Surface Chemistry

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Astringent Mouthfeel as a Consequence of Lubrication Failure

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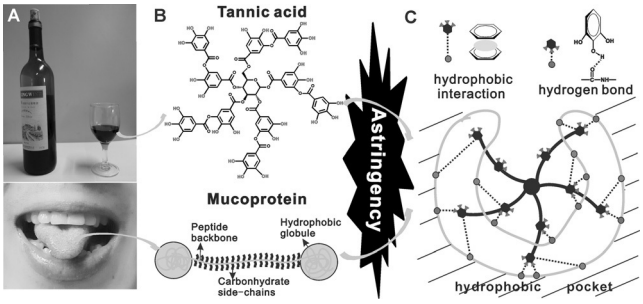
Abstract: *Herein, we systematically investigate the origin of astringent mouthfeel when we eat unripe fruits, drink coffee or tea, from the perspective of lubrication by simulating the dynamic weak interaction on the tongue with model protein (mucoprotein, MP) and polyphenolic compounds (tannic acid, TA). Astringency was due to the protein-mediated lubrication failure when encountering polyphenolic molecules that normally exist, for example in unripe fruits, coffee, tea. The underlying molecular mechanism of oral tribology is widely present in nature and enables us to engineer a tongue-like polyacrylamide composite hydrogel that exhibits high TA sensitivity and to develop a scientific strategy for catching slippery fish using TA-containing gloves. These results provide novel and useful insights into the failure of biological boundary lubrication on soft tissue surface with the adsorbed proteins.*

In general, oral astringency,[1,2] as a sensation of taste on the tongue when drinking wine and eating some unripe fruits, was revealed because of the polyphenol–protein complexation reaction.[3–7] This phenomenon has aroused huge scientific interest,[8–10] considering its potential application in both food nutriology and pharmacology.[11,12] However, this occurrence has never been explored from the perspective of tribology, despite the recent investigation on shear forces in porcine gastric mucin.[13] At this point, a detailed scientific investigation on interfacial lubrication is necessary to elucidate the in-depth mechanism underlying the common phenomenon.

Two chemicals secretory mucoprotein (MP) and extraneous tannic acid (TA) were selected to simulate the involved lubricious proteins and polyphenols analogs. TA, an organic polyphenol macromolecule,[14,15] is well known for its excellent anchoring and coordinating ability on various substrates.[16–18] It has considerable amounts in wine and is normally seen in unripe fruit. MP, which is secreted in oral cavity, has strong adsorption ability on various surfaces.[19–21] People have investigated the physical behavior of mucin under the biological boundary lubrication regime.[22–25] When mucin adsorbs on the surface of the human tongue, a molecular boundary lubricating layer arises, which endows the surface with low friction coefficient.

Red wine is rich in polyphenols including a large amount of TA molecules, whereas mucin abundantly exists on the tongue to form an effective boundary lubrication film as shown in Scheme 1 A. When wine is consumed, TA analog



Scheme 1. Molecular structure and underlying mechanism in astringency sensation. A) Optical images of tannic acid (TA)-rich dry red wine (top) and mucoprotein (MP)-rich tongue (bottom). B) Molecular structure of TA (top) and MP (bottom). C) Possible mechanism involving hydrophobic interactions and hydrogen bonding between TA and MP.

molecules (from the wine) rapidly associate with MP (on the tongue surface) and induce the sensation of astringency. At that moment, the tongue initially feels less slippery. The interaction may be attributed to the special molecular structures of TA and MP (Scheme 1 B). TA is enriched in phenolic hydroxy groups and phenyl rings,[16–18] whereas MP exhibits a monomeric dumbbell-like structure with both positive and negative charges and hydrophobic domains. When mixed, possible interaction mechanism between polyphenols and MP is proposed in the literature.[26,4,27] (RE: ref.4 was added while ref.27 was replaced by a new literature) Typically, both hydrophobic and hydrogen bonding interactions enable well decoration of the proteins surface with a less hydrophilic layer of polyphenol molecules. Given the synergistic and strong complexation interaction, hydrophobization of MP would occur, which results in protein aggregation and even precipitation.

This association process can be demonstrated by the in situ capturing the disturbing friction force between a soft ball (PDMS) and a mucin-adsorbed glass surface. (Simulation of the oral sensation through soft–soft contact in a tribological test gives the same trend, if not the same value. See Figures S10–12 in the Supporting Information.) The experiment setup was given in Figure S1 and the tests were performed at a constant shear velocity of 0.02 $m s^{-1}$ under

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the normal load of 300 g and under lubrication of a drop of liquid, through quantitatively tuning the amount of reactants at the microliter level. To eliminate the effect of concentration in boundary lubrication,[28] the MP concentration was kept constant for the entire experiment. Interfacial friction force decreased rapidly as 10 μL of MP (1 mg mL^{-1} MP solution) was injected into pure water (Figure 1 A). As

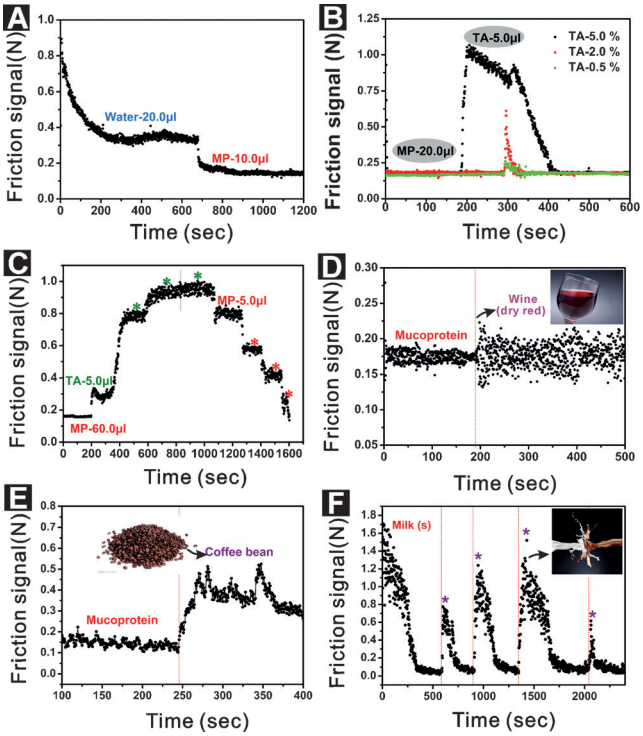

Figure 1. Friction force signals captured under constant load of 3 N at a sliding velocity of 0.02 m s^{-1} on glass surface. A) In situ trace signal of friction force captured after injection of 10 μL of 1 mg mL^{-1} mucoprotein (MP) solution into 20 μL of pure water. B) Friction signal after lubrication with 1 mg mL^{-1} MP solution and different dosages of tannic acid (TA) solution, particularly, 5.0% (black), 2.0% (red), and 0.5% (green). C) Gradient signal captured from the association between 1 mg mL^{-1} MP solution and 5% TA. D) Friction force signal captured after injecting wine solution into MP solution. E) Friction force signal captured after injecting 10 μL of coffee bean solution (10 mg mL^{-1}) into MP solution. F) Reversible friction force signal captured after continuously injecting 10 μL of coffee bean solution (10 mg mL^{-1}) into 10 μL of milk on the glass surface.

expected, the injected mucin would adsorbed onto both substrate and PDMS surfaces[29,30] and thus induced boundary/mixed lubrication between the two rubbing surfaces.[31] Similar molecular lubrication regimes[32–34] have been found in mucin-adsorbed surfaces in living systems.[35–37] Injecting the TA solution led to a fast rise of the friction force (Figure 1 B). Only TA does not lead to friction rise, but decrease, compared with pure water. TA molecules may stimulate the change in MP structure from a natural conformation to a distorted one; much of their hydrophobic groups would be exposed to the molecular surface and lead to dehydration of the adsorbed protein molecules that may collide with one another as they settle on the substrate.[38] In this case, the hydration lubrication layer on the MP-adsorbed surface is quickly damaged, leading to the remarkable rise in friction force. Once precipitated, the associated proteins would be wiped from the interface, and then the dissociated proteins would form new boundary lubrication film. As expected, this disturbance is sensitive to TA concentrations.[39] Increasing the dosage of TA resulted in a friction rise and longer time to restore the friction force to its original value (Figure 1 B). After reaching the highest value, the friction force decreased gradually to its original value. Successive supplementation of MPs after initiating the association would facilitate the formation of a new boundary lubricating layer. We observed a progressive reduction in friction force after the stepwise injection of MP solution, as shown in Figure S2. Continuous MP replacement from the regeneration of fresh salivary protein from glands explains why astringency is a temporary phenomenon and lubrication recovers momentarily. When the TA concentration was reduced, the friction map showed a similar trend but a slightly smaller amplitude (Figure S3). This observation suggested that the strength of astringency was directly related to the TA concentration. Figure 1 C shows the stepwise back-and-forth tuning of the friction force starting from 60 μL of MP (1 mg mL^{-1}) lubricating liquid. The friction force peaked after four successive injections of 5 μL of TA (5%), and gradually reverted to the boundary lubrication regime after four successive injections with 5 μL of MP solution (Figure 1 C).

After investigating the functional mechanisms with model compounds, experiments were performed using the actual systems. MP lubrication was indeed disturbed by in situ injection of red wine (Figure 1 D) as implied by an even larger friction force fluctuation, indicating the presence of TA or TA-type chemicals in red wine that may destroy the lubricating layer.[40] An apparent rise in the friction force signal was observed after the in situ injection of a coffee bean solution because of its high polyphenols content (Figure 1 E).[41,42] When we lubricated the said sliding pairs with milk (which is rich in proteins and fats), we observed a progressive reduction in the friction force (300 s running-in period) until a stable and low friction force level was reached (Figure 1 F). However, when 10 μL of a 10 mg mL^{-1} coffee bean solution was injected onto the interface, the friction force signal increased instantly. Similarly, a coffee bean solution presented a high friction force and encountered a sudden decrease by the addition of milk, and then went back to the original high friction force level when more coffee bean solution was injected (Figure S4). Milk, a kind of stable protein emulsion contributes to low friction orally perceived as smoothness and sliminess.[31,43,44] The injected coffee bean solution impaired the emulsion stability and subsequently destroyed the protein-generated thin lubricating layer, resulting in increased friction force.[28,45]

The interaction between MP and TA solutions was monitored by the changes in frequency and dissipation of a gold-coated quartz chip using a quartz crystal microbalance (QCM). As shown in Figure 2 A, pure water was successively delivered over the chip to achieve equilibrium (1), and then 1.0 mg mL^{-1} MP solution was injected into the QCM chamber (2). A significant decrease in frequency (78 Hz) and increase

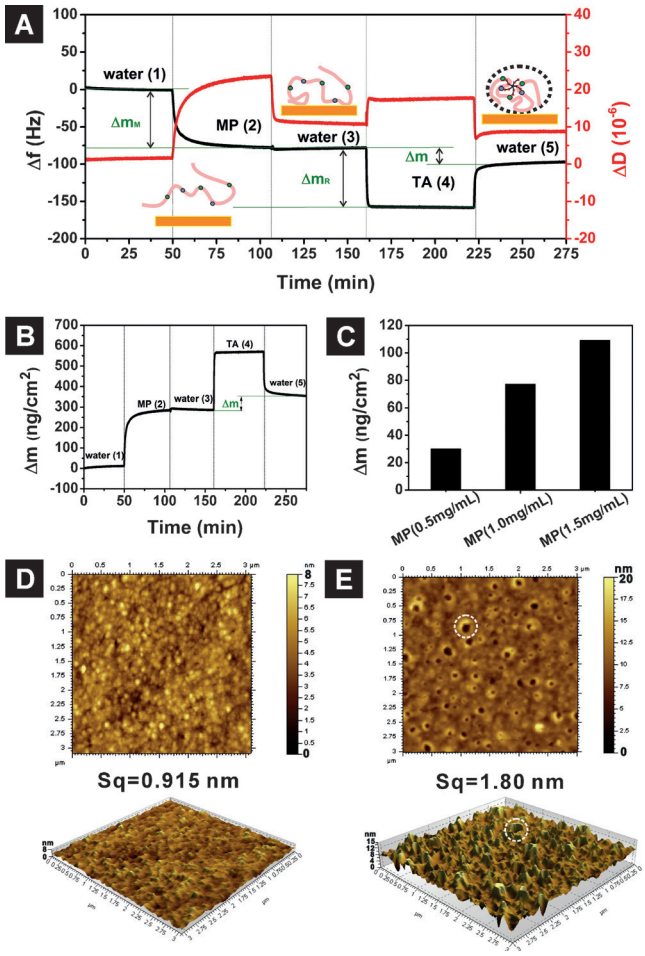

Figure 2. A) In situ monitoring association between MP and TA by QCM as the changes in frequency and corresponding dissipation of QCM chip in five successive processes, (MP conc. 1 mg mL^{-1}; TA conc. 5%). B) the calculated adsorption mass changes of process A. C) The net mass addition (Δm) in the protein absorption settlement process (4) at different MP concentrations and interaction with 5% TA solutions. D) AFM morphology captured on chip surface after MP absorption and subsequent rinsing with water. E) AFM morphology captured on chip surface after the adsorbed MP film was precipitated by TA molecules.

in dissipation indicated the strong absorption of MP on the chip. Almost no change in frequency was detected after washing with pure water, which indicated the strong absorption of MP on the surface. However, an evident decrease in dissipation caused by the removal of the viscous MP layer, as well as an increase in rigidity, was observed (3). After sequentially injecting 5% TA solution (4), the frequency decreased instantly to about 79 Hz, attributing to the association of the adsorbed MP layer with TA molecules. After flushing with water to remove the free TA molecules (5), the frequency apparently increased but remained lower than that of the MP absorption layer. Meanwhile, the net energy of dissipation decreased, which indicated that a more dense hydrophobic film was formed. In a control experiment, no change in energy dissipation was observed for single TA (5.0%) adsorption on the chip (Figure S5). The corresponding mass curve is given in Figure 2B, which shows that the net adsorption mass of MP is about 274.8 ng cm^{-2} and then increased by 279.0 ng cm^{-2} because of the association with TA molecules. After washing with pure water, the net mass adsorption value was calculated to be about 77.2 ng cm^{-2} (Δm), which corresponds to the strongly bonded molecules. As the MP concentration increased from 1.0 mg mL^{-1} to 1.5 mg mL^{-1} (Figure S6), or reduced to 0.5 mg mL^{-1} (Figure S7), similar trends of changes in frequency and energy dissipation were observed. The tested chips were imaged using atomic force microscopy (AFM). As shown in Figure 2D, after about 50 minutes of adsorption and rinsing with water for 60 minutes to remove free molecules, the MP molecules arranged into a flat, dense film on the chip surface with roughness of Sq = 0.915 nm. This step was followed by interaction with injected TA molecules, which caused the appearance of a large number of defects on the surface. In this case, the chip surface presented with a relative high roughness of Sq = 1.8 nm.

To imitate actual tongue conditions, we engineered MP into a polyacrylamide (PAM) hydrogel, which was envisioned to be sensitive to TA (Figure 3A). The obtained composite hydrogel kept highly flexible and presented low-friction in water (readily slid off fingers), but became very sticky after treatment with TA solution (Figure 3B). This weak interaction led to evident shrinkage of the gel network caused by dehydration, which was clearly demonstrated in the dynamic wetting process: 5 μL of water wet within only 1.60 s on the as-prepared hydrogel, but required 71.68 s for 5 μL of TAs (Figure 3C). The TA-MP interaction also influenced the mechanical strength of the hydrogel. As shown in Figure 3D, the MP-PAM hydrogel exhibited good elasticity but low elongation stress. After treatment with TA solution, the mechanical strength increased significantly concurrent with

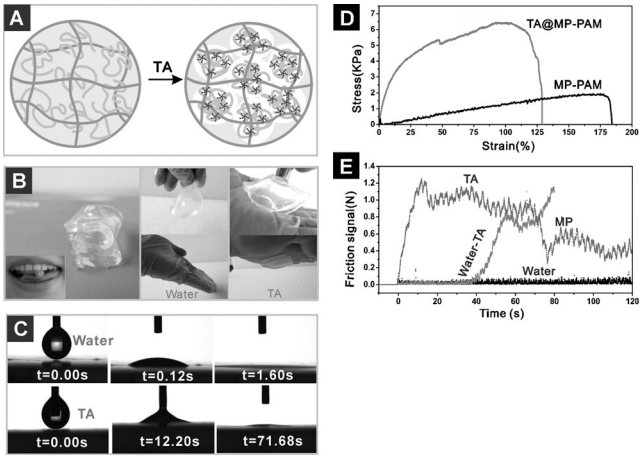

Figure 3. A) Schematic depiction of MP-incorporated PAM hydrogel (left) and subsequent interaction with tannic acid (TA; right). B) Optical images of the tongue-like MP-PAM hydrogel (left), showing ultra-slippery state of the wetted hydrogel (middle) and strong adhesion to TAs (right). C) Wetting 5 μL of water drop (top) and 5 μL of 5% TA drop (bottom) on surface of the composite hydrogel. D) Stress–strain curve before (MP-PAM) and after (TA@MP-PAM) treatment with TA. E) Friction signal of the composite hydrogel under pure water (water), after in situ TA injection (water-TA) and after complete wetting by TA (TA).

the reduction in elasticity. The MP-PAM hydrogel exhibited greater porosity, whereas the TA@MP-PAM hydrogel showed a more compact structure (Figure S8). This observation suggested the widespread TA-MP interaction that occurred and shrank the hydrogel. After sliding against PDMS, the interfacial friction force increased rapidly during TA addition (Figure 3E). Figure S9 demonstrates a weight (20 g) slide along hydrogel sheets (76 mm × 26 mm). The weight slid for a very short time (0.6 s) along the water-saturated hydrogel tilted at 8.8°. On hydrogel sheets with two halves treated separately with water and TA solution, the weight slid rapidly on the water-treated side, but stopped at the TA-treated side. The hydrogels fully treated with TA solution showed the longest steel weight travel time (> 120 s) even at a larger tilting angle (> 20°).

The epidermal layer of fishes secretes various kinds of biologically active proteins,[46] which provide an underwater natural defense system to escape easily because of the extreme slipperiness. This observation inspired research on whether the TA–protein interaction exists universally, and the development of materials that can facilitate catching a fish (Figure 4A). The surfaces of both "yellow catfish" and loach are very slippery in pure water (Figure 4B, black curve), but presented with high friction force after treatment by TA (Figure 4B, green curve). Thus, we hypothesized a similar functional mechanism as the above mentioned. Thus, the loach showed low friction force in the wet state and slid fast to the bottom of the quartz tube (length of 60 cm) in 1.03 s with an average calculated velocity $\bar{v}$ of 53.09 cm s^{-1} (Figure 4C). However, after wetting with 5% TA solution and the loach slid very slowly to the bottom in 8.53 s with $\bar{v}$ of 7.03 cm s^{-1} (Figure 4D). The two experiments simply implied that TA may interact with a number of different proteins through collective hydrogen bonding and hydrophobic interactions. Based on experimental observations, a glove that can release TA upon contact with objects was demonstrated. A yellow catfish was instantly caught with the glove (Figure 4E,F), which was almost impossible to accomplish with a normal glove (Figure 4G). It is found that the surface of the fish became white in comparison with the control fish (inset bottom right).

In summary, we experimentally explored, for the first time, astringency in drinking wine/coffee and eating unripe fruits from a tribological perspective by the in situ capture of disturbing signals of friction force. The underlying chemistry comprises the interaction between polyphenolic molecules and mucoproteins, MPs. Results showed that astringency arose from the temporary failure of the hydration boundary/mixed lubrication conferred by the adsorbed MP. Different biological creatures or organs use different proteins for specific functions, and the interactions of these proteins with polyphenols are widely existent in nature. We then translated these interactions to those of artificial materials with tunable friction property by engineering MP into PAM hydrogels to simulate the tongue. Consequently, the composite gels exhibited responsive friction to TA. Furthermore, the underlying science allowed us to design a strategy to catch slippery fish easily using TA-releasing gloves.

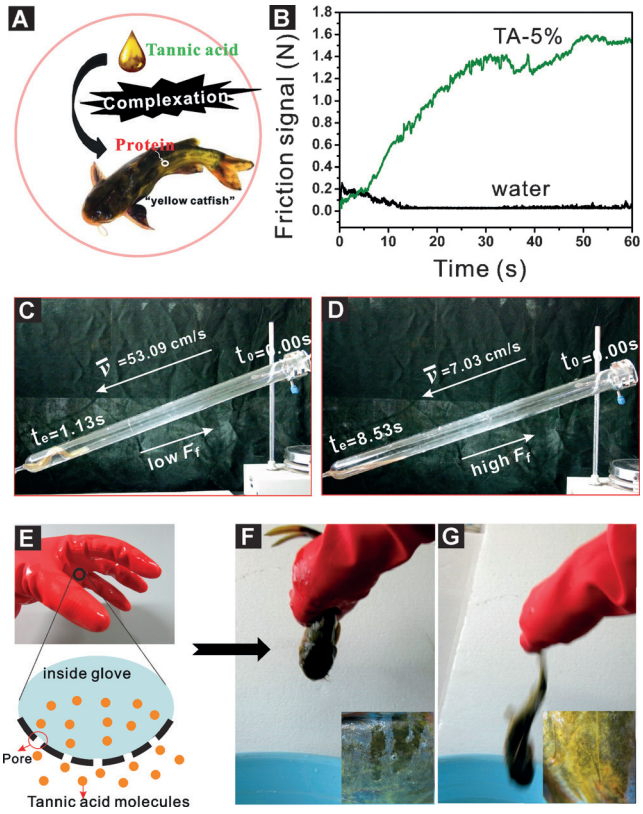

Figure 4. Responsiveness of a fish for tannic acid. A) Schematic depicting the mechanism of interaction between tannic acid (TA) solution and the protein-rich "yellow catfish" surface. B) In situ friction force signal of fish surface under pure water media (black curve) and TA media (green curve). C) Loach sliding test along a 60 cm quartz tube wetted with pure water and D) 5% TA solution. E) Scheme of catching fish using a TA-glove. F) Image showing successful catching of fish with TA-glove. G) Image showing failure of catching fish with normal glove.

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