63*(34), 7539-7545.

Mesquita, P. R., Piçarra-Pereira, M. A., Monteiro, S., Loureiro, V. B., Teixeira, A. R., & Ferreira, R. B. (2001). Effect of Wine Composition on Protein Stability. *American Journal of Enology and Viticulture, 52*(4), 324-330.

Mierczynska-Vasilev, A., & Smith, P. A. (2015). Current state of knowledge and challenges in wine clarification. *Australian Journal of Grape and Wine Research, 21*(S1), 615-626.

Mitropoulou, A., Hatzidimitriou, E., & Paraskevopoulou, A. (2011). Aroma release of a model wine solution as influenced by the presence of non-volatile components. Effect of commercial tannin extracts, polysaccharides and artificial saliva. *Food Research International, 44*(5), 1561-1570.

Mo, L., Chen, J., & Wang, X. (2019). A novel experimental set up for in situ oral lubrication measurements. *Food Hydrocolloids, 95*, 396-405.

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*(4), 537-543.

Murray, N. J., Williamson, M. P., Lilley, T. H., & Haslam, E. (1994). Study of the interaction between salivary proline-rich proteins and a polyphenol by 1H-NMR spectroscopy. *European Journal of Biochemistry, 219*(3), 923-935.

Nigen, M., Valiente, R. A., Iturmendi, N., Williams, P., Doco, T., Moine, V., Massot, A., Jaouen, I., & Sanchez, C. (2019). The colloidal stabilization of young red wine by Acacia senegal gum: The involvement of the protein backbone from the protein-rich arabinogalactan-proteins. *Food Hydrocolloids, 97*, 105176.

Nunan, K. J., Sims, I. M., Bacic, A., Robinson, S. P., & Fincher, G. B. (1998). Changes in Cell Wall Composition during Ripening of Grape Berries. *Plant Physiology, 118*(3), 783-792.

Ozawa, T., Lilley, T. H., & Haslam, E. (1987). Polyphenol interactions: astringency and the loss of astringency in ripening fruit. *Phytochemistry, 26*(11), 2937-2942.

Parpinello, G., Ricci, A., Arapitsas, P., Curioni, A., Moio, L., Rio Segade, S., Ugliano, M., & Versari, A. (2019). Multivariate characterisation of Italian monovarietal red wines using MIR spectroscopy. *4*, 741-751.

Pellerin, P., & Cabanis, J. C. (1998). Les Glucides. In Œnologie. *Fondements scientifiques et technologiques, 40–92. Flanzy C. (ed.). Lavoisier- Tec & Doc, Paris*.

Peñas, E., Di Lorenzo, C., Uberti, F., & Restani, P. (2015). Allergenic Proteins in Enology: A Review on Technological Applications and Safety Aspects. *Molecules, 20*(7), 13144-13164.

Pérez-Magariño, S., Martínez-Lapuente, L., Bueno-Herrera, M., Ortega-Heras, M., Guadalupe, Z., & Ayestarán, B. (2015). Use of Commercial Dry Yeast Products Rich in Mannoproteins for White and Rosé Sparkling Wine Elaboration. *Journal of Agricultural and Food Chemistry, 63*(23), 5670-5681.

Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology, 17*(11), 579-590.

Piombino, P., Pittari, E., Gambuti, A., Curioni, A., Giacosa, S., Mattivi, F., Parpinello, G. P., Rolle, L., Ugliano, M., & Moio, L. (2020). Preliminary sensory characterisation of the diverse astringency of single cultivar Italian red wines and correlation of sub-qualities with chemical composition. *Australian Journal of Grape and Wine Research, n/a*(n/a).

Ployon, S., Morzel, M., Belloir, C., Bonnotte, A., Bourillot, E., Briand, L., Lesniewska, E., Lherminier, J., Aybeke, E., & Canon, F. (2018). Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of bPRPs. *Food Chemistry, 253*, 79-87.

Poncet-Legrand, C., Doco, T., Williams, P., & Vernhet, A. (2007). Inhibition of Grape Seed Tannin Aggregation by Wine Mannoproteins: Effect of Polysaccharide Molecular Weight. *American Journal of Enology and Viticulture, 58*(1), 87-91.

Quijada-Morín, N., Williams, P., Rivas-Gonzalo, J. C., Doco, T., & Escribano-Bailón, M. T. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry, 154*, 44-51.

Ramos-Pineda, A. M., García-Estévez, I., Brás, N. F., Martín del Valle, E. M., Dueñas, M., & Escribano Bailón, M. T. (2017). Molecular Approach to the Synergistic Effect on Astringency Elicited by Mixtures of Flavanols. *Journal of Agricultural and Food Chemistry, 65*(31), 6425-6433.

Ramos-Pineda, A. M., García-Estévez, I., Soares, S., de Freitas, V., Dueñas, M., & Escribano-Bailón, M. T. (2019). Synergistic effect of mixture of two proline-rich-protein salivary families (aPRP and bPRP) on the interaction with wine flavanols. *Food Chemistry, 272*, 210-215.

Rauf, A., Imran, M., Abu-Izneid, T., Iahtisham Ul, H., Patel, S., Pan, X., Naz, S., Sanches Silva, A., Saeed, F., & Rasul Suleria, H. A. (2019). Proanthocyanidins: A comprehensive review. *Biomedicine & Pharmacotherapy, 116*, 108999.

Renard, C. M. G. C., Watrelot, A. A., & Le Bourvellec, C. (2017). Interactions between polyphenols and polysaccharides: Mechanisms and consequences in food processing and digestion. *Trends in Food Science & Technology, 60*, 43-51.

Ribeiro, J. M., Pereira, C. S., Soares, N. C., Vieira, A. M., Feijó, J. A., & Jackson, P. A. (2006). The contribution of extensin network formation to rapid, hydrogen peroxide-mediated increases in grapevine callus wall resistance to fungal lytic enzymes. *Journal of Experimental Botany, 57*(9), 2025-2035.

Journal Pre-proof

Riou, V., Vernhet, A., Doco, T., & Moutounet, M. (2002). Aggregation of grape seed tannins in model wine—effect of wine polysaccharides. *Food Hydrocolloids, 16*(1), 17-23.

Ruiz-Garcia, Y., Smith, P. A., & Bindon, K. A. (2014). Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydrate Polymers, 114*, 102-114.

Sarni-Manchado, P., Cheynier, V., & Moutounet, M. (1999). Interactions of Grape Seed Tannins with Salivary Proteins. *Journal of Agricultural and Food Chemistry, 47*(1), 42-47.

Saucier, C., Bourgeois, G., Vitry, C., Roux, D., & Glories, Y. (1997). Characterization of (+)-Catechin−Acetaldehyde Polymers: A Model for Colloidal State of Wine Polyphenols. *Journal of Agricultural and Food Chemistry, 45*(4), 1045-1049.

Schöbel, N., Radtke, D., Kyereme, J., Wollmann, N., Cichy, A., Obst, K., Kallweit, K., Kletke, O., Minovi, A., Dazert, S., Wetzel, C. H., Vogt-Eisele, A., Gisselmann, G., Ley, J. P., Bartoshuk, L. M., Spehr, J., Hofmann, T., & Hatt, H. (2014). Astringency Is a Trigeminal Sensation That Involves the Activation of G Protein–Coupled Signaling by Phenolic Compounds. *Chemical Senses, 39*(6), 471-487.

Schwarz, B., & Hofmann, T. (2008). Is there a direct relationship between oral astringency and human salivary protein binding? *European Food Research and Technology, 227*(6), 1693.

Scollary, G. R., Pásti, G., Kállay, M., Blackman, J., & Clark, A. C. (2012). Astringency response of red wines: Potential role of molecular assembly. *Trends in Food Science & Technology, 27*(1), 25-36.

Simoes Costa, A. M., Costa Sobral, M. M., Delgadillo, I., Cerdeira, A., & Rudnitskaya, A. (2015). Astringency quantification in wine: comparison of the electronic tongue and FT-MIR spectroscopy. *Sensors and Actuators B: Chemical, 207*, 1095-1103.

Singleton, V. L., & Noble, A. C. (1976). Wine Flavor and Phenolic Substances. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (Vol. 26, pp. 47-70): AMERICAN CHEMICAL SOCIETY.

Smith, P. A., McRae, J. M., & Bindon, K. A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. *Australian Journal of Grape and Wine Research, 21*(S1), 601-614.

Soares, S., Brandão, E., Guerreiro, C., Mateus, N., de Freitas, V., & Soares, S. (2019). Development of a New Cell-Based Oral Model To Study the Interaction of Oral Constituents with Food Polyphenols. *Journal of Agricultural and Food Chemistry, 67*(46), 12833-12843.

Soares, S., Brandão, E., Guerreiro, C., Soares, S., Mateus, N., & de Freitas, V. (2020). Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste. *Molecules, 25*(11), 2590.

Soares, S., Mateus, N., & de Freitas, V. (2012). Carbohydrates Inhibit Salivary Proteins Precipitation by Condensed Tannins. *Journal of Agricultural and Food Chemistry, 60*(15), 3966-3972.

Soares, S., Vitorino, R., Osório, H., Fernandes, A., Venâncio, A., Mateus, N., Amado, F., & de Freitas, V. (2011). Reactivity of Human Salivary Proteins Families Toward Food Polyphenols. *Journal of Agricultural and Food Chemistry, 59*(10), 5535-5547.

Sommer, S., Weber, F., & Harbertson, J. F. (2019). Polyphenol–Protein–Polysaccharide Interactions in the Presence of Carboxymethyl Cellulose (CMC) in Wine-Like Model Systems. *Journal of Agricultural and Food Chemistry, 67*(26), 7428-7434.

Springer, L. F., & Sacks, G. L. (2014). Protein-Precipitable Tannin in Wines from Vitis vinifera and Interspecific Hybrid Grapes (Vitis ssp.): Differences in Concentration, Extractability, and Cell Wall Binding. *Journal of Agricultural and Food Chemistry, 62*(30), 7515-7523.

Springer, L. F., Sherwood, R. W., & Sacks, G. L. (2016). Pathogenesis-Related Proteins Limit the Retention of Condensed Tannin Additions to Red Wines. *Journal of Agricultural and Food Chemistry, 64*(6), 1309-1317.

Thongkaew, C., Gibis, M., Hinrichs, J., & Weiss, J. (2014). Polyphenol interactions with whey protein isolate and whey protein isolate–pectin coacervates. *Food Hydrocolloids, 41*, 103-112.

Journal Pre-proof

Unterkofler, J., Muhlack, R. A., & Jeffery, D. W. (2020). Processes and purposes of extraction of grape components during winemaking: current state and perspectives. *Applied Microbiology and Biotechnology*, *104*(11), 4737-4755.

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*(16), 4020-4030.

Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M. J., Gawel, R., Williams, P., Waters, E., & Cheynier, V. (2004). Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Quality and Preference, 15*(3), 209-217.

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The polysaccharides of red wine: total fractionation and characterization. *Carbohydrate Polymers*, *54*(4), 439-447.

Villamor, R. R., Evans, M. A., Mattinson, D. S., & Ross, C. F. (2013). Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Research International, 50*(1), 38-45.

Villamor, R. R., Evans, M. A., & Ross, C. F. (2013). Effects of Ethanol, Tannin, and Fructose Concentrations on Sensory Properties of Model Red Wines. *American Journal of Enology and Viticulture, 64*(3), 342-348.

Villamor, R. R., & Ross, C. F. (2013). Wine Matrix Compounds Affect Perception of Wine Aromas. *Annual Review of Food Science and Technology, 4*(1), 1-20.

Vincenzi, S., Crapisi, A., & Curioni, A. (2014). Foamability of Prosecco wine: Cooperative effects of high molecular weight glycocompounds and wine PR-proteins. *Food Hydrocolloids, 34*, 202-207.

Waters, E. J., Alexander, G., Muhlack, R., Pocock, K. F., Colby, C., O'Neill, B. K., Hoj, P. B., & Jones, P. (2005). Preventing protein haze in bottled white wine. *Australian Journal of Grape and Wine Research, 11*(2), 215-225.

Waters, E. J., Pellerin, P., & Brillouet, J.-M. (1994a). A Saccharomyces mannoprotein that protects wine from protein haze. *Carbohydrate Polymers, 23*(3), 185-191.

Waters, E. J., Pellerin, P., & Brillouet, J.-M. (1994b). A Wine Arabinogalactan-protein That Reduces Heat-induced Wine Protein Haze. *Bioscience, Biotechnology, and Biochemistry, 58*(1), 43-48.

Watrelot, A. A., Le Bourvellec, C., Imberty, A., & Renard, C. M. G. C. (2013). Interactions between Pectic Compounds and Procyanidins are Influenced by Methylation Degree and Chain Length. *Biomacromolecules, 14*(3), 709-718.

Watrelot, A. A., Le Bourvellec, C., Imberty, A., & Renard, C. M. G. C. (2014). Neutral sugar side chains of pectins limit interactions with procyanidins. *Carbohydrate Polymers, 99*, 527-536.

Watrelot, A. A., Schulz, D. L., & Kennedy, J. A. (2017). Wine polysaccharides influence tannin-protein interactions. *Food Hydrocolloids, 63*, 571-579.

Will, F., Pfeifer, W., & Dietrich, H. (1991). The importance of colloids for wine quality.

Zhu, F. (2018). Interactions between cell wall polysaccharides and polyphenols. *Critical Reviews in Food Science and Nutrition, 58*(11), 1808-1831.

Zhu, F., Du, B., & Li, J. (2016). Aroma Compounds in Wine. *Grape and Wine Biotechnology*.

Journal Pre-proof

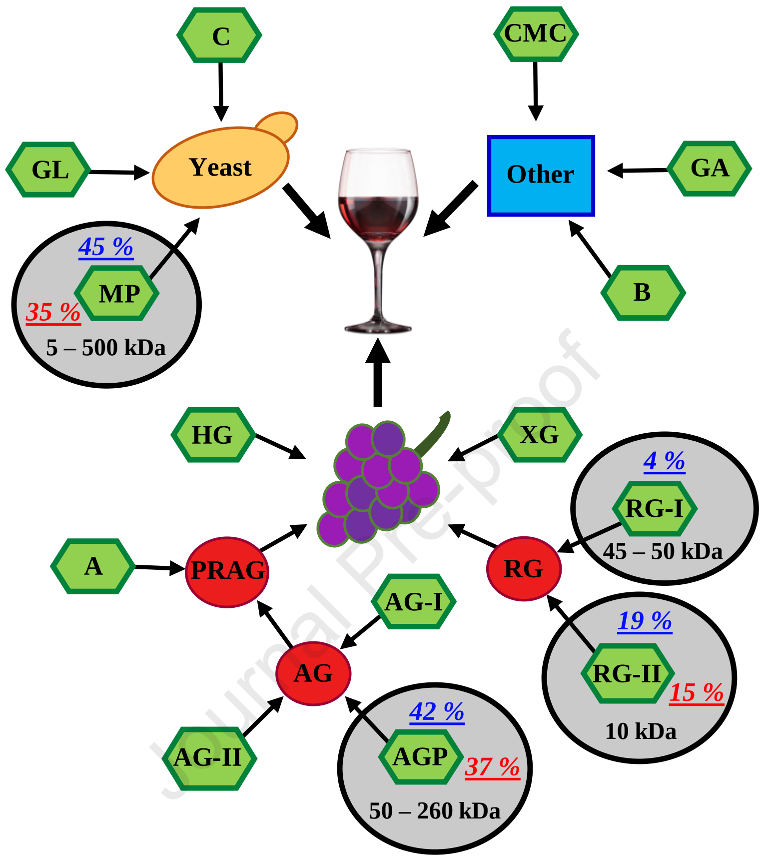

Figure 1. A summary illustration of wine polysaccharides and their respective origins. The green hexagons represent the polysaccharides, and the red circles show the families that these polysaccharides belong to. The polysaccharides highlighted by the grey circles are the most abundant in wine. The percentages represent their reported compositions in Carignan noir wine (Vidal, et al., 2003) in blue and in Tempranillo in red (Ayestarán, et al., 2004). The respective molecular weights of these polysaccharides are also given in kDa (Guadalupe, et al., 2014; Martínez-Lapuente, et al., 2019). A: Arabin, AG: Arabinogalactan, AG-I: Arabinogalactan-I, AG-II: Arabinogalactan-II, AGP: Arabinogalactan Protein, B: Bacteria, C: Chitin, CMC: Carboxymethylcellulose, GA: Gum Arabic, GL: Glucans, HG: Homogalacturonan, MP: Mannoprotein, PRAG: Polysaccharides Rich in Arabinose and Galactose, RG: Rhamnogalacturonan, RG-I: Rhamnogalacturonan-I, RG-II: Rhamnogalacturonan-II, XG: Xyloglucan.

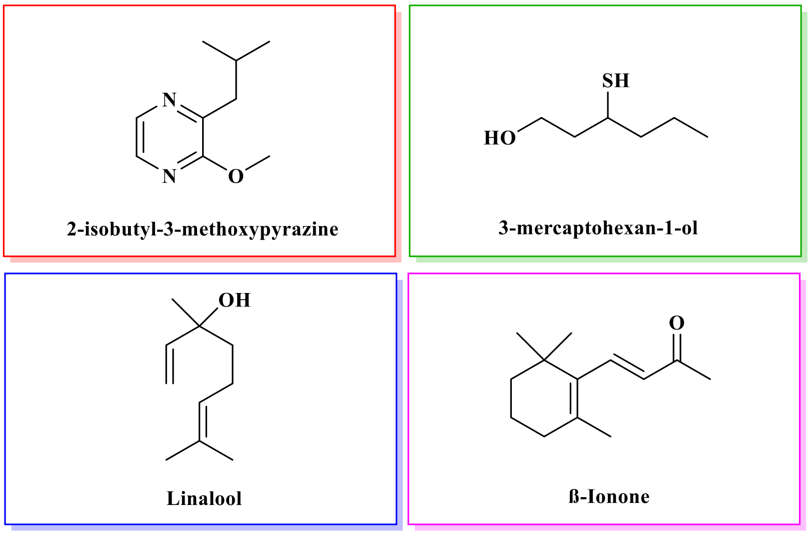

Figure 2. An example of four chemical classes of aroma compounds present in wines, illustrating the chemical structures of a methoxypyrazine, a sulfur compound, a terpene and an isoprenoid (Villamor & Ross, 2013).

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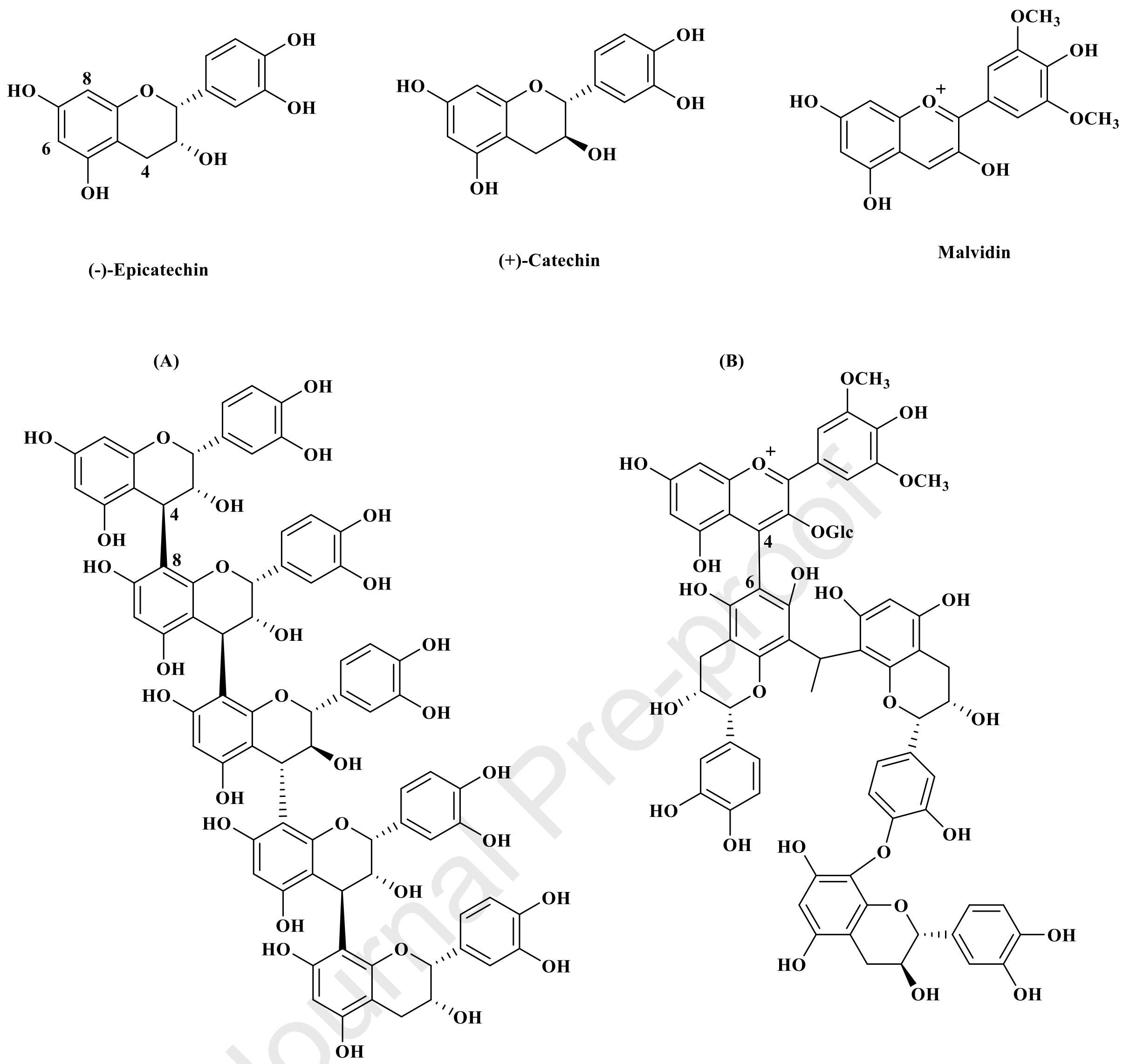

Figure 3. Grape seed and skin tannins undergo structural evolution during the winemaking and ageing processes. This figure illustrates an example of a more uniform, grape derived tannin (A) structure showing C4-C8 linkages, in comparison to a more complex wine tannin (B) structure showing the presence of C4-C6 linkages, with an example of some respective monomers above (Hanlin, et al., 2010; Smith, et al., 2015).

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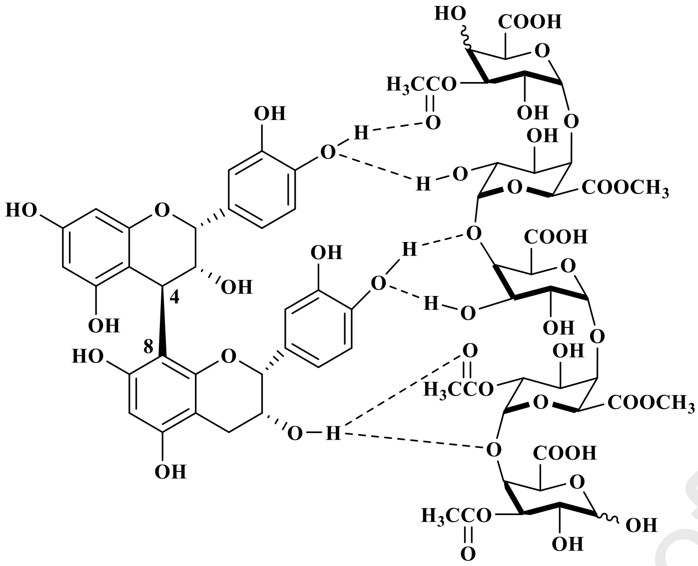

Figure 4. An example of the proposed hydrogen bonding that exists between the galacturonic acid backbone of pectic polysaccharides and monomeric units of the tannin structure (Hanlin et al., 2010).

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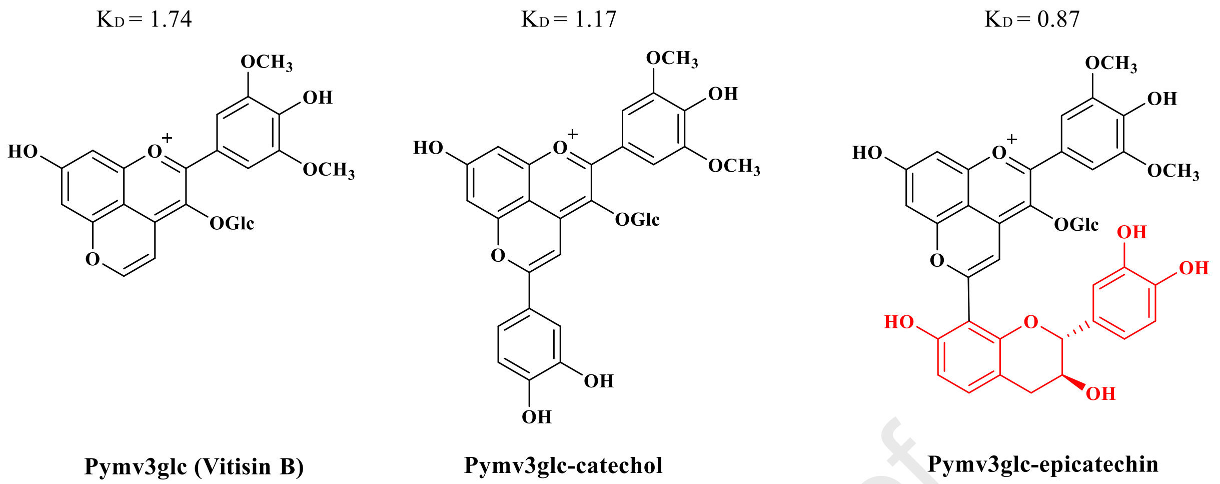

Figure 5. An illustration of the three polyphenols studied by Garcia Estevez *et al.* with their respective dissociation constants (K_D). The lower the K_D, the greater the binding affinity for the given protein, thus Pymv3glc-epicatechin, containing a "tannin structural unit" (shown in red) bound with the greatest affinity. Pymv3glc = Pyranomalvidin-3-*O*-Glucoside (García-Estévez et al., 2017).

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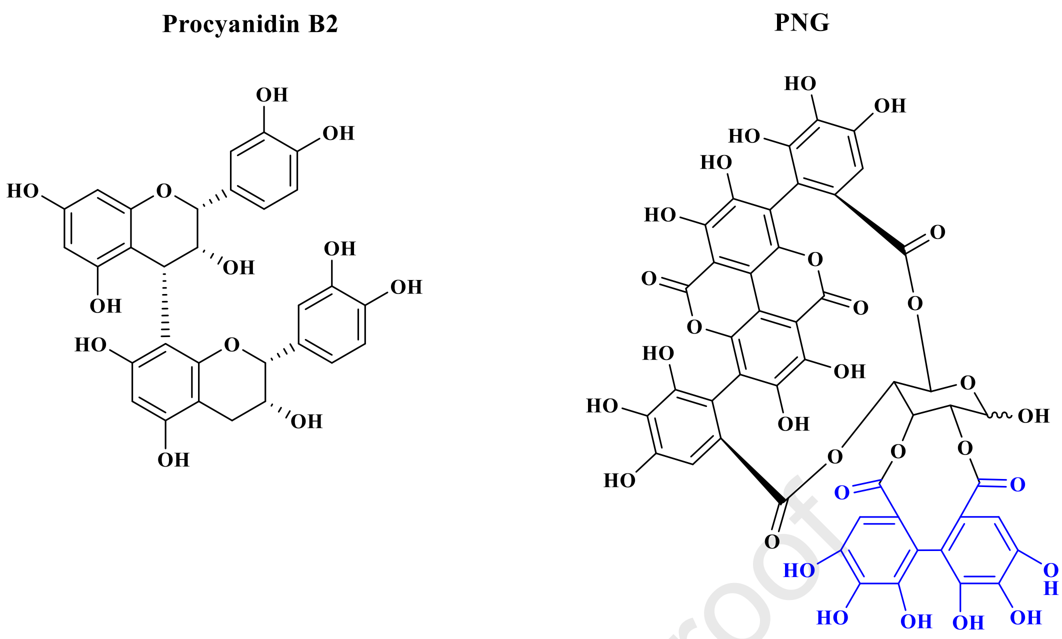

Figure 6. The structures of the two polyphenol compounds studied by Brandão *et al.* (Brandão et al., 2017). The blue highlights the hexahydroxydiphenoyl (HHDP) moiety, a constituent of some ellagitannins, a diverse type of hydrolysable tannins.

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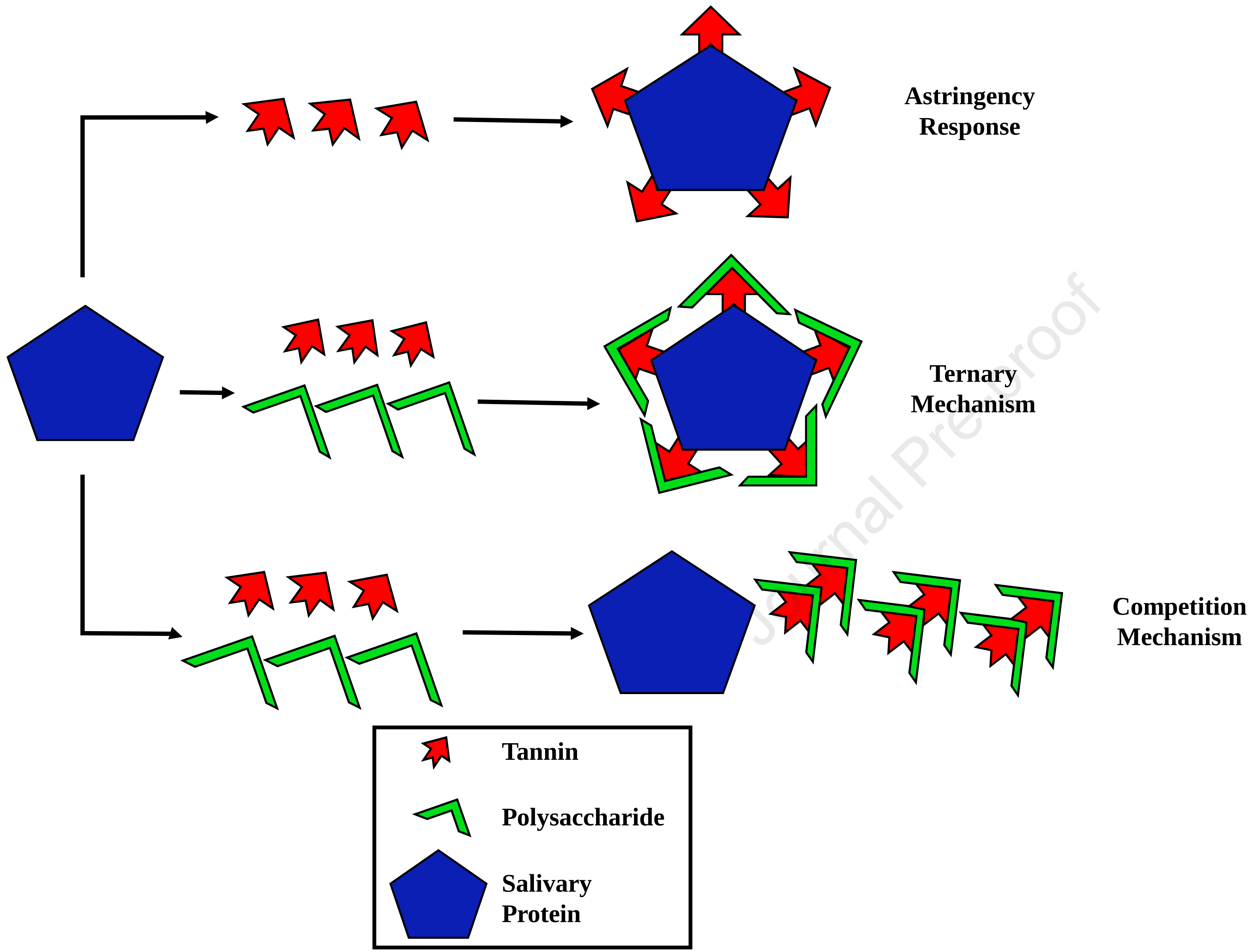

Figure 7. The proposed mechanisms for how polysaccharides interact with the protein-tannin complex to modulate the astringency response and perception. Adapted from Mateus *et al.* (Mateus et al., 2004), de Freitas *et al.* (de Freitas & Mateus, 2012).

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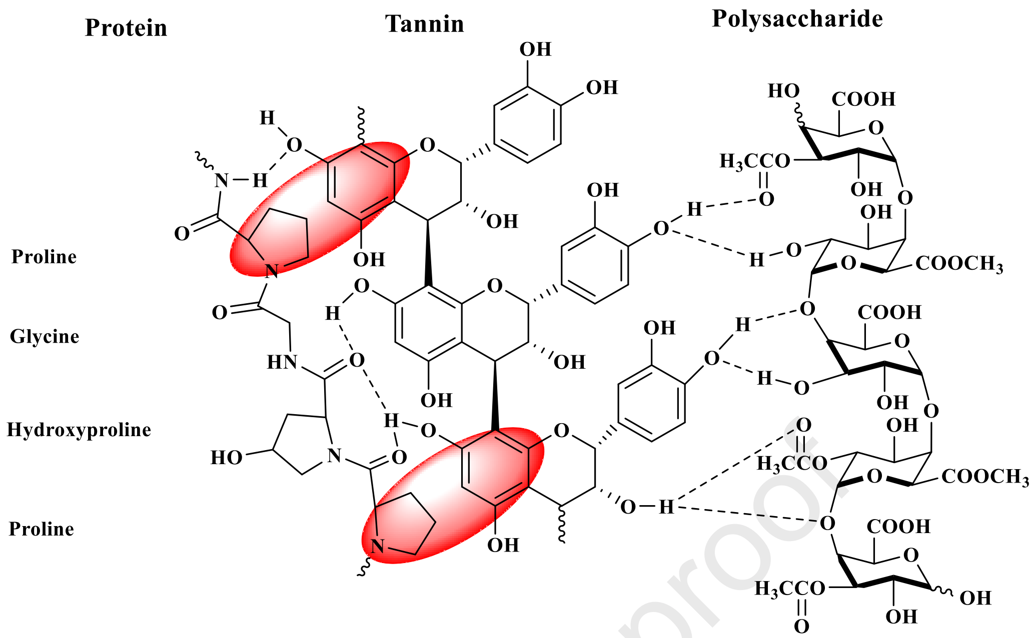

Figure 8. An example illustration of the proposed interactions between the polysaccharide-tannin-protein complex. Hydrogen bonding is represented by the dashed lines and the red circles highlight the potential areas for hydrophobic interactions. Adapted from Scollary *et al.* (Scollary et al., 2012*)* and de Freitas *et al. (*de Freitas & Mateus, 2012). Note that hydrophobic interactions between tannins and polysaccharides are hypothesised to exist but are not included in the diagram due to insufficient information.

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Highlights

1. Elevated glycoprotein concentrations can cause aroma retention.
2. Polysaccharides can improve foam stability in sparkling wines.
3. Physico-chemical parameters of cell wall polysaccharides and tannins can influence extractability during winemaking.
4. Interactions with endogenous tannin and protein complexes can influence the haze potential of wine.
5. Polysaccharides can play an important role in modulating the astringency of wine.

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Conflict and Declaration of Interest

Title: The interactions of wine polysaccharides with aroma compounds, tannins, and proteins, and their importance to winemaking.

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Conflict of Interest: none
Declarations of interest: none

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