Astringency Perception in a Red Wine Context – A Review

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ABSTRACT

Astringency is not considered one of the modern gustatory tastes. The understanding of astringency perception has evolved considerably over the past eighty years, but the exact mechanism is still undefined. This review aims to identify the current theory of astringency perception in the context of red wine. While astringency perception is a complicated mechanism that is not yet well understood, much research has focused on it. The studies discussed up to now have been done using red wine or wine-derived polyphenols. In a red wine context, astringency is driven by polyphenol concentration, galloylation and degree of polymerisation. Compounds that do not bind protein but are still strong astringent agonists are present in red wine. Among them, the role of polysaccharides and oligosaccharides is not clear, as these compounds are difficult to purify in quantities large enough for use in sensory studies. The literature strongly points to astringency being a compound perception. There is a stage of early protein binding, possibly as a “neutralising” effect for small amounts of dietary tannins with little actual perception, building into a more pronounced effect as tannin concentrations increase. A loss of lubrication in the oral cavity might announce the overpowering salivary protein capacity resulting from tannin concentrations, with direct detection of some astringent compounds once protein in the salivary fluid and the salivary pellicle are depleted.

KEYWORDS: Red wine, astringency, tannin, lubrication, oral cavity, salivary pellicle, salivary protein
INTRODUCTION

Astringency is not considered one of the five main tastes, but it is a significant part of the oral sensory experience as a sensation or mouthfeel detection. Significant astringency is tolerated (even considered a requirement) in some foodstuffs (e.g., tea, coffee and wine), but a defect in others (e.g., fruit). The understanding of astringency perception has evolved considerably over the past eighty years, but the exact mechanism is still undefined. This review seeks to identify the current theory of astringency perception in the context of red wine. However, a review of this nature is necessarily limited and, regrettably, it was not possible to discuss certain aspects, such as sensory methods, prediction models, repeated exposure effect, the role of isomeric compounds and viscosity.

THE BEGINNING OF A MECHANISM

While working on condensed tannins from plants at Cambridge in 1954, E.C. Bate-Smith posited that the rough and dry sensations attributed to astringent compounds were caused by particulate formation of salivary proteins (SP) and condensed tannins. The theory that astringency is caused by increased friction created by precipitates in saliva forms the basis of a central part of astringency understanding today. In 1973, Bate-Smith reported a method using haemoglobin as a replacement for SPs (which were too difficult to separate using the methods of the day) to determine the reactivity of different tannin classes, finding that haemoglobin protein precipitation increased as a tannin increased in polymerisation (E. C. Bate-Smith, 1973). Further work by Siebert et al. (1996) examined haze formed in solutions of proline-rich proteins (PRP) and the monomer tannic acid, showing that proteins and phenols each have a specific number of possible binding sites which allow the stoichiometric prediction of haze formation. Further work showed multidentate interaction between polyphenols and a synthetic, 19-residue PRP fragment. NMR studies of titrated reactions showed that larger and more complex polyphenols interacted more strongly with the PRP fragment.

Around the same time, Green (1993) reported that a sensory analysis focusing on astringency had identified various sub-qualities of astringency, including a drying sensation, roughness, puckering and graininess. The existence of sub-qualities would indicate that there is variation in the mechanics of astringency perception. Both authors argue that the loss of lubrication in the saliva layer caused by the loss of SPs is the driving factor of a multifaceted perception of astringency; i.e., the oral sensation is not caused by the presence or absence of precipitate, but rather by the reduced lubrication of the saliva. This line of research has also explored the accumulation of astringency perception with repeated exposure; an increasing sensation is seen as more likely being a diminishing of lubricating protein concentration than the accumulation of a precipitate that would be washed away with each intake.

While precipitation interaction between polyphenols and saliva proteins is a widely accepted explanation for astringency, work on green tea has shown that certain flavan-3-ol glycosides have a very low astringency detection threshold - much lower than similar catechins or theaflavins. Schwarz and Hofmann (2008) found that these same compounds had astringency responses that were not correlated with their ability to bind protein, eliciting a response with no protein interaction. Lee and Vickers (2012) have presented evidence to suggest that astringency perception is not linked to loss of lubrication. Different astringent agonists have been shown to precipitate different types of proteins from the saliva, some of which had no effect on the lubrication ability. However, even with clear evidence of polyphenol-protein interaction, as well as significant data indicating a real change in oral lubrication, the molecular mechanism through which agonists generate the astringency sensation remains unclear and could result from either chemoreceptors or trigeminal mechanoreceptors.

Saliva has a complicated role in the oral cavity, being involved in pH control, debris-clearing, enzyme delivery, and at least two mechanisms of lubrication. SPs in solution decrease friction between both the soft and hard palate and enamel surfaces in the oral cavity, but they also form a semi-permanent coating on all elements in the mouth. This pellicle provides a protective barrier between the tissue and the greater oral cavity, and provides an initial lubrication layer that interacts with saliva, greatly reducing intra-oral friction. It might interact with with astringent compounds after the population of unbound SPs has been depleted.

Once the layer has been stripped away, procyanidins have been shown to react with oral epithelial cells, following the relative depletion of SPs in solution (Soares et al., 2016). The effect of this interaction is unknown. Other gustatory tastes, such as sweet or sour, are detected by receptor proteins held in taste bud structures within this layer of epithelial cells underneath the salivary pellicle; however, astringency can still be detected when the nerve that carries these signals has been incapacitated, indicating that it is not a gustatory taste (Schobel et al., 2014). In the oral cavity, there is a second sensation detection apparatus connected to the brain through a separate pathway: the trigeminal nerve. In the same experiment, astringency perception was severely impaired by anaesthesia of the trigeminal nerve. Schöbel and colleagues showed galloyl-group sensitivity in rat cells taken from the trigeminal nerve and suggested the existence of the same in humans. This would help explain astringency perception that is not related to protein precipitation; however, the location of these trigeminal receptors is assumed to be in or under the epithelial cells, and passage of hydrophobic phenol groups is unlikely.

Recent results reported a correlation between transmembrane mucin MUC1 expression and trigeminal sensitivity. This mucin has two domains: a peptidic glycoprotein and a transmembrane domain, which appears to connect to a yet undefined signalling pathway. The peptidic domain is
completely extra-cellular and is bound to the transmembrane subunit via noncovalent interactions. The extracellular subunit is a repeated proline-, threonine- and serine-rich amino acid sequence with 20 to 120 repeats in the studied population. The authors postulate that the peptidic domain anchors the mucosal pellicle and that as tannin concentration increases, PRPs native to saliva are depleted first, and then the tannin begins to aggregate MUC1 mucins. At a specific point, the increasing friction causes the mucosal pellicle to cleave the peptidic domain from the transmembrane, ensuring the continued lubrication of the mouth while simultaneously triggering a not-yet-defined signalling mechanism to the brain. This proposed mechanism neatly accounts for difficult-to-model aspects of astringency perception, including individual sensitivity to astringency (possible relationship to number of repeated sequences of the peptidic domain), sub-qualities of astringency, such as those described as ‘velvety’ or ‘finely grained’ (the gradient of PRP-depletion rate by specific polyphenols), and non-saliva binding astringent agonists (possible non-friction related cleavage of the two domains).

**DEFINING THE SYSTEM: THE ORAL CAVITY, SALIVA, ASTRINGENTS AND THE DELIVERY MATRIX**

Astringency perception happens in the oral cavity in the presence of saliva, the astringent compound and the matrix in which the astringent was delivered. The sensation is acutely felt in the sublingual area, between the cheeks and gums on both the upper and lower jaw and laterally at the interface between the cheeks and the back of the tongue. To better understand proposed astringency mechanisms, it is helpful to understand the parts of the system in which astringency perception happens: the oral cavity, saliva, astringents and the delivery matrix.

**1. The oral cavity**

Taste signals are produced by G-protein coupled receptors and ion channels in the taste bud structures of the tongue and conveyed by the chorda tympani to the solitary nucleus of the brain via the glossopharyngeal and vagus nerves. In addition to these basic tastes, retronasal olfaction mechanisms detect aromas and other volatile food components. Somatosensory sensations provide information about heat, texture, particle size, rheological properties and some chemical aspects related to pungency and sharpness (e.g., horseradish), coolness (menthol) or irritation (chilli pepper). While olfaction is not to pungency and sharpness (e.g., horseradish), coolness (menthol) or irritation (chilli pepper). While olfaction is not conveyed by the chorda tympani to the solitary nucleus of the brain via the glossopharyngeal and vagus nerves. In addition to these basic tastes, retronasal olfaction mechanisms detect aromas and other volatile food components. Somatosensory sensations provide information about heat, texture, particle size, rheological properties and some chemical aspects related to pungency and sharpness (e.g., horseradish), coolness (menthol) or irritation (chilli pepper).

**2. Saliva**

Saliva is produced in various saliva glands spread across the mouth: parotid glands (upper jaw, near the second molar), submandibular glands (located on the floor of the mouth), sublingual glands (under the tongue, multiple duct outlets across the floor of the mouth) and the minor glands (spread throughout). Parotid glands produce serous saliva, while the submandibular and sublingual glands contribute both serous and mucous saliva. Serous saliva consists of a higher concentration of enzymes and proteins but no mucins. In contrast, mucous saliva has fewer proteins but a high percentage of mucins and other glycoproteins (Helmerhorst, 2012). The combined output of these glands is considered “whole saliva”, which is the focus of this review. Saliva has many functions in maintaining the health of the oral cavity and the digestive system. Saliva has a buffering capacity that maintains a pH in the mouth favourable for maintaining the enamel layer of the teeth, flushes the oral cavity clean after ingestion, serves as a primary enzymatic defence against bacteria and viruses, forms a protective coating for all elements in the oral cavity, and provides a solvent that bathes taste receptors with dissolved substances from food. Several salivary functions appear to be integral to the perception of astringency: the clearing of the oral cavity, formation of a protective coating, lubrication and interaction of proteins with astringent agonists.

**3. The oral cavity as a system**

The mechanical swallowing of saliva and foodstuff in the mouth is the primary mechanism of clearing the oral cavity. Saliva provides a flushing action. Saliva flow rates vary considerably between individuals, and the saliva production rate directly affects the dilution and clearing of gustatory stimuli from the mouth. Gibbins and Carpenter (2013) found that high-flow rate individuals experience faster decay curves of astringency intensity and experience lower overall intensity than low-flow individuals. In addition, saliva protein content, total volume and pH, for example, are also important factors that vary between individuals.

The surface of the oral cavity is covered with a mucosal pellicle comprising a thin, self-assembling layer of proteins, glycoproteins, mucins and carbohydrates, all of which are constantly present in saliva (see Figure 1). This layer is reported to be between 100-1000 nm, depending on formation time and individual variation. It contains mainly mucin, cystatins, amylases and PRPs, which are held onto the oral epithelial cells by covalent and non-covalent bonds (Ployon et al., 2018). This layer provides a lubricating coating that interacts with bulk saliva in the interstitial spaces to reduce friction.

The lubrication effect of saliva has two features the rheological limit and the tribological limit. Rheology refers to a system where two surfaces are separated by a thick layer of fluid (a layer greater in depth than any irregularities on the surfaces). Tribology takes over when the fluid layer becomes thin enough that the irregularities of the surface interact with each other. In the initial stages of ingestion, the free saliva in the mouth, called the bulk saliva, separates all surfaces in the mouth. Detected changes in lubrication are caused by dilution or interaction of saliva and foodstuff.
As ingestion proceeds and some foodstuff is swallowed, bulk saliva is then depleted, and the boundary layer between oral surfaces becomes thin enough for individual surface features of the epithelial layer (mostly filiform papillae) to come into direct contact with each other, causing a marked rise in the friction coefficient. Changes in lubrication at this level can be modified by hydrocolloids contained in the foodstuff, giving rise to sensations of creaminess, slickness or pastiness (Sarkar et al., 2019).

Almost three thousand proteins (including glycoproteins, like mucins) have been identified in saliva. Some of these proteins are involved in precipitation interactions, which are widely thought to be, at least in part, a major contributor to an astringency mechanism. PRPs are particularly prone to precipitation with polyphenols, but not exclusively. Different classes of precipitating proteins have been classified by the type of astringent agonist they interact with, including polyphenols, metal salts, high-isoelectric point basic proteins and poly-glucosamine. This topic will be explored in the discussion of the astringency mechanism theories hereafter.

4. Astringents as a class
All having the ability to react with proteins, astringents form a fairly diverse class comprising four subgroups: salts of multivalent cations (including Al, Cr, Zn, Pb, Ca and B), dehydrating compounds (e.g., alcohols and acetone), some organic and inorganic acids, and plant tannins.

Cationic salts can cross-link salivary and epithelial cell proteins, causing precipitation or agglomeration. These salts are used for flocculation to remove organic matter in water processing, and they have been shown to cause an astringency response in humans, although in concentrations many times higher than encountered in nature. Cationic salts are often perceived as bitter as well, and in food systems their bitter qualities are noted at concentrations far below the astringency concentration threshold. A notable exception is alum (KAl(SO$_4$)$_2$), or potassium aluminium sulphate, which is an ideal astringency standard with low bitterness perception (Yang and Lawless, 2005).

Dehydrating compounds, including ethanol, have an innate astringency in the classical definition, meaning they have a drying and tightening effect on tissues. Ethanol is widely used at high concentrations for sanitation purposes by causing cellular death by dehydration, lipid-layer dissolution and protein precipitation, but at the levels typically found in food and fermented beverages it does not cause protein precipitation alone. Fontoin et al. (2008) showed that increased levels of ethanol in model wine solutions (at a constant pH) caused a linear decrease in astringency. The authors suggested that there might be an increase in viscosity caused by increasing ethanol concentration that masks astringency; however, later work has shown that increased ethanol increases protein-tannin precipitate formation. This increase in precipitation may be explained by the increase in electrostatic attraction between protein particles due to the displacement of water molecules from the hydrophobic regions of the proteins by the ethanol. As water removal minimises hydrophobic interactions,
Acids have been shown to change astringency perception in sensory panels. Inorganic acids have a stronger effect than organic acids, but the impact of both seems to be inversely related to pH, which may reflect that stronger inorganic acids cause a more considerable reduction in pH at the same concentration (Fontijn et al., 2008). A shift in pH below the isoelectric point of proteins causes precipitation, a property often used for protein separations. This is a protein-protein interaction, with no involvement of another agglomerative agent (e.g., cationic salt) forming a cross-linkage. In wine, most proteins are between 20-30 kilodaltons, with relatively low isoelectric points (4.1<pI<5.8). These proteins have a net positive charge at wine pH (Ferreira et al., 2001). In a food system, a pure acid solution would rarely be introduced to the mouth, so the astringency caused by pH changes would be additive to other reactions, like tannin-protein cross-linking.

Plant tannins are the most familiar astringents—and for good reason. Bitterness is often confused with astringency (Kuhlman et al., 2022), possibly because one is often accompanied by the other in complex mixtures of plant metabolites. Plant phenols, which include condensed tannins (proanthocyanidins), hydrolysable tannins and other phenolic compounds, are the most widely distributed secondary metabolites in the plant kingdom. Secondary metabolites are compounds in plant cells that are not immediately essential for photosynthetic or respiratory function but are thought to be required or advantageous for species’ survival in the environment (Cheynier et al., 2013). Terrestrial plants have evolved alongside herbivores, and plants that can repel or disadvantage possible consumers are at an advantage. Polyphenols reduce the value of a feed stock to mammalian herbivores and omnivores by binding to enzymes, impeding necessary steps in digesting plant material (Barbehenn and Peter Constabel, 2011). Mammalian herbivores and omnivores produce relatively rare proline-rich SPs, which have a very high affinity for polyphenols—but carnivores do not. In some rodents, these specialised proteins are absent in the saliva until they are induced by a high polyphenol diet, where as in humans and ruminants, they are omnipresent and reflect the approximate levels of polyphenols found in their typical diets (Haslam, 1998). In this exploration of astringency, this specific co-evolution is of primary interest.

5. Phenolic compounds and their role in protein precipitation

If protein precipitation is accepted as the primary driver of astringency, then the primary polyphenols of interest must efficiently precipitate SPs. García-Estévez et al. (2018) have shown a good correlation between the ability of astringent compounds to precipitate proteins and their perceived astringency as evaluated by a sensory panel. This relationship has been studied using model proteins, such as bovine serum albumin (BSA) or gelatine, as well as with human SPs. Phenolic compounds are defined by an aromatic ring with at least one hydroxyl group. Simple phenols include only one ring; polyphenols include at least two. Polyphenols can bear two aromatic rings in a C6-C2-C6 conformation, like stilbenes, or a C6-C3-C6 structure, composed of two aromatic rings, one ring (A) attached to a heterocyclic pyran ring, and the second ring (B) attached by a covalent bond to the pyran ring, like flavonoids, as depicted in Figure 2.

The flavonoid group is diverse, including anthocyanins, flavones, flavanones, flavonols, flavan-4-ols and flavan-3-ols; the basic structure consists of a B ring attached to C6 of the pyran ring (also referred to as the C ring). Isoflavonoids differ in that the B ring is attached to C3, on the C ring, and neoflavonoids from C6. Flavan-3-ols are the building blocks of proanthocyanidins (also called condensed tannins) that are polymers of flavan-3-ols (de Freitas and Mateus, 2012). Because of their ubiquity in edible plants (especially wine and tea), flavan-3-ols and anthocyanins are the most studied in terms of astringency; however, other flavonoids have been shown to have astringent properties, including flavan-4-ols, chalcones and isoflavones (Bordénave et al., 2014). Most research focuses on the flavan-3-ol group, as it is by far the most common in the human diet.

Based on their polymer structure, tannins can be divided into two categories: hydrolysable tannins and condensed tannins (also called proanthocyanins). Hydrolysable tannins are not common in the diet of most mammals, but they are relevant to wine as they are extracted from wood employed in the winemaking process. Hydrolysable tannins form polymers around a central sugar alcohol, in which the

![FIGURE 2](image-url). Fundamental flavan-3-ol structure and various examples common in wine. R1 = H, R2 = OH, R3 = H, R4 =OH: (+)-catechin; R1 = OH, R2 = H, R3 = H, R4 =OH: (−)-epicatechin; R1 = H, R2 = OH, R3 = OH, R4 =OH: (+)-gallocatechin; R1 =OH, R2 = H, R3 = OH, R4 =OH: (−)-epigallocatechin.
FIGURE 3. General structure of proanthocyanidins. The middle flavan-3-ol structure repeats, creating condensed tannins of varying lengths. $R_1 = H$, $R_2 = H$: propelargonidin; $R_1 = OH$, $R_2 = H$: procyanidin; $R_1 = OH$, $R_2 = OH$: prodelphinidin.

FIGURE 4. Representation of interactions between proline-rich proteins (rendered in green) and condensed tannin (in black). Hydrogen bonds are represented by dotted lines, hydrophobic interactions by grey ovals. Only a small portion of either protein is represented. Adapted from de Freitus and Mateus (2012).
hydroxyl groups oxidise to form ester bonds with gallic acid, called gallotannins. Ellagitannins are similar, but adjacent galloyl ester groups are bonded by oxidative coupling as part of a larger polygalloyl D-glucose ester (Haslam, 2007). These structures are hydrolysed to their component parts, sugar alcohol and gallic acid (or ellagic acid in the case of a gallic acid dimer), giving rise to the name.

Condensed tannins have no central core but are rather a backbone chain of a monomeric flavan-3-ol, with some tannin structures exhibiting secondary branches also composed of linear flavan-3-ol chains. Condensed tannins, or proanthocyanidins, take two basic forms, A and B, which are polymers of the previously described flavan-3-ols: diastereomers (-)-epicatechin/(+)-catechin and galloylated derivatives of these subunits. Proanthocyanidin B has either an interflavanic bond at C8-C6 or C8-C4 that bonds two adjacent subunits, as shown in Figure 2.

Grapes and wine contain large concentrations of type B proanthocyanidins, which can form polymers that chain more than 35 subunits (Spranger et al., 2008) (see Figure 3). Besides the type of bond between subunits and the degree of polymerisation, proanthocyanidins present varying galloylation and hydroxylation patterns creating a wide diversity of condensed tannin structures which determine the degree of bitterness and astringency perceived (Soares et al., 2020). Theoretically, these polymers could be of infinite size, in which they are metabolised by the plant cell. Indeed, this is a major impediment to research, as extracting the total amount of condensed tannin from any complex plant material is limited by the solubility of tannins.

Phenolic compounds bind with proteins through both covalent and non-covalent interactions. The covalent binding of polyphenols and proteins results from a reaction between functional groups of the proteins, like amine and amide groups and quinone structures. An illustrated example of these interactions can be seen in Figures 4 and 5.

Flavonoid (and simple phenolic compounds) oxidation by chemical or enzyme means yields quinones (see Figure 6), usually created in foods by exposure to high heat, alkaline conditions, enzymatic oxidation, or other post-harvest handling or processing. Unlike non-covalent interactions, these covalent bonds are normally irreversible. Recent work has provided evidence of covalent bonding between several animal- and plant-based proteins and a variety of polyphenols, including epicatechin, quercetin, gallic acid and anthocyanins among others (Keppler et al., 2020).

The astringency perception of polyphenols in wine has been shown to have a strong positive correlation with tannin concentration (Ma et al., 2014), primary among other correlations observed between astringency and ethanol, polysaccharide or anthocyanin concentrations (Fontoin et al., 2008). Stereochemistry, galloylation/hydroxylation patterns,
molecular weight, degree of polymerisation, percentage of prodelphinidins and conformation all affect the ability of tannins to interact with proteins; however, a predictive rule remains elusive as each tannin-protein pairing is unique. Generally, increased tannin-protein interaction is favoured by a greater degree of polymerisation, higher molecular weight and increased galloylation (number of galloyl group substitutions) (de Freitas and Mateus, 2012). A possible correlation has also been found between the concept of tannin activity - or a measure of the enthalpy of interactions between a hydrophobic surface and a specific tannin - and astringency perception, possibly because higher tannin hydrophobicity could increase the attraction between tannins and proteins.

6. Phenol agonists that do not precipitate proteins

Protein precipitation has long been studied as the main mode of astringency, but certain agonists are significantly astringent but have no protein binding activity. Using taste dilution analysis of black tea, Scharbert et al. (2004) showed that several different flavon-3-ol glycosides have astringency detection thresholds a hundred times lower than aglycones. Later work by the same group quantified the amount of SPs bound by each compound. Flavanol glycosides, such as quercetin, bound no detectable protein but produced a threshold astringency response at very low concentrations, while gallic acids, procyanidin B2 and (+)-catechin bound very small amounts of protein but had similar thresholds to compounds (such as epigallocatechin gallate (EGCG) or catechin gallate) that bound 10-80 times the amount of protein. (Schwarz and Hofmann, 2008).

THE DELIVERY MATRIX

Astringency is not perceived in a vacuum. The astringent compound must be delivered to the oral cavity in some manner; only in a clinical research environment are these delivery vehicles well-defined. Food and drink are the normal matrices that cause an astringency response, and these are complex matrices made up of crude mixtures of simple and complex saccharides, lipids, amino acids and proteins, and various alcohols and volatile compounds. As any good cook knows, a sour dish can be tempered with a sweet ingredient or a dash more of salt; in the same manner, different compounds have been shown to enhance or reduce the perception of astringency. Astringency-moderating compounds common in human food matrices include organic acids, simple and complex sugars, lipids, ethanol, amino acids and proteins.

1. Lipids, proteins and sugars

Tannins consumed in the presence of vegetable oil are perceived as less astringent in sensory analysis (Saad et al., 2021). In the food bolus, lipids are droplets that maintain a hydrophobic interior while dispersed in a greater aqueous mass. Using deuterated lipids, NMR has been used to measure the ordering properties at the surface layer of the droplet, showing a strong interaction upon the introduction of tannins. Calculations of association constants show that the interaction of tannins with lipids in the food bolus is about one order of magnitude greater than with saliva proteins. Simply put, fats will compete with SPs as ligand partners for tannins, reducing the complexation of tannin-protein pairs when present (Dufourc, 2021). The “camembert effect”, or the reduction of astringency perception of a tannic red wine when drinking it with fatty cheese, is a clear example of this competition.

Alternatively, fat might replace the loss of lubrication that occurs when SPs bind with tannins. Fat was found to offset astringent attributes like drying, roughing and puckering in an experiment using cocoa, where samples with increased fat, but a constant concentration of cocoa, were perceived as less astringent (Hamada et al., 2020).

Proteins introduced to the food matrix can be considered competitors for SPs in tannin complexation. Most protein research has involved caseins and other milk proteins, as they
are common ingredients in prepared foods and are soluble in an aqueous solution. Casein has been shown to form complexes with EGCG, reducing the contact of EGCG with SPs. The same effect was seen with polyphenol-rich plant extracts mixed into milk, both with and without fat (Huang and Xu, 2021). Little work has been done on other food proteins that may interact with tannins. Mannoproteins will be discussed at length along with other carbohydrates.

Simple sugars have been shown to reduce astringency perception at concentrations over 5.0 %. Ishikawa and Noble (1995) analysed both maximum astringency intensity and time to maximum intensity perception in model and red wines but did not examine what mechanism might have caused this reduction. Earlier work by Harbertson et al. (2013) shows evidence that increased sucrose, fructose and glucose concentrations prevented the precipitation of polyphenol-protein complexes but did not prevent their formation. This effect only became significant at concentrations of sugar over 20 %. It remains unclear if this reduction in astringency perception is due to an increasing sensation of sweetness masking the astringency, as there is no evidence that simple sugars change the complexation of proteins and phenols.

2. Wine polysaccharides

Polysaccharides are polymers formed from monosaccharides. The most common monosaccharides in plants are arabinose, rhamnose, fucose, mannose, glucose, galactose, xylose, glucuronic acid and galacturonic acid. Wine polysaccharides are grouped into two families according to the provinice: plant-derived polysaccharides originating from grape cell walls, and microorganism-derived polysaccharides released by yeasts, bacteria and fungi. Grape polysaccharides include polysaccharides rich in arabinose and galactose (PRAGs), which include arabinogalactanactans, arabinogalactan proteins (AGPs, see Figure 7), rhamnogalacturonans type I (RG-I, see Figure 8) and type II (RG-II, see Figure 9) and homogalacturonans (Guadalupe and Ayestarán, 2007).

As a group, PRAGs are highly polydisperse, but these polysaccharides make up the pectic network of the primary cell wall and are thought to bind together structural polysaccharides and extensins. The structural polysaccharides include cellulose (α-(1-4)-linked glucose chains) and hemicelluloses, which are primarily xyloglucans (β-(1-4)-D-glucan backbone with ~75 % of the glucose residues carrying a xylose residues as shown in Figure 10).

These structural polysaccharides are prevalent in grape and must samples but are found in very low quantities in wine, as it is thought they precipitate during winemaking. Homogalacturonans, also found in abundance in fresh tissue, are found in very low quantities post-fermentation, most likely due to endogenous polygalacturonases (Guadalupe et al., 2015).

Mannoproteins, mannans and glucans are structural parts of the cell wall of yeast; mannoproteins produced by yeast are the second most abundant polysaccharide in wine.

**FIGURE 7.** Representation of an arabinogalactan protein polymer. Arabinose side chains composed of 1-4 monosaccharides. O-linked arabinogalactan-II glycan are substituted at R by any of the listed sugar sequences. Not all hydroxyproline residues (represented by the angles of the zig-zag chain) necessarily host a glycan sequence. AGPs are a very heterogeneous class of glycoprotein. Adapted from Ellis et al. (2010)
These glycoprotein polymers are highly polydisperse in size and have carbohydrate fractions of more than 90% (Guadalupe and Ayestarán, 2007). Polysaccharides from both families are found in red wine at around 42% AGPs, 35% mannoproteins, 19% RG-II and 4% RG-I (Vidal et al., 2003).

2.1 Polysaccharides compete with salivary proteins for tannin ligand partners

Polysaccharides can interrupt protein-tannin interactions by competing with the SPs for tannin ligand partners or by forming a ternary protein-tannin-polysaccharide complex (Mateus et al., 2004). This first evidence of competition by polysaccharides showed that polysaccharides that are able to develop a hydrophobic cavity in an aqueous solution prevent the formation of peptide-polyphenol (Gaffney et al., 1986). Gaffney and co-workers used cyclodextrins as model polysaccharides and caffeine to stand in for peptides. Other researchers have employed other model polysaccharides, such as pectin and gum Arabic, along with model proteins, including BSA, α-amylase and gelatine, which showed degrees of disruption via competition, supporting the hypothesis that hydrophobic cavities formed by polysaccharides encapsulate the aromatic rings of polyphenols, inhibiting their binding ability (Le Bourvellec et al., 2004).

The forces responsible for the interactions between tannins and polysaccharides are hydrogen bonding and hydrophobic interactions: both polysaccharide and tannin conformations are important, as they need the flexibility to interact (Watrelot et al., 2013). The polarity of individual polysaccharides is also important and related to their ionic charge. Soares and co-workers noted that ionic polysaccharides (e.g., pectin, polygalacturonic acid, xanthan) reduced the interaction between BSA and procyanidins more effectively than neutral polysaccharides (arabinogalactan, dextran). They also found that increasing the polarity of polysaccharides increased the disruption of BSA-tannin aggregation. Tannin structure was also important; low polymerised procyanidins were more easily targeted by all polysaccharides. This same study also found that while xanthan gum could encapsulate low mDP polyphenols, pectin was not. Carvalho et al. (2006) found that BSA-tannin inhibition by xanthan is more effective for smaller polyphenols of lower complexity.

Model proteins are problematic to work with in an astringency context, as their structures differ considerably
from saliva proteins. The theory of co-evolution maintains that protein amelioration of tannin compounds in mammal digestion - if one were to assume that this is the point of some saliva proteins - would have evolved as plants developed defence strategies that utilise tannins, making them very specific for plant tannins. In studies developed using SPs instead of model proteins, results indicated that pectin and polygalacturonic acid favour the formation of ternary SP-tannin-polysaccharide complexes (Soares et al., 2012).

Similarly, model polysaccharides do not exactly mimic those naturally present in a system, thus further work has focused on polysaccharides found in the most studied system: red wine. In experiments by Vidal and co-workers, RG-II has been shown to reduce astringency perception of a model wine, whereas AGPs and MPs had less effect. (Vidal et al., 2004).

2.2 Molecular approaches to studying protein-tannin complex inhibition

Carrying out research using purified wine polysaccharides is difficult, as extraction and identification are laborious; therefore, in some cases, information on the astringency moderation effect of these compounds is contradictory. In many cases, only the *in vitro* study of protein-tannin complex inhibition has been possible; the small amounts of polysaccharides that are feasible in purification are seldom sufficient for complementary sensory evaluation. In this context, several molecular approaches have been developed to probe the interactions between molecules, yielding useful results. Working with two SPs (α-amylase and IB8c, a PRP fragment) and grape seed tannins, and using light scattering, Carvalho et al. (2006) found that most acidic AGPs inhibit protein-tannin aggregates for both proteins, but RG-II only inhibits α-amylase. This study showed the importance of both the ionic character of the polysaccharide and the structure of the protein. Brandão and colleagues studied several interactions between saliva proteins and tannins in the presence of various polysaccharides, confirming that polysaccharides can act by competition with proteins or by forming a more soluble ternary structure. Ternary interaction between RG-II and AGPs was studied using two SPs, an aPRP and P-B peptides (PB), and procyanidin B2 (condensed tannin) and punicalagin (PGN, hydrolysable tannin). Both polysaccharides were able to reduce the interaction of SP and tannins; however, the extent of each polysaccharide’s ability to interrupt complexation depended on the specific ligand pairs. AGPs reduced the complexation of all pairs, and RG-II all pairs, except for PB-PBG. AGPs also had their weakest inhibitory effect on the PB-PBG ligand; the authors suggest this reflects the more apolar nature of these two compounds. RG-II, an acidic polysaccharide, does not have a competition effect (Brandão et al., 2017). It is unclear from this experiment how the polarity of these polysaccharides was determined. RG-II was generally more efficient in inhibiting SP-tannin complexation, but the presence of sodium ions in the solution negated this effect, suggesting that negatively charged regions of this epitope were neutralised by Na⁺ ions. Using complementary approaches to explain the mechanism, Brandão et al. (2020) suggested that both AGPs and RG-IIs can both compete to bind with tannins, but only AGPs participate in a ternary mechanism. More recently Kuhlman et al. (2023) and (Kuhlman et al., 2024) have shown that

**FIGURE 9.** Representation of a monomer rhamnogalacturonan-II glycan. RG-II are thought to be more homogeneous than other cell wall polysaccharides and to maintain a narrow molecular weight range. The dimer form of RG-II is caused by a boron molecule crosslinking the apiose monosaccharide found on chain A. Adapted from Barnes et al. (2021).
The relationship between grape polysaccharides extracted from grapes and astringency has also been investigated by Quijada-Morin et al. (2014) and Manjón et al. (2023). Polysaccharides extracted from white grape skin cells also showed inhibitory effects on a more complex matrix. Unpurified extracts from grape skins (hot water extraction and a chelating-solvent extraction) both showed the ability to inhibit complexation between a procyanidin dimer mixture and acidic PRPs (aPRPs), PBs and cystatins, but not with glycosylated PRPs (Brandão et al., 2020). It is interesting to note that, in general, polysaccharides in a mixture (as opposed to purified glycans) seemed to be synergistic in their inhibition of a complicated protein mixture like saliva. Manjón et al. (2020) found that mannoproteins from yeast cell walls with higher protein content were more likely to compete to bind with flavanols, while those with larger mannan content favoured ternary complex formation. Interestingly, in a sensory analysis, reduction of astringency perception was best correlated with the largest mannoprotein molecules; however, it was unclear whether size or make-up contributed the most to this effect, as the largest mannoproteins had neither the largest nor smallest protein content of the three mannoprotein samples. Different approaches to examining a molecular basis for this interaction support the findings that MPs and RG-IIs can alter the interactions of tannins and proteins in these experiments, using BSA as a model protein with varying wine-derived polysaccharides and condensed tannins (Lei et al., 2019).

3. Acid, pH, minerals and solvents

A discussion about astringency perception should also consider the fact that the environment in which astringency mechanisms function is bound by the limits of the food matrix and the oral cavity. Organic acids at normal levels found in food and drink have not been shown to contribute to astringency perception; however, pH is negatively correlated with astringency intensity. A wine at pH 3 would be more astringent than the same wine at pH 7. Water is the main solvent in food and the oral cavity, but ethanol is an important factor for wine and beer, which are major focuses of astringency study. Ethanol decreases astringency perception as its concentration rises, especially at typical wine concentrations (11-15 %) (Fontoin et al., 2008).

Mineral content effect on astringency is a relatively unstudied area. Mineral salts have been tested in the context of nutritional supplements, and most that are found naturally in food are not thought to add to astringency, except for zinc salts (with zinc iodide being the most potent agonist). Mineral interference in protein-tannin reactions appears to have not been studied. In wine, potassium, magnesium and sodium are the most common minerals. The presence of sodium ions has been shown to inhibit the ability of specific acidic polysaccharides to compete for tannin binding in tannin-protein interactions (Brando et al., 2020). Dissolved minerals in an aqueous environment can have a larger effect than initially assumed. In 1888, Franz Hofmeister, a protein scientist at the University of Prague, published research that noted a curious effect of different salts on protein precipitation. Hofmeister and co-workers found that different salts could induce precipitation of proteins at very different concentrations. While the exact concentrations varied depending on the protein, the order of precipitation strength appeared to be constant (Franz Hofmeister, 1888 as translated by Kunz et al., 2004). This ranking of the ability to precipitate the protein of each salt is still called the Hofmeister series. There is still disagreement about the exact mechanism of the Hofmeister effect, but the broad outline can be summarised as follows: a mass of pure water behaves like a collection of individual water molecules, but upon the introduction of an ion, this mass of water behaves like a mixture of two solutions. One solution is pure water, but the second “solution” is the number of water molecules each ion molecule “holds” due to its intrinsic charge. An ion that “holds” more water molecules around it reduces the amount of “free water” in the system, effectively increasing the ratio of the second solution in the mixture. In an aqueous protein solution at a specific concentration of ions, there is no longer enough free water to hydrate the protein, and therefore it precipitates (sometimes referred to as “salting out”) (Zavitsas, 2016).

FIGURE 10. Representation of a xyloglucan polysaccharide fragment. Xyloglucans are a heterogenous class of cell wall hemicellulose. Not every unit of the glucose polysaccharide carries a side chain, and not every side chain represented here will be present in every xyloglucan.
While protein precipitation is the most obvious effect, the Hofmeister effect changes other physical properties of a system, including surface tension, viscosity, disassociation constants and many varieties of non-covalent interplay, such as hydrostatic interactions, as well as substrate-specific changes, such as the presence of preferential ion binding sites in proteins and enzymes (Budroni et al., 2020). As far as the authors know, the effect of differing mineral compositions in food matrices on astringency perception remains unreported.

**TOWARDS A THEORY OF ASTRINGENCY PERCEPTION**

1. **Tannin-protein complexation**

   E.C. Bate-Smith first suggested in 1954 that astrinency was not a taste but a feeling, and the tactile nature of astrinency quickly became the accepted paradigm. He theorised that astrinency was the interaction and precipitation of glycoproteins in mucous secreted by the salivary glands (E. Bate-Smith, 1954). Bate-Smith thought this precipitation had two effects: a change in lubrication caused by the loss of proteins in saliva, and the adherence of the precipitates to the epithelial cells of the oral mucosa, where they form a residue. Theories followed that the precipitation of proteins caused dehydration of tissues, closure of saliva ducts or inhibition of the salivary gland, but all were shown to be unsupported as it was understood that protein precipitation increased saliva production (Bajec & Pickering, 2008). A two-stage model has evolved where polyphenol-protein interactions precede the binding of the aggregate to the epithelial proteins (Jöbstl et al., 2004).

   Much work has been done to explain the complexity of SP and polyphenol interactions, and while specific protein-polyphenol pairings can have very different behaviours, some trends have been found. Tannins that have a higher degree of polymerisation and more galloylation are more likely to interact with proteins. (de Freitas and Mateus, 2012) While not subject to linear polymerisation, hydrolysable tannins were more reactive with proteins when they were more galloylated (Ferrer-Gallego et al., 2012). The tannin interactions may also contribute to a more reactive polyphenol, yielding stronger hydrophobic interactions.

   Similarly, SP structure influences interaction with polyphenols. In general, globular proteins are less reactive than more open structures, such as coiled or intrinsically disordered proteins. Charged, polar amino acids, such as proline or glycine, promote disordered proteins. Charged, polar amino acids, such as hydrostatic interactions, as well as substrate-specific changes, such as the presence of preferential ion binding sites in proteins and enzymes (Budroni et al., 2020). As far as the authors know, the effect of differing mineral compositions in food matrices on astringency perception remains unreported.

   **ASTRINGENCY PERCEPTION**

   As SPs provide lubrication for the oral cavity, the loss of lubrication properties of saliva could be positively correlated with increased astrinergency perception in sensory panellists assessing red wines. Furthermore, observing microstructures formed by SPs and red wine samples showed extensive aggregate formation, suggesting severe protein depletion from oral surfaces. Recent work has explored the interaction of different classes of astrigent agonists, including small phenolic compounds, large polyphenols and cationic astringents, with mucin and PRPs. Small phenols and cationic agents did not cause a decrease in lubrication; however, small phenols did not cause aggregation of proteins, while cationic agents did, but these proteins were able to provide continuous lubrication even when interacting with the astrigent agent. Large polyphenols did cause aggregation and lubrication loss, but surprisingly mucin was shown to aggregate without a loss of lubrication, indicating PRPs are the most important contributors to saliva lubrication (Rudge et al., 2021).
2. Pellicle disruption

As the unbound saliva proteins are depleted, tannin interacts with the mucosal pellicle. The epithelium is the outermost layer of cells in the oral mucosa, on which the mucosal pellicle is formed, which is a thin layer of proteins that adhere to the cells themselves, forming a sort of barrier layer. The mucosal pellicle contains mucins, cystatins, amylases, IgA and aPRPs, and can be up to 100 nm in thickness (Ployon et al., 2018). Mechanoreceptors are specialised nerve cells that detect pressure, vibration and stretching located in the mucosa (Canon et al., 2018). Ployon et al. (2018) have suggested that oral epithelial cells can bind tannins and that tannin-protein interactions may change the structure of the mucosal pellicle. The work by Ployon and co-workers showed that two dietary tannins had a detrimental effect on this protein layer, causing increased aggregation of protein from the pellicle that increased with both concentration and galloylation of the tannin. Interestingly, protein aggregation from the pellicle was prevented when a bPRP was present. To date, all research has been done with epithelial cells grown in vitro and with induced pellicles created by allowing salivary mixtures to bond to various substrates.

3. Chemosensory detection

Schöbel and colleagues showed galloyl-group procyanidin concentration, galloylation, and a degree of polymerisation. However, it is also clear that it is the complex interacting nexus of the proteins, polysaccharides and polyphenols in the delivery matrix that determine the complex perception of astringency in red wine. Compounds that do not bind protein but are still strong astringent agonists are present in red wine. The effect of polysaccharides and oligosaccharides, two agonists also present in wine, is not clear, as these compounds are difficult to purify in quantities large enough for use in sensory studies. This relationship between specific polysaccharide epitopes and size is not yet understood, and there is evidence that while polysaccharides generally reduce astringency, oligosaccharides may increase it. From this review, the evidence strongly points to astringency being a compound perception. There is a stage of early protein binding, possibly as a “neutralising” effect for small amounts of dietary tannins with little actual perception, building into a more pronounced effect as tannin concentrations become larger. A loss of lubrication in the oral cavity might announce the overpowering SPs capacity by tannin concentrations, with direct detection of some astringent compounds once protein in the salivary fluid and the salivary pellicle are depleted.

FURTHER STUDY AND CONCLUDING REMARKS

While astringency perception is a complicated mechanism that is not yet well understood, much research is focused on it. The studies discussed up to this point have been done using red wine or wine-derived polyphenols. Red wine contains the following astringent agonists and astringency moderators: ethanol, phenols, from simple to complex condensed tannins, cell-wall and yeast-derived polysaccharides and oligosaccharides, simple sugars, small amounts of protein, small amounts of glycerol and other more complex alcohols, and traces of sodium, potassium and magnesium. In a red wine context, astringency seems to be driven by polyphenol concentration, galloylation, and a degree of polymerisation. However, it is also clear that it is the complex interacting nexus of the proteins, polysaccharides and polyphenols in the salivary fluid and the salivary pellicle are depleted.

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