Quantification of oral roughness perception and comparison with mechanism of astringency perception

THESIS

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By

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Abstract

Texture plays a vital role in food preference and acceptance, but oral tactile texture perception is incompletely understood. A wide body of information currently exists on tactile perception of the hands and fingertips. Tactile perception in the hands and finger tips is mediated by nerve fibers, both slowly adapting and fast adapting, which terminate in four different types of mechanoreceptors; Merkel cells, Meissner corpuscles, Ruffini endings, and Pacinian corpuscles. These same mechanoreceptors are present in the tongue, with the exception of Pacinian corpuscles. It therefore, should be possible to use the knowledge of tactile perception in the skin and translate this to a better understanding of tactile perception of the tongue and other oral surfaces.

In this study, individual sensitivities to oral tactile roughness are quantified, and the factors that may drive potential differences in sensitivity are examined. Additionally, a relationship between astringency and oral mechanisms of tactile roughness perception is investigated. If the sensation of astringency is purely the result of friction between oral surfaces due to decreased lubricity following astringent- protein complex precipitation, then an individual's sensitivity to tactile roughness, perceived by activation of oral mechanoreceptors, should be indicative of how sensitive they are to astringent stimuli. It was hypothesized that individuals highly sensitive to tactile roughness would also exhibit high sensitivity to astringent stimuli.

Detection thresholds and suprathreshold sensitivities were determined for surface roughness, epigallocatechin gallate (EGCG) astringency, and tannic acid (TA) astringency from 30 individuals. Roughness stimuli included 7 stainless steel coupons with surface roughness (R_a) ranging from 0.177-0.465µm, measured by optical profilometry, and five pieces removed from an electroforming comparator with surface roughness ranging from 0.51 to 22.8 µm. Astringent stimuli included 10mL samples, concentration ranges 0-0.7g/L (EGCG) and 0-1.0g/L (TA). DT was assessed for each stimulus set using the staircase method. Suprathreshold sensitivity was assessed by asking subjects to rate perceived roughness (comparator stimuli) or astringency (EGCG and TA) using the gLMS. Salivary flow rate, fungiform papillae count, and astringent food consumption data was collected.

Participants were divided in to high sensitivity to roughness (RHi, n=16) and low sensitivity to roughness (RLo, n=14) groups, which differed significantly in their roughness detection thresholds ($p<0.001$). RHi participants were able to detect significantly lower amounts of EGCG than RLo ($p<0.05$). A significant positive correlation was also seen between roughness suprathreshold and EGCG astringency suprathreshold. This relationship was not seen between roughness sensitivity and TA astringency. When participants were divided in to high (n=15) and low salivary flow (n=15), however, TA detection threshold was seen to be significantly more affected by salivary differences than EGCG, with low salivary flow participants performing significantly worse on the TA detection threshold task ($p=0.05$).

These findings demonstrate that there are quantifiable differences in individual ability to discriminate oral tactile roughness. This tactile sensitivity was notably found not to relate to fungiform papillae density. Findings also illustrate that astringency perception is influenced by at least two key factors; oral tactile roughness sensitivity and salivary differences, and the factor which predominates in importance is dependent on the nature of each astringent. Consumption data presents suggestions for ways in which roughness and astringency sensitivity may contribute to consumption and perceived pleasantness of complex food products like dark chocolate and red wine.

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Ch. 1 Literature Review

1.1 Texture and Tactile Perception

In recent years there has been a prolific increase in tactile research fueled by the advancement of recent technologies including touch-sensitive consumer products, touch-sensing robotics, and virtual reality and remote sensing (Skedung et al. 2013). Recent publications in prominent journals on the topic of tactile perception further underscore the emphasis being placed on its study (Scheibert et al. 2009, Mate et al. 2011, Wandersman et al. 2011, Adams et al. 2013). Despite the latest push in tactile research, a disproportionately small amount has been directed toward tactile perception in the oral cavity (Kravchuk et al. 2012). Texture plays a vital role in an individual's determination of both food quality and acceptability, which combine to have a lasting impact on individuals' food product preferences (Spence et al. 2013). In a survey completed by 140 professionals working in the field of chemical senses, Delwiche (2003) reported that temperature and texture were ranked highest ahead of color, appearance, and sound, in terms of what sensory cues these professionals believe were of the greatest degree of importance to food preferences. Though a number of studies have been done, general

understanding what drives texture preferences as well as perception difference among consumers remains incompletely understood and more research must be conducted in this area in order to remedy this (Kravchuk et al. 2012, Foegeding et al. 2015).

1.2 The Study of Texture in the Oral Cavity

The first studies on oral texture perception in the late 19th century were primarily focused on using texture as a means to correct or eliminate food defects (Szcesniak 2002). Basic instrumental testing strategies such as simple puncture tests or the use of a basic viscometer were paired with chemical analyses and some sensory testing and were used as quality control standards to prevent undesirable defects in food manufacturing (Bourne 1982). In the 1950s texture began to be looked at as its own subject worthy of study and better understanding (Szczesniak 2002). Today, instead of being just a yardstick for lack of defect, texture is acknowledged as an indicator of freshness, quality of food preparation, and a major factor in food product acceptance (Fig. 1.1; left hand panel).

1.2.1 Top-Down

The majority of the research that does exist in food texture research approaches the subject by way of sensory analysis of food texture of specific food matrix categories. This "top-down" strategy looks at texture of an entire food product and might study, for example, how slight alterations in a product's formulation have the side effect of altering texture and consumer preference. Of studies published in the *Journal of Texture Studies* between 2005-2010, only 8% of food texture studies addressed the physiological and

psychological parameters underpinning oral texture perception (Fig. 1.1; right hand panel) (Kravchuk et al. 2012).

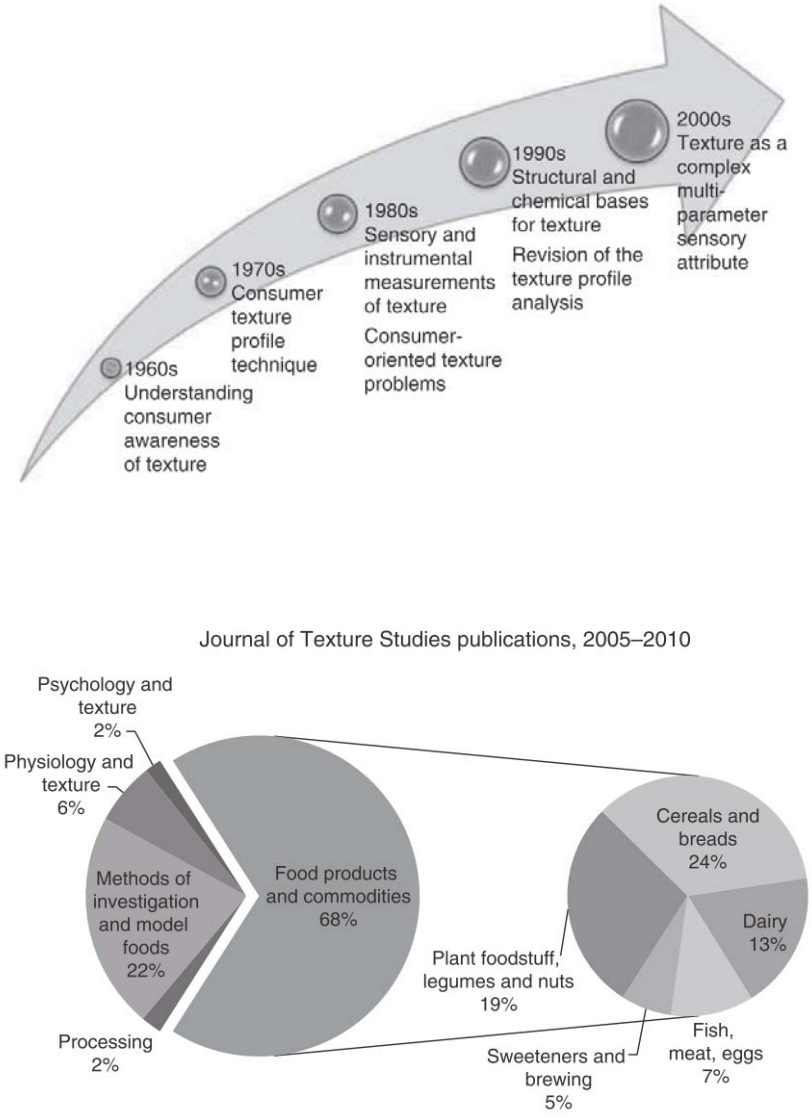

Figure 1.1 Trends in food texture research. Trends based on citations of publications by Journal of Texture studies founding editor Alina Szcsesniak (top) and distribution of food texture studies in Journal of Texture Studies from 2005-2010, borrowed from Kravchuk et al. 2012 (bottom).

A great deal of "top-down" sensory analysis studies are executed using trained sensory panels in which panelists are conditioned to associate a specific stimulus with a specific response. The most common methods for descriptive sensory analysis of texture are *Texture profile* (Bourne 1978) *and Qualitative Descriptive Analysis* or QDA (Stone and Sidel 1998). Both of these methods rely heavily on extensive training during which panelists are familiarized with specific sensations and instructed on the intensities of references (Kravchuk et al. 2012). The reasoning behind this is to treat the panelists as analytical equipment to measure textural attributes and to calibrate all members to identical intensity standards. This type of analysis, however, provides little to no information on individual texture perception (Piggott et al. 1998), nor does it relate perception to underlying physiological or psychological processing mechanisms.

Individual deviations from average panel behavior are equally as important and insightful as averaged textural responses and could contribute to a better understanding of consumer preferences (Kravchuk et al. 2012). Preoccupation with developing the ideal complete texture within a food matrix often disregards the lack of overall understanding of the influence and interaction of a food's surface microstructure, composition, and oral physiology, each of which contribute to the sensations perceived by an individual (Kravchuk et al. 2012).

1.2.2 Bottom-up

An alternative to the widely used "top-down" approach to look at texture would be a "bottom-up" approach which looks more closely at the individual texture modalities that combine to form a complete texture profile. Szczesniak (2002) defines texture as the

"sensory and functional manifestation of the structural, mechanical, and surface properties of food detected through the senses of vision, hearing, touch and kinesthetic (feeling movement)". Based on this definition, texture is a multi-parameter sensory characteristic; its perception is not based on a single attribute but rather is based on the combination of many, like chewiness, roughness, stretchiness (Szczesniak 2002). For this reason, it is logical to try to classify and understand modulators and perception of these individual modalities before rushing to trying to understand texture as a whole. We suggest that the "bottom-up" approach has an immense amount to contribute to the study of oral texture. A comprehensive understanding of the neurological mechanisms of texture perception is key to this building blocks approach.

1.3 Mechanoreceptors

Much of what is known physiologically about texture perception in the mouth is translated from findings in the hands (Foegeding et al. 2015). Texture in the glabrous skin of the hands is communicated to the brain through specialized mechanoreceptors innervating the skin at various depths connected to nerve fiber afferents which communicate the stresses and strains caused by tactile actions back to the somatosensory cortex of the brain for cognitive processing. It is accepted that there are two major classes of nerve fibers innervating the skin; slowly adapting (SA), and rapidly adapting(RA),

each subdivided in to two more specific types of nerve fibers (SAI, SAII; RAI, RAII). Each nerve fiber type is encapsulated by one of four receptor cell types, each of which respond characteristically to specific types of surface stresses and strains and are partially responsible for the attached fiber's adaptation characteristics. SAI fibers are associated with Merkel's disk receptors, SAII with Ruffini ending receptors, RAI with Meissner corpuscles and RAII with Pacinian corpuscles. In general terms, SA fiber types respond to sustained static stimulation while RA fiber types respond primarily to changes in stimulation. More specifically, Merkel disks are associated with responding to edges, points, and corner, while Ruffini endings are most closely associated with skin stretch. Meissner afferents and Pacinian corpuscles are associated with responding to general skin motion and vibration, respectively (Foegeding et al. 2015).

Surfaces of the oral cavity are innervated by the same nerve fibers as the skin of the fingers, with the possible exception of RAII and its Pacinian corpuscle mechanoreceptors, which are yet to be located in the oral surfaces (Johansson et al. 1988, Trulsson and Essick 1997, Bukowska et al. 2010). Both the tongue and the finger tips are innervated by a majority of rapidly adapting (RA) class nerve fibers, compared to the hairy skin of the back of the hand and face which is reported to be predominantly innervated by slow activing (SA) fibers (Trulsson and Essick 1997). The high density of small receptive fields associated with fibers innervating the anterior portion of the tongue suggest this surface as sensitive, if not more so, than the exceptionally sensitive glabrous skin on the finger tips (Johansson and Valbo 1983, Trulsson and Essick 1997). Most RAI and SAI receptive fields on the glabrous skin of the hand range in diameter from 2-8mm

(Johansson and Valbo 1983). Their spatial resolution on the glabrous skin of the hand allows spatial resolution of approximately 1.7mm, 4mm, and 8.33 mm in the finger tips, fingers, and palm, respectively. On the anterior portion of the tongue innervated by the lingual branch of the trigeminal nerve (CNV), RAI receptive fields have been found to have a median size of 1.80 mm^2 compared to 7.07 mm^2 for both those of the facial skin and vermilion of the lip and 7.06 mm^2 for the oral mucosa. Similarly, SA I receptive fields of the tongue have been found to be 0.80 mm^2 compared to 3.93, 7.07, and 3.14 mm^2 for the oral mucosa, vermilion, and facial skin, respectively (Bukowska et al. 2010). These receptors respond to the minute details on surfaces to which they are exposed and this information is communicated to the somatosensory cortex of the brain through the lingual branch of the trigeminal nerve (CNV). There, information processing results in conscious perception of a given textural surface. The signal patterns registered by SA and RA nerve fibers are integrated during higher processing in the brain to express the perception of specific basic textural modalities such as smoothness, roughness, or viscosity, all of importance to the eating experience. It is important to note that each mechanoreceptor type does not directly code a specific texture modality, rather each modality is coded by the combination of the signals resulting from multiple types of stresses and strains on a given receptive field or population of receptive fields (Foegeding et al. 2015). With this understanding, however, it is still of great interest to understand how this pooled receptor activity is combined to result in different textural modalities.

1.4 The Oral Cavity

Research on oral tactile perception going forward stands to benefit from a better understanding of the impact of factors external to a food's material properties (Kravchuk et al. 2012). Conventional instrumental texture measurements with their roots in quality control have often been inadequate in explaining the relationship between food structure and texture perception (Bourne 1982). Research by Engelen and Van Der Bilt (2008) found that differences in oral texture perception could be due, at least in part, to variations in oral physiology that could include oral sensitivity, tongue movements, temperature, and saliva composition (Fig 1.2).

There is only limited understanding on the impact of oral physiology in texture perception, but evidence has pointed towards fungiform papillae density and saliva as well as age as possible influences (Engelen and Van Der Bilt 2008).

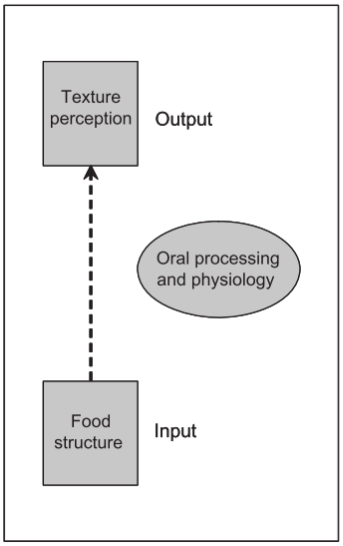

Figure 1.2 Illustration of oral processing and physiology as the missing link affecting texture perception in the oral cavity. Illustration taken from Englelen and Van Der Bilt 2008.

1.4.1 Fungiform Papillae

Ellis et al (1958) claimed to see that in the mammalian tongue (chimpanzee), a lose bundle of cholinesterase nerves can be seen in the fungiform papillae, some of which are seen to be connected to the nerve net innervated by the lingual nerve, a branch of the trigeminal cranial nerve which communicates tactile sensations from the anterior tongue to the brain. Similarly, Whitehead et al (1985) found the lingual nerve to terminate in the extragemmal and perigemmal tissue surrounding the taste buds, while the chorda tympani was seen to primarily innervate the intragemmal tissues, where gustation is localized (Fig 1.3) (Whitehead et al.,1985). This was interpreted to potentially implicate an association between lingual nerve innervation and taste papillae and suggest the taste bud structure as a site of tactile signal transduction. This is supported by their additional finding that high lingual nerve innervation has been found to occur in the anterior portion of the tongue, the area in which fungiform papillae density is also highest (Whitehead et al., 1985). Toyoshima et al (1987) see a moderate number of Merkel cells (the specialized mechanoreceptors typically found at the end of SAI nerve fibers) terminating in the fungiform papillae in primates. These findings have led many to speculate on a relationship between fungiform papillae density and increased mechanoreceptor innervation, leading to heightened oral texture perception.

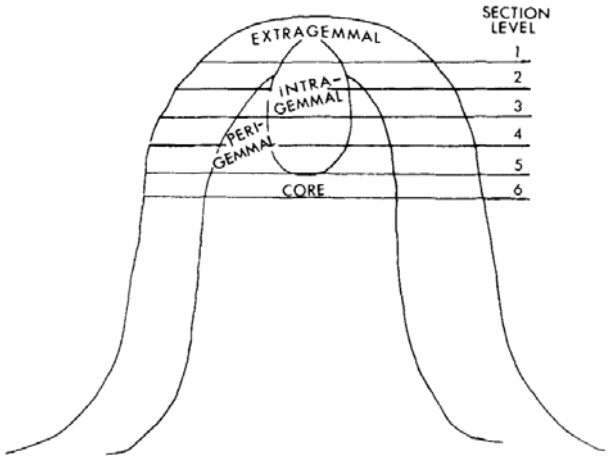

Figure 1.3 Diagram of transverse section levels of a fungiform papillae. Illustration borrowed from Whitehead et al 1985. "The terms "core" and "perigemmal" refer to locations immediately below the bud or along its sides, respectively."

While evidence for a positive correlation between sensitivity to tastants and increasing number of fungiform papillae have been found (Miller and Reedy 1990, Zuniga 1993), it still presently remains unclear whether a direct relationship exists between texture perception and density of fungiform papillae. Despite previously mentioned evidence indicating a relationship between lingual nerve termination in surrounding tissues of fungiform papillae, these nerve fibers have not yet been specifically identified as mechanoreceptive neurons. Though des Gachon et al. 2011 demonstrated that these fibers do not appear to be nociceptive due to their lack of expression of TRPAI, there still remains the possibility that these nerve endings are either thermoreceptive or mechanoreceptive. Further, even if they are mechanoreceptive, it is not clear that the mechanoreceptors in which the fibers terminate encompass all classes of mechanoreceptors. Thus it would be possible that the mechanoreceptors responsible for edge and point detection are located there, but not those responsible for roughness

sensitivity. Prutkin et al. (2000) found a positive correlation between two point detection threshold and average distance between fungiform papillae, and Essick et al. (2003) showed acuity of identification of plastic letters with the tongue to be greater in those with greater fungiform papillae density. Hayes et al. (2007) also found a relationship between increased creaminess ratings and higher fungiform papillae density. Conversely, Bakke (2008) more recently reported no relationship between fungiform papillae density and roughness perception. Because of this inconclusive evidence, the relationship between fungiform papillae density and tactile acuity of the tongue should be examined further to explore its validity.

1.4.2 Saliva

The tribological interactions between food, saliva, and the surfaces of the oral cavity area are a critical component to recognize in order to completely understand oral texture (Kravchuk et al. 2012). Both salivary flow volume and composition vary greatly from one individual to another (Bajec and Pickering 2008). From a volume and lubrication perspective alone, variations in saliva could affect oral tactile perception from a purely frictional standpoint (de Wijk and Prinz 2006). de Wijk and Prinz found that reductions in overall salivary volume lead to decreased lubrication, increased friction, and increased perception of roughness. Salivary volume, however, is only one component of saliva that must be considered. Saliva is made up of 99% water and the remaining approximately 1% is composed of various ions, enzymes and proteins. The composition of that 1% as well as overall salivary flow volume differ immensely depending on individual person, gland, type of saliva production (unstimulated vs. stimulated), and

even time of day (Engelen and Van Der Bilt, 2008). de Wijk and Prinz (2006) suggested that variations in lubricative properties of saliva could also be attributed by salivary composition, namely by variations in and interactions with mucin proteins, the large salivary proteins to which lubrication in the oral cavity is largely attributed.

When food is introduced into the mouth, the contribution of interactions of saliva with food to food texture and experience must be considered. Engelen et al. (2007) found that perception of flavor and mouth feel of a number of semi-solid foods is strongly correlated with total protein concentration and α-amylase activity in measured saliva for an individual. High concentrations of both α-amylase and proteins in an individual's saliva were found to correlate negatively with thickness perception of semi-solid food samples. Engelen proposed that the method of this negative correlation could be the decrease of viscosity of the product in the presence of high salivary protein concentration due to enzymatic breakdown or the proteins exerting some type of competitive action on binding to receptors in the oral mucosa. In the case of α-amylase, the reduced perception of viscosity was unsurprising due to the known starch breakdown activities of α-amylase, which results in reduction of viscosity. These hypothesized effects of the various attributes of saliva on lubrication, enzymatic breakdown, and competition on the oral mucosa could all have an effect on how a food's roughness is perceived.

1.4.3 Age

The effect of age on taste thresholds has been widely examined, and the majority of these studies strongly reinforce the idea of decreased sensitivity with age (Mojet et al. 2001). Thornbury et al. (1981), Woodward (1993), and Bangcuyo et al (unpublished

manuscript) have extended this decreased sensitivity to include tactile and thermal stimuli**.** Thornbury et al. (1981) measured tactile thresholds on the finger pads using a modified version of von Frey hairs and a forced choice procedure and confirmed the decrease of tactile acuity with age. It was proposed that this difference could be due to the changes in skin properties such as thinning and elasticity or a reduction of Meissner corpuscle density with age. Woodward (1993) supported these results with similar findings associating increased age with decreased sensitivity in the fingers and attributing this to changes in the nervous system as opposed to mechanical properties in the skin. Bangcuyo (unpublished manuscript) extended these findings to the tongue and found that in a letter identification task aimed at assessing lingual tactile sensitivity, panelists 40 years or older had significantly higher thresholds than those in their 20s.

1.5 Roughness

Roughness is a promising texture modality through which to study differences in oral sensitivity between individuals because of its extensive study in the fingers (Taylor et al. 1975; Yoshioka 2001, Depeault et al. 2009; Kappers et al. 2009; Skedung et al. 2013) and its relatability to a number of food related sensations. Studies by Essick et al. (2003) and Bangcuyo et al (unpublished manuscript) assessed oral tactile sensitivity differences in individuals using a letter recognition task which ultimately illustrated individuals ability to use edge and point detection to discriminate between letters of

difference sizes. By nature of the symbols being letters, however, this task also introduces a cognitive component such that results do not necessarily reflect only lingual tactile sensitivity. Tactile acuity on the tongue has also been studied through use of punctate stimuli in the form of von Frey hairs (Nachtsheim et al. 2013). Though this method eliminates potential cognitive biases of the letter strategy, single-point punctate stimuli are not particularly relevant to the types of textures frequently encountered by the tongue upon food consumption.

Roughness presents a basic texture modality that avoids any of these literary and cognitive biases. Howes (2014) describes surface roughness as the dominant factor in the exploration of surfaces by touch. Perception of sensations like grittiness and granularity, and astringency; all prominent qualities observed in foods, are likely perceived through mechanisms related to roughness, making it a potentially more relevant attribute to measure than punctate sensations via von Frey hair stimulation.

Bakke et al. 2008 attempted to differentiate oral roughness sensitivity among individuals using refined wheat and whole wheat breads staled to different levels. In order to assess roughness, panelists took a bite of a sample of bread, used their tongue to touch the bread to the roof of their mouth, and then immediately rated the roughness of the sample on a gLMS scale. Participants repeated this procedure for eight samples total staled to either 0, 1, 3, or 5 days. In this study, Bakke et al. found that roughness ratings increased alongside staling time in that fresh bread and day-old bread were both found to be significantly less rough than the same bread aged for 3 or 5 days, according to panelist gLMS report. Whole wheat breads were also reported to be significantly rougher than

refined breads. Fungiform papillae density, age, gender were also collected for each participant, as was bitterness, sweetness and PROP sensitivity. The effects of all 6 of these attributes on individual roughness intensity ratings were analyzed. Findings indicated that roughness sensitivity corresponded to PROP sensitivity, however, neither roughness sensitivity nor discrimination between samples of different roughness was found to be related to fungiform papillae density. A suggested explanation for this was the inadequacy of fungiform papillae density to accurately reflect overall mechanoreceptor innervation in the mouth (Bakke et al. 2008).

Though this study benefitted from the applicability of roughness to food systems, the use of gLMS intensity rating as a method to quantify roughness is unreliable and subjective. Additionally, staling is a minimally controlled product modification that likely results in changes to other textural properties in addition to roughness, like hardness or pliability. The possibility of co-variance in multiple textural dimensions could have additionally confounded this study design. This study provides a good starting point for the study of oral roughness sensitivity, but leaves much room for methodological improvement. By adopting instrumentation from the field of materials engineering, a more reliable method for quantification of roughness prior to assessment can be adopted.

1.5.1 Instrumentation

Instrumentation has long played a role in texture analysis. Since the 1960s, texture profile analysis using texture analyzers has been advocated by leaders in the field like Malcom Bourne (Kravchuk et al. 2012). Universal texture analyzers can be used to

collect objective information about food textures by applying forces that mimic various oral processes related to the textures being measured.

In their study of multidimensional oral perception of different types of materials, Howes et al. (2014) highlight that the use of material science data to predict tactile perception of materials is a promising analytical method for extension in to studies of oral sensation. The area of tactile roughness perception study has much to gain from the adaptation of materials science techniques to measure surface characteristics of stimuli. In Howes' study, the roughness of samples is quantified objectively prior to participant exposure using a surface profilometer. Both surface (contact) and optical (non-contact) profilometers are available to measure surface roughness with great precision either by running a specially designed stylus along the stimulus surface or by reflecting light on the test material and measuring the returned light pattern (Fig 1.4). For the optical profilometer, differences in height along the test surface result in variation in the pattern of light refraction back to the camera, allowing height differences to be measured. Using both surface and optical profilometers, the arithmetic average surface roughness (R_a), or the average height of peaks and valleys from a given midline, can be determined with great precision. These instruments allow quantification of roughness differences not obvious to the eye or touch and would allow the possibility for stimuli to be created that differ only very slightly in roughness, allowing for the determination of precise roughness detection thresholds differences among individuals. The use of these instruments could prove valuable to the study of both tactile and oral tactile roughness perception.

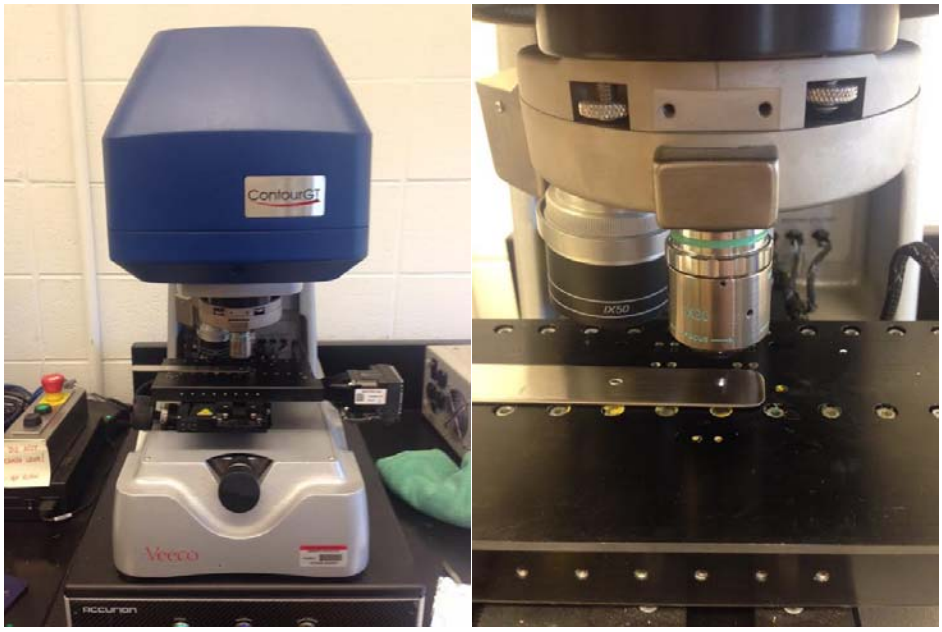

Figure 1.4 Optical profilometer. Image of a Veeco Contour GT optical profilometer. Allows, among many other functions, the precise and objective quantification of surface roughness.

1.6 Roughness and Astringency

An additional benefit to looking at oral texture perception through the lens of roughness sensitivity is its relatability to other sensations of interest, like astringency. Astringency is generally accepted as the 'drying', 'puckering', or 'roughing' sensation resulting from the exposure of oral surfaces to foods high in protein-binding polyphenol compounds and plays an important role in the sensory experience associated with consumption of a wide range of foods such as red wine, green tea, and chocolate (Jobstl et al., 2004; Bajec and Pickering 2008). Astringency has been linked to changes in natural lubrication of the salivary layer coating the oral surfaces (Kravchuk et al., 2012).

It is commonly accepted that astringent compounds interact with and bind proline-rich proteins (PRPs) and potentially other proteins in the saliva such as mucins (Bajec and Pickering 2008, Lee et al. 2012). The currently popular "lubrication" theory of astringency states that this binding and subsequent astringent-salivary protein complex formation further results in aggregation of these complexes and ultimately decreases salivary viscosity and strips the oral cavity of the mucosal protein layer responsible for lubrication (Bajec and Pickering 2008). The theory then asserts that once lubrication is reduced, the resulting increase in friction between oral surfaces stimulates mechanoreceptors to a greater degree than when lubricated, thereby resulting in the "drying" and "roughing" sensations experienced. By this definition, astringency is fundamentally a tactile sensation perceived in a manner closely resembling the perception of roughness.

Some conflicting studies exist, however, that suggest that astringency is more than a purely tactile sensation (Rossetti et al. 2009). Contradicting literature has shown results indicative of potentially differing pathways of astringency perception dependent on the identity and chemical structure of the astringent compound as well as of the involvement of additional mechanical or chemical interactions (Bajec and Pickering 2008).

Because of the potential interaction of astringency with many basic tastes, Bajec and Pickering (2008) advocate that the mechanisms underlying the perception of astringency should be studied further using "simple, single-component stimuli". Bajec and Pickering suggest that simple stimuli would also allow researchers to tease apart the

individual parts of this complex sensation and would more likely allow the acceptance of causative relationships between the stimuli and the resulting sensations. Stimuli that measure roughness could serve as one of these single-component stimuli contributing to a better understanding of astringency. If astringency is, at its core, a purely tactile precept resembling roughness, then stimuli measuring roughness sensitivity could contribute to differentiation between subpopulations of astringency sensitivity. Alternatively, if astringency mechanisms are more complex, then comparison between roughness and astringent sensitivity within individuals should help to tease the two apart if a potentially more complex mechanism is at play in the latter.

Ch. 2: Introduction

The importance of texture to the food consumption experience is at last being recognized and appreciated after years of serving as a measure for food quality control. Whereas a "top-down" approach looking at complex textures of food matrices is most often employed, there is great potential for increased attention towards a "bottom-up" approach. The latter would employ research strategies which look at texture perception at a more basic receptor and single textural modality level in an attempt to more closely characterize the contribution of pooled mechanoreceptor responses and then use understanding of basic textural modalities to build, from the bottom up, a complete picture of oral texture perception and preference differences among individuals. Beyond surface attributes of a given food, various oral physiological factors as well as age have all been implicated in texture and must be taken in to account as they likely contribute to differences in perception among individuals. The texture modality of roughness appears to provide a promising and, thus far, very infrequently used attribute for the characterization of oral tactile acuity. The potential applicability of roughness sensitivity to a better understanding of the sensation of astringency presents additional value.

In this study, we aimed to better understand individual differences in oral cavity texture perception by assessing individual differences in sensitivity to surface roughness. We further used the textural modality of roughness as a means to estimate the

contribution of the mechanoreceptors responsible for the perception of surface roughness to the sensation of astringency, using the astringent polyphenol compounds epigallocatechin gallate (EGCG) and tannic acid (TA). This was accomplished by assessing individual detection threshold and suprathreshold to surface roughness, EGCG astringency, and TA astringency. Information on fungiform papillae density, salivary flow volume, age and astringent food preferences was also collected. We hypothesized that individuals would vary in their abilities to detect differences in surface roughness, and that these differences may be reflected by differences in fungiform papillae density, salivary flow volume, or age. It was additionally hypothesized that individuals exhibiting high sensitivity to roughness, would also exhibit high sensitivity to astringent stimuli.

Ch. 3: Materials and Methods

3.1 Stimuli

3.1.1 Roughness

Detection threshold. Stimuli used for quantifying roughness sensitivity consisted of 7 coupons of stainless steel 316 measuring approximately 5.5cm in length by 1.5.cm in width (Fig 3.1c). Surface roughness values of the stimuli were varied by even, professional grinding with sandpapers of various grits. Following roughening, surface roughness of each stimulus was quantified using a ContourGT optical profilometer (Veeco Instruments Inc, Oyster Bay, NY) (Fig 3.1 a,b). Stimuli roughness (R_a) values included 0.177, 0.190, 0.206, 0.234, 0.276, 0.322, and 0.465 µm, denoted R0, R1, R2, R3, R4, R5, and R6, respectively.

Suprathreshold. A microfinish comparator (GAR Electroforming, Danbury, CT) was used to obtain roughness suprathreshold stimuli. Five stimuli were cut from comparator (Fig 3.1d) each with a final size of approximately 3.5 cm by 1.25 cm. Chosen stimuli roughnesses (R_a) included 0.51 µm, 3.05 µm, 7.62 µm, 14.22 µm, and 22.86 µm.

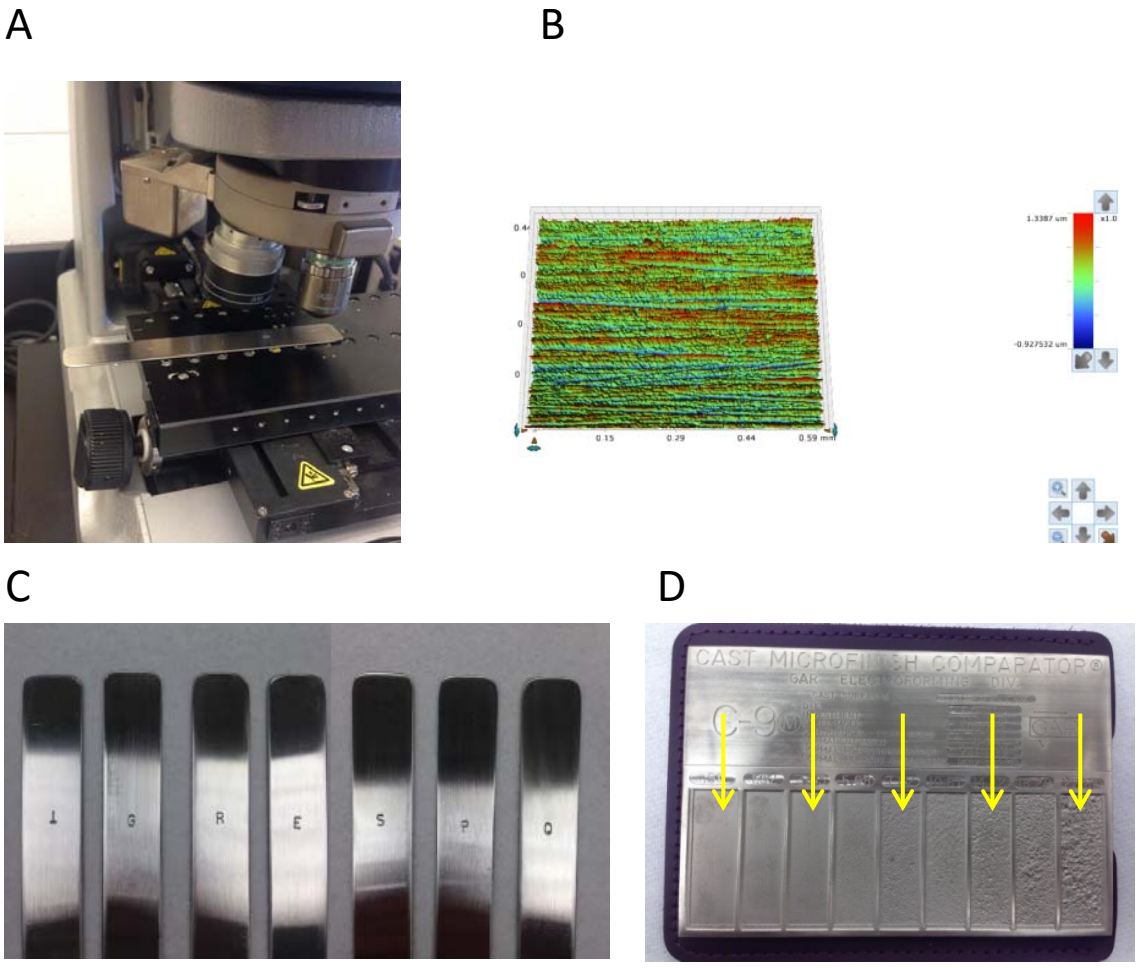

Figure 3.1 Roughness stimuli and measurement. Roughness of stimuli measured using optical profilmeter (a), magnified image of roughed surface of detection threshold stimulus R1 (0.190µm) obtained using optical profilometer (b) detection threshold stimuli; note letter labels not indicative of roughness order (c), and microfinish comparator included the 5 stimuli that were extracted from comparator for use as roughness suprathreshold stimuli (d).

3.1.2 Astringency

Detection Threshold. Two separate sets of astringent stimuli were prepared in distilled water using two different compounds; Epigallocatechin Gallate (EGCG, Teavigo, Taiyo International, Minneapolis, MN; Fig 3.2, left panel) and Tannic Acid

(TA, Sigma Aldrich, St. Louis, MO; Fig 3.2, right panel), were used to assess astringency detection thresholds. EGCG stimuli concentrations included 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 g/L EGCG while TA stimuli concentrations included 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 g/L TA. Tannic acid concentrations encompassed a wider range as it was found in preliminary testing that participant performance varied more greatly on this stimulus and so a slightly wider stimuli range was implemented as a precaution to accommodate this observation. Ten mL samples of each stimulus solution were portioned in to 2 oz. plastic portion cups (GFS, Grand Rapids, MI). The sensory profiles of both EGCG and TA included bitterness in addition to astringency. Thus, to prevent bitterness from acting as a cue for astringency intensity, 0.6mL 1.073 g/L of sucrose octaacetate (SOA, Sigma Aldrich, St. Louis, MO), a bitter compound, was added to each 10-mL sample to make bitterness equally intense across samples.

Suprathreshold. Stimuli were prepared again using EGCG and TA in distilled water to assess astringency suprathreshold. For these stimuli sets, TA concentrations included 0, 0.5, 1.0, 1.5, 2.0, and 2.5 g/L while EGCG concentrations included 0, 0.8, 1.4, 2.0, 2.6, and 3.2 g/L. No SOA was added to EGCG or TA solutions for suprathreshold assessments because the differences in bitterness between the samples were large and obvious. Subjects were instructed, however, to ignore the bitterness of the solutions and assign intensity ratings solely for the perceived astringency.

All astringent stimuli were prepared in the aforementioned concentrations within 48-hr of testing and stored at 4^{o}C. Stimuli were removed from refrigerator and allowed to equilibrate to room temperature at least 1 hr. prior to testing.

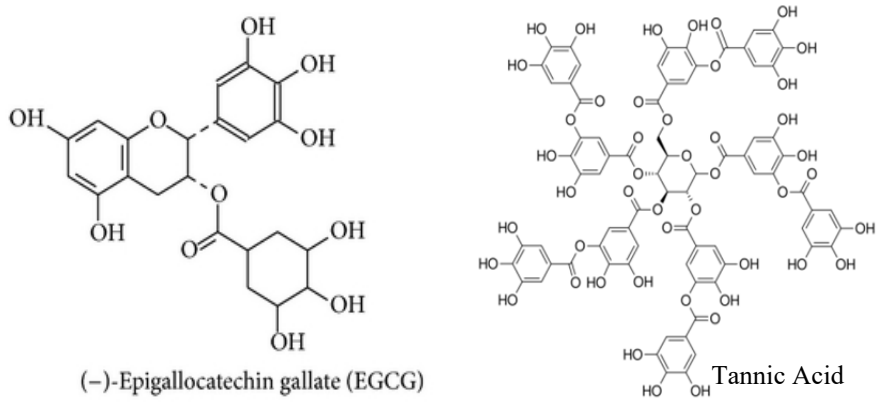

Figure 3.2 Astringent stimuli. Chemical structures of Epigallocatechin gallate (EGCG) (left) and Tannic Acid (TA) right.

3.2 Panelists

30 panelists (12 males, 18 females) with ages ranging from 21 to 60 were recruited from the Ohio State University campus and surrounding area. Participants were asked to refrain from drinking coffee or smoking one hour prior to testing. Participants came for two separate sessions, each 1 hr. in length, and were compensated $40 following completion of the second session. For a given panelist, sessions were no more than 3 days apart and were held at the same time of day to control for circadian effects on salivary flow. The data collected was approved by the local Institutional Review Board under written informed consent of the panelist.

3.3 Salivary Flow Collection

After written consent was obtained but prior to threshold testing, salivary flow was measured by having participants expectorate as much as possible in to a weighed sample collection cup for 2 minutes. This measurement was collected on both days of testing, weighed, and, averaged within each participant to obtain an approximate salivary flow volume in g/2 min.

3.4 Training

Panelists were briefly educated on the differences between astringency and bitterness to ensure that they were not confusing the two during the task. For each liquid sample in training and throughout testing, participants were instructed to put all 10 mL in their mouth, swish around all mouth surfaces for 3 seconds, expectorate sample into a spittoon, and then rub the tip of their tongue from side to side 3-5 times on their hard palate (the front portion of the roof of the mouth). For the first training sample, panelists were given 10mL of 0.7g/L aluminum sulfate (Sigma Aldrich, St. Louis, MO) in water. At 0.7g/L, aluminum sulfate expresses moderate astringency with negligible bitterness or sourness (Drobna et al. 2004). Panelists were informed that this sample was astringent and **not** bitter. Next, panelists received a sample of 0.6 mL 1.073g/L sucrose octaacetate (SOA) in 10mL of water and were told that this sample is bitter but **not** astringent. If the panelist was confident that he/she could distinguish between the two sensations at this point, they were provided with a practice pair of samples. In the pair, both samples were

bitter, but one was astringent in addition to bitter. They were told which sample was astringent, and which one was not, and if the panelist again expressed confidence in their ability to distinguish between the two sensations, then the training was ended. If panelists were unsure, they were able to repeat the training task. All 30 panelists indicated confidence in their ability to distinguish between bitterness and astringency following this training.

During training, participants were additionally familiarized with blinding goggles and were given the opportunity to practice licking roughness stimuli. During roughness stimuli testing, participants would be blinded using safety glasses wrapped in Para film. The use of blinding goggles was required because samples had been marked with letters during roughing that could allow identification. The blinding goggles eliminated visual acuity needed to distinguish the letter labels, but enabled the panelist to make out shapes well enough to receive stimuli when handed to them. During training and testing, panelists were instructed to lick roughness stimuli by moving their tongue in a circular motion, in order to avoid directional bias.

Panelists were also familiarized with the general Labeled Magnitude Scale (Bartoshuk et al. 2004). Participants were read 5 verbal examples of sensory stimuli and asked to point to where they believed they would fall on the gLMS scale as an introduction to its use. Verbal stimuli included; the brightness of this room, the pain of putting your hand under scalding water, the sweetness of cotton candy, the sourness of a lemon, and the loudness of a whisper. Panelists were not corrected on their placement of

verbal example stimuli. This gLMS acclimation procedure and examples were adapted from Bakke et al. 2008.

3.5 Detection Threshold Assessment

3.5.1 Roughness

In order to assess roughness sensitivity, roughness detection thresholds were obtained using the up-down staircase method (Fig 3.3). Stimuli were handed to participants in clothespin clips and clips were switched regularly to avoid the possibility of temperature inadvertently providing cues through the transfer of heat directly from hands to metal coupons. Participants were given two stimuli at a time, one always being the least rough, control R0 stimulus (0.177 μm) and the other of varied roughness's, beginning in the middle of the scale at R4 (0.276 μm). Individuals were asked to lick both stimuli by moving their tongue in a circular motion (to eliminate directional bias) on the stimuli surfaces. For each pair of stimuli, participants were asked to answer the question, "which stimulus is more rough?". If the panelists correctly identified the rougher stimulus, they were provided with the R0 stimulus again, paired with a less rough stimulus (e.g. R3 (0.206 μm)). Participants were again asked to identify the rougher stimulus. If the participant continued to correctly identify the roughest stimulus, the presented coupon continued to decrease in roughness. Conversely, if the participant answered incorrectly, they were presented with a rougher stimulus and again asked which was rougher. A "reversal" occurred when a panelist answered incorrectly following a

series of correct responses OR when a panelist answering correctly following one or more incorrect responses (thereby "reversing" the direction they had been moving up or down the stimuli staircase). The trial was finished when the panelist accrued 8 reversals. For detection threshold assessment, the geometric mean of the stimuli roughness (R_a) values at each of the 8 reversals was calculated and this value was determined to be the individuals "roughness detection threshold" (RDT). A full example trial is depicted in Figure 3.3.

In order to prevent participants from becoming overly familiar with stimulus R0 (which was always given in the stimulus pair), a distraction staircase was executed simultaneously from the other, rougher end with R6 as the reference stimuli (Fig. 3.4). In other words, in this distraction staircase, participants were given stimuli pair R6/R3 first, then if they correctly identified the rougher stimulus (R6), they were given R6/R4, while if they answered incorrectly, the distance between stimulus roughness was increased and they were given R6/R2; and so on and so forth. Trials alternated between the main staircase and the distraction staircase every other stimuli presentation. Data from the distraction staircase was not analyzed.

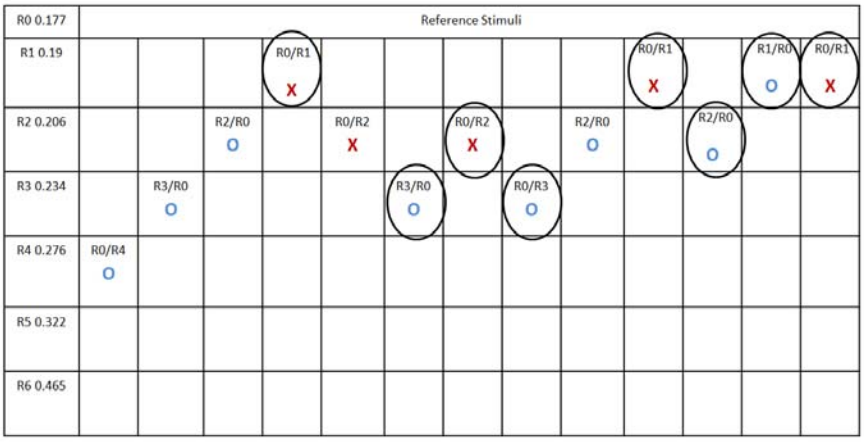

Figure 3.3 Staircase chart example. Stimuli presented to the left and right hands of the participant are left and right of the slash, respectively. Correct responses are marked with an "O", incorrect are marked with an "X". "Reversals" are circled. Roughness (R_a) at each of the 8 reversals were averaged together to calculate Roughness detection threshold.

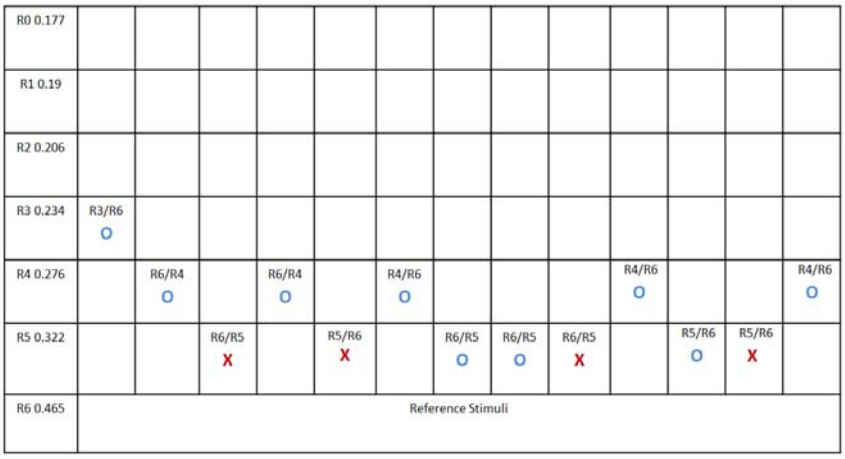

Figure 3.4 Distraction staircase example. Distraction staircase was executed in order to prevent panelist from becoming overly familiar with reference stimulus R0. Stimulus presentation alternated between Staircase and Distraction Staircase**.**

3.5.2 Astringency

A similar up-down staircase was used for astringency detection threshold. Participants were presented with a control stimulus (distilled water with SOA), as well as a stimulus with some added concentration of astringent. Order of presentation was

randomized. Panelists took the first sample into their mouth, swished, spit and assessed astringency as trained. They were then instructed to rinse with water and were provided with the second sample to assess. Following assessment of the second sample, panelists were asked to identify which of the two stimuli was the astringent stimulus. A 20-second interval between the rinse and evaluation of the next sample was maintained in an attempt to minimize carry-over astringency. The interstimulus interval was not able to be lengthened further due to memory effects which would come in to play in identifying the astringent sample of a given pair. Carry-over was occasionally expressed in later samples of the trial, so to account for this, DT values were calculated by averaging sample concentrations at first 5 reversals (instead of first 8 as in roughness). Testing set-up is depicted in Figure 3.5.

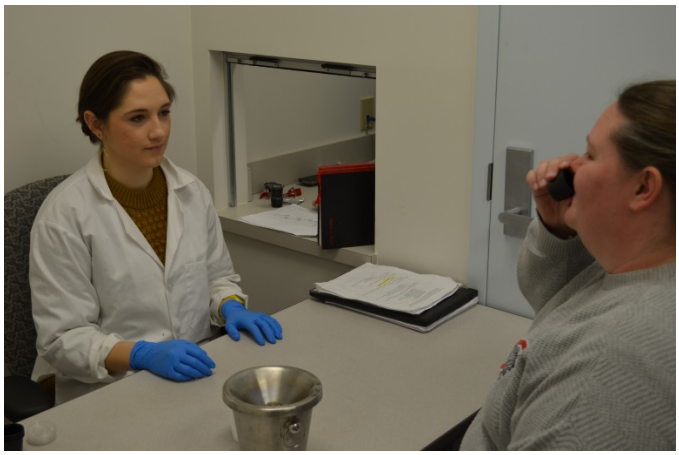

Figure 3.5 Detection threshold testing set-up. Depiction of panelist completing astringent detection threshold task. After swishing, panelist spit sample in spittoon (bottom left) and verbally reported to researcher which stimulus of the pair they thought had been astringent.

3.6 Suprathreshold Assessment

3.6.1 Roughness

Panelists were presented with each of the 5 suprathreshold roughness stimuli in randomized order and were instructed to lick each stimulus in a circular motion and rate the intensity of perceived roughness on the general Labeled Magnitude Scale (gLMS) (Bartoshuk et al. 2004)(Fig 3.6).

3.6.2 Astringency

Participants were presented with the 6 surprathreshold concentrations of EGCG or TA in randomized order and asked to rate intensity of astringency on the gLMS. Evaluations of the TA or EGCG samples were randomized across panelists and occurred in separate sessions.

Roughness and astringency suprathreshold ratings were collected on a laptop using Compusense Cloud Software.

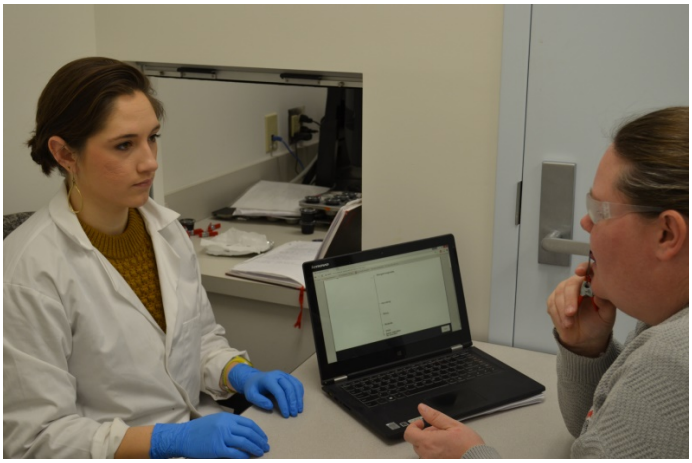

Figure 3.6 Suprathreshold testing set-up. Participant is depicted completing roughness suprathreshold gLMS assessment task. Lab goggles are covered in Para film to avoid visual bias and gLMS ratings are assigned by clicking on the gLMS scale where they believed the roughness intensity to fall using laptop mouse-pad.

3.7 Fungiform Papillae Count

Following completion of all threshold testing on the second day of testing, panelists' tongues were photographed in order to observe and quantify fungiform papillae density differences. The Denver Papillae Protocol (Nuessle et al. 2015) was followed closely in both photographing tongues as well as quantifying fungiform papillae. A 0.1% w/v blue dye solution (Sensient, Technologies, Milwaukee, WI) was applied to the anterior of the tongue and a filter paper circle with an inner diameter of 1.59 cm was placed on the tip of the participants tongue, to the left of the tongue's midline (Fig 3.7, left panel). A Nikon D5200 DSLR Camera (Tokyo, Japan) was used to capture tongue images using the macro setting. Papillae were counted using open source software, ImageJ (Fig 3.7, right panel) using the cell counter plug-in and adhering to the fungiform papillae criteria for size, color, and shape described by Nuessle et al. (2015). Fungiform

papillae were counted by two separate researchers. When counts from the investigators differed by more than 10%, researchers conferred to identify reasons underpinning this difference and recounted. Papillae counts from each investigator were then averaged together for final fungiform papillae count.

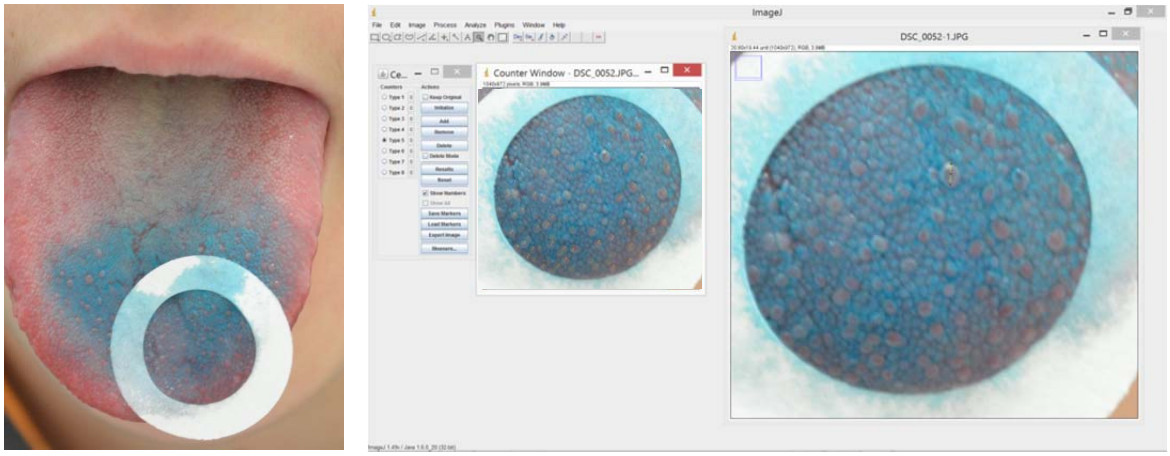

Figure 3.7 Fungiform papillae count. Painted tongue and filter paper placement (left), papillae counting using ImageJ (right).

3.8 Demographic and Consumption

Panelists completed a demographic and consumption questionnaire at the end of the second day of testing (Appendix D). In the questionnaire, participants were asked to rate their preference for a number of astringent foods and their less astringent counterparts. The food pairs were adapted from a survey used in a study by Tornwall et al (2011) and included; tea (with or without sweetener), banana (ripe or unripe), chocolate (milk or dark), wine (white or red). Participants were instructed to, “rate [their]

perceived pleasantness of the following foods and beverages", and were able to choose from response options; very unpleasant (-3), fairly unpleasant (-2), slightly unpleasant (-1), neither pleasant nor unpleasant (0), slightly pleasant (1), fairly pleasant (2), and very pleasant (3). For analysis, the pleasantness scores of the more astringent of the 4 food pairs (tea without sweetener, less ripe banana, dark chocolate, and red wine) were looked at individually as well as averaged across all four foods to obtain an overall astringency pleasantness score (Overall TBCW). These individual and overall consumption pleasantness scores were examined across both roughness sensitivity (RHi, RLo) and salivary flow (SalivaLo, SalivaHi).

3.9 Testing Schedule

The testing schedule was planned carefully in order to minimize carry-over effects and fatigue. Each session lasted for approximately 1hr. The testing schedule is depicted in Table 3.1. Astringent suprathreshold testing was always the last sensitivity task on each day because samples in this task had the highest astringencies and therefore greatest potential for carry-over.

Table 3.1 Schedule of panelist testing. It was randomized whether "astringent 1" or "astringent 2" were EGCG or TA for each participant. Astringent suprathreshold was always last the last sensitivity task on each day as it had the greatest potential for carry-over.

Day 1	Day 2
Consent Form Completion Saliva Collection Training Astringent 1-Detection Threshold Roughness Detection Threshold Astringent 1- Suprathreshold	Saliva Collection Training Astringent 2- Detection Threshold Roughness Suprathreshold Astringent 2- Suprathreshold Demographic and Consumption Tongue Picture

3.10 Data Analyses

Participants were divided into high roughness sensitivity (RHi; n=16) and low roughness sensitivity (RLo; n=14). When a panelist correctly differentiated RO from R1 7 or 8 times (out of 8 comparisons), they were placed in RHi. Panelists making two or more errors were placed in RLo. Panelists were also divided by salivary flow into high salivary flow (SalivaHi) (n=15) and low salivary flow (SalivaLo) (n=15) groups, divided equally at the median salivary flow value of 1.8g/2min.

Suprathreshold sensitivity was assessed by plotting log transformed gLMS intensity values to obtain Area Under the Curve (AUC) measurements for each panelist's stimuli intensity ratings. AUC was calculated using GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA).

Differences in EGCG, TA and Roughness thresholds and suprathreshold assessments were examined across Rough Hi/Lo and Saliva Hi/Lo group divisions by

independent samples t-test, binomial analysis, and analysis of variance (ANOVA). Data analysis was completed using IBM SPSS Statistics 23.0 (IBM Corporation, Armonk, NY).

Ch.4: Results

4.1 Detection Threshold Statistics

4.1.1 Roughness

Roughness detection thresholds where found by calculating the geometric mean of the roughness (R_a) of the first 8 reversals that a panelist accrued while completing the roughness detection threshold staircase task. Roughness detection thresholds ranged from 0.190 µm to 0.238 µm, with an average of 0.200 µm (Fig 4.1).

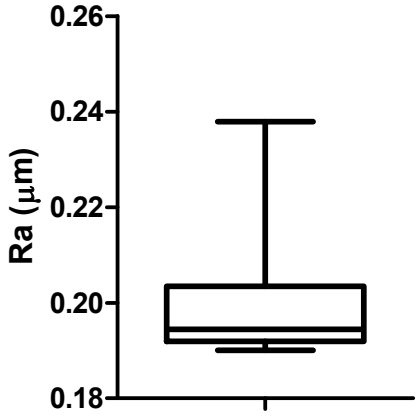

Figure 4.1 Distribution of roughness detection thresholds. A box and whisker plot of panelist roughness detection thresholds. Average surfaces roughness of stimuli (R_a) plotted on y-axis. Thresholds ranged from 0.190 to 0.238 µm with an average of 0.200 µm.

4.1.2 Astringency

Overall astringent detection threshold averages are displayed in table 4.1 in both w/v and molarity concentrations for both EGCG and TA. Based on w/v concentration, there is no significant difference in detection threshold between the two compounds when detection thresholds are averaged across all participants (paired t-test, $p=0.906$) (Fig 4.2a). However, when converted to molarity, TA detection threshold is significantly lower than EGCG detection threshold for the group (paired t-test, $p<0.001$))(Fig 4.2b). On an individual basis, out of the 30 total participants, a significant majority of participants (28 of 30; binomial $p<0.001$) exhibited lower detection thresholds for TA than they did for EGCG based on molarity. The ratio of EGCG DT to TA DT was calculated in molarity for each individual participant. The average EGCG DT: TA DT ratio was found to be 4.95 with a SEM of 0.614.

Table 4.1 Astringency detection threshold statistics. Detection thresholds represented as Mean +/- SEM for both EGCG and TA.

	TA		EGCG	
	DT	SEM	DT	SEM
Average DT (g/L)	0.361	0.041	0.376	0.032
Average DT [M]	2.12×10^{-4}	2.46×10^{-5}	7.70×10^{-4}	6.63×10^{-5}

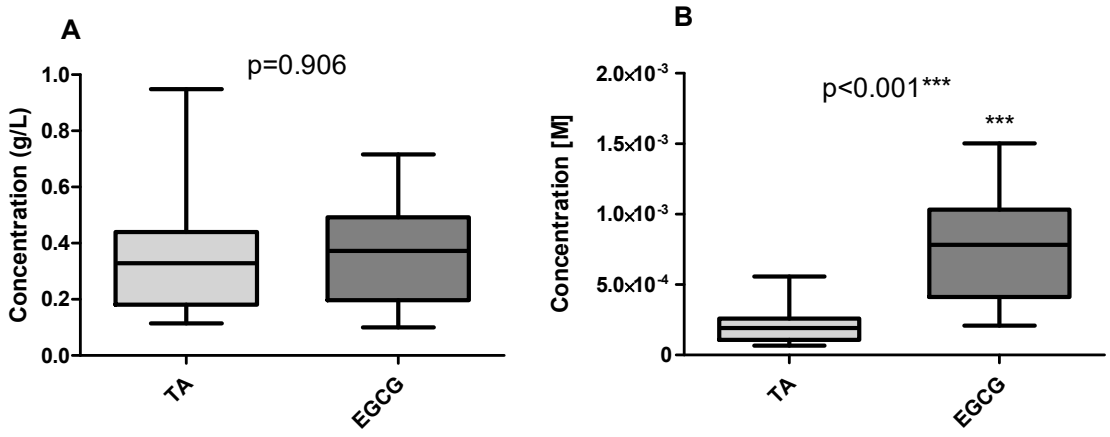

Figure 4.2 Distribution of astringency detection thresholds. Average astringency detection thresholds are depicted by box and whisker plot in both w/v concentration (a) and molarity (b). Concentration of astringent stimuli plotted on y-axis in g/L (a) and molarity (b).

4.2 Detection Threshold Analyses

4.2.1 High Roughness Sensitivity, Low Roughness Sensitivity

The majority of participants were able to correctly identify the second smoothest stimulus (R1) from the smoothest control stimulus (R0) on at least one occasion. Some individuals, however, were always or nearly always able to distinguish these two, while others had much more difficulty. Those who went down to the R0/R1 stimuli pair and correctly identified R1 as being rougher 8 times (perfect), or 7 times (1 miss), were put into the high roughness sensitivity group (RHi) (n=16), and individuals who missed more than this were placed in the low or "normal" roughness sensitivity group (RLo)(n=14). The average roughness detection threshold of RHi (0.192 μm) was significantly lower than that of RLo (0.209 μm) ($p < 0.001$) (Fig 4.3).

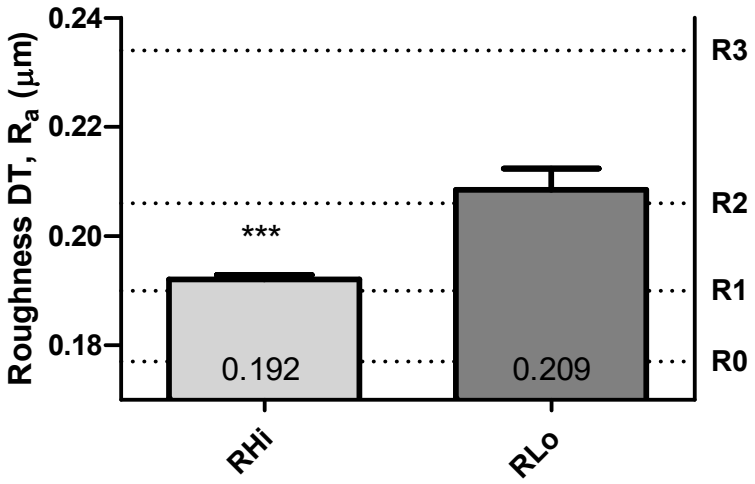

Figure 4.3 Roughness detection thresholds based on high roughness sensitivity (RHi) and low roughness sensitivity (RLo). Detection thresholds plotted as Mean +/- SEM. Surface roughness is plotted on the y-axis. Surface roughnesses of stimuli R0 through R3 are marked with dotted lines on right y-axis for reference.

Independent samples t-tests were run across the RHi/RLo divisions, using the test variables salivary flow, age, and fungiform papillae density. Notably, there was no significant difference found in fungiform papillae density between the high roughness sensitivity (RHi) and low roughness sensitivity (RLo) groups (p=0.951) (Fig 4.4). Effects of age and salivary flow approached significance and are worth noting due to the small sample size. Individuals in the high roughness sensitivity group (RHi) tended to be lower in age than those in the low roughness sensitivity group (RLo), averaging 26.4 (±1.15 SEM) and 31.6 (±2.89 SEM) years old, respectively (p=0.090). Individuals in the high roughness sensitivity group (RHi) also tended to exhibit relatively lower salivary

flow rates, averaging 1.86 (±0.20 SEM) g/2min versus 2.64 (±0.44 SEM) g/2min for the low roughness sensitivity group (RLo) (p=0.104).

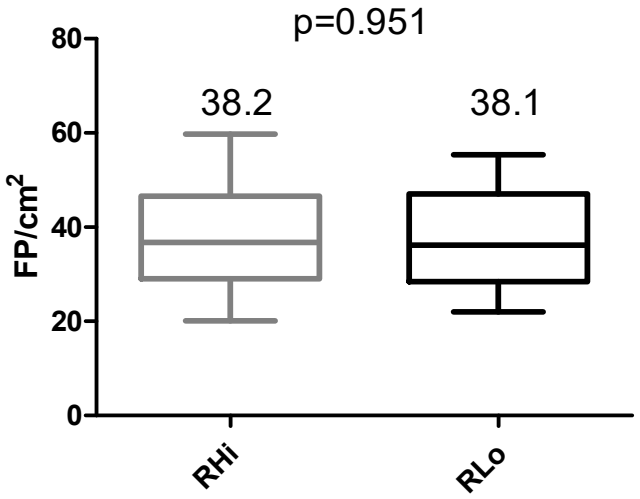

Figure 4.4 Fungiform papillae density across roughness sensitivity. Box and whisker plot of fungiform papillae density distribution for high roughness sensitivity (RHi) and low roughness sensitivity (RLo) groups with mean values displayed. The number of fungiform papillae counted per cm^2 is plotted on the y-axis.

An independent samples t-test was then run to compare sensitivity to both astringents, TA and EGCG, across the RHi/RLo roughness sensitivity division. This test revealed that those subjects exhibiting high roughness sensitivity also exhibited significantly greater sensitivity to EGCG astringency (Fig 4.5). Average EGCG detection thresholds for RHi and RLo were found to be $1.88x10^{-4}$ M (± $2.43x10^{-5}$ SEM) and $2.67x10^{-4}$ M (±$2.32x10^{-5}$ SEM) respectively ($p<0.05$).

No significant difference was seen across roughness sensitivity groups when looking at TA detection thresholds (Fig 4.6).

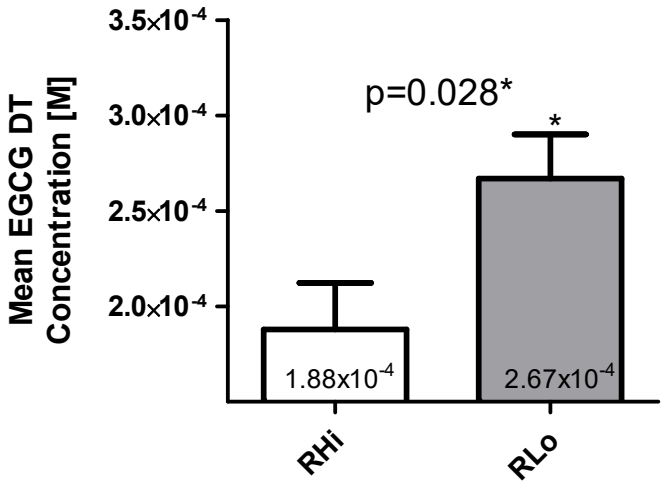

Figure 4.5 Epigallocatechin gallate (EGCG) detection threshold based on roughness sensitivity. EGCG detection threshold concentration plotted on y-axis in molarity by roughness sensitivity group (High roughness sensitivity (RHi), low roughness sensitivity (RLo)) shown as mean +/- SEM.

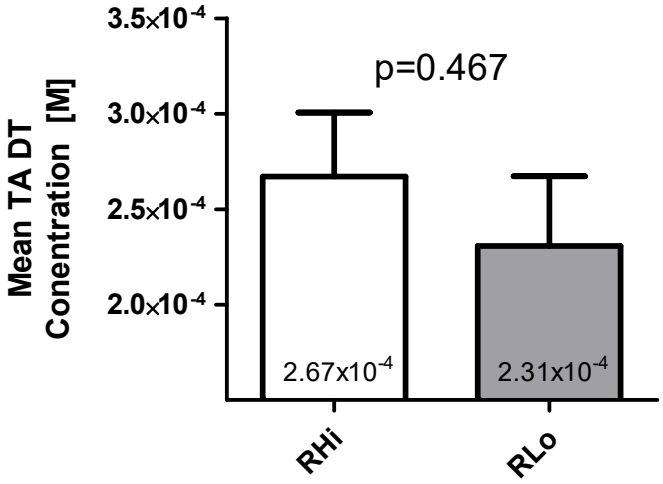

Figure 4.6 Tannic acid (TA) detection threshold based on roughness sensitivity. TA detection threshold concentration in molarity plotted on y-axis by roughness sensitivity group (High roughness sensitivity (RHi), low roughness sensitivity (RLo)) shown as mean +/- SEM.

4.2.2 High Salivary Flow, Low Salivary Flow

To look at the effect of salivary flow on roughness and astringency detection, individuals were divided in to two groups based on their measured salivary flow output. This division was made at the median observed salivary flow of 1.8 g/ 2min (Fig 4.7). Participants who produced less than this volume were assigned to SalivaLo (n=15), and those producing above were placed in SalivaHi (n=15).

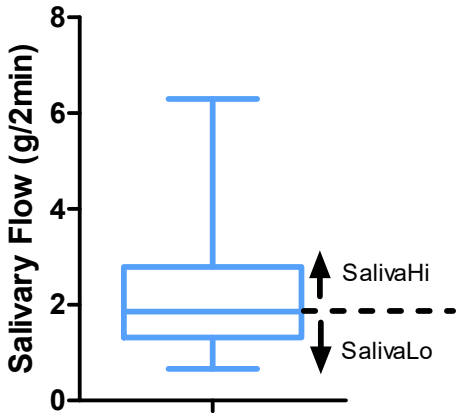

Figure 4.7 Salivary flow volume distribution. Salivary flow volume plotted on y-axis in g/2 min. Salivary flow volume ranged from 0.665 to 6.295. Median and mean values were 1.855 and 2.221 g/2min, respectively.

Using this division, independent samples t-tests were executed for test variables; roughness detection threshold, EGCG detection threshold and TA detection threshold. Based on the high and low salivary flow division, participants in the SalivaHi group, were found to be significantly more sensitive to TA astringency than those in the

SalivaLo group (Fig 4.8). Average TA DT values for SalivaHi and SalivaLo were 2.99x10-4 and 2.04x10-4, respectively (p=0.050). There was no significant difference in EGCG (Fig 4.9a) or roughness detection threshold (Fig 4.9b) on the basis of salivary flow (p=0.635, 0.504, respectively).

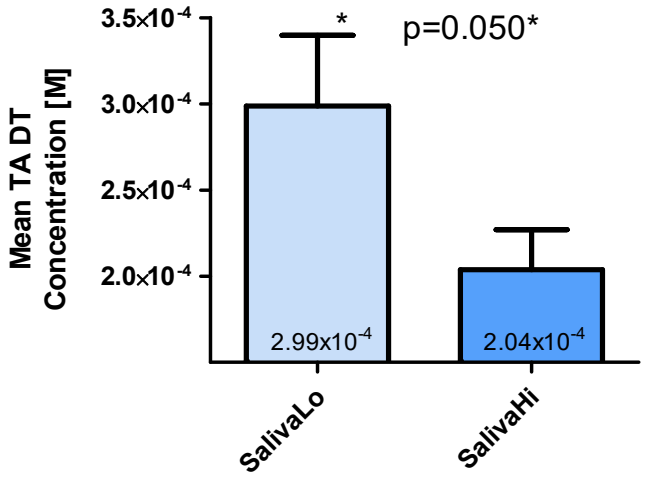

Figure 4.8 Tannic acid (TA) detection threshold based on salivary flow. TA detection threshold concentration plotted on y-axis by salivary flow group (high salivary flow (SalivaHi), low salivary flow (Salivalo)) shown as mean +/- SEM.

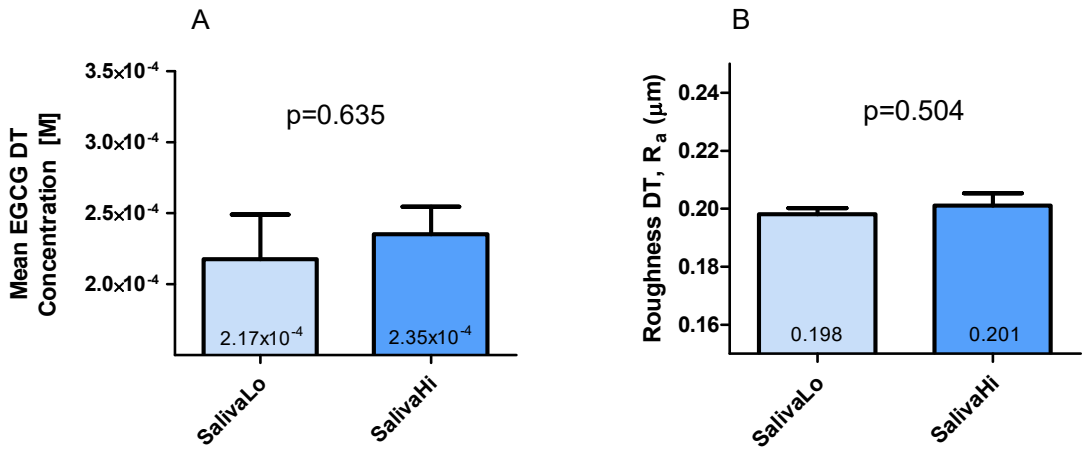

Figure 4.9 Epigallocatechin gallate (EGCG) and roughness detection thresholds based on salivary flow. EGCG detection threshold with stimuli concentration in molarity plotted on y-axis (a). Roughness detection threshold plotted with stimuli surface roughness on y-axis (b) by salivary flow group (High salivary flow (Saliva Hi) and low salivary flow (SalivaLo) shown as mean +/- SEM.

4.3 Suprathreshold

Suprathreshold roughness and astringency measurements collected using gLMS scores were log transformed and plotted as log transformed gLMS intensity scores on the y-axis vs. stimulus value in increasing intensity order on the x-axis, and the Area Under the Curve (AUC) was calculated for each set of suprathreshold ratings for each individual (Fig 4.10).

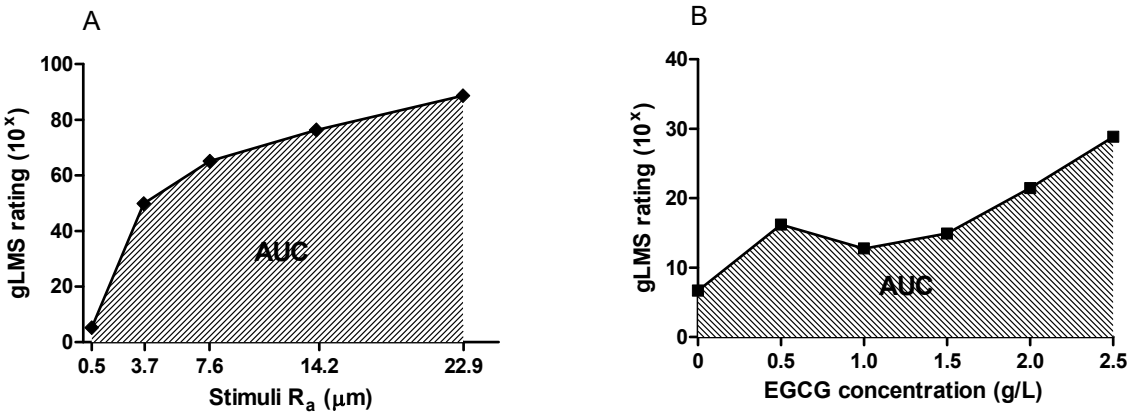

Figure 4.10 Example of Area Under the Curve (AUC) plots for suprathreshold data analysis. Reported gLMS intensity values were log transformed and plotted against the stimuli intensity (R_a or concentration). The total area under this plot was calculated to yield the area under the curve (AUC) for each participant's surpathreshold stimuli rating task. Example AUC plots for one participant are shown above for roughness(a) and EGCG(b).

Bivariate correlation statistical tests were run between EGCG suprathreshold AUC and rough suprathreshold AUC, TA suprathreshold AUC and rough suprathreshold AUC, and EGCG suprathreshold AUC and TA suprathreshold AUC. These analyses were only executed for the last 15 participants as the first 15 were not given a "0" stimulus with no astringent for astringent assessments, and the absence of this stimulus artificially inflated astringent AUC ratings for the first 15 participants. In the subsequent 15 participants, this oversight was corrected by providing a stimulus with no astringent ("0").

A significant correlation was found between EGCG AUC and Rough AUC ($r=0.602$, $p<0.05$) (Fig. 4.11). A significant correlation was also found between EGCG AUC and TA AUC ($r=0.562$, $p<0.05$) (Fig 4.12). No significant correlation was seen between TA AUC and Rough AUC ($r=0.197$, $p=0.50$) (Fig 4.13).

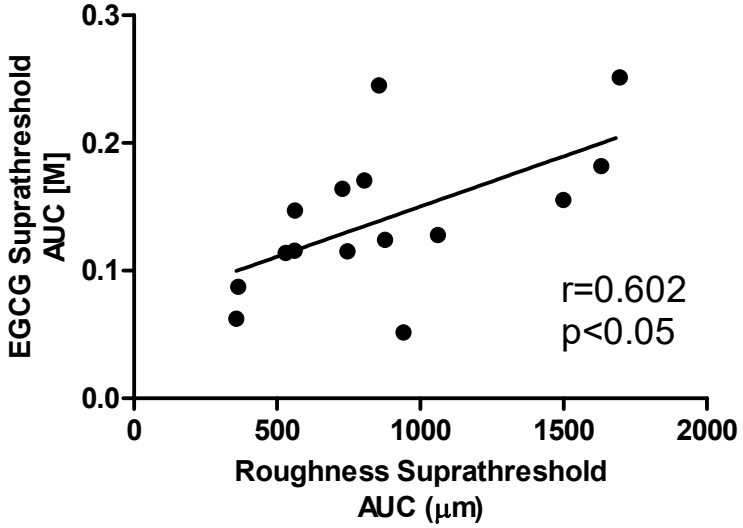

Figure 4.11 Correlation between Epigallocatechin gallate (EGCG) suprathreshold and roughness suprathreshold ratings. EGCG suprathreshold area under the curve (AUC) value plotted on y-axis versus roughness suprathrehold AUC plotted on x-axis for each participant. Significant correlation seen when EGCG suprathrehold AUC is plotted by roughness suprathreshold AUC ($r=0.602$, $p<0.05$).

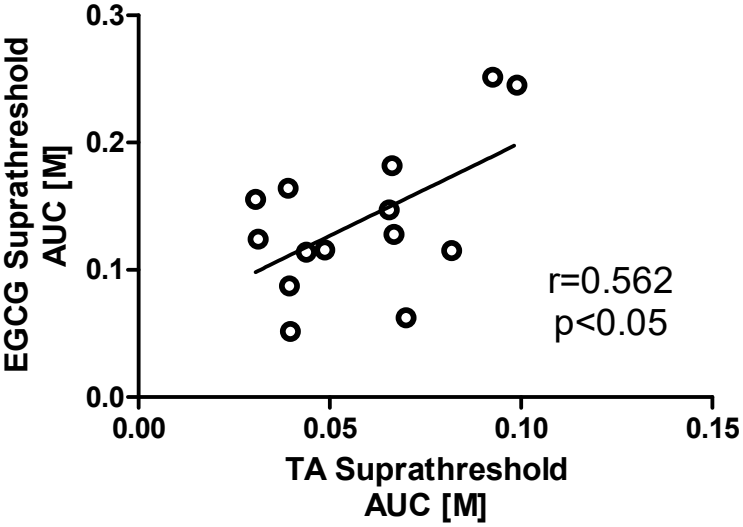

Figure 4.12 Correlation between Epigallocatechin gallate (EGCG) suprathreshold tannic acid (TA) suprathreshold ratings. Significant correlation seen between EGCG suprathreshold area under the curve (AUC) and TA Suprathreshold AUC ($r=0.582$, $p<0.05$)

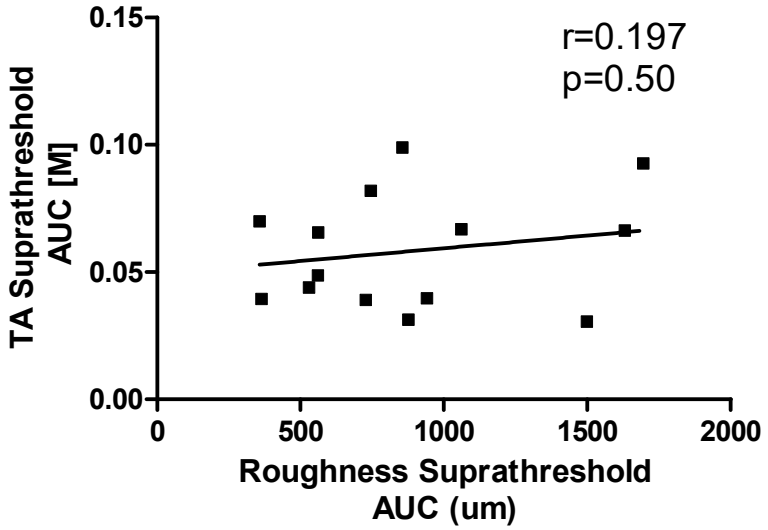

Figure 4.13 Tannic acid (TA) suprathreshold area under the curve (AUC) by roughness suprathreshold area under the curve (AUC). TA AUC plotted on y-axis vs roughness AUC on x-axis. No correlation seen between TA and roughness suprathreshold AUCs (r=0.197, p=0.50)

4.4 Consumption

Across roughness sensitivity groups, the astringent versions of banana (unripe), and chocolate (dark) were found to be significantly more pleasant by those highly sensitive to roughness (RHi) ($p<0.05$, $p<0.005$, respectively) (Fig 4.14). Overall astringency shows the same pattern of participants in RHi rating pleasantness of overall astringency, trending towards significance ($p=0.093$).

Across salivary flow groups, the astringent version of wine (red) as well as the overall astringency rating were both rated to be significantly more pleasant by high saliva producers (SalivaHi) than by lower saliva producers (SalivaLo) ($P<0.05$, $p<0.05$) (Fig

4.15). Tea is trending towards being significantly more well-liked by SalivaHi participants as well (p=0.113).

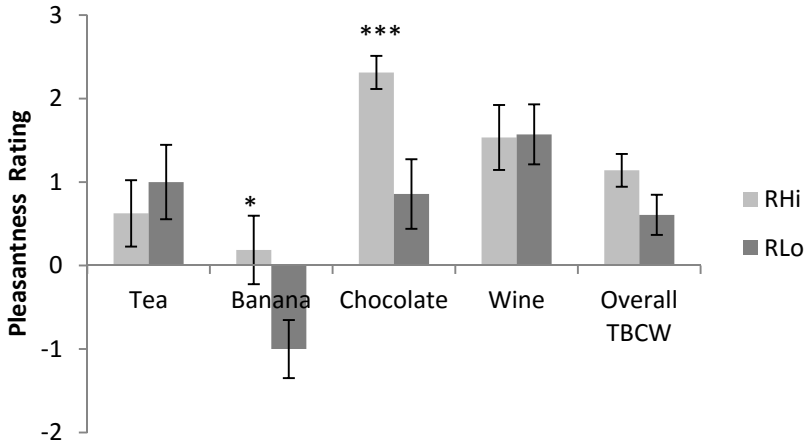

Figure 4.14 Astringent food pleasantness ratings by roughness sensitivity. Astringent food pleasantness ratings obtained from consumption questionnaire by astringent food examples. Pleasantness ratings plotted by roughness detection threshold sensitivity (high roughness sensitivity (RHi), low roughness sensitivity (RLo). Ratings represented as mean +/- SEM.

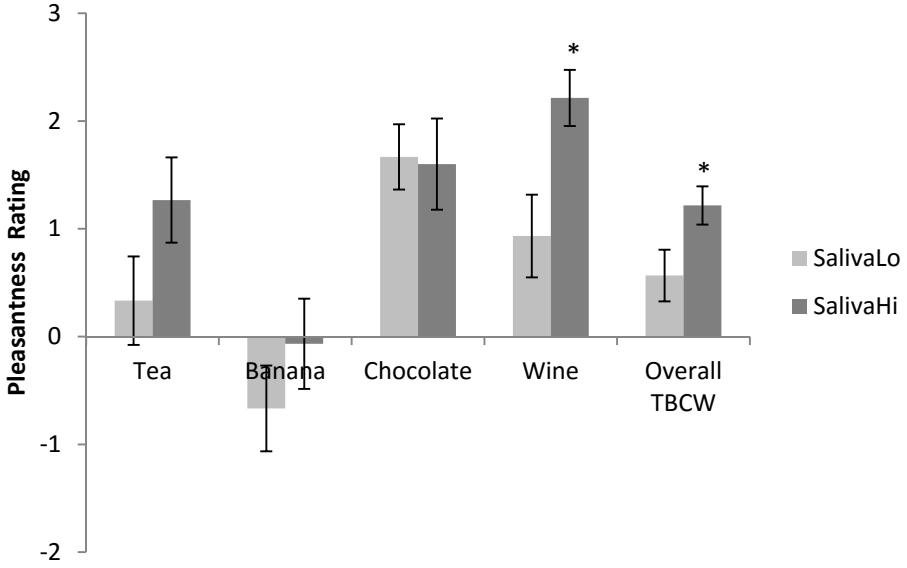

Figure 4.15 Astringent food pleasantness ratings by salivary flow. Astringent food pleasantness ratings obtained from consumption questionnaire plotted on y-axis by astringent foods on x-axis. Ratings plotted for both high salivary flow (SalivaHi) and low salivary flow (SalivaLo) groups. Ratings represented as mean +/- SEM.

Ch. 5: Discussion

5.1 Oral Tactile Acuity

This study provides additional support for the idea put forth previously that the human tongue exhibits exquisite tactile sensitivity (Trulsson and Essick 1997). The majority of participants in the present study were able to distinguish the stimuli pair with the smallest roughness differences, 0.013µm, at least once. The overall average surface roughness detection threshold was 0.20 µm. This suggests that, on average, participants would be able to distinguish between surfaces at 0.20 µm and 0.177 µm (R0), or a roughness difference of 0.023µm. This difference is very small and, although not tested directly, anecdotal evidence suggests that these stimuli were not easy to distinguish with the fingertips, famously regarded for their extreme sensitivity to tactile stimuli. Comparing sensitivity of the fingertips and tongue tip to these stimuli is a focus of current research efforts.

Previous studies have evaluated the size and force threshold of activation of mechanoreceptor receptive fields either in the fingers and hands (Johansson and Valbo 1983), or in the various mucosa and labial surfaces surrounding the mouth (Trulsson and Essick 1997, Bukowska et al. 2009). Results point towards the smallest receptive fields

and force thresholds being found on the dorsal surface of the anterior portion of the tongue, but an experiment directly comparing tactile sensitivity in the glabrous skin of the finger tips versus that in the oral mucosa is yet to be executed. Bukowska et al. (2010) compared their receptive field and sensitivity findings on the inner lip with previous findings on the hands in the literature, but these findings were extracted from previous studies as opposed to directly compared. The findings of this study underline the exquisite tactile acuity of the tongue and support the possibility of its superior tactile sensitivity and resolution compared to the hands and fingers. Further study should be made to directly compare tactile sensitivity in these two areas.

5.2 Roughness Sensitivity Differences

In this study it was demonstrated that there are in fact differences in individual sensitivity to roughness. Bakke et al. (2008) utilized solely the gLMS scale to ask panelists to rate the intensity of the roughness they perceived in breads at various stages of staling. Despite absolute anchors designed to minimize bias, procedures like this are still vulnerable to a degree of subjectivity and likely reflect relative, as opposed to absolute, measures of intensity . Because of the forced-choice, staircase threshold procedure used in the present study, the subjectivity of using subjective intensity scale rating alone has been reduced. Based on the detection threshold findings, there are quantifiable differences in individuals' abilities to differentiate varying degrees of surface roughness.

Stimuli in this study were carefully chosen from a number of prepared stimuli with the objective of having minimal overlap in their roughnesses when standard deviations of profilometer surface roughness measurements were considered. In doing so, we were able to ensure that there was no confusion between stimuli (eg. participants would not be presented with a pair of stimuli in which both were essentially the same roughness; there was always one stimulus of the pair that was definitively rougher). However, the stimuli used in this study were prepared by hand-sanding and, though it was done professionally for maximum consistency, if stimuli could be created with greater consistency and even further minimize deviation in roughness within a stimulus then it is likely that further differences could be elucidated in individuals' roughness detection abilities. Skedung et al. (2013) advocated the use of strain-induced surface wrinkling as a method to obtain controlled wavelength stimuli to fill the stimuli gap necessary to adequately test the tactile limits of the fingers. Stimuli like these could be useful in further exploring the oral tactile limits as well.

Previous studies have pointed towards fungiform papillae density, saliva, and age as potential influences of oral tactile sensitivity (Engelen et al 2008). In this study, as in Bakke et al 2008, tactile sensitivity in the form of sensitivity to detect different levels of roughness was found to be unrelated to fungiform papillae density. This notably contradicts earlier-mentioned theories of both Essick et al. (2003) and Hayes et al. (2007) who observed relationships between fungiform papillae density and tactile acuity, and suggested this supported the idea that fungiform papillae serve as an "array of sensors for detection of oral touch sensation," as put forth by Prutkin et al. (2000) (Essick et al.

2003). The lack of a relationship in our study could stem from differences in the tactile task; Essick was measuring tactile acuity using letter identification as the tactile task, while Hayes was measuring oral tactile acuity through creaminess perception. These tactile tasks differ significantly from the roughness tasks executed in both Bakke et al. (2008), as well as in the present study. It is worth noting that previous studies reporting lingual nerve innervation in the peri- and extragemmal tissues of fungiform taste papillae were designed to identify somatosensory termination but not specifically mechanoreceptors (Ellis et al. 1958, Whitehead et al. 1985). It is possible some of these nerve fibers are non-tactile (the lingual nerve communicates information from thermoreceptors and nociceptors in addition to mechanorecptors). des Gachon et al. (2013) showed evidence against these fibers communicating nociceptive information, but the possibility of these fibers terminating in thermoreceptors still very much exists. Alternatively, it is also possible that the types of fibers associated with fungiform papillae terminate primarily in mechanoreceptors that are involved in edge and point detection but not surface roughness. This possibility would explain a correlation of fungiform papillae density with a letter identification task (Essick et al. 2003) but not with a surface roughness task, as found in both Bakke et al. (2008) and the present study.

An alternative could also be, as Bakke suggested, the overall inadequacy of fungiform papillae density counting to accurately predict overall mechanoreceptor innervation in the mouth. The suggestion of a correlation between mechanoreceptive innervation and fungiform papillae density stems largely from a claim made by Prutkin et al (2000) that the two point discrimination distance on the tongue approximates the

distance between fungiform papillae on the tongue. However, studies supporting this claim since are limited and indirect and none conclusively present a reason for why a positive correlation would be found between fungiform papillae density and tactile acuity of the tongue (Essick et al. 2003, Hayes et al. 2007).

One oversight, in particular, is the potential contribution of other structures besides fungiform papillae; such as filiform, circumvallate, or foliate papillae, or alternative surfaces of the tongue, to tactile sensitivity in the oral cavity. Ellis reports in his 1958 examination of the mammalian tongue, that in the anterior portion of the tongue, almost every filiform papilla contains a subepithelial nerve ending, which he interprets to be either a Meissner's corpuscle or an end bulb of Krause. Perhaps these suggest a larger role of filiform papillae in tactile perception.

For this particular study, because we did not see a relationship between fungiform papillae and roughness sensitivity and discrimination, we were interested in looking at a potential link between the amounts of space between fungiform papillae on the dorsal surface of the tongue. The approximation of this space takes in to account the area of filiform papillae and the size of fungiform papillae, which a simple fungiform papillae count alone does not. For this approximation, the average diameter of 20 papillae within the defined 1.59cm filter paper circle on the tongue (Fig 3.7) was obtained. This average diameter was then multiplied by the total fungiform papillae count, and subtracted from the total area (1.99cm^2), and the resulting value was then taken as the "Non FP" (NFP) area. When sensitivity to roughness was looked at relative to fungiform papillae density in an independent t-test analysis, the p-value was 0.951 (Fig 4.4), strongly indicating no

difference between the two groups. When NFP was compared between roughness sensitivity groups though, the p-value went down to 0.290. Though this value is still relatively far from indicating a significant difference between sensitivity groups, this measure is much closer to revealing potential differences between the two sensitivity groups. The NFP calculation was a rough approximation and the sample size for this study was relatively small; both of these factors could have contributed to why this calculation had been unable to reveal differences. However, it is worth noting that this and other alternative methods to estimate mechanoreceptive innervation besides fungiform papillae counting may be more valuable and should be considered in future studies.

Age and saliva were found to have near significant influence on roughness sensitivity. Though these differences were not statistically significant, because of the relatively small sample size, the visible trends are still worth noting. Individuals in the high roughness sensitivity group (RHi) tended to be lower in age and produce lesser volumes of saliva in a 2 minute collection period than those in the low roughness sensitivity group (RLo). The age observation is consistent with previous findings indicative of decreased sensitivity with age (Mojet et al. 2001, Thornbury et al. 1981, Woodward 1993, and Bangcuyo et al. (unpublished manuscript)). The salivary trend that high roughness sensitivity individuals tended to exhibit relatively low salivary flow rates is consistent with the suggestion by deWijk and Prinz (2006) that decreased lubrication results in increased friction and directly increased perception of roughness.

5.3 Astringency: EGCG vs Tannic Acid

Two structurally different astringents were used to measure astringency detection threshold. Based on w/v concentration alone, there was no difference in detection threshold between the two, but when compared by molarity, tannic acid has a significantly lower detection threshold on average than EGCG. This is relatively unsurprising as there have been multiple reports citing increased affinity of larger, polymerized polyphenols for salivary proteins, attributing this feature to their multidentate nature which allows a single molecule to simultaneously bind many salivary proteins (Jobstl et al. 2004, Baxter et al. 1997, Charlton et al. 2002, Bajec and Pickering 2008). This would explain why, on a molarity basis, average detection threshold was significantly lower for tannic acid than for EGCG in the present study. Differences in binding strategies have also been cited. Condensed tannins have been suggested to employ hydrogen bond formation as the driving force of interaction, while hydrolysable tannin interaction has been seen to be often driven by hydrophobic binding (Hagerman et al. 1998, Baxter et al. 1997; Jobstl et al. 2004, Charlton et al. 2002). These binding differences could also contribute to decreased molar detection threshold of tannic acid compared to that of EGCG.

On an individual basis, the ratio of EGCG detection threshold to TA detection threshold was also calculated for each participant on the basis of molarity. The average EGCG detection threshold to TA detection threshold ratio was 4.95 (SEM 0.61). Galloyl rings have been implicated as playing a significant role in the affinity of tannin-protein binding, with the degree of galloylation found to have a direct relationship with affinity

for proteins for hydrolysable tannins (Baxter et al. 1997, Charlton et al. 2002). Of the compounds used in the present study, EGCG has one prominent external galloyl ring, and TA has five. Therefore, the findings from this study further support the importance of galloyl rings in tannin-protein affinity.

5.4 Roughness and Astringency

In this study, roughness sensitivity is seen to have a strong relationship with EGCG astringency sensitivity for both detection thresholds and suprathreshold perception. This suggests that the sensation elicited by astringent chemical compounds shares similarity to the sensation elicited by a stimulus that is solely mechanical. Though previous studies of astringency have implicated mechanoreceptors as the ultimate mechanism underpinning astringency perception (Trulsson and Essick 1997, deWijk and Prinz 2006, Bajec and Pickering 2008), to our knowledge, this is the first study to directly demonstrate the contribution of the mechanoreceptors involved in perceiving roughness to the sensation of astringency.

The fact that we did not see a stronger correlation in sensitivity to both astringent stimuli could result from a number of factors. First, the perception of roughness, using our roughness detection threshold stimuli, recruited the sole involvement of receptors on the dorsal surface of the anterior portion of the tongue. Detection of astringency, on the other hand, involved the entire surface of the tongue as well as additional surfaces in the oral cavity such as the roof of the mouth, which participants were instructed to rub their

tongue against when perceiving astringency, and the gums and cheeks, which also were exposed to the stimuli and assisted in evaluation of stimuli astringency. The involvement of these additional oral surfaces adds an element which differentiates the roughness stimuli from the chemical astringents and could easily result in sensitivity discrepancy between stimuli within an individual. An alternative for direct comparison would be to develop roughness stimuli which could be manipulated by the whole oral cavity and then expectorated. An additional method that would limit, but not completely eliminate these evaluation differences would be to apply the astringent directly to the surface of the tongue with an applicator and have the participant only rub their tongue on their hard palate to perceive the astringency. This strategy would still involve the roof of the mouth, a surface that is unexplored in terms of mechanoreceptive force threshold and receptive field size, as an additional mouth surface in the perception of astringency, but would not involve exposure to as many additional oral surfaces as the strategy utilized in the present study.

Another factor that can result in perceptual differences between the roughness and astringency stimuli is saliva. The direct preliminary interaction of salivary protein with astringent stimuli and the involvement of this interaction in creating the sensation of astringency is a key difference between roughness and astringency.

In this study, a relationship is seen between roughness detection abilities and astringency for EGCG, but not for tannic Acid. A relationship is seen, however, between tannic acid astringency detection and salivary flow. This tastefully illustrates the idea that there are (at least) two key factors at play in the perception of astringency; oral tactile

sensitivity to roughness, and saliva, and that the importance of each factor appears dependent on the astringent stimuli. From our results, it could be hypothesized that oral tactile roughness sensitivity plays a more significant role in EGCG detection threshold while salivary flow appears to play the more significant role in tannic acid astringency detection.

5.5 Saliva and Astringency

Salivary flow and composition differ greatly across individuals (Fischer et al. 1994, Bajec and Pickering 2008). In the present study alone, the unstimulated saliva produced by a single individual in two minutes ranged from just 0.6 g to almost 6.5 g. Differences in salivary flow result in differences in salivary volume, salivary pH, and protein composition of this saliva in the mouth (Fischer et al. 1994). In this study, tannic acid astringency detection threshold was found to differ significantly by salivary flow while EGCG astringency detection threshold was not. We hypothesize this susceptibility of TA astringency to salivary flow rate, especially as compared to EGCG, may reflect specific chemical properties that make it more sensitive to changes in salivary volume, pH, and protein composition. Two possible explanations for this discrepancy in astringents could be differences in binding mechanisms or differences in pKa between the two compounds; both resulting from structural differences. It has been suggested that binding between condensed tannins and proteins is initiated primarily by hydrogen binding while binding between hydrolysable tannins can be much more reliant on

hydrophobic binding (Hageman et al. 1998). Individual differences in saliva could affect one form of bond formation to a greater degree than the other which could account for why detection of tannic acid astringency is more impacted by saliva. Additionally, salivary pH typically falls around 7.2. The pka of EGCG is 7.75, while the pka of tannic acid is 10. Because of this, tannic acid would require much greater buffering action in the oral cavity than EGCG and salivary buffering capacity differs greatly with salivary flow (Fischer et al. 1994).

In the present study, we see that individuals in the high flow salivary group have significantly lower tannic acid astringency detection thresholds. A possible explanation is that individuals in the high salivary flow group were more sensitive to the astringency of tannic acid. Individuals with higher salivary flow are likely to have greater overall salivary protein content (Dawes 1969). If this is the case, it is a possibility that high salivary flow individuals had more proteins with which tannic acid was able to bind, resulting in greater precipitation and aggregation of astringent-salivary protein complexes and a greater resulting overall reduction in lubrication, with greater increase in friction being ultimately conveyed by the oral mechanoreceptors.

An alternative explanation may be found in potential carry-over effects. Ishikawa and Noble (1995) found that individuals with low salivary flow perceived astringency later and rated it higher on the intensity scale than high flow individuals during astringency rating of red wines. It was proposed that these differences were the result of high-flow individuals being able to more efficiently restore lubrication in the oral cavity following exposure to astringent stimuli (Ishikawa and Noble 1995, Lesschaeve and

Noble 2005). On the surface, our findings seem contradictory. However, in our study we were repeatedly providing individuals with astringent stimuli with a 20s interval between rinsing and the next stimulus. We did not see many instances of carry-over, as by the nature of the staircase procedure we were most frequently operating just above or below an individuals' astringency detection threshold. However, there were a few instances where minimal carry-over was reported. If carry-over occurred in low-salivary flow individuals without their noticing or expressing its existence, these subjects may have had a more difficult time overcoming any potential carry-over effects and had a more difficult time with subsequent evaluations, raising their overall detection thresholds.

The salivary flow measurement taken in this study is relatively crude, and because it was a volume measurement alone, it is difficult to infer salivary protein composition. A valuable amendment in future study would be to collect and analyze the concentrations of the different proteins found in the saliva and compare these specific concentrations to astringency sensitivity.

5.6 Consumption

The food consumption data reinforces the idea of the duality of astringency in that the sensation appears to me prominently influenced by two factors; salivary flow volume and oral roughness sensitivity. Depending on the astringent food, significant differences in pleasantness rating were seen based on high/low roughness sensitivity or high/low salivary flow divisions. These differences were seen most notably for chocolate and red

wine. Individuals that were found to be more sensitive to roughness, and also EGCG, rated dark chocolate, which has substantially more astringent character than milk chocolate, to be much more pleasant than less sensitive individuals. Similarly, those with high salivary flow rates, that were also seen to be more sensitive to tannic acid, rated red wine, which has much greater astringent character than white wine, as significantly more pleasant than those with low salivary flow rate.

The polyphenol content of dark chocolate predominates in monomer flavan-3-ols and their dimers, trimers, and oligomers (Miller et al., 2009). These structures make up 60% of the polyphenol content of the cocoa bean; mainly composed of monomeric epicatechin, and dimeric and trimeric procyaninin B-2, B-5, and C-1 (Haslam 1998). Though up to 90% of the total polyphenol content present in the cocoa bean is converted to red-brown colorants during the fermentation process, dark chocolate retains a much greater percentage of these than milk chocolate. Dark chocolate retains an average of 145.8 mg of flavan-3-ols per 40 g chocolate serving compared to 27.4 mg for a 40 g serving of milk chocolate (Miller et al., 2009). These catechin and epicatechin flavan-3-ol based structures share common structural features to the condensed flavan-3-ol used in this study, epigallocatechin gallate (EGCG). Individuals sensitive to roughness, which was seen to correspond to greater EGCG sensitivity in this study, are the group that reported a significantly greater preference for dark chocolate. In this case, it is possible that because of this roughness sensitivity, which appears to underpin EGCG sensitivity, individuals are able to pick up on the subtle astringency contributed by the relatively high

astringency threshold flavan-3-ols of chocolate (Haslam, 1998) and find that this additional characteristic contributes depth to the flavor, increasing their appreciation.

The polyphenolic content of red wine is also dominated by flavan-3-ols whereas in white wines, flavan-3-ols and their related proanythocyanidins, are virtually unseen because of the minimal contact that they have with the phenol-rich grape skin and seeds (Haslam 1998). Per liter, on average, red wines express around 200mg of caftaric acid and non-flavonoids, around 400 mg of anthocyanins, and then around 800 mg of a combination of both monomeric and oligomeric phenolic flavan-3-ols (Haslam 1998). Red wines aged in oak barrels, however, do have the additional possibility to express ellagi- and gallotannins imparted on the wine from the wood (Glabasnia and Hofmann 2006). These hydrolysable tannins are more structurally similar to the polymeric hydrolysable tannic acid compound utilized in this study. The high saliva group, which we also found to be the group best able to detect the astringency of tannic acid, reported wine to be significantly more pleasant than their low saliva counterparts. This again may be able to be interpreted as an increased ability to perceive, and therefore, greater appreciate the extra tactile component contributing to the overall mouthfeel of the product. Alternatively, in the case that carry-over explains the tannic acid sensitivity results, it is also possible that low saliva individuals take less pleasantness from the astringency of red wine because of an inability to rid themselves of the sensation quickly enough that it becomes too overwhelming.

These findings further underscore the overall conclusions of this study and the great importance that both tactile sensitivity and salivary flow and composition play in

astringency perception. If lower tannic acid detection thresholds were due to higher sensitivity (as opposed to carry-over effects) then the consumption based on both roughness sensitivity and salivary flow are both consistent with the idea that individuals most sensitive to astringency are the ones that find it most pleasant. This would be an interesting finding as one might hypothesize that perceiving greater astringency could be unpleasant to some, and these results would appear to support the opposite. It is possible that because of increased perception, individuals sensitive to astringency in these foods are able to appreciate the somatosensory experience that this sensation imparts on the food, and this allows them a greater appreciation over those less able to perceive this.

Ch. 6: Conclusion

In this study, a novel approach and stimuli were used to evaluate oral roughness sensitivity. This study improved upon previous investigations of oral tactile acuity by looking at individual sensitivity differences through the lens of a textural modality—roughness—that is directly applicable to foods, both in its relation to grittiness and graininess and in its contribution to astringency. Previous studies aimed at specifically addressing oral roughness sensitivity were improved upon by roughing stimuli in a controlled manner which minimized confounds, by measuring stimulus roughness using objective instrumental means, and by implementing a quantitative testing method that is more informative of sensitivity differences within and between individuals. The present study is also able to draw connections between sensitivity of the controlled stimuli to sensitivity of astringent compounds important in food texture.

We notably did not find a relationship between tactile roughness sensitivity and fungiform papillae density. This leads us to believe that the suggestion of fungiform papillae as an array of sensors for tactile perception on the tongue should be re-examined. In addition to the present work, other studies have not found a relationship between sensitivity to a tactile dimension and fungiform papillae density. These results suggest

that fungiform papillae density does not provide a strong indicator of tactile sensitivity, at least for tactile sensitivity for the sensation texture modality of surface roughness. The contribution of other structures in the tongue to oral tactile sensitivity, such as filiform, foliate, and circumvallate should be considered.

We found a marginally significant relationship of oral tactile roughness sensitivity with both age and salivary flow. Individuals with high roughness sensitivity tended to be younger in age and have relatively low salivary flow rates. The age trend is in accordance with findings of sensory decline with age observed in previous studies.

Presently, we also demonstrated a relationship between oral roughness sensitivity and astringency imparted by the flavan-3-ol epigallocatechin gallate (EGCG). This provides support for the theory that astringency is prominently tactile driven. The correlation of these sensations suggests that the same mechanoreceptors that are activated during stimulation with purely tactile stimuli are similarly activated during friction between de-lubricated oral surfaces, ultimately resulting in the sensation known as astringency. The mechanism of astringency being the result of mechanoreceptors stimulation has been suggested but has not been directly illustrated prior to the present study.

There was no pronounced relationship seen between oral roughness sensitivity and tannic acid astringency, however, a relationship was alternatively seen between tannic acid sensitivity and salivary flow. This finding, paired with the relationship between roughness sensitivity and EGCG sensitivity, leads us to hypothesize that sensitivity to astringency is driven by (at least) two factors; oral roughness sensitivity and

salivary flow and composition, and that the factor by which it is principally driven is compound dependent. The results collected from our food consumption questionnaire appear to support this hypothesis in that individuals were seen to prefer certain astringent foods in accordance with either their roughness sensitivity or salivary flow.

In future studies, stimuli with even more precisely controlled roughness variation allowing smaller differences between stimuli would be beneficial to uncover more specific sensitivity differences among individuals. The field would also benefit from a study which directly compares the tactile sensitivity of the fingers to that of the tongue, through multiple different textural modalities (e.g. edge and point, punctate, roughness) to acknowledge potential modality dependent superiority of one area of the other. Finally, in looking at sensitivity to astringency on the basis of salivary flow, it would be advantageous in the future to measure protein composition in addition to simple volume. It is possible that this plays an interesting and more important role in astringency perception than salivary flow volume itself.

The applicability of roughness to food matrices makes these findings very translatable to the industry. During product development, instead of creating one texture for a product, it may worthwhile to keep in mind that sensitivity to roughness, and likely thereby preference for different textures, may be better satisfied by multiple products that span the roughness continuum, due to apparent sensitivity differences across the population.

Astringency, like oral tactile roughness sensitivity, is also not yet completely understood. By providing support for mechanoreceptive activation underpinning the

mechanism of astringency, we hope to contribute to the body of research aimed at improving its understanding. Many polyphenolic compounds have positive antioxidant potential, but cannot be added to foods in amounts that take advantage of this because of their overwhelming astringency in these quantities. A better understanding of the step-by-step mechanism underlying astringency will allow the development of improved techniques to mitigate negative aspects and potentially allow incorporation of some of these valuable compounds in quantities that will benefit the consumer.

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Appendix A: IRB Approval

Behavioral and Social Sciences Institutional Review Board

Office of Responsible Research Practices
300 Research Administration Building
1960 Kenny Road
Columbus, OH 43210-1063

Phone (614) 688-8457
Fax (614) 688-0366
www.orrp.osu.edu

June 18, 2015

Protocol Number:	**2013B0277**
Protocol Title:	**FLAVOR INTERACTIONS AND THE IMPACT ON TEXTURE ASSESSMENT AND ORAL TACTILE SENSITIVITY, Christopher Simons, Food Science & Technology**
Type of Review:	Continuing Review – expedited
IRB Staff Contact:	Jenna Mowls-Hutkowski Phone: 614-688-2208 Email: mowls-hutkowski.1@osu.edu

Dear Dr. Simons,

The Behavioral and Social Sciences IRB **APPROVED BY EXPEDITED REVIEW** the above referenced research. The Board was able to provide expedited approval under 45 CFR 46.110(b)(1) because the research meets the applicability criteria and one or more categories of research eligible for expedited review, as indicated below.

Date of IRB Approval:	June 17, 2015
Date of IRB Approval Expiration:	June 17, 2016
Expedited Review Category:	7

Request for changes dated May 28, 2015 (Add Brianne Linne, Elka del Portal, Michael Arato and Robert Kaufman as key personnel).

If applicable, informed consent (and HIPAA research authorization) must be obtained from subjects or their legally authorized representatives and documented prior to research involvement. The IRB-approved consent form and process must be used. Changes in the research (e.g., recruitment procedures, advertisements, enrollment numbers, etc.) or informed consent process must be approved by the IRB before they are implemented (except where necessary to eliminate apparent immediate hazards to subjects).

This approval is valid for **one year** from the date of IRB review when approval is granted or modifications are required. The approval will no longer be in effect on the date listed above as the IRB expiration date. A Continuing Review application must be approved within this interval to avoid expiration of IRB approval and cessation of all research activities. A final report must be provided to the IRB and all records relating to the research (including signed consent forms) must be retained and available for audit for at least 3 years after the research has ended.

It is the responsibility of all investigators and research staff to promptly report to the IRB any serious, unexpected and related adverse events and potential unanticipated problems involving risks to subjects or others.

This approval is issued under The Ohio State University's OHRP Federalwide Assurance #00006378. All forms and procedures can be found on the ORRP website – www.orrp.osu.edu. Please feel free to contact the IRB staff contact listed above with any questions or concerns.

Michael Edwards, PhD, Chair
Behavioral and Social Sciences Institutional Review Board

Appendix B: Panelist Consent Form

The Ohio State University Consent to Participate in Research

Study Title: Flavor interactions and the impact on texture assessment and oral tactile sensitivity

Researcher: Christopher T. Simons, Ph.D.

Sponsor: None

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate.

Your participation is voluntary.

Please consider the information carefully. Feel free to ask questions before making your decision whether or not to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form.

Purpose:
We are interested in how flavor information is processed to create the sensations elicited when smelling and eating foods. The overall purpose of this study is to gain insight into how perceptions and liking of food change as taste, smell, spiciness, temperature and texture components are modulated individually or in combination. In addition, we believe tactile sensitivity of the tongue contributes to texture perception. To gain insight as to how oral texture information is processed, different aspects of tactile sensitivity of the tongue will be measured. This information will be used to determine if tactile sensitivity of the tongue and the perception and liking of different food textures are linked.

Procedures/Tasks:
In some cases, you will be asked to evaluate the intensity and liking of tastes, flavors and/or textures from various model food systems or food products. After tasting each sample, you will be asked to rate how strong you perceive the taste, flavor or texture attribute. Similarly, you will also be asked how much you like or disliked the sample.

In some cases, we may be interested in how sensitive your tongue is to different textural attributes. There are several ways we may test this. In some cases, we may blindfold you and touch various locations on your tongue tip with a thin nylon monofilament. You will be asked to identify the side of your tongue touched by the monofilament. We will also ask you to rate the intensity of this stimulus. We will assess your sensitivity before and after we apply a flavor to your tongue. The flavor may have a taste (e.g. sour or bitter) or it may be spicy or cooling. We will tell you what the flavor is before applying it to your

tongue with a cotton swab. The second way we may assess your tongue's sensitivity is by having you rate the roughness of various surfaces using your tongue tip. The temperature of these surfaces may vary from cold to hot and so we will ask you not to keep your tongue in contact with them for longer than 15 sec at a time. We may also have you rate the roughness of various surfaces after we apply a flavor to your tongue. The flavor may have a taste (e.g. sour or bitter) or it may be spicy or cooling. The final way we may assess your tongue's sensitivity to tactile stimuli is by having you identify raised alphabetical letters affixed to a holder using only your tongue tip. The temperature of the holder may vary from cold to hot and so we will ask you limit contact between your tongue and the letters to 15 sec or less. In some cases we may ask you to identify letters after pre-treating your tongue with a flavor that may have a taste (e.g. sour or bitter) or may be spicy or cooling.

Duration:
Participation in this experiment will take no more than 60 min per session. In some cases, you may be asked to return to the laboratory at a subsequent time for further testing. In such instances, you will be notified prior to the onset of the first experimental session so you can decide if you want to participate.

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

Risks and Benefits:
The food and flavor products that you will evaluate are comprised of ingredients that have been approved for use in foods by the United States Food and Drug Administration. In some cases, the products may contain spicy or cooling compounds that elicit burning or cooling sensations, respectively. You may experience mild discomfort associated with these sensations. Typically, these sensations disappear within approximately 10 min. In some cases, we may ask you to place your tongue on a temperature probe that can be heated or cooled. If your tongue remains in contact with the heated probe for longer than 1 min, you may receive a minor burn. We will ask that you keep your tongue in contact with this probe for no longer than 15 sec at any given time.

You will receive no direct benefit for participating in this study. However, the insight gained from your participation will give us a better idea of how various food attributes are processed by the brain to influence food perception and liking.

Confidentiality:
All information will be stored in a secure computerized database. At the onset of the experimental session, you will be asked to provide general demographic information including age, gender and ethnicity. In some cases additional information regarding

eating and dietary habits may be obtained. These data will be collected using secured computerized data acquisition software or, on occasion, paper ballot. Data collected from paper ballots will be input into a secure computer at the earliest convenience and the paper ballot destroyed.

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law. Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor, if any, or agency (including the Food and Drug Administration for FDA-regulated research) supporting the study.

Incentives:
You will receive either course credit or a gift card in the amount of $20 per hour. At the conclusion of data collection, you can choose to be compensated with a gift card or course credit. In the event that you participate in an experiment that requires returning to the laboratory for multiple sessions, you will receive compensation at the end of each session.

Participant Rights:

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

Contacts and Questions:
For questions, concerns, or complaints about the study, or you feel you have been harmed as a result of study participation, you may contact the Principal Investigator, Christopher T. Simons at (614) 688-1489 or simons.103@osu.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form**.**

Appendix C: Demographic Questionnaire

Please type in your age below.

Please verify your ethnicity by selecting the corresponding option below.

- White, Caucasian
- Black, African American
- American Indian or Alaska Native
- Hispanic or Latino
- Asian, Pacific Islander
- Other
- Prefer not to answer

Please verify your gender by selecting the corresponding option below.

- Male
- Female
- Other

Are you a cigarette smoker? If yes, when was the last time that you smoked?

- Yes
- No

Appendix D: Consumption Questionnaire

Please rate your perceived pleasantness of the following foods and beverages.

Tea with sweetener

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

Tea without sweetener

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

Very ripe banana

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

Not very ripe banana

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

Milk chocolate

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

Dark chocolate

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

White wine

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say

Red wine

Very unpleasant	Fairly unpleasant	Slightly unpleasant	Neither pleasant nor unpleasant	Slightly pleasant	Fairly pleasant	Very pleasant	Unknown product/Cannot say