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Review

Wine tannins, saliva proteins and membrane lipids☆

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ABSTRACT

Polyphenols have been part of human culture for about 6000 years. However, their mode of action in relation to wine tasting while eating is only beginning to be understood. This review, using analytical techniques and physicochemical concepts, attempts to summarize current knowledge and present an integrated view of the complex relationship between tannins, salivary proteins, lipids in food and in oral membranes. The action of tannins on taste sensations and astringency depends on their colloidal state. Although taste sensations are most likely due to interactions with taste receptors, astringency results from strong binding to proline-rich salivary proteins that otherwise lubricate the palate. Tannins disorder non-keratinized mucosa in mouth, possibly perturbing taste receptor function. The 10–15% ethanol present in wines potentiates this action. Cholesterol present in large quantities in keratinized mucosa prevents any disordering action on these oral membranes. Polyphenols bind strongly to the lipid droplets of fatty foods, a situation that reduces the astringency perceived when drinking a tannic wine, the so-called "camembert effect". Based on binding constants mainly measured by NMR, a comprehensive thermodynamic model of the interrelation between polyphenols, salivary proteins, lipids and taste receptors is presented.

1. Introduction

Wine tannins, saliva proteins and lipids can be found together, particularly when drinking wine while eating. The feelings that everyone can experience, have been known for thousands of years all over the world, but remained mysterious. In fact, drinking and eating occupies a special place in the collective imagination. The Egyptians, Greeks and Romans celebrated Osiris, Dionysus and Bacchus. Plato's banquet, the wedding of Cana, the writings of Rabelais, Apollinaire and Rimbaud celebrate the benefits of wine and food. Jars dating back 6000 years containing tartaric acid and tannins, suggesting that the peoples of the South Caucasus already knew how to make wine, have just been found [1]. The first Pharaohs used to be buried with hundreds of amphorae containing wine. The Gauls drank it pure and adapted to the land the grape varieties imported by the Romans at the beginning of our era: in Bordeaux, *vitis biturica*, the ancestor of Cabernet, in Burgundy, *vitis allobrogica*, that of Pinot Noir, Syrah and Chardonnay. Wine making, however, remained random for a long time, and the wine would not keep well unless some additives such as resin or spices were added. This posed a problem for its marketing, such as the famous "claret" consumed at the court of the King of England in the 15th century. Indeed, at the beginning of the 14th century, the wine from the Bordeaux vineyards began to be exported to the countries of Northern Europe and particularly to England. It was a primeur wine that was drunk during the year but whose poor stability induced a transformation during transport. It was under the impetus of Napoleon III and thanks to the discovery by Louis Pasteur in 1866 of the roles of yeasts in alcoholic fermentation that the production of wines that were stable over time became possible. More recently, the expression "French paradox" [2–5] has been used to explain the resistance of the Mediterranean peoples to cardiovascular disease thanks to the action, among other things, of polyphenols and unsaturated fatty acids, which are very abundant in red wines and in Mediterranean food. For just over a century, we have started understanding the physical and chemical mechanisms that occur naturally between the molecules that make up wine during its production, and between these molecules and the taste receptors during tasting.

All the elements for making wine in a natural way are contained in the bunch of grapes. It contains more than 1000 chemical compounds already identified, some of them in large quantities: sugars, water, organic acids, polyphenols that give the wine its color, and yeasts and bacteria that allow fermentations to take place. Wine making is based on a series of steps combining natural chemical and physical processes.

The objective of this minireview is not to describe the processes of

☆ A tribute to Prof Felix M Goñi, University of the Basque Country, SpainA world-renowned food and wine amateur

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Abbreviations

DMPC	1,2-myristoyl-*sn-glycero*-3-phosphocholine
CH	cholesterol
TG	triglycerides
FA	fatty acids
MAS	magic-angle sample spinning
MLV	multilamellar vesicle
NMR	nuclear magnetic resonance
C	catechin
EC	epicatechin
EGCG	epigollocatechin galate
EGC	epigallocathechin
B1	epicatechin-(4β-8)-catechin
B2	epicatechin-(4β-8)-epicatechin
B3	catechin-(4a-8)-catechin
B4	catechin-(4α-8)-epicatechin
C2	catechin-(4α-8)-catechin (4α-8)-cathechin
$IB7_{14}$	14-residue long PRP
$IB9_{37}$	37-residue long PRP
CMC	critical micellar concentration
ETH	ethanol
K_a	association constant

wine making but rather trying to summarize what is known about interactions between components in wine, especially polyphenols and alcohol (10–15%, v/v), and molecules present in the mouth (saliva proteins, lipids in the oral cavity, taste receptors) and food. These interactions are most of the time related to the 5 organoleptic perceptions (salty, bitter, sugar, acid, umami) and to astringency. This molecular approach will allow understanding, on physical grounds, how these feelings can be modulated by tannins, proline rich proteins (PRP) and lipids.

2. Wine tannins as colloidal particles

Tannins are products of vegetable origin and have been known since the history of ancient Greece where they were used in the transformation of animal skins into leather. They were called "vegetable tannins", although the term polyphenol seems more appropriate today [6]. Nowadays, plant polyphenols benefit from a recognition by the general public because of their abundance in fruits, seeds, vegetables food and derived beverages, such as wine and tea, which are consumed on a regular basis. They have been declared beneficial to human health because of their ability to eliminate free radicals generated by human activity and have been shown to be very useful in reducing the risk of degeneration and diseases occurring during aging. Polyphenols are a very important class of molecules ubiquitous in nature. Three classes of polyphenols have been reported: a) condensed tannins, *e.g.,* polymers of catechin or epicatechin, for instance abundantly found in red wines and tea, b) gallo- and ellagitannins (hydrolysable tannins), found in trees and c) phlorotannins contained in algae (for a review see [6]). They are broadly defined as compounds containing hydroxylated benzene rings that were assumed to be highly water-soluble due to the presence of numerous OH groups [7]. As we will see below, the water solubility may be toned down as certain polyphenols concentrations in wine and tea are of several g/L and are no longer soluble.

As shown in Fig. 1, polyphenols can indeed fall into the category of colloids: below their critical micelle concentration in water (CMC), they appear as free molecules in solution, above, they aggregate into colloidal particles of several nanometers in diameter. Only a few CMC values have been reported, using NMR and molecular modeling, for the monomers and polymers of catechin and epicatechin, tannins present in wine [9]. Very different behaviors were observed for the monomers and oligomers. The monomers self-associate with a high affinity constant to form micelles at low CMC values (1–5 g/L). These micelles undergo a time-dependent coalescence process to form hazes and ultimately precipitates. For dimers and trimers, self-association also occurs at higher CMC values (10–20 g/L) to form small micelles (<5 nm) that remain stable for long periods of time. The presence of about 10% ethanol, as in wines, increases their CMC value by about 50%, *i.e.*, increases their solubility, and decreases the micelle size [9]. As it will be seen below, interactions with other molecules, such as saliva proteins, may depend on the molecular availability of polyphenols: their affinity for other

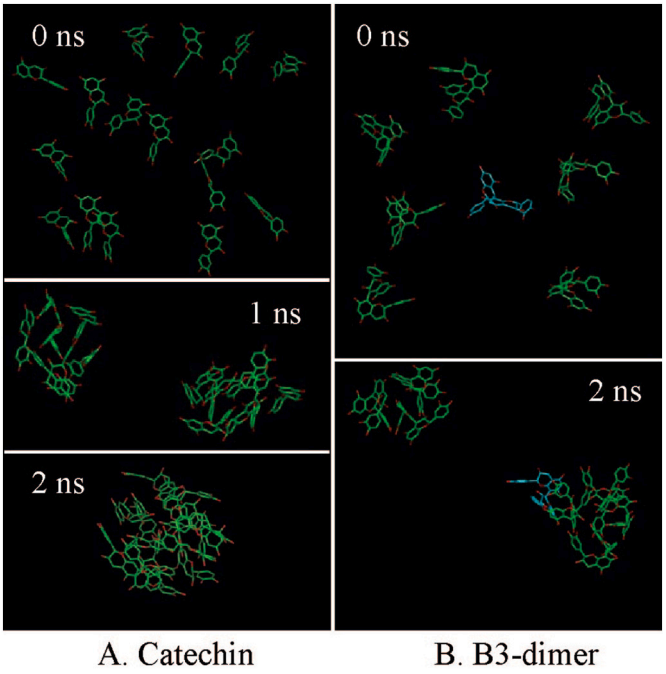

Fig. 1. The time course of polyphenol association as micelles in a water-ethanol solution. (Bottom, A) View of a micelle composed of 15 catechins obtained after 2 ns molecular dynamics (MD) simulation. (B) View of a micelle composed of 5 B3 dimers obtained after a 2 ns MD. The starting conditions (monomers dispersed in solution) are indicated in the upper panels. The molecules are represented by sticks with carbon in green and oxygen in red. Solvent, hydrogen atoms and H-bonds have been omitted for clarity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
(From [8].)

molecules will depend whether monomers or aggregated tannins are present in the hydroalcoholic medium. It has been shown that the origin of this change in solubility as a function of the state of aggregation depends on the intrinsic dynamic properties of tannins. For polymers of polyphenols, there are additional movements (inter-monomer rotations for example), which promote conformations that reduce the contact of the benzene rings with water and provide more OH groups for hydration [10]. If the colloidal state of tannins in water may modulate taste feelings (*vide infra*), the stability of colloidal particles may be a very useful property. It has indeed been recently reported that quercetin, a wood polyphenol, with antioxidant and various pharmacological properties could be delivered together with a polymer under the form of nm nanoparticles [11].

3. Saliva proteins

Human produce between 1 and 2 L of saliva per day. The volume depends on circadian rhythms and on food ingestion [12]. Oenologists who ingest a lot of polyphenols during tasting can produce 2 times more saliva. Saliva is essentially composed of water (99%), electrolytes, proteins, glycolipids, carbohydrates, enzymes, *etc.* [13,14]. The proportions of these various components vary from one individual to another and according to external stimuli [15–17]. Saliva acts as an antimicrobial and a lubricant, protecting the oral mucosa and teeth. It buffers the environment, helps digest food and allows us to enjoy the taste of food. A study of the saliva proteome identified above 2500 different proteins [18]. Their average concentration is *ca.* 3.5 mg/mL [19] and can fluctuate with circadian rhythms, diet and between individuals.

A person who is used to drinking red wine will have a better ability to produce proteins involved in the sensation of astringency such as proline-rich proteins, PRP [21], *vide infra*. Other proteins are involved in maintenance of ionic calcium concentration, antimicrobial action or protection of oral tissues against degradation by proteolytic activity [22]. Proline-rich proteins may represent up to 40% of the whole saliva proteome [23]. Of all the salivary proteins, basic PRPs do not yet have any known biological function other than lubricating properties. Whereas enzymes and other proteins have globular structures made of alpha helices, beta sheets and disordered strands, PRP proteins have unusual 3D structures in water-ethanolic solutions: they are rather opened with a high proportion of helices of type II (PPII) also called polyproline helices. Such helices, as shown in Fig. 2 right, for one of the PRP proteins (IB9) are more compact that alpha helices with 4.4 residue per turn. The proportion of such helices in human saliva PRPs may reach 40–50%, roughly corresponding to the amount of Proline residues in the sequence. Interestingly, protein structure representation according to the lipophilic potential calculation, showing hydrophilic and hydrophobic surfaces, highlights the fact that the "upper" side of IB9 (as shown in Fig. 2 left) offers its hydrophilic face (blue) rather opened and ready to bind tannins (*vide infra*).

4. Lipids in mouth and food

Lipids are essential components of all biomembranes. In the context of taste, lipids of the oral cavity and of ingested food are of principal interest. The term oral mucosa is used to define all the tissues lining the inside of the mouth (including the tongue). There are different types of oral mucosa depending of their function and location in the oral cavity. The masticatory mucous membranes are found in the gingiva and hard palate, covering 25% of the oral surface. They have a keratinized epithelium and help in the mechanical compression of food. The border mucous membranes cover the mucosal side of the lips, the inside of the cheeks, the floor of the mouth and the soft palate, and cover 60% of the oral cavity. They have a non-keratinized epithelium, and are considered a prime target for the delivery of therapeutic molecules. So-called specialized mucous membranes represent 15% of the oral surface and are located on the dorsal surface of the tongue and have keratinized and non-keratinized regions and host most of lingual papillae [24]. The lipid composition of oral mucosa depends whether they are keratinized or not. As shown in Fig. 3 the mucous membranes are dominated by phospholipids and sterols. Interestingly the amount of cholesterol may reach 40% in keratinized tissues whereas it is about 25% in non-keratinized. Sphingolipids represent *ca.* 15% in both tissues.

It has been reported that water permeability appears to be lower in keratinized regions (epidermis, gingiva, and palate) compared to non-keratinized regions (palate floor). However, the water permeability of the oral mucosa is one order of magnitude higher than that of the human skin. This indicates clearly that the oral cavity presents very different regions where adsorption/diffusion of molecules such as polyphenols may be very differential. Lipids in food represent approximately 20% by mass of all nutrients (carbohydrates, lipids and proteins). They often take the form of lipid droplets, also known as adiposomes, *i.e.*, lipid-rich cellular organelles that regulate the storage and hydrolysis of neutral lipids. They also serve as a reservoir for cholesterol for the formation and maintenance of membranes. Lipid droplets are present in all eukaryotic organisms and store a large proportion of the lipids in mammalian adipocytes. Lipid droplets are considered to be highly dynamic organelles that play a very important role in the regulation of intracellular lipid storage. The role of lipid droplets outside of lipid and cholesterol storage has recently begun to be elucidated and includes a close association with inflammatory responses, cancer and atherosclerosis. However, little is known about the role of lipid droplets in taste. The dietary lipids consumed by humans are mainly composed of triglycerides (90%), smaller amounts of phospholipids (1–10%), cholesterol (mainly in esterified form) and other minor lipid compounds such as fat-soluble vitamins and carotenoids [26,27].

5. Wine tannins interact with saliva proteins: the origin of astringency

From a physico-chemical point of view, the first step in wine tasting is the interaction (or not) of a hydroalcoholic solution of tannins with the proteins of saliva. The second step is the interaction with the membranes of the oral cavity and the taste receptors embedded in them. As polyphenols may appear as monomers or associated under a colloidal/micelle form their interaction with saliva PRP has been followed from both starting states. Polyphenol monomers (Catechin (C), Epicatechin (EC), Epigollocatechin Galate (EGCG)), dimers of catechin-epicatechin (B1, B2, B3, B4) or trimers of catechin (C2) have been synthesized [28] and applied to synthetic PRP of different sizes in solution ($IB7_{14}$, $IB9_{37}$) [29,30]. Globally, all polyphenols interact with PRP

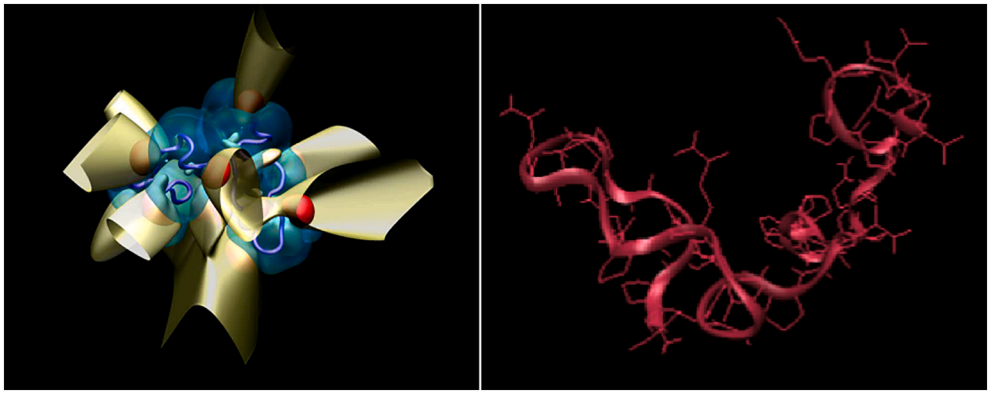

Fig. 2. Representation of the Proline-Rich Protein $IB9_{37}$ 3D structure obtained by molecular dynamics under NMR constrains. The right-hand side structure displays a classical purple ribbon representation where solvent and hydrogens are not shown, for clarity. 4 type II (polyproline) helices can be seen. The left-hand side show a different view of the same 3D structure where the atomic lipopotential has been calculated. Blue surfaces represent the hydrophilic side of the protein, red (barely seen because in the back) represent lipophilic surfaces. The yellow surface stands for the interface. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Adapted from [20].

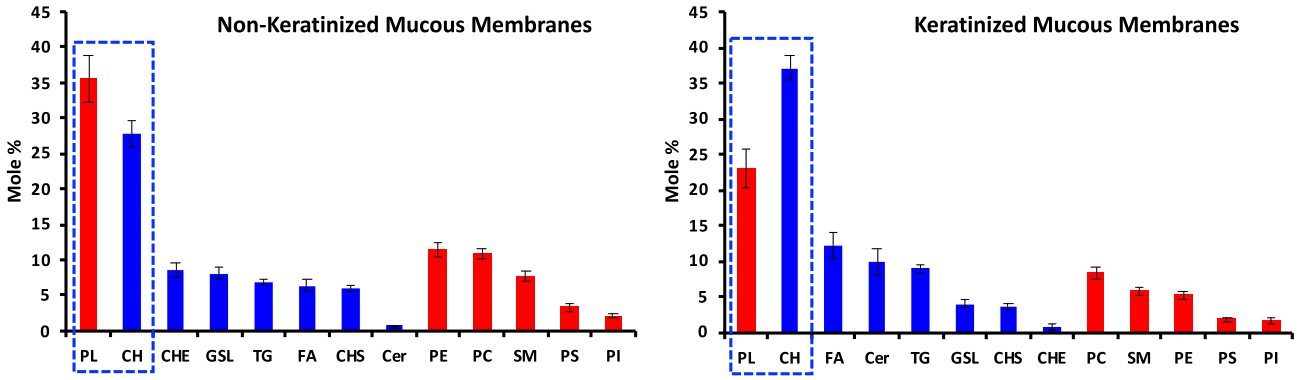

Fig. 3. Representation of the molar lipid composition of non-keratinized (left) and keratinized (right) oral mucosa. PL: Phospholipids, CH: Cholesterol, CHE: Cholesterol esters, GSL: Glucosylsphingolipids, TG: Triglycerides, FA: Fatty acids, CHS: Cholesterol sulphate, Cer: Ceramides, PE: Phosphatidylethanolamine, PC: Phosphatidylcholine, SM: Sphingomyelin, PS: Phosphatidylserine, PI: Phosphatidylinositol. The two major lipids and fatty chains are shown in a blue frame. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
From [25].

with affinities that depend on CMC (critical micelle concentration), tannin degree of polymerization and protein size. Binding can readily be followed with liquid-state ^{1}H NMR by monitoring the chemical shift change of PRP resonances of protons close to tannins [20,31]. Using a classical binding scheme, binding constants, K_a, and numbers of tannins associated per protein can be obtained. Two main situations can be distinguished: below and above the CMC of each polyphenol. Below the CMC, affinity to PRP roughly follows the degree of polymerization, *i.e.*, one may rank the polyphenol affinity to, for instance $IB7_{14}$, as C2 > B2 > B4 > B1 > B3 > C> > EC. About the same ranking is observed for another longer PRP, $IB9_{37}$, with however greater binding constants: size matters, the larger the PRP, the better affinity for polyphenols. It is noteworthy that dimers or trimers investigated so far have different affinities towards the same PRP: this is due to different 3D spatial configurations, favoring or not the binding to the PRP hydrophilic groove. It is also interesting to note that when tannins (below their CMC) bind to proteins, this occurs on the hydrophilic side, through hydrogen bonding interactions between tannins hydroxyls and carbonyl groups of proline residues (Fig. 4 left). The stoichiometry of the complex is of 2–3 tannins per PRP. The latter forms a small colloid of a few nm size. When tannins interact with PRP, above their CMC, an entire tannin micelle interacts non-specifically with the protein (Fig. 4 right). This may lead in some cases to large complexes of micrometric-millimetric size, which can be detected by eye inspection. This is usually observed when oenologists taste very young wines that have not been filtered by "collage" (fining), *i.e.*, the application of egg albumin (from egg whites) to filter by sedimentation tannins in excess. Albumin is not a PRP but nonetheless binds tannins when added in excess. We have indeed verified using a non-PRP saliva protein, Histatin-3, that the binding affinity to tannins is two orders of magnitude lower than $IB9_{37}$ [32]. The very different tannin-PRP assemblies shown in Fig. 4 may explain the so-called astringency, *i.e.*, the dry mouth sensation when drinking very tannic wines: the PRPs, which usually lubricate the palate, are strongly complexed by the polyphenols and the lubricating action is inhibited. Fortunately, this sensation disappears quickly when the saliva is renewed after a few minutes.

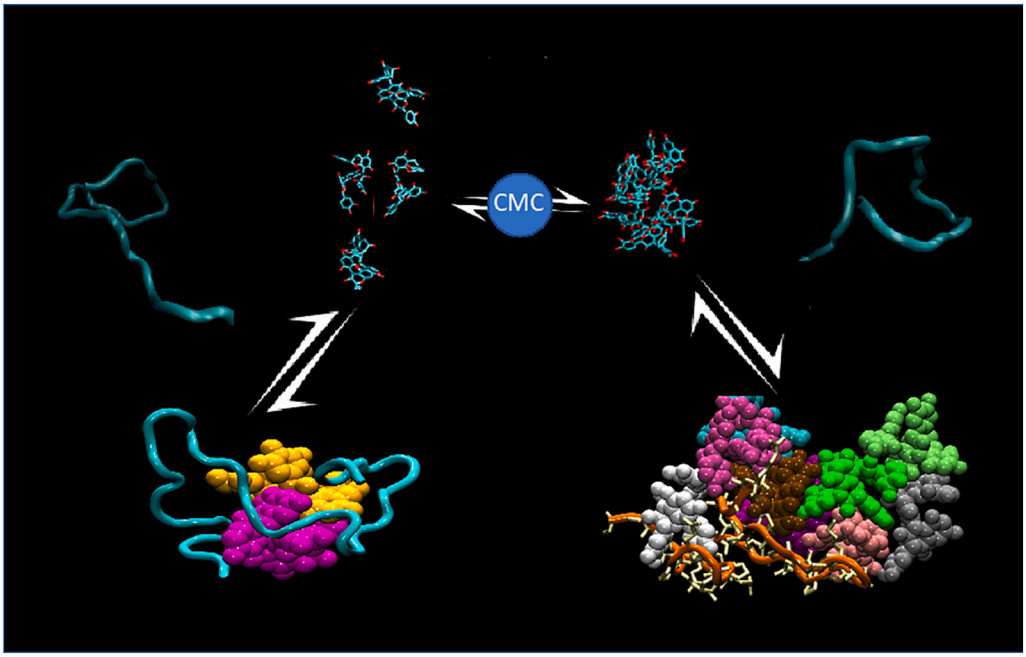

Fig. 4. The two modes of complexation of tannins with Proline Rich Proteins from human saliva. Below the CMC (left), tannins are monomeric, and specific interactions occur by hydrogen bonding between 2 to 3 tannins (atoms represented by VdW volumes) and the proline residues of PRP (blue ribbon). Above the CMC (right), an entire tannin micelle binds non-specifically to the PRP (brown ribbon). The left-hand pathway is assumed to illustrate "velvet" tannins and the right-hand pathway "hard" or astringent tannins leading to precipitation of the complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Adapted from [9].

6. Wine tannins interact with mouth and food lipids: the camembert effect

The aim of this section is to report on polyphenol/lipid interactions in the context of wine tasting or wine consumption during meals. Association of flavanols with lipids should be favored due to their high octanol/water (O/W) partition coefficients [33]. Available measured values for catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) are respectively, 2.4, 2.4, 0.3, 48.0 and 12.1, and are consistent with a membrane location. As taste receptors are embedded in lipid membranes of the oral cavity, the possible disturbance of their function due to binding/alteration of membrane fluidity is of interest as it may modulate all feelings experienced during tasting. Multilamellar vesicles (MLV) of saturated phospholipids with cholesterol in low and high amounts have been used to mimic the very different keratinized situations accounting for the different geographical compositions of membranes within the oral cavity (see Section 4). Membrane microfluidity (order parameters) has been assessed by the well-known deuterium solid-state NMR method using deuterated lipids [34]. It is reported that monomeric (C, EC, ECG, EGCG) and dimeric polyphenols (B1) act as disordering the membrane core: lowering the order-disorder phase transition of membranes made with a single phospholipid (Fig. 5B) and decreasing the chain ordering in the fluid phase; dimers produce a greater effect. In the presence of low amounts of cholesterol, the disordering effect is still perceived and is dose dependent, the more tannin, the greater disorder induced. For high amounts of cholesterol, as in keratinized mucous membranes of the oral cavity, the disordering effect is barely perceived and no longer depends on the polyphenol dose (Fig. 5C). Cholesterol appears as a membrane "protectant" agent against membrane perturbations [35]. This situation is understandable on the basis of high-resolution NMR and molecular modeling experiments. Tannins upon interaction with membranes localize at the interface (Fig. 5A), in contact with phospholipid head groups. As cholesterol is known to condensate the lipid chains (increase their lateral packing) [36], it is understandable that tannins could be expelled from the interface, therefore losing their disordering action on membranes. This situation is interesting from the point of view of taste receptors: depending upon their location in non-keratinized or keratinized mucous membranes they could be affected or not by membrane disordering as produced by tannins. Membrane disordering has been shown to be synonymous with membrane thinning [37–39], a situation that would destabilize the taste receptors anchoring in membranes and therefore perturb their function. Taste receptors in cholesterol-rich membranes, such as in rafts, would be protected from such an effect. As polyphenols produce disordering effects that depend on their structure and degree of polymerization, their action on lipid membranes with low cholesterol content can be expected to be selective.

The potential interaction with lipid droplets of the food bolus is also important as it could compete with the astringency-related tannin/salivary protein associations (*vide supra*). Lipid droplets have been modelled by nanometric bicelles, a mixture of long and short chain phospholipids, which have the advantage of presenting a hydrophobic interior as in natural lipid droplets [41]. Oil-in-water emulsions have also been prepared using DMPC as a stabilizer of the oil droplet in water, the amphiphilic lipid covering the micrometric oil droplet surface. The use of deuterated lipids in both cases allowed solid-state NMR measuring the ordering properties at the interface. For both lipid droplet model systems, a strong interaction is observed upon application of tannins. Isotropic nanoscale bicelles transform into hexagonal phases and micrometric emulsions coalesce and increase in size. The local ordering probed by DMPC at the surfaces/interfaces shows the same disorder pattern as observed in multilamellar vesicles (*vide supra*). Interestingly, the strong interaction of tannins with model lipid droplets clearly indicates that fat, when ingested by drinking tannin-containing wine, can easily compete with saliva proteins. This suggests that the astringent sensation promoted by the tannins binding the PRPs in saliva can be strongly attenuated by the interaction of the polyphenols with the lipids in food [42]. This is the well-known "camembert effect": consumption of full-fat cheese attenuates the dryness in the mouth produced by young, strong tannic wines.

7. The effect of ethanol

Natural wines contain between 10 to 15% alcohol in volume, *i.e.*, between 80 to 120 g of ethanol per liter of wine. In comparison, the tannin content does not exceed 5 g/L. This quite elevated concentration of ethanol has several impacts. The first effect concerns the concentration at which tannins associate to form micelles. The tannin CMC is of the order of 2–14 g/L (depending of tannin nature and degree of polymerization) in the absence of alcohol whereas it increases to 5–22 g/L in the presence of 10% EtOH. This clearly indicates that ethanol helps

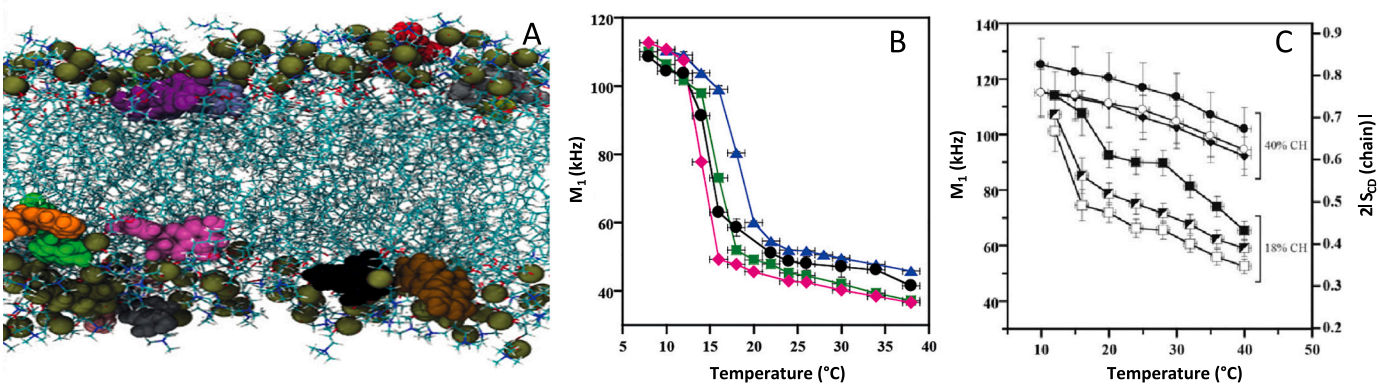

Fig. 5. Polyphenols on lipid membranes. A) Molecular dynamics of an excess water-DMPC membrane system in the presence of catechin molecules. Lipid-phosphates (tin color) and catechins (other colors) are depicted using VdW spheres, lines (grey, blue, red) are used for remaining lipid atoms. Water molecules are not represented for clarity. B) DMPC membrane ordering (lipid chains) as a function of temperature. Plain lipid (blue triangles), +C (11 mol%, black circles), ETH (12% v/v, green squares), +ETH + C (12% v/v, 11 mol%, pink diamonds). C) DMPC cholesterol-doped membrane thermotropism in the presence of two doses of catechin (1/20 and 1/8, C/lipids) and 2 percentages of cholesterol (18 and 40%). Filled squares and circles: DMPC/CH; empty symbols: +C 11 mol%; filled diamonds and half-filled squares: + C at 5 mol%. On double y-axis is plotted twice the chain order parameter; a value of 1 represents full order, lipid chains in all trans configuration as in a solid; 0 represent complete disorder with all conformations equiprobable as in liquids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Adapted from [34,35,40].

"solubilizing" tannins, more molecular species will be available to bind specifically to saliva proteins and disfavor the astringency effect [9].

Ethanol also interacts with lipidic membranes, although its partition coefficient (−0.31) favors a twice higher concentration in water. As shown in Fig. 5, 12% EtOH (v/v) disorder membranes in a way similar to 11 mol% cathechin [35]. However, the ethanol/lipid molar ratio is about 10 in the fully hydrated sample, meaning that there are about 4 ethanol molecules per lipid (considering the O/W partition coefficient). The number of polyphenols per lipid is only *ca.* 0.1. This quick calculation indicates that tannins are about 40 times more efficient for membrane disordering. It is interesting to see that ethanol and polyphenol disordering on membranes is additive (see Fig. 5B): action of polyphenols in a wine-like medium promotes greater membrane disorder.

8. From physicochemistry to wine tasting

How the above studies may account for the various feelings that one can experience when drinking wine while eating? The Fig. 6 tries to summarize the many body complexes that may occur in the mouth. Even though there are 6 bodies for interaction, the Fig. 6 oversimplifies the real phenomena occurring upon wine tasting with food. In addition, sugars have been omitted, considering that the binding of low molecular mass sugars has little effect on polyphenol complexation [43]. Ethanol has been also neglected as it mildly potentiates interactions (increase tannin CMC and membrane disorder, *vide supra*). Arrows and numbers on Fig. 6 give an idea of the interaction strength by reporting the association constant range in M^{-1}. We have included a partner not yet described above; the taste receptors that are embedded in mucous membranes. Studies on these receptors are scarce and it is only known that they fall into several categories associated to the 5 essential feelings, those which taste the bitter have been identified [44–46]. Polyphenols can act as activators or inhibitors of taste receptors, which in turn will direct the information towards the brain for interpretation as salty, bitter, sugar, acid, umami feelings. These sensations may be seen as the integration of several different receptor transmissions to the brain. Unfortunately, there is little information on 3D structure and binding constants with polyphenols. As the binding affinity of small molecules to membrane receptors, like opioid receptors, is known to be in the range of 1–100 nM [47], we took 10^6 M^{-1} as the lower crude estimate for the association constant, K_a, of tannins to taste receptors. The Fig. 6 tells us that interactions first depend on tannin CMC. The co-association of tannins [9] to form micelles is however the lowest of all association constants measured. Nonetheless it serves as a basis to explain astringency, the mouth dryness, a situation experienced when tannins above their CMC directly interact with PRP to form visible precipitates (Fig. 6 upper right). Interaction with lipids of the food bolus is about one order of magnitude greater than that with saliva proteins [34,35,40,48]. This indicates that lipid droplets (Fig. 6 center left) are good competitors of saliva PRP [43].

Organoleptic tests were conducted on this basis, with a group of about 80 educated panelists drinking edible plant tannins with or without rapeseed, grape seed or olive oil. Principal component analysis of the results concluded that astringency disappeared completely when drinking tannins after drinking rapeseed and grape seed oils and that astringency was replaced by a fruity sensation after drinking olive oil

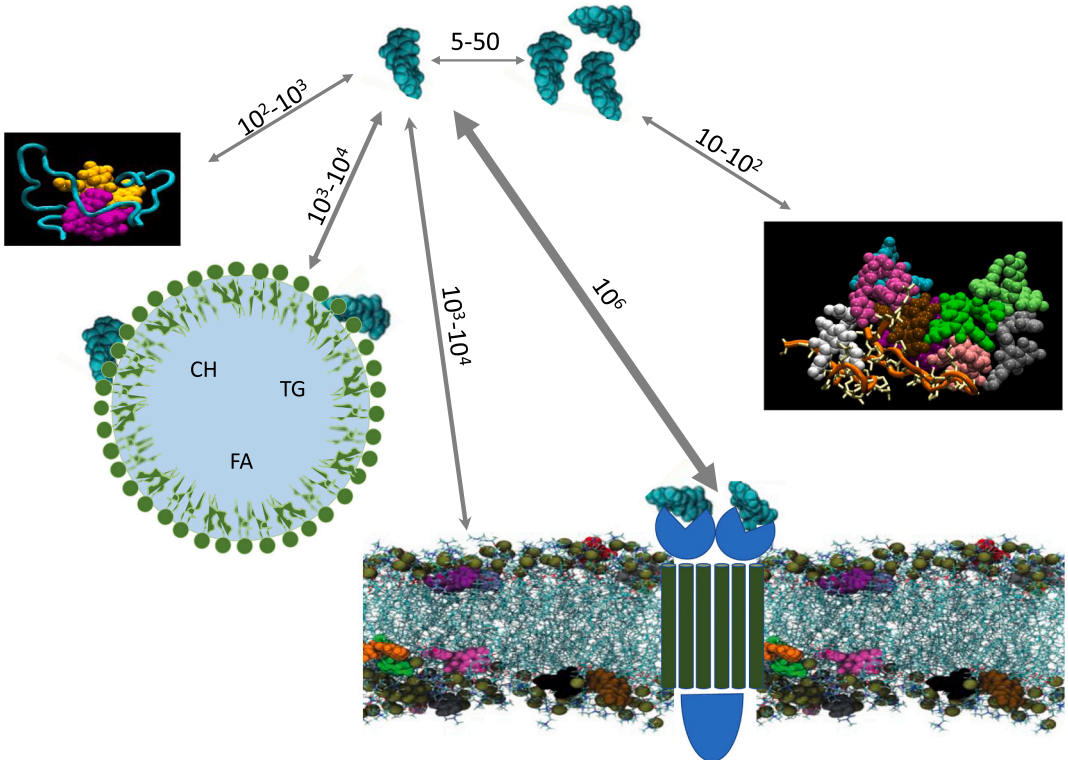

Fig. 6. A thermodynamic view of the many equilibria occurring during the interaction of polyphenols in the course of wine tasting while eating. Wine tannins are in equilibrium between monomers and micelles (top). They can interact with proline-rich proteins in saliva to lead, depending on their CMC, to nanoscale micelles (top left) or large precipitates (top right). When eating, they can also interact with lipid droplets (middle left), *i.e.*, micrometer-sized spherical reservoirs of fat containing cholesterols (CH), triglycerides (TG) and fatty acids (FA). Tannins bind to and disorder the phospholipid interface. Tannins can also bind to the mucous membranes (bottom) of the oral palate which contain taste receptors (green-blue sketch). Binding takes place both to lipids at the membrane surface and on receptor specific sizes. Tannins disorder non-keratinized membranes (low cholesterol content) which can alter the response of taste receptors. Tannin atoms are represented in VdW spheres. The arrows represent the numerous thermodynamic equilibria, the thickness of the arrows and the numbers (in M^{-1}) represent the strengths of the various interactions (range of association constants, K_a, taken from [9,20,29,34,35] and estimated from [47]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[42]. This clearly accounts for the attenuation of astringency when eating fatty foods and may be recognized as the "camembert effect": wines appear less astringent when consumed with fatty cheese or fatty foods. The last interaction described in Fig. 6 is that of tannins with the mucous membranes of the oral cavity. Although we do not have figures available for receptor binding, it is believed that this interaction dominates all others. At the same time, the binding of tannins to membrane lipids is important and can lead to receptor dysfunction due to the disordering effect on the membranes. Of course, this depends on the location of the receptor in keratinized *versus* non-keratinized mucosa. Receptors located in cholesterol-rich membranes will be much less affected by such membrane loosening. Fig. 6 shows a thermodynamic view of some of the events that may occur in the mouth during the consumption of fatty foods and tannic drinks. It should not be considered complete as the kinetics of these interactions is not known. In the long term, thermodynamics will prevail, but it may well be that the interaction of tannins with salivary proteins occurs very rapidly and sequesters almost all of the available tannins in the mouth, leading to the first impression of dryness, followed in the long term by other more subtle sensations perceived by the receptors.

9. Conclusion

In this small review we have presented some of the aspects of the complex exchanges and complexes that may occur in our mouth when drinking tannic wine while eating. Using analytical approaches such as solid-state and liquid-state NMR, mass spectrometry, light scattering, molecular modeling, chemical synthesis of tannins and saliva proteins, use of labelled lipids rendered possible to perform titration experiments at the molecular level and obtain the 3D atomic structure and dynamics of the tannin-protein-lipid assemblies. Stoichiometry of complexes, sizes, internal dynamics, sites for binding and binding constants could be obtained to reveal the strength and the nature of binding events. The situation is however far from complete since other natural components have to be taken into account. Although sugars and ethanol were not found to significantly alter the above complexes, other compounds may affect the 6-body problem. In addition, there is great variability in the structure and size of tannins and salivary proteins and more of these compounds should be studied including in mixtures to monitor synergistic effects. Further efforts should also be devoted to the characterization at the molecular level of the structural dynamics and binding constants of tannin-receptor complexes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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