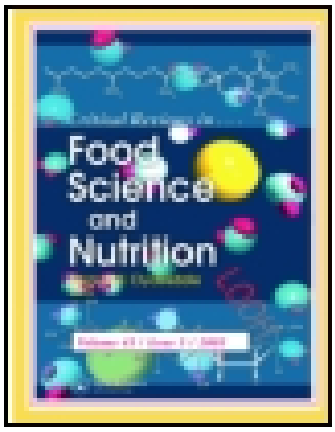

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Sensorial Properties of Red Wine Polyphenols: Astringency and Bitterness

Susana Soares, Elsa Brandão, Nuno Mateus & Victor De Freitas

Accepted author version posted online: 21 Apr 2015.

SENSORIAL PROPERTIES OF RED WINE POLYPHENOLS: ASTRINGENCY AND BITTERNESS

Susana Soares*, Elsa Brandão, Nuno Mateus and Victor de Freitas

* Corresponding author

<CFN>Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn</CFN><TI>This to be the bottom CFN on opening page</TI>

ABSTRACT

Polyphenols have been the subject of numerous research over the past years, being referred as the nutraceuticals of modern life. The healthy properties of these compounds have been associated to a natural chemoprevention of 21st century major diseases such as cancer and neurodegenerative diseases (e.g. Parkinson's and Alzheimer's). This association led to an increased consumption of foodstuffs rich in these compounds such as red wine. Related to the ingestion of polyphenols are the herein revised sensorial properties (astringency and bitterness) which are not still pleasant. This review intends to be an outline both at a sensory as a molecular level of the mechanisms underlying astringency and bitterness of polyphenols. Up-to-date knowledge of this matter is discussed in detail.

Keywords: bitter taste receptors, proline-rich proteins, salivary proteins, tannins

POLYPHENOLS

Polyphenols are a large family of metabolites that result from the secondary metabolism of plants. These compounds are widely present in vegetables, fruits and derived products (*e.g.* red wine and green tea) being responsible for major organoleptic properties (color and taste attributes such as bitterness and astringency).

Several health benefits have been associated to these compounds, namely prevention towards cancer, allergies, cardiovascular, Parkinson and Alzheimer's diseases, among others (Ramassamy 2006, de la Iglesia et al. 2010).

The well-known French Paradox that appeared in 1992 (Renaud and de Lorgeril 1992), is a classic example. The French population showed a low incidence of cardiovascular diseases despite the high consumption of saturated fat and tabaco. This fact was attributed to a regular and moderate consumption of red wine.

Presently, these positive health effects have been related to the strong antioxidant capacity of polyphenols but also to much more complex effects related to cell signaling.

Polyphenols are classically divided in two main families: non-flavonoids and flavonoids (Figure 1). The first ones are a large family of compounds including mainly small molecules such as benzoic and cynamic acids. However, the most important family of polyphenols in food is the flavonoids. This family is structurally very diverse, derived from a central core, the flavanic nucleus (Figure 1). Flavonoids are divided in several classes according to the oxidation degree and substitution pattern of ring C. The main classes of flavonoids include anthocyanins, flavan-3-ols, flavones, flavonols, among others (Figure 1).

Anthocyanins are responsible for the color of several fruits and vegetables such as red fruits and derived products such as red wine. They exhibit colors between red and blue depending on the pH. They are responsible for the initial color of red wine but also by the changes that occur progressively during red wine ageing as a result of their condensation with flavanols and/or with smaller compounds, such as pyruvic acid, vinylphenol or glyoxylic acid.

In fruits, anthocyanins are always in their glucoside form but in other foodstuffs they may occur as an algycon, i.e., without the sugar moiety. In most cases, sugars are linked in O-3 position, but they could also be linked in O-5 and O-7.

The main interests in anthocyanins rely not only on their diverse colors enabling their application in the food and cosmetic industry, but also on their antioxidant capacity attractive to the pharmaceutical and cosmetic industry.

Flavan-3-ols is one of the most abundant class of polyphenols in nature. These compounds differ in the stereochemistry of carbon 3 of ring C and in the hydroxylation degree of ring B. They have a monomeric unit of (+)-catechin (C) or (-)-epicatechin (EC) and they can associate forming polymers called proanthocyanidins or condensed tannins. Polymers with up to 80 units have been reported in grape skin tannins (Souquet et al. 1996). They can also be esterified with gallic acid, thereby including epigallocatechin or epigallocatechin gallate.

An important designation for some polyphenols is the so called tannins. Tannins are a group of polyphenols structurally very diverse that share the ability to interact and precipitate proteins. They are usually divided in condensed and hydrolysable tannins (Figure 1), with the first ones, as referred above, being oligomers of catechins and the last ones esters of monosaccharides with gallic acid or oligomers of gallic/ellagic acids.

The ability to precipitate proteins is at the origin of positive and negative effects of these compounds. One of the major characteristic associated to tannins and further discussed below, is the widely known astringency sensation. Besides its involvement in astringency, tannin/protein interactions have also biological importance regarding the absorption, metabolism and bioactivities of polyphenols in human body. So, the study of this interaction has been the scope of numerous research groups and the recent literature has given great advances and significant knowledge on this interaction as reviewed herein.

For example, the work done by Canon and colleagues (Canon et al. 2013) suggests that the aggregation of salivary proteins by tannins in the mouth could have a physiological function, such as the regulation of the amount of free tannins in the ingested food and drinks. Moreover, it is known that the interaction between polyphenols and proteins affect the antioxidant capacity of polyphenols (Arts et al. 2002) but this interaction could also be responsible for the transport of polyphenols across the human body in order to exert their antioxidant action (Diniz et al. 2008, Xiao and Kai 2012).

As referred, one of the major characteristic associated to tannins is the astringency sensation which is related to red wine quality. Red wine tannins consist mainly on condensed tannins extracted from grapes and subsequently structurally modified during winemaking and ageing. A small percentage of hydrolysable tannins are extracted from oak barrels or chips during ageing or can be added during winemaking by the addition of enological tannins (Sarneckis et al. 2006). However, due to their low amounts in red wine, these tannins are not probably major contributors to the astringency sensation.

Considering the structural diversity of tannins from red grapes, it is well established that the average size of skin tannins is much larger than that of seed tannins. Tannin extracts from skins have mean degrees of polymerization around 30 (Souquet et al. 1996) compared with 10 in extracts from seeds (Prieur et al. 1994). Skin tannins combine with polysaccharides and protein and contribute to the softness and roundness, but can impart herbaceous notes if fruit is not ripe. Seed tannins give structure to the wine but can also impart excessive astringency.

Condensed tannins from grape skins are the first to be extracted in the fermentation process whereas grape seed and flesh tannins are extracted later and increasing with maceration time. Winemakers often employ several techniques (e.g., maceration time, temperature and cap management) to maximize the extraction of tannins and pigments from grapes into the must. However, when the extraction of these compounds is deemed insufficient, winemakers often add enological tannins to improve wine quality.

Besides the influence of all these factors concerning red wine polyphenols, it is also important to refer that tannins and anthocyanins are both relatively unstable species that can undergo various types of chemical reactions such as spontaneous cleavage of interflavan bonds in acidic medium, as well as acetylation and oxidation (Fulcrand et al. 2006). The complex interactions between these compounds and other molecules are a challenge for red wine composition analysis and consequently for the study of red wine quality sensorial parameters such as astringency.

ASTRINGENCY

Astringency is a tactile sensation perceived on the human palate and has been defined as a complex group of sensations involving dryness, tightening and shrinking of the oral surface and

puckering sensations of the oral cavity. As referred before, it results from the ingestion of food products rich in tannins being a quality parameter of red wine and of its mouth-feel (Kennedy et al. 2006, Mercurio et al. 2010).

Wine tannin quality is dependent on the maximum intensity of the mouth feel, total duration and time taken to reach maximum intensity, as well as the extent of mouth drying and mouth roughness. The spectrum of subtle differences in astringency sensations was compiled as a 'red wine mouth-feel wheel' (Gawel et al. 2000), which include descriptors as 'powder' through to 'adhesive' and 'aggressive'. Astringent sensations of wine are considered pleasant when balanced with other factors including alcohol and sugar content (Fischer et al. 1994, Boselli et al. 2004). Higher concentrations of tannins and acids compared with sugar result in a highly astringent wine that is considered 'harsh', 'unripe' or 'green', and conversely, higher concentrations of sugars can result in a wine that may be described as 'thick' or 'flabby' (Kennedy 2008).

As astringency influences the overall quality of red wine, the knowledge of the structure/activity relationship on the sensory properties as well as the mechanisms underlying astringency development are important aspects of winemaking. The understanding of these aspects will enable growers and winemakers to have more control over the characteristics of the produced wine.

Presently, the possible mechanisms for astringency development are controversially discussed by the scientific community with several mechanisms being proposed. As astringency is considered to be a tactile sensation, some authors point that it could result from altered salivary lubrication (Rossetti et al. 2008, Rossetti et al. 2009). However Lee and Vickers (Lee and Vickers 2012) had

provided evidences that the loss of salivary lubricity is not likely to be a central mechanism of astringency. Other authors suggest that astringency could be detected by increased activation of receptors located within the mucosa, like other primary tastes such as bitterness. However, astringency increases upon repeated exposure (Dinnella et al. 2009), in contrast to taste sensations, suggesting that it involves mechanical rather than chemosensory processes. Moreover, astringency perception occurs on nongustatory mucosal surfaces and requires tissue movement to be perceived, in agreement with a tactile mechanism involving an increase of mouth friction. Another hypothesis suggest that astringency could be related to interactions between tannins and oral epithelial cells (Payne et al. 2009).

In the past years, several research groups studied the molecular basis of astringency development using model bioassays with pure/isolated proteins, namely proline-rich proteins (PRPs) and tannins. Several techniques have been used for this study, namely SDS-PAGE, spectrophotometry, nephelometry, NMR, DLS (dynamic light scattering) and mass spectrometry. However, there are some difficulties in correlating the perceived astringency to a single physical-chemical phenomenon.

The most established mechanism for astringency involves the interaction between tannins and proteins. Since 1954, when Bate-Smith (Bate-Smith 1954) proposed that astringency results from the interaction of tannins with salivary proteins (SP) in the mouth, it has been generally accepted and supported by numerous works along the years that astringency is due to the tannin-induced interaction and/or precipitation of salivary PRPs in the oral cavity.

A recent work (Sun et al. 2013) studied the reactivity of polymeric proanthocyanidins toward SP by SDS-PAGE and Folin-Ciocalteau and correlated with the astringency perceived by a sensory

panel. These authors observed that salivary proteins were more precipitated by the compounds that were perceived as more astringent (polymeric procyanidins). Also Rinaldi and co-workers (Rinaldi et al. 2012) that evaluated the astringency of red wines by several methods showed a significant correlation between the precipitation of salivary proteins and the perceived astringency. In fact, a recent work (Canon et al. 2013) has shown a correlation between the thresholds for the formation of aggregates *in vitro* to the perceived astringency. In this work, the authors studied the aggregation between a PRP from saliva (IB5) and epigallocatechin gallate (EGCG) and find out that the ranges of concentration for the perception of EGCG astringency (0.2 to 0.4 mM) were remarkably close with those where aggregation and precipitation take place (0.2 mM EGCG for 0.21 mM IB5). Moreover, these authors have demonstrated that this aggregation process readily leads to massive aggregates when tannins concentrations exceed the threshold.

Furthermore, it seems that astringency intensity and development could be related to modifications of different SP. Soares and co-workers (Soares et al. 2011) who studied the astringency of red wine supplemented with oligomeric procyanidins either by *in vitro* experiments (interaction with saliva) as by *in vivo* analysis (sensorial evaluation), showed that for low concentration of tannins the decrease of aPRPs and statherin is correlated with astringency intensity, with these proteins having a high relative complexation and precipitation towards condensed tannins comparatively to the other SP. However, for higher concentrations of tannins the relative astringency between wines seems to correlate to gPRPs changes. This work showed for the first time that the several families of SP could be involved in different stages of the astringency development.

The relation between astringency and tannin/protein interaction has also been studied using model proteins instead of SP. Also in these cases, it was shown a correlation between tannin/protein precipitation and perceived astringency. Llaudy and collaborators (Llaudy et al. 2004) have shown that the astringency evaluated by a sensory panel was correlated to the precipitation of gelatin and ovalbumin. Also, Kennedy and co-workers (Kennedy et al. 2006), who assessed this relationship in 40 red wines using various analytical methods, showed that protein precipitation was the method with the best correlation with astringency.

Although most of the literature supports this correlation, there are also mixed reports showing that some astringent molecules are unable to precipitate different proteins. Rossetti and collaborators (Rossetti et al. 2008) observed that (-)-epicatechin was unable to precipitate proteins of parotid saliva, despite being tested at the concentration that is perceived to be just as astringent as epigallocatechin gallate (EGCG). Regarding (-)-epicatechin it is important to refer that is not very soluble so the tested concentrations could not be very high. Also, Schwarz and Hofmann (Schwarz and Hofmann 2008) who compared the human sensory threshold concentrations and the salivary protein binding activity of a series of astringent polyphenol stimuli, revealed evidences that the quantity of the non-bound "free" astringent stimulus in the saliva liquid might be more closely related to the sensory perception of astringency than the amount complexed or precipitated by proteins. They observed significant protein binding for EGCG, GCG, GC and CG with high sensory thresholds of more than 200 µmol/L, while other compounds (N- and O-glycosides) did not show any protein binding activity, despite exhibiting extraordinarily low sensory threshold concentration (*e.g.*, less than 0.001 and 0.0003 µmol/L).

The formation of soluble aggregates between hydrolysable tannins and gelatin *in vitro* has also been shown to correlate to an astringent sensation *in vivo*, suggesting that precipitation is not necessary to induce an astringent sensation (Obreque-Slier et al. 2010). Overall, this work supports the hypothesis that astringency correlates better with tannin/protein interaction than with tannin/protein precipitation.

Astringency is such a complex sensation that there are evidences that its mechanism could differ among classes of astringents, which complicates the study of this sensation. Lee and co-workers (Lee and Vickers 2012) studied the effect of different astringents (tannins, alum and hydrochloric acid) on SP precipitation and observed that tannins and alum (a sensory standard for astringency) induced mainly the precipitation of PRPs but hydrochloric acid did not precipitate these proteins. However, alum also appeared to have an affinity for many of the higher molecular weight proteins, such as mucins, while tannins did not.

The influence of tannins structure on astringency has also been examined through many studies (Noble 1990, Brossaud et al. 2001, Vidal et al. 2003). A recent work done by Sun and collaborators (Sun et al. 2013) showed that polymeric procyanidins (mean dp of 25.2) were more astringent than oligomeric procyanidins (mean dp of 7.2). The fact that increasing the dp of proanthocyanidins increase astringency has been supported by other works (de Freitas and Mateus 2002, Vidal et al. 2003). Regarding the effect of the galloylation degree there are some mixed reports. In general, galloyl groups are thought to increase astringency. The work performed by Yu and co-workers (Yu et al. 2014) which studied the astringency of green tea by reconstruction/omission experiences by sensory evaluation showed evidences that EGCG is the main contributor to astringency in green tea. Indeed, also Scharbert and co-workers (Scharbert et

al. 2004) found that EGCG has a lower astringency threshold than the other species of catechins. The same authors also found that (−)-catechin gallate has a significant contribution to astringency. However, despite this effect of galloyl groups, gallic acid by itself has been found to not contribute significantly to astringency. Oppositely, Vidal and co-workers observed that an increase in galloylation was responsible for a decreasing coarse perception of the proanthocyanidins.

Other authors (Quijada-Morín et al. 2012) observed that astringency was more affected by the subunit composition of procyanidins than by the total concentration or the average degree of polymerization. Higher proportions of epicatechin subunits in extension position and gallocatechin subunits in terminal positions were shown to increase astringency. Oppositely, the amount of EGC in both extension and terminal positions was negatively correlated with astringency.

Regarding more other classes of polyphenols such as anthocyanins there is a lack of information. Vidal and collaborators (Vidal et al. 2004) showed that free anthocyanins, like the coloured tannin-like polyphenolic compounds from wine and pomace, do not contribute to wine astringency.

Besides tannins structure, individual variability and sensitivity also affects extremely astringent perception. Saliva viscosity, flow rate and proteic composition vary between individuals and the two latter have been shown to have a significant effect on perceived astringency. Higher concentrations of particular saliva proteins and lower flow rate of saliva have been shown to generally increase the sensation of astringency. Dinella and co-workers (Dinnella et al. 2010) studied the individual responses to astringency and individual salivary protein characteristics.

They divided subjects in 3 groups: low, medium and high responding subjects according to variations in protein concentration and haze-forming capacity. These authors observed that high responsive subjects had a strong decrease in all PRP, while low responding subjects had no decrease. In another work of the same group (Condelli et al. 2006) it was observed that astringency intensity perception was inversely related to the saliva flow rate.

As referred throughout this review, several research groups studied the molecular basis of astringency using bioassays models with pure/isolated PRPs (or similar proteins) and tannins. However, few works have used whole saliva to study the onset of astringency.

Despite the numerous works done on this subject, there is scarce experimental evidence about the relative affinity of different families of SP for tannins, in a competitive/associative medium (like whole saliva). In fact, the first studies that analyzed the interaction of different SP present in simultaneous (saliva) with polyphenols were done by Kallithraka and co-workers (Kallithraka et al. 1998). They analyzed human saliva before and after the interaction with polyphenols ((+)-catechin, (-)-epicatechin, procyanidin B2, or procyanidin C1) by HPLC. However, the SP involved in the interaction were not identified.

The work done by Soares and co-workers (Soares et al. 2011) brought important insights in this area. These authors developed a chromatographic and proteomic approach (HPLC-DAD, ESI-MS, SDS-PAGE, and MALDI-TOF-MS) adapted to study the affinity of different families of SP present in saliva with grape seed procyanidins. First, the SP present in human saliva were identified and then they studied their interaction with procyanidins. The results showed that condensed tannins interact and precipitate firstly with aPRPs and statherin and only then with

histatins, gPRPs and finally bPRPs. These results are opposite to what was expected since bPRPs have been referred as the most reactive PRPs toward tannins.

In summary, the major literature supports that tannin/protein interaction and/or precipitation is likely to be a great contributing factor to the development of astringency, but some other mechanisms could also be present.

TANNIN/PROTEIN INTERACTIONS

Several works suggest that tannin/protein interactions are governed by hydrophobic interactions and the formed complexes are then stabilized by hydrogen bonds. These complexes could be soluble or insoluble. Reversible tannin/protein interactions lead to the formation of soluble complexes in solution via non-covalent forces. Both aromatic nuclei and hydroxyl groups of the aromatic ring of phenolic compounds provide the main binding sites for tannin/protein interaction. Murray and colleagues used NMR to study this interaction and based on their results these authors suggest stacking between the phenolic rings of the proanthocyanidins and proline residues with hydrogen bonding between a galloyl ring and the pyrrolidine ring face containing the Cα proton.

Regarding the influence of proteins on this interaction, one important feature is the primary structure of the protein. Wroblewski and collaborators (Wroblewski et al. 2001) observed by NMR that the binding of tannins to a modified histatin (*i.e.*, histatin with the same residues but disposed randomized) was significantly reduced when compared to the original histatin.

It is well supported that tightly coiled globular proteins (such as amylase and albumins) show less affinity for tannins when compared to proteins that have a loose conformation (de Freitas

and Mateus 2001). Moreover, proteins with high affinity for tannins have relatively high molecular weight.

It is also well recognized that proteins rich in proline residues (such as PRPs) have significant affinity for tannins. In PRPs, the main binding sites are the ones with a sequence of proline residues, being the first residue of a sequence Pro-Pro the preferred one. According to this idea, Canon and co-workers (Canon et al. 2013) have recently shown that for IB-5 interaction with EGCG, the number of binding sites is n = 8, which matches the number of short proline repeats on the polypeptide chain. Also, Cala and colleagues (Cala et al. 2012) found that the binding sites related to specific association are located in the same hydrophilic domains involving proline residues.

Despite this, there are evidences showing that it is not possible to occur any interaction between tannins and proline residues if the stereochemical environment of the surrounded residues is not suitable for the interaction (Richard et al. 2005). So, it seems that proline residues besides their ability to interact with tannins induce more open conformations to the proteins leading to an exposure of other residues that could also bind to tannins, in comparison to other globular and compact proteins.

It is important to refer that the work done by Hagerman and Butler showed that proline and similar amino acids presented comparable affinities for tannins as other non-cyclic amino acids (such as glycine or alanine) (Hagerman and Butler 1981). This could mean that the high affinity of tannins for PRPs is not to the heterocyclic ring of proline but is more related to the structural open and flexible conformation of proteins induced by the presence of proline residues.

Glycosilated proteins also have affinity and selectivity towards tannins. It has been suggested that the oligosaccharide moiety of these proteins has the ability to maintain the protein structure in a relatively open conformation and is also responsible for the kinetic partitioning between folding and aggregation. Sarni-Machado and co-workers (Sarni-Manchado et al. 2008) have demonstrated that for low tannin concentration, tannin/glycosylated PRP interactions led to complexes that are more soluble than the counterpart non-glycosylated PRP that formed insoluble complexes. These authors suggested that, at low concentration of tannins, the precipitation could be inhibited by the low hydrophobicity as a result of glycosylation. At high tannin concentration, precipitation may be enhanced by the increased hydrophobicity as a result of increased binding of tannins. So the interaction with tannins reduced the apparent stabilizing effect of glycosylation. Also, Soares and co-workers (Soares et al. 2011) showed that glycosylated PRPs have affinity to interact with tannins.

Regarding tannins, the factors that influence their interaction with proteins are similar to the referred before for astringency. Several structural features of tannins influence their ability to interact with proteins namely molecular weight, stereochemistry, interflavanic bond, projection of hydroxyl groups and presence of galloyl groups (Ricardo-da-Silva et al. 1991, Kawamoto et al. 1995, Baxter et al. 1997, de Freitas and Mateus 2001, Soares et al. 2007, Canon et al. 2010).

In general, the binding affinity increases with the molecular weight of tannin compounds (Sarni-Manchado et al. 1999, Sun et al. 2013). The presence of galloyl groups is also a critical feature for the tannins ability to bind to proteins, with higher number of these groups leading to higher interaction. The position of these groups is an important factor even though the number is the more determinant one. De Freitas and collaborators (de Freitas and Mateus 2001) observed that

esterification of a galloyl group to the C(3) hydroxyl function of (-)-epicatechin or to the epicatechin moiety of procyanidin dimer B2 increased binding to proteins. However, this was not a strong effect for the dimer, probably as a result of the expected "closed" structure of B2-3'-*O*-gallate. Also, Kawamoto and co-workers (Kawamoto et al. 1995) who studied the interaction between a galloylglucose molecule and bovine serum albumin (BSA), observed that the position of the galloyl group in the glucose core also affects the relative affinity but the effect is smaller than that of the number. The relative affinity toward BSA was penta> tetra- > tri- ,di-, monogalloylglucose molecule.

Regarding the interflavanoid bond, one work that studied this aspect showed that procyanidin dimers linked through a C(4)-C(8) bond had consistently greater interaction with proteins than their counterparts with a C(4)-C(6) linkage (de Freitas and Mateus 2001).

According to several authors (Bacon and Rhodes 1998, Soares et al. 2007, Obreque-Slier et al. 2010), hydrolysable tannins are more reactive toward proteins than condensed tannins being influenced in a similar way by the structural features referred previously.

The model currently accepted for tannin/protein interactions was proposed by Jobstl and collaborators (Jobstl et al. 2004) in which the interaction occurs by three stages (Figure 2): (i) the simultaneous binding of the multidentate polyphenols to several sites on the free protein whose structure evolves from a loose and extended conformation to a more compact one; (ii) as the polyphenol concentration increases, polyphenols cross-link different protein molecules leading to dimerization; and at last (iii) aggregation into larger particles that finally precipitate.

This model of aggregation has been supported by several works along the years. The work done by Cala and co-workers (Cala et al. 2012) confirmed the first stage of the recognition process

between a PRP and tannin in which a structural rearrangement of the peptide occurs. They studied the interaction between a fragment of PRP IB-9 and procyanidins B1, B3 and C2 and observed that IB-9 changes its conformation when it interacts with procyanidins, surrounding up to three procyanidins when its length is sufficient. Also, Canon and colleagues (Canon et al. 2011) observed a transition from an extended form of IB5 to a more compact structure as the number of tannins bound to the protein increased.

The second and third stages of the model are also sustained by some works (Cala et al. 2012, Canon et al. 2013). In these works the authors observed that after specific association, and above tannin critical micellar concentration (around 10 mM for procyanidin dimers or trimers), nonspecific interactions are predominant. So, procyanidin molecules first attach the peptide as a whole in the specific hydrophilic sites of the peptide and thereafter develop interactions randomly with the hydrophobic part of the peptide. In this case, procyanidins can bridge two or more different peptides leading to the formation of a network that further precipitates.

Besides the influence of protein and tannin structure, tannin/protein interaction is affected by several other factors, such as temperature, pH of the solution, ionic strength and presence of carbohydrates.

EFFECT OF CARBOHYDRATES ON ASTRINGENCY AND TANNIN/PROTEIN INTERACTIONS

The first report of the inhibitory effect of carbohydrates in tannin/protein interactions was made several years ago by Haslam and colleagues and concerned the proposed mechanisms for the astringency loss during fruit ripening: as the cellular structure softens during fruit ripening, there is an increase in water-soluble pectin fragments that could prevent the formation of aggregates

between fruit tannins and salivary proteins in the mouth, leading to a modified astringency response (Ozawa et al. 1987). After these initial ideas, several studies showed that the astringency of tannins, as well as their interaction with proteins, is in fact reduced by the addition of carbohydrates (Luck et al. 1994, Haslam 1998, Mateus et al. 2004, Quijada-Morín et al. 2014). Carbohydrates are frequently used in food industry as food colloids (gums) and are also naturally present in several food products, thereby affecting their astringent sensation. Therefore, this effect of carbohydrates in protein/tannin interaction has a great impact in the perception and choice of foodstuffs.

Despite the knowledge that carbohydrates inhibit protein/tannin interactions, the study of this inhibition is still intricate because it includes three complex variables (ternary system). In addition, carbohydrates are very complex and large molecules and for most of them there are no defined structure and/or molecular weight. So, most of the literature on this subject consists mainly on studies involving model carbohydrates such as pectin, cyclodextrin, xanthan, arabic gum, as well as model proteins (bovine serum albumin (BSA), α-amylase and gelatin) (Luck et al. 1994, de Freitas et al. 2003, Carvalho et al. 2006, Soares et al. 2009).

Two mechanisms have been proposed to explain this inhibitory effect of carbohydrates: (I) carbohydrates form ternary complex protein/polyphenol/carbohydrates, which enhances solubility in an aqueous medium; (II) there is a molecular association in solution between carbohydrates and polyphenols hence competing for protein aggregation.

These two mechanisms have been supported by different works. The results obtained by Gaffney and co-workers (Gaffney et al. 1986) support the competition mechanism. These authors studied the interaction between β-cyclodextrin with methylgallate and trigalloylglucose (polyphenols) in

the presence and absence of caffeine (used as a model of proteins) by microcalorimetry and NMR. The results showed that cyclodextrin was able to develop in aqueous media a secondary structure containing hydrophobic cavities that can scavenge the hydrophobic rings of the polyphenolic substrate. This strongly supports the view that the inhibitory effect of this carbohydrate is via encapsulation of the 'phenolic end' of the galloyl ester groups.

The ability of cyclodextrin to encapsulate polyphenols has been observed by other authors (Jullian et al. 2007, Fernandes et al. 2014).

There were also some evidences that cyclodextrin as well as arabic gum exert their effect by the competition mechanism (Soares et al. 2009). The authors of that work used fluorescence quenching, nephelometry and dynamic light scattering (DLS) techniques to study the effect of different carbohydrates in the aggregation of α-amylase with procyanidin fractions. They observed that arabic gum and β-cyclodextrin reduced the quenching effect of procyanidin fractions on amylase fluorescence. These carbohydrates inhibit protein/polyphenol aggregation by association with polyphenols, competing with protein aggregation. They also reduce the size and the formation of insoluble aggregates.

Regarding the mechanism of ternary complex formation, the same authors also had evidences that pectin exerts its effect in this way. Oppositely to arabic gum and β-cyclodextrin, pectins do not affect the quenching of amylase fluorescence by procyanidins fractions. This suggests that procyanidins still interact with protein although the size and turbidity of the solution decreases. Also, Luck and co-workers (Luck et al. 1994) have shown that soluble pectins, galactomannans and carrageenans inhibit the PGG precipitation by proline-rich gelatine and sodium caseinate.

When considering this inhibitory effect of carbohydrates on the interaction of tannins with salivary proteins, there is still little information. There is a lack of information in which way the different SP and carbohydrates affect the inhibition. One work was performed and focused on the effect of different carbohydrates arabic gum (AG), pectin and polygalacturonic acid (PGA) on the interaction between SP and a grape seed fraction (condensed tannins) by HPLC and DLS (Soares et al. 2012). The results showed that pectin was the most efficient in inhibiting tannin/protein precipitation, followed by AG and PGA. The results also suggested that pectin and PGA exert their effect by formation of a ternary complex protein/polyphenol/carbohydrate, while AG competes with proteins for tannin binding (competition mechanism).

It seems that carbohydrates action on tannin/protein interaction is also governed by hydrophilic and hydrophobic interactions and as described for tannin/protein interaction the structure and size of the compounds involved are very important (de Freitas et al. 2003, Quijada-Morín et al. 2014).

Ionic carbohydrates such as pectin, xanthan, polygalacturonic acid and gum arabic have shown to be more effective in disrupting the aggregation between BSA and procyanidins than neutral sugars such as β-cyclodextrin, arabinogalactan, dextran and glucose.

Wines also have carbohydrates and the major ones are arabinogalactan proteins (AGP) and rhamnogalacturonan II (RGII), pectic polysaccharides that originate from grape cell walls and mannoproteins (MP) that are produced by yeast during fermentation (Doco et al. 2000). AGP and MP are neutral carbohydrates while RGII have acidic character. These carbohydrates also influence the perceived astringency of wines but their effect is also associated to their structure and chemical properties in particular to their ionic character. It was reported by sensory studies

that RGII significantly decrease the attributes associated with the astringency of a wine model solution in absence and presence of procyanidins, while the neutral wine polysaccharide fraction containing AGP and MP had less effect on reducing the ratings for these attributes (Vidal et al. 2004, Vidal et al. 2004). However, Carvalho and collaborators (Carvalho et al. 2006) observed in *in vitro* experiments a different trend for these wine polysaccharides. These authors studied the effect of these polysaccharides on the interaction between condensed tannins and salivary proteins (α-amylase and IB8c, a PRP) by light scattering and they observed that the effect of RGII depend on the protein: it was relatively effective in preventing aggregate formation between α-amylase and tannin but favored the formation of aggregates between IB8c and tannin. Regarding AGP the most effective in the inhibition of aggregate formation between both proteins and condensed tannins is the one with the stronger ionic character. In fact, the most neutral fraction of AGP seemed to have the reverse effect on the aggregation between protein and tannin increasing the aggregates. This effect is relatively small on the case of α -amylase and very evident in the case of IB8c.

SALIVA AND SALIVARY PEPTIDES (SP)

Whole saliva represents a mixture of the secretions of the major (submandibular, sublingual, and parotid) and minor salivary glands, together with the crevicular fluid, bacteria, and cellular debris. The secretions from the different glands have been shown to differ considerably and to be affected by day time, diet, age, gender, several disease states, and pharmacological agents.

Percentage contributions of the different salivary glands during unstimulated flow are estimated to be: 20% from parotid, 65% from submandibular, 7% to 8% from sublingual, and less than

10% from numerous minor glands (Edgar 1990). Different forms of stimulation, eating or drinking, also cause the different salivary glands to become activated to different extents (Edgar 1990). For example, the parotid gland increases its proportion to the total whole saliva to more than 50% during stimulation, and taste also has been shown to lead to a greater level of stimulation than chewing.

In general, saliva is composed of electrolytes including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates, small organic compounds, immunoglobulins, enzymes, proteins (namely amylase and seric albumin) and peptides.

With respect to the SP, some of the most abundant peptides have been grouped into four structurally related major classes namely histatins, PRPs, statherin and cystatins. PRPs are one of the dominant classes of SP and are usually further divided in three families according to their acidic/basic characteristics: basic PRPs (bPRPs) have mainly basic residues, acidic PRPs (aPRPs) have the first 30 residues composed mainly by aspartic and glutamic acid and glycosylated PRPs (gPRPs) are bPRPs that have carbohydrates in their structure.

As their name evidences and has been referred previously, PRP are proteins rich in proline residues that are presently called intrinsically unstructured proteins, proteins with a randomly coiled structure. Three amino acids account for 70−80% of the total residues of these proteins (approximately 40% proline, 21% glycine, and 17% glutamine residues) and they are organized in repeated domains (3 to 5, depending on the considered PRP) (Bennick 1982). These proteins lack of any stable tertiary structure.

Most of all SP have well defined important biological functions in saliva. The role of aPRPs is linked to the dental mineralization process providing a protective and reparative environment for

dental enamel and may be also relevant modulators of bacterial colonization of the teeth (Castagnola et al. 2011). gPRPs are associated to lubrication of oral cavity and for bPRPs it has been proposed that one of their functions is to bind tannins, preventing their toxic effects in the gastro-intestinal tract .

Regarding histatins they are secreted by the parotid, submandibular and sublingual glands. They are a family of small (3-4 kDa), histidine-rich peptides making these peptides slightly basic. In contrast to PRPs, they contain no proline residues except for a single residue in histatin 1. These peptides have a defensive role against salivary pathogens, antifungal activity and are important for mineralization and wound-healing (Oppenheim et al. 1988, Troxler et al. 1990).

Statherin is secreted by the parotid gland, being abundant in tyrosine residues and phosphorylated at two residues (Ser2 and Ser3). This peptide shows great affinity for calcium phosphate minerals, contributing to the stabilization of these minerals. In fact, statherin is able to inhibit precipitation and crystal growth of hydroxyapatite from supersaturated solutions of calcium phosphate and serves as a lubricant to protect the tooth (Douglas et al. 1991).

BITTERNESS AND BITTER COMPOUNDS

Bitterness is one of the five basic tastes. Its recognition and aversion to it are thought to protect the organism against the ingestion of poisonous food compounds, which are often bitter.

This taste is perceived by activation of the human bitter taste receptors, TAS2Rs, encoded by the TASTE 2 Receptor (TAS2R) gene family that codes for ~25 taste receptors (TAS2Rs) in humans, which are G protein-coupled receptors. The TAS2R are expressed in a specific subset of

taste receptor cells (TRC) localized in the oral cavity in taste buds that are embedded in the epithelium of the gustatory papillae on the tongue and palate.

TAS2Rs are proteins with 290-330 amino acid residues and exhibit high similarity in their primary structure suggesting that different receptors could recognize compounds structurally diverse. Actually, it is necessary that this happens because humans have only 25 TAS2Rs for the detection of hundreds of bitter compounds that are not only numerous as highly structural diverse, including peptides, amino acids, lactones and phenols, among others. Apparently, individual TAS2Rs are activated by a battery of both structurally related and unrelated bitter compounds, while preserving selectivity for chemical groups and even stereo-selectivity.

In order to facilitate studying the chemical features associated with bitterness, presently there is a bitter database (BitterDB). BitterDB currently contains more than 550 compounds that were cited in the literature as bitter, offering information regarding their molecular properties, different compound identifiers (e.g., CAS registry number, IUPAC systematic name), and indication of compound source, among other information.

An extremely interesting feature of these receptors is that there are recent reports about expression of TAS2Rs in non-gustatory tissues, suggesting that these proteins may have additional functions apart from detection of taste (Behrens and Meyerhof 2011). Of the extraoral tissues expressing TAS2Rs, the gastrointestinal tract has received much attention since mounting evidence indicates that after being ingested, bitter tastants might play important regulatory roles in digestive and metabolic processes. TAS2Rs were also found in human airway smooth muscle tissue and the effect of bitter tastants on function of human bronchi is currently being studied (Morice et al. 2011).

An important aspect regarding bitterness is the individualized perception of different bitter compounds, that is, humans vary greatly with regard to bitterness perception of some bitter compounds. This remarkable individuality has been associated to single-nucleotide polymorphisms of the 25 TAS2Rs that lead mainly to altered amino acid sequences (haplotypes) (Drayna 2005, Kim et al. 2005). This might affect dietary habits profoundly and ultimately lead to consequences for an individual's health.

Although bitterness is usually an unpleasant taste, in the case of some beverages such as red wine and beer, it is a necessary attribute when present with a moderate intensity. This taste is usually assessed by sensory analysis, being thought and supported by the literature that in the case of red wine it is mainly induced by polyphenol compounds (*e.g.* tannins).

However, the limited available data regarding polyphenol's structure/bitterness relationship are rather inconsistent. In general, the few works that studied the bitterness of polyphenol compounds, such as polymeric fractions of tannic acid and tannins, as well as flavan-3-ol monomers, dimers, and trimers, demonstrated that larger molecules tend to be less bitter and more astringent. Peleg and co-workers (Peleg et al. 1999) found that (-)-epicatechin was more bitter than the stereoisomer (+)-catechin and that these both were more bitter than the procyanidin trimers, catechin-(4-8)-catechin-(4-8)-catechin and catechin-(4-8)-catechin-(4-8)-epicatechin. Robichaud and colleagues (Robichaud and Noble 1990) found that tannic acid, commercial hydrolysable pentagalloylglucose-rich tannin, was more bitter than both (+)-catechin and a grape seed extract rich in polymeric procyanidins. However, other reports have shown that bitterness of polyphenols increases with molecular weight. Hufnagel and Hofmann (Hufnagel and Hofmann 2008) found that procyanidin dimers and a trimer were more bitter than (-)-

epicatechin. These authors also demonstrated by taste reconstruction and omission experiments that the bitterness of red wine could be induced by subthreshold concentrations of phenolic acid ethyl esters and flavan-3-ols.

Besides these inconsistencies, there is also insufficient knowledge about the bitter taste of polyphenol compounds that do not belong to the tannin class but that are widely present in nature, such as anthocyanins.

In an attempt to better understand structure/bitterness relationship and to overcome the inconsistencies of sensory panels, the TAS2Rs activated by various polyphenol compounds were identified in a recent work (Soares et al. 2013). These authors used heterologous expression of TAS2R to successfully measure TAS2R activation by purified bitter chemicals that are normally present in food and beverage sources such as red wine, beer hops, cheese, and soy products. These kinds of studies are actually blooming in the bitterness research field (Pronin et al. 2004, Kohl et al. 2013, Ji et al. 2014).

The six polyphenol compounds tested by these authors [(-)-epicatechin, procyanidin dimer and trimer, malvidin-3-glucoside, cyanidin-3-glucoside and PGG] were administered, at the highest possible concentrations, by bath application to the 25 different receptor-expressing cell populations. In these experiments it was shown that receptors TAS2R4, TAS2R5 and TAS2R39 responded to (-)-epicatechin. Receptors TAS2R5 and TAS2R39 were also sensitive to PGG and TAS2R5 was also activated by procyanidin trimer. Finally, it was found that malvidin-3-glucoside elicited signals specifically in cells transfected with DNA for TAS2R7.

The agonist-receptor pairs display an interesting activation pattern. Firstly, different compounds activate the same receptor. For example (-)-epicatechin and PGG activate TAS2R39 whereas (-)-

epicatechin, PGG and procyanidin trimer activate TAS2R5. Secondly, different receptors are activated by the same compound. This is evidenced by (-)-epicatechin which activates TAS2R4, TAS2R5 and TAS2R39 and PGG which stimulates TAS2R5 and TAS2R39. These results agree with the combinatorial activation patterns of TAS2Rs previously reported. Furthermore, due to the structural diversity of the tested compounds, these data have also implications for structure-activity relationships. They suggest that the catechol and/or galloyl group (which has only one more hydroxyl group than catechol) are critical features (although not essential) for the interaction of polyphenol compounds with TAS2R5. Firstly, the compounds that activated this receptor, (-)-epicatechin, procyanidin trimer and PGG have at least one of these groups while the other tested compound, malvidin-3-glucoside, did not. Secondly, procyanidin trimer and PGG that possess three ortho-catechol groups and five galloyl groups, respectively, activate TAS2R5 at 100-fold lower concentrations than does (-)-epicatechin which has only one ortho-catechol group. However, it is evident that the presence of these groups is not sufficient for the responsiveness of this receptor since procyanidin dimer (which has two ortho-catechol groups) does not activate this receptor. Nonetheless, it seems that, when a compound activates TAS2R5, the presence of these groups could be essential for higher responses/interaction. The importance of the hydroxyl groups and other structural elements in bitter receptor activation is also referred by Roland and co-workers (Roland et al. 2011), who have studied the activation of receptor TAS2R39 by the soy (iso)flavonoids. In a previous work these authors concluded that the presence of three hydroxyl groups in the isoflavonoid ring system, which resembles that of the polyphenols, seemed to be more favorable for TAS2R39 activation than the presence of fewer hydroxyl groups. In a more recent work and with a wide range library of compounds (Roland et

al. 2013), the same authors observed that small structural changes had different effects on receptor activation. The structural characteristics for an (iso)flavonoid to activate TAS2R39 (and/or TAS2R4) were determined to be composed of two (or three) hydrogen bond donor sites, one hydrogen bond acceptor site, one hydrophobic ring structure, and one aromatic ring structure.

Notably, the above chemicals were the first natural bitter compounds found for TAS2R5, as this specific receptor responded so far only to the synthetic compound phenantroline, and may therefore be the only TAS2R that is activated ("specific") by natural tannins.

Another structure-activity highlight observed concerns the presence of glucose residues, which seems to be important for TAS2R7. Whereas malvidin-3-glucoside activated TAS2R7, its aglycon form (without glucose residue) failed to do so, at least at the tested concentrations.

Although this result for malvidin-3-glucoside opposes those of a sensory study that showed that this anthocyanin does not taste bitter, these two data sets appear not to be comparable since they have distinct experimental conditions (namely pH and solution composition) that could influence the perceived bitterness and receptor responses differently. The sensory study evaluated the bitterness of malvidin-3-glucoside in a mixture with cyanidin-3-glucoside made in a solution saturated with tartaric acid (pH 3.6) and containing ethanol. It is also important to mention that anthocyanins, co-exist in solution in different forms because of their well-known pH dependence; at low pH (1-2) they are essentially present in the red cationic form (AH+), but as the pH increases rapid proton transfer reactions occur leading to the formation of blue quinonoidal bases (A and A-) and chalcones.

The data demonstrate that the half maximal effective concentrations (EC_{50}) values obtained vary from the µM to the mM range. While the EC_{50} values for PGG at both TAS2R5 and TAS2R39 are ≥ 8.5 and ≥ 6.6 µM, respectively, those for (-)-epicatechin are ~1000 fold higher and vary between 3.2 and 3.8 mM. Like the EC_{50} values for PGG, those for malvidin-3-glucoside and procyanidin trimer are also in the micromolar range, being 12.6 µM at TAS2R7 and 35.6 µM at TAS2R5, respectively.

Besides these pronounced differences in the EC_{50} values, the observed threshold concentrations, defined as the lowest concentration that resulted in receptors activation, are also largely different. Whereas PGG induced robust receptor responses already at a concentration of 3.0 µM at both TAS2R5 and TAS2R39, a 300-1000 fold higher concentration of (-)-epicatechin was required to evoke receptor responses at TAS2R4, TAS2R5, and TAS2R39. For malvidin-3-glucoside, the lowest concentration that induced detectable receptor responses were 6.0 µM at TAS2R7 and for procyanidin trimer 30.0 µM at TAS2R5.

Overall, the EC_{50} values obtained for the different compounds vary 100-fold with the lowest values for PGG and malvidin-3-glucoside compounds suggesting that they could be significant polyphenols responsible for the bitterness of fruits, vegetables and derived products even if they are present at very low concentrations.

CONCLUDING REMARKS AND GUIDELINES FOR FUTURE WORKS

Over the past years, there has been an increasing concern about eating well, about consumption of foodstuffs rich or supplemented with natural antioxidants in particular polyphenols, all in favor of a healthy ageing and natural chemoprevention of important diseases such as cancer, Parkinson and Alzheimer's. In fact, polyphenols have been referred as nutraceuticals of modern life.

However, associated to the ingestion of polyphenols are the reviewed sensorial properties which are not always pleasant. In this way, the study of polyphenols' astringency and bitterness is extremely important for the food industry especially for wine and fruit juice producers. This review intends to be an overview both at a sensory as a molecular level of the mechanisms underlying astringency and bitterness of polyphenols. As referred previously, astringency is a very complex sensation and although most of the literature supports that tannin/salivary protein interaction and/or precipitation is likely to be a great contributing factor to its development, some other mechanisms could also be present. So, future works are expected to rely on studying the contribution of the different proposed mechanisms to the overall astringency. As major guidelines for future works it is expected (I) to study the contribution of each family of SP to the overall astringency and/or to the different subqualities of astringency; (II) to study the contribution of mechanoreceptors and/or identify which mechanoreceptors are involved in astringency perception; (III) to study the contribution of oral cells in astringency perception; (IV) in which way the presence of food carbohydrates influence the previous topics.

Oppositely to astringency, the origin and perception of bitterness taste is well understood. However, the structure/bitterness pattern of polyphenols and of other bitter compounds is not

well known as well as the influence of other compounds in polyphenols bitter taste. Also, the differences on bitterness perception between different individuals require further studies.

Therefore, although important and numerous research has been done in the past years in polyphenols astringency and bitterness sensory attributes and significant knowledge has been acquired, there are still many questions to be answered on this matter.

References

Arts, M. J. T. J., Haenen, G. R. M. M., Wilms, L. C., Beetstra, S. a. J. N., Heijnen, C. G. M., Voss, H.-P. and Bast, A. (2002). Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. *J. Agric. Food Chem.* **50**: 1184-1187.

Bacon, J. R. and Rhodes, M. J. C. (1998). Development of a competition assay for the evaluation of the binding of human parotid salivary proteins to dietary complex phenols and tannins using a peroxidase-labeled tannin. *J. Agric. Food Chem.* **46**: 5083-5088.

Bate-Smith, E. C. (1954). Astringency in foods. *Food* **23**: 124.

Baxter, N. J., Lilley, T. H., Haslam, E. and Williamson, M. P. (1997). Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry* **36**: 5566-5577.

Behrens, M. and Meyerhof, W. (2011). Gustatory and extragustatory functions of mammalian taste receptors. *Physiol. Behav.* **105**: 4-13.

Bennick, A. (1982). Salivary proline-rich proteins. *Mol. Cell. Biochem.* **45**: 83-99.

Boselli, E., Boulton, R. B., Thorngate, J. H. and Frega, N. G. (2004). Chemical and sensory characterization of DOC red wines from marche (Italy) related to vintage and grape cultivars. *J. Agric. Food Chem.* **52**: 3843-3854.

Brossaud, F., Cheynier, V. and Noble, A. C. (2001). Bitterness and astringency of grape and wine polyphenols. *Aust. J. Grape Wine R.* **7**: 33-39.

Cala, O., Dufourc, E. J., Fouquet, E., Manigand, C., Laguerre, M. and Pianet, I. (2012). The colloidal state of tannins impacts the nature of their interaction with proteins: The case of salivary proline-rich protein/procyanidins binding. *Langmuir* **28**: 17410-17418.

Canon, F., Ballivian, R., Chirot, F., Antoine, R., Sarni-Manchado, P., Lemoine, J. R. M. and Dugourd, P. (2011). Folding of a salivary intrinsically disordered protein upon binding to tannins. *J. Am. Chem. Soc.* **133**: 7847-7852.

Canon, F., Giuliani, A., Paté, F. and Sarni-Manchado, P. (2010). Ability of a salivary intrinsically unstructured protein to bind different tannin targets revealed by mass spectrometry. *Anal. Bioanal. Chem.* **398**: 815-822.

Canon, F., Paté, F., Cheynier, V., Sarni-Manchado, P., Giuliani, A., Pérez, J., Durand, D., Li, J. and Cabane, B. (2013). Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EgCG. *Langmuir* **29**: 1926-1937.

Carvalho, E., Mateus, N., Plet, B., Pianet, I., Dufourc, E. and De Freitas, V. (2006). Influence of wine pectic polysaccharides on the interactions between condensed tannins and salivary proteins. *J. Agric. Food Chem.* **54**: 8936-8944.

Carvalho, E., Póvoas, M. J., Mateus, N. and De Freitas, V. (2006). Application of flow nephelometry to the analysis of the influence of carbohydrates on protein-tannin interactions. *J. Sci. Food Agric.* **86**: 891-896.

Castagnola, M., Cabras, T., Vitali, A., Sanna, M. T. and Messana, I. (2011). Biotechnological implications of the salivary proteome. *Trends Biotechnol.* **29**: 409-418.

Condelli, N., Dinnella, C., Cerone, A., Monteleone, E. and Bertuccioli, M. (2006). Prediction of perceived astringency induced by phenolic compounds II: Criteria for panel selection and preliminary application on wine samples. *Food Qual. Prefer.* **17**: 96-107.

De Freitas, V., Carvalho, E. and Mateus, N. (2003). Study of carbohydrate influence on protein-tannin aggregation by nephelometry. *Food Chem.* **81**: 503-509.

De Freitas, V. and Mateus, N. (2001). Structural features of procyanidin interactions with salivary proteins. *J. Agric. Food Chem.* **49**: 940-945.

De Freitas, V. and Mateus, N. (2002). Nephelometric study of salivary protein-tannin aggregates. *J. Sci. Food Agric.* **82**: 113-119.

De La Iglesia, R., Milagro, F. I., Campión, J., Boqué, N. and Martínez, J. A. (2010). Healthy properties of proanthocyanidins. *BioFactors* **36**: 159-168.

Diniz, A., Escuder-Gilabert, L., Lopes, N., Villanueva-Camañas, R., Sagrado, S. and Medina-Hernández, M. (2008). Characterization of interactions between polyphenolic compounds and human serum proteins by capillary electrophoresis. *Anal. Bioanal. Chem.* **391**: 625-632.

Dinnella, C., Recchia, A., Fia, G., Bertuccioli, M. and Monteleone, E. (2009). Saliva characteristics and individual sensitivity to phenolic astringent stimuli. *Chem. Senses* **34**: 295-304.

Dinnella, C., Recchia, A., Vincenzi, S., Tuorila, H. and Monteleone, E. (2010). Temporary modification of salivary protein profile and individual responses to repeated phenolic astringent stimuli. *Chem. Senses* **35**: 75-85.

Doco, T., Williams, P., Moutounet, M. and Pellerin, P. (2000). Les polysaccharides du vin. *Bull. OIV* **73**: 785-792.

Douglas, W. H., Reeh, E. S., Ramasubbu, N., Raj, P. A., Bhandary, K. K. and Levine, M. J. (1991). Statherin: A major boundary lubricant of human saliva. *Biochem. Biophys. Res. Commun.* **180**: 91-97.

Drayna, D. (2005). Human taste genetics. *Annu. Rev. Genomics Hum. Genet.* **6**: 217-235.

Edgar, W. M. (1990). Saliva and dental health. Clinical implications of saliva: Report of a consensus meeting. *Br. Dent. J.* **169**: 96-98.

Fernandes, A., Ivanova, G., Brás, N. F., Mateus, N., Ramos, M. J., Rangel, M. and De Freitas, V. (2014). Structural characterization of inclusion complexes between cyanidin-3-o-glucoside and β-cyclodextrin. *Carbohydr. Polym.* **102**: 269-277.

Fischer, U., Boulton, R. B. and Noble, A. C. (1994). Physiological factors contributing to the variability of sensory assessments: Relationship between salivary flow rate and temporal perception of gustatory stimuli. *Food Qual. Prefer.* **5**: 55-64.

Fulcrand, H., Dueñas, M., Salas, E. and Cheynier, V. (2006). Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.* **57**: 289-297.

Gaffney, S. H., Martin, R., Lilley, T. H., Haslam, E. and Magnolato, D. (1986). The association of polyphenols with caffeine and α- and β-cyclodextrin in aqueous media. *Chem. Commun.*: 107-109.

Gawel, R., Oberholster, A. and Francis, I. L. (2000). A 'mouth-feel wheel': Terminology for communicating the mouth-feel characteristics of red wine. *Aust. J. Grape Wine R.* **6**: 203-207.

Hagerman, A. E. and Butler, L. G. (1981). The specificity of proanthocyanidin-protein interactions. *J. Biol. Chem.* **256**: 4494-4497.

Haslam, E. C. (1998). Maturation - changes in astringency. **In**: Pratical polyphenolics: From structure to molecular recognition and physiological action. pp. Cambridge University Press

Hufnagel, J. C. and Hofmann, T. (2008). Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **56**: 9190-9199.

Ji, M., Su, X., Su, X., Chen, Y., Huang, W., Zhang, J., Gao, Z., Li, C. and Lu, X. (2014). Identification of novel compounds for human bitter taste receptors. *Chem. Biol. Drug Des.*

Jobstl, E., O'connell, J., Fairclough, J. P. A. and Williamson, M. P. (2004). Molecular model for astringency produced by polyphenol/protein interactions. *Biomacromolecules* **5**: 942-949.

Jullian, C., Miranda, S., Zapata-Torres, G., Mendizábal, F. and Olea-Azar, C. (2007). Studies of inclusion complexes of natural and modified cyclodextrin with (+)-catechin by NMR and molecular modeling. *Bioorg. Med. Chem.* **15**: 3217-3224.

Kallithraka, S., Bakker, J. and Clifford, M. N. (1998). Evidence that salivary proteins are involved in astringency. *J. Sens. Stud.* **13**: 29-43.

Kawamoto, H., Nakatsubo, F. and Murakami, K. (1995). Quantitative determination of tannin and protein in the precipitates by high-performance liquid-chromatography. *Phytochemistry* **40**: 1503-1505.

Kennedy, J. A. (2008). Grape and wine phenolics: Observations and recent findings. *Cien. Inv. Agr.* **35**: 107-120.

Kennedy, J. A., Ferrier, J., Harbertson, J. F. and Des Gachons, C. P. (2006). Analysis of tannins in red wine using multiple methods: Correlation with perceived astringency. *Am. J. Enol. Vitic.* **57**: 481-485.

Kennedy, J. A., Saucier, C. and Glories, Y. (2006). Grape and wine phenolics: History and perspective. *Am. J. Enol. Vitic.* **57**: 239-248.

Kim, U., Wooding, S., Ricci, D., Jorde, L. B. and Drayna, D. (2005). Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci. *Hum. Mutat.* **26**: 199-204.

Kohl, S., Behrens, M., Dunkel, A., Hofmann, T. and Meyerhof, W. (2013). Amino acids and peptides activate at least five members of the human bitter taste receptor family. *J. Agric. Food Chem.* **61**: 53-60.

Lee, C. A. and Vickers, Z. M. (2012). Astringency of foods may not be directly related to salivary lubricity. *J. Food Sci.* **77**: S302-S306.

Llaudy, M. C., Canals, R., Canals, J. M., Rozes, N., Arola, L. and Zamora, F. (2004). New method for evaluating astringency in red wine. *J. Agric. Food Chem.* **52**: 742-746.

Luck, G., Liao, H., Murray, N. J., Grimmer, H. R., Warminski, E. E., Williamson, M. P., Lilley, T. H. and Haslam, E. (1994). Polyphenols, astringency and proline-rich proteins. *Phytochemistry* **37**: 357-371.

Mateus, N., Carvalho, E., Luís, C. and De Freitas, V. (2004). Influence of the tannin structure on the disruption effect of carbohydrates on protein-tannin aggregates. *Anal. Chim. Acta* **513**: 135-140.

Mercurio, M. D., Dambergs, R. G., Cozzolino, D., Herderich, M. J. and Smith, P. A. (2010). Relationship between red wine grades and phenolics. 1. Tannin and total phenolics concentrations. *J. Agric. Food Chem.* **58**: 12313-12319.

Morice, A. H., Bennett, R. T., Chaudhry, M. A., Cowen, M. E., Griffin, S. C. and Loubani, M. (2011). Effect of bitter tastants on human bronchi. *Nat. Med.* **17**: 775.

Noble, A. C. (1990). Bitterness and astringency in wine. **In**: Bitterness in foods and beverages. pp. 145-158. Elsevier. Amsterdam.

Obreque-Slier, E., López-Solís, R., Peña-Neira, Á. and Zamora-Marín, F. (2010). Tannin–protein interaction is more closely associated with astringency than tannin–protein precipitation: Experience with two oenological tannins and a gelatin. *Int. J. Food Sci. Technol.* **45**: 2629-2636.

Oppenheim, F. G., Xu, T., Mcmillian, F. M., Levitz, S. M., Diamond, R. D., Offner, G. D. and Troxler, R. F. (1988). Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. *J. Biol. Chem.* **263**: 7472-7477.

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

Payne, C., Bowyer, P. K., Herderich, M. and Bastian, S. E. P. (2009). Interaction of astringent grape seed procyanidins with oral epithelial cells. *Food Chem.* **115**: 551-557.

Peleg, H., Gacon, K., Schlich, P. and Noble, A. C. (1999). Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric.* **79**: 1123-1128.

Prieur, C., Rigaud, J., Cheynier, V. and Moutounet, M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **36**: 781-784.

Pronin, A. N., Tang, H., Connor, J. and Keung, W. (2004). Identification of ligands for two human bitter T2R receptors. *Chem. Senses* **29**: 583-593.

Quijada-Morín, N., Regueiro, J., Simal-Gándara, J., Tomás, E., Rivas-Gonzalo, J. C. and Escribano-Bailón, M. T. (2012). Relationship between the sensory-determined astringency and the flavanolic composition of red wines. *J. Agric. Food Chem.* **60**: 12355-12361.

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

Ramassamy, C. (2006). Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: A review of their intracellular targets. *Eur. J. Pharmacol.* **545**: 51-64.

Renaud, S. and De Lorgeril, M. (1992). Wine, alcohol, platelets, and the french paradox for coronary heart disease. *The Lancet* **339**: 1523-1526.

Ricardo-Da-Silva, J. M., Cheynier, V., Souquet, J.-M., Moutounet, M., Cabanis, J.-C. and Bourzeix, M. (1991). Interaction of grape seed procyanidins with various proteins in relation to wine fining. *J. Sci. Food Agric.* **57**: 111-125.

Richard, T., Vitrac, X., Merillon, J. M. and Monti, J. P. (2005). Role of peptide primary sequence in polyphenol-protein recognition: An example with neurotensin. *Biochim. Biophys. Acta - General Subjects* **1726**: 238-243.

Rinaldi, A., Gambuti, A. and Moio, L. (2012). Application of the SPI (saliva precipitation index) to the evaluation of red wine astringency. *Food Chem.* **135**: 2498-2504.

Robichaud, J. L. and Noble, A. C. (1990). Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* **52**: 343-353.

Roland, W. S. U., Van Buren, L., Gruppen, H., Driesse, M., Gouka, R. J., Smit, G. and Vincken, J.-P. (2013). Bitter taste receptor activation by flavonoids and isoflavonoids: Modeled structural requirements for activation of hTAS2R14 and hTAS2R39. *J. Agric. Food Chem.* **61**: 10454-10466.

Roland, W. S. U., Vincken, J.-P., Gouka, R. J., Van Buren, L., Gruppen, H. and Smit, G. (2011). Soy isoflavones and other isoflavonoids activate the human bitter taste receptors hTAS2R14 and hTAS2R39. *J. Agric. Food Chem.* **59**: 11764-11771.

Rossetti, D., Bongaerts, J. H. H., Wantling, E., Stokes, J. R. and Williamson, A. M. (2009). Astringency of tea catechins: More than an oral lubrication tactile percept. *Food Hydrocolloids* **23**: 1984-1992.

Rossetti, D., Yakubov, G. E., Stokes, J. R., Williamson, A. M. and Fuller, G. G. (2008). Interaction of human whole saliva and astringent dietary compounds investigated by interfacial shear rheology. *Food Hydrocolloids* **22**: 1068-1078.

Sarneckis, C. J., Dambergs, R. G., Jones, P., Mercurio, M., Herderich, M. J. and Smith, P. A. (2006). Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. *Aust. J. Grape Wine R.* **12**: 39-49.

Sarni-Manchado, P., Canals-Bosch, J. M., Mazerolles, G. and Cheynier, V. (2008). Influence of the glycosylation of human salivary proline-rich proteins on their interactions with condensed tannins. *J. Agric. Food Chem.* **56**: 9563-9569.

Sarni-Manchado, P., Cheynier, V. and Moutounet, M. (1999). Interactions of grape seed tannins with salivary proteins. *J. Agric. Food Chem.* **47**: 42-47.

Scharbert, S., Holzmann, N. and Hofmann, T. (2004). Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *J. Agric. Food Chem.* **52**: 3498-3508.

Schwarz, B. and Hofmann, T. (2008). Is there a direct relationship between oral astringency and human salivary protein binding? *Eur. Food Res. Technol.* **227**: 1693–1698.

Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W. and De Freitas, V. (2013). Different phenolic compounds activate distinct human bitter taste receptors. *J. Agric. Food Chem.* **61**: 1525-1533.

Soares, S., Mateus, N. and De Freitas, V. (2007). Interaction of different polyphenols with bovine serum albumin (BSA) and human salivary α-amylase (HSA) by fluorescence quenching. *J. Agric. Food Chem.* **55**: 6726-6735.

Soares, S., Mateus, N. and De Freitas, V. (2012). Carbohydrates inhibit salivary proteins precipitation by condensed tannins. *J. Agric. Food Chem.* **60**: 3966-3972.

Soares, S., Sousa, A., Mateus, N. and De Freitas, V. (2011). Effect of condensed tannins addition on the astringency of red wines. *Chem. Senses*.

Soares, S., Vitorino, R., OsóRio, H., Fernandes, A., Venâncio, A., Mateus, N., Amado, F. and De Freitas, V. (2011). Reactivity of human salivary proteins families toward food polyphenols. *J. Agric. Food Chem.* **59**: 5535-5547.

Soares, S. I., Gonçalves, R. M., Fernandes, I., Mateus, N. and De Freitas, V. (2009). Mechanistic approach by which polysaccharides inhibit α-amylase/procyanidin aggregation. *J. Agric. Food Chem.* **57**: 4352-4358.

Souquet, J. M., Cheynier, V., Brossaud, F. and Moutounet, M. (1996). Polymeric proanthocyanidins from grape skins. *Phytochemistry* **43**: 509-512.

Sun, B., Sá, M. D., Leandro, C., Caldeira, I., Duarte, F. L. and Spranger, I. (2013). Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *J. Agric. Food Chem.* **61**: 939-946.

Troxler, R. F., Offner, G. D., Xu, T., Vanderspek, J. C. and Oppenheim, F. G. (1990). Structural relationship between human salivary histatins. *J. Dent. Res.* **69**: 2-6.

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

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V. and Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food Agric.* **83**: 564-573.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V. and Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta* **513**: 57-65.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V. and Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chem.* **85**: 519-525.

Wroblewski, K., Muhandiram, R., Chakrabartty, A. and Bennick, A. (2001). The molecular interaction of human salivary histatins with polyphenolic compounds. *Eur. J. Biochem.* **268**: 4384-4397.

Xiao, J. and Kai, G. (2012). A review of dietary polyphenol-plasma protein interactions: Characterization, influence on the bioactivity, and structure-affinity relationship. *Crit. Rev. Food Sci. Nutr.* **52**: 85-101.

Yu, P., Yeo, A. S.-L., Low, M.-Y. and Zhou, W. (2014). Identifying key non-volatile compounds in ready-to-drink green tea and their impact on taste profile. *Food Chem.* **155**: 9-16.

FIGURE 1

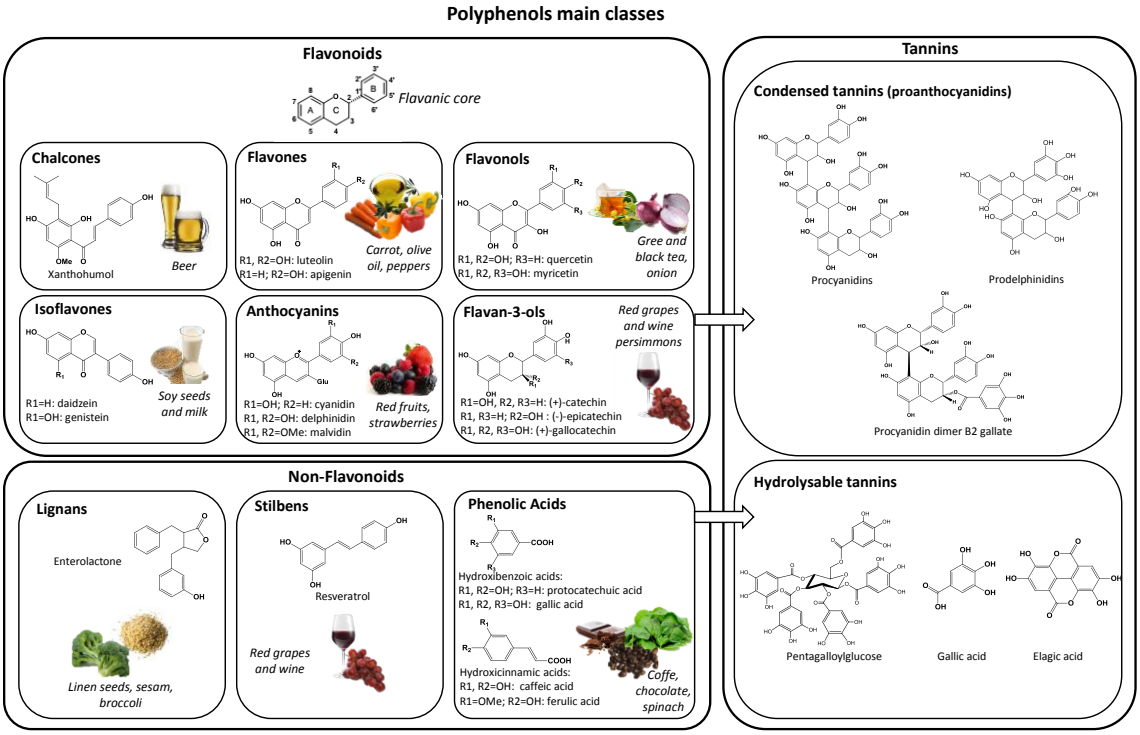

Figure 1. Main classes of polyphenol compounds showing some representative compounds of each class as well as some major food were these classes occur.

FIGURE 2

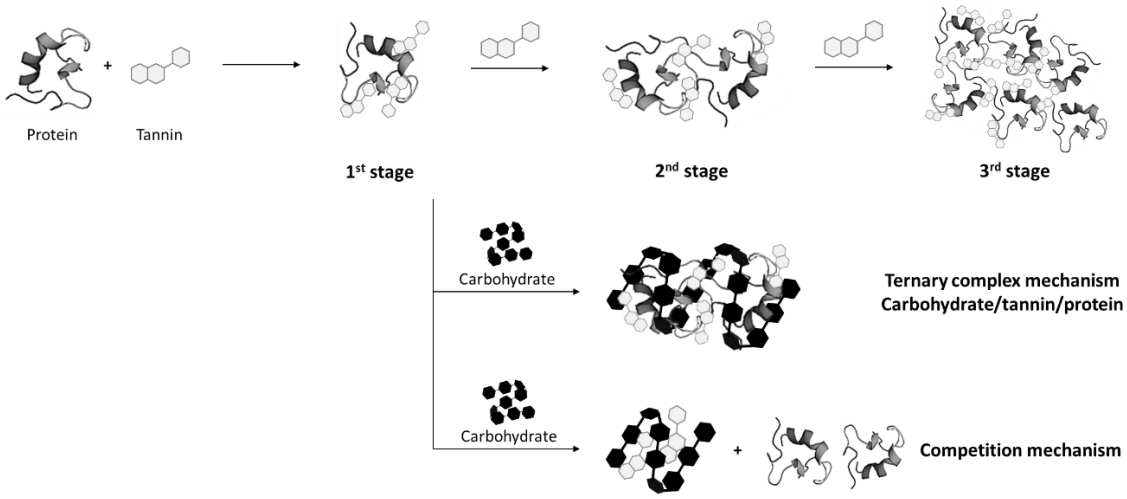

Figure 2. Schematic representation of the third stages of tannin/protein aggregation and of the two proposed mechanisms by which carbohydrates inhibit tannin/protein interaction.