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ALTERNATIVE MECHANISMS OF ASTRINGENCY – WHAT IS THE ROLE OF SALIVA?

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Astringency, lubrication, mucosal pellicle, saliva

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ABSTRACT

Astringency is described as a "dry puckering-like sensation" in the mouth following consumption of tannins including tea polyphenols. The current model describing astringency is based on precipitation of salivary proline-rich proteins by polyphenols and/or altered salivary lubrication. Because dryness from astringency is detected by oral tissues this suggests other interactions, possibly through direct alteration of the lubricating mucosal pellicle, which may also expose the oral mucosa below. A loss of mucosal lubrication is likely to be fundamental in astringency development and it seems likely that astringent stimuli alter the salivary bulk, saliva rheology and the saliva pellicle leading to an increase of friction in the oral cavity.

PRACTICAL APPLICATIONS

For the consumer, high levels of astringency in foods may lead to them becoming unpalatable. However, as the health benefits of many plant-based astringent molecules, such as polyphenols, have become more apparent there is a desire to increase their levels in the diet. Currently, because of the complexity of astringency and the likelihood of multiple mechanisms occurring simultaneously, there is still a gap in knowledge regarding how these mechanisms affect each other and how these lead to altered mouthfeel after consumption of astringent stimuli. A good understanding of astringency development in the oral cavity may lead to an advancement of mechanisms that may prevent the astringency without reducing levels of the astringent molecules in food.

WHAT IS ASTRINGENCY?

Astringency occurs when the oral cavity is exposed to astringent molecules (Green 1993). More specifically, it is defined as "the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins" by the American Society for Testing of Materials (ASTM 2004). These astringent molecules are commonly plant-based products, most commonly tannins, present in fruits and leaves or bark. The most common form of exposure to these products worldwide is through drinking tea, which contains a group of tannins known as polyphenols. Other astringent compounds include acids and metal alums (Bajec and Pickering 2008) and other dehydrating agents such as alcohols (Lee *et al.* 2012), which can also create the subjective feeling of astringency. Frequently, the feeling of astringency is felt alongside bitterness or sourness (Lee and Lawless 1991); however, these taste perceptions are likely to be a result of another mechanism.

Astringency is not thought to arise like the prototypical five tastes: sweet, sour, salty, bitter, and umami (Delwiche 1996), which evoke responses on the taste buds through G-protein-coupled receptors and ion channels (Chaudhari and Roper 2010). Simon *et al.* 1992, has shown that tannic acid, an astringent stimulus, can inhibit ion transport via

amiloride-inhibitable channels in isolated epithelial cells (Simon *et al.* 1992). However, astringency is considered by most to be a tactile sensation, detected by increased activation of mechanoreceptors, located within the mucosa, presumably because the loss of lubrication increases friction (Breslin *et al.* 1993) and the force required to move tissues such as the tongue around the mouth. However, there has been some evidence in rodents that the tongue's lingual nerve, which detects tactile sensations, along with heat and pain, is not affected by astringent stimuli (Schiffman *et al.* 1992). This is unlike the chorda tympani nerve, which transmits anterior tongue tastes in a concentration-dependent manner (Schiffman *et al.* 1992).

The most established mechanism of astringency involves the interaction of tannins with specific salivary proteins (Baxter *et al.* 1997; Charlton *et al.* 2002). This has developed from the observations that proline-rich proteins (PRPs) and histatins readily interact with tannins (Bennick 2002). Initially these interactions cause soluble aggregates but with more tannins these become insoluble and precipitate (Baxter *et al.* 1997). How these aggregates cause the sensation of dryness is unclear. Two mechanisms have been proposed – that the interaction of tannins causes a disruption of the lubricating salivary film which covers all oral surfaces causing friction in the oral cavity, or that this leads to exposure of the oral mucosa allowing tannin–protein aggregates to interact directly with oral tissue possibly through receptors. It is also possible that the free tannins alone interact directly with the mucosa/receptors after disruption of the pellicle by tannin–protein aggregates.

Originally, the feeling of astringency was thought to be a protective deterrent to discourage consumption of these plants and other products (Mehansho *et al.* 1987; Robbins *et al.* 1991). Tannins were thought to have a harmful effect on humans (Lu and Bennick 1998) and are believed to reduce the nutritional value of foods by reducing intestinal adsorption (Jobstl *et al.* 2004). They have been shown to inhibit digestive enzymes (Naz *et al.* 2011). Thus salivary protein adsorption could prevent toxicity of the high-molecular weight tannins, which is believed to be the primary role of basic PRPs in saliva (Mehansho *et al.* 1987; Jobstl *et al.* 2004), it may also help reduce their effects on oral mucosa (Horne *et al.* 2002). Reaffirming this protective aspect of PRPs, in some species PRP stimulation has been seen to increase upon regular consumption of tannins in the diet (Mehansho *et al.* 1985).

However, it is now known that there are several potential physiological benefits from the consumption of polyphenols (Cooper 2012), and we are now encouraged to consume some of these tannins, including polyphenols in tea. Epigallocatechin gallate (EGCG), a major component of green tea, is known to have anti-inflammatory properties (Zhong *et al.* 2012) and along with other tannins has anticancerous effects and antioxidant activity (Kim *et al.* 2012; Narotzki *et al.* 2012). Therefore tannin consumption in moderation is likely to be an important part of a healthy lifestyle. Understanding astringency mechanisms caused by such stimuli may enable us to increase their concentration in foods without making tea and other products too astringent, because it is the astringency that prevents the greater ingestion of polyphenols. Ares *et al.* 2009, explored the possibility of using a sweeter taste to lower astringency perception with sucrose and an artificial sweetener, which had a certain level of success depending on the astringent compound (Ares *et al.* 2009). They also found milk and a fat-mimetic, polydextrose, inhibited astringency. This was speculated to be due to the milk–proteins/polydextrose complexion with polyphenols, which provides competition for the salivary proteins and would therefore lower astringency, through hydrogen binding (Plug and Haring 1993).

NORMAL MOUTHFEEL

To understand the development of astringency, it is important to understand how the normal mouthfeel is developed and maintained. The normal feeling is mostly a tactile feeling with no abrasion between rubbing surfaces (e.g., tongue on palate) aided by hydrated surfaces maintained by a thin film of saliva.

Whole mouth saliva (WMS) is produced by the three main salivary glands: sublingual, submandibular and parotid, along with many minor salivary glands located throughout the oral mucosa. Saliva consists of over 99% water, the remainder containing a mixture of organic and inorganic substances (Engelen *et al.* 2007). This includes a variety of electrolytes, immunoglobulins, proteins, enzymes, highly glycosylated mucins and other substances such as urea and ammonia (Humphrey and Williamson 2001). There is large variability of salivary flow rates among individuals. Normal flow rate is accepted to be greater than 0.2 mL/min for unstimulated saliva. During periods of eating or chewing, the whole salivary flow rate may be twice as high as the resting rate. As well as a higher flow rate, eating or drinking also causes the different salivary glands to become activated to different extents. For example the parotid gland increases its proportion of the total WMS from 30 to 70% (Sas and Dawes 1997). Taste has been shown to lead to a greater level of stimulation than chewing alone (Watanabe and Dawes 1988). The increased salivary flow during tasting and chewing food helps return mouthfeel back to "normal" and protect the oral cavity from any adverse effects of the food. For example, acidic beverages stimulate the greatest flow from the parotid gland

and return the pH back to normal faster than resting saliva (Millward *et al.* 1997) protecting the teeth from acid-erosion.

The importance of saliva as an oral lubricant for the normal mouthfeel is well demonstrated by people who suffer from xerostomia, the feeling of a severely dry mouth. These subjects have a reduced salivary flow rate of <0.1 mL/min (Nederfors 2000). This can be caused by diseases such as Sjögren's syndrome or as the result of radiation treatment for cancer or as a side effect of prescribed drugs (Narhi 1994). However, there are a number of people who perceive that they suffer from a "dry mouth," yet have a normal salivary flow, which could be a result of dehydration (Atkinson *et al.* 2005) or could be a result of abnormal salivary physical properties. Although saliva is composed of 99% water, it does not behave like water. It is the salivary proteins and ions that create the physical properties of saliva that allow it to play an essential role in the maintenance of a normal mouthfeel. Some of the more abundant proteins in the saliva include salivary mucins MUC5B and MUC7, as well as the secretory immunoglobulin A (sIgA), acidic, basic and glycosylated PRPs (gPRPs), statherin, histatin, carbonic anhydrase VI and amylase.

Salivary mucins MUC5B and MUC7, produced by submandibular and sublingual glands, and gPRPS are essential for the lubricating properties of saliva because of their structure (Slomiany *et al.* 1996; Inoue *et al.* 2008; Boze *et al.* 2010). MUC5B, the higher molecular weight mucin (>1,000 kDa), is composed of multiple highly glycosylated covalently linked subunits. MUC7 has a lower molecular weight (200–300 kDa) and is a single glycosylated peptide chain (Slomiany *et al.* 1996; Wickstrom *et al.* 2000). Mucins have low solubility, while being highly viscous and elastic with a stringy adhesive quality (Inoue *et al.* 2008). These mucins are also believed to be involved in adsorbed protein layer at the cell–liquid interface of saliva, the mucosal pellicle, which creates the lubricating layer.

Salivary PRPs account for approximately 70% of parotid saliva (Kauffman and Keller 1979) and are classed as acidic, basic and gPRPs, although both the acidic and basic PRPs may be glycosylated to some degree. The primary role of the basic PRPs is thought to be tannin binding, which could be crucial in the development of astringency (Jobstl *et al.* 2004). Like statherin and histatin, acidic PRPs are thought to be important for the pellicle development on teeth (Campese *et al.* 2009). gPRPs are also seen to precipitate some tannins (Asquith *et al.* 1987; Boze *et al.* 2010), and because of their structure are thought to contribute to salivary lubrication (Chan and Bennick 2001) along with salivary mucins. These two functions of gPRPs (the ability to interact with tannins and their lubricating properties) could have an impact on each other with regard to mouthfeel following consumption of tannins.

SALIVA RHEOLOGY

Oral health and physiochemical processes present in the oral cavity can be affected by the rheological properties of saliva and lubrication is essential to prevent abrasion and damages to surfaces in the mouth (Stokes and Davies 2007). Lubricating properties of saliva are dependent on saliva's viscosity, its ability to resist deformation, and how this is changed by shear rate, which can affect its efficacy (Waterman *et al.* 1988). Shear rate can increase to high levels when swallowing and speaking, which can decrease viscosity (van Aken *et al.* 2011). If saliva becomes too viscous, this can also inhibit its ability to lubricate the mouth, and lead to oral diseases such as caries or periodontitis (Inoue *et al.* 2008). Different rheological properties have been identified in saliva produced by the different salivary glands, with mucin-rich submandibular/sublingual secretions being most viscous and viscoelastic, and parotid saliva secretions being the least viscous and viscoelastic saliva. These various salivary secretions contribute to rheology of WMS and contribute to its viscoelasticity and extensional rheology, aiding in the maintenance of a normal mouthfeel. Viscosity is reported to be lower in chewing stimulated whole saliva, where parotid saliva contribution would have been increased, compared with unstimulated WMS (Inoue *et al.* 2008).

SALIVA FILM

The salivary film is a layer of saliva coating all surfaces in the mouth. This salivary film can be measured and has varying thickness on different surfaces in the mouth (Pramanik *et al.* 2010), but on average, a thickness of 70–100 μm (Collins and Dawes 1987). The velocity of the film also varies throughout the mouth depending on location and changes in saliva stimulation. When unstimulated, the velocity of the saliva film between the lingual and mandibular incisors is 8 mm/min, but following chewing gum can increase to 300 mm/min (Dawes 2008). The velocity of this film can contribute to the friction in the mouth and alter the lubricating properties of the film, explained later.

At the point where air meets the salivary liquid interface, studies by Waterman *et al.* 1988, have shown that a protein layer is adsorbed. Proctor *et al.* showed statherin to be a predominant salivary protein present in this layer (Proctor *et al.* 2005). This protein layer can affect the saliva and the salivary film's response to shear stress. At low levels of shear stress, a substance with yield-stress appears solid-like because of its elastic behavior, but acts like a viscous liquid when exposed to high shear stresses (Holterman *et al.* 1990). This characteristic yield-stress/shear–thinning

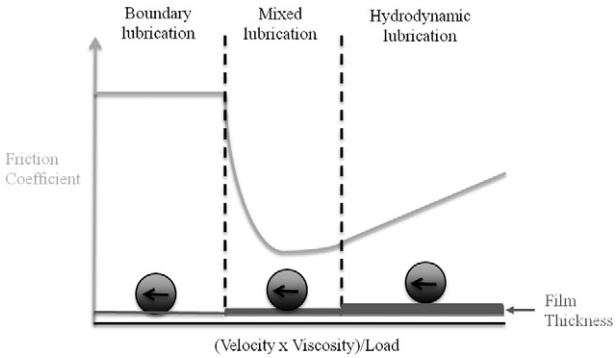

FIG. 1. STRIBECK CURVE SHOWING CHANGES IN FRICTION AND HOW THE SALIVA FILM THICKNESS COULD BE ALTERED DEPENDING ON THE FILM VELOCITY AND VISCOSITY AND HOW THIS IS AFFECTED BY LOAD. THE TYPE OF LUBRICATION IS INDICATED ABOVE THE GRAPH. FIGURE ADAPTED FROM COLES *ET AL*. (2010)

behavior is seen with submandibular saliva and WMS, but not with parotid saliva and so may be due to the presence of the mucins in the former.

The salivary film plays a major role in lubricating the oral cavity, and this can be significantly affected by the consumption of food and drinks (Chen and Stokes 2012). The high viscoelasticity of the salivary film, because of proteins including mucins (Coles *et al*. 2010) among others, can play a large role in its response to consuming food and drink and lead to changes in lubrication. Figure 1, a Stribeck curve, shows how film thickness and friction between surfaces can change in the mouth. The salivary film dominates the interactions of surfaces, where the friction coefficient is a function of fluid viscosity and shear velocity (Coles *et al*. 2010). Surfaces in close contact undergo boundary lubrication, where in the mouth, the salivary film needs to have a low surface energy (Coles *et al*. 2010), proteins need to be well retained or replaced easily by saliva after shearing. At this point, the film is not thick enough to prevent contact and the film velocity too low to prevent the load causing high friction. However, it has been shown that lubricating properties can still be present following the removal of bulk viscoelastic properties with regards to boundary lubrication (Bongaerts *et al*. 2007), where surfaces are in contact and salivary film is thin (Coles *et al*. 2010; Chen and Stokes 2012).

Mucins are likely to be crucial here to maintain this lubrication as they bind strongly to surfaces and are both sterically and electrostatically repulsive when in formed layers (Coles *et al*. 2010). These proteins will affect which type of lubrication regime will dominate following food and drink (see Fig. 1). Introduction of food or drink can increase fluid film viscosity and velocity, and may reduce load pushing the lubrication into the mixed state on the graph, where the salivary film builds up and prevents abrasion causing a significant decrease in friction, although this can build up again if hydrodynamic lubrication is reached where the film has continued to thicken and introduces fluid drag. This can be seen with some fatty/oily products that can introduce viscosity, which can result in hydrodynamic friction on the lubricating surfaces in the mouth (van Aken *et al*. 2011).

SALIVARY MUCOSAL PELLICLE

Part of the saliva's protective properties stem from its ability to form a pellicle, a proteinaceous coating on the surfaces in the oral cavity. On teeth, this is called the acquired enamel pellicle and is well characterized for its protective functions (Lendenmann *et al*. 2000; Hannig *et al*. 2008). On the mucosa a pellicle also forms (Bradway *et al*. 1989) with salivary proteins directly binding to the oral epithelium and being stabilized by protein cross-linking (Bradway *et al*. 1992). This provides a surface for the more mobile salivary film to traverse. At the most basic level the salivary pellicle functions as an actual physical barrier, and like the salivary film, one of its most essential functions is its contribution to lubrication, helping to prevent abrasion between surfaces, improving swallowing abilities and contributing to a normal mouthfeel (Humphrey and Williamson 2001; Hannig *et al*. 2005). This protective pellicle consists of multiple salivary proteins including mucins MUC5B and MUC7, statherin, sIgA, carbonic anhydrase VI and cystatin S (Cardenas *et al*. 2007).

The mucosal pellicle forms within minutes of exposure of saliva to the oral cavity (Dickinson and Mann 2006; Hannig and Hannig 2009). Proteins such as statherin and PRPs are thought to initiate this pellicle formation on the teeth (Bradway *et al*. 1992; Yao *et al*. 2000). This may be further aided by the enzyme transglutaminase. Transglutaminase has been shown (Bradway *et al*. 1989, 1992) to be present within the oral cavity and Lamkin *et al*. (1995) demonstrated the presence of a protein that they think represents a cross-link between a basic PRP and statherin, catalyzed by transglutaminase (Lamkin *et al*. 1995). Yao *et al*. (2000) also showed that transglutaminase was able to catalyze a cross-link between acidic PRP-1 (24 kDa) and statherin (8 kDa) *in vitro* (Yao *et al*. 2000). These studies suggest that some of the tannin-interacting PRPs are present within the mucosal pellicle.

How salivary mucins become bound to the epithelial surface is not clear. Possibly, they entangle with cell membrane-bound mucins such as MUC1 or other proteins (Coles *et al*. 2010). Salivary MUC5B is a gel-forming mucin (Cone 2009) and calcium has been shown to aid gel formation of MUC5B from saliva by creating protein cross-links (Raynal *et al*. 2003). On a hydrophobic surface Macakova *et al*. (2010) have shown that a pre-absorbed saliva film can be formed. Along with small salivary proteins, the

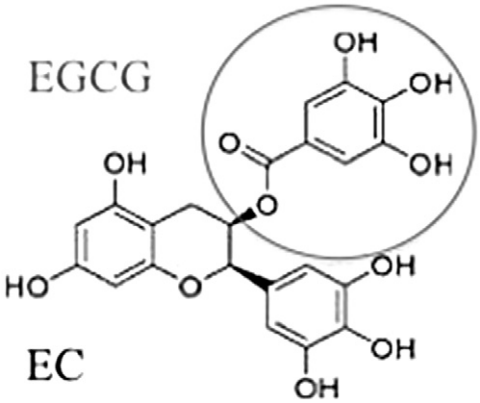

FIG. 2. EC AND EGCG, WHICH DIFFER IN STRUCTURE BECAUSE OF THE ADDITION OF A GALLOYL GROUP (CIRCLED) ON EGCG
EC, epicatechin; EGCG, epigallocatechin gallate.

nonglycosylated regions of mucin form a dense anchoring layer with protruding hydrated viscoelastic layer of glycosylated proteins (Macakova *et al.* 2010). This could provide an essential barrier to particles and debris that could damage the oral cavity and contributes to the normal lubricious mouthfeel (Yakubov *et al.* 2009). However, a crucial point to make here is that this protein layer can collapse irreversibly in the presence of de-ionized water, which in the mouth could lead to a severely altered mouthfeel (Macakova *et al.* 2010).

MUC7 may further aid in this pellicle process by forming heterotypic complexes with sIgA and lactoferrin, which may improve their incorporation into the pellicle layer (Biesbrock *et al.* 1991; Soares *et al.* 2004). IgA is required for immune protection in the oral cavity (Phalipon *et al.* 2002) and is commonly secreted as sIgA. The secretory component attached to IgA could also aid in its incorporation into the mucosal pellicle and could protect against proteolytic activity (Ma *et al.* 1998).

CURRENT UNDERSTOOD MECHANISMS OF ASTRINGENCY

Green tea alone contains several polyphenols, which are known to be astringent: EGCG, EGC, epicatechin gallate (ECG) and EC. The structure of some of these catechins can be seen in Fig. 2. These molecules have been studied as model polyphenols to understand the perception of astringency using taste panels. These astringents have dose-dependent astringent qualities (Rossetti *et al.* 2009), with increasing astringency as the number of galloyl rings increases as indicated in Fig. 2 (Rossetti *et al.* 2009; Narukawa *et al.* 2010).

These tea polyphenols and other astringent molecules are currently believed to cause the feeling of astringency through aggregation and precipitation of salivary proteins (Jobstl *et al.* 2004), particularly PRPs (Lu and Bennick 1998) and histatins (Naurato *et al.* 1999), which have both shown the ability to easily form complexes with polyphenols (Soares *et al.* 2011). More recently, this has been shown with the salivary mucins MUC5B and MUC7 (Gambuti *et al.* 2006; Lee *et al.* 2012). This mechanism is summarized in Fig. 3, and involves the polyphenols binding to the salivary proteins, which causes them to aggregate together,

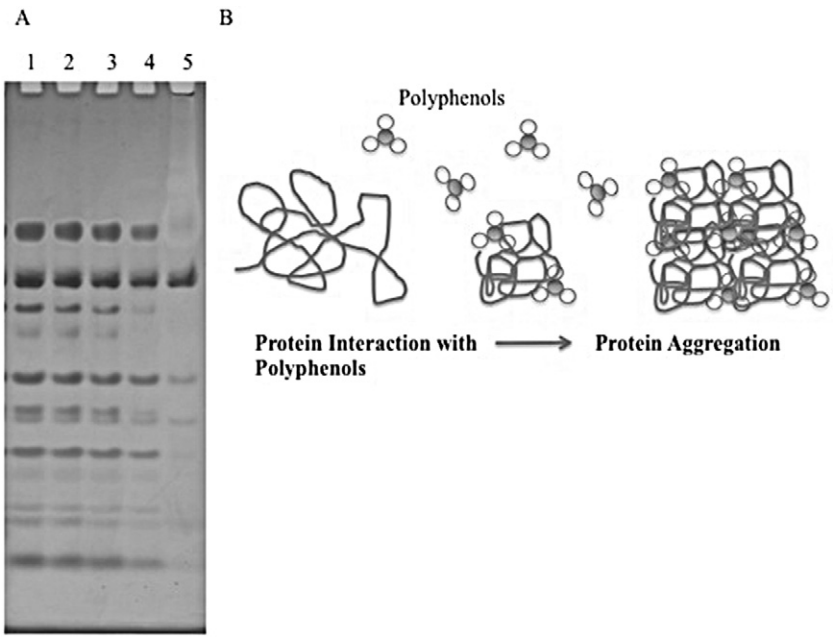

FIG. 3. (A) NONREDUCED GEL ELECTROPHORESIS SHOWING INCUBATION OF PS WITH WATER AND DIFFERENT POLYPHENOLS
Lanes show: 1-PS, 2-PS with water, 3-PS with EC 5 mg/mL, 4-PS with EGC 2 mg/mL, 5-PS with EGCG 2 mg/mL. (B) Diagram shows compaction and aggregation of salivary proteins upon polyphenol binding. EC, epicatechin; EGCG, epigallocatechin gallate; PS, parotid saliva.

ultimately leading to their precipitation out of saliva. These interactions are thought to occur due to hydrophobic forces and the development of hydrogen bonds (Bennick 2002). These polyphenol–protein precipitates may themselves add to a drying feeling in the mouth through a gritty feeling or interruption of the salivary film. However, this alone does not appear to fulfill the astringent feeling described as constriction of the epithelium and strong dry puckering feeling.

There are also mixed reports showing that some astringent molecules are unable to precipitate different proteins, which can be seen in Fig. 3A. EC was unable to precipitate the proteins in the parotid saliva, despite being used at the concentration that is perceived to be just as astringent as the EGCG (Rossetti *et al.* 2009). However, this disagrees with work by Kielhorn who suggests that EC is not perceived to be as astringent as tannic acid, and in fact suggest it causes a bitter taste (Kielhorn and Thorngate 1999). Lee *et al.* have also shown this year that PRPs were not precipitated by acid (HCl) and that the tannin they used was unable to precipitate mucin (Lee *et al.* 2012). Therefore, protein aggregation and precipitation is likely to just be a contributing factor to the development of astringency.

SALIVA AND POLYPHENOLS

As previously mentioned, basic PRPs and histatins are known to be significantly precipitated by tannins. PRPs have an extended structure allowing them to bind more than one astringent molecule and the structure of the precipitate formed can vary because of astringency molecules bound (Monteleone *et al.* 2004; Canon *et al.* 2010). These tannin-PRP complexes are usually insoluble and can be maintained in environments similar to those through the digestive tract (Lu and Bennick 1998). Basic PRPs are not thought to be important for saliva's lubricating properties unlike gPRPs (although some basic PRPs are glycosylated). Initially it was thought that without the removal of its carbohydrate side chains through deglycosylation, gPRP showed no binding affinity with tannins (Lu and Bennick 1998). However, this seems to be largely dependant on the tannin used (see Fig. 3). Lee *et al.* (2012) and others (Soares *et al.* 2011), have shown precipitation of the glycosylated PRPs as well basic PRPs and acidic PRPs by both tannin and alum, but not with acid (Lee *et al.* 2012). MUC5B and MUC7 are also both shown to be precipitated by alum and acid, but not by tannin by Lee *et al.*, which opposes work by others (Gambuti *et al.* 2006). This indicates that there are likely to be more astringent mechanisms occurring in the mouth and protein precipitation is not the only cause, but does highlight that proteins essential for lubrication and normal saliva rheology, such as mucins and gPRP as mentioned earlier, may be altered and affect their ability to maintain a normal mouthfeel.

SALIVARY FILM AND PELLICLE CHANGES BECAUSE OF POLYPHENOLS

This review has tried to describe saliva as a multi-modal system – it exists as a fluid in the mouth and an absorbed layer on surfaces. Few studies concerning astringency have made that distinction. If the PRPs and histatins are important in the perception of astringency their removal from the mouth should decrease the perceived astringency. In a simple experiment by the authors the opposite was found. When volunteers washed out their mouths with water (to remove salivary PRPs) the perception of a tea solution increased (Nayak and Carpenter 2008). This counter-intuitive result suggests that the mucosal pellicle is more important than the salivary film for detecting astringency. Differences in subjects responses to astringents and flow rate also suggest that the salivary concentration of proteins interacting with astringent molecules is not so important (Dinnella *et al.* 2010). Together these studies suggest that the salivary film has a protective or inhibitory role whereas the mucosal pellicle is where the astringent feeling is developed.

Saliva easily forms a film or pellicle on different surface types, which is a mechanism that can be used to aid the understanding of how astringent compounds interact with these films. These can be studied using different techniques including using a quartz crystal microbalance with dissipation (Yao *et al.* 2010), du Nouy ring rheometer (Rossetti *et al.* 2008), surface plasmon resonance (Macakova *et al.* 2010), ellipsometry (Joiner *et al.* 2004) and mini traction machine (Rossetti *et al.* 2009).

Ellipsometry studies have shown that tea polyphenols can incorporate themselves into the salivary film already pre-adsorbed onto hydroxyapatite and indicated that subsequent attempts to add more saliva after tea polyphenol incorporation led to an easily removed film unlike the original saliva layer, which was more firmly adhered (Joiner *et al.* 2004). Lee *et al.* has also shown that tannins and astringent molecules were unable to remove significant levels of mucin from the oral cavity (Lee *et al.* 2012). This work has been re-affirmed by Yao *et al.* who also showed a strong formation of EGCG adlayers to a salivary film, which showed a greater affinity to WMS films as opposed to parotid saliva films. However, work completed by McColl *et al.* has shown that EGCG when mixed with a mucin layer improved its adsorption to a surface and also slowed its desorption (McColl *et al.* 2009). These data sets indicate that the salivary film and pellicle in the mouth could be altered by astringent molecules affecting their properties and leading to an altered mouthfeel. It may also indicate that in the mouth astringent compounds may increase pellicle development, which could lead to decreased friction between surfaces and improved mouthfeel after an astringent

solution. Thus, it may be as important to examine changes to mouthfeel after an astringent solution as during.

Studies looking at the interfacial properties of the salivary film have also shown the ability of polyphenols and other astringent compounds to alter the salivary film leading to changes in its rheological and lubricating properties. Recently, Rossetti *et al.* in 2008 showed that at low concentrations EGCG increased the shear elasticity of the saliva film, which is believed to indicate that the tannin is absorbing at the air liquid interfaces (Rossetti *et al.* 2008). However, the interfacial shear elasticity of a salivary film was significantly reduced when WMS was mixed with EGCG at a higher concentration or citric acid (Rossetti *et al.* 2008), which is likely to be due to protein aggregation disrupting the salivary film, indicating that there is likely to be reduced lubrication ability of saliva following astringent compound consumption. Mucin has also shown to have a reduced spreading effect when mixed in a layer with EGCG (McColl *et al.* 2009). However, another astringent polyphenol EC, which has no galloyl ring yet is perceived as astringent (Rossetti *et al.* 2009), shows no alteration of the elasticity of the salivary film (Rossetti *et al.* 2008) or reduction of salivary lubrication (Rossetti *et al.* 2009). More recently, Lee *et al.* have shown that a tannin–saliva mix has resulted in increased friction compared with a water–saliva mix when measured instrumentally (Lee and Vickers 2012). However, this was not detectable by subjects testing this between their fingers, although I would question if the technique would be sensitive enough for this result to be conclusive. This shows the complexity of astringency and re-affirms the point that there may be multiple mechanisms involved in astringency development.

RECEPTORS AND THEIR ROLE IN ASTRINGENCY

As mentioned earlier, astringency is a complex sensation and usually accompanied by other tastes including bitterness (Rossetti *et al.* 2008). The development of some taste sensations, such as bitterness, involves receptors in the mouth and it is possible that these mechanisms could be involved in the development of astringency. Likewise, it is also possible that the mechanisms work in conjunction through exposure of the receptors on the mucosa through stripping/alteration of pellicle by astringent compounds. Tea polyphenols, particularly those including the galloyl ring, have been shown to activate human bitter receptors hTAS2Rs (Narukawa *et al.* 2011). Further works have indicated that EGCG activates transient receptor protein channel, resulting in a Ca^{2+} response, which the authors suggested could occur on the tongue and contribute to the astringent taste in the mouth (Kurogi *et al.* 2012). It is possible that free tannins, not interacted with salivary proteins, directly affect these receptors, which may also include a 67 kDa lamin receptor shown to interact with EGCG (Schwarz and Hofmann 2008).

The delivery of the astringent stimuli could also affect its perception. Chemical irritations to the mouth such as heat can trigger a response from nociceptors that are within the mucosa or buried just below the epithelium surface (Green 1996). This is generally a delayed effect that may decrease the sensation of taste in the mouth. However, as the likely mechanism of astringency involves a tactile sensation, as previously mentioned (Bajec and Pickering 2008), it could be that heat can reduce its intensity, but this could also be counteracted by movement in the mouth (Green 1996). In 1993, Breslin *et al.*, showed that just the taste of astringent molecules on the tongue led to alum being perceived as less astringent when compared with a mixture with a strong taste. However, when exposed to an alternative oral surface where there would be movement against another, the astringent perception was reversed indicating the importance of the movement and surface interaction (Breslin *et al.* 1993). This is most likely to involve the mechanisms suggested earlier where lubrication may be reduced because of interactions with the saliva and the salivary mucosal pellicle leading to reduced lubrication. The tongue also has mechanoreceptors, both superficial slow-adapting and fast-adapting receptor units (van Aken 2010), which may be more likely to respond to this mechanism than any gustatory receptors because of friction from reduced lubrication (Breslin *et al.* 1993).

PERCEPTION AND INDIVIDUAL VARIATION

A large factor in the development of astringency in the oral cavity is going to be due to individual perception. The source of the astringent molecule itself will also have an effect on its perception depending on how it alters saliva stimulation (Dinnella *et al.* 2009) and how this affects movement in the mouth (Navazesh and Kumar 2008). Individuals will also vary in their response to stimuli and how quickly they can restore saliva back to its normal composition (Dinnella *et al.* 2009, 2011).

Total salivary protein concentration has shown no correlation with astringency perception (Kallithraka *et al.* 2001). Despite PRPs showing greater aggregation levels with astringency stimuli in astringency, PRP-rich parotid saliva protein levels have also shown no correlation with astringency (Guinard *et al.* 1997). However, Kallithraka *et al.*, in 2001, did show a positive correlation with the reduction of hydrophilic PRP in saliva following astringent stimuli, which may indicate a crucial protein that could result in a greater level of astringency when it is at lower levels in

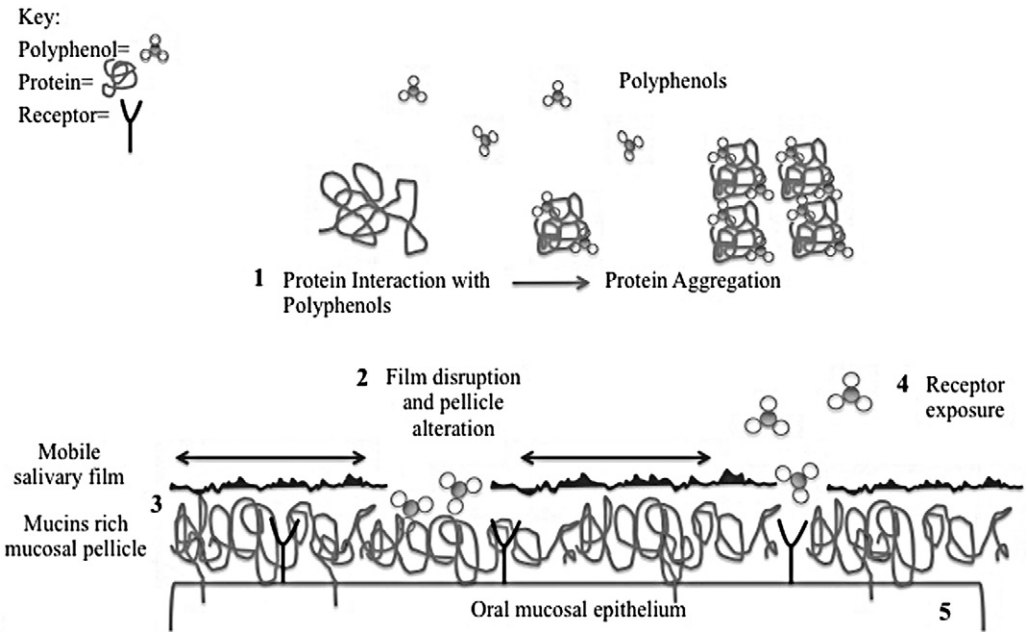

FIG. 4. POSSIBLE MECHANISMS OF ASTRINGENCY OCCURRING SIMULTANEOUSLY IN THE ORAL CAVITY: 1. AGGREGATION OF SALIVARY PROTEINS CREATING GRITTINESS, 2. SALIVARY FILM DISRUPTION, 3. REDUCED SALIVARY LUBRICATION, 4. POSSIBLE EXPOSURE OF RECEPTORS, 5. NOCICEPTORS/MECHANORECEPTORS OR NERVE INNERVATION

saliva. More recently, Dinnella *et al.*, in 2010, showed that volunteers who had a higher response to astringent stimuli corresponded to a reduction in gPRP because of aggregation (Dinnella *et al.* 2010).

Research is yet to show any difference in astringency perception dependent on age or gender (Sowalsky and Noble 1998; Michon *et al.* 2009). Presumably the difficulties of working with children have prevented the studies that might show their increased sensitivities to taste, texture and probably astringency, which are obvious to most parents. The elderly have been shown to have a lower taste perception for sweetness than younger adults (Kennedy *et al.* 2010), although again, the evidence for increased liking of astringency in the older population seems lacking.

SUMMARY

Clearly astringency is a complex sensation and it is likely that multiple mechanisms are occurring simultaneously (see Fig. 4). Precipitation of salivary proteins, in particular the PRPs, is clearly not the only mechanism in astringency development, because not all astringency compounds cause salivary protein precipitation and not all agents that cause salivary protein precipitation cause astringency.

A physical interaction mediated by loss of lubrication appears to be an important factor in the development of astringency. This may be due to protein interaction/precipitation effects on bulk WMS, but there are increasing number of studies to indicate that the mucosal pellicle has an important part to play as seen in Fig. 4. The polyphenol/protein aggregates may disrupt the salivary film and alter lubrication, or this may be due to the free polyphenol that is not aggregated. This in turn could also lead to exposure of the pellicle and its ability to protect and lubricate the epithelium may be reduced. Lastly, this could also expose receptors that could aid in the feeling of astringency or reduced lubrication could trigger responses from mechanoreceptors.

How astringent molecules alter the physical properties of the salivary film and pellicle over time also seems to be important. Often only single time points are taken from experiments when clearly from a consumer point of view astringent solutions are enjoyed because they alter the mouthfeel (from thirst, for example) to a state of improved hydration/lubrication after drinking (McEwan and Colwill 1996). Thus, the after-effects of astringent compounds represent an important future area of research in understanding astringency.

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