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Oral tribology: Update on the relevance to study astringency in wines

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Abstract

Oral tribology is emerging as a new paradigm to quantify friction and lubrication of food-saliva mixtures in the oral mucosa. Recently, oral tribology has captured research attention in quantifying wine astringency, a characteristic "dryness feeling", which strongly impacts consumer preference. Hence, this paper aims to provide a concise review of oral tribology in the context of wine astringency. Firstly, the important roles of "biolubricant" saliva, salivary proteins and current tribo-pairs used in oral tribology measurements are reviewed. Then, we have discussed the key mechanisms of wine astringency involving polyphenol-salivary protein interactions (hydrogen bonding, hydrophobic interactions), rupture of the lubricating salivary film and oral sensation of discrete particles. Studies employing Stribeck curve analysis and microstructural characterization to understand polyphenol-salivary protein interactions are reviewed. Finally, we highlighted the need for bio-relevant tribo-pairs, simulated oral conditions and tribology-sensory correlation, before such quantification can be used to characterize wine astringency at a commercial level.

Keywords: Oral tribology; Wine; Astringency; Mucin; Saliva; Lubrication; Tannins; Proline-rich proteins

Introduction

Astringency is defined as "the complex of sensation due to shrinking, drawing or puckering of the oral epithelium as a result of exposure to substances, such as alums or tannins"[1]. Astringency is a very frequent sensory experience perceived upon consumption of various food and beverages, such as some unripe fruits (e.g. persimmon, chokecherry), soy-based foods, green and black tea, some herbs and spices (e.g. turmeric, marjoram, sage) and red wine. In

wine, astringency can be associated with different components, such as metals ions, alcohols, organic acids, but polyphenols are generally agreed to play the most important role. These polyphenols in wine come from the grapes (hydroxybenzoic and hydroxycinnamic acids, flavonol glycosides, flavan-3-ols/procyanidins, and stilbenes) and the oak barrels (hydrolysed tannins) in which the wine is stored for ageing.

Particularly, astringency is an important wine texture quality parameter. Till now, wine astringency research has mainly focused on identifying appropriate analytical methods, such as chromatography[2]. Although chromatographic tests have enabled successful identification of the relevant wine components that cause astringency, they do not allow quantifying the intensity or the evolution of the "astringent feeling". That is why, the gold standard method of assessment of the astringency in wine is "tasting" by trained sensory panels using set of reference compounds and descriptors[3]. However, training a sensory panel is time-consuming and expensive. Furthermore, astringency is a complex sensory attribute as it builds in intensity over repeated exposure. Thus, it is difficult to clean the mouth between the samples with astringent components, latter can cause fatigue in sensory panel members and consequently assessments errors[4, 5].

From mechanistic viewpoint, the term astringency comes from the Latin phrase "ad stringere" meaning 'to bind, which is believed to be related to the ability of astringent substances, such as wine polyphenols to bind to and precipitate salivary proteins[5]. Although there have been several hypotheses on interactions between wine polyphenols and salivary proteins, the predominant mechanism by which solutions containing polyphenols are perceived as astringent is still not clear. Using psychophysical methods, Green[6] suggested that oral friction is the key underlying physiological mechanism behind the sensation of astringency. This oral friction has been postulated to be resulting from the loss of oral mucosal lubrication of the salivary film, on exposure to the polyphenol components[7]. Therefore, "tribology" i.e. the

science of friction, wear and lubrication appears as a promising approach that can be used quantify coefficient of friction in oral environment, former has gathered recent research attention in understanding astringency perception.

Oral tribology is the study of friction and lubrication between two interacting surfaces, such as teeth–teeth, tongue–palate, tongue–teeth, tongue–food, lips, lips–food, bolus–palate, food particles–oral surfaces that are in relative motion in the oral cavity[8, 9]. Coefficient of friction and its relation to sensory smoothness and slipperiness in food research domain was first detailed by Kokini and co-workers[10] in 1977. The term "lubrication" as a determinant of food bolus formation and swallowing was used by Hutchings and Lillford[11, 12] after nine years. Lubrication in mouth was proposed to be dependent on saliva coating the oral surfaces before eating. Post food consumption, the changing properties of food and its interaction with the in-mouth environment was hypothesized to be the driver of oral lubrication. However, it is only recently that there has been an upsurge in research efforts in oral tribology, which can be evidenced by a power-law behaviour in the distribution of citations received by scientific papers over the last 10 years (Figure 1). Particularly, there has been some recent efforts to relate oral friction to sensory characteristics of "astringency"[13-16], latter is an important quality characteristic in wine.

Hence, this review is aimed to provide a concise update on studies employing oral tribology as a quantitative tool to predict wine astringency. Firstly, we have provided a brief introduction on oral tribology with respect to definition and relevance of the tribo-pairs (i.e. pair of materials used to create the contact surfaces), load (i.e. tongue pressure against the hard upper palate) and chemistry of the "biolubricant" saliva. Then we have specifically focussed on wine and its components (polyphenols), which interact with saliva. Finally, we have provided an update of how tribology has been used as a tool to determine the loss of salivary lubricity on exposure to wine polyphenols and highlighted the research gaps in this area.

Oral tribology

The key parameter of tribology measurement is the friction coefficient, calculated as the ratio of the measured friction force against the normal load (Figure 2a)[8, 9, 17-19]. When two surfaces are in the relative motion at a steady speed of V, the frictional force (F_R) can be expressed as $F_R = \mu \times F_L$, where μ is the friction coefficient (dimensionless) and F_L is the normal force. Lubrication is a surface property, and the magnitude of μ thus depends on the surface roughness and geometry of the interacting surfaces as well as nature of lubricant. A typical tribometer with ball on a rotating disc configuration during sliding is illustrated in Figure 2b.

The friction coefficient is dependent on the lubricant film thickness (δ) between the two moving surfaces and is typically presented in a Stribeck curve (Figure 2c)[17]. The distinct friction scenarios that can occur between the tongue and palate is represented by three different regimes: the boundary regime, the mixed regime and the hydrodynamic regime. Details of these regimes can be found in previous reviews[8, 17].

Role of tribo-pairs and loads

In order to understand the complex oral system (oral surfaces, saliva or saliva-wine mixtures as the lubricants), researchers have used different metallic, crystalline, polymeric and animal tissue-based tribo-pairs to mimic the topologies of real human tongue and oral palate. Pin-on-disc, ball-on-dics tribometers with tribo-pairs made up of steel[20], tetrafluorethylene and zirconia[21], glass[22] surfaces in a sliding or rotating configurations have been used. However, as one might imagine, contrasting to these surfaces, oral surfaces may vary significantly from highly keratinized bony palate to soft and rough tongue with papillae being in of order 20-100 μm[12, 23].

Innovative approaches, such as everted dried dead tongues of pigs/ piglets have been also used in tribometers to represent human tongue surfaces[12, 24]. Besides ethical constraints, lack of

information about surface chemistry and biological heterogeneity of using animal tissues, papillae of the dried pig tongue ex vivo was not firm and erect during tribology measurements, which might be attributed to the biochemical changes (post-mortem) or dehydration process. Furthermore, the dead animal tissues were less hydrophobic and lubricating as compared to the living surfaces[12, 25]. It is also worth recognizing that the diameter of the hairs of the human filiform papillae (27 μm) is larger than that of the pig tongue (18 μm)[26]. Hence, the surface roughness of these dried animal tissue surface used in the tribology measurement was not representative of the real human tongue surface. Hence, the friction measurement interpretation for human tongue needs to be taken with precaution.

Instead of "hard" metallic surfaces and animal tissues, soft elastomeric substrates, such as polydimethysiloxane (PDMS) that can be deformed by contact pressure are currently preferred as tribo-pairs[19, 27, 28]. Although tongue surface is significantly rougher than smooth PDMS surfaces, PDMS surfaces can be modified in deformability, roughness and hydrophobicity to represent tailored oral surfaces. For example, the hydrophobicity of PDMS surfaces can be tuned using plasma oxidation, surface coating with functional groups or layer-by layer[29-31].

"Loads" in oral tribology context can be defined as the normal force that the tongue exerts on the hard upper palate. As compared to typical mechanical engineering context, a lower range of loads (1-10 N) has been used in oral tribology studies[19, 27]. Measurements of the loads of the tongue against the upper hard palate generally ranges from 0.01-90 N[32]. It is worth noting that the tongue pressure distribution is not uniform across different parts of the tongue-oral palate contacts and the load distribution might also vary with time[33]. Tongue pressure might also differ depending upon the population used for study, for instance, elderly population show significantly lower tongue pressures than younger adults group[34-37]. Hence, oral tribology study

for a particular wine consumer group needs to be carried out at a range of relevant loads rather than a single-point load to represent different oral conditions.

Saliva: The potent "bio-lubricant"

Saliva is composed of water (99.5%), proteins (highly glycosylated mucins, proline-rich proteins and enzymes, such as α-amylase) (0.3%), and inorganic substances (0.2%) with pH around 6.8[38-40].

Formation of salivary mucosal pellicle

Salivary mucosal pellicle is a viscoelastic gel that protects the oral mucosa from mechanical and chemical damages, such as exposure to microorganism, toxic materials, environmental insult, dehydration of oral mucosal epithelium and lubrication. The most prominent constituent of oral pellicles are mucins, a high molecular weight glycoprotein[41 42]. As Figure 3 shows, salivary mucosal pellicle comprises of two phases, an immobile pellicle retained on epithelial cells (membrane associated mucins: MUC1, MUC3, MUC4, MUC12) and a mobile salivary film (secreted soluble mucins: MUC2, MUC5A, MUC5B, MUC6, MUC7)[43-46].

The MUC5B (high molecular weight) and MUC7 (low molecular weight) are the most important glycoproteins with regards to lubrication. Saliva is secreted to maintain saliva pellicle thickness of ~70-100 μm[47], but vary depending upon the oral location. The oral mucosa where saliva pellicle is created is generally hydrophobic until the salivary proteins bind. Upon adsorption to the tongue (hydrophobic), glycoproteins tend to bind with their hydrophobic sites towards the tongue, whilst hydrophilic sites point outwards for water retention. Salivary film reduces the "μ" in oral surfaces. Using AFM, human salivary pellicles have been shown to reduce the μ by a factor of 20 between hard contact surfaces[48], having $\mu \approx 0.02$ i.e. two orders of magnitude lower than that of water[49].

Use of saliva in oral tribology studies

Use of saliva is becoming popular in oral tribological measurements in food research as saliva is a key "biolubricant" that can reduce "μ" significantly within the human oral surfaces. However, such lubricating properties of saliva (ex vivo) can vary significantly depending upon stimulation (unstimulated, mechanical, acid), collection (protein-binding properties and air exposure) and usage (immediate use, freeze-thaw-induced precipitation)[50, 51]. Also, within an individual, salivary protein amount varies and acidic and glycosylated proline-rich-proteins PRPs (gPRPs and aPRPs) may vary significantly throughout the day and is highly dependent on the type of food ingested[52]. Other factors influencing interactions with wine are pH, buffering capacity and concentrations of calcium and phosphate in saliva, latter shows huge variation over a day in unstimulated whole saliva[53] and even depends on how saliva has been handled after collection[51].

The friction coefficient of stimulated and unstimulated saliva measured between two mucosal surfaces using loads (0.34-2.20 N) showed decrease of μ with increase in load and speed for both types of saliva[54, 55]. The differences in μ were due to the protein content and rheological properties of saliva, particularly, stimulated saliva produced by sublingual and submandibular gland had a higher protein content and lower viscosity as compared to unstimulated saliva[54].

Saliva also changes its composition along the salivary film (Figure 3), and until now, the "mobile salivary phase" has only been studied. However, the most important lubricating proteins (MUC5B and MUC7) still remain attached to the mucosal epithelia even if the salivary film is ruptured. As these mucins may be important to understand "astringency", it might be worth to consider collecting saliva from parotid glands or gently scraping the immobile salivary pellicle from the oral surfaces of the participants after ethics approval for tribological measurements.

Finally, the use of "artificial saliva" i.e. fluid mimicking the ionic composition, mucin and rheological properties of unstimulated human saliva has been quite common due to its ease of preparation and reproducibility[38-40]. However, the term "artificial saliva" has been argued by several authors as there has been no bio-mimetic that accurately simulates all of the properties of saliva[50]. In a recent study by Laguna and coworkers[19], μ of artificial saliva was measured in a PDMS-PDMS ball-dics set-up and the Stribeck pattern was found to be similar to real human saliva (unstimulated)[54]. Hence, use of at least mucin in a mimicked ionic composition can be a good starting point to understand wine-saliva interaction as compared to that without consideration of any aspects of salivary lubrication.

Wine and astringency

Wines, derived from fermented grapes [56] (Vitis Vinifera) are essentially composed of 80-85% water, 9-20% ethanol and other minor compounds, such as phenolic compounds, esters, acids, nitrogenous compounds, volatiles, lipids, mineral salts etc. A well balanced-wine should contain optimum primary taste components (i.e. balance between sweetness and acidity), tactile elements (i.e. astringency) and flavour[57]. Among the different textural attributes, astringency has been considered to be one of the most important sensory characteristic in red wines.

Since astringency can be perceived in the mouth where no taste receptors are present, it is considered to be tactile rather than a taste stimulation, contrary to the initial speculations[6]. Different phenolic compounds show different affinities towards human salivary proteins[58]. Polyphenols with extended structure have been reported to have higher affinity to PRPs[58, 59]. In other words, smaller polyphenols can bind with one phenolic ring, whilst larger polyphenols interact in a multi-dentate fashion, occupying two or three consecutive prolines increasing the degree of salivary protein precipitation. Despite the chemical differences in phenolic components, the astringency of polyphenols mixtures with different structures, such as,

phenolic acids and catechins were perceived to be of same astringency by a trained sensory panel[60]. The total phenolic content of wines depends on many factors and it can vary from 900-1400 mg/L in young red wines and 1600-2500 mg/L in aged red wines[61]. Astringency feeling evolves during aging, and it is generally higher in young wines and decreases over time, "softening" the wine. This is caused presumably by the soluble pectin fragments, associated with the grapes that might inhibit protein-tannin interactions and pectin might aggregate or encapsulate the tannins making the latter unavailable to the salivary proteins[62, 63].

Three different mechanisms of wine astringency has been hypothesized that complement each other: protein precipitation, rupture of the lubricating salivary film and formation of mouth debris[64] (Figure 3). Firstly, wine polyphenolic compounds form complexes with salivary proteins, specially PRPs[65] due to hydrophobic interactions and hydrogen bonding, precipitating the salivary proteins and decreasing its viscosity[66], latter affecting the integrity of the salivary film. Hydrogen bonding occurs between hydroxyl groups of phenolic compounds and carbonyl and amide group of the salivary protein, whereas hydrophobic interactions occur between the benzoic ring of phenolic compounds and the apolar side chains of amino acids such leucine, lysine or proline in the salivary proteins[62] (Figure 3). The rupture of the lubricating saliva film activates the mechanoreceptors, located within the mucosa connected with the trigeminal nerve that then transmits to brain the perception of astringency[67]. Furthermore, the increase in precipitated salivary proteins and other debris in saliva increases the sense of "discrete particles" in the mouth, which essentially relates to roughness and oral friction[23]. Due to the strong correlation between astringency perception and formation of insoluble salivary protein-wine polyphenol complexes, research has focused in finding analytical methods for quantification/qualification of these complexes. In the next section, we only focus on recent studies that used Stribeck curves to quantify astringency.

Relevance of oral tribology to unravel wine astringency

Salivary proteins are widely separated from each other due to mutually repulsive forces of negatively charged mucins[38] at neutral pH in saliva, latter is a highly diluted system[23]. However, when tannic acid was added, large flocs appeared in saliva (approx. 300 μm) (Figure 4a)[23]. In red wines-saliva mixtures, similar aggregates have been recently observed using light and transmission electron microscopy (Figure 4b)[14]. Furthermore, the microstructure of such aggregates varied depending upon the wine type and their polyphenol composition, specifically proanthocyanidin (grape skin) and tannin (seeds). Cabernet Sauvignon wines presented densely-packed aggregates whereas Carménère, Merlot wines showed smaller aggregates with much more open structure (Figure 4b)[14]. However, irrespective of the type of wines[14], wine-saliva mixtures showed a significantly higher μ as compared with human saliva in the boundary regime using a PDMS-steel contact surfaces (Figure 4c). Authors reported a high correlation (R^2=0.93) between μ and sensory "astringency" at a sliding speed of 0.075 mm/s linking astringency to salivary protein depletion by wine polyphenols.

In a separate study, tribological analysis in a soft PDMS ball/model mucin-adsorbed glass surface[16] indicated that astringency may arise from the temporary failure of the boundary lubrication of the adsorbed mucins by tannic acid. This loss of boundary lubrication showed concentration dependency on tannic acid. Authors suggested that interaction with tannic acid molecules might result in the change in conformation and hydration of adsorbed mucin, both leading to the marked rise in friction force. This is in agreement with a previous report, where "chemically pure" polyphenol (epigallocatchin gallate) appeared to partially deplete the thin lubricating human salivary film (mechanically stimulated whole saliva) from the smooth PDMS-PDMS contact surfaces in a tribological experiment performed at 37 °C[15]. This induced an increase in μ and was correlated to a certain extent with the astringency perception.

Besides phenolic compounds, there are other components in wine, which can also contribute to astringency perception. For example, tartaric acid present in wines are known to lower the pH of wine significantly, which precipitates the salivary proteins[2] as well as increase the binding affinities of the salivary proteins with polyphenols. In contrast, the presence of ethanol in wine has been reported to modify the degree of hydrogen bonding between polyphenols and salivary proteins. This tend to modify the degree of protein folding and solubility of tannins[68]. Another key component in wines i.e. glycerol has been associated with oiliness, persistence and mellowness[69]. Interestingly, tribological measurements of aqueous solutions of glycerol in steel tribo-pairs (ball/ disc) have suggested glycerol to be a potential "green lubricant" with its lubricating properties being better than those of rapeseed oil. Hence, contribution of wine components other than polyphenols in astringency should not be underestimated and the complex interplay of polyphenol, pH, ethanol, glycerol in wine astringency needs further investigation from tribological viewpoint[70].

Conclusions

In summary, astringency studies in wine essentially rely on sensorial methods so far. Interaction between polyphenols in wine and salivary proteins is generally considered to be the main mechanism inducing astringency sensation. Oral tribology is a relatively recent approach that has been used to quantitatively study the loss of lubricity of saliva on exposure to polyphenols. Measurement of coefficient of friction of wine and specific polyphenols at certain sliding speeds have shown some correlation with sensory perception of astringency. This shows potential of oral tribology measurement as a promising quantitative tool for analysing astringency perception. However, lubrication is a surface property. Hence, the friction coefficient not only depends on the mechanical properties of the lubricant (e.g. saliva) but also on the surfaces used in tribology measurement to represent the tongue and the upper palate.

Currently, the contact surfaces used in oral tribology range from steel to glass to PDMS. The key requirement is the accurate development of bio-relevant tribo-contact surfaces that effectively represent the soft, micro-patterned tongue and bony upper palate surfaces. Use of accurate loads representative of real human tongue pressure values when consuming polyphenol-rich food need to be used in such measurements. Use of relevant tribo-pairs and loads need to be standardized across different laboratories to have comparable results. Most importantly, these quantitative friction measurements need appropriate correlation with sensory perception using trained sensory panel, before such quantification can be of use to characterize astringency in wine and other polyphenol rich foods at a commercial level.

Notes on Contributors

Dr. Laura Laguna works for the Spanish National Research Council (CSIC) at the Institute of Food Research (CIAL, Madrid). Over the last years, her research has been focused on the study of food oral processing, nowadays with special emphasis on studying the sensory perception of wine components.

Dr. Anwesha Sarkar is a Lecturer of Food Colloids at the University of Leeds. She has more than 36 scientific papers and 4 patents. Research interests: colloidal design (emulsion, emulsion gels, microgel, particles, protein complexes, coacervates), oral tribology, lubrication in soft contacts, multi-scale structural analysis, in vitro digestion.

Disclosure statement

No potential conflict of interest was reported by the authors.

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