# THE MOUTHFEEL OF BLACK TEA BEVERAGES

By

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I never thought I would stay in Nottingham for more than 6 months and I give thanks to God for bringing me here and seeing me through the PhD.

# DEDICATION

Dedicated to my parents, my brother and Ola

# ABSTRACT

The mouthfeel and texture of food and beverage products play an important role in consumer liking. However, to date these key sensory properties have received limited attention from researchers and our understanding of texture and mouthfeel perception in food remains restricted. For tea, a popular beverage enjoyed around the globe, astringency is the most frequently highlighted mouthfeel attribute. Since black tea is commonly consumed with milk and sugar, research investigating the mouthfeel of tea as impact by these added ingredients is of commercial interest.

The aim of this PhD research was to gain a better understanding of the mouthfeel perception of black tea beverages using sensory and instrumental methods. In order to achieve this, a model system consisting of black tea instant powder, sugar, and fat (added as oil-in-water emulsion) was developed which allowed for the independent variation of the design factors (tea, sugar, fat).

Firstly, a mouthfeel lexicon was developed using Quantitative Descriptive Analysis (QDA), and the impact of tea, sugar and fat on the perception of selected attributes was assessed by a trained panel (n=10) using a Design of Experiments approach to generate predictive polynomial models from a D-optimal design. Furthermore, the temporal perception of astringency and bitterness over multiple sips of tea was assessed by a trained panel (n=9) using Time Intensity measures. The effect of sweetness and viscosity from sucrose was decoupled using sweeteners and thickeners and the effect of sugar on astringency and bitterness perception was investigated in more detail. Once the mouthfeel perception of black tea

beverages was explored, instrumental methods were deployed to reveal possible correlations between instrumental parameters and mouthfeel perception. Two instrumental methods were used: a force plate to measure the frictional and vibrational behaviour of tea samples, whilst an accelerometer was used to measure vibrations related to tongue movements using a technique called "acoustic tribology".

The results showed that besides astringency, fat-driven attributes, such as "thickness", "slipperiness" and "mouth coating", play a significant role in the mouthfeel perception of black tea beverages. Predictive polynomial models revealed the complex effects of key ingredients on the mouthfeel of tea. The results showed that tea was not a significant factor ($p>0.05$) for the attributes "thickness" and "mouth coating", whereas for all other attributes all three design factors affected perception significantly ($p<0.001$). It was found that astringent and bitter intensity was reduced by the addition of fat and sugar in a similar fashion. However, when evaluated over time, it was found that astringency and bitterness had distinctly different temporal profiles in tea, illustrating the importance of temporal ratings. A build-up in astringency and bitterness intensity with increasing number of sips gave further insight into the perception of both attributes during tea consumption, representing a normal tea drinking behaviour. Furthermore, the data indicated that sugar reduces astringency due to its sweetness and not the rise in viscosity.

The results from the force plate experiment showed that the addition of 5% fat significantly reduced the friction coefficient of tea samples ($p<0.0001$) and that an addition of 10% fat did not reduce the friction coefficient further ($p=0.97$). It was also shown that the friction coefficient

did not vary significantly between tea levels ($p=0.324$). The results also revealed that fat-driven attributes were negatively correlated to friction and that astringency was a complex precept, which was difficult to predict using instrumental methods, resulting in a poor correlation between friction coefficient and astringency ($R^2=0.16$). The results from the "acoustic tribology" experiment indicated that mouthfeel was linked to measured vibrations caused by tongue movements. Furthermore, differences in tea composition resulted in different oral vibrations, and it was observed that for example "thick" is positively correlated to low frequency vibrations.

The results of this research on the perceived mouthfeel of tea provide much needed insight into this key sensory property of tea. These results will be further useful for product developers interested in producing ready-to-drink tea beverages where mouthfeel is likely to be a critical factor for consumer liking and commercial success.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| TRC | Taste Receptor Cell |
| PRP | Proline-Rich Proteine |
| ATP | Adenosine Triphosphate |
| PROP | 6-n-proylthiuracil |
| QDA | Quantitative Descriptive Analysis |
| TI | Time-Intensity |
| AuC | Area under Curve |
| $I_{Max}$ | Maximum Intensity |
| $T_{Max}$ | Time of Maximum Intensity |
| ANOVA | Analysis of Variance |
| PCA | Principal Component Analysis |
| MFA | Multiple Factor Analysis |
| SD | Standard Deviation |
| CV | Coefficient of Variance |
| GAE | Gallic Acid Equivalent |
| rpm | Revolutions per Minute |
| kPa | Kilopascal |
| µm | Micrometre |
| nm | Nanometre |
| mm | Milimetre |
| $s^{-1}$ | Per Second |
| mPa·s | Milipascalseconds |
| N | Newton |
| g | Gram |
| l | Litre |
| ml | Mililitre |
| % | Percent |
| °C | Degree centigrade |
| ° | Degree |
| S | Second |
| min | Minute |
| h | Hour |
| Hz | Hertz |

# 1 Introduction

## 1.1 Structure of the Thesis

This thesis is organised into six chapters namely a general introduction and four results chapters followed by overall conclusions. All four results chapters include a brief introduction specific to the research reported in the respective chapter, a methods section, a results and discussion section followed by the conclusions drawn from the research. The thesis is structured in this way to prepare for publication of the different parts of this research after the publication embargo requested by the industrial sponsor of this PhD has been lifted. Therefore, some aspects in each chapter introduction are repeated. However, where methods have been repeated, these have been references, as opposed to being repeated to avoid continuous repetition for the reader. In line with publication guidelines of journals selected for publication, Chapters 2 and 3 feature a separate results and a separate discussion section whereas these two sections haven been combined in Chapter 4 and 5.

## 1.2 Literature Review

### 1.2.1 Black Tea

*1.2.1.1 The Importance of Tea*

Tea is one of the most enjoyed and popular drinks in the world and is consumed by two-third of the world‘s population (Sinija et al., 2007). Tea was first discovered by the herbalist and Chinese emperor Shen Nung almost 4000 years ago (Bond, 2011). Via the Silk Road and the Spice Route, tea made its way into the Middle East and into the rest of Europe.

Tea arrived in the United Kingdom in 1610 for the first time (Bond, 2011). Since then, tea has become the national drink of the British with about 80 % of British consumers drinking tea every day, which adds up to 165 million cups (UK Tea Association, 2017). In the UK, tea is usually consumed with milk, and one third of the consumers add sugar to their cup of tea (UK Tea Association, 2017), illustrating the importance of both ingredients.

Tea has been considered to have many positive benefits such as improving the mood and increasing relaxation and concentration. Although consumers have historically associated tea consumption with certain psychological benefits, only in recent years have scientists been able to provide some evidence of these benefits (Einother & Martens, 2013). For example, it was found that the consumption of black tea may improve attention and self-reported alertness (De Bruin et al., 2011; Hindmarch et al., 1998). Increased attention span as a result of tea consumption has generally been attributed to caffeine and theanine, and studies which investigated the impact of caffeine or theanine on performance support these findings in black tea (Einother & Martens, 2013). Tea is also thought to have a positive impact on mood. Recently, it was shown that positive mood states lead to improved creative problem solving compared to neutral mood states (Davis, 2009). It was reported that tasting a cup of commercially available ice tea increased problem solving skills compared to tasting bottled water or no consumption of any drink (Isen et al., 2004). Furthermore, Steptoe and Wardle (1999) and Bryan et al. (2012) found indications that subjects who consume more tea feel less tired and reported better work performance more often than subjects who drank less tea. However, this only applied when tea was consumed without milk and/or

sugar and could be an indication that additives may play a role in how tea impacts tiredness and work performance.

Besides its positive impact on mood, tea is also known to promote good health. A number of studies have investigated whether tea or tea polyphenol consumption can improve or prevent illnesses such as cancer, cardiovascular diseases and diabetes (Baker et al., 2007; Khan & Mukhtar, 2007; Lorenz et al., 2007; Luczaj & Skrzydlewska, 2005). Researchers have identified both (-)-epigallocatchin-3-gallate (green tea) and theaflavin-3,3´-digallate (black tea) as the two most effective anti-cancer compounds found in tea (Khan & Mukhtar, 2007).

#### *1.2.1.2 The Manufacturing Process of Black Tea*

Tea is brewed from the leaves of two varieties of the plant *Camellia sinensis* - *assamica* and *sinensis*, both originating from China and Southeast Asia (Balentine et al., 1997). All teas originate from the same plant but different manufacturing processes give different teas their characteristic aroma and taste (see Figure 1-1). For black tea, which was used for this research, the tea leaves are plucked, then withered, rolled, oxidized and dried for a final time. The tea leaves can be picked in four different ways: imperial, fine, normal or coarse. The imperial method, mainly used for white teas, yields the most valuable product as just the tip (bud) of the tea plant is taken. The fine method is most frequently used and involves collecting the top bud and the first two leaves by hand. The normal method is very similar to the fine method, but it is less refined and selective, which means that sometimes three or four leaves are picked. The coarse method is done by machine and

up to six leaves may be picked from the tea plant (UK Tea Academy, 2017).

After picking the leaves, they are transported and processed in a nearby processing facility, starting with the second stage of production known as "withering". During withering which usually takes place indoors, the moisture content of the tea leaves is reduced from 75 – 80 % to 55 – 70 %. This process can take up to 16 hours and leads to the concentration of polyphenols in the tea leaves.

Withering is followed by either rolling or cutting of the leaves. Leaves are rolled when a so-called orthodox manufacture is followed. Here, leaves are rolled most commonly by machines or for high quality tea by hand. The breaking of the leaves leads to the break up the cells and prepares them for oxidation. This style of manufacture produces large pieces of leaf, medium-sized broken pieces and some small dusty particles. If the leaves are cut, the CTC method is used. CTC stands for "cut, tear, curl" or "crush, tear, curl". The withered tea leaves are minced in rolling drums with teeth and shredded into very fine particles, mostly used in tea bags. Both processes aim to reduce the leaf size and also to ensure a level of homogeneity in the tea leaves.

The withered and rolled leaves are subsequently oxidised in order to produce complex polyphenols which give black tea its characteristic flavour. Oxidation usually takes place in oxidation chambers and depending on the method and temperature can take between 40 min to 3 hours. The process of oxidation can also be wrongly referred to as fermentation. However, oxidation describes the reaction of enzymes of the

tea leaves in presence of oxygen whereas fermentation refers to bacteria-induced reactions in the tea leaves, often in the absence of oxygen. Black tea is fully oxidised whereas oolong tea is partially oxidised. The most common fermented tea commercially available is puerh tea.

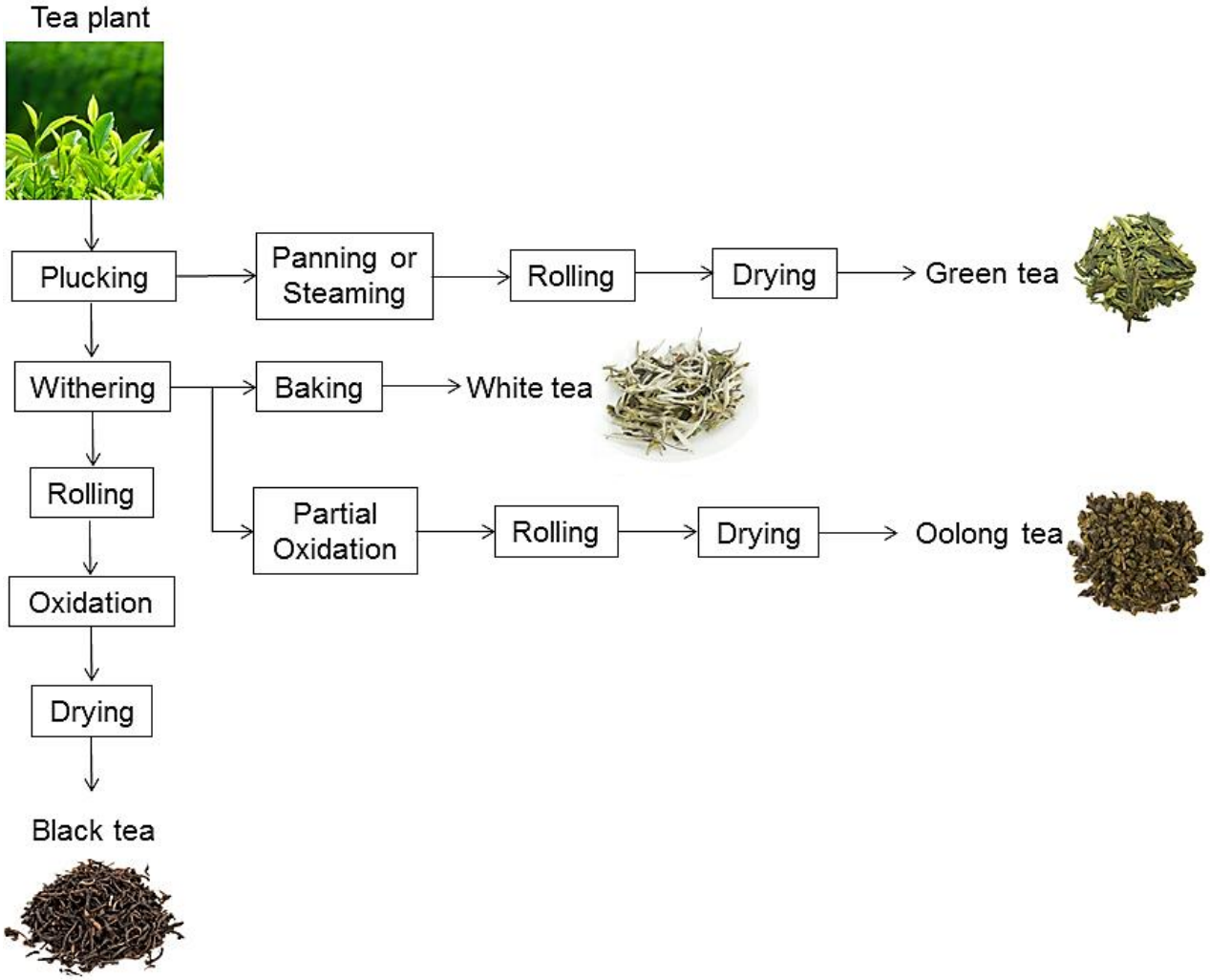



*Figure 1-1: Tea manufacture chart for black tea, green tea, white tea and oolong tea. Adapted from UK Tea Academy (2017)*

The final process, also known as "firing" is an essential drying process used to stop the oxidation of the tea leaves and to further reduce the moisture content of the tea leaves to approximately 2.5 – 3.5 % (Wright, 2007).

The way tea is consumed has changed dramatically in the last 100 years. Originally tea was consumed as loose tea leaves, later on it changed to blended teas, packet teas, tea bags and finally to instant teas, ready-to-drink teas and flavoured teas (Sinija et al., 2007).

Throughout this research, a powdered instant black tea was used. The first instant tea was produced in England from black tea in 1940. Instant tea powder is a fully soluble solid material that emerged as a new and fast-growing product in many countries, especially in Asian countries where ready-to-drink teas are common (Someswararao & Srivastav, 2012). Instant tea is presently produced via hot or cold water extraction from the tea infusion or by spray/freeze-drying of the concentrated brew of extracted tea leaves (Ye et al., 2014).

*1.2.1.3 The Composition of Black Tea*

Tea has a unique composition including a wide range of catechins, theaflavins and other polyphenols and also theanine, an amino acid that is virtually unique to tea (apart from the fungus Bay bolete) (Einother & Martens, 2013). The composition of solid extracts from black tea can be found in Table 1-1.

*Table 1-1: Composition of a solid extract from black tea. Source: (Khan & Mukhtar, 2007).*

| Compound | Concentration [%] |
|---|---|
| Catechins | 10 – 12 |
| Theaflavins | 3 – 6 |
| Thearubigins | 12 – 18 |
| Flavonols | 6 – 8 |
| Phenolic acids | 10 – 12 |
| Amino acids | 13 – 15 |
| Methylxanthines | 8 – 11 |
| Carbohydrates | 15 |
| Proteins | 1 |
| Mineral matter | 10 |
| Volatiles | <0.1 |

Catechin, theaflavins and thearubigins are part of a group called polyphenols. Polyphenols are secondary plant metabolites produced by two main synthetic pathways - the shikimate pathway and the phenylacetate pathway, and over 8000 polyphenolic structures are currently known (Lattanzio, 2013). Natural polyphenols range from simple molecules such as phenolic acids to highly polymerized compounds such as tannins. Flavonoids are the most common and wildly distributed group of plant phenolics. Figure 1-2 represents the basic structure of the flavonoid molecule, a diphenylpropane ($C_6$ — $C_3$ — $C_6$) which consists of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle.

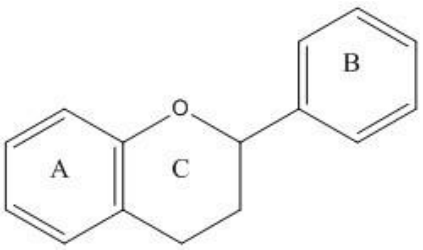



*Figure 1-2:Basic structure of a flavonoid - adopted from Bravo (1998)*

Commercially available black teas in the UK have a total polyphenol content of 80.5 to 134.9 mg/g of dry matter and a catechin content of 5.6 to 47.5 mg/g of dry matter (Khokhar & Magnusdottir, 2002). For instant black teas, total polyphenol contents between 350 mg/g and 440 mg/g of dry matter were observed (Bryans et al., 2007; Salah et al., 1995).

Catechin and epicatechin are the main flavanols in many fruits, whereas gallocatechins, epigallocatechin and epigallocatechin gallate are mainly found in grapes and more importantly in tea (Manach et al., 2004). The chemical structure of these simple polyphenols is shown in Figure 1-3. Catechins belong to the group of flavonoids which are monomeric constituents of condensed tannins (Balentine et al., 1997). The sensory properties of catechins are described as bitter and astringent (Millin et al., 1969).

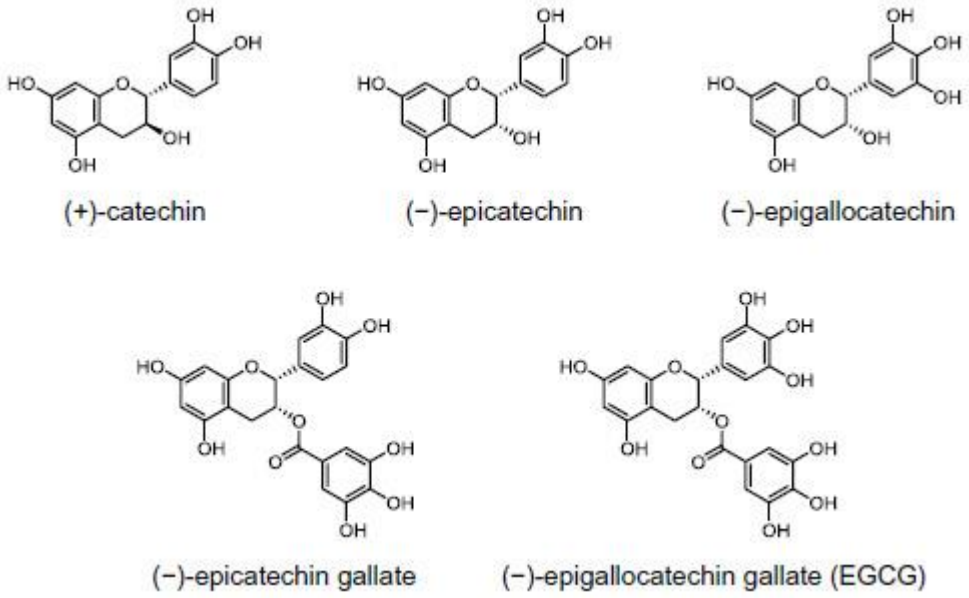



*Figure 1-3: Chemical structure of simple tea polyphenols.*

The oxidized polyphenols which are produced during the oxidation process of tea are mainly theaflavins and thearubigins (Bravo, 1998). Theaflavins are a group of flavonoid bioploymers that exhibit a bright orange-red colour in solution. The total theaflavin content in black tea leaves can vary between 3 and 6 %. Theaflavins are known to contribute to the astringency of tea (Balentine et al., 1997). Pure crystalline theaflavin is perceived to have a "very fierce astringency" and solutions have been described as distinctly tea-like (Millin et al., 1969).

During the tea manufacturing process, the content of catechins reduces by 85 % and only approximately 10 % can be identified as theaflavins and theaflavic acids. The fraction left after a simple solvent extraction is water-soluble and thought to be the brown to black pigments of tea (Balentine et al., 1997). These compounds have historically been named "thearubigens". Until today, it remains a challenge to identify the chemical structure of individual thearubigens and only little progress has been made (Haslam, 2003). Both, thearubigens and theaflavins are responsible for briskness, brightness, strength and colour of black tea (Bhuyan et al., 2015).

### 1.2.2 Taste perception

The enjoyment of a cup of tea in general depends on its sensory properties, which include aroma, taste, flavour and mouthfeel. The tongue plays a key role in sensing different tastes and houses three types of gustatory papillae that house taste buds: circumvallate, foliate, and fungiform papillae (see Figure 1-4). Each gustatory papilla is organised into taste buds which consist of clusters of 50 to 150 polarized, neuroepithelial taste receptor

cells (TRC). Taste buds have a diameter of 50 – 70 µm and are located just below the surface epithelium (Smith & Margolskee, 2001). The fourth type of papillae, the cone-shaped filiform papillae have no function as chemical sensors or taste buds (Stokes et al., 2013).

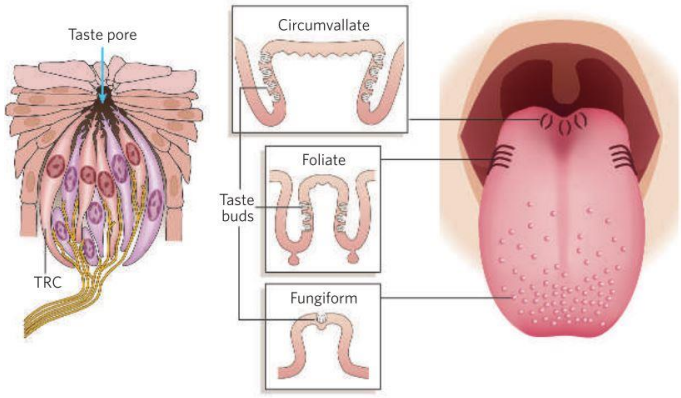



*Figure 1-4: Taste buds (left) are composed of 50 - 150 polarized, neuroepithelial taste receptor cells (TRC). Taste buds can be found across different papillae. Circiumvallate papillae at the back of the tongue contain thousands of taste buds. Foliate papillae at the posterior lateral edge of the tongue contain a dozen to hundreds of taste buds. Fungiform papillae found on the anterior two-thirds of the tongue contain one or a few taste buds. Source: Chandrashekar, Hoon, Ryba, and Zuker (2006).*

To date, it is agreed that humans perceive and distinguish five basic tastes: sweet, sour, salty, umami and bitter with the latter one thought to be the most complex and least understood (Behrens et al., 2007; Drewnowski, 2001). The different tastes are mediated by taste receptor proteins that reside on the surface of TRC within the taste buds of the tongue. Studies have shown that sour and salty tastes depolarise TRCs by directly interacting with ion channels. Amino acids, sugars and other compounds perceived as sweet, and most bitter tasting compounds activate G-protein-coupled receptors (GPCRs) (Kim et al., 2004). TRCs detect chemicals in the oral cavity and transmit this information to the gustatory nerve fibers of the facial and glossopharyngeal nerves using ATP (adenosine

triphosphate) as the key neurotransmitter (Finger et al., 2005). The neurosignals are transmitted to the thalamus, the sensory relay centre for all senses except olfaction. Signals are sent to the "gustatory" cortex comprising of the anterior insula and frontal operculum.

Bitterness is a characteristic taste of polyphenol-rich foods such as tea (Ma et al., 2014). Bitter perception plays an important role as many naturally toxic substances taste bitter to humans. This suggests that the bitter transduction evolved as a defence mechanism against harmful substances (Chandrashekar et al., 2000). However, a recent study by Nissim et al. (2017) suggests that bitterness might not be a sufficient indicator for toxicity. Bitter tasting compounds in food cover a wide range of different chemical compounds including amino acids and peptides, esters and lactones, methylxanthines (caffeine), sulfimides (saccharin), organic and inorganic salts, flavonoids and other classes of polyphenols (Drewnowski, 2001). Due to the diversity of bitter tasting compounds and the wide array of different functional groups represented by these compounds, it is likely that different mechanisms and receptors exist for perceiving bitterness. Currently, at least two bitter transduction mechanisms are known. The bitter taste transduction response is initiated when bitter chemicals bind to the bitter receptors of the TAS2R family (see Figure 1-5). Activated TAS2Rs free the Gαβγ heterotrimeric protein into Gβγ and Gα subunits. One pathway may involve a G-protein that stimulates enzyme-activated IP3, which leads to a release of calcium from cell stores. The increase of calcium in the cells activates the transient receptor potential channel, leading to the influx of sodium ions and subsequently cell depolarization. The combination of elevated calcium ions and membrane depolarization results

in neurotransmitter release. ATP is secreted to gustatory afferent glossopharyngeal nerve fibres and ultimately generates a nerve signal in the brain recognised as bitter taste (Ma et al., 2014). Another mechanism for the perception of bitterness may involve G-protein, α-gustducin, which activates enzyme phosphodiesterase to decrease intracellular cAMP. Furthermore, direct blocking of $K^+$ channels by bitter tastants may also occur (Drewnowski, 2001). A number of receptors facilitate the transduction of bitterness sensation, with approximately 25 – 30 receptors belonging to the TAS2 receptor gene family identified as key bitter taste receptors in mammals (Meyerhof, 2005). These receptors can be found in all circumvallate and foliate papillae (Adler et al., 2000).

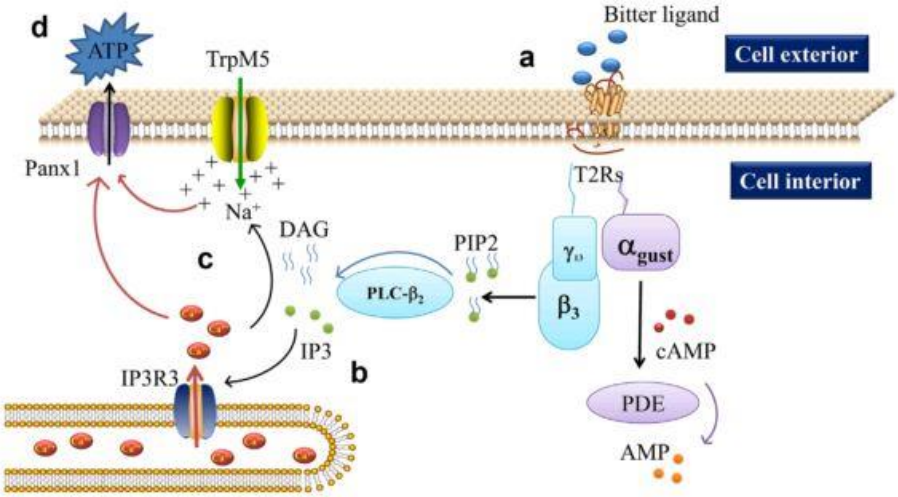



*Figure 1-5: Illustration of bitterness transduction mechanisms: a) bitter ligands active TAS2Rs which split a G-protein. b) Released Gβγ activates PLC-β2 to generate IP3, initiating the release of $Ca^{2+}$ from intracellular stores. c) TrpM5 is activated by elevated $Ca^{2+}$ to flow in $Na^+$, resulting in a depolarised cell. d) elevated $Ca^{2+}$ and depolarised cell leads to ATP release which transmits nerve signal to brain. Source: Ma et al. (2014).*

### 1.2.3 Mouthfeel

*1.2.3.1 Definition*

Defining mouthfeel precisely is difficult and several definitions have been proposed to describe it. In the ISO-standard "Sensory analysis-Vocabulary" (ISO 5492:2008) mouthfeel is defined as: "the tactile sensations perceived at the lining of the mouth, including the tongue, gums and teeth". The first of two Canadian definition proposals to revise the ISO-standard define mouthfeel as: "related to the perception of moisture and fat content of a food by tactile receptors in the mouth cavity and may also be concerned with the lubricating properties of the product" (Folkenberg et al., 1999). In the second Canadian proposal mouthfeel is defined as: "a mixed experience derived from sensations in the mouth that relate to physical (e.g. density, viscosity, particulate) or chemical (e.g. astringency, cooling) properties of a stimulus" (Folkenberg et al., 1999). Significantly, the second Canadian definition suggests that mouthfeel is a multi-faceted sensation which includes different attributes. Szczesniak (1979) classed different mouthfeel attributes into different categories which show what a wide range of physical sensations are covered by the descriptor "mouthfeel" (see Table 1-2). It has been reported "that trained panelists differentiate the physical sensations as texture properties and the chemical sensations as flavor properties" (Folkenberg et al., 1999). Mouthfeel is commonly used to describe the tactile properties of semi-solid and liquid foods and beverages, whereas texture is used in reference to solid foods (Guinard & Mazzucchelli, 1996).

*Table 1-2: Classification of mouthfeel properties for beverages. Source: Szczesniak (1979).*

| **Category** | **Typical words** |
|---|---|
| Viscosity-related terms | Thin, thick, viscous |
| Feel on soft tissue surfaces | Smooth, pulpy, creamy |
| Carbonation-related terms | Bubbly, tingly, foamy |
| Body-related terms | Heavy, watery, light |
| Chemical effects | Astringent, burning, sharp |
| Coating of oral cavity | Mouth coating, clinging, fatty, oily |
| Resistance to tongue movement | Slimy, syrupy, pasty, sticky |
| Afterfeel – mouth | Clean, drying, lingering, cleansing |
| Afterfeel – physiological | Refreshing, warming, thirst-quenching, filling |
| Temperature-related | Cold, hot |
| Wetness-related | Wet, dry |

A review on the mouthfeel of tea and astringency perception will follow in this literature review. However, firstly the role of mechanoreceptors and saliva will be explored.

### *1.2.3.2 The Role of Mechanoreceptors*

Besides taste receptors, the oral cavity houses mechanoreceptors which are designed to detect any physical/mechanical stimulus exerted on the tissue surface and are directly responsible for sensing food texture (Upadhyay et al., 2016). Four different types of mechanoreceptors are known and each responds differently to external stimuli (see Figure 1-6). The four mechanoreceptors can be grouped into slowly and rapidly adapting fibres with the slowly adapting (SA) fibres (Merkel receptors (SA1) and Ruffini cylinders (SA2)) responding as long as the stimulus is presented; and the rapidly adapting (RA) fibres (Meissner corpuscles (RA1) and Pacinian corpuscles (RA2)) responding to stimulation with a burst of firing at the beginning and end of stimulation (Upadhyay et al., 2016).

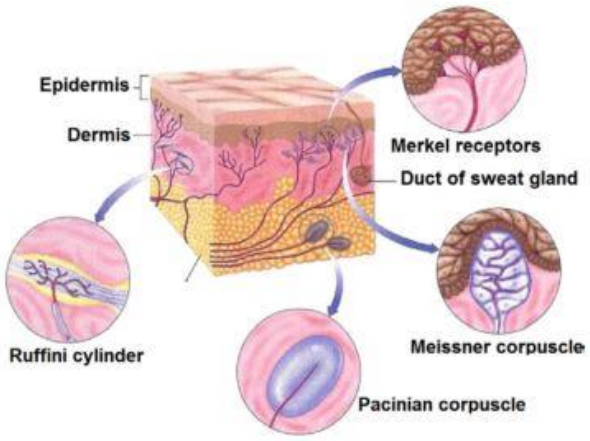



*Figure 1-6: Four different types of mechanoreceptors. Source: Chen (2014).*

Merkel receptors are disc-shaped, slowly-adapting and located near the border between epidermis and dermis. They are responsible for detecting low frequency stimuli (0.3 – 3 Hz) related to static pressure, indentations, form, size and surface characteristics. Meissner corpuscles are stacks of flattened cells and are located in the dermis just below the epidermis with a single nerve fibre winding through these cells. They are rapidly adapting, low threshold mechanoreceptors and respond to stimuli with a frequency range of 3 – 40 Hz. They can detect skin movements associated with touch. The third type of mechanoreceptors are Ruffini cylinders which are branched fibres inside a cylindrical capsule, located in the dermis. These receptors are slowly adapting receptors which are sensitive to skin deformation and respond to high frequency stimuli (15 – 400 Hz). They sense the stretch of organs to determine the motion and shape of an object. The last type of mechanoreceptors are Pacinian corpuscles located in the subcutaneous fat and formed in an onion-like capsule surrounding a nerve fibre. They respond to relatively high frequency stimuli (10 – 500 Hz) (Upadhyay et al., 2016).

From these four types of mechanoreceptors, all except the Pacinian corpuscles are found in the mouth (Engelen & Van der Bilt, 2008). Rapidly adapting mechanoreceptors are more sensitive to sudden changes and are more likely to dominate sensation in a dynamic environment such as eating and swallowing (Chen, 2014).

*1.2.3.3 The Role of Saliva*

Saliva is a most valuable oral fluid that is often taken for granted (Humphrey & Williamson, 2001). The functions of saliva in the oral cavity are numerous and include the initial breakdown of food, impacting flavour release, the dilution of flavours and tastes, precipitation of proteins by tannins, oral health protection, and lubrication of the oral tissues (Engelen et al., 2007). 90% of whole saliva is produced by three major salivary glands (see Figure 1-7): parotid, submandibular and sublingual (Navazesh & Kumar, 2008). The remaining 10% are produced by minor salivary glands which are scattered throughout the mouth.

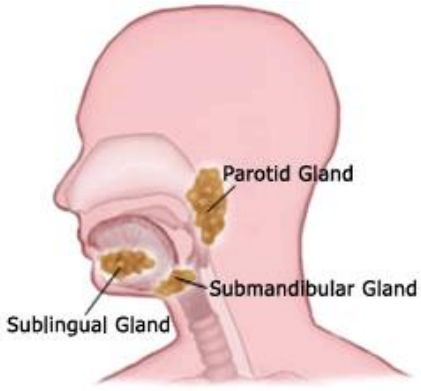



*Figure 1-7: Illustration of three major salivary glands: parotid, submandibular and sublingual. Minor salivary glands are scattered throughout the oral cavity. Source: [http://medicalterms.info/anatomy/Salivary-Glands](http://medicalterms.info/anatomy/Salivary-Glands)*

Saliva is composed of approximately 99% water and 1% proteins and salts, including sodium, potassium, calcium, magnesium, bicarbonate, and

phosphates (Humphrey & Williamson, 2001). Furthermore, immunoglobulins, proteins, enzymes, mucins and nitrogenous products such as urea and ammonia can be found in saliva. One class of salivary proteins is of special interest since they are thought to contribute to the perception of astringency: the proline-rich proteins (PRP). PRP are one of the main protein families secreted from the parotid and submandibular glands. They are characterized by their highly repetitive structure of approximately 19 residues of proline, glycine and glutamine, which alone accounts for 70 – 80 % of the total amino acid content. Three types of PRPs exist; basic, acidic, and glycosylated accounting for 23 %, 30 %, and 17 % of the total protein in parotid saliva; with PRPs overall comprising 70 % of salivary proteins (Kauffman & Keller, 1979).

The average daily amount of whole saliva produced by a healthy individual varies between 1 and 1.5 litres (Humphrey & Williamson, 2001). Resting or unstimulated saliva is mainly produced by submandibular glands, whereas the parotid glands mainly contribute to stimulated saliva. Although minor salivary glands do not contribute much volume to whole saliva, they play a significant role in the lubrication and protection of the oral mucosa due to their mucous secretion.

Saliva flow can vary greatly between individuals and across the day (Rantonen & Meurman, 2009). A normal flow rate of unstimulated saliva is any flow rate above 0.1 ml/min. For stimulated saliva, the flow rate increases to 0.2 ml/min for a healthy individual. On average, unstimulated saliva flow is 0.3 ml/min and the flow rate for stimulated saliva can reach a maximum of 7 ml/min, therefore contributing as much as 80% to 90% of daily salivary production (Humphrey & Williamson, 2001). Several

studies have tried to correlate saliva flow rate and saliva composition to mouthfeel and texture perception (Engelen et al., 2007; Janssen et al., 2007; Kallithraka et al., 2001). These studies are discussed in detail in section 1.2.5.3 where correlation between instrumental methods and mouthfeel perception are presented.

## 1.2.4 Astringency perception

*1.2.4.1 Definition and Mechanism*

Astringency is a characteristic mouthfeel of polyphenol-rich products, such as red wine and tea (Bate-Smith, 1954) and is commonly described as a drying, roughing and puckering sensation of the tongue (Gawel et al., 2001; Rossetti et al., 2008). Some researchers suggest that astringency is not a single perceptual phenomenon but a complex percept summarizing a number of subtle tactile sensation, so-called astringent "sub-qualities" (Bajec & Pickering, 2008a). Lee and Lawless (1991) developed a language describing these sub-qualities and suggested that "drying", "pucker feeling", "roughing" and overall astringency may not be totally interchangeable. Gawel et al. (2000) developed a mouthfeel wheel for red wine and listed 33 sub-qualities of astringency. However, it is questionable how many of these attributes are not highly correlated and if they are relevant for consumers, especially in beverages and food products with little astringency present.

It is widely accepted that astringency is a tactile phenomenon (Breslin et al., 1993). Green (1993) suggested that astringency is tactile and results from an increase in oral friction coefficient due to a decrease in salvia lubrication. The word "astringent" comes from the Latin *ad stringere*,

meaning “to bind” (Joslyn & Goldstein, 1964) and refers to the ability of astringent compounds to bind and precipitate proteins. The proline-rich proteins in saliva are known to have a high affinity for polyphenols (Prinz & Lucas, 2000). A large number of researchers have investigated the interactions between polyphenols and salivary proteins in order to understand the mechanism of astringency perception. Salivary proteins can covalently bind to the oral mucosal cells and form a layer surrounding the soft structure of the mouth. The thinnest layer with less than 10 µm is found on the hard palate (Collins & Dawes, 1987). Usually the thickness of saliva is reported as a single value in the range of between 70 and 100 µm. When tannins pass by this layer of saliva proteins, they interact with them and form insoluble tannin-protein precipitates. This leads to an increase in friction and a loss of lubrication. However, the formation of a soluble tannin-protein complex was also observed (Kallithraka & Bakker, 1998), indicating a wide range of interactions between proteins and polyphenols. Charlton et al. (2002) introduced a 3-stage model for interactions between tannins and proteins (see Figure 1-8). First, hydrophobic associations between the planar surface of the tannin aromatic rings and hydrophobic sites of proteins forming soluble complexes are observed. At the same time, hydrogen bonding aids the stabilisation of the complex formed (OH-groups of tannins and CH-, $NH_2$-groups of proteins) to form colloidal size complexes. Then the protein-tannin complexes self-associate via further hydrogen bonding to produce soluble, large protein-tannin complexes that then aggregate into an insoluble sediment. At the end the aggregated complexes, known to have a diameter

between 80 to 500 nm (Charlton et al., 2002), are large enough to form insoluble sediments and precipitate from the solution.

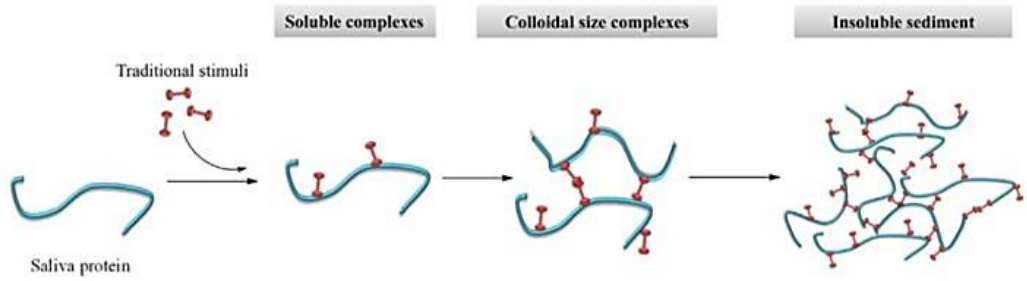



*Figure 1-8: Schematic representation of 3-stage model of the interaction between stimuli and proteins proposed by Charlton et al. (2002). Adapted from Ma et al. (2014).*

Later on, it was suggested by Gibbins and Carpenter (2013) that astringency is so complex that it is likely that multiple mechanisms are occurring at the same time (see Figure 1-9 for illustration of the possible mechanisms). The precipitation of salivary proteins is not the only mechanism in astringency development, since it has been observed that not all astringent substances cause salivary protein precipitation (Lee et al., 2012; Rossetti et al., 2009). An important factor that contributes to the development of astringency is thought to be the loss of lubrication. The loss of lubrication could be caused by protein interaction or precipitation between tannins and salivary proteins. However, there is an increasing numbers of studies showing that the mucosal pellicle has an important part to play (Lee et al., 2012; Nayak & Carpenter, 2008; Rossetti et al., 2008). The disruption of the salivary film and alteration of lubrication may be caused by polyphenol/protein aggregates or free polyphenols that are not aggregated. The disruption of the salivary film could expose the pellicle

lying underneath and its ability to protect and lubricate the epithelium. Furthermore, it could lead to exposed receptors that could contribute to the perception of astringency or a reduced lubrication could trigger responses from mechanoreceptors (Gibbins & Carpenter, 2013).

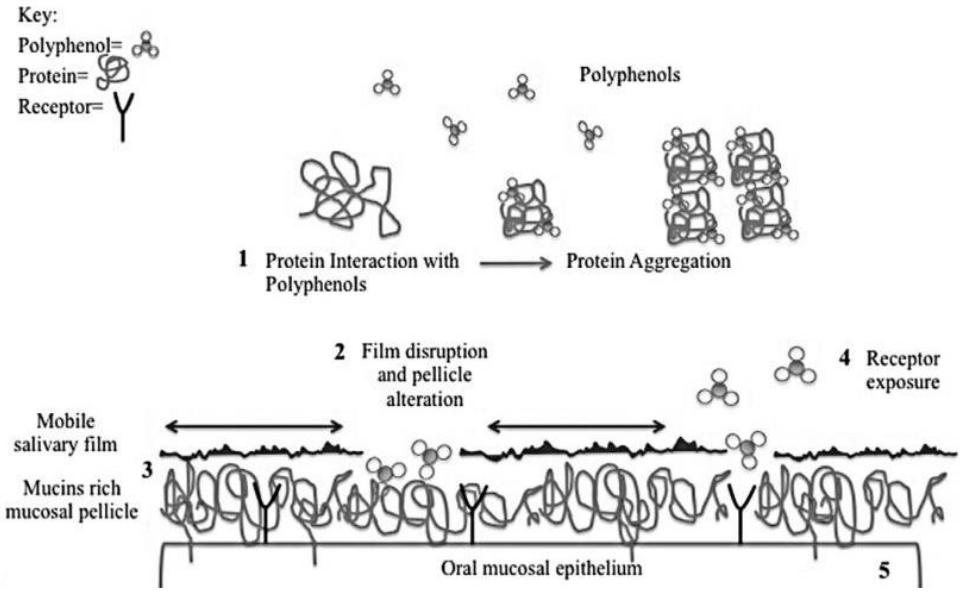



*Figure 1-9: possible mechanisms of astringency occurring simultaneously: (1) aggregation of salivary proteins creating grittiness, (2) salivary film disruption, (3) reduced salivary lubrication, (4) possible exposure of receptors, (5) mechanoreceptors or nerve innervation. Source: Gibbins and Carpenter (2013).*

### *1.2.4.2 Physiological Factors influencing Astringency Perception*

#### *1.2.4.2.1 Saliva Flow*

Since saliva is an important factor in the perception of astringency, a number of studies have investigated possible correlations between saliva flow rate and astringency perception. Dry crackers, acids and astringent substances are the most potent elicitors of saliva flow and Fischer (1990) showed that saliva flow from the parotid salivary gland increased while subjects sipped wine. This rapid increase in saliva flow rate peaked 20 s after consumption and then rapidly decreased.

Studies have found that subjects with lower saliva flow rates perceive the maximum intensity of astringency later, more intensely and for a longer

time (Fischer et al., 1994; Ishikawa & Noble, 1995; Peleg et al., 1999). However, several studies also found no correlation between saliva flow rate and astringency perception (Corregelongue et al., 1999; Peleg et al., 1998; Smith et al., 1996). Horne et al. (2002) studied the relationship between the formation of turbidity in saliva-tannin mixtures and astringency perception. They observed that haze development and astringency perception were negatively correlated and a higher haze development lead to lower perception of astringency. This suggests that an increased protein content results in more haze protecting the oral tissues against the direct attack of tannins (Horne et al., 2002). The saliva protein composition was thought to be an important characteristic which could be linked to astringency perception as shown by Kallithraka et al. (2001). They found correlations between the relative concentrations of individual proteins and Time-Intensity (TI) data, whereas no correlation was found between total protein concentration and TI data. This suggests that the protein composition of saliva might be more important to astringency than the overall protein concentration (Kallithraka et al., 2001).

#### *1.2.4.2.2 PROP Taster Status*

PROP (6-n-propylthiouracil) is a bitter tasting substance that can only be perceived by certain individuals. The ability to taste PROP is determined by three polymorphisms (A49P, V262A, I296V) in the TAS2R38 bitter-taste receptor gene (Melis et al., 2017). Individuals are broadly divided into three groups depending how intensely they perceive PROP. Supertasters perceive PROP to have a high bitter intensity, whereas taster (or medium

tasters) still perceive PROP as bitter but at a lower intensity. For non-tasters, PROP is tasteless or barely detectable (Melis et al., 2017).

Besides variation in the perception of PROP, these three taster group have been found to differ in the density of fungiform taste papillae and number of taste pores per taste buds (Bajec & Pickering, 2008b). Supertasters are known to have a higher density and more taste pores in comparison to non-tasters with reduced density of fungiform taste papillae. This physiological difference could explain why supertasters perceive most tastes as more intense compared to non-tasters.

A number of studies have tried to link PROP taster status and astringency perception but available results are conflicting. Studies which did not find a correlation between PROP taster status and astringency perception include studies conducted on wine (Ishikawa & Noble, 1995), acids (Sowalsky & Noble, 1998), soymilk (Corregelongue et al., 1999), alum and phenolic compounds (Peleg et al., 1998; Peleg & Noble, 1999; Smith et al., 1996; Thorngate III & Noble, 1995). However, Pickering et al. (2004) found that supertasters and medium tasters were more sensitive to bitterness and astringency in red wine than non-tasters. Further work by Pickering and coworkers (Pickering & Robert, 2006) found that supertasters rate the "overall astringency" in red wines lower than non-tasters, however supertasters rated all astringent sub-qualities higher, indicating that maybe supertasters are able to distinguish better between more subtle astringency qualities than non-tasters.

*1.2.4.3 Astringency in Tea*

Tea is a classic example of an astringent beverage due to its high content of polyphenols and in previous studies on tea, astringency is the only mouthfeel attribute reported (Koch et al., 2012; Lee & Chambers, 2007). Thus far, the majority of research has focused on the aroma and flavour descriptors of tea. Lee and Chambers (2007) developed a lexicon for green tea using 138 green tea samples from different countries. The lexicon comprises 31 attributes including two mouthfeel attributes: astringent and tooth-etch. Astringency was described as "the drying, puckering sensation on the tongue and other mouth surfaces" (Lee & Chambers, 2007). The attribute "tooth-etch" was defined as "a chemical feeling factor perceived as a drying/dragging when the tongue is rubbed over the back of the tooth surface". The description of both attributes indicates that they are closely related, and possibly describe the same mouthfeel. This is also confirmed by the reference used for both attributes, which was different solutions of alum ranging from 0.03% to 0.2%.

To the authors knowledge, the lexicon by Lee and Chambers (2007) is the only peer-reviewed and published lexicon on tea brewed from the leaves of *Camellia sinensis*. Koch et al. (2012) developed a lexicon and sensory wheel for rooibos tea, which is brewed from the leaves of *Aspalathus linearis*, the rooibos plant. In their research, mouthfeel was described by using the attributes soft/smooth, astringent, flat/bland and harsh astringency. Some of these descriptors describe astringent sub-qualities, such as "soft/smooth", "round", and "subtle". As reference for astringency, a 0.7% alum solution was used (Koch et al., 2012).

A number of methods have been used to study astringency perception, however, to date, sensory methods are thought to be the most effective methods. Besides using discrimination tests to distinguish between samples with different astringency and rating astringency on a scale, temporal methods have been used to capture the perception over time. Astringency is known to be dynamic in nature and therefore has a time course profile which is best captured by temporal sensory methods (Ishikawa & Noble, 1995; Lee & Lawless, 1991; Robichaud & Noble, 1990; Valentová et al., 2002). For tea, it was reported that the maximum intensity of astringency for Indian and Ceylon tea was reached after 4 and 10 s after swallowing, respectively; and that the perceived astringency after the consumption of one sip of tea disappeared entirely after 2 min (Valentová et al., 2002).

*1.2.4.4 Interactions between Polyphenols and Milk*

Astringency of tea is caused by the polyphenols in the tea leaves and the addition of milk, which is very popular in the UK (UK Tea Association, 2017), reduces the astringency of the tea during consumption (Brown & Wright, 1963). It is well known that milk proteins and polyphenols interact (Brown & Wright, 1963; Hasni et al., 2011; Rashidinejad et al., 2016; Yuksel et al., 2010). Caseins, the major milk proteins are phosphoproteins that precipitate from milk at pH 4.6 at 30°C and comprise of $\alpha_{S1}$-, $\alpha_{S2}$-, β-, γ- and κ-caseinate (Farkye & Shah, 2015). The major caseins exist in strong association with themselves and in the form of spherically shaped colloidal particles, known as casein micelles (Yuksel et al., 2010).

Polyphenols, such as catechins form insoluble complexes with milk proteins (Hasni et al., 2011). Hasni et al. (2011) investigated the interaction between α- and β-caseins with tea polyphenols (catechin, epicatechin, epigallocatechin and epigallocatechin gallate) using spectroscopic methods as well as molecular modelling. They found that the casein conformation was altered by polyphenols with a major reduction of α-helix and β-sheet and an increase of random coil and turn structure suggesting further protein unfolding.

When phenolic compounds interact with proteins, some proteins, especially proline-rich proteins can be precipitated. Proline groups e.g. in β-casein have a strong affinity for hydroxyl-groups of catechins (Arts et al., 2002). A reduced availability of hydroxyl-groups can affect the electron donation capacity of catechins, which might be related to a reduction of antioxidative activity of tea in the presence of milk. Yuksel et al (2010) characterised the binding between green tea flavonoids and milk proteins using fluorescent probe binding and isothermal titration calorimetry. A decrease in the number of surface hydrophobic sites in the presence of green tea flavonoids for all casein concentrations was observed. This decrease in protein surface hydrophobicity was explained by hydrogen bonding between milk protein and green tea flavonoids.

### 1.2.5 Instrumental Methods used to measure Mouthfeel

In order to better understand mouthfeel, instrumental methods have been used to generate analytical data with the further aim of correlating these results with those acquired from sensory studies. Sensory studies are time-consuming and expensive, therefore instrumental methods which can

predict perceived texture and mouthfeel are advantageous. There are three major instruments which can be used for this purpose: the texture analyzer, the rheometer and the tribometer. The texture analyser is a versatile instrument which can for example be used to measure force needed to break a food product. The analytical data obtained using the texture analyser has been shown to have a good correlation for texture attributes of solid food, e.g. force needed to cut a extruded snack was correlated to hardness and adhesiveness (Paula & Conti-Silva, 2014). The rheometer is used to measure elastic and viscous behaviour of semi-solid and liquid products. The principles behind viscosity measurements will be explained in the next section. Finally, tribological devices, either purpose-built or commercially available ones, are commonly used to measure friction and lubrication properties of semi-solid and liquid products. These parameters are thought to be closely related to mouthfeel attributes such as creamy, smooth, and rough/astringent. The application of tribology in food research has received a lot of attention from researchers in recent years. Section 1.2.5.2 will discuss the physical principles of tribology in more detail.

*1.2.5.1 Rheology*

Rheology is the science of deformation and flow and in the simplest case, the rheological property of interest is elasticity for solid bodies and viscosity for fluids (Borwankar, 1992). Since tea is of liquid nature, the concept of viscosity will be discussed in detail.

The concept of viscosity was introduced by Issac Newton, who proposed that a force is necessary to shear a fluid film (Bhushan, 2013). To describe

the concept of viscosity a two-plate-model is used. A liquid rests between two parallel plates (see Figure 1-10) with a distance (h) between them. Initially the system is at rest but at the time t=0, the upper plate is set in motion in z-direction at a constant velocity by applying a force (F), while the bottom plate is kept stationary (Sahin & Sumnu, 2006). The force applied creates a tangential stress (force per unit area) called shear stress (τ). Therefore, the movement of the upper layer drags the lower layer, creating a velocity gradient called shear rate ($\dot{\gamma}$) in the process. The following relationship applies (Equation 1):

$$\tau = \frac{F}{A} = \eta\dot{\gamma} \qquad (1)$$

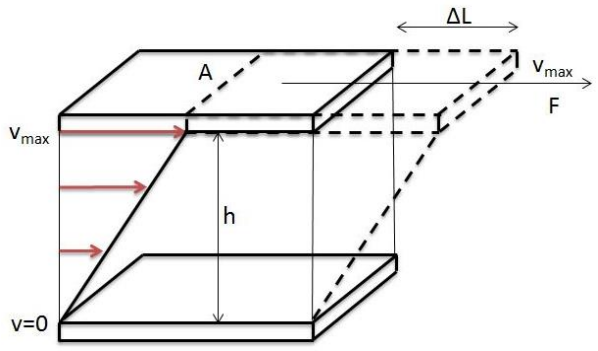



*Figure 1-10: Two-Plate-Model: A is the area of the plate [m²], h is the distance between the two plates [m], F is the force applied [N], ΔL is the deflection [m], v is the velocity [m/s]. Adapted from He (2013).*

Viscous fluids are categorised into Newtonian and non-Newtonian fluids, depending on their behaviour under stress. For Newtonian fluids, the shear stress (τ) is directly proportional to the shear rate ($\dot{\gamma}$) and the proportionally constant (η) is the viscosity of the fluids (see Figure 1-11B). Most liquids that contain more than 90% water follow Newtonian behaviour e.g. tea, coffee and milk. For non-Newtonian fluids the viscosity is a

function of shear rate because their viscosity increases or decreases depending on the shear rate.

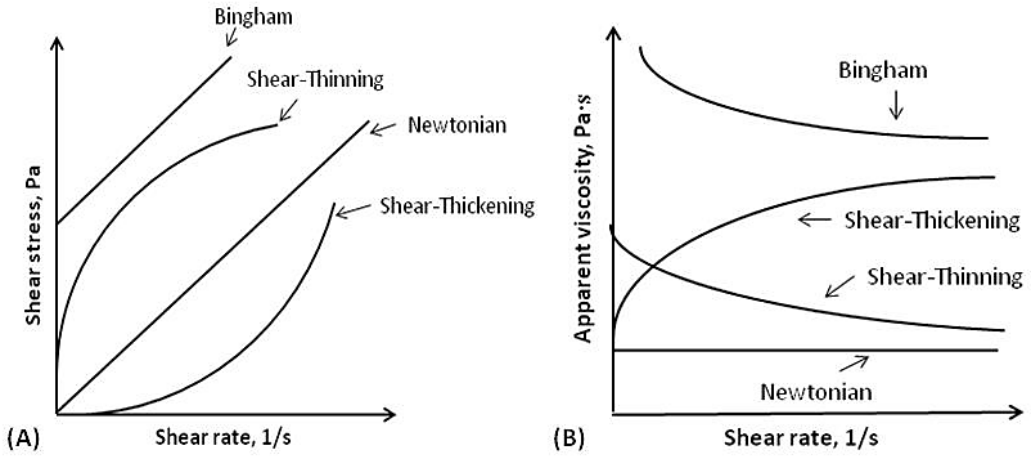



*Figure 1-11: Flow curves and viscosity curves of different fluids. Source: Bhushan (2013)*

Non-Newtonian fluids show a shear-thinning or shear-thickening behaviour. For shear-thinning fluids the friction between layers decreases with increasing shear rate. The shearing straightens out the entangled and long-chain molecules, which leads to reduced viscosity. Fruit and vegetable products such as apple sauce, banana purée, and concentrated fruit juices are good examples of shear-thinning fluids in food systems (Borwankar, 1992). Shear-thickening fluids are fluids with increasing apparent viscosity by increasing shear rate. Corn starch suspensions follow shear-thickening behaviour. If stress is applied e.g. fast stirring with a spoon the apparent viscosity increases and the suspension becomes difficult to stir. When less force is applied (gentle stirring) the solution appears water-like because the apparent viscosity decreases. Furthermore, a third category of fluids so-called Bingham fluids are known, needing to overcome a yield point to become liquid. A typical example of a Bingham plastic fluid is toothpaste.

If the applied stress is below the yield stress it will not flow out of the tube under gravity but upon squeezing, the stress applied exceeds the yield stress and the toothpaste flows like a Newtonian fluid. Mayonnaise, tomato paste and ketchup are further examples (Borwankar, 1992).

Viscosity is measured with viscometers and commonly three different types of viscometer are known: the capillary flow viscometer, the falling ball viscometer and rotational viscometer (Bhushan, 2013). In this research a rotational viscometer was used for viscosity measurement. The principle of a rotational viscometer is that the force that is required to turn an object in a fluid indicates the viscosity of this fluid. A rotational viscometer consists of two main parts, the sample-filled cup which stands still and the measuring bob that is immersed in the sample and rotates. The viscosity is proportional to the motor torque necessary to move the measuring bob against the fluid‘s viscous forces. The speed required to turn the measuring bob is pre-set and the torque is measured. Depending on the samples and the tests performed there are different geometries available for measuring rheological properties with a rotational viscometer, as shown in Figure 1-12. For liquid samples the concentric cylinder and the double gap cylinder are commonly used. The concentric cylinder (Figure 1-12A) is used for low-viscosity liquids and is very useful for temperature sweeps as the small exposed surface gives little evaporation. For samples with an even lower viscosity, a double gap cylinder (Figure 1-12B) can be used as it increases low stress measurement capabilities compared to concentric cylinder.

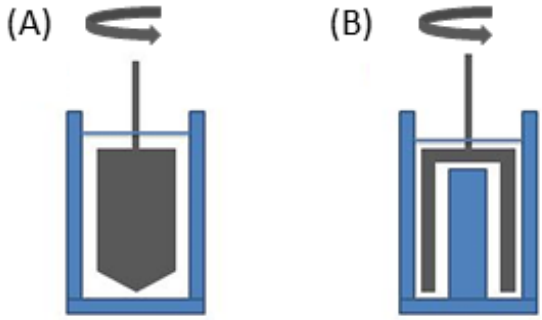



*Figure 1-12: Examples of different measurement set-ups to measure the viscosity of liquid samples: (A) Concentric Cylinder, (B) Double Gap Cylinder.*

### *1.2.5.2 Tribology*

The word "tribology" originates from the Greek work *tribos* meaning rubbing or sliding (Stokes, 2012). Tribology is the study of friction between two interacting surfaces and includes wear, tear and lubrication. Leonardo Da Vinci introduced the concept of the friction coefficient as the ratio of the friction force to normal load, however his work remained unpublished for a long time (Bhushan, 2013). In 1699, the French physicist Guillaume Amonton rediscovered the rules of friction and postulated two empirical laws.

Friction is the resistance to motion during sliding that is experienced when one solid body moves tangentially over another with which it is in contact (see Figure 1-13). The friction force acts directly opposite to the direction of motion. Two types of friction exist: dry friction and fluid friction. Dry friction describes the friction force that exists when two dry surfaces move relative to one another, whereas fluid friction refers to the friction force between adjacent layers in fluids that are moving at different velocities to each other. For two moving bodies which are in contact with each other, static and dynamic friction force can be measured. Static friction is the

force needed to initiate the motion of one body and dynamic friction, also called kinetic friction, is the force needed to maintain the relative motion.

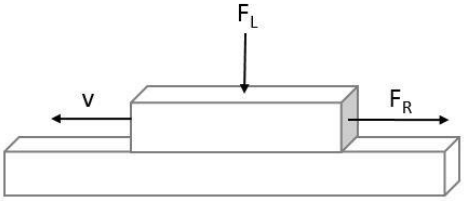



*Figure 1-13: Representation of friction between two interacting surfaces with $F_L$ = normal force, v = sliding speed, $F_R$ = force of friction. Arrows indicate directions of forces. Adapted from Prakash, Tan, and Chen (2013).*

For the contact between two solid surfaces, the empirical laws of Amonton and Coulomb can be applied. The first Amonton`s law states that the friction is directly proportional to the normal load (see Equation 2).

$$F_R = \mu F_L \tag{2}$$

$F_R$ = friction force in [N], μ = friction coefficient (dimensionless), $F_L$ = normal force or the surface load in [N].

The friction coefficient is independent of normal load and can cover a wide range for dry friction (from 0.05 to 10).

The second Amonton`s law states that the friction force is independent of the apparent area of contact between the contacting bodies. Therefore, two bodies, regardless of their physical size, have the same friction coefficient. Solid surfaces contain irregularities from the prescribed geometrical form which means that when two surfaces are placed in contact, surface roughness causes contact to appear at discrete contact spots. The sum of areas of all these contact spots is the real area of contact and for most materials will only be a small fraction of the apparent area of contact (Bhushan, 2013).

The third law which describes friction is Coulomb`s law which states that the kinetic friction force (or the friction coefficient) is independent of the sliding velocity once motion starts. The friction coefficient between two dry surfaces is constant with increasing load but if a surface film is present, the friction coefficient may not be constant anymore. When this happens, the friction coefficient is no longer a constant but a function of surface properties, surface load, moving speed as well as the lubricant properties, for example, viscosity (Prakash et al., 2013). If a surface film is present between two surfaces, it is commonly observed that the friction coefficient increases with increasing load. At high load, the surface film breaks, exposing the surface leading to an increased friction coefficient. Coulomb`s law is not generally valid as the friction coefficient of kinetic friction decreases with increasing sliding velocity. The decrease is usually small, with the friction coefficient changing a few percent for a change in velocity of an order of magnitude.

From a sensory perspective, tribology can be used to understand the physical properties of food during oral processing where foods are squeezed and sheared. This process results in constantly changing mechanical and physiochemical properties of the food. In the mouth, there are numerous "interacting surfaces in relative motion" for example teeth, tongue-palate, tongue-teeth, teeth-food, tongue-food, lips, lips-food and so on (Stokes, 2012). The most important interacting surfaces are probably tongue-palate and tongue-food since food is squeezed between these surfaces during oral processing (Chen & Stokes, 2012). Rheology is insufficient to describe the flow behaviour and surface-related mouthfeel attributes (Selway & Stokes, 2013). Attributes which are driven by in-

mouth lubrication can be explained by measuring the frictional response of food and saliva when being rubbed between oral surfaces (Kokini et al., 1977). A study by Selway and Stokes (2013) showed that commercially available dairy products such as custard and yoghurt can have similar rheological properties but very different friction coefficient, thus highlighting the importance of tribological measurements.

Lubrication behaviour of food can be visualised using the Stribeck curve (see Figure 1-14). The friction coefficient is plotted against a combined parameter of viscosity, speed and load. The combination of fluid viscosity $\eta$ (Pa·s), relative speed of surface movement v (m/s), and surface load $P_L$ (Pa) gives a unit of length (m), which resembles the thickness of the lubricant film between the two moving surfaces (Prakash et al., 2013). The Stribeck curve is typically divided into 3 different regimes: boundary regime, mixed regime and hydrodynamic regime which all represent different interactions/friction scenarios between two surfaces and in the case of oral processing, different amounts of food between the tongue and palate (Stokes, 2012).

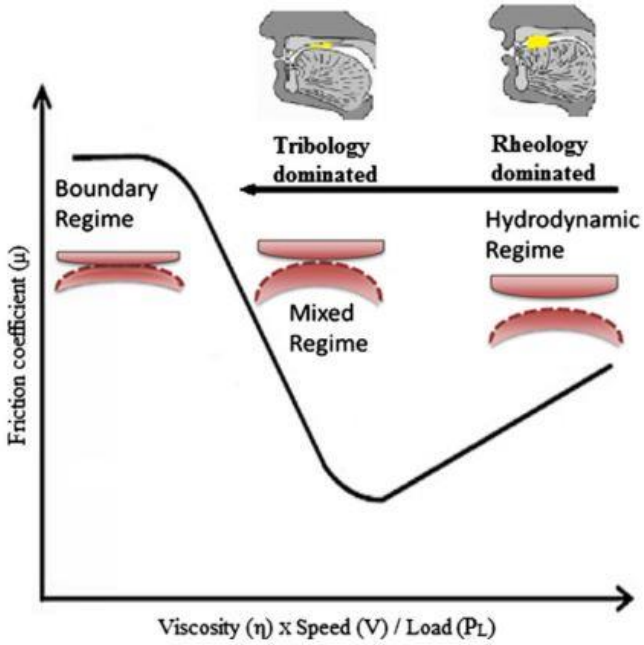



*Figure 1-14: Illustration of a Stribeck curve where the friction coefficient is plotted against the combined parameters of viscosity, entrainment speed and surface load. Source: Prakash et al. (2013).*

- In the boundary regime only low speeds occur and the hydrodynamic pressure is insufficient to separate the two moving surfaces, e.g. palate and tongue, furthermore fluid entrainment is negligible and an "immobile" layer is present on the tongue and palate surfaces. The load is carried by the contacting asperities and is dependent on the surface and interfacial film properties at the molecular scale. It is thought that this regime is closely related to the human perception of astringency and slipperiness (Prakash et al., 2013).
- The mixed regime lies between the boundary and hydrodynamic regime. The hydrodynamic pressure is high enough to partly separate the two rubbing surfaces. In this regime, friction is determined by both, the bulk properties and the boundary film. The friction coefficient reaches its minimum in this regime and, with

either increased asperity contact or decreased thickness of the lubricant layer, the friction coefficient will increase (Prakash et al., 2013). The lubricant thickness of Newtonian fluids in the boundary and mixed regime is estimated to be below 1 μm (Selway & Stokes, 2013).

- In the hydrodynamic regime the lubricant film is entrained and hydrodynamic pressure is high enough to fully separate both surfaces, with the film thickness expected to range from 500 nm to 2μm (de Vicente, Stokes, & Spikes, 2005). This lubrication regime is also sometimes called fluid-film or thick-film lubrication. The film thickness and friction generated in this regime depend on the viscosity of the food. In this regime, sensory thickness is perceived and related to the viscosity of the food (Prakash et al., 2013; Upadhyay et al., 2016).

Besides the forces developed when two surfaces are in motion, friction also causes acoustic signals including oscillations and vibrations (Zahouani et al., 2009). Friction sound can be observed in a wide range of situations e.g. brakes of squealing cars, music instruments such as violins, the sound radiated by glass when it is rubbed by moist finger and so on (Zahouani et al., 2009). Friction noise is usually classified in two categories: mechanical instabilities and roughness sound (Akay, 2002). Mechanical instabilities can be for example the squeaking of a door. These stick-slip instabilities are generated under strong contact pressure. The roughness noise is caused by dynamical contact of two rough surfaces. Small shocks between antagonist asperities generate vibration into the solids which, in turn, are responsible for the resonated sound. This is observed under light contact

pressure. This second type of friction noise has been related to the noise caused when rubbing a finger on an object (Zahouani et al., 2009). This sound can be measured as shown by van Aken (2013) who recorded noise when rubbing the finger across different surfaces. He also recorded sounds originating from the mouth when rubbing the tongue against the palate (van Aken, 2013).

#### *1.2.5.3 Correlation between Instrumental Data and Mouthfeel Perception*

A large body of work have tried to identify instrumental measurements which are linked to the perception of different sensory attributes in food and beverages. This section will focus on various studies which have tried to link mouthfeel perception with data collected using instrumental methods. Some texture attributes, especially mechanical ones such as hardness, show excellent correlations with instrumental parameters e.g. those measured by the texture analyser (Szczesniak, 2002). However, mouthfeel attributes of semi-solid and liquid foods and beverages are more difficult to correlate with instrumental parameters, due to the complex oral processing involved in sensing these attributes.

Since astringency has been linked to an increase in friction, numerous studies have tried to link astringency perception and friction coefficient (Brossard et al., 2016; Rossetti et al., 2009; Vardhanabhuti et al., 2011). Rossetti et al. (2009) directly probed the impact of astringent compounds on the lubricating properties of saliva by measuring the friction coefficient between a ball-disc sliding contact. It was found that epigallocatechin gallate (EGCG) solutions increased the friction coefficient significantly due to a depletion of lubricating salivary proteins. An increase in concentration

of EGCG also increased the friction coefficient more rapidly. The observed increase in friction was in line with astringency perception, however for epi-catechin (EC), another common tea polyphenol, no change in lubricating properties of the saliva was observed, although it is perceived to be astringent. Vardhanabhuti et al. (2011) used the same experimental set up as Rossetti et al. (2009) to investigate the impact of β-lactoglobulin on the lubricating properties of saliva. It was found that β-lactoglobulin at pH 3.5 and 7.0 increased the friction coefficient in a manner which is related to astringency perception. Increasing β-lactoglobulin concentration at pH 3.5 caused a rapid increase in friction, however for the highest concentration at 10% the friction coefficient was found to be higher than water but below the values observed for lower protein concentrations. They concluded that tribology tests are not representative of the dynamic in-mouth system and that a simple relationship between friction and sensory astringency cannot be found for all conditions (Vardhanabhuti et al., 2011). Only one study found a correlation between friction coefficient and astringency perception. Brossard et al. (2016) investigated possible correlations between friction coefficient and perceived astringency of different wines. They found a correlation between friction coefficient and astringency perception for four types of wine mixed with saliva (1:1) at a low sliding speed of 0.075 mm/s.

Astringency is the dominant mouthfeel attribute for polyphenol-rich foods, however there is another group of mouthfeel attributes which has also received a lot of attention from researchers - those of liquid and semi-solid foods such as emulsions (Hollowood et al., 2007; van Aken et al., 2011; Vingerhoeds et al., 2008) and custards (de Wijk et al., 2003; Engelen &

Van der Bilt, 2008; Kilcast & Clegg, 2002). Mouthfeel attributes of emulsions and semi-solid foods include "thickness", "smoothness", "fattiness", "mouth coating" and "creaminess". Most of these mouthfeel attributes are driven by the presence or absence of fat, fat content, type of fat and droplet size (van Aken et al., 2011). An extensive body of work has investigated how these mouthfeel attributes can be measured instrumentally (Ares et al., 2012; Camacho et al., 2015; Chojnicka-Paszun et al., 2012; de Wijk et al., 2006; de Wijk et al., 2003; Houska et al., 1998; Janssen et al., 2007).

Since tea is often consumed with milk, an oil-in-water emulsion representing a non-dairy creamer was added in this research to understand how this affects mouthfeel perception. An emulsion is a dispersion of droplets of one liquid in another one with which it is incompletely miscible (IUPAC 1972). There are two types of emulsions: oil-in-water or water-in-oil emulsions. In an oil-in-water emulsion small spherical oil droplets are dispersed in water, the continuous phase, whereas in a water-in-oil emulsion small spherical water droplets are dispersed in an oil phase. Emulsifiers are used to create stable emulsions which maintain a stable droplet size over time. Emulsifiers are surface-active molecules which absorb to the surface of freshly formed droplets, forming a protective layer which prevents the droplets from coming close enough together to aggregate, thereby ensuring their stability for a period of time (Mc Clements, 1999). Most emulsifiers are amphiphilic molecules meaning they have polar and nonpolar regions. Commonly used emulsifiers in the food industry include amphiphilic proteins, small-molecule surfactants and phospholipids. Milk proteins, such as sodium caseinate, used in this

research are widely used to stabilize emulsion because they are known to be rapidly adsorbed at oil-water interfaces (Srinivasan et al., 2001). Depending on the droplet size of the dispersed phase, an emulsion can be characterized as micro emulsion with a droplet size of 100 nm to a few micrometers or nano emulsion with a droplet size of 1 – 100 nm (Kale & Deore, 2016). The size of fat globules in milk can vary between 100 - 1500 nm (Lu et al., 2016).

Early on, rheological measurements were used to understand the relationship between viscosity and perceived thickness (Kokini et al., 1977). In the mouth, a liquid is squeezed between the tongue and the palate and then sheared during the back and forth motion of the tongue (Kokini, 1987). For Newtonian fluids, the perceived thickness was found to be proportional to the square root of viscosity meaning only a four-fold change in viscosity results in a two-fold change of perceived thickness. For non-Newtonian fluids the gap between the tongue and the palate is estimated in order to calculate the effective shear rate in the mouth. Since the viscosity of non-Newtonian fluids is shear rate dependent, efforts were made to identify the best shear rate viscosity to correlate with perceived thickness. Cutler et al. (1983) further proved the correlation between viscosity and perceived thickness. In their study, the best correlation was found at a shear rate of 10 $s^{-1}$. However, Akhtar et al. (2005) and Koliandris et al. (2010) recorded the best correlation between viscosity and perceived thickness at a shear rate of 50 $s^{-1}$ and 500 $s^{-1}$, respectively. These contradicting results may be due either to different criteria of subject selection and experimental set up or the different rheological nature of the samples.

In emulsions, fat is known to behave as a filler, contributing significantly to viscosity (van Aken et al., 2011). This viscosity increase leads to an increase of perceived thickness and creaminess. Oil droplets of emulsions can become incorporated into the mucous oral coating which increases the perception of attributes such as "creamy", "fatty", "slippery mouthfeel" and "mouth coating after-feel" (de Wijk & Prinz, 2006). Although it was found that "creamy" or "fatty" perception is related to the apparent viscosity of the product, the sensory properties of emulsions cannot be fully described by bulk rheology (Chojnicka-Paszun et al., 2012). Tribological measurements showed that friction coefficient of milk samples with a fat content above 1% correlated well with the perception of creaminess. The best correlations were obtained for speeds below 200 mm/s that correspond to a speed comparable with the speed in the mouth (Chojnicka-Paszun et al., 2012). In custards it was also observed that zero or low fat content resulted in high ratings of "roughness" and low rating of "creaminess". A number of studies have tried to link sensory attributes of custards with instrumental measurements. de Wijk and Prinz (2005) observed that custards with a higher fat content gave lower friction and are perceived to be less rough and creamier. Furthermore, smaller droplets which are less deformable than larger ones reduced friction and increased the perception of creaminess. However, as observed by Akhtar et al. (2005) increasing the droplet size from 0.5 to 2 μm in oil-in-water emulsions did not significantly influence the perception of "thickness" and "creaminess". Friction increases with increasing fat droplet size, decreasing fat content, increasing particle size and sharper particle shapes (spherical vs irregular shape). Increased friction also typically resulted in increased

sensations of roughness, prickling, dry and heterogeneity and significantly decreased sensations of creaminess, fattiness, stickiness, slipperiness and smoothness (de Wijk & Prinz, 2005).

Mouth coating, an after feel, describes the coating of the oral cavity (Szczesniak, 1979). Mouth coating is considered to be a desirable property if the beverage is generally perceived to have a pleasant flavor and mouthfeel, however if the beverage is unpleasant, mouth coating can become an undesirable attribute since it makes it more difficult to clear the oral cavity of the unpleasant beverage (Withers et al., 2013). Although no direct correlation with perceived mouth coating was carried out, Pivk et al. (2008) observed that the residual amount of lipids measured in the oral cavity increased with increasing oil intake. The same trend was described in sensory studies, where fatty mouthfeel or mouth coating increased with the fat content.

In a study by Engelen et al. (2007) the effect of saliva composition on texture perception of semi-solids was investigated. They found that subjects with a lower protein concentration in their saliva perceived the slipperiness to be more intense. A lower protein concentration was also linked to a smaller decrease in viscosity and therefore a more viscous product might stick better to the hard oral surfaces, leading to a slippery sensation. Furthermore, the fatty after feel was less strongly perceived by subjects with a higher protein concentration in their saliva. Alpha-amylase activity was found to be correlated to a number of attributes. Subjects with a high α-amylase activity perceived the thickness of starch-based custard to be lower since the α-amylase breaks down the starch, resulting in lower

viscosity. Subjects with a lower α-amylase activity perceived slipperiness as stronger and a high α-amylase activity decreased creamy after-feel.

## 1.3 Aims and Objectives

The aims of this research were to:

1. Understand which mouthfeel attributes are important for black tea beverages and how mouthfeel and taste are impacted by additives such as fat and sugar. It is hypothesized that mouthfeel and taste are linked to compositional factors.
2. Explore different instrumental methods to test whether these provide new insights into the mechanisms of mouthfeel perception and data that correlates to mouthfeel perception. It is hypothesized that instrumental methods can be used to predict mouthfeel perception of tea samples.

The specific objectives to achieve these aims were to:

O1) Develop a black tea beverage model system where tea, fat and sugar content can be varied independently.

O2) Develop a mouthfeel lexicon which defines and describes key mouthfeel attributes for tea beverages.

O3) Generate predictive models which reveal how tea, fat and sugar impact individual mouthfeel attributes

O3) Understand the temporal perception of bitterness and astringency over multiple sips of tea which reveals how tea, fat and sugar impact the two attributes.

O4) De-couple the effect of sweetness and thickness on astringency and bitterness perception

O5) Utilize a force plate in order to measure the frictional and vibrational behavior of different tea samples and correlate to mouthfeel perception.

O6) Develop a method including protocol and data analysis to measure the sounds from the mouth when sampling tea in-vivo using the "acoustic tribology" and correlate to mouthfeel perception.

# 2 Understanding the Impact of Tea, Fat and Sugar Content on the Mouthfeel of Black Tea Beverages

## 2.1 Introduction

The liking of food is a complex process which is affected by taste, aroma, flavour and texture and also physiological, psychological and biological factors (Engelen & de Wijk, 2012). Much of the research investigating perception and liking has focussed on the impact of taste, aroma and flavour. However, research over the last decade has recognised the importance of texture and mouthfeel on consumer liking (Szczesniak, 2002). Therefore, it is of great value to understand the impact of food ingredients on the perception of mouthfeel. Texture is commonly used to refer to properties of solid products, whereas mouthfeel includes all tactile properties of semi-solid and liquid products, from the time when the food/beverage is placed in the mouth until it is swallowed (Guinard & Mazzucchelli, 1996).

In order to better understand the sensory perception of texture and mouthfeel, the development and definition of appropriate terms which allow for their characterisation in food and beverage products is paramount. Sensory lexicons are used as a tool to communicate sensory properties of food products across diverse audiences (Lawless & Civille, 2013). The key stages of a lexicon's development include preparation and development. In the preparation stage, suitable panellists are selected, and samples are chosen which represent the entire product space. In the later development stage, terms describing the product are generated and

defined, which is followed by selecting appropriate references, development of evaluation protocols and a further clarification of terms (Lawless & Civille, 2013). Tea is one of the most pleasant and popular beverages in the world (Alasalvar et al., 2013), however, published lexicons describing its properties are limited to green tea (Lee & Chambers, 2007), honeybush tea (Theron et al., 2014) and rooibos tea (Koch et al., 2012), and mainly focus on their flavour properties. The mouthfeel attributes described in the above studies include "tooth-etching", "harsh astringent", "flat/bland" and soft/smooth" and as reference different alum solutions were commonly used. The mouthfeel of tea remains poorly understood and reported attributes are mostly limited to those which describe the sensation of "astringency"/"dryness".

The way black tea is consumed around the globe varies significantly, with some countries using high quantities of milk and sugar, whereas others do not add any milk (Sharma et al., 2008). Tea is not only prepared fresh but ready-to-go tea using instant tea powder is becoming increasingly popular (Someswararao & Srivastav, 2012). These ready-to-go tea beverages, consumed both hot and cold, usually contain high quantities of sugar and a creamer which may elicit different mouthfeels in comparison to fresh preparations. The addition of milk will introduce fat, protein and lactose in varying levels depending on the type. Other sources of fat commonly added to black tea are creamers, which can be dairy or non-dairy (Sharma et al., 2008). It has been shown that with increasing fat content in emulsions, the perception of attributes such as "fatty", "creamy" and "coating afterfeel" increases (Vingerhoeds et al., 2008), whereas other research showed that perceived astringency decreases with increasing fat content

(des Gachons et al., 2012). Sugar increases the sweetness of the beverages but also affects perceived thickness and has also been found to decrease astringency in wine (Ishikawa & Noble, 1995). Therefore, it is likely that the addition of fat or sugar will decrease astringency perception and the addition of both could have a synergistic effect decreasing perceived astringency further. The impact on other mouthfeel attributes requires investigation. Therefore, research is needed to improve our knowledge of mouthfeel perception and the impact different ingredients may have on the consumer sensory experience. In this study, the impact of added sugar and fat on the mouthfeel of black tea was investigated. Fat was added as an oil-in-water emulsion with de-odourised coconut oil as dispersed phase, representing a non-dairy creamer. The use of this emulsion allowed control over droplet size, and enabled the addition of fat in a controlled way. Furthermore, using an emulsion with a higher fat content than milk enables us to produce a design space which covers a wider range of fat contents. Besides the addition of fat, the emulsion contains the emulsifier sodium caseinate, a dairy protein, which is commonly used in creamers (Gardiner, 1977). Since milk and creamers are commonly added to tea, the presence of sodium caseinate in the emulsion represents the milk and creamers in a better way than using an emulsifier which is usually not present in milk. It is known that caseinates and tea polyphenols interact (Hasni et al., 2011; Kartsova & Alekseeva, 2008; Yuksel et al., 2010) which might affect mouthfeel perception, especially astringency perception as shown by Ares et al. (2009). With the addition of 10 % fat also 0.8 % of sodium caseinate are added to the tea which is equivalent to the amount of casein present in 30 ml milk.

The aim of the study was firstly to develop a much needed, novel mouthfeel lexicon for black tea beverages which can be used to communicate mouthfeel attributes in the tea industry. In the current study a black tea model system was developed This allowed each design factor to be systematically varied to reflect the global tea market and was used to develop this specific mouthfeel lexicon. Secondly, the impact of varying concentrations of key ingredients such as fat, sugar and tea on perceived mouthfeel was investigated using a trained sensory panel. Understanding the impact of these key ingredients will drive the development of novel and potentially healthier products.

## 2.2 Materials and Methods

### *2.2.1 Model Tea System and Experimental Design*

A model system approach was adopted, allowing all factors (black tea, sucrose and fat) to be systematically controlled, varied independently, and investigated separately. The design space was used to design samples containing between 0.1 – 1 g/100 g instant black tea powder (provided by Unilever, UK), 0 – 10 g/100 g sucrose (Tate and Lyle Granulated Sugar, UK), and 0 – 10 g/100 g fat (deodorised coconut oil, provided by Unilever, UK), added as an oil-in-water emulsion. The processing conditions of the oil-in-water emulsion are described in section 2.2.2. A D-optimal design was chosen over a factorial design, to minimise the sample number for assessment whilst maintaining the ability to produce predictive models. The D-optimal design consisted of 28 samples from the design space, including three experimental replicate samples and three centre points, giving the model greater predictability.

### *2.2.2 Sample Preparation*

The oil-in-water emulsion was processed in batches of 400 g, which were then combined before addition to the tea. Each batch of emulsion contained 20 g/100 g deodorized coconut oil, 1.6 g/100 g sodium caseinate (Bacarel & Co, UK) and 78.4 g/100g distilled water. As illustrated in Figure 2-1, sodium caseinate was dissolved in distilled water at 80 °C for 15 min. Melted coconut oil was added to the casein-water mixture and processed using a high-shear blender (Silverson Machines Ltd, Chesham, UK) for 90 s at 5000 rpm. The coarse emulsion was then transferred into a high pressure homogeniser (Panda 2K, Niro Soavi S.p.A, Italy) and processed at 110 kPa for 2 passes. The emulsion was stored for a maximum of 5 days at 4 °C until it was used during sensory evaluation. Storage for a maximum of 5 days was found to be possible, following positive microbiological tests which proved that the emulsion was safe for consumption (data not shown).

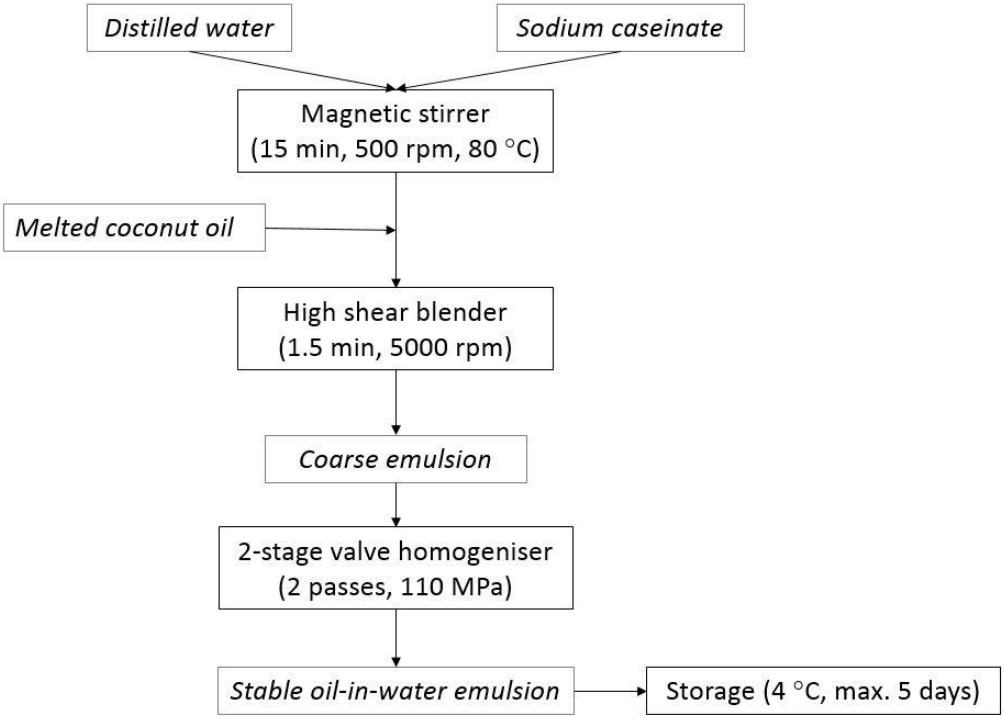



*Figure 2-1 Schematic illustration of the emulsion process*

All samples were prepared a maximum of 2 h in advance of the sensory session. First, black tea powder was dissolved in hot water (70 °C) and sucrose was added while the tea was still hot to ensure complete dissolution. The samples were prepared in Duran bottles and shaken until the sugar was dissolved. To add the emulsion, the tea was cooled down to a temperature of 50 – 60 °C. Samples were stored at room temperature until the beginning of the session. Before the samples were filled into amber glass vials for sensory assessment, each bottle was shaken to ensure a homogeneous mixture of the tea sample.

### *2.2.3 Model System Characterisation*

The droplet size of the emulsion was determined after dilution in distilled water by laser diffraction with a particle size analyser LS13320 (Beckman

Coulter Ltd, High Wycombe, UK). The emulsion showed a reasonably symmetrical distributed droplet size (see Appendix 1) with an average droplet size below 200 nm ($D_{3,2}$ = 177 nm). Flow characteristics of the tea samples were measured at 40 °C using MCR 301 Rheometer (Anton Paar Ltd, St Albans, UK) and a double gap cylinder geometry. All samples showed Newtonian behaviour and shear viscosity at 50 $s^{-1}$ ranged from 0.66 mPa·s for black tea to 1.46 mPa·s for samples containing the maximum of tea, fat and sugar, see Table 2-8 for numerical values.

Total polyphenol content was determined via a colometric method using Folin-Ciocalteu reagent following ISO 14502 – 1(2005) . Gallic acid monohydrate was purchased from MP (UK), sodium carbonate from Acros Organic (UK) and Folin-Ciocalteu reagent was obtained from Merck, Germany. The instant black tea, assessed in triplicate, was found to have a total polyphenol content of 192±14.9 mg GAE/g dry matter.

### *2.2.4 Sensory Evaluation*

Ethical approval for this study was granted by the Medical Ethics Committee of the University of Nottingham (Q13032015). All panellists gave written informed consent to participate in this study and received a disturbance allowance for their participation. Data was collected using Compusense Cloud (Compusense, Canada). Testing was performed at room temperature (20±2°C) under Northern Hemisphere daylight in facilities according to ISO 8589:2007.

*2.2.4.1 Panel Screening*

Subjects were screened to ensure that they were able to discriminate between relevant samples and articulate perceived taste and mouthfeel sensations. The screening consisted of two parts: an online questionnaire and one screening session. The questionnaire included general health questions as well as questions concerning tea consumption habits. 28 subjects completed the questionnaire of which 12 were naïve subjects; the remaining 16 subjects were part of an established and experienced panel. 22 subjects were invited to participate in the two-hour screening session. The screening session comprised of a basic taste recognition test to determine any sensory impairment, difference tests to determine discrimination abilities and descriptive tests to assess the capability to articulate perception. A point system was used to aid with panel selection. Emphasis was given to the sensations and abilities which are important for this study, for example, the ability to recognise bitterness and astringency and the ability to discriminate different levels of thickness and bitterness.

*2.2.4.2 Attribute Generation and Panel Training*

13 panellists scored above 65% in screening tests and were invited to take part in the study, of which 10 agreed. A modified Quantitative Descriptive Analysis (QDA) approach (Stone et al., 1974) was used to generate attributes for the mouthfeel lexicon and subsequently evaluate samples. This approach was chosen to explore all mouthfeel attributes using the panellist`s own vocabulary to generate an initial list. The method relies on consensus to develop a complete list of attributes and aims to avoid attribute dumping. The attributes describing the mouthfeel of the model system were generated by the panel in two 2 h sessions. The initial list of

attributes was produced through a projective mapping. Panellists were given 8 samples which represent the corners/extremes of the design space plus one replicate sample and were asked to sort them on a piece of paper. In the first projective mapping exercise the panellists were asked to do a global napping (overall sensory perception) and in the second sorting exercise the panellists were instructed to group samples according to their mouthfeel (partial napping). Samples grouped close together are perceived to be similar whereas samples far apart from each other are very different. The panellists were also instructed to describe the samples using descriptive terms. After a group discussion, an initial list of attributes was composed.

The training, which included the establishment of evaluation protocols, was conducted over a total of 17 x 2 h sessions. During training, panel performance (discriminative ability and reproducibility) was monitored. During the first sessions, the panellists were presented with examples which represented the attributes and appropriate definitions and evaluation protocol were discussed. It was also established which attributes were relevant for tea beverages and which attribute described the same mouthfeel. In this way, the number of attributes was reduced. Once important attributes were identified, and evaluation protocols were established, the panel was trained to rank-rate the attributes on an unstructured 10-point scale with anchors (0 = low, 10 = high). Different samples from within the design space were presented and the panel used rank-rating to score their mouthfeel. The rank-rating scores were discussed in order to identify if the panel agreed on the ratings. In the final stages of the training, the panel rated samples and was given feedback

using Compusense as an indication if their scores were different to the panel mean.

*2.2.4.3 Sample Evaluation*

The set of 28 samples (see Table 2-1) was rated in triplicate. A continuous, unstructured line scale was used with anchor points labelled at "0" and "10", where 0 represented low and 10 represented high for the respective attribute. The samples were presented monadically according to a randomised balanced, blocked design. 30 ml samples were served in 30 ml amber vials labelled with randomised three-digit codes. Amber vials enabled the appearance differences between the samples to be disguised. The samples were heated in a water bath for 15 min to ensure a serving temperature of 50 ± 1 °C, which was determined by the panel to be a pleasant drinking temperature for tea. At the beginning of each session the panellists received two reference samples: 1 % tea labelled as "thin, bitter, astringent" and 0.1 % tea with 10 % fat and 10 % sugar labelled as "thick, sweet, slippery, mouth coating" as agreed during initial attribute training sessions. Training revealed a first position effect and to avoid this, a dummy sample (0.2 % tea) was used and served as the first samples to every panellist in each session but the data was disregarded for analysis purposes. This particular tea sample was chosen as dummy sample because it was rated medium for astringency and bitterness and therefore a midrange sample to start the evaluation. A total of 15 samples were evaluated in one session. Between each sample the panellists were asked to cleanse their palate using crackers (Rakusen`s Limited, UK), honeydew melon (local retailer) and water (Evian, Danone Waters, France), and a

four-minute break was given. Honeydew melon has been used as a palate cleanser for black tea (Wright, 2007) and the panel found it particularly helpful to cleanse the palate after fatty samples were consumed. After the evaluation of 5 and 10 samples the panellists were given a 10 min break. The panellists were asked to consume the whole sample in two sips. After each sip, three attributes were evaluated as agreed in training.

*Table 2-1: Samples rated in triplicate by the panel. * indicates experimental replicates.*

| | **Composition** | | |
|---|---|---|---|
| Sample | Tea [%] | Fat [%] | Sugar [%] |
| 1 | 0.1 | 10 | 0 |
| 2* | 0.1 | 0 | 10 |
| 3* | 0.1 | 0 | 10 |
| 4 | 0.1 | 6.67 | 10 |
| 5 | 0.1 | 0 | 3.33 |
| 6* | 0.1 | 5 | 0 |
| 7* | 0.1 | 5 | 0 |
| 8 | 0.1 | 10 | 6.67 |
| 9 | 0.33 | 7.5 | 2.5 |
| 10 | 0.33 | 2.5 | 5 |
| 11 | 0.4 | 3.33 | 10 |
| 12* | 0.4 | 0 | 0 |
| 13* | 0.4 | 0 | 0 |
| 14 | 0.55 | 10 | 10 |
| 15* | 0.55 | 5 | 5 |
| 16* | 0.55 | 5 | 5 |
| 17* | 0.55 | 5 | 5 |
| 18 | 0.78 | 2.5 | 2.5 |
| 19 | 0.78 | 7.5 | 7.5 |
| 20 | 0.7 | 10 | 0 |
| 21 | 0.7 | 0 | 10 |
| 22 | 1 | 0 | 0 |
| 23 | 1 | 10 | 10 |
| 24 | 1 | 3.33 | 10 |
| 25 | 1 | 10 | 3.33 |
| 26 | 1 | 5 | 5 |
| 27 | 1 | 6.67 | 0 |
| 28 | 1 | 0 | 6.67 |

*2.2.5 Data Analysis*

Panel performance was assessed using PanelCheck (version 1.4.2, Norway). Repeatability and discrimination ability of the panel were evaluated by assessment of samples in duplicate, visualised in a p-MSE plot. The mean squared error (MSE) indicates the reproducibility of a panellist and should be as small as possible. The p-value indicates if a panellist is able to discriminate between samples for a given attribute. Interaction plots were interrogated from three different sessions to determine the extent of general agreement for different attributes.

Once the panel showed a good panel performance, the sample set was evaluated in triplicate. 3-factor analysis of variance (ANOVA) (panellist, sample, session) with interaction followed by Posthoc test (Tukey) was conducted for each attribute to identify significant differences between samples.

To establish the relationship between variations in sensory attributes and composition (tea, fat and sugar) predictive, polynomial models were generated through multiple regression analysis using Design Expert (version 7, Minneapolis, USA). Non-significant terms which were determined by ANOVA, were removed. To provide evidence that the predictive models are valid, different statistical values were determined including $R^2$, Adj $R^2$, Pred $R^2$ and Adeq Precision. $R^2$ is a measure of the variation within the data set, which is explained by the model; a value close to 1 shows that the model explains most of the variation. The adjusted $R^2$ (Adj $R^2$) should be close to the $R^2$ value to show that there are only terms in the model which are significant and add value. The predictive $R^2$ (Pred $R^2$) value should be close to the Adj $R^2$ value and explains variation

in the model. Adequate Precision measures the signal to noise ratio and a value >4 indicates adequate model discrimination.

To evaluate the predictive ability of the models, a validation study was carried out. A validation set consisting of 7 samples (see Table 2-2) from within the design space was evaluated by the panel in duplicate after the initial evaluation was completed. The validation set represented the full range of compositional factors, but these samples were not part of the original model data set. Predictive vs. actual values were plotted and correlations between the actual mean panel intensity values from the validation set and predicted values from the model were calculated.

*Table 2-2: Composition of samples used to validate model equations.*

| Tea [%] | Fat [%] | Sugar [%] |
|---|---|---|
| 0.1 | 0 | 5 |
| 0.2 | 0 | 0 |
| 0.55 | 5 | 10 |
| 0.8 | 2 | 6 |
| 1.0 | 10 | 0 |
| 1.0 | 0 | 10 |
| 1.0 | 2 | 0 |

To help visualise the resulting data, interaction plots were used to illustrate interactions between two design factors and contour plots were used for interactions including all three design factors. The plots were obtained from Design Expert. Relationships between attributes and ingredients are displayed using Principal Component Analysis (PCA) produced by XLSTAT (version 19.01). Design factors (tea, fat, sugar) were included as supplementary data.

## 2.3 Results

### *2.3.1 Panel Performance*

To illustrate the improvement of the panel throughout the training, Figure 2-2 shows profile plots of four attributes in three training sessions. Mouth coating, sweet, bitter and astringent are shown as examples. Mouth coating, together with thick, was an attribute that the panel understood and defined easily and as can be seen from the profile plots, good agreement between the panellists was observed throughout training sessions 10, 13 and 15, with improved agreement in session 15. For sweetness a similar trend was observed and it was found that this attribute was easy to evaluate. Bitterness and astringency were attributes which were more difficult for the panel to evaluate. The profile plots of session 10 show many panellist*sample interactions, indicating that the panel does not agree on the bitterness and astringency perception of certain samples. Therefore, dedicated sessions training the panel on these attributes were required, including sessions where samples were rank-rated and feedback of the expected scores was given. Training was deemed to be effective as the interaction plots in session 13 and 15 show a much better agreement between the panellists. This was confirmed by the increased p-values of sample*panellist interactions shown in Table 2-3. An increase in p-values shows that sample and panellist interaction are less important, indicating a better agreement of the panel. Furthermore, p-MSE values improved from session 10 to 15 session. All panellists were able to discriminate between samples ($p<0.05$) and their repeatability improved. This was not just observed for bitterness and astringency but for all attributes. A final panel performance check was undertaken prior to evaluation of the final

sample set and showed that p-MSE values improved on average by 50% from session 10 to session 17 (data not shown).

*Table 2-3: Sample*Panellist interaction for 3 selected session. Increasing p-values indicate improved panel agreement.*

| | Sample * Panellist Interaction p-values | | |
|---|---|---|---|
| **Attribute** | **Session 10** | **Session 13** | **Session 15** |
| **Bitter** | 0.008 | 0.006 | 0.024 |
| **Sweet** | 0.031 | 0.054 | 0.062 |
| **Mouth coating** | 0.009 | 0.024 | 0.097 |
| **Thick** | 0.013 | 0.031 | 0.068 |
| **Slippery** | 0.008 | 0.028 | 0.056 |
| **Astringent** | 0.001 | 0.02 | 0.045 |

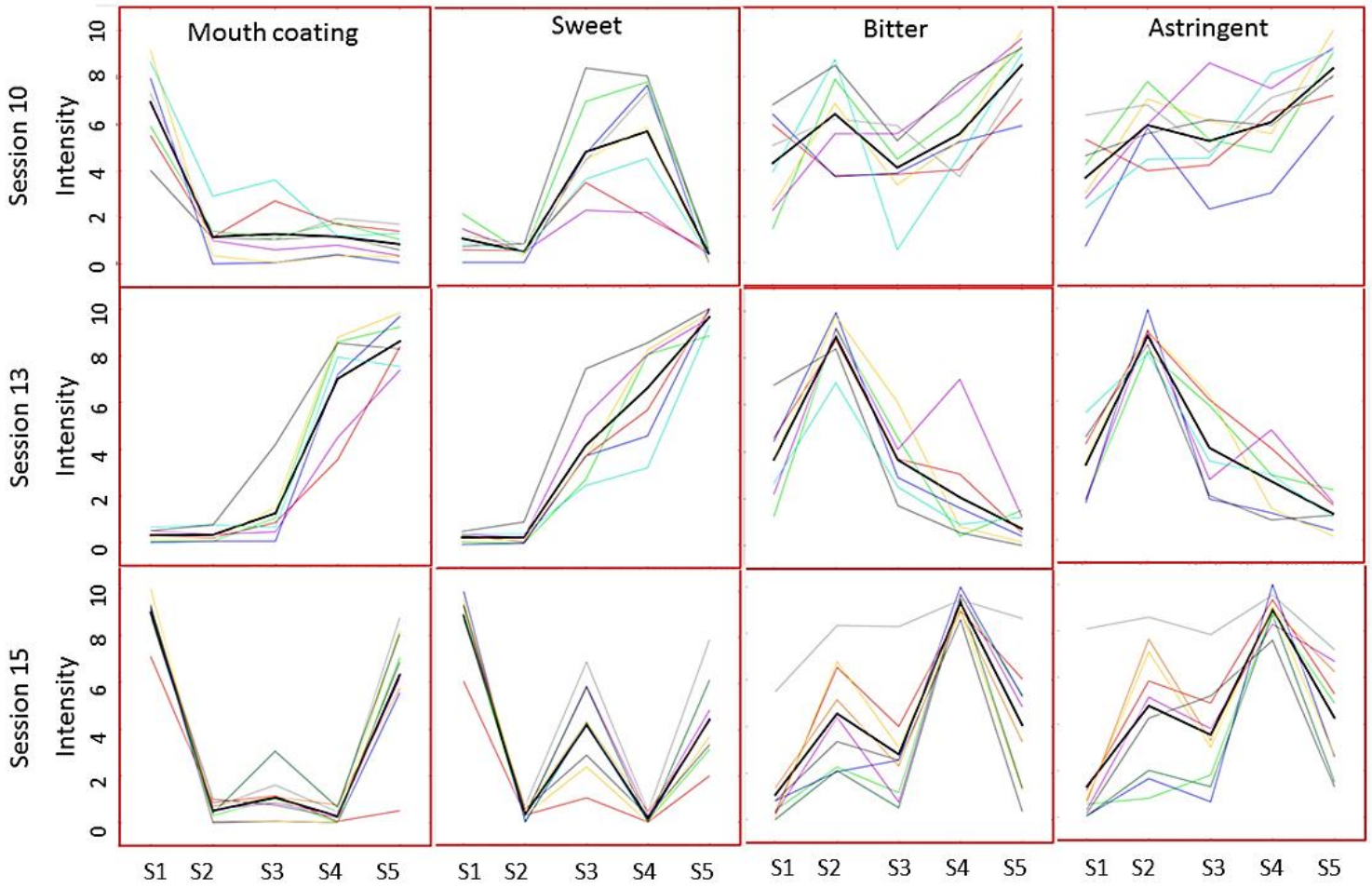



*Figure 2-2: Illustration of improvement of panel agreement of 3 selected sessions for 4 attributes (mouth coating, sweet, bitter and astringent). In each session a selection of different tea samples with sugar and fat addition were rated on a 10-point scale. Different colour show the performance of different panellists. Black line shows panel mean score.*

### *2.3.2 Mouthfeel Lexicon Development*

Table 2-4 shows the initial list of attributes which were summarised after the projective mapping task and group discussion. The list shows that a number of attributes were used to describe the rough/dry mouthfeel. These attributes included dry, astringent, mouth puckering, numbing and furry. Watery, light, thick, heavy and viscous referred to the thickness/viscosity of the samples, whereas the remaining attributes describe mouthfeel that appeared to be related to fat perception e.g. sticky, creamy, oily mouthfeel, mouth coating. Several attributes were also used to describe the general mouthfeel of the samples e.g. smooth and silky.

*Table 2-4: List of initial attributes generated by the panel through Napping.*

| **Attributes** | |
|---|---|
| dry | creamy |
| astringent | fatty |
| mouth puckering | oily mouthfeel |
| numbing | mouth watering |
| furry | mouth coating |
| feeling thirsty | teeth coating |
| watery | creamy layer |
| light | milky |
| thick | smooth |
| heavy | silky |
| viscous | soft |
| sticky | full bodied |
| claggy | rounded |
| slippery | cleansing |
| | lingering |

Further group discussions and training identified the key attributes for black tea beverages and the complete lexicon including definitions, evaluation protocols and references can be found in Table 2-5. For the model system, the panel agreed on mouthfeel attributes which described the mouthfeel of tea during consumption (thickness, slipperiness) and after

swallowing, commonly called "after-feel" (mouth coating, astringency). Although the focus of this study was mouthfeel, the basic tastes of initial sweetness and initial bitterness were included to avoid attribute dumping, which occurs when panellists perceive an attribute which they are not asked to rate and then express this on a scale intended for a different attribute (Kemp et al., 2009). Bitterness and astringency are attributes which can easily be confused by panellists, so the evaluation of both aimed to minimize confusion. Furthermore, it was also of interest to observe if mouthfeel perception changes in relation to taste perception and vice versa, i.e. cross-modal interactions.

The panel discussed further attributes, e.g. "smooth" which was defined as "the mouthfeel in the absence of particles" and "mouth-watering" described as "the sensation of saliva-formation after swallowing". During training, the panel was not able to discriminate between samples for smoothness, and mouth-watering was not perceived by all panellists; consequently, these attributes were removed for sample evaluation.

For all sensory attributes and samples, panel means, standard deviations (SD) and results of Tukey`s multiple comparison analysis are shown in Table 2-5. 3-way ANOVA was performed on the panel mean data of three replicates. For all sensory attributes, ANOVA showed that the intensity varied significantly ($p<0.05$) among samples with different composition. The Tukey test revealed good separation between samples, indicating a good level of discrimination between the samples across all attributes.

*Table 2-5: Lexicon of attributes used to evaluate the mouthfeel of the model tea system, including definitions, evaluation protocols and training references*

| **Attribute** | **Definition** | **Evaluation protocol** | **Reference** |
|---|---|---|---|
| Thickness | Resistance of a liquid to flow | Evaluated prior swallowing by moving tongue up and down | Yoghurt, whole fat milk |
| Mouth coating | Layer that remains in mouth after swallowing | Evaluated immediately after swallowing (layer of product on cheeks and mouth roof) | Single cream, mayonnaise |
| Slipperiness | Lack of friction on the roof of the mouth while sample is consumed, sample`s ability to lubricate | Evaluated prior swallowing by moving tongue across the roof of the mouth | Single cream |
| Astringency | Sensation of a dry, puckering tongue | Evaluated 15 sec after swallowing by moving tongue behind teeth and assessing dryness of the tongue | Pomegranate juice<br>Soya yoghurt |
| Initial Sweetness | Taste of sweetness | Evaluated prior to swallowing | Sucrose solution |
| Initial Bitterness | Taste of bitterness | Evaluated prior to swallowing | 90 % cocoa chocolate, caffeine solutions, grapefruit |

### *2.3.3 Model Equations and Model Validation*

The impact of tea, fat and sugar on the key mouthfeel attributes was evaluated and model equations were obtained which can be used to predict the perceived mouthfeel for samples within the design space that were not included within the evaluation set. Table 2-7 shows the predictive equations for all key attributes and also statistical values describing the fit of the model, e.g. statistics describing the model fit (adequate precision) and predictive capability (Adj and Pred $R^2$ values). High $R^2$ values indicate that all models explain the majority of variation within the data set. The values for adjusted $R^2$ are close to $R^2$ and show that only significant terms are included in the model. Predicted $R^2$, which is close to Adj. $R^2$, indicates that the model is able to predict a response value with good accuracy. The signal to noise ratio, indicated by the adequate precision value is higher than 4 showing a high level of model discrimination.

The validation set of samples evaluated by the panel showed good agreement between actual and predicted values as demonstrated by an average difference between predicted and actual scores of 0.6 on the sensory scale for all 7 samples. Attained $R^2$ values between 0.99 and 0.80 (see Figure 2-3) indicate good agreement between the experimental mean panel sensory scores and the predicted values generated by the models for all attributes.

The significant model terms in Table 2-7 reveal which factor impacts each attribute. A number in the tea, fat and sugar columns signifies a significant impact on that attribute. A positive number denotes a positive impact on perception and a negative number denotes a negative impact on perception. The higher the number, the higher the impact. Therefore, it

can be seen from Table 2-7, which factors were contributing to each attribute. The key factor impacting thickness, mouth coating and slipperiness attributes was fat. Tea content was the main driver for perceived bitterness and astringency and, not surprisingly, sugar content impacted perceived sweetness positively.

*Table 2-6: Mean panel scores (from 10 panellists, 3 replicates) and standard deviation (SD) for each attribute. Tukey Post hoc grouping are shown by superscript letters, samples which share a common letter are not significantly different, p<0.05. (Table continued on next page)*

| Composition | | | Attributes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sweet | | Thick | | Slippery | | Mouth coating | | Bitter | | Astringent | |
| Tea [%] | Fat [%] | Sugar [%] | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 0.1 | 10 | 0 | $0.59^{HI}$ | 0.67 | $6.89^{DEF}$ | 2.06 | $6.79^{BCDE}$ | 2.63 | $7.55^{CDEF}$ | 2.21 | $3.71^{FGHI}$ | 2.95 | $3.09^{GHI}$ | 2.42 |
| 0.1 | 0 | 10 | $8.91^{A}$ | 0.93 | $3.40^{GH}$ | 2.07 | $3.61^{G}$ | 3.01 | $1.01^{H}$ | 1.09 | $1.59^{J}$ | 2.18 | $1.84^{I}$ | 1.75 |
| 0.1 | 0 | 10 | $8.99^{A}$ | 0.94 | $3.83^{G}$ | 2.21 | $3.97^{FG}$ | 3.16 | $1.33^{H}$ | 1.47 | $1.92^{HIJ}$ | 2.47 | $1.99^{I}$ | 1.97 |
| 0.1 | 6.67 | 10 | $9.18^{A}$ | 1.09 | $8.83^{AB}$ | 1.32 | $8.56^{A}$ | 1.52 | $9.05^{AB}$ | 1.01 | $1.32^{J}$ | 1.69 | $1.89^{I}$ | 2.20 |
| 0.1 | 0 | 3.33 | $4.28^{CDE}$ | 1.91 | $2.25^{HIJ}$ | 2.14 | $1.76^{H}$ | 2.49 | $0.83^{H}$ | 0.81 | $2.07^{HIJ}$ | 2.47 | $2.17^{HI}$ | 2.37 |
| 0.1 | 5 | 0 | $0.59^{HI}$ | 0.76 | $6.11^{F}$ | 2.72 | $6.95^{ABCDE}$ | 2.58 | $7.14^{EFG}$ | 2.41 | $3.97^{EFG}$ | 2.95 | $3.69^{FGH}$ | 2.30 |
| 0.1 | 5 | 0 | $0.64^{HI}$ | 0.84 | $6.41^{EF}$ | 2.74 | $6.23^{CDE}$ | 2.64 | $7.60^{CDEF}$ | 2.42 | $3.78^{FGH}$ | 2.84 | $4.21^{EFG}$ | 2.46 |
| 0.1 | 10 | 6.67 | $8.30^{AB}$ | 1.22 | $8.35^{ABCD}$ | 1.47 | $8.24^{AB}$ | 2.06 | $8.76^{ABCD}$ | 1.33 | $1.89^{IJ}$ | 2.69 | $1.77^{I}$ | 1.95 |
| 0.33 | 7.5 | 2.5 | $2.50^{FG}$ | 1.88 | $7.21^{CDEF}$ | 1.85 | $7.13^{ABCDE}$ | 2.36 | $7.55^{CDEF}$ | 2.07 | $2.70^{GHIJ}$ | 2.68 | $3.19^{GHI}$ | 2.45 |
| 0.33 | 2.5 | 5 | $5.30^{CDE}$ | 2.06 | $7.03^{CDEF}$ | 1.49 | $6.83^{BCDE}$ | 2.29 | $7.02^{EFG}$ | 2.18 | $2.14^{GHIJ}$ | 2.40 | $2.77^{GHI}$ | 2.36 |
| 0.4 | 3.33 | 10 | $8.74^{AB}$ | 0.15 | $8.49^{ABC}$ | 0.97 | $7.82^{ABC}$ | 2.65 | $8.88^{ABC}$ | 0.40 | $2.32^{GHIJ}$ | 2.72 | $1.79^{I}$ | 2.42 |
| 0.4 | 0 | 0 | $0.14^{HI}$ | 1.07 | $0.72^{J}$ | 1.25 | $1.56^{H}$ | 2.41 | $0.36^{H}$ | 1.00 | $7.18^{BC}$ | 2.71 | $6.95^{BC}$ | 1.85 |
| 0.4 | 0 | 0 | $0.13^{HI}$ | 0.16 | $0.93^{IJ}$ | 1.01 | $1.47^{H}$ | 2.40 | $0.37^{H}$ | 0.47 | $7.68^{B}$ | 2.30 | $7.09^{B}$ | 1.96 |
| 0.55 | 10 | 10 | $9.21^{A}$ | 1.00 | $9.11^{A}$ | 1.20 | $8.00^{AB}$ | 2.15 | $9.27^{A}$ | 0.93 | $1.96^{HIJ}$ | 2.67 | $1.91^{I}$ | 2.32 |
| 0.55 | 5 | 5 | $5.49^{C}$ | 2.12 | $7.35^{BCDEF}$ | 1.59 | $7.76^{ABC}$ | 1.59 | $7.82^{BCDEF}$ | 1.38 | $2.37^{GHIJ}$ | 2.49 | $2.78^{GHI}$ | 2.50 |
| 0.55 | 5 | 5 | $4.77^{CD}$ | 2.43 | $7.65^{ABCDEF}$ | 1.28 | $7.65^{ABCD}$ | 2.04 | $7.87^{ABCDEF}$ | 1.83 | $2.65^{GHIJ}$ | 3.21 | $2.77^{GHI}$ | 2.86 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.55 | 5 | 5 | 5.03$^{CD}$ | 2.06 | 7.16$^{CDEF}$ | 1.47 | 7.24$^{ABCDE}$ | 1.84 | 7.37$^{DEFG}$ | 1.76 | 2.05$^{HIJ}$ | 2.24 | 2.58$^{GHI}$ | 1.98 |
| 0.78 | 2.5 | 2.5 | 1.52$^{GH}$ | 1.46 | 6.24$^{F}$ | 2.42 | 5.60$^{EF}$ | 2.83 | 5.96$^{G}$ | 2.79 | 5.72$^{CDE}$ | 3.23 | 5.36$^{CDEF}$ | 2.53 |
| 0.78 | 7.5 | 7.5 | 8.32$^{AB}$ | 1.32 | 8.50$^{ABC}$ | 1.06 | 7.98$^{AB}$ | 1.74 | 8.68$^{ABCD}$ | 1.33 | 2.65$^{GHIJ}$ | 3.32 | 2.21$^{HI}$ | 2.11 |
| 0.7 | 10 | 0 | 0.72$^{HI}$ | 0.80 | 7.45$^{BCDEF}$ | 2.20 | 7.18$^{ABCDE}$ | 2.44 | 8.00$^{ABCDEF}$ | 2.24 | 5.33$^{CDEF}$ | 3.53 | 5.29$^{CDEF}$ | 2.45 |
| 0.7 | 0 | 10 | 7.88$^{AB}$ | 1.59 | 2.70$^{GH}$ | 2.20 | 2.46$^{GH}$ | 2.66 | 1.10$^{H}$ | 1.41 | 5.65$^{CDE}$ | 3.47 | 5.84$^{BCDE}$ | 2.99 |
| 1 | 0 | 0 | 0.10$^{I}$ | 0.14 | 0.75$^{J}$ | 1.04 | 0.97$^{H}$ | 1.70 | 0.30$^{H}$ | 0.48 | 9.57$^{A}$ | 0.74 | 8.90$^{A}$ | 1.75 |
| 1 | 10 | 10 | 8.65$^{AB}$ | 1.38 | 9.11$^{A}$ | 0.92 | 8.04$^{AB}$ | 1.90 | 9.12$^{AB}$ | 1.05 | 2.48$^{GHIJ}$ | 2.95 | 2.24$^{HI}$ | 2.41 |
| 1 | 3.33 | 10 | 7.52$^{B}$ | 1.83 | 7.24$^{CDEF}$ | 2.00 | 6.79$^{BCDE}$ | 2.50 | 6.82$^{FG}$ | 2.77 | 3.97$^{EFG}$ | 3.57 | 3.78$^{FGH}$ | 2.52 |
| 1 | 10 | 3.33 | 3.31$^{EF}$ | 1.84 | 7.92$^{ABCDE}$ | 1.47 | 7.14$^{ABCDE}$ | 2.11 | 8.37$^{ABCDE}$ | 1.71 | 3.56$^{FGHI}$ | 2.94 | 3.31$^{GHI}$ | 2.45 |
| 1 | 5 | 5 | 3.84$^{DEF}$ | 2.42 | 7.16$^{CDEF}$ | 1.37 | 6.64$^{BCDE}$ | 1.88 | 7.52$^{CDEF}$ | 1.85 | 5.14$^{DEF}$ | 3.33 | 5.23$^{DEF}$ | 2.69 |
| 1 | 6.67 | 0 | 0.57$^{HI}$ | 0.84 | 6.42$^{EF}$ | 2.13 | 5.99$^{DE}$ | 2.49 | 6.84$^{FG}$ | 2.79 | 5.88$^{BCD}$ | 3.15 | 6.26$^{BCD}$ | 2.34 |
| 1 | 0 | 6.67 | 5.31$^{C}$ | 2.74 | 2.49$^{GHI}$ | 1.73 | 1.60$^{H}$ | 1.87 | 0.87$^{H}$ | 0.85 | 5.90$^{BCD}$ | 3.27 | 6.91$^{BCD}$ | 2.70 |
| **p-values (sample effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (subject effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (session effect)** | | | 0.077 | | 0.693 | | 0.390 | | 0.420 | | 0.112 | | 0.271 | |



*Table 2-7: Predictive equations generated for attributes. Fat² and Sugar² are quadratic terms in the model equation*

| **Significant model terms** | | | | | | | | **Model statistics** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Attribute** | **Intercept** | **Tea** | **Fat** | **Fat²** | **Sugar** | **Sugar²** | **Tea × Fat** | **$R^2$** | **Adj $R^2$** | **Pred $R^2$** | **Adeq Precision** |
| Thickness | 1.16 | | 1.52 | -0.1 | 0.23 | | | 0.96 | 0.95 | 0.94 | 36.65 |
| Mouth coating | 0.3 | | 2.05 | -0.13 | 0.14 | | | 0.96 | 0.95 | 0.94 | 33.21 |
| Slipperiness | 1.83 | -0.84 | 1.52 | -0.1 | 0.18 | | | 0.96 | 0.95 | 0.94 | 36.08 |
| Bitterness | 4.50 | 5.29 | -0.34 | 0.03 | -0.84 | 0.05 | -0.39 | 0.94 | 0.93 | 0.89 | 31.92 |
| Astringency | 4.19 | 5.09 | -0.05 | | -0.76 | 0.05 | -0.38 | 0.92 | 0.9 | 0.86 | 27.84 |
| Sweetness | 0.68 | -1.08 | 0.09 | | 0.85 | | | 0.97 | 0.97 | 0.96 | 42.94 |

$R^2$ – measure of variation in data set explained by the model
Adj $R^2$ – Values close to $R^2$ indicate there are only terms in the model which are significant and add value
Pred $R^2$ – Value should be close to Adj $R^2$ and explains variance in the model
Adeq Precision – measures signal to noise ratio. Values > 4 indicate adequate model discrimination

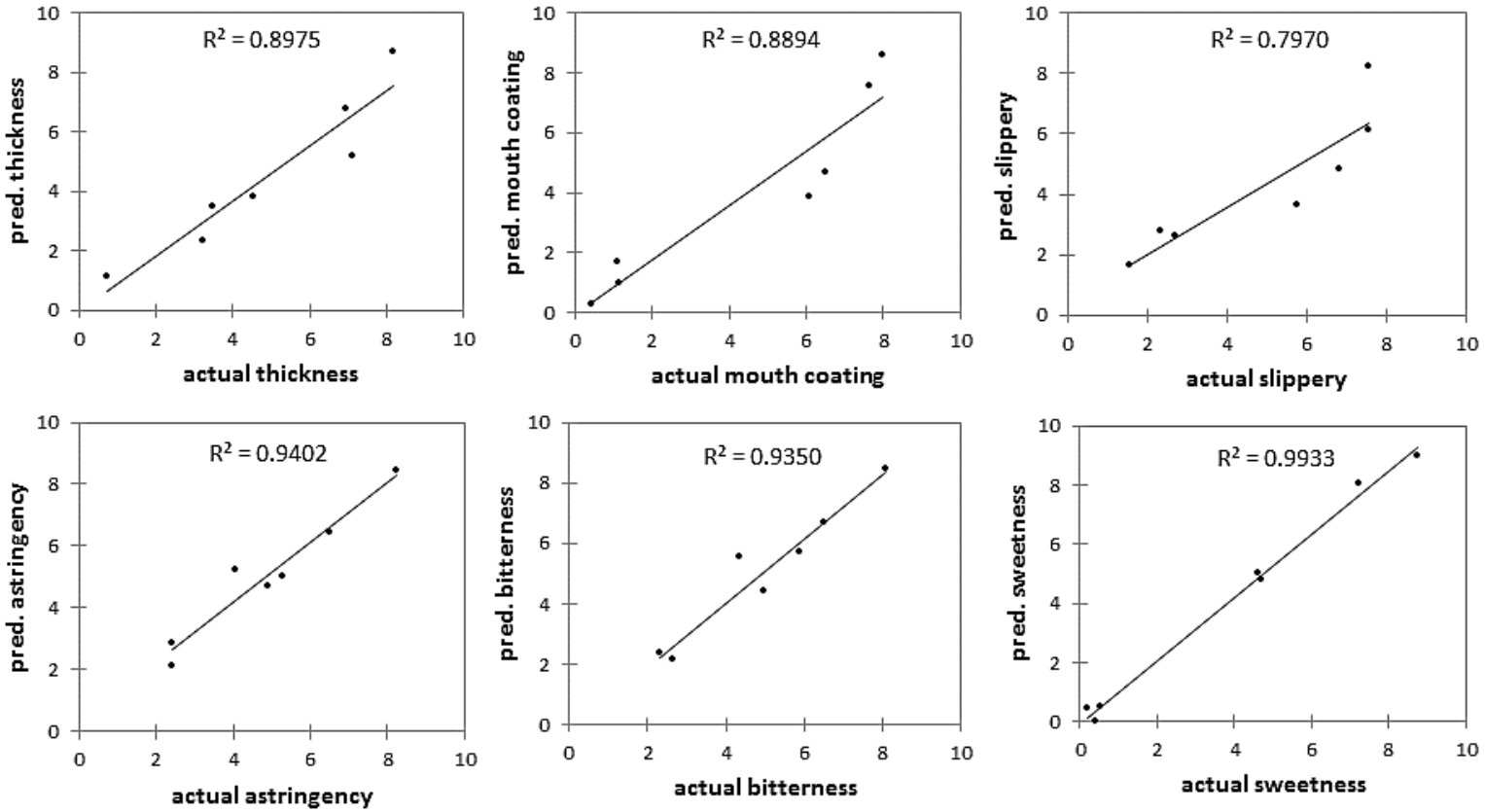



*Figure 2-3: Predicted vs actual scores for perceived mouthfeel and taste attributes for the 7 samples used for the model validation (see Table 2-2 to sample composition)*

### *2.3.4 Impact of Tea, Fat and Sugar on Mouthfeel and Taste Perception*

The interaction and contour plots illustrate the interaction between design factors and are presented in Figure 2-4 to Figure 2-8. These plots represent the predictive models, not data points from the data set. The equation in Table 2-7 shows that perceived thickness is not significantly influenced by tea content, since tea concentration is not included as a significant factor in the model. Therefore, the interaction plot in Figure 2-4A illustrates the relationship between fat and sugar. The major driver of perceived thickness in tea was fat, followed by sugar. The samples perceived to be the thickest contained a combination of high concentrations of fat and sugar. The perceived thickness reached a plateau at approximately 7 % fat, indicating that increasing the fat content further did not have a significant impact on thickness perception. This observed plateau appears to be independent of sugar addition. An impact of sugar on perceived thickness was also observed, since the addition of 10 % sugar increased the perceived thickness independent of fat addition. The perception of mouth coating increased with increasing fat content as shown in Figure 2-4B in a linear fashion, however a plateau was reached at approximately 7% fat. Sugar increased the perception of mouth coating and it appears that sugar has more impact at higher fat levels than at lower fat levels as shown in Figure 2-4B. Tea was not found to have a significant impact on the perception of mouth coating, shown also by the model equation where tea is not included as a significant factor (see Table 2-6).

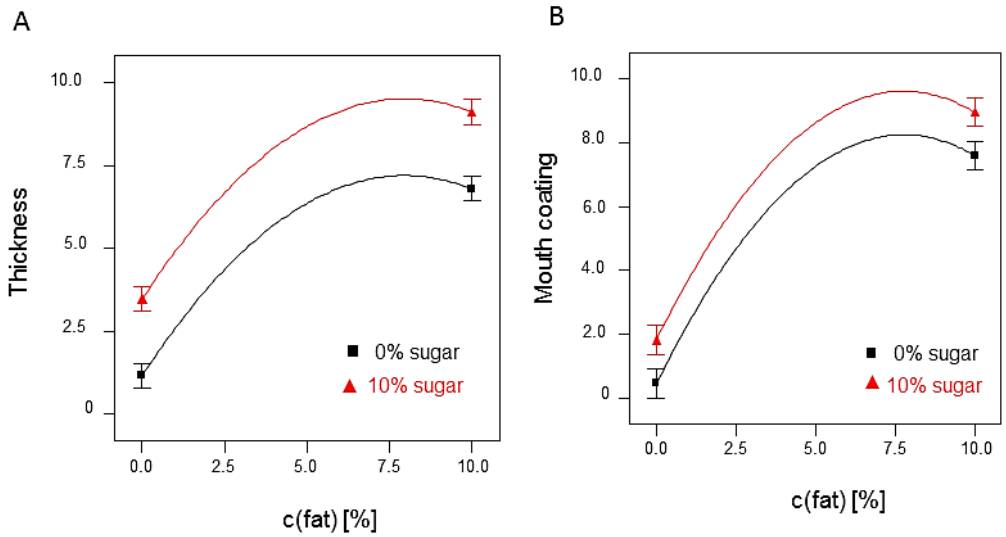



*Figure 2-4: Interaction plots for (A) perceived thickness and (B) mouth coating for varying fat contents and two sugar levels (0 and 10%). Error bars represent 95% least significant difference (LSD) intervals and points with not overlapping error bars are significant different.*

The effect of tea content on slipperiness is presented in Figure 2-5 and the equation can be found in Table 2-7. The displayed contour plots show two variables - fat and sugar concentrations on the x and y axis respectively for a) low (0.1%) and b) high tea levels (1.0%). The contour lines show the predicted sensory score on a 10-point scale for a specific combination of fat and sugar at a given tea content. The key driver for the perception of slipperiness was fat, with increasing fat concentration corresponding to increased slipperiness perception. This increase was almost linear until a fat concentration of around 5 % was reached. Further increases in fat content increased slipperiness, but at a slower rate until a plateau was eventually reached at around 7.5% fat. The effect of sugar on slipperiness was found to be smaller than that of fat, but sugar addition also increased perception. Comparing Figure 2-5A (low tea level) to Figure 2-5B (high tea level) it was found that high tea reduced slipperiness by one point on the sensory scale since the maximum score of slipperiness for low tea was 9 and 8 for high tea.

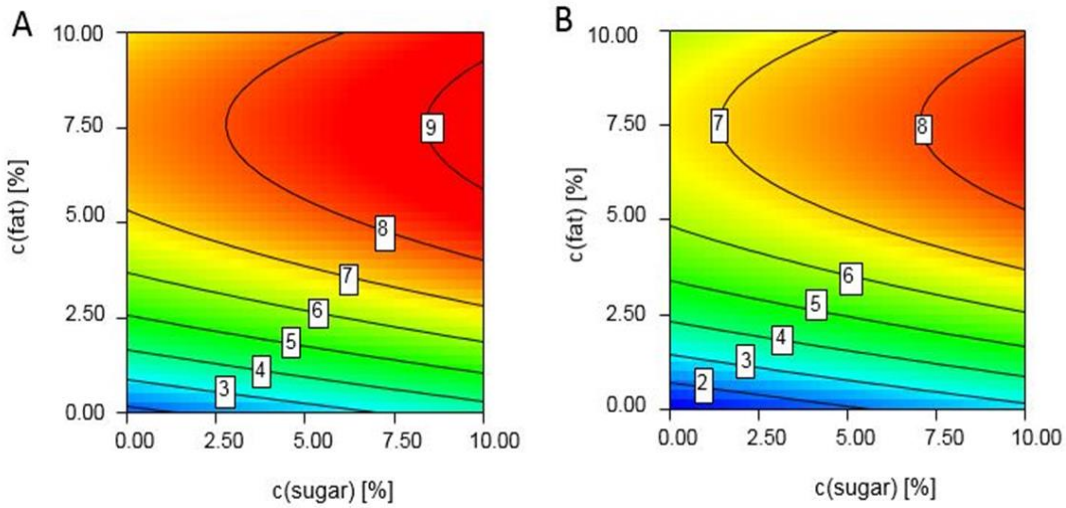



*Figure 2-5: Contour plots for perceived slipperiness in relation to concentrations of fat and sugar at (A) low tea level, (B) high tea level*

Figure 2-6 and Figure 2-7 show the interactions between sugar and fat on perceived bitterness and astringency at low (0.1% tea), medium (0.55% tea) and high (1% tea) tea levels. Firstly, for bitterness and astringency an increase in perception was observed with increasing tea level. The impact of fat and sugar on bitterness and astringency appear to be similar and both attributes are the only ones evaluated in this study where an interaction term was significant. The interaction term shows that the relationship between the impact of fat on astringency and bitterness was different at different tea levels. At a higher tea level, the addition of fat reduced astringency and bitterness to a greater extent than at lower tea levels. The addition of 10% sugar to low tea reduced bitterness and astringency more than the addition of 10% fat and the same was observed for a medium tea level. However, at high tea level, the addition of 10 % fat reduced the perceived bitterness to a greater extent compared to 10% sugar. The same trend was found for astringency, with 10% fat reducing astringency by approximately 4 points on the sensory scale while the same

level of sugar only reduced perceived astringency by 2.5 points on the sensory scale. Both bitterness and astringency are most effectively suppressed by a combination of high sugar and high fat levels; with this combination leading to significantly lower perception than the addition of either high levels of fat or sugar independently.

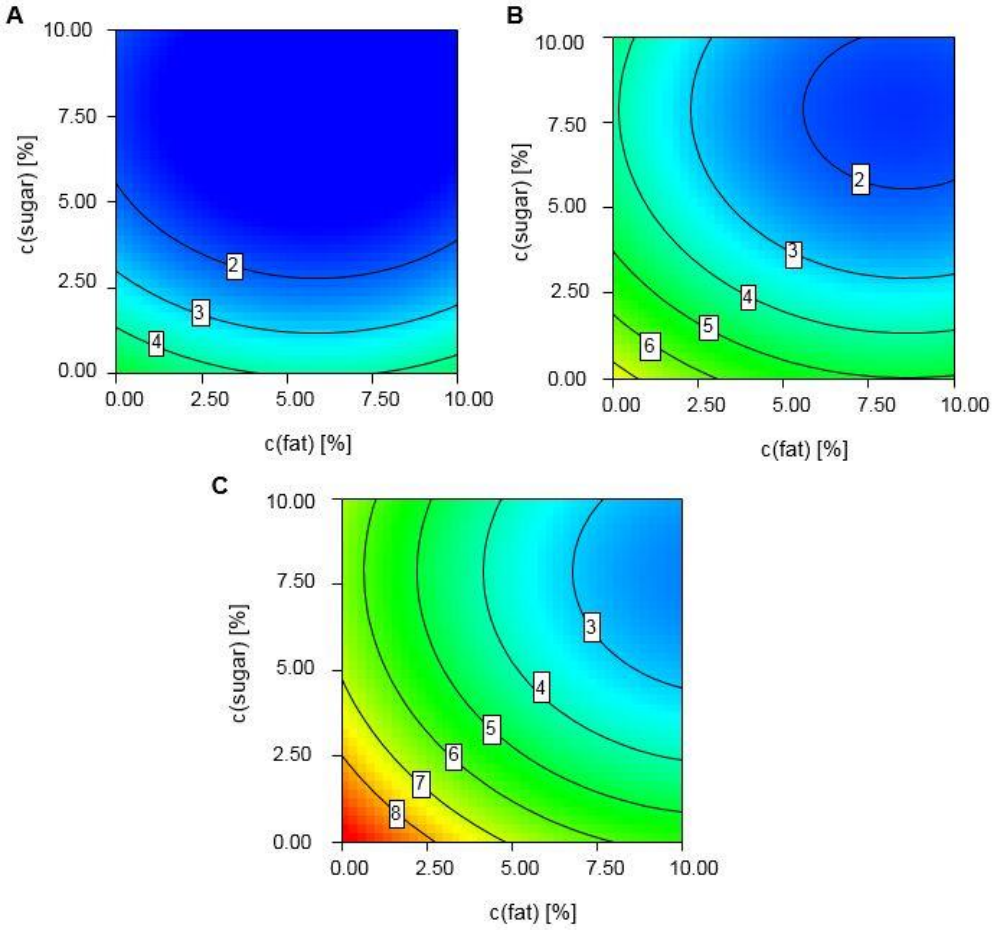



*Figure 2-6: Contour plots for perceived bitterness at different tea levels; (A) 0.1% tea, (B) 0.55% tea, (C) 1.0% tea*

Model equations for both bitterness and astringency (see Table 2-7) include similar factors with the only difference being that for bitterness "fat$^2$" was a significant factor whereas for astringency it was not, this explains the more curved contour lines in Figure 2-6. This difference results in slightly

different scores for astringency and bitterness depending on the sample composition.

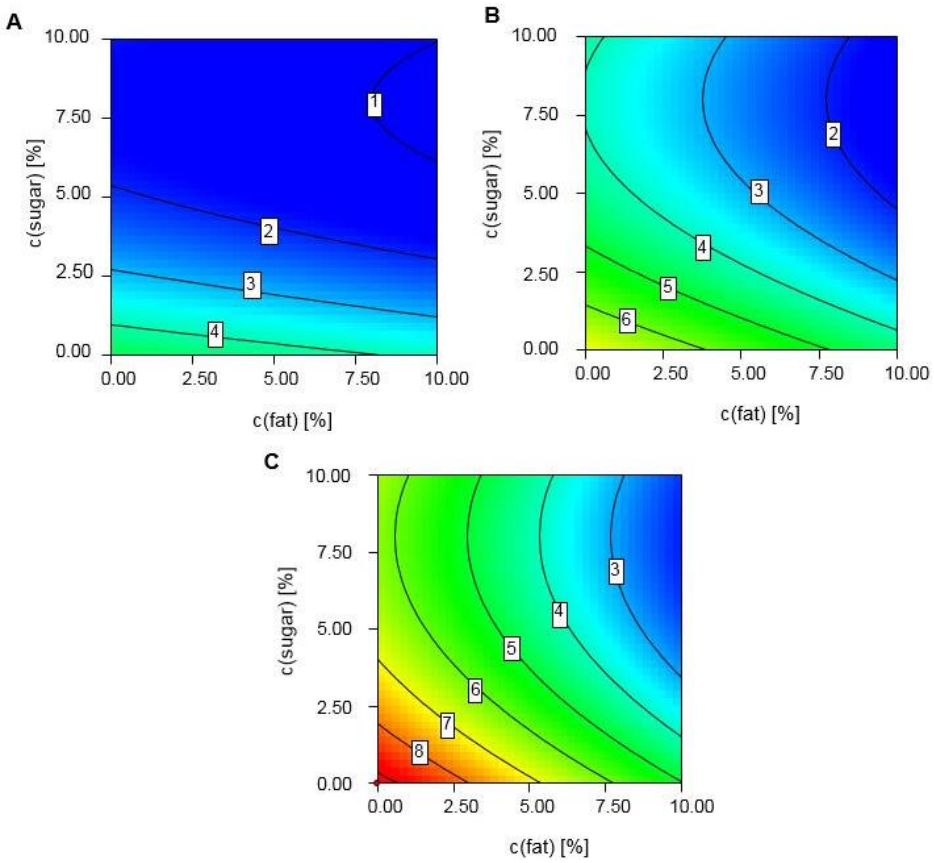



*Figure 2-7: Contour plots for perceived astringent at different tea levels; (A) 0.1% tea, (B) 0.55% tea, (C) 1.0% tea*

The effect of varying levels of tea, sugar and fat on perceived sweetness is presented in Figure 2-8 as contour plots. Not surprisingly, with increasing sugar content the perceived sweetness increases almost linearly; but sweetness did not only depend on sugar content. The concentration of tea and fat also affected perceived sweetness. With increasing tea concentration, the perceived sweetness was reduced by approximately 1 point on the sensory scale as shown when comparing the maximum score in Figure 2-8A with Figure 2-8B. Increasing fat concentration enhanced

sweetness in this model system. For example, at a constant sugar content of 5%, the impact of increasing fat content (up to 10%) increased perceived sweetness by almost 1 point on the sensory scale.

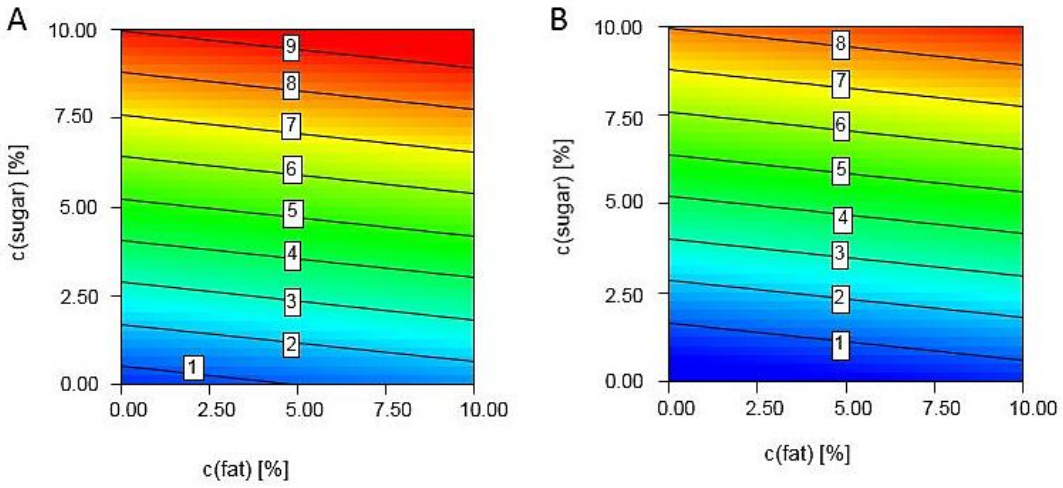



*Figure 2-8: Contour plots for perceived sweetness at different tea levels, (A) 0.1% tea (low tea) and (B) 1% tea (high tea)*

### *2.3.5 Relationships between Mouthfeel and Taste Attributes*

A principal component analysis (PCA) was performed to visualise correlations between attributes. Design factors were overlaid as supplementary variables. The PCA in Figure 2-9 shows that astringency and bitterness are highly positively correlated and negatively correlated to sweetness. It was also found that tea concentration is somewhat positively correlated to astringency and bitterness. However, the correlation was weak with R = 0.479 for tea and astringency and R = 0.472 for tea and bitterness. As expected, sugar concentration and sweetness perception were highly correlated (R = 0.975). The remaining mouthfeel attributes thickness, lubrication and mouth coating were positively correlated which each other and fat content was found to impact these attributes most.

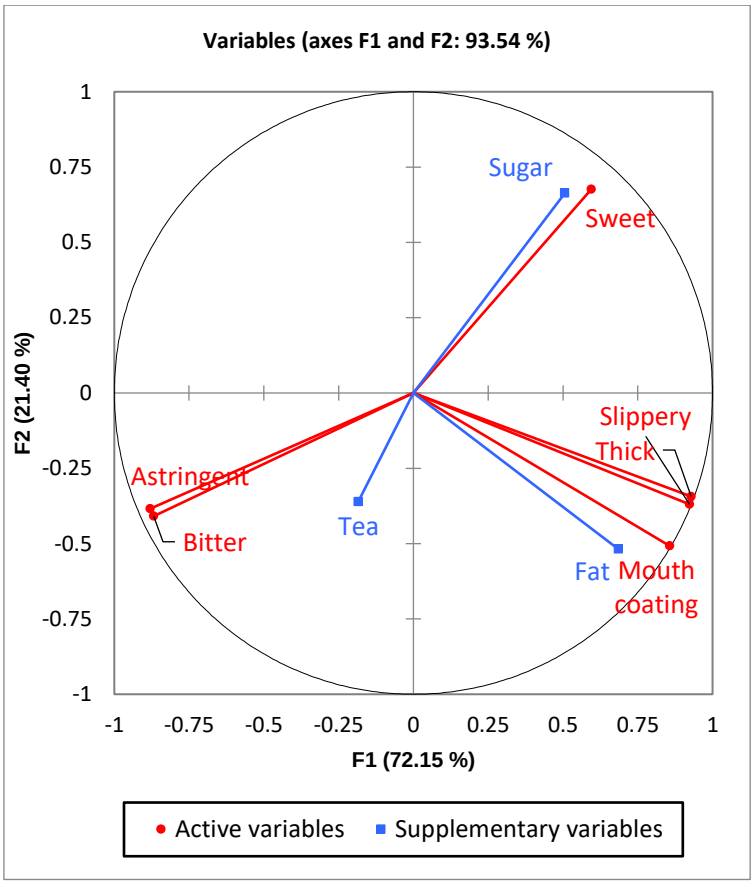



*Figure 2-9: PCA plot with attributes as active variables and design factors as supplementary variables. The PCA illustrates which attributes are related and by which ingredient they are driven.*

### *2.3.6 Relationship between measured Viscosity and perceived Thickness*

The viscosity of the tea sample which were evaluated by the panel was measured (see details in section 2.2.3) and the data is shown in Table 2-8. Using this data, a predictive polynomial model was calculated, and it was found that all three design factors (tea, fat, sugar) were included in the model and shown to significantly contribute to viscosity. The model equation can be found in equation 3 and the model had an $R^2$ of 0.99 (Adj. $R^2$ = 0.99, Pred. $R^2$ = 0.98, Adeq Prec = 64.79), proving its accuracy and validity.

*Table 2-8: Viscosity of tea samples measured at 40ºC and 50 $s^{-1}$ using a MCR301 rheometer with a double gap cylinder geometry.*

| Sample | Composition | | | Viscosity at 50 $s^{-1}$ [mPa·s] |
|---|---|---|---|---|
| | Tea [%] | Fat [%] | Sugar [%] | |
| 1 | 0.1 | 10 | 0 | 1.11 |
| 2* | 0.1 | 0 | 10 | 0.88 |
| 3* | 0.1 | 0 | 10 | 0.86 |
| 4 | 0.1 | 6.67 | 10 | 1.24 |
| 5 | 0.1 | 0 | 3.33 | 0.70 |
| 6* | 0.1 | 5 | 0 | 0.83 |
| 7* | 0.1 | 5 | 0 | 0.81 |
| 8 | 0.1 | 10 | 6.67 | 1.37 |
| 9 | 0.33 | 7.5 | 2.5 | 1.00 |
| 10 | 0.33 | 2.5 | 5 | 0.82 |
| 11 | 0.4 | 3.33 | 10 | 0.66 |
| 12* | 0.4 | 0 | 0 | 1.02 |
| 13* | 0.4 | 0 | 0 | 0.71 |
| 14 | 0.55 | 10 | 10 | 1.42 |
| 15* | 0.55 | 5 | 5 | 0.92 |
| 16* | 0.55 | 5 | 5 | 0.94 |
| 17* | 0.55 | 5 | 5 | 0.97 |
| 18 | 0.78 | 2.5 | 2.5 | 0.79 |
| 19 | 0.78 | 7.5 | 7.5 | 1.14 |
| 20 | 0.7 | 10 | 0 | 1.01 |
| 21 | 0.7 | 0 | 10 | 0.93 |
| 22 | 1 | 0 | 0 | 0.70 |
| 23 | 1 | 10 | 10 | 1.46 |
| 24 | 1 | 3.33 | 10 | 1.03 |
| 25 | 1 | 10 | 3.33 | 1.12 |
| 26 | 1 | 5 | 5 | 0.95 |
| 27 | 1 | 6.67 | 0 | 0.86 |
| 28 | 1 | 0 | 6.67 | 0.81 |

$$\textbf{\textit{Viscosity}} = 0.653 + 0.046 \cdot tea + 0.021 \cdot sugar + 0.019 \cdot fat - 0.016 \cdot tea \cdot fat + 0.002 \cdot sugar \cdot fat + 0.003 \cdot fat^2 \quad (3)$$

To further understand how perceived thickness and measured viscosity are correlated, Figure 2-10 displays the relationship between both factors. A trendline was fitted through the data and the highest correlation between perceived thickness and measured viscosity was obtained when a quadratic function was fitted ($R^2$ = 0.735), whereas with a linear function an $R^2$ of

0.592 was obtained (data not shown). The figure also shows that with increasing measured viscosity, perceived thickness increases in a linear manner until a viscosity of approximately 1 mPa·s. At a higher viscosity the perceived thickness of the tea samples increased in a slower manner, resulting in an almost plateau-like shape of the curve, which is best explained through a quadratic function.

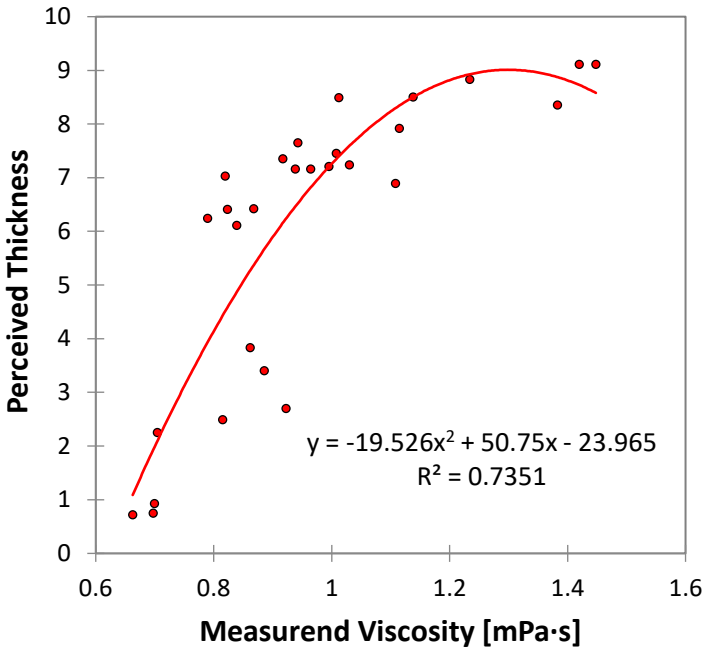



*Figure 2-10: Correlation between perceived thickness and measured viscosity. Data from perceived thickness can be found in Table 2-6 and the data for measured viscosity is shown in Table 2-8.*

## 2.4 Discussion

### *2.4.1 Mouthfeel Lexicon of Tea Beverages*

This research successfully developed a novel mouthfeel lexicon which describes and defines the mouthfeel characteristics of black tea beverages. Basic tastes, bitterness and sweetness, were included to avoid attribute dumping and furthermore it was of interest to understand the relationship between bitterness and astringency as well as the effect of other mouthfeel

attributes on taste perception. The attributes "thickness" and "slipperiness" referred to mouthfeel perception during tea consumption, whereas "astringency" and "mouth coating" described the after-feel of the tea. The number of attributes included in the lexicon are in-line with the number of attributes identified by other researchers of milk beverages (Adhikari et al., 2010; N`Kouka et al., 2004). During panel training other attributes such as "smooth" and "mouth-watering" were discussed but found not to discriminate between the samples set and were consequently removed. However, it is possible that these mouthfeel attributes could be relevant to a different sample set.

### *2.4.2 Perceived Thickness of Tea in Relation to Fat and Sugar Content*

The perceived thickness of the model system was driven by fat and sugar concentration, with fat having the greater impact. At low to medium fat contents (0 – 5 %), the perceived thickness increased linearly and then reached a plateau at approximately 7 % fat. This may be a perception threshold, meaning that at higher levels of fat, a greater viscosity increase is required to cause a perceivable increase in thickness. This was confirmed by plotting perceived thickness against measured viscosity as shown in Figure 2-10 which showed that a higher viscosity, perceived thickness increased in a slower fashion. This phenomenon is well-known and early work by Kokini (1987) gave the simple rule of thumb that a four-fold change in viscosity results in only a two-fold change in sensory thickness which could explain the plateau observed in this study.

Tea was not found to have a significant impact on perceived thickness suggesting that an increase in tea powder which is associated with tea flavour and other compositional factors, for example polyphenols, does not contribute to perceived thickness. However, it was observed that tea concentration significantly ($p<0.0001$) contributed to measured viscosity. All the samples within the model system design space were characterised by Newtonian flow behaviour, and there was a measurable difference in viscosity for different tea concentrations with 0.1% tea having a viscosity of 0.66 mPa·s and 1% tea having a viscosity of 0.70 mPa·s. The results suggest that these differences were not at a magnitude that could be perceived by the panel, which is reflected by the model equation for perceived thickness.

### *2.4.3 Perceived Mouth coating in Relation to Fat and Sugar Content*

Mouth coating was defined as the extent of layer of sample remaining in the mouth after swallowing. The key driver for this attribute was fat and it is therefore likely that a layer of fat droplets is formed on the oral cavity after swallowing. In the absence of sugar, and with increasing fat concentration, the perception of mouth coating increased linearly until 7% fat where it reached a plateau. This observation is in line with finding of Vingerhoeds et al. (2008) who investigated the effects of emulsion composition and structure on sensory perception of low-viscosity emulsions. They observed that the after-feel "coating", defined as fatty coating on tongue, lips or cheeks, increased significantly between 0 and 5% sunflower oil, but the increase from 5 to 10 % oil was not significant. In fact, there was no significant difference between 5, 10 and 20% oil, only

the highest fat content, 40%, was perceived to be significantly more mouth coating. The exact mechanism of how this layer in the oral cavity is formed and how it is structured remains unclear. Pivk et al. (2008) assessed the lipid layer using a mouth rinse method and fluorescence. They were able to quantify the lipids and measure the thickness of the layer in mouth. They hypothesized that the amount of lipid deposited on the oral surface is related to mouthfeel perception. This seems a plausible explanation assuming that mouth coating is caused by oil droplets covering the whole oral cavity. Once the whole oral cavity is covered, possibly by several layers of oil droplets, it could be assumed that the addition of more oil droplets, forming further layers will only slightly increase the perception of mouth coating. The knowledge that mouth coating perception reaches a plateau can be used to create products which contain less fat but are still perceived to be mouth coating, if this is found to be a desirable attribute.

### *2.4.4 Perceived Slipperiness in Relation to Fat, Sugar and Tea Content*

In early research by Kokini et al. (1977) it was shown that "thickness", "smoothness" and "slipperiness" are key descriptors of the mouthfeel of liquids and that slipperiness involves a combination of viscous and frictional forces. In the present study, the panel defined "slippery" as the lack of friction when the tongue moves across the roof of the mouth during consumption, and fat level affected slipperiness the most, possibly caused by the lubrication properties of the emulsion, reducing the friction in mouth. At a low tea level, fat was able to reduce the friction more effectively than at a high tea level, where it may be the increase in

polyphenols contributing to a higher oral friction, leading to a lower perception of slipperiness.

### *2.4.5 Perceived Bitter and Sweet Taste and the Effect of Fat*

Bitterness and sweetness are known to mutually suppress each other and the mixture of a bitter and a sweet stimuli is perceived as less sweet or bitter than the sum of the individual taste intensities (Kroeze & Bartoshuk, 1985). This was observed in this research, where for example, the highest tea concentration with a bitterness score of 9 on a 10-point scale, was significantly reduced by the presence of sugar. This interaction is mainly caused by a central cognitive effect, known as mixture suppression (Keast & Breslin, 2002). In the presence of high levels of fat, perceived sweetness increased. The effect of fat on the perception of sweetness has been studied by Hollowood et al. (2007) who found that an increase in oil increased the perceived sweetness in their oil-in-water emulsions, which is in-line with findings in this study. An increase in oil content reduces the aqueous phase and consequently increases the sugar concentration in the aqueous phase, meaning more tastant is available to be transported to taste receptors (Hollowood et al., 2007).

Both fat and sugar reduced the perceived bitterness of the model system with fat showing a greater impact on bitterness reduction than sugar at the highest tea level. Fat and sugar also increased the viscosity of the model system. An increase in viscosity can reduce the mass transport of tastants resulting in less tastant reaching the receptors (Hollowood et al., 2007). However, a study by Pripp et al. (2004), which investigated the effect of

viscosity on bitterness of olive oil found that viscosity had very little impact (Pripp et al., 2004). A possible mechanism for bitterness suppression with increasing fat could involve the interference of fat droplets with the transport of polyphenols on the tongue, consequently leading to less bitter tastants reaching the taste receptors. Furthermore, the emulsifier used in this research (sodium caseinate) might be involved in suppressing bitterness as it is well known that casein and polyphenols interact (Hasni et al., 2011). A reduced availability of polyphenols due to their binding with sodium caseinate could lead to reduced bitterness and astringency perception. Although, bitterness and astringency are two different attributes with bitterness being a basic taste and astringency being a tactile perception, both attributes are closely related for tea beverages. Most tea polyphenols are reportedly perceived as not only astringent but also bitter (Lee & Lawless, 1991) and increasing the tea content also increased perceived bitterness and astringency in this study. Bitterness and astringency have been referred to as a "twin sensation" (Lea & Arnold, 1978) and both are often confused sensorially. This research showed that bitterness and astringency are affected by the addition of fat and sugar in a similar way, adding to evidence that they are closely related. However, during QDA, the perception of an attribute is rated at one time point. Bitterness and astringency are both known to be perceived differently over time (Lee & Lawless, 1991; Peleg et al., 1999). It is possible that the impact of tea, fat and sugar on astringency and bitterness perception when evaluated using temporal methods is different to when evaluated at one time point.

### *2.4.6 Perceived Astringency in Tea as influenced by Sugar and Fat*

The perception of astringency on the tongue caused by tea polyphenols is well-documented in the literature (Bate-Smith, 1954; Rossetti et al., 2009; Wismer & Goonewardene, 2004). It is therefore not surprising that increasing tea level increased the perception of astringency. Here, sugar reduced the perceived astringency of tea significantly. The total polyphenol content of the instant black tea powder used was 192 mg Gallic acid equivalent (GAE)/g dry matter, which gives a total of 1920 mg GAE/L for a 1 w/w % tea solution. To reduce the perceived astringency (e.g. from a sensory score of 9 to 7) 41 g/L sucrose was required. This translates to approximately 2 teaspoons of sugar in a cup of tea (180 ml) using this specific black instant tea powder. This is comparable with findings from Ishikawa and Noble (1995), who determined that a sucrose concentration greater than 50 g/L was necessary to significantly suppress the astringency of red wine which had a total polyphenol content of 780 and 1890 mg GAE/L. There are several possibilities as to the potential mechanism of astringency reduction via sugar addition in tea. Firstly, the decrease in perceived astringency could be due to a cognitive process where the sweetness of the sugar masks the astringency, which is then perceived to be lower than without sugar. Furthermore, hydrophilic sucrose could interfere with the hydrogen bonding between polyphenols and proteins in saliva and thereby reduce astringency (Ishikawa & Noble, 1995). The addition of sugar also increases the measured viscosity of the tea sample and it was shown by Peleg and Noble (1999) that an increase in measured viscosity leads to a decrease in astringency perception. However, at this

point it is not possible to conclude which mechanism reduces astringency perception.

Fat also reduced the perception of astringency; the opponency of fat and astringent sensation is well known (des Gachons et al., 2012). However, little is known about the mechanism behind this suppression. The addition of an emulsion to the tea led to an increase in viscosity which could also lead to an increase in oral lubrication. Furthermore, it is also possible that the layer of fat droplets is the significant factor by increasing oral lubrication or disturbing the interaction between polyphenols and salivary proteins. Results of this study showed that the perception of astringency and bitterness in tea is significantly affected by the addition of sugar and fat. Therefore, a reduction of fat and sugar for health reasons would increase bitterness and astringency perception which might have a negative impact on consumer liking. To investigate this further, temporal methods could be used to explore perception over time and gain insight into the mechanism of how astringency and bitterness are suppressed by fat and sugar and why fat is more effective than sugar for this purpose.

### *2.4.7 Relationship between Attributes and Impact of Ingredients*

It was found that bitterness and astringency are highly correlated, which is expected as both have been named "twin attributes" (Lea & Arnold, 1978) and astringent foods are usually also perceived to be bitter. However, it is interesting to note that the correlation between bitterness or astringency and tea concentration is only weak, R = 0.472 and 0.479, respectively. This shows that bitterness and astringency perception was

not solely linked to tea concentration. It might be more appropriate to correlate bitterness and astringency perception to the concentration of certain tea polyphenols.

Slipperiness, thickness and mouth coating are positively correlated to each other and also to fat concentration, which is expected as fat is the key factor for the perception of these three attributes. Furthermore, slipperiness, which was defined as the lack of friction when moving the tongue across the palate is not strongly negatively correlated to astringency, which is defined as the sensation of a dry tongue. This shows that both attributes do actually describe different mouthfeel sensations and not just the opposite of the same sensation as for example thick and thin. Sweetness and sugar concentration are highly correlated and negatively correlated to bitterness and astringency attributes. This emphasises the antagonistic perception of sweetness/bitterness and sweetness/ astringency.

## 2.5 Conclusions

For the first time, a specific lexicon of the mouthfeel of black tea beverages was developed. Four key mouthfeel attributes were defined for black tea and evaluation protocols were set up. Besides mouthfeel, the perception of sweetness and bitterness was also evaluated with an aim of decoupling the complex relationship between bitterness and astringency and to avoid attribute dumping during sensory assessment. However, the impact of each component on bitterness and astringency perception produced similar predictive models when using a descriptive profiling technique. Therefore, further investigations using temporal methods to decouple these attributes

further and provide insightful detail regarding the impact of components on mouthfeel perception is required.

A mouthfeel lexicon for black tea beverages is an important step for the global tea industry to enable the same language to be used when describing black tea beverages. The corresponding impact of different concentrations of fat and sugar at low, medium and high tea concentrations showed that the perception of mouthfeel is complex and significantly affected by the various factors investigated. Tea concentration was found to have a significant impact on perceived astringency, slipperiness, bitterness and sweetness but not on mouth coating and thickness. Increasing tea concentration led to increased bitterness and astringency and caused a slight decrease in perceived sweetness and slipperiness. The addition of fat to tea increased the perception of slipperiness, mouth coating and thickness, but decreased perceived astringency and bitterness. Sugar had the greatest impact on sweetness but also suppressed bitterness and astringency, and enhanced thickness, slipperiness and mouth coating. A better understanding of mouthfeel and the impact of ingredients on key mouthfeel attributes is of importance since consumers are keen to consume healthier products and but not willing to compromise on sensory enjoyment. Understanding the mechanism behind bitterness and astringency reduction due to fat and sugar will help to create products which are less bitter or astringent but also healthy. This research represents one of many steps required to further our understanding of the mouthfeel of tea beverages.

# 3 Multiple-sip temporal Character of Astringency and Bitterness in Black Tea Beverages

## 3.1 Introduction

Tea (*Camellia sinensis*) is a popular beverage, which, after water, is the second most consumed drink in the world (Sharma et al., 2008). Black tea is commonly drunk in the United Kingdom, with tea drinkers consuming up to 3 – 4 cups per day (Ryan & Petit, 2010). Black tea is rich in polyphenols which contribute to its bitterness and astringency (Thorngate III & Noble, 1995). Both sensations are commonly found in the same foods and have been previously coined "twin sensations" (Lea & Arnold, 1978). Bitterness is a basic taste and is perceived through taste receptor cells in the taste buds on the tongue (Ma et al., 2014). Astringency is thought to be perceived as a consequence of the precipitation of salivary proteins in the presence of astringent compounds (e.g. polyphenols). This precipitation causes an increase in oral friction, which is associated with an increase in roughness/astringency within the oral cavity.

Cultural habits impact the way black tea is consumed in different countries; in Ireland, Canada, India and the United Kingdom milk is usually added, whereas in Japan and China tea is mostly consumed without milk (Bandyopadhyay et al., 2012; Sharma et al., 2008). It is also known that some countries consumed their tea with cream or use a creamer (dairy or non-dairy versions available). Milk is known to decrease the perception of astringency (Ares et al., 2009) by one or several of the following mechanisms: 1) fat droplets might interfere with the formation of protein-

polyphenol precipitates; 2) fat droplets form a layer between tongue and palate to reduce friction; 3) caseins (milk proteins) are known to bind polyphenols (Hasni et al., 2011) which could therefore contribute to a reduced astringency perception. In addition to milk, one-third of British tea drinkers like their tea with sugar (UK Tea a Association, 2017). Sugar and other sweeteners are well known to suppress bitterness (Ley, 2008) via a taste-taste interaction which has been studied extensively (Ares et al., 2009; Kroeze & Bartoshuk, 1985; Roy, 1992) and has been shown to be due to a central cognitive effect (Keast, 2008; Keast & Breslin, 2002). Studies have also shown that sugar reduces astringency (Ishikawa & Noble, 1995). The mechanism of which is not fully understood but could be due to a cross modal interaction caused by the increase of perceived sweetness, an interference of hydrophilic sucrose with hydrogen bonding between polyphenols and proteins in the saliva or an increase in oral lubrication through the viscosity of sucrose (depending on the amount of sugar added) (Ishikawa & Noble, 1995). In order to understand how astringency perception of black tea is modified due to sugar addition, both sweetness and viscosity need to be considered individually.

Both, bitterness and astringency show a time-dependent behaviour and it is well known that perceived astringency intensity builds up slowly after consumption and persists for a long duration (Bajec & Pickering, 2008a). Therefore, temporal sensory methods such as Time Intensity (TI) can be used to measure the time-dependant character of attributes and multi-sip protocols can be used to capture perceived astringency and bitterness during consumption. In the study presented here, astringency and bitterness perception was measured using a multi-sip TI method, where

the change in intensity of a given sensory attribute is recorded over time. The intensity is recorded together with the corresponding time coordinates and this relationship is displayed in the form of a TI-curve (Liu & MacFie, 1990).

The temporal perception of astringency and bitterness in beverages has been studied by different researchers and different observations have been made (Ishikawa & Noble, 1995; Lee & Lawless, 1991; Robichaud & Noble, 1990; A. K. Smith et al., 1996; Valentová et al., 2002). Ishikawa and Noble (1995) found that astringency in red wine increased in a linear manner until a maximum intensity was reached at approximately 15 s after consumption. After a short plateau astringency perception starts to decrease and disappears completely after 50 s. Previous studies have used a trained panel to decouple astringency and bitterness perception and found that the time to reach maximum intensity was longer for bitterness (18.3 s) than for astringency (16.0 s) which indicates that both attributes might be perceived through different mechanisms (Robichaud & Noble, 1990). However, in a study by Peleg et al. (1999), the time to reach the maximum intensity did not vary between bitterness and astringency. Currently, the temporal perception of bitterness and astringency for tea has not been studied in great detail, although individual tea polyphenols were investigated (Peleg et al., 1999) and none have captured build-up over multiple sips.

In the past decade, texture and mouthfeel perception have become of great interest to food researchers as it has been recognised that texture and mouthfeel can impact on the liking of food and beverages (Guinard & Mazzucchelli, 1996). Astringency and bitterness can impact liking

negatively, however at the right level they also contribute to the complexity and enjoyment of beverages and foods (Ares et al., 2009; Drewnowski, 2001); therefore a better understanding of astringency and bitterness perception in key beverages such as tea is necessary.

The aim of this study was 1) to train a panel to decouple astringency and bitterness in black tea beverages and rate each attribute using TI method; 2) to investigate the temporal profile of astringency and bitterness across different tea strengths and for individual sips; 3) to understand the impact of key ingredients on the temporal profile of astringency and bitterness; and 4) to decouple sweetness and viscosity. In order to investigate the impact of key ingredients on astringency and bitterness perception, a tea model system consisting of tea, sugar and fat was used.

## 3.2 Materials and Methods

### *3.2.1 Tea Model System and Sample Preparation*

The sample set chosen represented a factorial design space with three variables (tea, fat and sugar). Three tea levels (0.1 % (low), 0.55 % (medium), and 1.0 % (high)) were achieved by dissolving appropriate amounts of instant black tea powder in water and sugar (sucrose) and/or fat were added at a level of 5 %. To achieve a more robust design an experimental replicate was included (0.55 % tea, 5 % fat, 5 % sugar). Fat was added to the system in the form of an oil-in-water emulsion. The oil-in-water emulsion was processed as described in Chapter 2 section 2.2.2. The emulsion was stored for a maximum of 5 days at 4 °C before it was used for sensory evaluation.

Samples were prepared by dissolving the relevant amount of tea powder in hot distilled water (70 °C). For samples with sugar, sucrose (Tate & Lyle, UK) was added while the tea was still hot to ensure complete dissolution. The emulsion was added to the tea after cooling to a temperature of approximately 50 °C.

*3.2.1.1 Decoupling the Impact of Sweetness and Viscosity*

In order to understand the impact of sweetness (independent of viscosity) on bitterness and astringency perception, various samples were prepared using high intensity sweeteners to produce a similar sensorial perception as the addition of 5 % sucrose. Preliminary bench testing showed a mixture of sweeteners (cyclamate, acesulfame K, saccharin) gave a similar sensorial profile to 5 % sucrose. Different concentrations of these sweeteners (cyclamate, acesulfame K, saccharin) were prepared and added to medium tea (0.55 %). These samples were compared against medium tea with 5% sucrose sample by 10 subjects following the "A, not A" method (Kemp et al., 2009). The evaluated samples and their composition can be found in Table 3-1. Before the test, subjects were presented with the example of "A" and "not A" and asked to familiarise themselves with both samples. 0.55% tea with 5% sucrose and the three potential matches were assessed by the subjects in duplicates.

*Table 3-1: Samples evaluated following the "A, not A" method by 10 subjects.*

| Sample | Tea [g/l] | Sucrose [g/l] | Cyclamate [g/l] | Acesulfame K [g/l] | Saccharin [g/l] |
|---|---|---|---|---|---|
| "A" | 5.5 | 50 | | | |
| "not A" | 5.5 | | 0.2 | 0.2 | |
| 1 | 5.5 | | 0.1 | 0.15 | 0.05 |
| 2 | 5.5 | | 0.1 | 0.1 | 0.05 |
| 3 | 5.5 | | 0.05 | 0.1 | 0.025 |

The impact of viscosity increase through the addition of 5% sucrose was mimicked with the addition of a thickener. Low-molecular Dextran (Meito Sangyo Co., Japan), was chosen because it shows a Newtonian-like flow behaviour. The quantity of Dextran added was determined by measuring physical viscosity at 40 °C and a shear rate of 50 $s^{-1}$ using a rotational rheometer (MCR 301, Anton Paar) and once physical viscosity was matched, perceived thickness was checked using a rank-rating method with 9 panellists.

### *3.2.2 Sensory Evaluation*

Table 3-2 shows the composition of the samples evaluated by the sensory panel. Tea samples were freshly prepared 1 h in advance of each sensory session. Each sample was served in volumes of 20 ml in 30 ml amber glass vials. The vials were then placed in water baths for 15 min to achieve a serving temperature of 50±4 °C prior to consumption. 40 ml of each sample were consumed during one evaluation session.

*Table 3-2: Composition of samples evaluated by the panel (n=9) for temporal astringency and bitterness perception using a multi-sip TI method. * -an experimental replicate*

| | Tea [%] | Fat [%] | Sugar [%] |
|---|---|---|---|
| **Low Tea** | 0.1 | 0 | 0 |
| | 0.1 | 5 | 0 |
| | 0.1 | 0 | 5 |
| | 0.1 | 5 | 5 |
| **Medium Tea** | 0.55 | 0 | 0 |
| | 0.55 | 5 | 0 |
| | 0.55 | 5 | 5 |
| | 0.55 | 5 | 5 |
| | 0.55* | 5 | 5 |
| | 0.55 | 1.2% dextran | |
| | 0.55 | mixture of sweeteners | |
| **High Tea** | 1.0 | 0 | 0 |
| | 1.0 | 5 | 0 |
| | 1.0 | 0 | 5 |
| | 1.0 | 5 | 5 |

Nine panellists with prior experience of sensory assessments and who attended the sensory study described in Chapter 2 participated in this study. This study was granted ethical approval by the Medical Ethics Committee of University of Nottingham (I12072016 SoBC SSC). All panellists gave written and informed consent to participate and were offered a disturbance allowance for their participation. Data was collected using Compusense Cloud (Compusense, Canada) and the study was performed at room temperature (20 ± 2 °C) under Northern Hemisphere daylight in facilities according to ISO 8589:2007.

Astringency and bitterness were evaluated in separate sessions with astringency being evaluated first. Evaluating the attributes separately helped the panel to focus on the attribute of interest. Before the first evaluation of astringency, the panel attended 3 x 2 h training sessions where they were familiarised with: the test protocol, the definition of astringency and the use of the 10-point, unstructured scale, where 0 = low and 10 = high. After training, the panel evaluated a number of samples and their performance was evaluated. Further training was provided where necessary and final performance checks were made before the panel assessed the final sample set in quadruplicate, resulting in 60 evaluations. Samples were presented in two blocks in order to assist with sample preparation and samples in each block were presented in a randomised order. Furthermore, to help the panel in the usage of the scale, they were given a medium tea at the beginning of each session and told that it represented the middle of the scale for the respective attribute as confirmed previously in the training sessions. A similar process was followed for bitterness evaluation.

The evaluation protocol was set up in the preliminary phase of the experiment, which revealed that 4 sips of tea were sufficient to show a modulation in astringency and bitterness perception within a sample. A schematic representation of the multi-sip TI protocol can be found in Figure 3-1. The panellists received one 30-ml vial containing 20 ml sample at the beginning of the evaluation (time point = 0 min). Panellists were instructed to take one sip and hold the tea sample in the mouth for 5 s prior to swallowing (a prompt to swallow was given via instructions on the screen). The panellists started to rate the perceived intensity for the respective attribute for 60 s. The panellists were then instructed to consume the second sip from the first vial one minute after the first sip. Sips 3 and 4 were consumed 2 min and 3 min respectively after the start of the evaluation, and were served in a new vial. This was necessary to ensure a consistent serving temperature of 50±4 °C. The second vial was served with the lid open, as to avoid interrupting the panellists during the evaluation. Astringency and bitterness were constantly rated over a total of 5 min and a break of 7 min was given between samples to ensure a complete recovery of the mouth/palate. Crackers (Rakusen`s Limited, UK), honeydew melon (sourced from a local supermarket), and water (Edivan, Danone, France) were provided for palate cleansing.

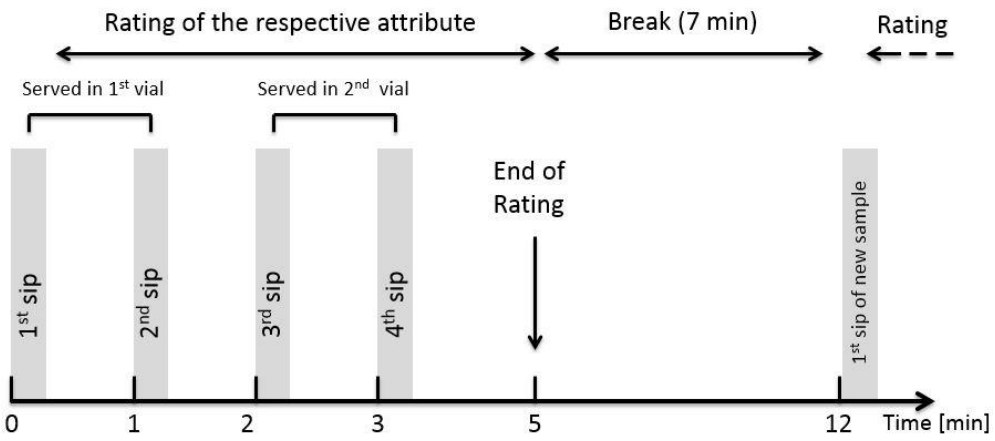



*Figure 3-1: Schematic representation of the multi-sip TI protocol. Illustration shows the time points at which each sip was consumed and at what time the next sample was presented.*

### *3.2.3 Data Analysis*

Panel performance was monitored and checked for interactions between panellists and replicates. Once the panel scored astringency and bitterness consistently, the sample set was evaluated. Several parameters were extracted from the TI curves using a macro provided by Compusense. An illustration of the extracted parameters can be found in Figure 3-2. The definitions of the extracted parameters of the TI curves are as follows (Compusense, 2015):

- $I_{Max}$: maximum intensity reached for the attribute of interest after the 4$^{th}$ sip of tea; $I_{Max1}$, $I_{Max2}$, $I_{Max3}$ to describe the maximum intensity for individual sips (sip 1, 2 & 3) will also be discussed.
- $T_{Max}$: time required to reach the maximum intensity, which was reached after the 4$^{th}$ sip of tea; $T_{Max1}$, $T_{Max2}$, $T_{Max3}$ to reach maximum intensity for individual sips (sip 1, 2 & 3) was also determined.
- AuC: Area under curve represents the overall perception of the attribute in question over the whole 5 min time frame of the evaluation (area under 4 sips). $AuC_1$, $AuC_2$, $AuC_3$ represent the area

under the curve for individual sips. AuC is calculated using the trapezoid method. Trapezoid rule: divide curve into series of trapezoids, each with area = (average height) x (width). Sum the areas of the strips.

- Initial delay: defines the time from start to the first point where the panellist detects the attribute.
- Increasing angle: describes how fast the sensation increases upon consumption in the early stages of the evaluation and is calculated as the area (in degrees) of ascent from the first recorded value to the maximum intensity. The increasing angle was also calculated for individual sips.
- Decreasing angle: describes how fast the sensation diminished towards the end of the evaluation and is calculated as the angle (in degrees) of descent from maximum intensity to the last recorded value. The decreasing angle was also calculated for individual sips.

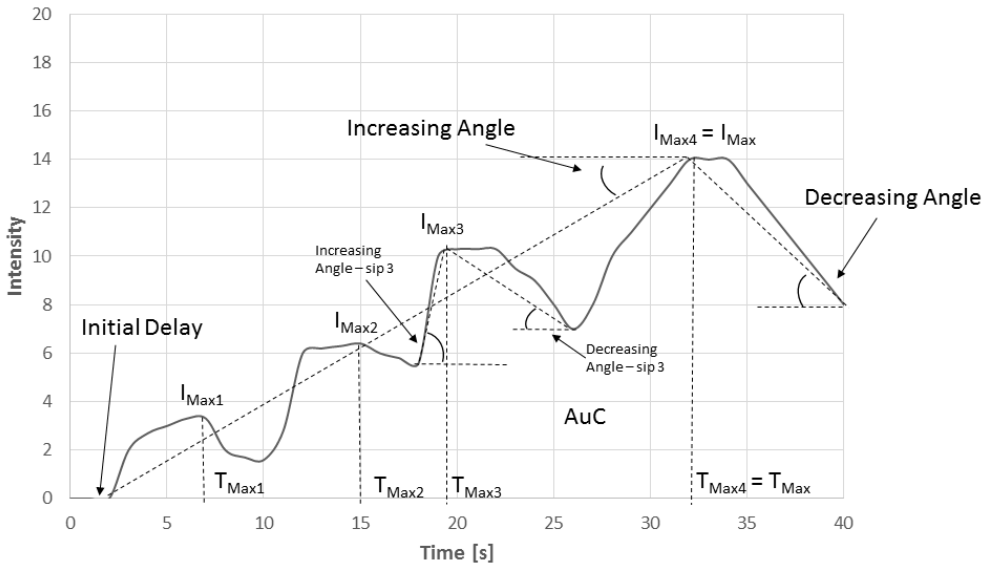



*Figure 3-2: Schematic illustration of parameters extracted from TI curves. An example of the increasing and decreasing angle of individual sips is given for sip 3. Parameters were extracted for all 4 sips. The data can be found in Table 3-4 to Table 3-7.*

Values for maximum intensity ($I_{Max}$), time at $I_{Max}$ ($T_{Max}$), area under curve (AuC), increasing angle, decreasing angle and initial delay of all tea samples can be found in Table 3-4 (for astringency) and Table 3-6 (for bitterness). In order to investigate the impact of multiple sips, each parameter was extracted for each sip of low, medium and high tea with no sugar or fat added and are shown in Table 3-5 and Table 3-7. A 2-way ANOVA (sample, replicate with panellist as random factor) was used to determine significant differences between samples and replicates, followed by Tukey Post-Hoc test, to identify which samples and replicates were significantly different from each other for each TI parameter using XLSTAT (version 2015.5).

In order to "match" the sweetness of medium tea with 5% sucrose and medium tea with a mixture of sweeteners an "A-not-A" test was conducted. Similarities between samples were calculated using the R-index, which is used as a measure of discrimination (Kemp et al., 2009). An R-index of 0.5 indicated that two samples are confusable, whereas an R-index of 1 shows that two samples are not confusable. The results will be discussed in section 3.3.3.4. The panel also rank-rated samples for perceived thickness and the instrumental viscosity was measured. ANOVA, followed by Tukey Post-Hoc test was used to identify significant differences between samples ($p<0.05$).

Fat and sugar were added to tea individually but also in combination. In order to understand if the impact of fat and sugar on astringency and bitterness is an additive effect, the impact when fat and sugar were added individually was calculated and compared to the impact of the simultaneous addition of fat and sugar. The TI parameters $I_{Max}$ and AuC of astringency

and bitterness were compared to each other. The impact of the addition of 5% fat on $I_{Max}$ or AuC was calculated by assuming a value of 100% for tea without any additional ingredients. The observed change in $I_{Max}$ or AuC when fat was added, was expressed in percentage with a negative sign indicating a decrease through the addition of fat. The same was done for teas with added sugar and added fat & sugar. These changes all represent the observed changes in the temporal profile of astringency and bitterness through the addition of fat or sugar and fat & sugar. The calculated impact of fat & sugar [%] was calculated by simply adding the observed changes of individually added fat and sugar together. The difference between the calculated impact of fat & sugar and the observed impact of fat & sugar was then compared. The results can be found in Table 3–8 .

## 3.3 Results

### *3.3.1 Panel Performance*

ANOVA results revealed that replicates had no significant effect ($p>0.05$) on the TI parameters of astringency and bitterness, however a significant subject*session interaction was observed. Another way to check for consistent rating is the addition of an experimental replicate. In this study, a sample in the middle of the design space (0.55 % tea, 5% fat, 5 % sugar) was chosen. Multiple comparison test revealed that, although the experimental replicate scored slightly higher for $I_{Max}$ and AuC than the test sample, no significant differences existed, indicating consistency across sessions (see Table 3-4 and Table 3-6 for Post-hoc groupings). However, inter-panel variability was observed. Figure 3-3 shows TI-curves of all panellists for perceived astringency of low (0.1% tea), medium (0.55%

tea) and high tea (1% tea) and illustrates the variation in perception. Visual inspection of the curves shows that the panellists can be split into 4 groups based on their perception. The temporal perception of the first group (panellist 1, 4 and 8) shows a stepwise increase of astringency with each sip. Furthermore, after reaching $I_{Max}$ for each sip, astringency intensity plateaus until the next sip is consumed. For group 2 (panellist 2 and 9), astringency increased sharply and after reaching $I_{Max}$ decreased sharply until the next sip was consumed. Panellist 9 perceived an increase in astringency with increasing number of sips, whereas panellist 2 did not perceive such an increase. Group 3 (panellist 3 and 5) perceived an overall increase in astringency with increasing number of sips, however it seems that for these panellists, astringency continued to increase between each sip and did not show a plateau-like behaviour as for group 1. Group 4 (panellist 6 and 7) were less discriminating of astringency between tea levels. However, panellist 7 perceived a step-wise increase of astringency with increasing number of sips.

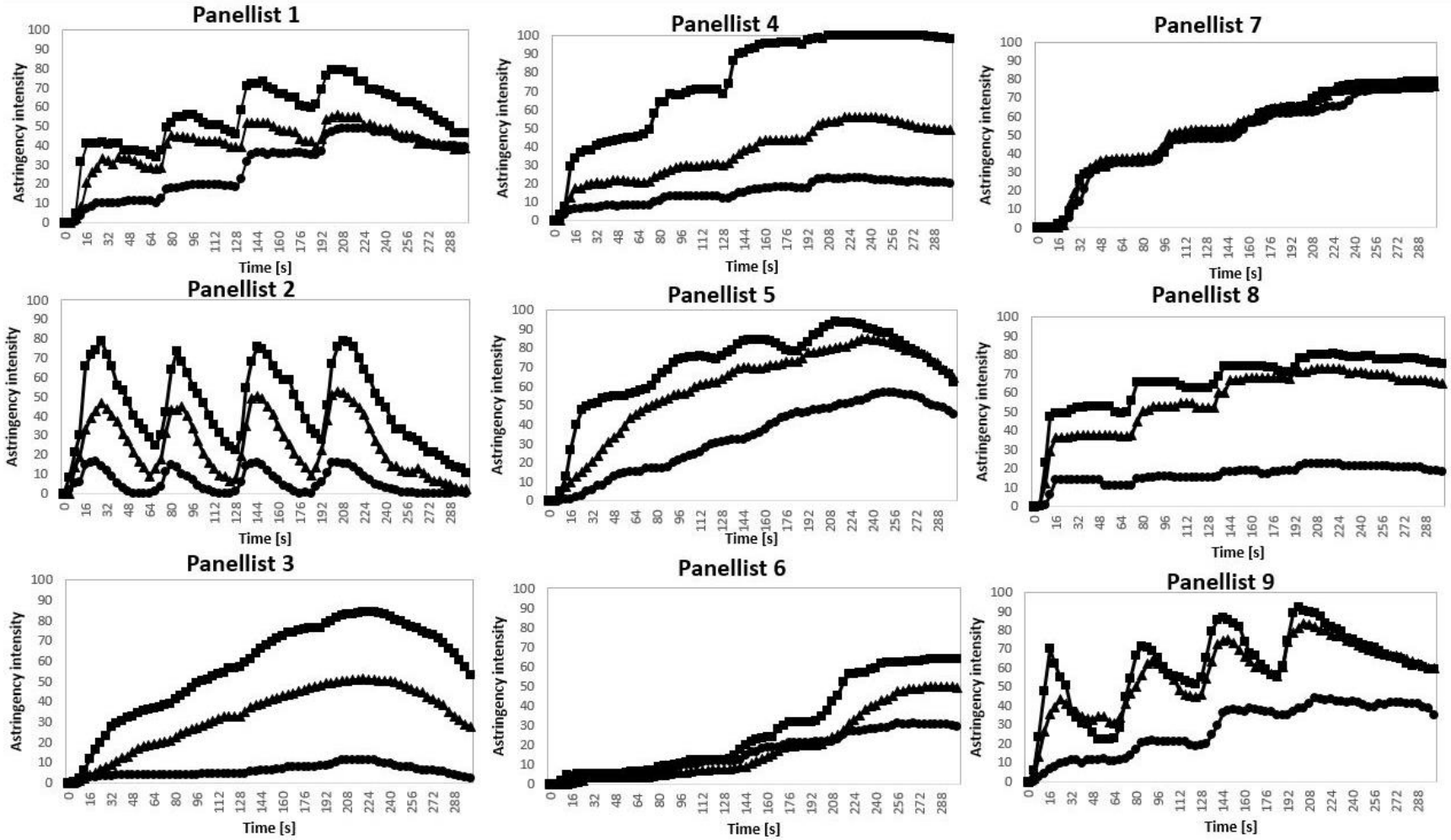



*Figure 3-3: TI curves for astringency perception of 3 different tea samples for all panellists (data from 4 replicates).* ■ *= high tea (1% tea),* ▲ *= medium tea (0.55% tea),* ● *= low tea (0.1% tea)*

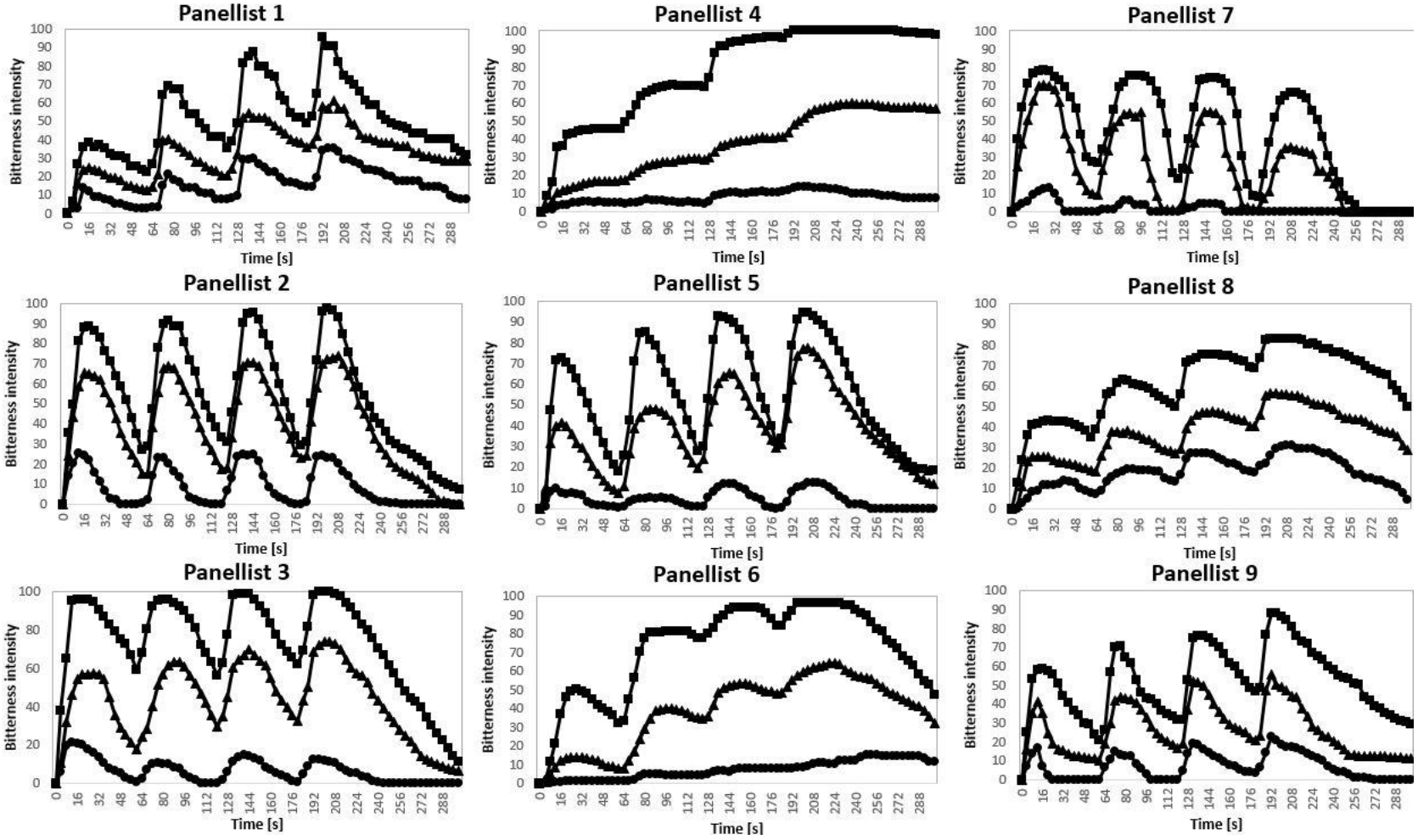



*Figure 3-4: TI curves for bitterness perception of 3 different tea samples for all panellists (data from 4 replicates). ■ = high tea (1% tea), ▲ = medium tea (0.55% tea), ● = low tea (0.1% tea)*

To further investigate the observation of different astringency perception, a 2-factor ANOVA (panellist and sample) with interaction was performed on the area under curve data. The ANOVA revealed that as well as panellist and sample, the interaction between panellist and sample was highly significant ($p<0.0001$). ANOVA was followed by Tukey`s Post Hoc test and it was observed that most panellists showed good discriminative abilities for the three tea levels (parameter = AuC) (see Table 3-3). However, for panellist 7 the Post Hoc grouping revealed no significant differences in AuC for the three tea levels (see Table 3-3), which confirms the visual observation that this panellist is not able to discriminate between different levels of astringency. This shows that although each panellist was consistent within themselves, the perception of astringency differed between individuals. However, it is also important to note that for all discriminating panellists, astringency increased with increasing tea content and no cross-over interactions were observed for these three tea levels, showing agreement.

*Table 3-3: AuC for 3 tea levels (0.1, 0.55, 1%). The grouping shows which panellists were not able to discriminate between different tea levels. Panellists*Samples sharing the same letter are not significantly different to each other (p>0.05, post-hoc Tukey).*

| Astringent | | | | Bitter | | | |
|---|---|---|---|---|---|---|---|
| **Panellist** | **Tea [%]** | **AuC** | **Grouping** | **Panellist** | **Tea [%]** | **AuC** | **Grouping** |
| 1 | 0.1 | 1476 | H | 1 | 0.1 | 4682 | IJKL |
| 1 | 0.55 | 12181 | BCDEFG | 1 | 0.55 | 10083 | FGHI |
| 1 | 1 | 16443 | ABCD | 1 | 1 | 15298 | DEF |
| 2 | 0.1 | 4525 | GH | 2 | 0.1 | 2515 | JKL |
| 2 | 0.55 | 8283 | DEFGH | 2 | 0.55 | 11937 | EFG |
| 2 | 1 | 14551 | ABCDEF | 2 | 1 | 16594 | BCDE |
| 3 | 0.1 | 1695 | H | 3 | 0.1 | 1883 | JKL |
| 3 | 0.55 | 9893 | CDEFGH | 3 | 0.55 | 13113 | DEFG |
| 3 | 1 | 17224 | ABCD | 3 | 1 | 22217.5 | AB |
| 4 | 0.1 | 4790 | GH | 4 | 0.1 | 2391.5 | JKL |
| 4 | 0.55 | 11598 | BCDEFG | 4 | 0.55 | 11298.5 | EFGH |
| 4 | 1 | 22013 | A | 4 | 1 | 23511 | A |
| 5 | 0.1 | 9885 | CDEFGH | 5 | 0.1 | 1342 | KL |
| 5 | 0.55 | 17982 | ABC | 5 | 0.55 | 11587.5 | EFGH |
| 5 | 1 | 21326 | A | 5 | 1 | 16618.5 | BCDE |
| 6 | 0.1 | 5080 | GH | 6 | 0.1 | 2174.5 | JKL |
| 6 | 0.55 | 6314 | EFGH | 6 | 0.55 | 11719 | EFG |
| 6 | 1 | 8654 | DEFGH | 6 | 1 | 21735.5 | ABC |
| 7 | 0.1 | 14798 | ABCDE | 7 | 0.1 | 533 | L |
| 7 | 0.55 | 15062 | ABCDE | 7 | 0.55 | 7515 | GHIJK |
| 7 | 1 | 16146 | ABCD | 7 | 1 | 13079 | DEFG |
| 8 | 0.1 | 5611 | FGH | 8 | 0.1 | 5392.5 | HIJKL |
| 8 | 0.55 | 16931 | ABCD | 8 | 0.55 | 11209 | EFGH |
| 8 | 1 | 19913 | AB | 8 | 1 | 18564 | ABCD |
| 9 | 0.1 | 8505 | DEFGH | 9 | 0.1 | 2032 | JKL |
| 9 | 0.55 | 17058 | ABCD | 9 | 0.55 | 7995.5 | GHIJ |
| 9 | 1 | 18334 | ABC | 9 | 1 | 15746 | CDEF |

The TI-curves of all panellists showing the temporal perception of bitterness are displayed in Figure 3-4. Compared to astringency, all panellists were able to discriminate between the three tea levels according to their bitterness (see Table 3-3 for groupings) and can be split into 2 groups based on their perception. The first group (panellists 1, 2, 3, 5, 7 and 9) perceived bitterness to sharply increase after the consumption of the first sip until $I_{Max}$ was reached. Bitterness intensity did not remain at

the maximum intensity for long and showed a sharp decrease until the next sip was consumed. With increasing number of sips bitterness increased somewhat, however this was not observed for all panellists. Group 2 (panellists 4, 6 and 8) perceived bitterness at a slower rate, resulting in flatter peaks. 2-way ANOVA (see Table 3-3) with interactions confirmed significant differences between panellist, samples and an interaction between both factors. However, the Post Hoc grouping revealed that all panellists were able to discriminate between the three tea levels (parameter = AuC). Comparing the temporal profiles of bitterness in tea, one can see that there are perceptual differences between individuals or differences in scale usage, but panellists followed the same trend when rating these three tea levels, as bitterness increases with increasing tea level.

It was found that panellist 7 had difficulties discriminating between the three tea levels according to their temporal astringency perception, however, the panellist was able to discriminate between the three samples according to their bitterness. Furthermore, it was found that removing panellist 7 from the data analysis did not affect the results. Considering these points, it was decided to analyse the data from all 9 panellists.

It has been shown by Fischer et al. (1994), Ishikawa & Noble (1995), and Peleg et al. (1999) that saliva flow rate can affect the temporal perception of astringency. The unstimulated saliva flow rate of all panellists can be found in Appendix 2. A correlation between unstimulated saliva flow rate and Area under the curve showed that there is no correlation between saliva flow rate and astringency perception (data not shown). However, it was found that panellist 2, the subject with the high unstimulated saliva

flow rate, and panellist 4, the subject with the lowest unstimulated saliva flow rate, perceive astringency over time in a different manner. For future studies, it might be of interest to correlate astringency perception to stimulated saliva flow rate, since the consumption of tea increases saliva flow, or to focus on the quantification of specific salivary proteins which have shown to be correlated to astringency perception (Kallithraka et al., 2001).

### *3.3.2 Temporal Profile of Astringency & Bitterness and the Impact of Tea Strength*

#### *3.3.2.1 Impact of different Tea Strength on temporal Astringency Perception*

Averaged TI parameters as well as Post-hoc groupings for temporal astringency perception of different tea samples are presented in Table 3-4 and Table 3-5. The figures displayed in the following sections represent panel average data. The perception of astringency for teas of different strength (low, medium, high) over the consumption of multiple sips is displayed in Figure 3-5A. In the following sections, low tea refers to a tea concentration of 0.1% tea, medium tea to a tea concentration of 0.55% tea and high tea to a tea concentration of 1% tea. All three tea levels showed a similar temporal profile which increased in intensity with increasing tea level. After the consumption of the first sip of tea, astringency increased and reached the maximum intensity for this sip. Once the maximum intensity was reached, perceived astringency maintained a plateau until the next sip was consumed. The same pattern was found for the remaining three sips.

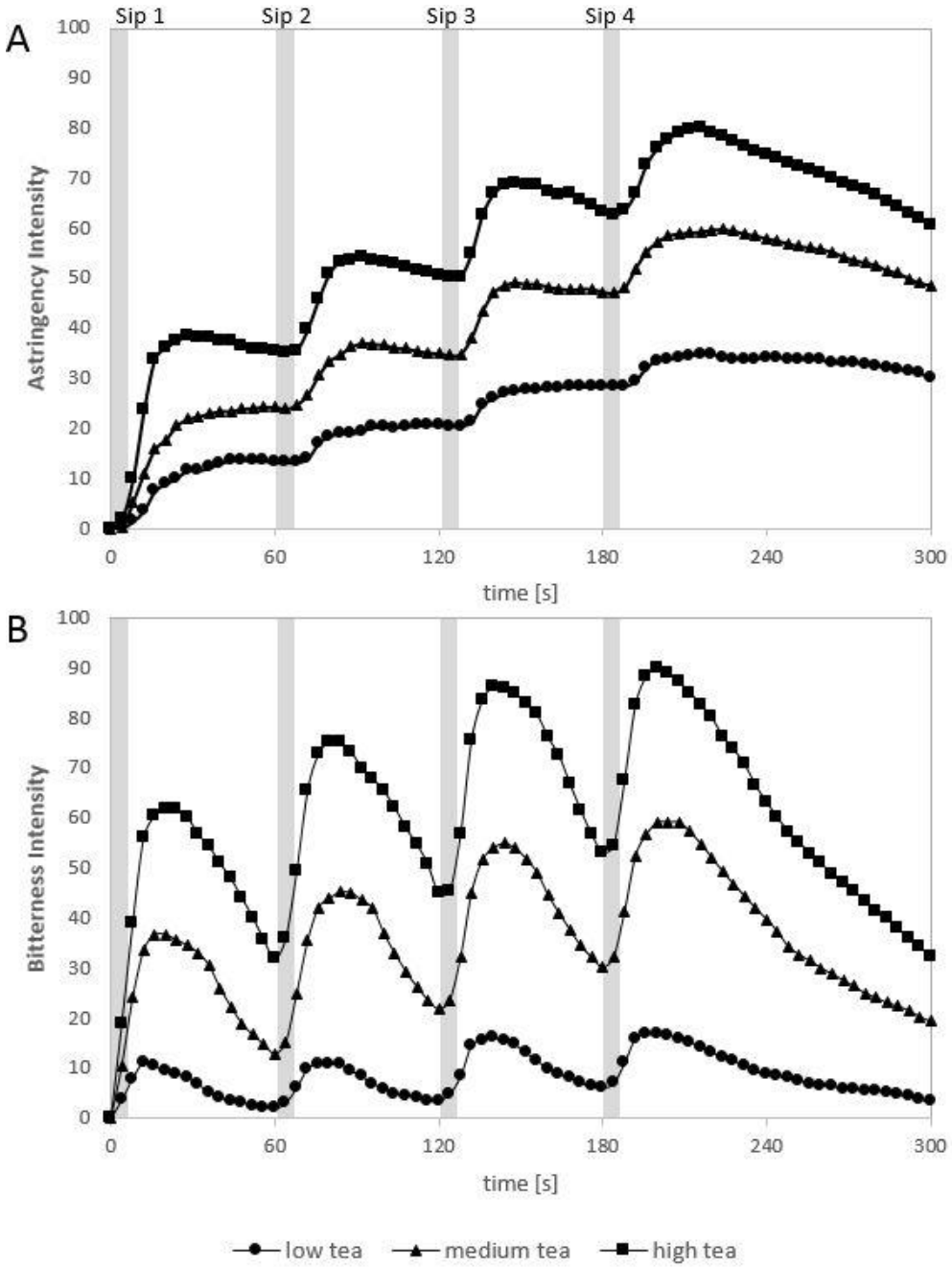



*Figure 3-5: Effect of tea strength and multiple sips consumption on the temporal profile of (A) astringency, (B) bitterness. The TI curve represents the average of all panellists (n = 9) across 4 replicates. Sips are taken at 0s, 60s, 120s and 180s as indicated by vertical grey bars; Intensity of the attribute (% of the graphical scale). See Table 3-4 and Table 3-6 for Post-hoc groupings*

*Table 3-4: Mean TI-parameters for astringent for each sample varying in composition. All parameters were extracted from a total of 4 sips of each sample. Samples sharing a common letter within a parameter are not significantly different ($p<0.05$).*

| | Samples | | | $I_{Max}$ [%] | | $T_{Max}$ [s] | | AuC | | Initial Delay [s] | | Increasing Angle [°] | | Decreasing Angle [°] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tea [%] | Fat [%] | Sugar [%] | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Low Tea | 0.1 | 0 | 0 | $39.7^{EFGH}$ | 26.8 | $203.9^{A}$ | 65.0 | $6968^{EFG}$ | 5575 | $11.0^{AB}$ | 10.3 | $12.1^{CDEF}$ | 11.1 | $8.1^{C}$ | 7.1 |
| | 0.1 | 5 | 0 | $38.5_{FGH}$ | 20.7 | $214.4^{A}$ | 55.8 | $6891^{EFG}$ | 4453 | $9.8^{B}$ | 9.2 | $11.0^{DEF}$ | 9.8 | $13.1^{ABC}$ | 12.8 |
| | 0.1 | 5 | 5 | $34.4^{GH}$ | 20.8 | $213.6^{A}$ | 62.7 | $5943^{FG}$ | 4239 | $10.8^{AB}$ | 7.8 | $9.9^{EF}$ | 10.3 | $10.8^{BC}$ | 12.0 |
| | 0.1 | 0 | 5 | $32.1^{H}$ | 23.1 | $213.2^{A}$ | 48.3 | $5424^{G}$ | 4567 | $14.1^{AB}$ | 15.1 | $8.8^{F}$ | 8.0 | $12.9^{ABC}$ | 11.6 |
| Medium Tea | 0.55 | 0 | 0 | $64.4^{BC}$ | 19.1 | $215.3^{A}$ | 45.9 | $12122^{BC}$ | 5143 | $7.9^{B}$ | 6.7 | $16.6^{ABCDE}$ | 9.1 | $13.0^{ABC}$ | 11.3 |
| | 0.55 | 5 | 0 | $56.2^{CD}$ | 17.8 | $213.0^{A}$ | 58.2 | $10631^{CD}$ | 4254 | $8.1^{B}$ | 6.9 | $16.6^{ABCDE}$ | 11.3 | $14.8^{AB}$ | 10.7 |
| | 0.55 | 5 | 5 | $35.3^{GH}$ | 17.0 | $214.4^{A}$ | 56.7 | $5840^{FG}$ | 3169 | $17.6^{A}$ | 21.7 | $10.8^{DEF}$ | 11.0 | $10.6^{BC}$ | 9.6 |
| | 0.55 | 5 | 5 | $42.8^{DEFGH}$ | 22.5 | $205.1^{A}$ | 74.4 | $7492^{EFG}$ | 4507 | $12.7^{AB}$ | 8.6 | $13.1^{BCDEF}$ | 10.8 | $10.5^{BC}$ | 9.6 |
| | 0.55 | 0 | 5 | $50.7^{CDEF}$ | 22.0 | $220.4^{A}$ | 43.7 | $9288^{CDE}$ | 4712 | $8.8^{B}$ | 8.0 | $12.8^{BCDEF}$ | 6.0 | $10.6^{BC}$ | 9.7 |
| | 0.55 | Dextran | | $64.2^{BC}$ | 19.6 | $209.2^{A}$ | 60.3 | $11990^{BC}$ | 4668 | $7.3^{B}$ | 7.6 | $18.8^{ABC}$ | 12.5 | $15.0^{AB}$ | 13.3 |
| | 0.55 | Sweetener | | $52.9^{CDE}$ | 23.3 | $201.9^{A}$ | 68.0 | $9704^{CDE}$ | 5069 | $10.4^{B}$ | 10.8 | $17.5^{ABCD}$ | 15.6 | $10.7^{BC}$ | 9.7 |
| High Tea | 1.0 | 0 | 0 | $84.5^{A}$ | 18.4 | $208.9^{A}$ | 59.5 | $17162^{A}$ | 4869 | $7.6^{B}$ | 14.7 | $22.9^{A}$ | 14.2 | $14.2^{ABC}$ | 11.8 |
| | 1.0 | 5 | 0 | $62.7^{C}$ | 19.3 | $224.8^{A}$ | 36.6 | $11865^{BC}$ | 4737 | $8.7^{B}$ | 9.1 | $15.6^{ABCDEF}$ | 5.0 | $15.2^{AB}$ | 12.6 |
| | 1.0 | 5 | 5 | $48.1^{DEFG}$ | 19.1 | $225.4^{A}$ | 52.7 | $8557^{DEF}$ | 4031 | $10.9^{AB}$ | 8.2 | $13.2^{BCDEF}$ | 10.6 | $13.4^{ABC}$ | 11.2 |
| | 1.0 | 0 | 5 | $77.4^{AB}$ | 16.6 | $218.3^{A}$ | 47.1 | $14567^{AB}$ | 4823 | $7.6^{B}$ | 6.1 | $19.7^{AB}$ | 11.2 | $18.8^{A}$ | 14.1 |
| p-values (sample effect) | | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| p-values (subject effect) | | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| p-values (session effect) | | | | 0.205 | | 0.579 | | 0.493 | | 0.870 | | 0.902 | | 0.885 | |

*Table 3-5: Mean TI parameters for astringent for samples varying in tea strength. All parameters were extracted from a single sip of tea (sips 1-4), showing the change in each parameter as more sips are consumed. Samples sharing a common letter within a parameter are not significantly different (p<0.05).*

| Sample | | | $I_{Max}$ [%] | | $T_{Max}$ [s] | | AuC | | Increasing Angle [°] | | Decreasing Angle [°] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sip | Tea [%] | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| low tea | 1 | 0.1 | $16.5^{H}$ | 16.0 | $35.4^{D}$ | 15.9 | $552^{G}$ | 562 | $23.0^{DEF}$ | 21.0 | $10.1^{ABC}$ | 15.6 |
| | 2 | | $23.1^{GH}$ | 19.4 | $93.2^{C}$ | 18.1 | $17444^{FG}$ | 16955 | $18.8^{EF}$ | 17.6 | $6.8^{C}$ | 12.6 |
| | 3 | | $31.5^{FG}$ | 22.9 | $156.7^{B}$ | 18.7 | $48291^{EF}$ | 39572 | $19.3^{EF}$ | 16.9 | $8.0^{BC}$ | 12.8 |
| | 4 | | $39.7^{EF}$ | 26.9 | $224.1^{A}$ | 34.6 | $178914^{C}$ | 142567 | $17.5^{F}$ | 14.2 | $9.7^{ABC}$ | 9.3 |
| medium tea | 1 | 0.55 | $28.9^{FGH}$ | 17.0 | $38.4^{D}$ | 16.6 | $1047^{FG}$ | 704 | $36.5^{B}$ | 22.2 | $9.1^{BC}$ | 18.5 |
| | 2 | | $40.6^{EF}$ | 20.5 | $101.0^{C}$ | 14.7 | $31777^{FG}$ | 17705 | $23.8^{CDEF}$ | 16.5 | $11.6^{ABC}$ | 20.1 |
| | 3 | | $52.8^{CDE}$ | 20.9 | $159.4^{B}$ | 16.4 | $84325^{DE}$ | 39393 | $27.7^{BCDE}$ | 18.9 | $11.2^{ABC}$ | 17.5 |
| | 4 | | $64.4^{BC}$ | 19.1 | $222.6^{A}$ | 30.2 | $302550^{B}$ | 119933 | $25.6^{BCDEF}$ | 18.9 | $14.2^{ABC}$ | 12.7 |
| high tea | 1 | 1 | $48.2^{DE}$ | 24.3 | $36.0^{D}$ | 18.4 | $1887^{FG}$ | 970 | $50.1^{A}$ | 25.7 | $16.4^{AB}$ | 24.2 |
| | 2 | | $59.8^{CD}$ | 22.0 | $97.8^{C}$ | 15.1 | $47937^{EF}$ | 18048 | $32.6^{BCD}$ | 21.3 | $14.5^{ABC}$ | 19.8 |
| | 3 | | $73.9^{AB}$ | 21.3 | $153.3^{B}$ | 16.2 | $121143^{D}$ | 39480 | $34.4^{BC}$ | 19.9 | $18.1^{A}$ | 21.2 |
| | 4 | | $84.5^{A}$ | 18.3 | $223.4^{A}$ | 32.7 | $398858^{A}$ | 118948 | $28.5^{BCDE}$ | 20.6 | $15.7^{AB}$ | 13.6 |
| **p-values (sip effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | 0.337 | |
| **p-values (sample effect)** | | | <0.0001 | | 0.124 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (subject effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (session effect)** | | | <0.020 | | 0.354 | | 0.251 | | 0.843 | | 0.814 | |

Area under Curve (AuC) is a measure for the overall perception of astringency and was found to be significantly different ($p<0.05$, see Table 3-4) for all tea levels (low, medium, high) which shows that the overall intensity of astringency increases with increasing tea concentration. This was as expected as with increasing tea strength, the concentration of astringent components in the tea will increase as well.

After the fourth sip of tea was consumed data collection continued for a further two minutes, which allowed the slow decrease of astringency to be captured. The perceived intensity of astringency did not decrease back to zero suggesting that it takes a long time for astringency to fade and the palate to recover. The decreasing angle (range 8 – 14°, see Table 3-4) did not differ significantly for these three tea levels. Interestingly, the increasing angle of high tea (23°) was significantly greater than for low tea (12°), which means that astringency perception increased faster for high tea than for low tea and is therefore tea level dependent. Both, increasing and decreasing angle are related to $I_{Max}$ of the fourth sip and do not take previous sips into account.

*3.3.2.2 Comparing Astringency Perception of individual Sips*

Table 3-5 shows the parameters extracted from the TI curves for individual sips of teas varying in strength. Consuming multiple sips of tea had a significant impact on astringency intensity, which generally doubled from sip 1 to sip 4 at each tea strength. $I_{Max}$ of the first sip ($I_{Max1}$) to the fourth sip ($I_{Max}$) increased by 58% for low tea, 55% for medium tea and 43% for high tea. In addition, differences between individual sips were observed and it was found that perceived astringency intensity ($I_{Max}$) for low tea

varied significantly between sips, with the first sip being significantly less astringent than sip 3 and 4 (see $I_{Max}$ in Table 3-5). The same pattern was found for medium and high tea showing a build-up in astringency perception which gives an insight into how astringency perception changes over the consumption of one cup of tea.

The decreasing angle for all sips of different tea strength ranged from 7 to 18°, with generally greater decreasing angles being observed for high tea than for low tea. The same trend was found for the increasing angle, ranging from 18 to 50° with higher increasing angles observed for high tea. This confirms that astringency decrease and increase are concentration dependent.

#### *3.3.2.3 Impact of different Tea Strength on temporal Bitterness Perception*

The temporal profile of bitterness in black tea is displayed in Figure 3-5B and TI parameters as well as Post-hoc groupings can be found in Table 3-6. After the consumption of the first sip, bitterness perception increased rapidly until a perceived maximum was reached. Once this maximum was reached, bitterness perception decreased sharply until the next sip was consumed. The AuC varied significantly ($p<0.05$, see Table 3-6) between the three tea levels, with low tea (0.1% tea) having the smallest area under the curve as it is perceived to be the least bitter compared to medium (0.55%) and high tea (1%) without additives. This is not surprising as many polyphenols are known to be bitter and it is expected that bitterness increases with increasing tea strength as shown by descriptive profiling (Chapter 2).

After the fourth sip of tea was consumed the data collection continued for two minutes, which allowed the slow decrease of bitterness to be captured. The perceived intensity of bitterness did not decrease back to zero at higher tea concentrations. This suggests that it takes a longer time for bitterness to fade and the palate to recover at higher tea concentrations. The decreasing angle (see Table 3-6) is an indicator for how quickly bitterness perception decreases after the last sip was consumed and it varied between 16 – 31°. Bitterness decreases quickest for high tea having the greatest decreasing angle of 31°. The decreasing angle for astringency was much smaller, with a range of 8 - 18°, showing that perceived astringency decreased much slower after each sip of tea compared to bitterness. The increasing angle for bitterness ranged from 20 to 32° and was the greatest for high tea, indicating that the bitterness increases quicker for high tea than for low and medium tea. Furthermore, the increasing angle differed between astringency and bitterness, with bitterness showing a faster increase than astringency (increasing angle median: 18° (bitter) versus 13° (astringent)).

*3.3.2.4 Comparing Bitterness Perception of individual Sips*

TI parameters extracted for the bitterness perception of individual sips can be found in Table 3-7. It was observed that with increasing number of sips, bitterness intensity increases. The parameter illustrating this increase is $I_{Max}$, which increased by 27 % for low tea, 35 % for medium tea and 29 % for high tea from the first sip to the fourth sip, showing a build-up during consumption. This build-up was smaller than that observed for astringency. For low tea, ANOVA shows that $I_{Max}$ was not significantly

different ($p>0.05$) between any of the sips, indicating that the increase in bitterness was only small, even though 4 sips were consumed. For medium and high tea significant differences ($p<0.05$) were found between the four sips analysed (see Table 3-7). ANOVA also revealed that 3 or 4 sips of the medium tea are not significantly different ($p>0.05$) to one sip of the high tea indicating that bitterness build-up over time.

The increasing angle did not vary between different sips of the same tea strength, however with increasing tea level the increasing angle of each sip increased. This trend shows that the increase in bitterness perception is impacted by tea concentration to a greater extend that it is by the number of sips. The decreasing angle also did not vary significantly between different sips of the same tea strength and it was found that, in general, the decreasing angle increases with increasing tea strength. As observed for the increasing angle, the decreasing angle depends more on the tea concentration than the number of sips consumed.

*Table 3-6: Mean TI curve parameters for Bitter for each sample varying in composition. All parameters were extracted from a total of 4 sips of each sample. Samples sharing a common letter within a parameter are not significantly different (p<0.05).*

| | Samples | | | $I_{Max}$ [%] | | $T_{Max}$ [s] | | AuC | | Initial Delay [s] | | Increasing Angle [°] | | Decreasing Angle [°] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tea [%] | Fat [%] | Sugar [%] | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Low Tea | **0.1** | **0** | **0** | $23.1^{FG}$ | 13.1 | $134.7^{ABC}$ | 84.2 | $2550^{EF}$ | 2071 | $6.4^{AB}$ | 15.7 | $20.0^{B}$ | 24.5 | $12.1^{G}$ | 9.9 |
| | **0.1** | **5** | **0** | $25.6^{EF}$ | 16.7 | $125.9^{BC}$ | 80.7 | $3174^{DEF}$ | 2850 | $4.4^{AB}$ | 5.3 | $18.9^{B}$ | 20.3 | $11.3^{G}$ | 9.4 |
| | **0.1** | **5** | **5** | $16.7^{FG}$ | 14.1 | $126.3^{BC}$ | 76.3 | $1464^{F}$ | 1545 | $8.7^{AB}$ | 14.2 | $14.6^{BC}$ | 20.6 | $11.7^{G}$ | 13.6 |
| | **0.1** | **0** | **5** | $12.6^{G}$ | 10.1 | $155.2^{ABC}$ | 75.0 | $1156^{F}$ | 1186 | $16.2^{A}$ | 48.3 | $6.8^{C}$ | 13.7 | $13.4^{EFG}$ | 14.2 |
| Medium Tea | **0.55** | **0** | **0** | $66.9^{B}$ | 14.7 | $174.8^{A}$ | 66.5 | $10717^{B}$ | 3209 | $0.8^{B}$ | 1.9 | $23.2^{AB}$ | 19.2 | $24.6^{ABC}$ | 13.4 |
| | **0.55** | **5** | **0** | $40.3^{CD}$ | 18.1 | $149.3^{ABC}$ | 70.6 | $5615^{C}$ | 3296 | $4.6^{AB}$ | 13.2 | $17.3^{BC}$ | 17.1 | $18.0^{CDEFG}$ | 12.7 |
| | **0.55** | **5** | **5** | $22.7^{FG}$ | 18.1 | $140.1^{ABC}$ | 77.4 | $2814^{F}$ | 3062 | $12.1^{AB}$ | 26.4 | $14.7^{BC}$ | 19.8 | $12.1^{G}$ | 11.1 |
| | **0.55** | **5** | **5** | $19.2^{FG}$ | 14.1 | $120.3^{C}$ | 81.7 | $2040^{F}$ | 1997 | $8.2^{AB}$ | 21.3 | $15.5^{BC}$ | 20.5 | $12.7^{FG}$ | 11.5 |
| | **0.55** | **0** | **5** | $41.6^{CD}$ | 15.6 | $165.9^{AB}$ | 68.5 | $5375^{CD}$ | 2839 | $3.1^{AB}$ | 3.7 | $17.5^{BC}$ | 17.6 | $19.8^{BCDEF}$ | 12.3 |
| | **0.55** | **Dextran** | | $69.7^{B}$ | 17.3 | $177.9^{A}$ | 62.2 | $11179^{B}$ | 4595 | $1.1^{B}$ | 2.1 | $22.9^{AB}$ | 17.0 | $25.0^{ABC}$ | 12.8 |
| | **0.55** | **Sweetener** | | $47.1^{C}$ | 18.1 | $170.9^{A}$ | 68.2 | $6644^{C}$ | 3520 | $4.3^{AB}$ | 11.5 | $18.3^{BC}$ | 19.3 | $20.4^{BCDE}$ | 11.5 |
| High Tea | **1.0** | **0** | **0** | $93.9^{A}$ | 7.1 | $148.0^{ABC}$ | 68.4 | $18152^{A}$ | 3880 | $0.4^{B}$ | 1.3 | $32.1^{A}$ | 20.6 | $31.3^{A}$ | 12.2 |
| | **1.0** | **5** | **0** | $62.6^{B}$ | 15.8 | $171.2^{A}$ | 69.2 | $10405^{B}$ | 3671 | $1.0^{B}$ | 2.2 | $23.6^{AB}$ | 19.3 | $22.3^{BCD}$ | 12.5 |
| | **1.0** | **5** | **5** | $34.5^{DE}$ | 17.8 | $143.3^{ABC}$ | 75.2 | $4738^{CDE}$ | 3253 | $3.6^{AB}$ | 3.8 | $18.4^{BC}$ | 18.6 | $16.7^{DEFG}$ | 11.5 |
| | **1.0** | **0** | **5** | $72.8^{B}$ | 19.3 | $169.1^{AB}$ | 65.8 | $11763^{B}$ | 4698 | $1.1^{B}$ | 2.6 | $25.9^{AB}$ | 19.3 | $26.6^{AB}$ | 14.4 |
| **p-values (sample effect)** | | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (subject effect)** | | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (session effect)** | | | | 0.357 | | 0.0001 | | 0.398 | | 0.834 | | 0.646 | | 0.705 | |

*Table 3-7: Mean TI parameters for bitter for samples varying in tea strength. All parameter were extracted from a single sip of tea (sips 1-4) showing the change in each parameter as more sips are consumed. Samples sharing a common letter within a parameter are not significantly different (p<0.05).*

| Sample | | | $I_{Max}$ [%] | | $T_{Max}$ [s] | | AuC | | Increasing Angle [°] | | Decreasing Angle [°] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sip | Tea [%] | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Low Tea | 1 | 0.1 | $14.3^{F}$ | 10.5 | $19.1^{F}$ | 10.6 | $356^{G}$ | 316 | $33.4^{D}$ | 25.9 | $25.0^{BC}$ | 24.0 |
| | 2 | | $13.6^{F}$ | 9.3 | $78.2^{E}$ | 8.2 | $6853^{G}$ | 5897 | $31.0^{D}$ | 21.1 | $18.7^{C}$ | 16.6 |
| | 3 | | $17.2^{F}$ | 11.5 | $140.6^{C}$ | 10.2 | $20038^{FG}$ | 15358 | $31.0^{D}$ | 22.8 | $20.3^{C}$ | 16.6 |
| | 4 | | $2^{F}$ | 14.2 | $199.7^{AB}$ | 11.4 | $53122^{DEF}$ | 55385 | $33.6^{D}$ | 24.0 | $16.4^{C}$ | 11.6 |
| Medium Tea | 1 | 0.55 | $40.8^{E}$ | 23.2 | $22.2^{F}$ | 12.2 | $1527^{G}$ | 919 | $56.2^{ABC}$ | 22.6 | $32.9^{AB}$ | 22.5 |
| | 2 | | $49.1^{DE}$ | 16.8 | $87.4^{D}$ | 12.8 | $33690^{EFG}$ | 11659 | $54.0^{ABC}$ | 18.4 | $33.3^{AB}$ | 21.1 |
| | 3 | | $57.3^{CD}$ | 15.9 | $144.4^{C}$ | 11.1 | $79937^{D}$ | 23253 | $53.5^{BC}$ | 22.6 | $32.2^{AB}$ | 19.7 |
| | 4 | | $66.9^{C}$ | 18.0 | $204.6^{A}$ | 12.1 | $216034^{B}$ | 94635 | $52.1^{C}$ | 19.9 | $26.3^{BC}$ | 15.5 |
| High Tea | 1 | 1 | $65.2^{C}$ | 23.7 | $20.9^{F}$ | 10.2 | $2818^{G}$ | 1104 | $65.8^{A}$ | 18.2 | $39.7^{A}$ | 19.8 |
| | 2 | | $78.5^{B}$ | 14.4 | $81.8^{DE}$ | 9.9 | $60777^{DE}$ | 13390 | $64.4^{AB}$ | 14.5 | $38.9^{A}$ | 22.4 |
| | 3 | | $88.1^{AB}$ | 12.6 | $140.4^{C}$ | 10.3 | $134986^{C}$ | 29851 | $61.2^{ABC}$ | 17.7 | $39.8^{A}$ | 23.1 |
| | 4 | | $93.9^{A}$ | 12.3 | $196.4^{B}$ | 8.6 | $345672^{A}$ | 123858 | $56.1^{ABC}$ | 25.3 | $35.1^{AB}$ | 14.8 |
| **p-values (sip effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | 0.120 | | 0.001 | |
| **p-values (sample effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (subject effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |
| **p-values (session effect)** | | | 0.084 | | <0.0001 | | 0.116 | | 0.584 | | 0.565 | |

### 3.3.3 The Impact of Key Ingredients

#### *3.3.3.1 The Impact of Fat on the temporal Perception of Astringency and Bitterness*

The impact of fat on the temporal perception of astringency and bitterness is displayed in Figure 3-6 and the TI parameters can be found in Table 3-4 and Table 3-6. The open symbols in the TI-curves indicate tea with 5% fat addition. The addition of 5% fat to tea impacted perceived astringency intensity, however the shape of the temporal profile did not change. The result (see mean data and Post-hoc grouping in Table 3-4) show that fat significantly reduced ($p<0.05$) the temporal perception of astringency at high tea level. The AuC decreased significantly and multiple comparison test (Table 3-4) revealed that the addition of 5% fat to high tea reduced perceived astringency to a similar intensity perceived when medium tea without fat addition was consumed. For medium tea, AuC (see Table 3-4) decreased when fat was added, however, this did not result in a significant reduction ($p>0.05$). For low tea, fat did not significantly reduce temporal astringency and this can be seen in Figure 3-6A where both curves followed a similar profile. Low tea was not perceived as particularly astringent originally and therefore the addition of fat was not expected to have a significant impact on the temporal perception of astringency at this level.

The addition of 5% fat also had a significant impact on perceived bitterness, which significantly decreased AuC as can be seen in Figure 3-6B. However, this effect was not the same at all tea levels. The Post-hoc grouping (see Table 3-6) revealed that for low tea, the addition of fat did not lead to significant decrease in bitterness intensity ($p>0.05$). A significant decrease ($p<0.05$) was found at medium and high tea levels; the AuC decreasing by 47% for medium tea and for high tea, the decrease

was 43%. The addition of 5% fat at the high tea level decreased bitterness intensity to a similar level of medium tea without fat addition, as was also observed for astringency.

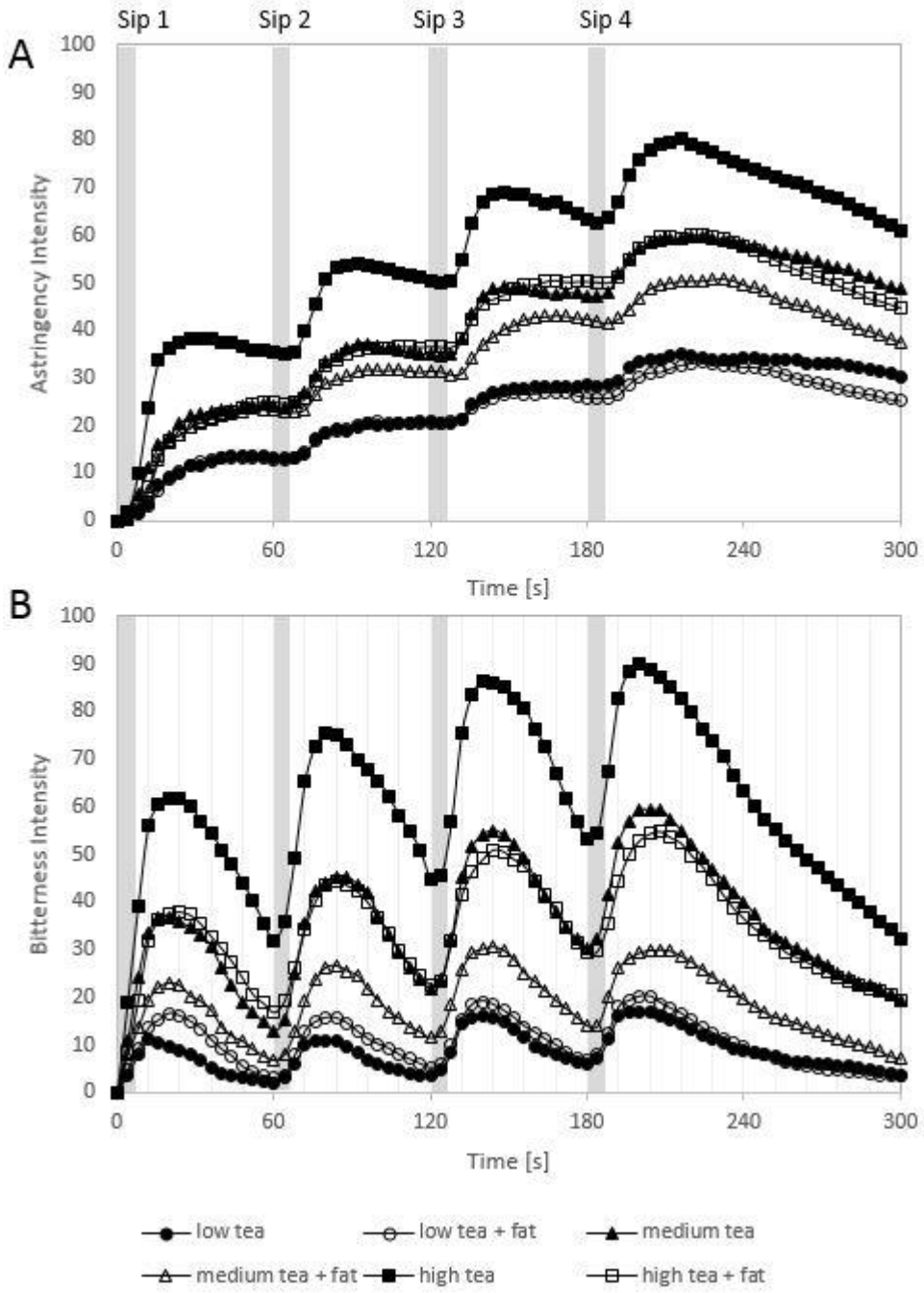



*Figure 3-6: Effect of fat (5 %) on the temporal profile of (A) astringency and (B) bitterness. The TI curve represents the average of all panellists (n = 9) across 4 replicates. Sips were taken at 0s, 60s, 120s and 180s as indicated by vertical grey bars; Intensity = intensity of the attribute (% of the graphical scale). See Table 3-4 and Table 3-6 for Post-hoc groupings.*

#### *3.3.3.2 The Impact of Sugar on the temporal Perception of Astringency and Bitterness*

Figure 3-7A shows the impact of 5% sugar on the perception of astringency. The addition of 5% sugar decreased the AuC for each tea level, however these decreases were not significant ($p>0.05$, see Table 3-4). This means that the addition of 5% sugar did not decrease perceived astringency at a significance level of 0.05% but the overall trend shows that sugar reduced astringency.

The addition of 5% sugar to tea had a greater impact on the perception of bitterness than astringency. For high tea, AuC reduced by 55% when 5% sugar was added ($p<0.05$, see Table 3-6). A significant impact ($p<0.05$) of sugar was also found in medium tea, where the AuC was halved. The addition of 5% sugar to high tea meant that 4 sips of this tea were perceived to be as bitter as 4 sips of tea at medium strength. For low tea, no significant change in AuC was observed, however a decrease was measured.

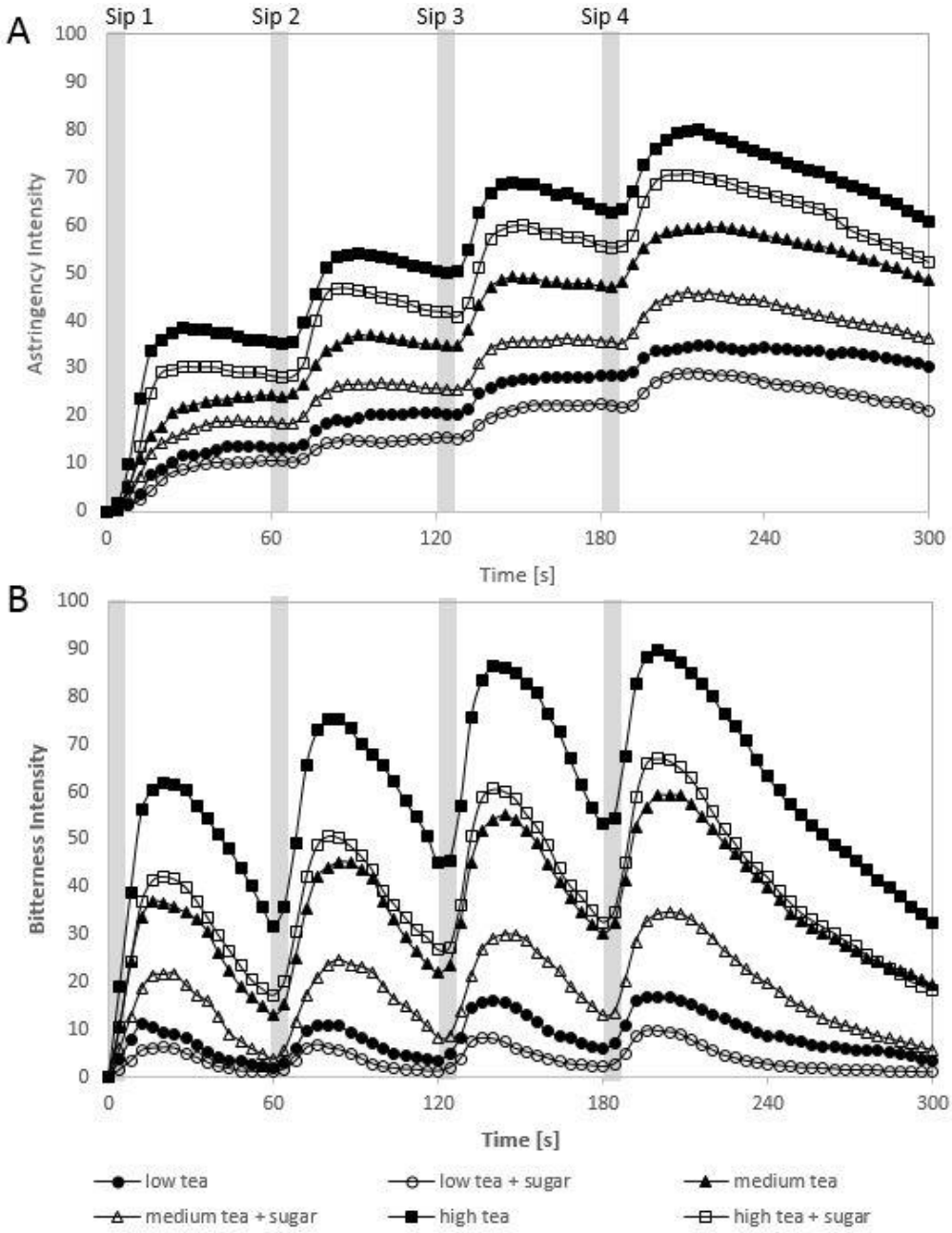



*Figure 3-7: Effect of sugar (5%) on the temporal profile of (A) astringency and (B) bitterness. The TI curve represents the average of all panellists (n = 9) across 4 replicates. Sips were taken at 0s, 60s, 120s and 180s as indicated by vertical grey bars; Intensity = intensity of the attribute (% of the graphical scale). See Table 3-4 and Table 3-6 for Post-hoc groupings.*

### *3.3.3.3 The Impact of Fat & Sugar on the temporal Perception of Astringency and Bitterness*

The impact of the addition of 5% fat & sugar when added together on the temporal perception of astringency and bitterness is displayed in Figure 3-8. The addition of both 5% fat & 5% sugar had the greatest impact on

perceived astringency and bitterness compared to the sole impact of either 5% fat or 5% sugar. For astringency, AuC significantly decreased in medium and high tea ($p<0.05$) when fat & sugar were added. AuC decreased by 45% at medium tea level and 50% at high tea level (see Table 3-4). This decrease in AuC shows, that both high tea and medium tea with fat & sugar addition were perceived to be similar in astringency to both low tea samples. The addition of fat & sugar did not significantly ($p>0.05$) reduce the astringency perception for low tea.

The addition of fat & sugar to high and medium tea decreased bitterness significantly ($p<0.05$). AuC decreased by 74% for both tea levels (see Table 3-6). The reduction in AuC resulted in a similar bitterness perception for medium tea with fat & sugar, low tea and low tea with fat & sugar. High tea with fat & sugar was reduced to a similar level of bitterness as low tea (see AuC groupings in Table 3-6). The bitterness of low tea was not significantly reduced ($p>0.05$) by the addition of both fat & sugar, which is not surprising as perception was low for this sample when consumed without additives.

Table 3–8 shows the combined impact of fat & sugar when added together in tea in comparison to the individual impact of both. For astringency, it was observed that for AuC the calculated impact of fat & sugar on low tea was higher than the observed one. For the astringency of medium and high tea, the calculated impact of fat & sugar was smaller than the observed impact. For bitterness the differences between calculated and observed impact of fat & sugar were in general greater. The observed reduction of the AuC of low tea with added fat & sugar was greater than the calculated reduction. For medium and high tea, the calculated impact of fat & sugar

on bitterness was greater than the observed impact. This is further evidence to support the view that there are different perceptual mechanisms for astringency and bitterness. However, the results are not consistent across tea levels, making it difficult to draw definite conclusions.

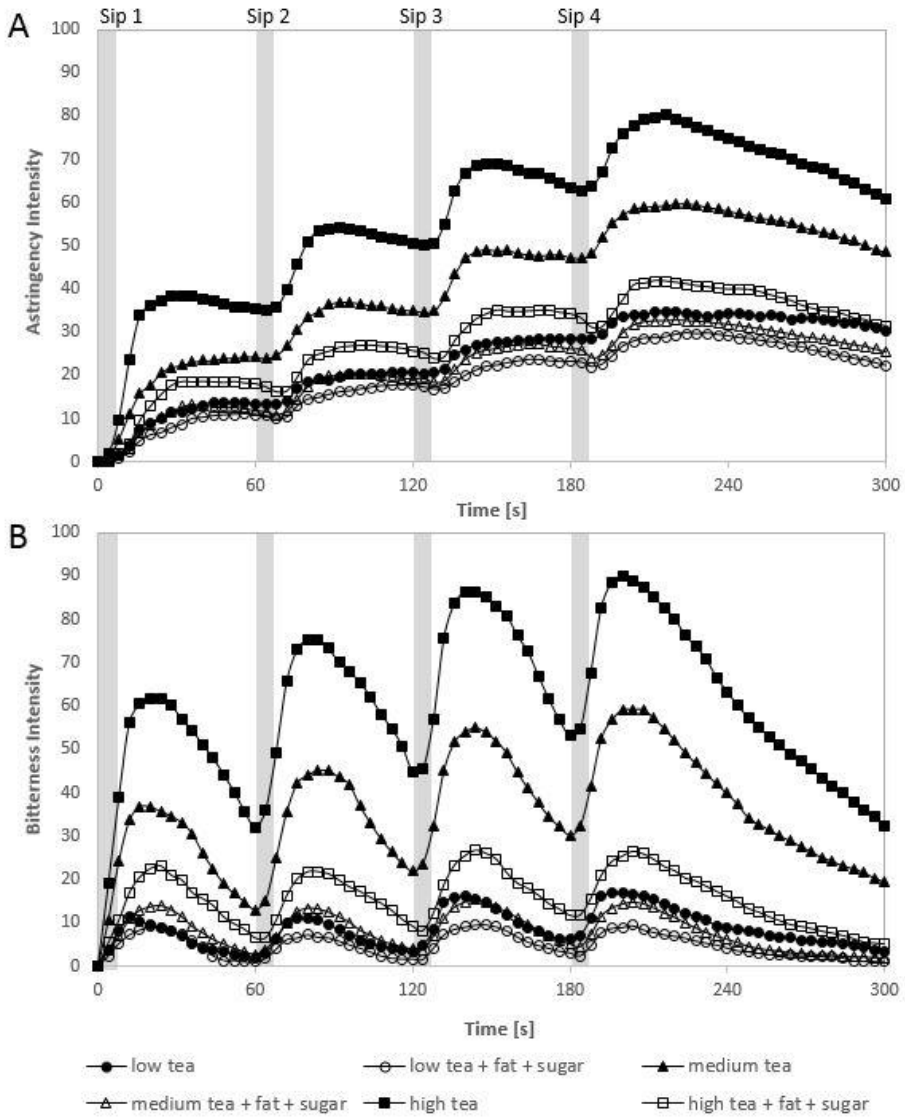



*Figure 3-8: Effect of fat (5 %) & sugar (5 %) on the temporal profile of (A) astringency, (B) bitterness. The TI curve represents the average of all panellists (n = 9) across 4 replicates. Sips are taken at 0s, 60s, 120s and 180s as indicated by vertical grey bars; Intensity = intensity of the attribute (% of the graphical scale). See Table 3-4 and Table 3-6 for Post-hoc groupings.*

*Table 3-8: The impact of fat, sugar and fat & sugar on astringency and bitterness parameters (AuC). Cal. impact of fat & sugar represents the calculated impact of fat & sugar by adding the impact of sugar and the impact of fat together. Obs. Impact of fat & sugar is the observed effect of the combination. The difference indicates the difference between calculated and observed impact of fat and sugar. Large differences between calculated and observed values indicate that factors are not additive.*

| | **Tea level** | **Impact of sugar [%]** | **Impact of fat [%]** | **Cal. impact of fat & sugar [%]** | **Obs. impact of fat & sugar [%]** | **Difference** |
|---|---|---|---|---|---|---|
| Astringency AuC | Low | -22.2 | -1.1 | -23.3 | -14.7 | -8.6 |
| | Medium | -23.4 | -12.3 | -35.7 | -45 | +9.3 |
| | High | -15.1 | -30.9 | -46.0 | -50.1 | +4.1 |
| Bitterness AuC | Low | -54.7 | +24.5 | -30.2 | -42.6 | +12.4 |
| | Medium | -49.9 | -47.6 | -97.5 | -77.4 | -20.1 |
| | High | -35.2 | -42.7 | -77.9 | -73.9 | -4.0 |

*3.3.3.4 Decoupling the Effects of Sugar – Sweetness and Viscosity*

Sucrose is added to tea to increase sweetness, however the viscosity is also increased as shown in Figure 3-9. Therefore, to investigate the impact of sweetness and viscosity independently, samples at medium tea level were prepared using 1) a low molecular weight dextran and 2) high intensity sweeteners to decouple the effects of sucrose addition. Samples were compared against medium tea and medium tea with 5% sucrose. The shear viscosity of each sample was measured using a rotational rheometer and the samples were compared for their overall sensory perception using the "A, not A" test and for perceived thickness using a rank-rating test.

The results of the "A-not-A"-test showed that a mixture of 0.1 g/l Cyclamate, 0.15 g/l Acesulfame K and 0.05 g/l Saccharin matched the sensorial profile of 5 % sucrose best, as indicated by a R-index close to 0.5 (R= 0.54). Figure 3-9 shows the results for the shear viscosity measurement and shows that the addition of sweeteners did not change viscosity significantly ($p>0.05$) in comparison to medium tea. The addition of dextran at 1.2% delivered a viscosity that was not significantly different to the viscosity measured when 5% sucrose was added ($p>0.05$).

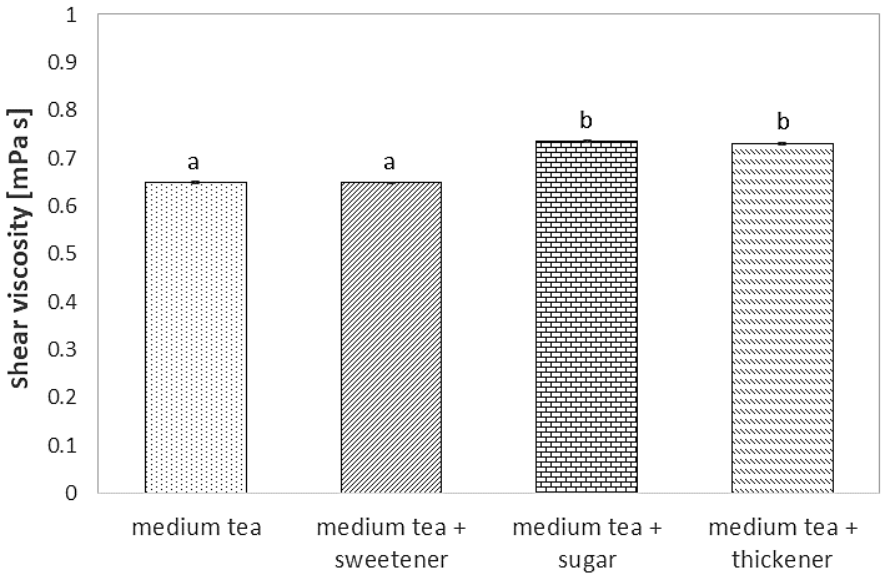



*Figure 3-9: Measured shear viscosity of medium tea samples (n = 3) at 50 $s^{-1}$. Samples sharing the same letter are not significantly different (Tukey PostHoc, $p<0.05$).*

Perceived thickness of the samples was checked using a rank-rating test and results are shown in Figure 3-10. The results showed that the tea with sweetener was not significantly different to medium tea without any added ingredients. However, medium tea with sugar was perceived to be significantly more "thick" than the viscosity matched tea with thickener. This suggests that perceived sweetness of the sample with sugar or sweetener can impact perceived thickness.

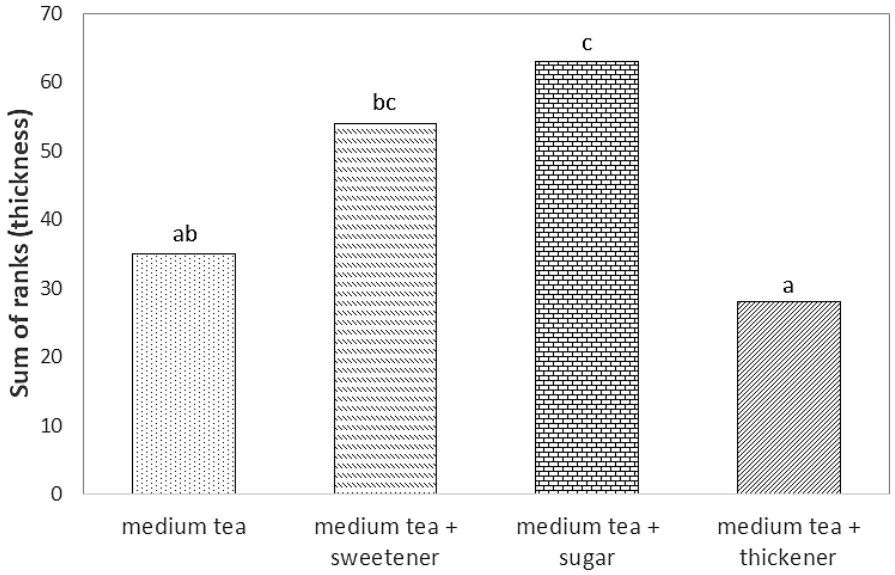



*Figure 3-10: Results of ranking test for 4 tea samples. The figure shows the sum of ranks and samples sharing the same letter are not significantly different (Tukey PostHoc p<0.05).*

The temporal perception of astringency and bitterness of the 4 samples was evaluated by the panel (results shown in Figure 3-11A & B). Although the samples were matched for viscosity, the addition of the thickener did not significantly alter the perception of astringency and bitterness, as can be seen in Figure 3-11A and B. However, the addition of a mixture of sweeteners decreased astringency to a similar level as 5% sugar addition. For bitterness, a similar result was observed. This indicates that perceived sweetness is the key factor driving the decrease in astringency and bitterness perception and not the concomitant increase in viscosity with sugar addition.

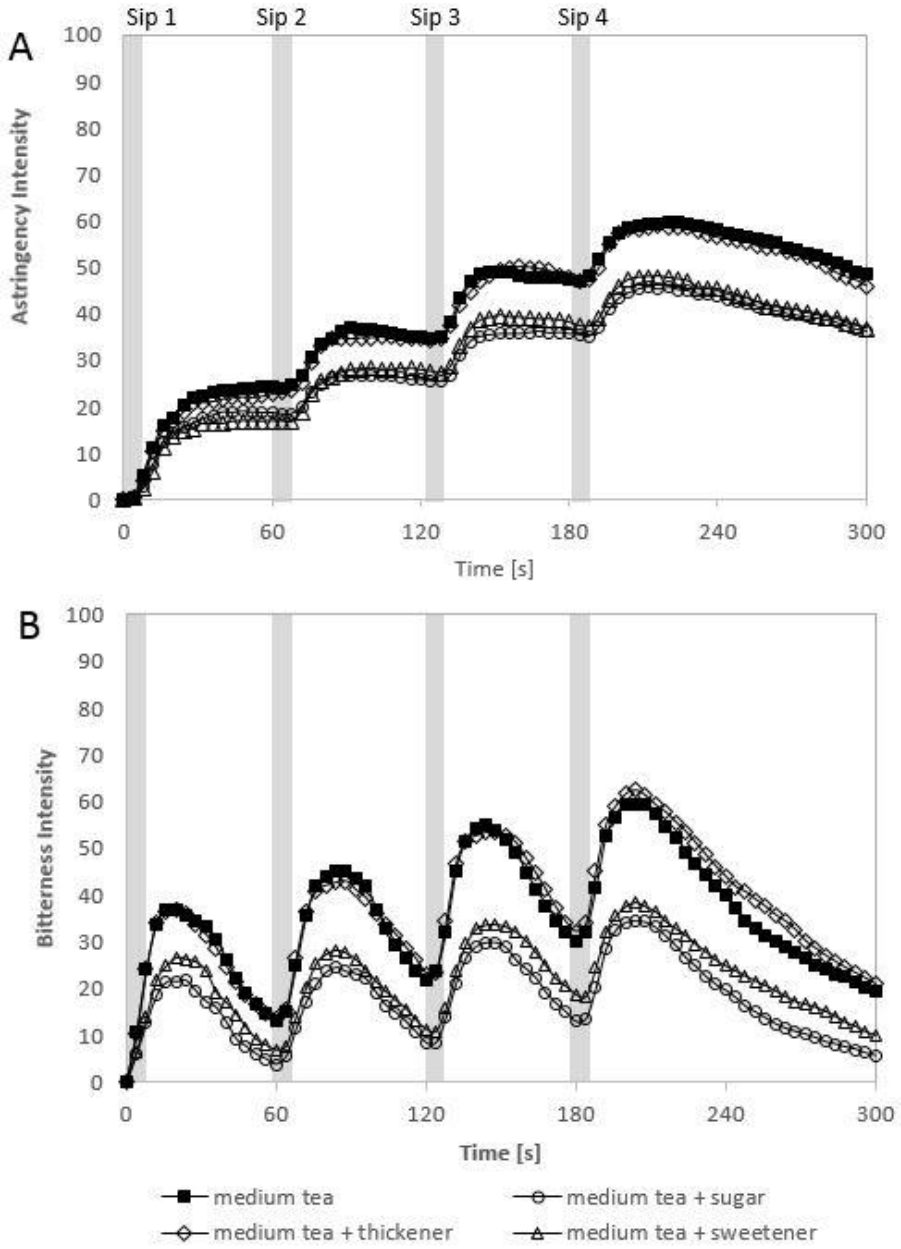



*Figure 3-11: The effect of sugar, thickener and sweeteners on the temporal profile of (A) astringency, (B) bitterness. The TI curve represents the average of all panellists (n = 9) across 4 replicates. Sips were taken at 0s, 60s, 120s and 180s as indicated by vertical grey bars; Intensity = intensity of the attribute (% of the graphical scale). See Table 3-4 and Table 3-6 for Post-hoc groupings.*

## 3.4 Discussion

### *3.4.1 The Impact of Tea strength on temporal Perception of Astringency and Bitterness*

The key finding of this study is that the temporal profiles for the twin-sensations astringency and bitterness are remarkably different. For astringency, perception increased to a maximum intensity and plateaued

whereas for bitterness once the maximum intensity was reached, a sharp and quick decrease in bitterness perception was observed. Increasing and decreasing angle indicate the rise and fall in perception for attributes. It was found that both were smaller for astringency than for bitterness. This shows that bitterness is perceived faster but also fades quicker than astringency. The difference in this temporal perception might lie in the different mechanisms through which both attributes are perceived. Bitterness is perceived by stimulating taste receptors whereas astringency is thought to be a tactile sensation whereby mechanoreceptors are stimulated (Ma et al., 2014). The findings of this study indicate that either the mechanoreceptors are stimulated longer than the taste receptors or that the brain adapts faster to the sensation of bitterness than to astringency resulting in a longer perception of astringency in comparison to bitterness. It was also observed that with increasing number of sips, perceived astringency and bitterness increased in intensity. This build-up was found for each tea sample, however bitterness showed a smaller build-up than astringency, which further indicated different mechanisms.

The time to reach the maximum intensity ($T_{Max}$) was one parameter that discriminated between astringency and bitterness with $T_{Max}$ being reached quicker for bitterness than for astringency. This finding is in contrast with findings from Robichaud and Noble (1990) who observed that the maximum bitterness intensity of phenolic compounds in wine was reached later than the maximum astringency intensity (Robichaud & Noble, 1990). In their study, the panellists were asked to rate astringency and bitterness in the same session, which might account for the difference found here. In

the current study panellists were trained to focus on one attribute and this was the only attribute measured for every sample at the time.

### *3.4.2 The Impact of Fat on the temporal Perception of Astringency and Bitterness*

The addition of an oil-in-water emulsion to produce a fat content of 5% in the tea beverage led to reductions in astringency and bitterness perception for all tea levels with the greatest reduction being observed for samples with the greatest intensities of measured attributes (medium and high tea levels). The emulsion seems to interfere with the mechanisms of astringency and bitterness perception. Astringency is thought to be of a tactile nature and might arise because interactions between salivary proteins and polyphenols increase friction. Fat droplets might interfere with the formation of the precipitates and thereby reduce astringency. Furthermore, it is also possible that fat droplets reduce the arising friction by forming a layer between tongue and palate, which could cause a mouth coating layer. Besides the fat content, it is also possible that the emulsifier used in this study (sodium caseinate) affected astringency perception. In a study by Ares et al. (2009) it was observed that milk with 0% fat reduced perceived astringency to a similar level as a milk with 3.2 % fat. They suggested that this was down to milk proteins interacting with the polyphenols of the investigated plant extracts. The formation of complexes between polyphenols and milk proteins leads to a reduction in polyphenols available to interact with salivary proteins, ultimately leading to a reduced astringency (Ares et al., 2009). This mechanism might explain why

astringency perception is reduced by the addition of an oil-in-water emulsion with sodium caseinate as emulsifier.

Several approaches are commonly proposed to account for bitterness suppression in foods (Keast, 2008): physio-chemical interactions in food, oral peripheral physiological interactions with receptor cells and central cognitive mixture suppression (via taste-taste interactions). Keast (2008) modified the bitterness of caffeine solutions using e.g. milk with different fat content (0, 2, 4 %). They observed a significant decrease in perceived bitterness between caffeine solutions containing 0 and 4 % fat milk (Keast, 2008). It is well known that fat emulsions reduce bitterness: a study used quinine-HCl, a lipophilic bitter compound, and observed a partition into the fat phase which reduced the concentration in the aqueous phase, resulting in a decrease in bitterness perception (Metcalf & Vickers, 2002). However, other work (Ares et al., 2009) has suggested that the reduction in bitterness through the addition of a fat emulsion can be explained by the interactions between milk protein and polyphenols (Ares et al., 2009). Like the reduction of astringency through the addition of an emulsion, the reduction in bitterness might be caused by several mechanisms: an interaction between polyphenols and proteins but also a reduction in friction due to the droplets itself.

### *3.4.3 The Impact of Sucrose, Thickener and Sweetener on the temporal Perception of Astringency and Bitterness*

The addition of 5% sucrose to tea reduced astringency and bitterness, however this reduction was greater for bitterness than for astringency where the reduction was not significant ($p>0.05$). The reduction of

bitterness through the addition of a sweetener or sugar is well known. It has been reported that this reduction is caused by a central cognitive effect (Keast, 2008; Keast & Breslin, 2002).

The fact that sucrose reduces astringency seems less understood and more complex. Valentovà et al. (2002) investigated temporal astringency perception of wine, tea and other beverages during ingestion and for up to 100 s after swallowing. The impact of sucrose on astringency of red wine was investigated and it was shown that 6% sucrose reduced perceived astringency over the whole intensity range. This previous work carried out by Valentová et al. (2002) is in agreement with the results observed in our study, where 5% sucrose reduced astringency for different tea strengths. The mechanism behind the reduction of astringency through sugar appears to be complex. Lyman and Green (1990) proposed that an increase in salivary volume caused by sugar leads to the reduction of astringency. This could be explained by three mechanisms (Lyman & Green, 1990): (i) increased saliva secretion means additional availability of proteins which might reverse the precipitation process of polyphenols and salivary proteins, (ii) increasing saliva flow helps to wash away polyphenols in the oral cavity and/or provides new proteins to replace the precipitated salivary proteins, (iii) sucrose increases the viscosity and provides lubrication to help mask astringency. The impact of the third proposed mechanisms was investigated by the addition of a thickener to tea. The results showed that increased viscosity had no impact on astringency and bitterness perception, indicating that this mechanism does not explain the impact of sucrose on astringency and bitterness. However, the non-existing impact of viscosity increase on astringency and bitterness is in contrast to

observations made by Smith et al. (1996) who found that increased viscosity yielded in a significant decrease in maximum intensity and total duration of astringency, although maximum intensity and duration of bitterness were not affected. In their study, Smith et al. (1996) increased the viscosity of grape tannin solutions from 2 to 45 mPa·s which is a much larger increase in viscosity than in the here presented study (viscosity of tea/tea with sweetener: 0.65 mPa·s, viscosity of tea with sugar/ thickener: 0.73 mPa·s), which might explain the different observations on astringency and bitterness perception.

It needs to be considered that although the tea with dextran was viscosity-matched to tea with sucrose, perceived thickness between these two samples differed and tea with sweetener was perceived to be of similar thickness as tea with sucrose, whereas tea with dextran was perceived to be thinner. This suggests that sucrose and sweeteners increase perceived thickness, whereby sucrose also increases the thickness in a physical manner whereas the increase in perceived thickness through sweeteners might be due to a logical error. Understanding this phenomenon is difficult and to our knowledge, only one study has investigated the effect of sugar/sweeteners on perceived thickness (Theunissen & Kroeze, 1995). Unfortunately, the results of that study were inconclusive, with one experiment indicating that sugar and sweeteners decreased perceived viscosity whereas the other experiment found no effect (Theunissen & Kroeze, 1995).

The addition of the sweeteners proved to be an effective way of reduction astringency and bitterness to the same extent as 5% sucrose. This proves that the addition of a sweetener can replace sugar and help to create low-

sugar products. Lyman and Green (1990) used aspartame to match the sweetness of sucrose and investigated the effect of both sugar and aspartame on astringency and bitterness perception. They found that sucrose reduced astringency and bitterness of tannic acid more effectively than aspartame and that aspartame had no impact on salivary flow. They therefore hypothesised that sucrose reduced astringency and bitterness due to an increased salivary flow or viscosity increase. The results of our study show that both sucrose and artificial sweeteners reduced astringency and bitterness in a similar manner, and therefore an increase in salivary flow caused by sucrose may not be the sole reason for this observation. We hypothesise that the sweetness of sucrose and sweeteners is the main reason for a reduction in astringency and bitterness. Furthermore, this seems to be due to cognitive progress (taste-taste or cross-modal interactions) rather than physical interactions.

### *3.4.4 The Impact of Fat & Sugar on the temporal Perception of Astringency and Bitterness*

The addition of 5% fat and 5% sugar, added combined, had the greatest impact of astringency and bitterness, in comparison to adding fat or sugar separately. Our study suggests that fat reduces astringency and bitterness through a physical mechanism, e.g. an interaction between polyphenols and proteins; whereas sugar seems to reduce the perception of both attributes by a cognitive mechanism. Both mechanisms work together to reduce astringency and bitterness.

The observed impact of fat & sugar on astringency and bitterness was compared to a calculated impact of fat & sugar (calculated from the

observed impact of fat and sugar) added separately. The effects of fat & sugar on astringency and bitterness were not consistent across the three evaluated tea levels and therefore it is difficult to conclude if fat and sugar when added together have an additive effect.

## 3.5 Conclusions

In this study, the impact of fat and sugar added at 5% at varying levels of tea (low, medium, high) on the temporal perception of astringency and bitterness were evaluated, using a multi-sip temporal method. During the consumption of 4 sips of tea, astringency and bitterness increased with increasing tea level. Although both attributes showed build-up over sips, the temporal profile attained clearly distinguished between these so called "twin sensations". Astringency increased until a maximum intensity was reached and then plateaued until the next sip was consumed; whereas bitterness increased sharply but was followed by a steep decrease until the next sip was consumed. Sugar and fat reduced both astringency and bitterness intensity resulting in significant decreases in $I_{Max}$ and AuC parameters. The addition of fat & sugar showed the greatest decrease in astringency and bitterness perception, however this effect only seemed to be additive for astringency. In order to understand the impact of sugar on perception, samples with thickener and sweeteners were evaluated and it was shown that sweeteners decreased the perception of astringency and bitterness as effectively as sugar, whereas the addition of a thickener did not impact the temporal perception of both attributes. This indicates that sugar reduces astringency and bitterness via a cross-modal and taste-taste

interaction related to sweetness delivery rather than a physical interaction, related to viscosity.

In addition to the impact of varying key ingredients, the difference between individual sips for low, medium and high tea levels were investigated. To the author`s knowledge there is no published data showing the temporal profile of astringency and bitterness over multiple sips of tea. For both attributes a clear build-up over the consumption of four sips was observed with the build-up for astringency being greater than for bitterness. The data also gives an insight in how the consumption of tea of low astringency or bitterness can increase over time.

This study has increased the understanding of the temporal perception of astringency and bitterness in black tea beverages which can be used to develop products with reduced fat and sugar content whilst minimising the perception of bitterness and astringency which are often thought of as negative attributes to consumers. Fat emulsions seemingly reduce the perception of astringency and bitterness in tea by a physical mechanism, but more research is required to understand the exact mechanism which will further inform how fat-replacements in beverages can be achieved successfully. Measuring the temporal perception of black tea beverages over multiple sips has given insight into the perceptual changes of astringency and bitterness as possibly perceived when consuming a cup of tea.

# 4 Using a force measuring Plate to understand the frictional and vibrational Differences between Tea Samples

## 4.1 Introduction

The results of the sensory study presented in Chapter 2 showed that mouthfeel attributes such as astringency, mouth coating, slipperiness and thickness are important for black tea beverages. In order to further understand mouthfeel, the frictional and vibrational behaviour of different tea samples were explored using a force measuring plate. Method development, results and discussion in context of the sensory data are presented in this chapter.

Astringency is a characteristic mouthfeel of polyphenol-rich products such as red wine and tea (Bate-Smith, 1954). It is commonly described as drying, roughing and a puckering sensation felt in the mouth which reduces the ability to lubricate – leading to a perception of increased oral friction (Gawel et al., 2001; Rossetti et al., 2008; Upadhyay et al., 2016). The word "astringent" comes from the Latin *ad stringere*, meaning "to bind" (Joslyn & Goldstein, 1964) and refers to the ability of astringent compounds to bind and precipitate proteins. It has widely been accepted that astringency is a tactile phenomenon (Breslin et al., 1993) but the mechanism behind how astringency is elicited still remains unclear. A common explanation for the perception of astringency is that this tactile sensation emerges due to crosslinking of polyphenols with proline-rich salivary proteins found between and above the epidermal cells of the

mucosal tissue in the mouth which leads to an increase in oral friction that is sensed as an increase in roughness (Rossetti et al., 2008).

Since it is hypothesised that astringency is related to an increase in oral friction, potential links between perceived astringency and a rise in friction have been investigated in several studies (Brossard et al., 2016; Rossetti et al., 2009; Vardhanabhuti et al., 2011). As mentioned previously, friction is the force that resists the relative motion of two surfaces sliding against each other, such as tongue and palate. There are three empirical laws that describe friction between dry surfaces. Amonton's laws (Bhushan, 2013) state that the force of friction is directly proportional to the applied load and independent of the apparent area of contact. A useful parameter to characterise the forces between two dry surfaces is the friction coefficient, μ. It is calculated as the ratio of the friction force between two surfaces and the force pressing them together (normal load) (see chapter 1 section 1.2.5.2) and therefore the effect of load is eliminated. Coulomb's law of friction states that kinetic friction, the friction between moving surfaces, is independent of the sliding velocity. The friction coefficient is dependent on the roughness of the surface and the geometric nature of surface asperities, with rough surfaces resulting in a higher friction coefficient than smoother surfaces (Prakash et al., 2013). In the presence of a lubricant, the friction coefficient is an effective indication of lubrication between the two interacting surfaces; when a lubricant is present, the friction coefficient is no longer a constant but a function of surface properties, surface load, moving speed as well as the lubricant properties, for example viscosity (Prakash et al., 2013). Three distinctly different lubrication regimes are known: the boundary regime, the mixed regime and the hydrodynamic

regime. In the boundary regime, the two surfaces are partially in contact with each other and an "immobile" layer of lubricant covers them. In this regime the friction coefficient is not dependent on load, speed or lubricant viscosity (de Hoog et al., 2006). In the mixed regime, the gap between the two surfaces increases due to the entrainment of some lubricant and subsequently the friction coefficient decreases. At high velocity or low load the hydrodynamic regime is reached, and the lubricant film fully separates the two surfaces and the friction coefficient increases. In order to transition from the boundary regime to the mixed regime, a decrease in load or an increase in speed is required, which encourages the entrainment of fluid.

In the case of beverage consumption, the boundary and mixed regimes have been identified as the most relevant regimes (Malone et al., 2003). It was argued that most mouthfeel attributes are perceived when rubbing the tongue against the palate which creates a small gap between both surfaces. However, some mouthfeel attributes such as thickness would be driven by the rheological properties of the sample, thus the hydrodynamic regime would be more relevant since in this regime surface properties are neglectable. Rossetti et al. (2009) investigated the impact of the tea polyphenols epi-gallocatchingallate (EGCG) and epi-catechin (EC) on the lubricating properties of saliva in the boundary regime at a sliding speed of 5 mm/s. In the experiment by Rossetti et al. (2009), 100 µl of saliva was adsorbed to PDMS substrates and a Mini Traction Machine was used to perform the tribological measurements. The Mini Traction Machine uses a ball-on-disc contact to create a sliding contact between two surfaces at various speeds and loads, and measures the frictional forces between the ball and the disc. Rossetti et al. (2009) found a significant increase in

friction coefficient with EGCG due to depletion of the lubricating proteins. However, EC did not alter the lubricating properties of the salivary film, even though it was perceived to be similarly astringent (Rossetti et al., 2009). They therefore concluded that the depletion of salivary proteins is not the only factor contributing to astringency perception and that astringency is unlikely to be a purely tactile percept (Rossetti et al., 2009). Vardhanabhuti et al. (2011) used the same experimental set up as Rossetti et al. (2009) to investigate the impact of β-lactoglobulin on the lubricating properties of saliva. They applied two different load and speed regimes: 1 N and 5 mm/s, and 3 N and 20 mm/s. β-lactoglobulin at pH 3.5 and 7.0 increased the friction coefficient in a manner which was related to astringency perception. Increasing β-lactoglobulin concentration at pH 3.5 caused a rapid increase in friction. At the highest concentration of 10%, however, the friction coefficient was higher than for water but below the values observed at lower protein concentrations. They concluded that tribology tests are not representative of the dynamic in-mouth system and that a simple relationship between friction coefficient and astringency perception cannot be found for all conditions (Vardhanabhuti et al., 2011). Finally, Brossard et al. (2016) used a purpose-built tribometer to investigate possible correlations between the friction coefficient and perceived astringency of different wines. The tribometer was built using a commercial texture analyser with additional fittings to create a ball-disc contact (Chen et al., 2014). At sliding speeds ranging from 0.025 to 10 mm/s they found a correlation between friction coefficient and astringency perception for four types of wine mixed with saliva (1:1) at low sliding speed (0.075 mm/s). This appears to be the only published study reporting

a positive correlation between friction coefficient and astringency perception.

In a bid to gain further insights into how friction and vibration parameters are linked to perceived mouthfeel and the effect of tea, fat and sugar on the measured parameters, a "force plate" was used to record the frictional and vibrational behaviour of different tea samples. The force plate was developed by Andrew Hopkinson (Unilever PLC) to measure the forces and torques acting on a plate caused when a finger moves across the surface of a sample mounted on a plate. From these measurements, the position and normal load of the finger on the surface, as well as lateral friction forces were calculated. The force plate is designed to measure vibrations related to touch perception. Hopkinson et al. (2008) have previously used the force plate to explore the friction forces related to aspects of tactile perception in sensory studies of different creams. In this PhD research, 18 samples containing different levels of tea, fat and/or sugar were evaluated. Samples were mixed with saliva and rubbed across a textured Bioskin with the index finger at different speeds and loads and results are discussed in the context of the sensory data on these samples (see Chapter 2 and 3).

## 4.2 Materials and Methods

### *4.2.1 Force Plate*

*4.2.1.1 Set-up and Principle of Operation*

The force plate used in this study was operated as follows: a sheet of Bioskin, a synthetic skin, (140 mm x 50 mm) was placed on the force plate which recorded the forces and torques exerted. The Bioskin (Beaulax Co, Japan) was made from polyurethane and is commonly used for skin-care

and make-up testing. Using this synthetic skin as one of the two surfaces is thought to give friction readings which are more representative to the friction present in the mouth compared to steel or glass surfaces commonly used for tribological measurements. Steel and glass surfaces have smooth surfaces which are different to the textured oral surfaces. The Bioskin is not smooth but somewhat textured and is hypothesised to represent the oral surfaces in a better way. The average surface roughness of the Bioskin is 13 μm (data provided by Simon Johnson, Unilever PLC). The Bioskin is therefore smoother than the surface of the tongue which was found to have surface roughness of 65 μm for healthy adults (Uemori et al., 2012), however, the Bioskin is less elastic than the tissue of the tongue and therefore it might be more appropriate to compare the Bioskin to the tissue of the hard palate. The fingertip used as the second surface in this experiment is more elastic than the Bioskin and might therefore be more similar to the tongue.

For the assessment, a subject spread a liquid sample, such as tea in this research, across the Bioskin followed by rubbing at various speeds and loads using their index finger (see Figure 4-1). A screen was used to provide live feedback about the speed and load applied to guide the subject to populate the evaluation space appropriately. A load range of 0.05 to 2 N and a speed range of 20 to 200 mm/s were covered during the sample assessment. It has been shown that the tongue moves at speeds of up to 200 mm/s and can apply normal loads from 0.01 N up to 90 N (Stokes et al., 2013), however the pressure distribution is not uniform and some parts of the tongue may exert high loads while others may not be in contact at all. Prinz et al. (2007) used loads varying from 0.34 to 2.20 N and speeds

varying from 0.1 to 700 mm/s in a study where they investigated the load dependency of the friction coefficient of the oral mucosa. The speed and load range used in this PhD research also represents a range that is possible to cover when rubbing with a finger. Covering lower or higher loads and speeds was less reproducible.



*Figure 4-1: Force plate with Bioskin, as used for sample assessment*

The measuring plate of the force plate was connected to six force transducers and was calibrated at the beginning of the study. During calibration the responses of all six force transducers to each of six different known forces and torques was recorded. Known forces and torques were applied by either placing weights on the force plate or by hanging weights on a thread over a pulley to apply defined horizontal forces. The 36 responses were transformed into a six-by-six calibration matrix using matrix inversion. During each actual measurement the calibration matrix was used in real time to convert the six measured voltages from the transducers into three forces ($F_x$, $F_y$, $F_z$) and three torques ($T_x$, $T_y$, $T_z$) (Hopkinson et al., 2008) that are shown in Figure 4-2.

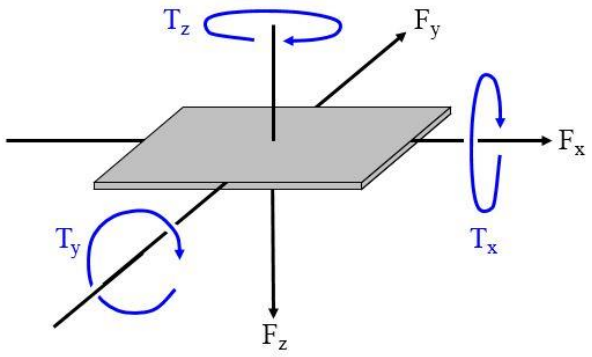



*Figure 4-2: Illustration of measured forces and torques during force plate analysis where Fz = force in the vertical axis (= load) and Fy and Fx the force in the horizontal axes. Source: Greenaway (2010).*

The position of the subject´s finger from the centre of the plate was calculated by using the normal load ($F_z$) and the torque data from the horizontal axes ($T_x$ & $T_y$) (Hopkinson et al., 2008), since the position can be calculated by dividing the torque data by the normal load (see Equations 4 & 5 ).

$$x\,[m] = \frac{T_y\,[Nm]}{F_z[N]} \tag{4}$$

$$y\,[m] = \frac{T_x\,[Nm]}{F_z\,[N]} \tag{5}$$

The speed, defined in Equation 6, at which the finger was moved was then calculated from the derivative of the position with time (Greenaway, 2010).

$$v = \frac{\Delta s}{\Delta t} = \frac{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}{(t_2 - t_1)} \tag{6}$$

Friction was defined as the magnitude of the force in the xy-plane and calculated as:

$$Friction = \sqrt{F_x^2 + F_y^2} \tag{7}$$

In addition to friction, vibrations were measured using proprietary sensing methods. The vibration data was filtered into medium frequency (88 – 354 Hz) and high frequency (354 – 1414 Hz) bands. Each of the frequency bands covered two octaves and were chosen to cover the range which is audible and in which the mechanoreceptors are active.

### *4.2.2 Materials*

Tea samples were prepared in advance according to the description in chapter 2, section 2.2.2. The oil-in-water emulsion used, to add fat to the tea, was prepared following the description in chapter 2, section 2.2.2. Tea samples with sweetener or thickener were prepared according to the description in chapter 3, section 3.2.1.1.

Aqueous solutions of quinine (Fisher, UK) and tannic acid (Sigma, UK) were made by mixing the appropriate amount of quinine or tannic acid with distilled water until the powdered substances were completely dissolved.

### *4.2.3 Samples*

The samples used in the force plate study were selected from the set of samples used in the Time-Intensity evaluation (see chapter 3, section 3.2.2) to enable comparison between measured parameters such as friction and vibration amplitude with perceived astringency. These samples were also part of the experimental design space in the mouthfeel lexicon study (see chapter 2) therefore allowing the sensory scores of other mouthfeel attributes, for example mouth coating and thickness, to be compared to instrumental data. Further samples were also included to help

understand the impact of fat and sugar for example 10% fat or sugar with medium tea (0.55%). To investigate if predominantly bitter and predominantly astringent samples would cause different friction forces and vibration amplitudes, an aqueous quinine solution (0.15%) and an aqueous tannic acid solution (0.075%), respectively, were added to the sample set. All samples used in the main force plate study, conducted after the pilot experiment described in the next section, are shown in Table 4-1.

*Table 4-1: Samples assessed in the force plate experiment. Each sample was prepared to a weight of 100 g.*

| Tea [%] | Fat [%] | Sugar [%] |
|---|---|---|
| 0.1 | 0 | 0 |
| 0.1 | 5 | 0 |
| 0.1 | 0 | 5 |
| 0.1 | 5 | 5 |
| 0.55 | 0 | 0 |
| 0.55 | 5 | 0 |
| 0.55 | 10 | 0 |
| 0.55 | 0 | 5 |
| 0.55 | 0 | 10 |
| 0.55 | 5 | 5 |
| 1.0 | 0 | 0 |
| 1.0 | 5 | 0 |
| 1.0 | 0 | 5 |
| 1.0 | 5 | 5 |
| 0.55 | 1.2 % Dextran | |
| 0.55 | Sweetener | |
| 0.15 % Quinine | | |
| 0.075 % Tannic acid | | |

## 4.3 Sample Assessment and Protocol

### *4.3.1 Pilot Experiment*

A pilot experiment was first conducted to assess whether the force plate was a suitable equipment to evaluate the friction and vibration behaviour of tea samples. In this short study, five tea samples (0.1% tea, 0.1% tea with 10% fat, 1% tea, 1% tea with 10% fat, 1% tea with 10% sugar) were assessed without saliva by two subjects in four replicates. The five samples

chosen represented the lowest and highest tea levels used in this research as well as containing high levels of fat or sugar as extremes of the variables of interest.

First, 1 mL of sample was added onto the Bioskin with an Eppendorf pipette and spread over an area of 140 mm x 50 mm for 15 s by the subject with the index finger of their dominant hand and no data was collected during this period of spreading. Before starting each measurement, baseline force, torques and vibrations were measured during which the subject did not touch the force plate or moved. The baseline force and torques were subtracted from the subsequent data. After 5 s of baseline measurement, the sample was rubbed with the index finger at different speeds and loads for 100 s. Load and speed were varied so as to cover a load range of 0.05 to 2 N and a speed range of 20 to 500 mm/s which is identical to the load and speed range used in the later main force plate study.

### *4.3.2 Saliva/Tea Ratio Determination*

Salivary proteins are thought to play a key role in astringency perception and interact with polyphenols as discussed in section 1.2.4.1; therefore, saliva was added to the tea samples in the main study to better replicate the actual in-mouth situation, while the pilot study was conducted without saliva. Whole unstimulated saliva was collected from one individual using the drooling method (Navazesh & Kumar, 2008). The subject sat still, leaned their head forward and provided saliva in a tub for 15 min. No food was consumed for 2 h prior to saliva collection. Saliva was stored on ice and used for a maximum of 2 to 3 h. The assessor used their own saliva to carry out the force plate measurement.

The ratio between tea and saliva was selected based on the following experiment. 0.1% tea and unstimulated whole saliva were mixed on the Bioskin at a ratio of 5:1 (0.5 mL tea and 0.1 mL saliva) using a pipette tip and compared to 1% tea mixed with saliva at the same ratio by rubbing both samples with the index finger of the left and the right hand. Both tea samples perceived as similarly rough and there was no difference in audible noise detected by the assessor during the rubbing. This indicated that with this level of saliva it might not be possible to distinguish between the two extreme tea levels used in the sample set. The experiment was repeated using lower ratios of tea to saliva: 5:2 (0.25 mL tea and 0.1 mL saliva) and 7:2 (0.35 mL tea and 0.1 mL saliva). It was possible to feel differences with both ratios whereby the mixture based on 1% tea was perceived as rougher. Furthermore, flocculation or haze, indicating an interaction between polyphenols and salivary proteins (Horne et al., 2002), was observed at the mixing ratio of 5:2 with 1% tea but not with 0.1% tea. Conversely, when the experiment was carried out using the 7:2 mixing ratio, no flocculation or haze was observed. Therefore, since a flocculated microstructure has previously been reported for expectorated tea samples (Nayak & Carpenter, 2008), the lower mixing ratio of 5:2 (0.25 mL tea and 0.1 mL saliva) was chosen for the main force plate experiment.

### *4.3.3 Evaluation Protocol*

Tea samples were prepared 30 min before the start of the experiment to a mass of 100 g. In total, 6 replicates per tea sample were measured, with 2 replicates per sample analysed in one session. During one session, 6 - 7 different tea samples were evaluated in duplicate, resulting in 12 – 14

experiments per session, using the same saliva collected prior. The sample order was randomised to avoid bias. Although the tea samples were prepared hot, since the force plate was not equipped to be heated, the tea samples cooled down to room temperature when they were rubbed across the Bioskin.

The evaluation protocol for the force plate experiment included 4 episodes comprising two combined dispensing and mixing episodes and two rubbing episodes, lasting in total 240 s (see Figure 4-3 for schematic illustration). During the first combined dispensing and mixing episode, 0.1 mL of saliva was pipetted onto the Bioskin followed by 0.25 mL of sample. Saliva and sample were then mixed with the index finger of the dominant hand and spread across the Bioskin for 30 s followed by the first 90 s rubbing episode for data collection. During the first rubbing episode, the subject moved the index finger of the dominant hand across the Bioskin at varying speeds (20 – 500 mm/s) and loads (0.05 – 2 N). In the second dispensing and mixing episode a further 0.25 mL of sample was added onto the Bioskin. The sample was mixed with the index finger of the left hand and spread across the Bioskin for 30 s before the second rubbing episode and data collection period was started. The same protocol as for the first rubbing episode was followed. The second rubbing episode, which included addition of further tea but not saliva, was introduced to investigate if the instrumental parameters were affect by sample volume.

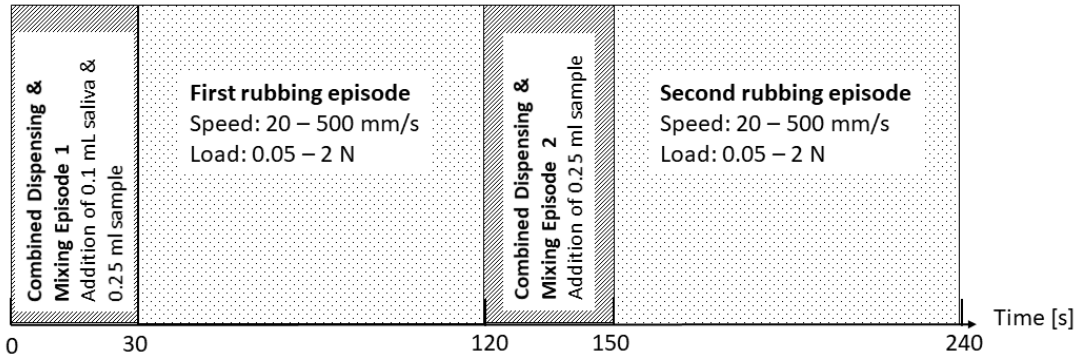



*Figure 4-3: Schematic overview of the force plate protocol used for each sample*

After each assessment the Bioskin was removed and thoroughly cleaned using warm water and a bactericidal dish detergent (Jeyes C41, Alliance UK) diluted with 5 parts water followed by drying using paper tissue before the next sample was assessed.

## 4.4 Data Analysis and Typical Data Plots

The protocol for checking the quality of data collected with the force plate equipment is introduced in this section together with the data processing method to generate graphs presented in the results section of this chapter. Furthermore, the results from the pilot study, validating the suitability of the force plate to analyse the friction properties of tea samples, are reported. The plots presented in this chapter were generated with the data visualisation software Tableau (Tableau Software Inc., version 10.1).

### *4.4.1 Data Quality*

Section 4.2.1.1 showed the calculation of the position of the finger on the force plate from force and torque data. As an example, Figure 4-4 shows the position data for one of the samples (0.55% tea 5% sugar, one

replicate) which is typical for samples assessed in this study. The different colours in the plot illustrate the two different rubbing episodes (orange = first rubbing episode, blue = second rubbing episode) and it is evident that the sample was spread across the majority of the Bioskin (visualised by the dotted rectangle in the plot). Furthermore, the finger did not touch the force plate outside the Bioskin since no data points were registered outside the boundary of the Bioskin.

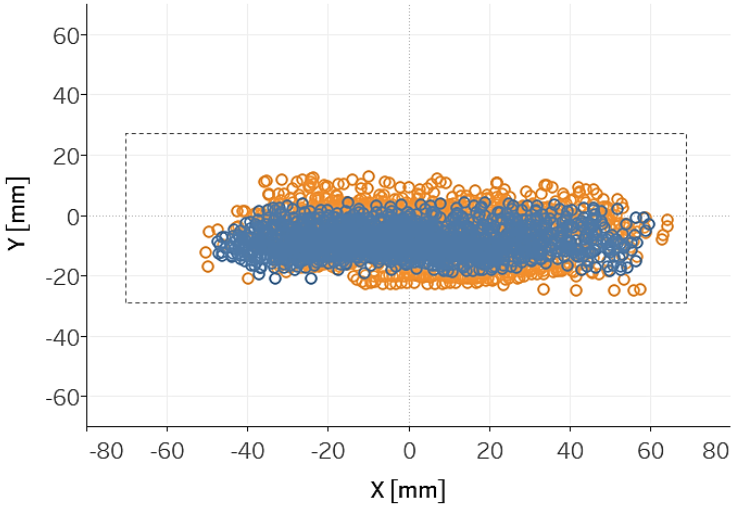



*Figure 4-4: Calculated position of the subject's finger (x, y) from the centre of the plate. The dotted line represents the area of the Bioskin over which the samples could be rubbed. Individual points represent data collected over an interval of 50 ms and were collected for one sample and one replicate. Orange = first rubbing episode, blue = second rubbing episode.*

Speed was calculated from the change of the position of the finger over time, so it was important that the position measured by the force plate was accurate. The load range used in this study included very low loads (down to 0.05 N). At these low loads it was possible that vibrations might affect the calculated position. This was checked by plotting an XY-plot for very low loads; 0.1 to 0.4 N. If the data followed a smooth motion as one would expect when the finger stroked in a continuous motion across the Bioskin,

the accuracy of the position was adequate. This was confirmed for all of the data acquired in this study.

Figure 4-5 shows a load versus speed plot for one of the samples (0.55% tea, one replicate). The load-speed range was well populated, which meant that there was enough data to be analysed. The grid lines indicate the data limits that were used in further analysis, therefore any data outside these boundaries was discarded. Data within the load range of 0.07 to 2 N and the speed range of 25 to 400 mm/s was analysed.

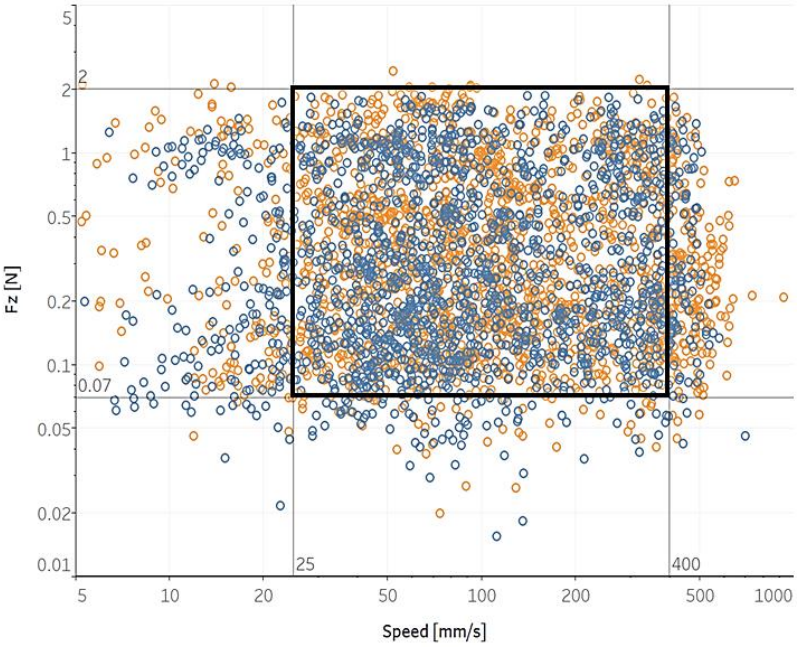



*Figure 4-5: Collected data for one sample (0.55% tea) and one replicate depending on load and speed. The data within the boundaries of the lines was used for the analyses (load range: 0.07 – 2N, speed range: 25 – 400 mm/s). Orange = first rubbing episode, blue = second rubbing episode*

The data was log-distributed, therefore all the dependent and independent variables were log-transformed before plane fitting was conducted. Plane fitting involved computing a plane that best fits the data points by minimising the sum of the quadratic distances between the plane and the

points, see Figure 4-6 for illustration. Proprietary data reduction methods were used to convert this large data set into subsets for further analysis. Friction, friction coefficient and vibration amplitude were then determined for five load-speed regimes (see Table 4-2) using plane fitting on the reduced data set. The first load-speed regime covered most of the load and speed applied whereas the other regimes focused on either low or high load/speed. Plane fitting was used to determine the value of a dependent variable, such as friction, friction coefficient and vibration amplitude, at certain values of independent variables, for example speed = 200 mm/s, load = 0.5 N. Least Square Regression was applied and was performed by computing the Eigenvalues and Eigenvectors associated with the distribution of the points using proprietary software.

*Table 4-2: Load-Speed ranges from which the main force plate data analysis was conducted.*

| Load-Speed Regime | Regime Name | Load [N] | Speed [mm/s] | Origin Load [N] | Origin Speed [mm/s] |
|---|---|---|---|---|---|
| 1 | General | 0.07 – 2 | 25 – 400 | 0.3 | 100 |
| 2 | Low Speed | 0.1 – 2 | 10 – 50 | 0.3 | 30 |
| 3 | Low Load | 0.05 – 0.15 | 20 – 500 | 0.1 | 100 |
| 4 | High Load | 1 – 2 | 20 – 500 | 1.5 | 100 |
| 5 | High Speed | 0.1 – 2 | 250 – 500 | 0.3 | 350 |

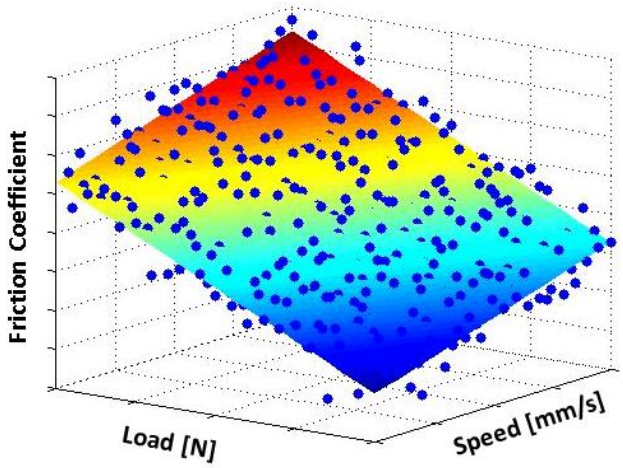



*Figure 4-6: Illustration of plane fitting for friction coefficient in dependency of load and speed. Source: https://uk.mathworks.com/matlabcentral/fileexchange/36353-planefit?s_tid=gn_loc_drop*

The data from the plane fits was then filtered and planes which were based on less than 8 data points were discarded. Means across replicates for friction, friction coefficient and vibration amplitude in different regimes were calculated and plotted on log-scales. Instead of plotting the data across a range of different loads and speeds using a scatter plot, data could now be plotted using a bar plot which shows the means of, for example, the friction coefficient for a selected load and speed using data from a certain load-speed regime (see Table 4-2).

Using the data from the pilot study, a 3-way ANOVA (sample, replicate, subject) with interactions followed by a Post-hoc test (Tukey) was performed to identify significant differences in friction coefficient or vibration amplitude between subject and samples. A 2-way ANOVA (sample, replicate) followed by Post-hoc testing (Tukey) was carried out on the data from the main experiment to identify significant differences in friction coefficient or vibration amplitude between samples. In order to identify linear correlations between mouthfeel perception and instrumental

parameters, Pearson correlation tests were carried out using XLSTAT (version 19.01). $R^2$-values range between 0 to 1 and a value closer to 1 indicates a stronger linear correlation between two parameters.

### *4.4.2 Typical Plots*

The aim of this section is to illustrate how the parameters measured with the force plate were impacted by different factors. The dependency of friction, friction coefficient and vibration amplitude on load and speed are discussed as well as the observed dependencies found across all samples. The data shown in this section was acquired in the pilot study and shows data of one subject unless stated otherwise. All of the plots shown in this section show the data before plane fitting, for a range of loads and speeds.

The relationship between friction and load, and friction and speed are illustrated in Figure 4-7A and B respecively. Figure 4-7A shows a strong dependency of friction on load; as, for all samples, with increasing load the friction increased. It was also observed that the friction differed across samples as, for example, 0.1% tea with 10% fat had a lower friction than the other two samples (0.1% tea and 1% tea with 10% sugar) across the whole load range. This is an indication that the force plate can be used to discriminate between tea samples of different composition. Figure 4-7B shows the friction data for the whole range of speed (20 – 400 mm/s) but for a limited range of load (0.5 – 1 N). Friction was strongly dependent on load, so plotting friction versus speed over a wide load range would increase the variation in the data. The plot shows that friction did not depend strongly on speed, since the friction only slightly decreased with

increasing speed. This is commonly observed as described by Bhushan (2013).

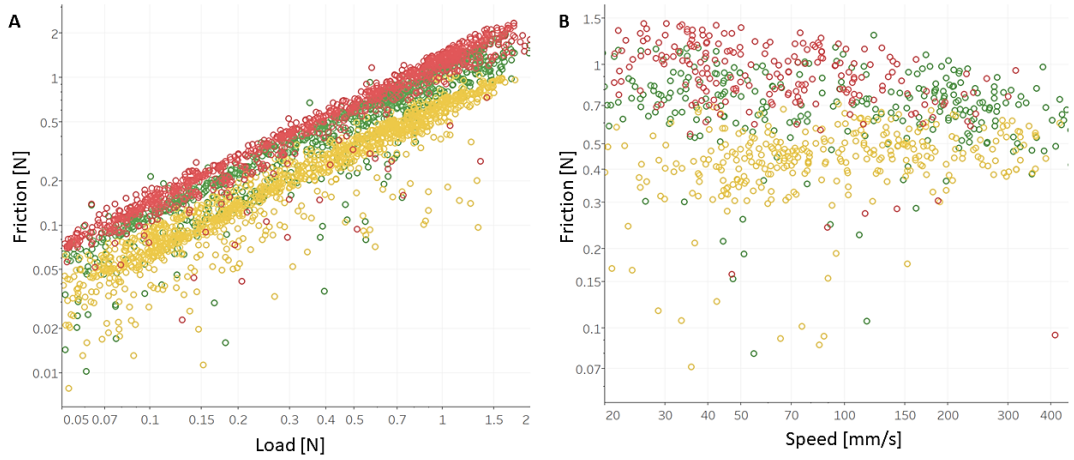



*Figure 4-7: Impact of (A) load and (B) speed on friction of 3 selected samples (yellow: 0.1% tea with 10% fat, red: 0.1% tea, green: 1% tea with 10% sugar. (B) shows data for a load range of 0.5 – 1 N. Raw data from one subject and one replicate of each sample.*

The friction coefficient is an important variable in tribological measurements and Figure 4-8A and B illustrate the impact of load and speed, respectively, on the friction coefficient. With increasing load the friction coefficient decreased by approximately 20% over the entire load range assessed for the selected samples (see Figure 4-8A). This 20% decrease is representative of other samples. Since the friction coefficient is calculated as the ratio of friction to load, a small decrease in friction coefficient with increasing load implied that friction increased at high loads. Given that the friction coefficient depended less on load than friction, it was more appropiate to visualise the effect of speed on friction coefficient than on friction as the variation due to load is reduced.

The impact of speed on friction coefficient seemed to be more complex (see Figure 4-8B). For two samples, 0.1 tea and 1% tea with 10% sugar,

the friction coefficient decreased with increasing speed by approximately 25% at all assessed speeds. However, for 0.1% tea with 10% fat the friction coefficient increased only at high speeds (> 100 mm/s). This could be an indication that samples passed through different lubrication regimes depending on the speed applied. The differences related to sample composition are discussed in the Results and Discussion section of this chapter.

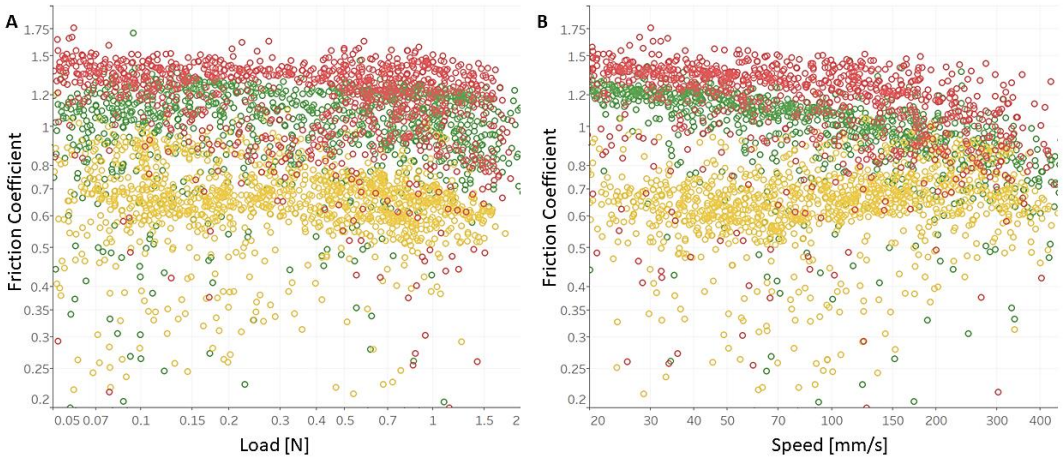



*Figure 4-8: Impact of (A) load and (B) speed on friction coefficient of 3 selected samples (yellow: 0.1% tea with 10% fat, red: 0.1% tea, green: 1% tea with 10% sugar). Raw data from one subject and one replicate per sample.*

The impact of load and speed on the vibration amplitude at high frequency (353 – 1414 Hz) can be found in Figure 4-9A and B, respectively. With increasing load, vibration amplitude increased for all of the samples, with little differences observed between samples. Furthermore, in comparison to the friction-load dependency, the vibration amplitude-load data was more scattered. This variation in the data was due to the vibration depending on both load and speed and, in the plot shown a wide range of speed was considered contributing to the variance in the data. However, the dependency of vibration amplitude on load was strong. The

dependency of vibration amplitude at high frequencies on speed was similar to the dependency on load and with increasing speed, vibration amplitude increased. At medium frequency (88 – 353 Hz) a similar dependency on load and speed was observed. High and medium frequency vibration amplitudes showed similar trends between samples (see Figure 4-10 as an example), only high frequency data are discussed in the Results and Discussion section.

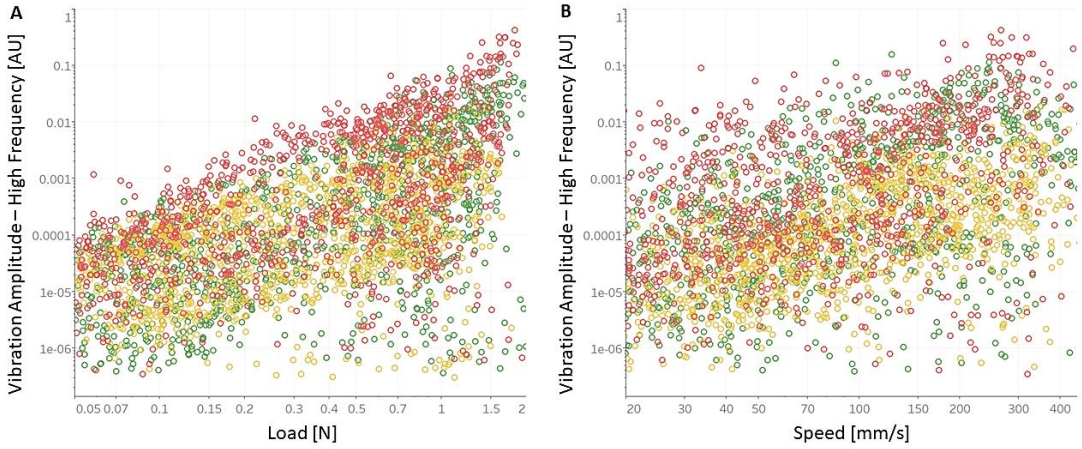



*Figure 4-9: Impact of (A) load and (B) speed on vibration amplitude (high frequencies) of 3 selected samples (yellow: 0.1% tea with 10% fat, red: 0.1% tea, green: 1% tea with 10% sugar). Raw data from one subject and one replicate per sample.*

*Figure 4-10: Illustration of similar trends between vibration amplitude of medium and high frequency*

### *4.4.3 Variation in Friction Data between different Individuals*

In order to understand if the friction and vibration amplitude data was dependent on the subject conducting the experiment, data from the two subject pilot study was analysed. The results shown in Figure 4-11 clearly demonstrate that subject 1 produced data with a lower friction coefficient than subject 2, for all of the five samples and over the whole load range (0.05 – 2 N). A 3-factor ANOVA (sample, replicate, subject) with interactions followed by Post-hoc testing (Tukey) confirmed that the differences between subjects were significant for all instrumental parameters measured, with subject 1 continuously producing lower value (see Table 4-3 for Post-hoc groupings). Both subjects conducting the pilot study used the index finger of their dominant hand whereby subject 1 was right-handed and subject 2 was left-handed. The consistently higher friction coefficient data generated by subject 2 might have been due to different skin topography and properties and the angle at which the finger was held when rubbing a sample across the Bioskin. However, the trends indicating differences between samples were the same for both subjects. It was also observed that the differences between samples were greater than the differences between the two subjects. The objective of this study was to understand and explore differences between samples and not focus on differences between subjects. Furthermore, the vast amount of data and the complex data analysis was outside the scope of this PhD; as, in order to carry out an experiment with a high statistical power, a larger number of subjects would have been required. Hence, the main study presented herein was carried out by one subject (subject 2).

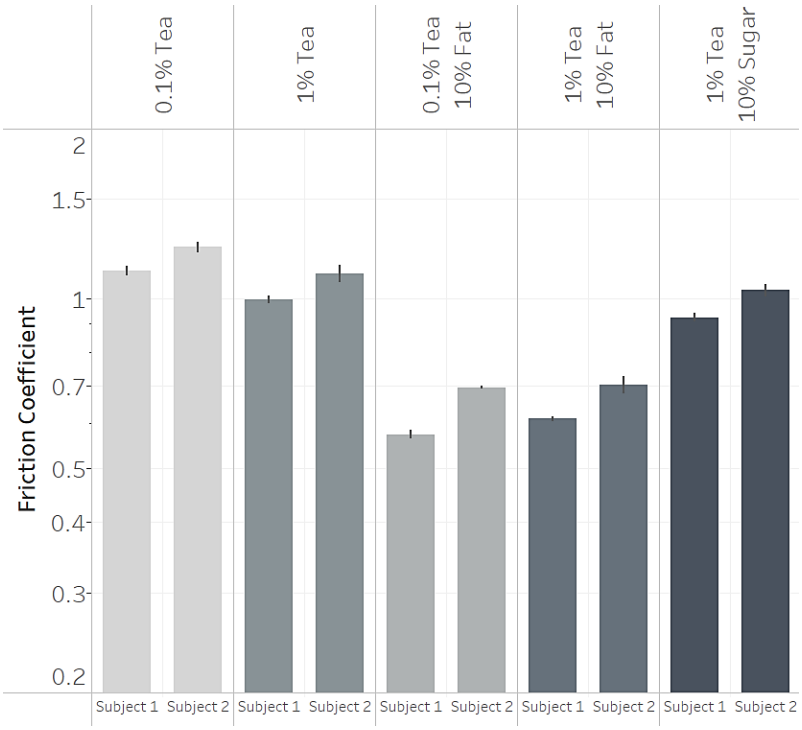



*Figure 4-11: Friction coefficient of different samples for two subjects. Data obtained from the general regime, without saliva addition and shows the mean data of 4 replicates. Error bars represent the standard deviation.*

*Table 4-3: Mean data from pilot study showing significant differences ($p<0.05$) between subjects. Significant differences were determined following a 3-way ANOVA (sample, replicate, subject) with Post-hoc testing (Tukey).*

| | Friction [N] | | Friction Coefficient | | Medium Frequency [AU] | | High Frequency [AU] | |
|---|---|---|---|---|---|---|---|---|
| **Subject** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| 1 | $0.261^{A}$ | 0.07 | $0.867^{A}$ | 0.23 | $3.38E\text{-}04^{A}$ | 1.64E-04 | $2.96E\text{-}04^{A}$ | 1.64E-04 |
| 2 | $0.306^{B}$ | 0.08 | $1.012^{B}$ | 0.25 | $7.30E\text{-}04^{B}$ | 6.72E-04 | $5.24E\text{-}04^{B}$ | 4.52E-04 |
| p-values (subject) | <0.0001 | | <0.0001 | | <0.0001 | | 0.018 | |

# 4.5 Results and Discussion

## *4.5.1 Impact of Ingredients on Friction Coefficient and Vibrational Amplitude*

### *4.5.1.1 Quinine, Tannic acid and Tea*

Figure 4-12 shows the mean friction coefficient for quinine, tannic acid and three tea levels (0.1%, 0.55% and 1% tea) for both rubbing episodes of the main study with saliva (see Table 4-4 and Table 4-5 for mean data and Post-hoc groupings). The friction coefficients shown in various figures in

section 4.5.1.1 to 4.5.1.4 were determined for the general regime (load range: 0.07 – 2 N, speed range: 25 – 400 mm/s), see section 4.4.1 for details. Quinine (0.15%) had the lowest friction coefficient and was significantly different ($p<0.001$) to all other samples. The friction coefficient for 0.1%, 0.55% tea and 1% tea was significantly higher than the friction coefficient of quinine but there was no significant difference ($p=0.324$) between the three tea levels, however there was a trend showing that medium and high tea have a higher friction coefficient than low tea. Tannic acid (0.075%) had the highest friction coefficient, which was significantly higher than quinine and 0.1% tea ($p=0.032$). It was observed that for all samples, friction coefficient increased slightly in the second rubbing episode. Since the change in friction coefficient between the first and second rubbing episode was very small for all beverage samples studied, only the data from rubbing episode 1 are discussed from here on.

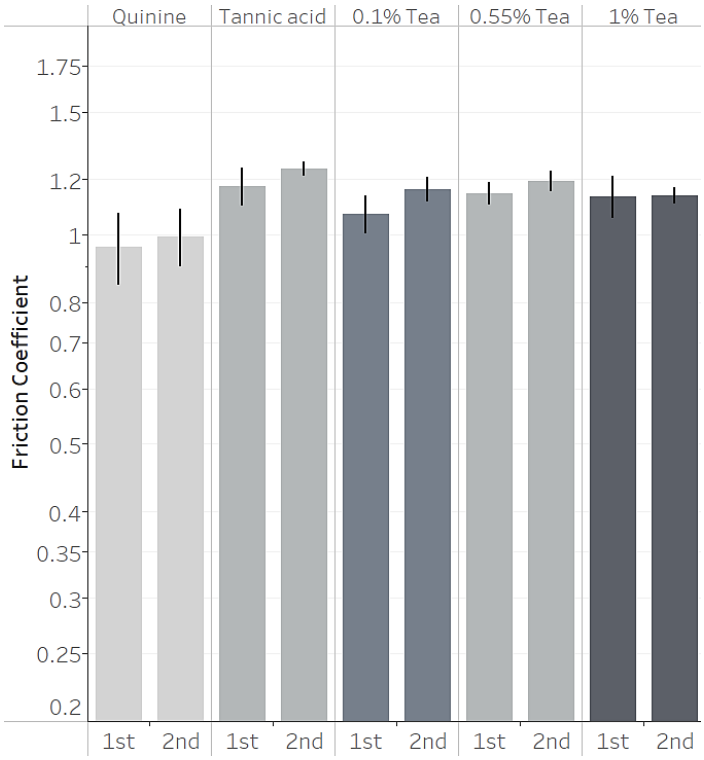



*Figure 4-12: Friction coefficients for quinine, tannic acid and different tea levels for both rubbing episodes. Data shows the mean friction coefficient of 6 replicates and error bars represent the standard deviation.*

The vibration amplitudes at high frequency for quinine, tannic acid and different tea levels are shown in Figure 4-13. Quinine had the lowest vibration amplitude, however unlike with the friction coefficient, tannic acid did not show the highest vibration amplitude. Tannic acid, medium and high tea concentrations had a similar vibration amplitude and the low tea concentration had the highest vibration amplitude. No significant differences ($p=0.063$) between the vibration amplitude of these samples was observed (see Table 4-4).

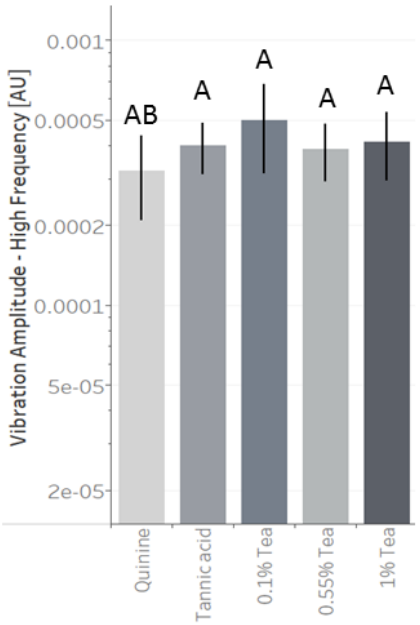



*Figure 4-13: Vibration amplitude (high frequency band) for quinine, tannic acid and different tea levels. Data shows mean vibration amplitude of 6 replicates from the first rubbing episode and error bars represent the standard deviation. Samples sharing the same letter were not significantly different (p>0.05).*

In order to understand differences in friction coefficient between samples, it is important to understand how the addition of saliva affected the friction coefficient and which lubrication regime prevailed. Figure 4-14 showing the friction coefficient plotted against load for 0.1% tea with and without saliva addition clearly demonstrates that in the load range investigated the friction coefficient for tea with and without saliva was similar.

*Table 4-4: Mean data from the first rubbing episode (6 replicates, with addition of saliva). Samples sharing the same letters are not significantly different (p>0.05) as determined by 2-way ANOVA (samples, replicates) with Tukey PostHoc*

| Sample | | | Friction [N] | | Friction Coefficient | | Medium Frequency | | High Frequency | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tea [%]** | **Fat [%]** | **Sugar [%]** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SE** |
| 0.1 | 0 | 0 | $0.321^{ABC}$ | 0.022 | $1.073^{ABC}$ | 0.067 | $7.21E\text{-}04^{A}$ | 2.28E-04 | $5.01E\text{-}04^{A}$ | 1.86E-04 |
| 0.1 | 5 | 0 | $0.216^{EF}$ | 0.011 | $0.718^{EF}$ | 0.037 | $2.57E\text{-}04^{B}$ | 4.42E-05 | $1.72E\text{-}04^{C}$ | 4.32E-05 |
| 0.1 | 5 | 5 | $0.209^{F}$ | 0.004 | $0.703^{F}$ | 0.013 | $3.14E\text{-}04^{B}$ | 1.23E-04 | $1.58E\text{-}04^{C}$ | 2.98E-05 |
| 0.1 | 0 | 5 | $0.306^{CD}$ | 0.014 | $1.028^{CD}$ | 0.041 | $7.91E\text{-}04^{A}$ | 1.40E-04 | $4.11E\text{-}04^{A}$ | 8.69E-05 |
| 0.55 | 0 | 0 | $0.343^{ABC}$ | 0.012 | $1.149^{AB}$ | 0.043 | $6.53E\text{-}04^{A}$ | 6.75E-05 | $3.88E\text{-}04^{A}$ | 9.49E-05 |
| 0.55 | 10 | 0 | $0.217^{EF}$ | 0.015 | $0.726^{EF}$ | 0.049 | $2.41E\text{-}04^{B}$ | 5.61E-05 | $1.50E\text{-}04^{C}$ | 4.22E-05 |
| 0.55 | 0 | 10 | $0.329^{ABC}$ | 0.009 | $1.101^{ABC}$ | 0.031 | $6.70E\text{-}04^{A}$ | 1.13E-05 | $4.52E\text{-}04^{A}$ | 6.58E-05 |
| 0.55 | 5 | 0 | $0.221^{EF}$ | 0.004 | $0.741^{EF}$ | 0.016 | $2.96E\text{-}04^{B}$ | 5.86E-05 | $1.48E\text{-}04^{C}$ | 2.15E-05 |
| 0.55 | 5 | 5 | $0.221^{EF}$ | 0.010 | $0.739^{EF}$ | 0.036 | $2.58E\text{-}04^{B}$ | 3.04E-05 | $1.55E\text{-}04^{C}$ | 2.05E-05 |
| 0.55 | 0 | 5 | $0.348^{A}$ | 0.013 | $1.165^{A}$ | 0.045 | $7.54E\text{-}04^{A}$ | 1.69E-04 | $5.12E\text{-}04^{A}$ | 1.89E-04 |
| 0.55 | 1.2% Dextran | | $0.311^{BCD}$ | 0.010 | $1.042^{BCD}$ | 0.028 | $7.06E\text{-}04^{A}$ | 1.31E-04 | $3.65E\text{-}04^{A}$ | 9.82E-05 |
| 0.55 | Sweetener | | $0.327^{ABC}$ | 0.011 | $1.088^{ABC}$ | 0.038 | $6.63E\text{-}04^{A}$ | 6.42E-05 | $3.21E\text{-}04^{A}$ | 3.37E-05 |
| 1 | 0 | 0 | $0.340^{AB}$ | 0.023 | $1.136^{ABC}$ | 0.079 | $5.51E\text{-}04^{A}$ | 1.67E-04 | $4.15E\text{-}04^{A}$ | 1.20E-04 |
| 1 | 5 | 0 | $0.235^{E}$ | 0.007 | $0.789^{E}$ | 0.024 | $3.36E\text{-}04^{B}$ | 9.42E-05 | $1.91E\text{-}04^{BC}$ | 5.80E-05 |
| 1 | 5 | 5 | $0.229^{EF}$ | 0.003 | $0.767^{EF}$ | 0.011 | $2.99E\text{-}04^{B}$ | 4.88E-05 | $1.82E\text{-}04^{C}$ | 1.60E-05 |
| 1 | 0 | 5 | $0.338^{ABC}$ | 0.006 | $1.127^{ABC}$ | 0.021 | $5.82E\text{-}04^{A}$ | 5.54E-05 | $3.70E\text{-}04^{A}$ | 4.34E-05 |
| Quinine | | | $0.287^{D}$ | 0.033 | $0.962^{D}$ | 0.114 | $5.78E\text{-}04^{A}$ | 2.45E-04 | $3.23E\text{-}04^{AB}$ | 1.14E-04 |
| Tannic acid | | | $0.351^{A}$ | 0.023 | $1.175^{A}$ | 0.074 | $6.16E\text{-}04^{A}$ | 1.31E-04 | $4.00E\text{-}04^{A}$ | 8.86E-05 |
| **p-value (sample effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |

*Table 4-5: Mean data from the second rubbing episode (6 replicates, with addition of saliva). Samples sharing the same letters are not significantly different (p>0.05) as determined by 2-way ANOVA (samples, replicates) with Tukey PostHoc.*

| Sample | | | Friction [N] | | Friction Coefficient | | Medium Frequency | | High Frequency | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tea [%]** | **Fat [%]** | **Sugar [%]** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| 0.1 | 0 | 0 | $0.348^{AB}$ | 0.015 | $1.164^{ABC}$ | 0.047 | $1.18E\text{-}03^{AB}$ | 5.32E-04 | $6.94E\text{-}04^{A}$ | 2.30E-04 |
| 0.1 | 5 | 0 | $0.214^{DE}$ | 0.007 | $0.713^{EF}$ | 0.024 | $2.69E\text{-}04^{D}$ | 6.40E-05 | $1.59E\text{-}04^{C}$ | 2.77E-05 |
| 0.1 | 5 | 5 | $0.207^{E}$ | 0.006 | $0.691^{F}$ | 0.021 | $2.81E\text{-}04^{D}$ | 7.06E-05 | $1.33E\text{-}04^{C}$ | 2.68E-05 |
| 0.1 | 0 | 5 | $0.330^{B}$ | 0.013 | $1.096^{C}$ | 0.045 | $1.29E\text{-}03^{A}$ | 4.25E-04 | $6.61E\text{-}04^{A}$ | 1.55E-04 |
| 0.55 | 0 | 0 | $0.357^{AB}$ | 0.013 | $1.197^{AB}$ | 0.041 | $9.84E\text{-}04^{ABC}$ | 1.43E-04 | $5.95E\text{-}04^{AB}$ | 6.23E-05 |
| 0.55 | 10 | 0 | $0.217^{DE}$ | 0.012 | $0.722^{EF}$ | 0.042 | $2.30E\text{-}04^{D}$ | 1.46E-05 | $1.33E\text{-}04^{C}$ | 2.80E-05 |
| 0.55 | 0 | 10 | $0.335^{B}$ | 0.016 | $1.117^{BC}$ | 0.057 | $8.89E\text{-}04^{ABC}$ | 1.45E-04 | $5.93E\text{-}04^{AB}$ | 5.02E-05 |
| 0.55 | 5 | 0 | $0.219^{DE}$ | 0.004 | $0.734^{EF}$ | 0.014 | $3.28E\text{-}04^{D}$ | 3.47E-05 | $1.70E\text{-}04^{C}$ | 2.47E-05 |
| 0.55 | 5 | 5 | $0.220^{DE}$ | 0.007 | $0.738^{EF}$ | 0.025 | $2.72E\text{-}04^{D}$ | 3.86E-05 | $1.59E\text{-}04^{C}$ | 2.27E-05 |
| 0.55 | 0 | 5 | $0.356^{AB}$ | 0.012 | $1.189^{ABC}$ | 0.042 | $1.05E\text{-}03^{ABC}$ | 2.56E-04 | $7.05E\text{-}04^{A}$ | 3.48E-04 |
| 0.55 | 1.2% Dextran | | $0.334^{B}$ | 0.016 | $1.126^{BC}$ | 0.051 | $9.74E\text{-}04^{ABC}$ | 1.85E-04 | $4.68E\text{-}04^{AB}$ | 6.09E-05 |
| 0.55 | Sweetener | | $0.344^{AB}$ | 0.014 | $1.158^{ABC}$ | 0.042 | $1.01E\text{-}03^{ABC}$ | 1.73E-04 | $4.68E\text{-}04^{AB}$ | 1.72E-05 |
| 1 | 0 | 0 | $0.343^{B}$ | 0.009 | $1.142^{BC}$ | 0.032 | $7.41E\text{-}04^{BC}$ | 2.00E-04 | $5.62E\text{-}04^{AB}$ | 1.23E-04 |
| 1 | 5 | 0 | $0.225^{D}$ | 0.005 | $0.753^{E}$ | 0.018 | $3.41E\text{-}04^{D}$ | 8.09E-05 | $1.84E\text{-}04^{C}$ | 3.81E-05 |
| 1 | 5 | 5 | $0.223^{DE}$ | 0.006 | $0.745^{EF}$ | 0.017 | $3.15E\text{-}04^{D}$ | 6.28E-05 | $1.73E\text{-}04^{C}$ | 1.67E-05 |
| 1 | 0 | 5 | $0.331^{B}$ | 0.005 | $1.102^{BC}$ | 0.021 | $6.57E\text{-}04^{C}$ | 1.09E-05 | $4.30E\text{-}04^{AB}$ | 7.05E-05 |
| Quinine | | | $0.299^{C}$ | 0.028 | $0.996^{D}$ | 0.094 | $8.00E\text{-}04^{BC}$ | 3.35E-04 | $4.05E\text{-}04^{B}$ | 1.50E-04 |
| Tannic acid | | | $0.374^{A}$ | 0.011 | $1.245^{A}$ | 0.030 | $7.70E\text{-}04^{BC}$ | 2.05E-04 | $4.80E\text{-}04^{AB}$ | 9.21E-05 |
| **p-value (sample effect)** | | | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | |

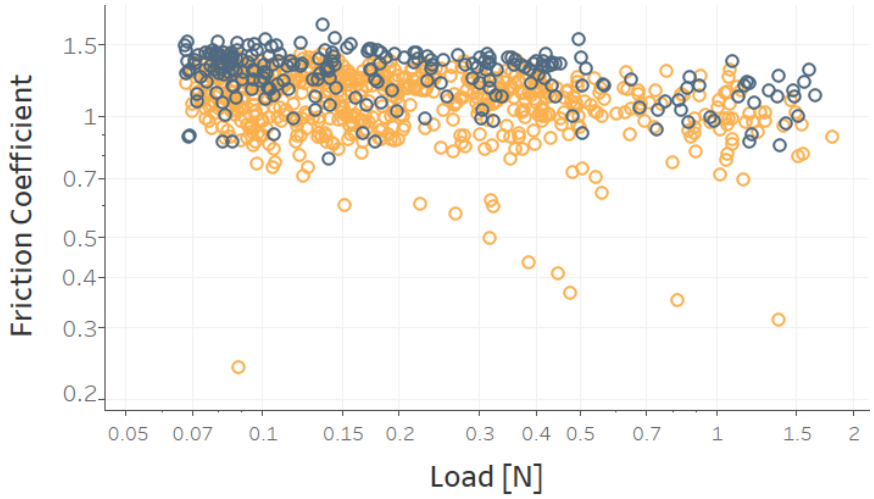



*Figure 4-14: Friction coefficient vs load for 0.1% tea for different substrate pre-treatment. Blue: without saliva, yellow: with saliva. Graph shows raw data.*

It is well known that astringent substances such as tannic acid and tea interact with and bind to salivary proteins (Horne et al., 2002; Nayak & Carpenter, 2008) forming insoluble agglomerates with a diameter between 80 and 500 nm (Charlton et al., 2002). The formation of insoluble agglomerates when rubbed over the Bioskin would lead to an increase in friction compared to tea without saliva. However, this was not observed in this experiment suggesting that tea polyphenols and salivary proteins do not form insoluble agglomerates, possibly due to the low solids content of the tea.

The similarity in friction coefficient is somewhat surprising for another reason. Saliva is known to have lubricating properties (Engelen et al., 2007) and therefore it could be expected that tea with saliva has a lower friction coefficient than tea without saliva. Although, there is a trend suggesting that saliva reduces the friction coefficient, this decrease is not great. It is therefore possible that the dilution of saliva suppressed its lubricating properties.

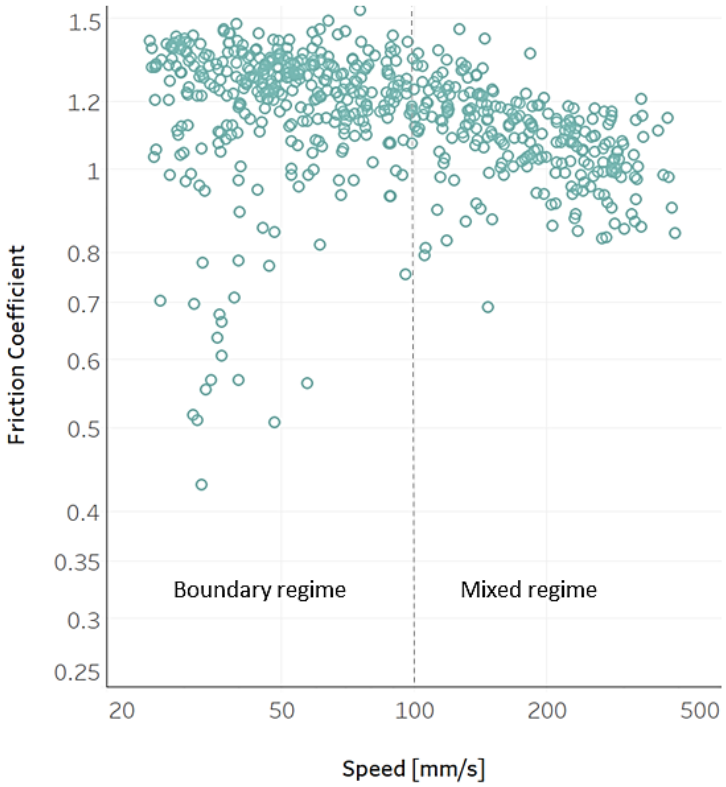



*Figure 4-15: Friction coefficient plotted against speed for 0.55% tea showing the prevailing lubrication regimes: the boundary regime and the hydrodynamic regime. The plot shows raw data.*

In order to understand which lubrication regime prevailed for the samples discussed in this section, the friction coefficient of 0.55% tea was plotted against speed and the relationship between friction coefficient and speed show in Figure 4-15 is representative for the samples discussed in this section. Three lubrication regimes are known: the boundary regime, the mixed regime and the hydrodynamic regime. In the boundary regime with increasing speed the friction coefficient remains constant, whereas in the mixed regime the friction coefficient decreases with further increased speed. In the hydrodynamic regime, the friction coefficient increases with increasing speed. Figure 4-15 shows the friction coefficient over a wide range of speed. At low speeds, the friction coefficient remains constant whereas at high speeds (from 100 mm/s onwards) a decrease in friction

coefficient is observed, therefore the lubrication regimes prevailing for the samples are the boundary and the mixed regime. Since the boundary regime is a relevant lubrication regime of the samples discussed in this section, the friction coefficient of the samples is determined by the surface properties. In the boundary regime, the gap between the finger and the Bioskin is minimal, with the finger directly rubbing across the Bioskin and pushing the liquid film aside. The Bioskin has a textured surfaces and the asperities are found to be in the micrometre range, which is much bigger than any polyphenols and salivary proteins. This explains also why the friction coefficient for different tea levels is very similar. Furthermore, when more tea is added to the existing tea-saliva mixture as done for the second rubbing episode, only a small increase in friction coefficient was observed. This shows that the further dilution of saliva did not affect the friction coefficient since the surface properties of the interacting surfaces did not change. In the mixed regime, some of the tea-saliva mixed is entrained between the finger and the Bioskin and the presence of a liquid film leads to a small reduction of the friction coefficient which is not affected by the tea concentration.

For vibration amplitude, only small differences between samples were observed (see Figure 4-13) and none were significant (see Table 4-4). It was hypothesised that noise, arising due to an increase in friction, could be used to differentiate between different samples. However, the results in Figure 4-13 show that the vibration amplitude cannot be used to differentiate between different tea levels or bitter and astringent samples.

*4.5.1.2 Fat*

Figure 4-16A shows the impact of 5% fat addition on the friction coefficient for low, medium and high tea concentrations and the impact of 10% fat addition to medium concentration tea. For each tea level, the addition of 5% fat significantly reduced the friction coefficient by approximately 30% ($p<0.0001$). Interestingly, it was observed that the addition of 5% fat reduced the friction coefficient of the high concentration tea less than for low and medium tea concentrations; although this was not significant ($p=0.97$). For medium tea, not only 5% fat but also 10% fat were added to investigate the impact of different levels of fat on friction. It was found that the addition of 10% fat did not alter the friction coefficient to a greater degree compared to 5% fat. It shows that the lubricating properties of fat have reached their maximum efficiency at 5% and the addition of a further 5% fat does not enhance the lubricating properties.

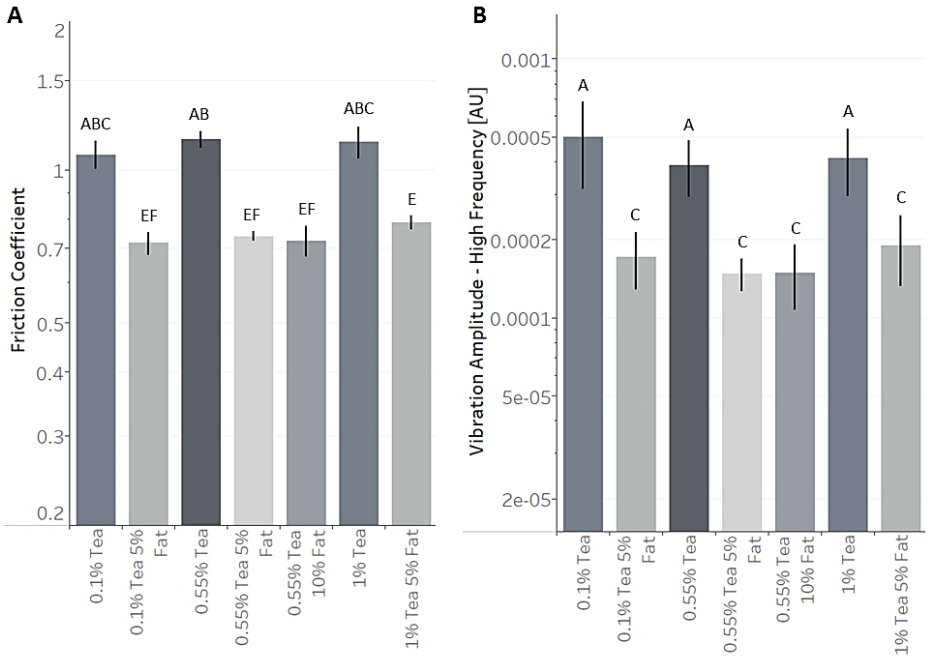



*Figure 4-16: (A) Impact of fat addition on the friction coefficient for different tea levels. (B) Impact of fat addition on the vibration amplitude (high frequency) at different tea levels. Data shows the mean values of 6 replicates of the first evaluation episode and error bars represent the standard deviation. Samples sharing the same letter are not significantly different ($p>0.05$).*

An explanation for the drop in friction coefficient when fat is added to the tea could be that the oil droplets in the oil-in-water emulsion break due to the shear forces applied when rubbing the tea sample across the Bioskin. This would lead to the oil being partially deposited onto the Bioskin and lubricating it. However, in this case there would be a greasy layer of oil on the Bioskin at the end of the experiment which would have been observed. This was not the case. It might still be of interest to measure the friction coefficient of melted coconut oil and compare it to the friction coefficient of the emulsion.

The addition of fat to tea could change the lubrication regime, which would explain the reduction in friction coefficient. Figure 4-17 shows the friction coefficient depending on speed for 0.55% tea with 5 or 10 % fat addition. In contrast to 0.55% tea (see Figure 4-15), the friction coefficient for 0.55% tea with added fat increases at high speeds, meaning that tea samples with fat transition from the mixed regime into the hydrodynamic regime. In the mixed regime, the friction coefficient reaches its minimum and with increasing speed, the lubrication regime changes to the hydrodynamic regime, where the friction coefficient increases. In the mixed regime, the interacting surfaces (finger and Bioskin) start to separate and entrain the tea with fat. As the speed increases, more liquid becomes entrained and the hydrodynamic pressure increases which helps to fully separate the interacting surfaces, as found in the hydrodynamic regime. The oil droplets of the emulsion cover the rough surface of the Bioskin and therefore contribute to the increase gap between finger and Bioskin. The oil droplets form a layer, covering the topography of the Bioskin, which results in a decrease of friction coefficient. For 0.55% tea

with 10% fat the same behaviour of the friction coefficient with increasing speed was observed, indicating that an increase in oil droplets does not increase the gap between finger and Bioskin, therefore resulting in the same friction coefficient as 5% fat.

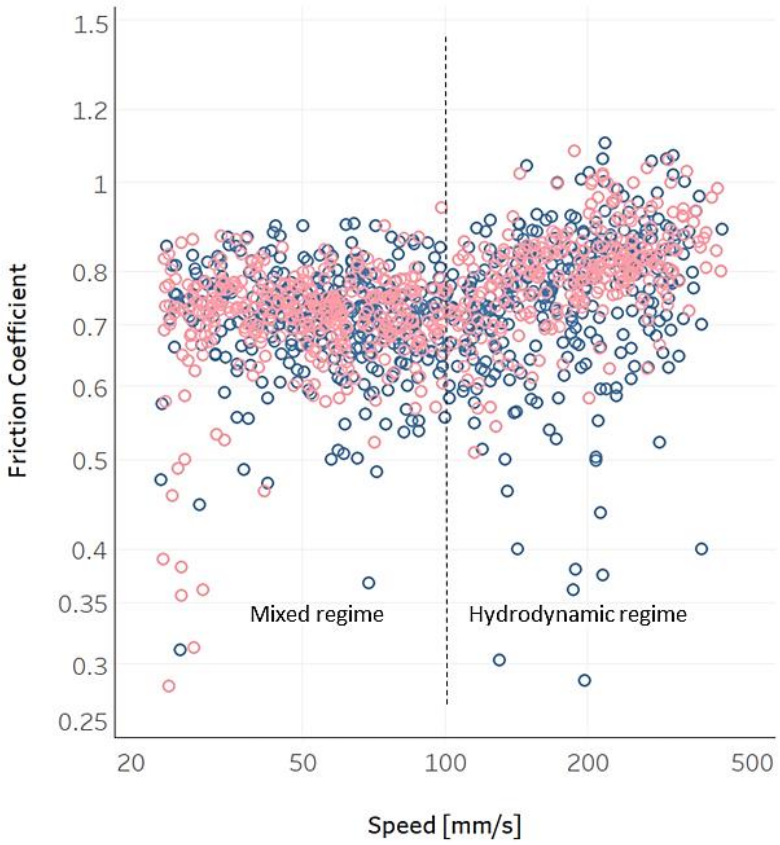



*Figure 4-17: Friction coefficient vs speed for 0.55% tea & 10% fat (in pink) and 0.55% tea & 5% fat (in blue). The plot shows raw data.*

The impact of fat addition on the vibration amplitude of high frequencies is shown in Figure 4-16B. The same trend as the friction coefficient was observed, with the addition of fat significantly reducing the vibration amplitude. The reduction in vibration amplitude may be due to two reasons: the addition of fat decreased the vibration amplitude due to increased lubrication resulting in less energy dissipation, which in turn leads to reduced friction noise; or, the fat reduces the interaction between

the topography of the Bioskin and the finger print when rubbing the finger across the force plate in comparison to a thinner liquid, e.g. tea.

#### *4.5.1.3 Sugar, Sweetener and Thickener*

The impact of sugar addition on the friction coefficient and high frequency vibration amplitude is displayed in Figure 4-18. Unlike with the addition of fat, the impact of sugar on the friction coefficient has no clear trend and no significant differences ($p=0.945$) between samples were observed (see Post-hoc grouping in Figure 4-18A and Table 4-4). The vibration amplitude changed very little when sugar was added to tea with no significant differences being observed (see Post-hoc grouping in Figure 4-18B and Table 4-4). Sugar is not known to interact with polyphenols or salivary proteins and therefore a change in friction compared to pure tea was not expected. Furthermore, although sugar increased the viscosity of the samples, a reduction in friction coefficient was not expected since only samples with viscosities greater than 100 mPa·s are known to lead to a reduced friction (de Wijk & Prinz, 2005). The samples used in this study had a viscosity range of 0.66 mPa·s to 1.46 mPa·s (see Table 2-8), which is below the viscosity which reduces friction. The results therefore support the hypothesis from the TI study (Chapter 3) that sugar reduced astringency via a cognitive process (cross-modal interaction) and not a physical process, like decrease in friction or viscosity increase.

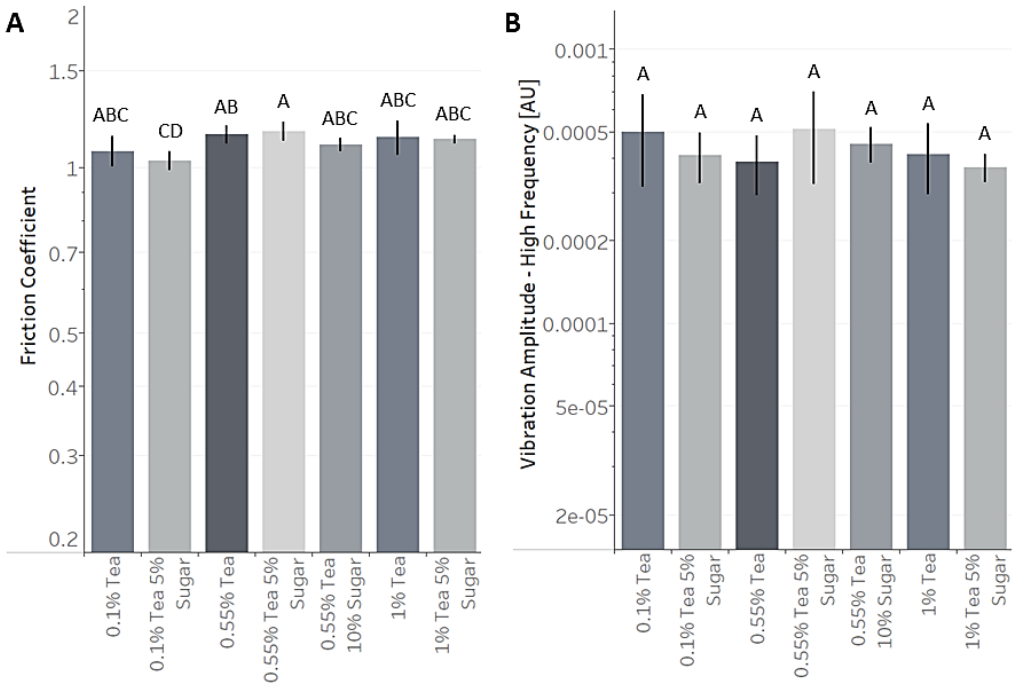



*Figure 4-18: (A) Friction coefficient for tea with sugar addition, (B) Vibration amplitude (high frequency band) for tea with sugar addition. Data shows the mean values for 6 replicates from the first rubbing episode and error bars represent the standard deviation. Samples sharing the same letter are not significantly different (p>0.5).*

In order to further understand the impact of sugar on friction behaviour, tea with sweetener and tea with thickener were used to de-couple the effect of sweetness from the viscosity increase caused by sugar addition. A tea with thickener (1.2% dextran) was prepared with the same viscosity as tea with 5% sugar and a tea with sweeteners (0.01% Cyclamate, 0.015% Acesulfame K, 0.005% Saccharin) was prepared with the same sweetness as tea with 5% sugar (see chapter 3 section 3.2.1.1 for detailed description). Figure 4-19A shows the friction coefficient for medium concentration tea, alone, with different sugar levels (5 and 10%) or with sweetener or thickener addition. It was found that the addition of sugar, sweetener or thickener did not significantly reduce the friction coefficient when compared to 0.55% tea (p>0.71).

For high frequency vibration amplitude, tea with sweetener showed the lowest mean vibration amplitude and tea with sugar had the highest mean value; although no significant difference was found ($p>0.05$). The addition of the thickener had no significant impact on the vibration amplitude compared to medium tea.

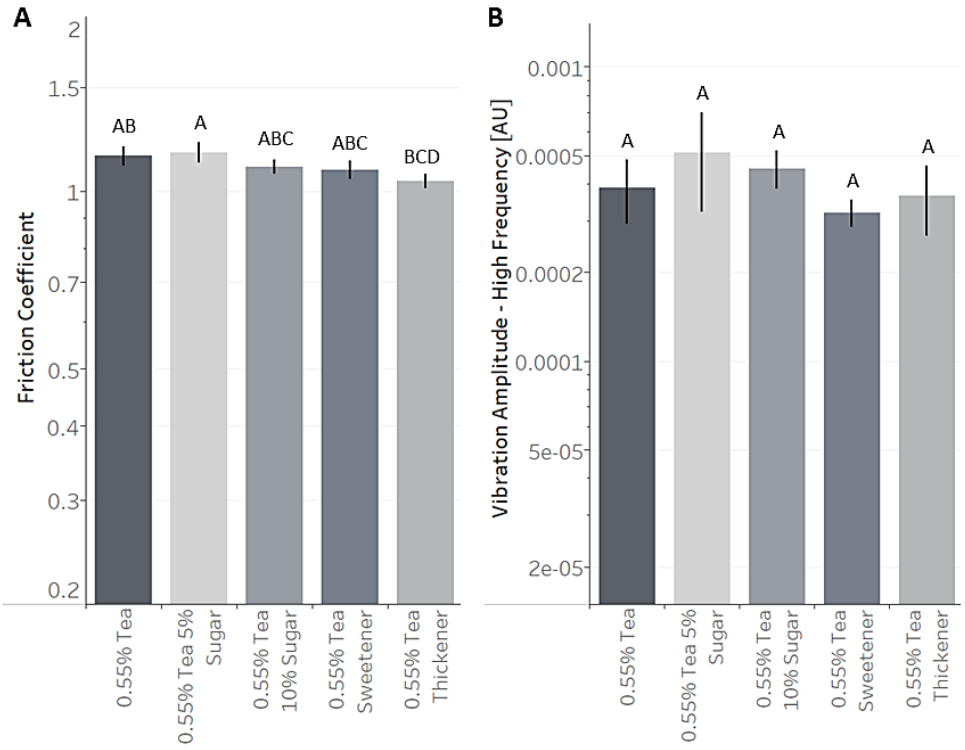



*Figure 4-19: (A) Friction coefficient for medium tea with different additives (sugar, sweetener, thickener). (B) Vibration amplitude (high frequency band) for medium tea with different additives (sugar, sweetener, thickener). Data shows the mean values of 6 replicates from the first rubbing episode and error bars represent the standard deviation. Samples sharing the same letter are not significantly different ($p>0.05$).*

#### *4.5.1.4 Fat and Sugar*

The impact of adding 5% fat in combination with 5% sugar, to tea, on the friction coefficient and high frequency vibration amplitude, is shown in Figure 4-20. The addition of both fat and sugar to tea significantly reduced both the friction coefficient and vibration amplitude ($p<0.0001$) (see Table 4-4). Since fat has been shown to decrease friction and vibration (see

chapter 4, section 4.5.1.2), it was expected that when fat is added with sugar, this decrease in friction coefficient and vibration amplitude would be maintained. Although sugar itself had little impact on the instrumental parameters as discussed in section 4.5.1.3, there may be a synergistic effect when combined with fat as previously observed in the sensory studies.

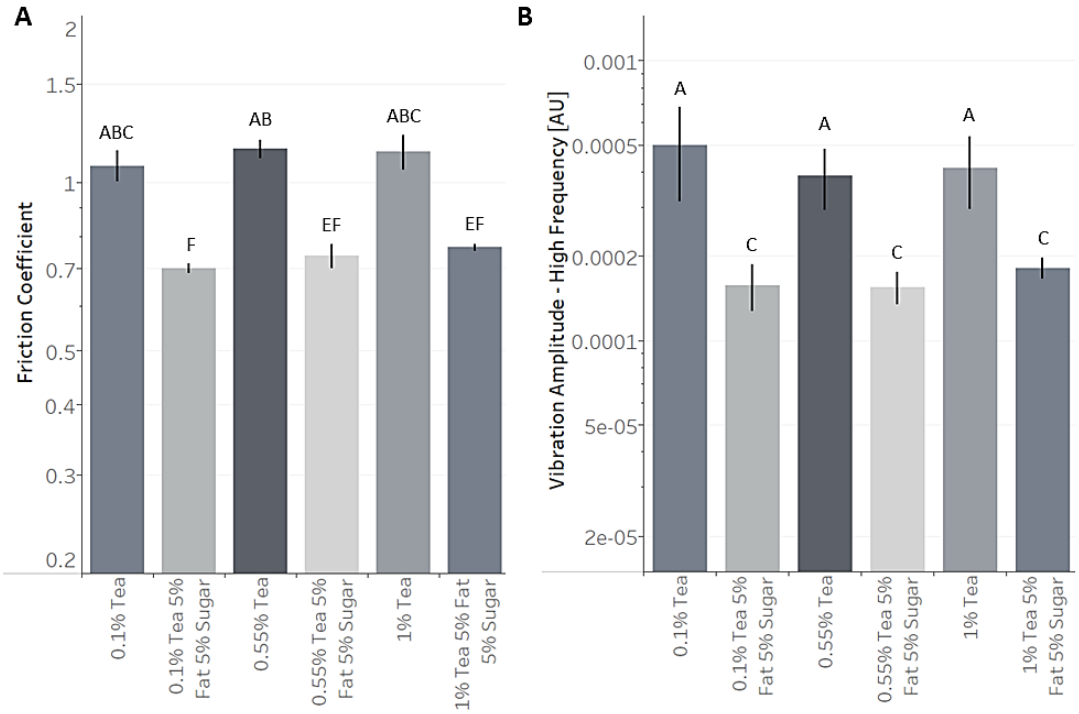



Figure 4-20: (A) Friction coefficient for different tea levels with fat & sugar addition. (B) Vibration amplitude (high frequency band) for different tea levels with fat & sugar addition. *Data shows the mean values of 6 replicates from the first rubbing episode and error bars represent the standard deviation. Samples sharing the same letter are not significantly different (p>0.05).*

Figure 4-21 show the impact fat, sugar and fat & sugar added together had on the friction coefficient. The figure illustrates that the friction coefficient decreased in a similar manner when fat or fat and sugar were added to tea, showing that fat is the driving factor in reducing the friction coefficient ($p=0.95$) and no synergistic effect between fat and sugar was found.

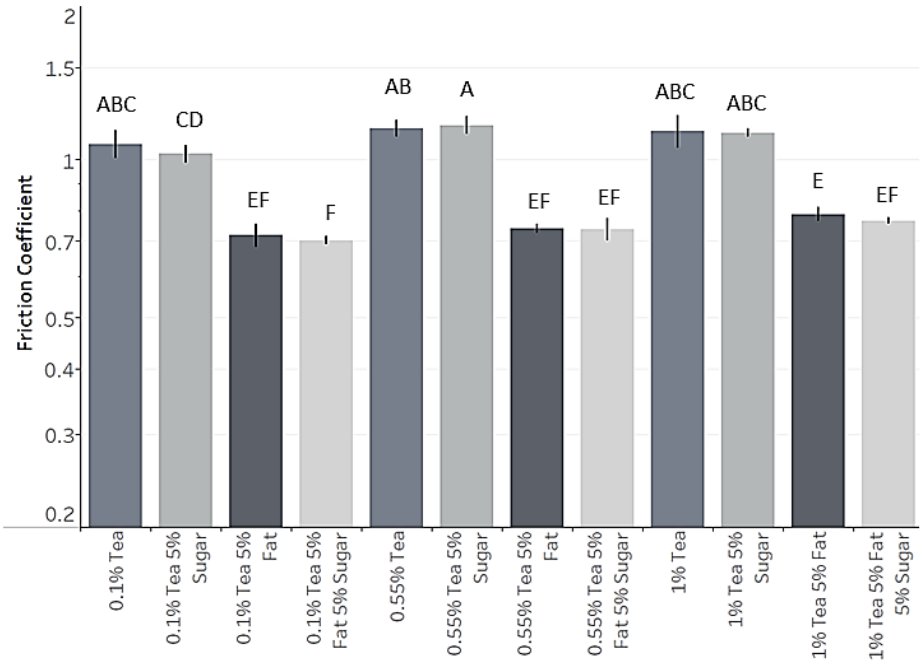



*Figure 4-21: Illustration of the impact of fat, sugar and fat & sugar on the friction coefficient of tea. Data shows the mean values of 6 replicates from the first rubbing episode and error bars represent the standard deviation. Samples sharing the same letter are not significantly different (p>0.05).*

### *4.5.2 Correlation between Friction Coefficient and Vibration Amplitude*

The results discussed in the previous sections have shown that friction coefficient and vibration amplitude show similar trends and differences between samples. In this section the data of both parameters is correlated to prove the observed correlation. Figure 4-22 shows the friction coefficient plotted against the vibration amplitude for all tea samples. A Pearson correlation test showed a strong correlation between friction coefficient and high frequency vibration amplitude, with an $R^2$-value of 0.908. The plot shows that all tea samples containing fat have lower values for both parameters compared to tea samples without fat. Therefore, it can be concluded that fat is the key variable in separating the samples according to their physical properties such as frictional and vibrational behaviour.

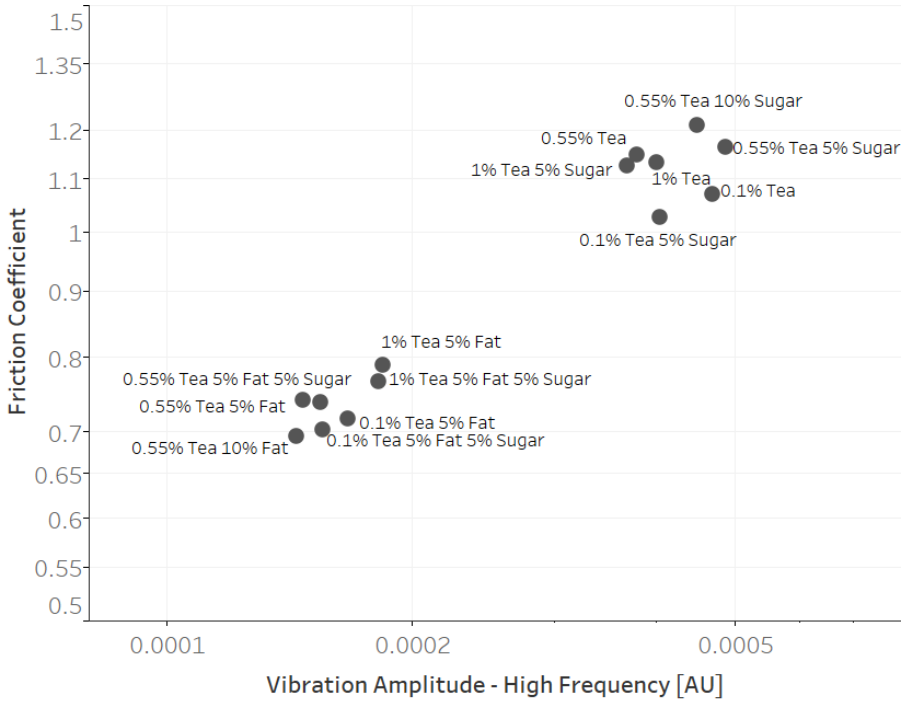



*Figure 4-22: Friction coefficient vs vibration amplitude for all tea samples assessed with the force plate.*

### *4.5.3 Correlation between Mouthfeel Perception and Instrumental Data*

The previous sections have discussed the differences of friction coefficient and vibrations amplitude of different samples, focusing on the impact the sample composition had. Now, these instrumental differences will be linked to sensory perception. In section 4.5.4 similarities between mouthfeel perception and instrumental data are discussed, focusing on trends. Then section 4.5.5 will focus on the results of a quantitative correlation, conducted using statistical methods, to support the trends found in section 4.5.4.

### *4.5.4 Qualitative Correlation between Mouthfeel Perception and Instrumental Data*

The sensory study carried out in chapter 2 assessed the mouthfeel properties of tea samples with varying tea, fat and sugar content. Tannic

acid and quinine were not assessed by the panel. Starting with the three different tea levels, results from the sensory study (see chapter 2) show that all three tea levels have different astringency scores. The lowest tea level (0.1% tea) scored 4.7 on a 10-point scale, medium tea (0.55% tea) scored 7 and the highest tea level (1.0% tea) reached a score of 9.3. If astringency perception is linked to friction or vibration an increase in measured friction coefficient and vibration amplitude would be expected with increasing tea level. However, no significant differences were measured for either friction coefficient or vibration amplitude between different tea concentrations. This discrepancy between perceived astringency and measured friction could be an indication that the force plate is not the right device or not sensitive enough to differentiate between different levels of astringency. However, quinine which is known to be non-astringent had a lower friction coefficient than astringent samples. This shows that there is a rise in friction coefficient when astringent samples are evaluated, supporting the studies which found a rise in friction for astringent compounds (Brossard et al., 2016; Rossetti et al., 2009).

The addition of an oil-in-water emulsion (fat) to tea significantly reduced the friction coefficient and vibration amplitude, compared to tea without emulsion addition. The results of the sensory study (see chapter 2) showed that the addition of an oil-in-water emulsion also decreased the perception of astringency. This shows that for samples containing fat, the friction coefficient is a good indicator for perceived astringency. However, the perceived astringency scores varied between different levels of tea with the same fat content, whereas the friction coefficient did not greatly differ.

Doubling the fat content leads to a 15% reduction in the perceived astringency, as the addition of 5% fat to 0.55% tea resulted in a score of 5.7 on a 10-point scale whereas the addition of 10% fat resulted in a score of 4.4. But, as stated in section 4.5.1.2, only a small difference in friction coefficient was observed, therefore the friction coefficient does not illustrate the effect 10% fat has on perceived astringency. However, this plateau reached by the friction coefficient when either 5 or 10% fat were added to tea is similar to the perception of mouth coating and thickness observed in the first sensory study (see chapter 2); after the addition of, approximately, 7% fat to tea, perceived mouth coating and thickness did not increase further. This could suggest that the friction coefficient might be related to other mouthfeel attributes, rather than just astringency.

The addition of sugar did not affect the friction coefficient measured using the force plate whereas the sensory studies showed that sugar reduced perceived astringency. However, results from the Time-Intensity study (see chapter 3 section 3.3.3.4) indicate that the reduction in astringency might be due to a cognitive process rather than a physical interaction between sugar and polyphenols, sugar and saliva or an increase in viscosity. Therefore, the results of this experiment give further evidence that sugar reduces astringency via a cross-modal interaction and not a physical interaction e.g. due to a viscosity increase or changes in saliva flow rate.

The last section also explored the impact of adding fat and sugar together on the friction coefficient and vibration amplitude. A significant decrease for both parameters was observed when fat and sugar were added to tea, but the reduction is similar to that observed when fat alone was added to

tea. The results from the sensory studies showed adding fat and sugar together, reduces perceived astringency to a greater extent than the addition of either fat or sugar alone. For high concentration tea, fat with sugar reduced astringency to a score of 4.6 in comparison with 6.7 for 5% sugar addition and 7.1 when 5% fat was added. The same trend was observed for medium and low tea levels. This discrepancy between instrumental data and sensory data shows that the friction coefficient is able to indicate a general reduction in perceived astringency through fat addition, however more subtle changes in astringency perception were not reflected by a change of friction coefficient or vibration amplitude. However, it also needs to be taken into consideration that during the sensory studies tea samples were served at 50 °C whereas in this force plate experiment tea samples were assessed at room temperature, resulting in a difference in physical stimuli.

### *4.5.5 Quantitative Correlation between Sensory and Instrumental Data*

In order to understand if a linear correlation between perceived mouthfeel and measured friction and vibration amplitude exists, a Pearson correlation test was carried out. The sensory data used in this correlation was obtained from the predictive mouthfeel model equations generated in the first study (see chapter 2 section 2.3.3). Using the data from the predictive mouthfeel model equations also allowed for the correlation between fat-driven attributes and instrumental data which is thought to be of interest since the addition of fat had the greatest effect on instrumental parameters. For the correlation test, samples containing quinine, tannic acid, sweetener or thickener were discarded, leaving only samples with tea alone and with fat

and/or sugar as sensory scores were only collected for these samples. To correlate mouth coating, thickness and slipperiness to instrumental data, tea samples containing sugar alone were not included since the sensory perception for these parameters was driven by the fat content (see chapter 2 section 2.3.4). However, with astringency, samples with both fat and sugar were used to correlate sensory and instrumental data as the sensory perception was affected by both ingredients (see chapter 2 section 2.3.4).

The results in Table 4-6 show the Pearson coefficient between perceived mouthfeel and the friction, friction coefficient and vibration amplitudes (high and medium frequencies). $R^2$-values in bold indicate a strong linear correlation between a mouthfeel attribute and an instrumental parameter.

*Table 4-6: $R^2$- and p-values determined with Pearson Correlation test. For slippery, mouth coating and thick tea samples containing fat and different levels of tea were considered. For astringency tea samples containing different levels of tea, fat and sugar were considered.*

| | **Friction [N]** | | **Friction Coefficient** | | **Vibration amplitude (Medium Freq.) [AU]** | | **Vibration amplitude (High Freq.) [AU]** | |
|---|---|---|---|---|---|---|---|---|
| | **$R^2$** | **p** | **$R^2$** | **p** | **$R^2$** | **p** | **$R^2$** | **p** |
| Slippery | **0.993** | <0.0001 | **0.987** | <0.0001 | **0.891** | <0.0001 | **0.926** | <0.0001 |
| Mouth coating | **0.976** | <0.0001 | **0.975** | <0.0001 | **0.926** | <0.0001 | **0.956** | <0.0001 |
| Thick | **0.976** | <0.0001 | **0.971** | <0.0001 | **0.921** | <0.0001 | **0.954** | <0.0001 |
| Astringent | 0.167 | 0.147 | 0.162 | 0.192 | 0.008 | 0.761 | 0.045 | 0.467 |

Strong correlations were observed between the perception of slipperiness, mouth coating and thickness with all listed instrumental parameters. Figure 4-23 shows a graphical representation of the correlation between mouthfeel attributes and the friction coefficient; therefore, in addition to the strength of the correlation, described by the $R^2$-value in Table 4-6, Figure 4-23 shows the direction of the correlation. For all three strongly correlated attributes, perception decreased with increasing friction

coefficient, showing a negative correlation between fat-driven mouthfeel attributes and friction coefficient. This negative correlation was also observed between these three mouthfeel attributes and the other instrumental parameters. However, it is important to notice that the strong correlation between friction coefficient and the fat-driven mouthfeel attributes is caused by two clusters of data with no data being found in the middle of the experimental design space.

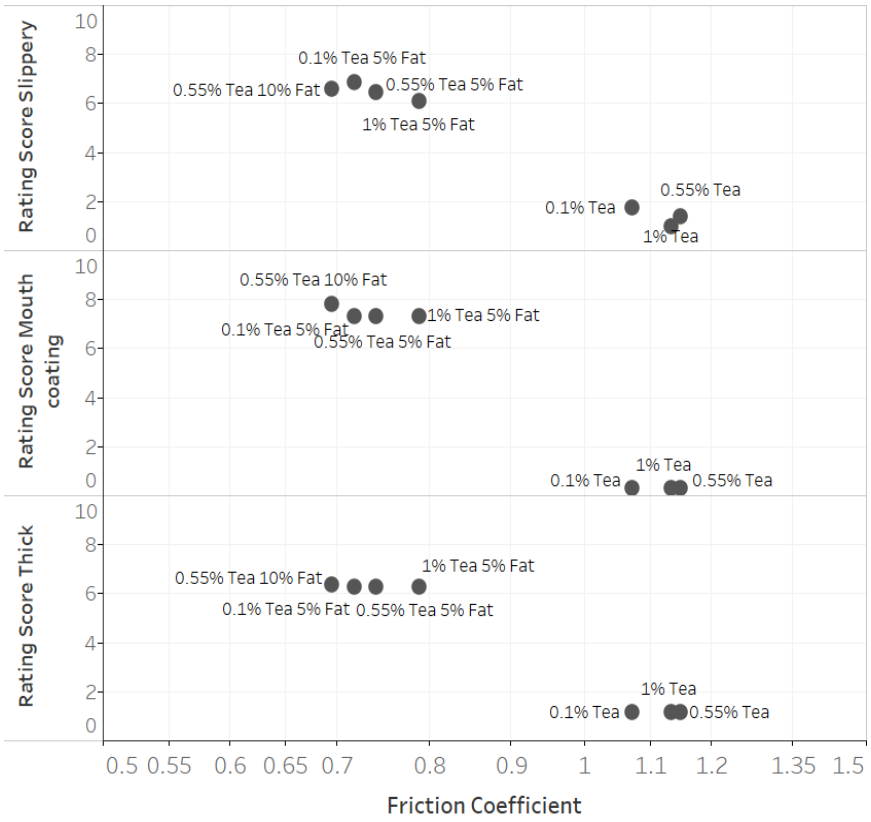



*Figure 4-23: Rating scores for perceived slipperiness, mouth coating and thickness vs friction coefficient.*

The reason for the strong correlation between the perception of these mouthfeel attributes and the instrumental parameters is the role of fat. Fat was the main driver which influenced the perception of slipperiness,

thickness and mouth coating; furthermore, fat was found to have the greatest impact on the parameters measured using the force plate.

The $R^2$-values in Table 4-6 show weak, if any, correlations between perceived astringency and all of the instrumental parameters. However, since it was suggested that sugar reduces astringency due to a cognitive process (cross-modal interaction), a correlation using all samples might not be appropriate leading to false-negative results. In order to investigate if there is an underlying correlation for some tea samples, Figure 4-24 shows the astringency and friction coefficients for all tea samples. Focusing on the effect of fat first (highlighted by the blue lines), it is observed that the addition of fat reduces astringency as well as the friction coefficient. However, astringency is reduced by fat to a greater extent than the friction coefficient. The effect of tea can be evaluated on three examples: rising tea concentration on its own, rising tea concentration when 5% fat are present and rising tea concentration is presence of 5% fat and 5% sugar; all highlighted through brown lines in Figure 4-24. In all three examples it was found that with increasing tea concentration perceived astringency increases to a much greater extent than the friction coefficient. This shows that the friction coefficient changes in a similar way to the perceived astringency, however the magnitude of this change is much smaller than the perceived change. Therefore, more subtle differences which can be perceived, would not be captured by the change in friction coefficient.

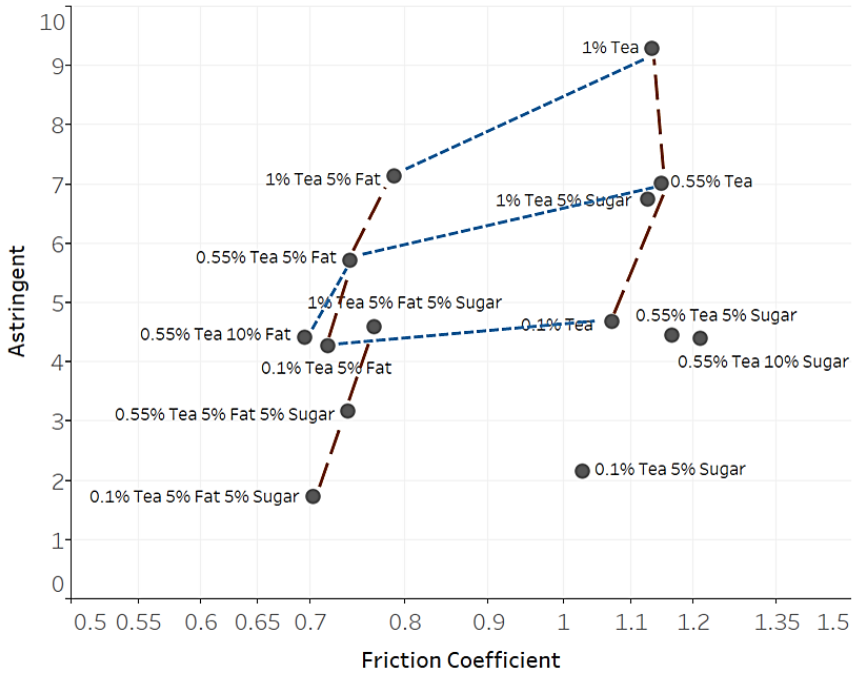



*Figure 4-24: Illustration of correlation between astringency perception and friction coefficient. Blue line: effect of fat; brown line: effect of tea*

The results show that the relationship between friction coefficient and astringency is more complex, supporting the findings of other studies (Rossetti et al., 2009; Vardhanabhuti et al., 2011). A positive correlation between friction coefficient and astringency ratings for tannic acid in wine was reported by Brossard et al. (2016), with an $R^2$-value of 0.93 at sliding speed below 0.5 mm/s, but this appears to be unique. Brossard et al. (2016) used a purpose-built tribometer where a ball-disc-contact was created using a commercial texture analyser with an attachment. This difference in experimental set-up between Brossard et al. (2016) and the here presented study could explain why Brossard et al. (2016) found a correlation between friction coefficient and astringency perception and our study did not observe this correlation.

In the study presented in this thesis a saliva to beverage (in this case tea) ratio of 2:5 was used which is different to the ratio used by Brossard et al.

(2016) which was 1:1 (saliva to astringent substance). Their reasoning for using this ratio was that astringency is perceived after swallowing and therefore, the amount of sample should be similar to the amount of saliva. This highlights the importance of the saliva/sample ratio and to date there seems to be no agreement between researchers on which ratio to use.

The results obtained by Brossard et al. (2016) showed a correlation between astringency and friction coefficient at low sliding speeds of 5 mm/s. So far, the results presented in chapter 4.5.1 were gained from a general regime which covered low and high speeds as well as low and high loads. In order to investigate if a better correlation between perceived astringency and friction and vibration amplitude exists, the data was split into 4 regimes as previously described in Table 4-2: low speed, high speed, low load and high load. The results for the correlation test are displayed in Table 4-7. Since strong correlations between slippery, mouth coating and thick with the instrumental parameters in the general regime were observed, it is expected that correlations in the other regimes can be found too. Focusing first on slippery, mouth coating and thick, it can be observed that the friction coefficients of in all regimes except the high speed regime were highly correlated to the perception of these attributes. A good correlation was found for the high frequency vibration amplitude in general regime, high speed regime and low load regime.

For astringency, it was found that the friction coefficient of the high speeds regime seemed to have the best correlation, however the $R^2$-value was still only around 0.4, indicating a weak relationship. No correlation was found between astringency and high frequency vibration amplitude in any of the load/ speed regimes.

Table 4-7: $R^2$-values for perceived mouthfeel and measured parameters in different speed and load regimes. μ = Friction coefficient, V.A.(H) = Vibration amplitude (high frequency). Values in red are the lowest, whereas values in green are the highest.

| | **General** | | **Low speed** | | **High speed** | | **Low load** | | **High load** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Speed range** | **25 – 500 mm/s** | | **10 – 50 mm/s** | | **250 – 500 mm/s** | | **20 – 500 mm/s** | | **20 – 500 mm/s** | |
| **Load range** | **0.07 – 2 N** | | **0.1 – 2 N** | | **0.1 – 2 N** | | **0.05 – 0.15 N** | | **1 – 2 N** | |
| | **μ** | **V.A.(H)** | **μ** | **V.A.(H)** | **μ** | **V.A.(H)** | **μ** | **V.A.(H)** | **μ** | **V.A.(H)** |
| **Astringent** | 0.16 | 0.05 | 0.16 | 0.13 | 0.41 | 0.21 | 0.16 | 0.2 | 0.09 | 0.01 |
| **Slippery** | 0.99 | 0.93 | 0.98 | 0.67 | 0.87 | 0.95 | 0.99 | 0.94 | 0.91 | 0.59 |
| **Mouth coating** | 0.98 | 0.96 | 0.98 | 0.7 | 0.83 | 0.93 | 0.97 | 0.93 | 0.94 | 0.67 |
| **Thick** | 0.98 | 0.95 | 0.98 | 0.69 | 0.81 | 0.93 | 0.97 | 0.93 | 0.94 | 0.94 |

## 4.6 Conclusions

For the first time, a force plate was used to investigate the frictional and vibrational differences of different tea samples, containing sugar and fat, and was found to be a suitable piece of equipment to measure friction and vibration. The results showed that the addition of fat, either alone or in combination with sugar, had the greatest impact on friction coefficient and vibration amplitude, reducing both parameters when compared to tea alone. The addition of sugar did not greatly impact friction coefficient or vibration suggesting that astringency reduction due to sugar is caused by a cross-modal interaction. Different levels of tea were found to have similar friction coefficients showing that the dilution of the saliva with tea resulted in the loss of lubricating properties of saliva. Quinine was found to have a lower friction coefficient than tannic acid or tea. The results show that the force plate is able to discriminate between tea samples with and without fat as well as show the differences between astringent and non-astringent samples, but is not able to predict subtle differences in astringency perception.

The correlation between instrumental and sensory data showed linear correlations between fat-driven attributes such as mouth coating, thickness and slipperiness, and the measured instrumental parameters; therefore, the force plate can be used to discriminate between samples containing different amounts of fat and might be able to predict the intensity of fat-driven attributes. However, no linear relationship between friction measurements and perceived astringency was found in any regime. This is a further indication that astringency is a complex sensation and

simple friction measurements are not able to fully predict astringency perception.

# 5 "Acoustic Tribology" and its links to Mouthfeel Perception

## 5.1 Introduction

It has been known for some time that the sound produced during eating is related to texture perception. Most studies published in literature focused on texture attributes such as crispy and crunchy for example of baked products (Çarşanba et al., 2017; Zadeike et al., 2018) or apples (Piazza & Giovenzana, 2015). In most studies a microphone was used to detect the sounds of the sample breaking (see Duizer (2001) for a review).

However, whilst it has been investigated how texture of solid food and sound might be related, only one study appears to have been carried out to link mouthfeel of liquid foods and sound using "acoustic tribology". The concept of "acoustic tribology" was introduced by van Aken (2013) and was described as "the measurement of acoustic emissions of vibrations which are involved in the sensory perception of touch and measured *in-vivo* (for actual biological surfaces)". Touch is sensed by mechanoreceptors which are embedded within the epithelial tissue of the mucosal surfaces of the mouth. As previously mentioned in chapter 1, two different groups of mechanoreceptors have been identified: slowly adapting and rapidly adapting mechanoreceptors (Upadhyay et al., 2016). Slowly adapting mechanoreceptors are Merkel disks and Ruffini cylinders, and Meissner corpuscle and Pacinian corpuscle are rapidly adapting mechanoreceptors. The latter mechanoreceptor has not been found in the oral cavity (Engelen & van der Bilt, 2008). Merkel discs and Meissner corpuscles are buried closely beneath the surfaces, whereas Ruffini corpuscles are buried deeper

within the skin. Slowly adapting mechanoreceptors sense forces which are constantly applied to the surface whereas rapidly adapting mechanoreceptors are known to be sensitive to force variations. Rapidly adapting mechanoreceptors do not directly signal the force exerted on the epithelial surface. Instead, they signal the deformation of the surrounding tissue. When the tongue rubs across the palate, the surface of the tongue is deformed. Since the tissue is viscoelastic, the deformation imparts oscillation to the tissue, which is detected by the mechanoreceptors. These oscillations or vibrations produce an acoustic signal which can be measured.

van Aken (2013) placed a microphone on the outer surface of the upper incisors of a subject who held it in place by hand to record acoustic signals produced when rubbing the tongue across the palate. In his one-subject study, different dairy samples were consumed and rubs at a frequency of 3 rubs per second were recorded for 10 s whilst breathing was stopped. The results show the acoustic signal or the integrated signal over a frequency range of 200 – 3000 Hz. Unfortunately, the study does not give details about the number of replicates carried out for each recording and no statistical analysis of the results is evident. The acoustic signal is not only dependent on the properties of the substrate but also the properties and movements of the oral surfaces (van Aken, 2013). The time-dependent behaviour of the acoustic signal was observed for recording after saliva was rubbed across the palate. The acoustic signal was somewhat larger after a prolonged rubbing of the tongue, lasting up to 4 s (van Aken, 2013). However, since biological surfaces are used, such variations may be linked to variations which can also be observed in

sensory evaluations, such as time-dependent changes of the surface, dilution and mixing with saliva.

The results of the study showed that milk with varying fat content changed the acoustic signal. For milk with the lowest fat content, an increase in acoustic signal compared to saliva was observed and with increasing amount of fat the acoustic signal decreased over the whole frequency range. The integrated signal for milk with different fat content showed that at a fat content of 1% or more the signal was similar to saliva (see Figure 5-1). van Aken (2013) suggested that for these samples, the tongue surface was lubricated by an adherent layer of mucus, aggregated emulsion droplets and free fat.

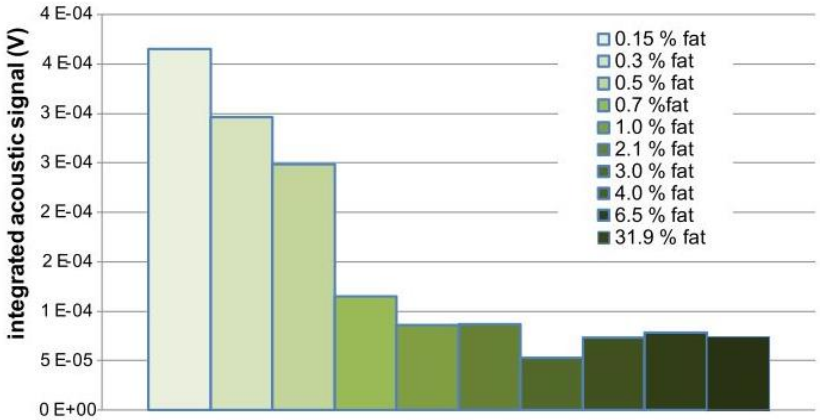



Figure 5-1: Integrated acoustic signals for a range of milk samples. With increasing fat content, the acoustic signal decreases. Saliva is typically in the range of 0.8 - $12 \cdot 10^{-4}$ V. Source: van Aken (2013).

van Aken (2013) concluded that the sensation of smoothness, lubricity and creaminess but also the opposed sensations like roughness, astringency and dryness of food are likely to be related to acoustic signals. However, no sensory study was conducted to validate this conclusion. Furthermore, it is unclear if acoustic signals contribute to mouthfeel perception or if the

tactile sensation which is sensed simultaneously to the noise is more important.

The aim of the present study was to measure the vibrations which arise when rubbing the tongue against the palate and to link the characteristics of these vibrations to the mouthfeel attributes of the samples. In order to do so, a method was developed to measure the vibrations when rubbing the tongue against the palate. A number of artefacts were identified and a data analysis procedure was developed to remove these. Three subjects consumed six samples for which the vibrations related to the tongue rubbing were recorded and the subjects rated the perception of selected attributes. The successful development of this method would contribute a measurement which might be related to mouthfeel perception using an *in-vivo* method.

## 5.2 Materials and Methods

### *5.2.1 Set-up and operational principal*

To measure vibrations caused by rubs of the tongue, an accelerometer (PCB Pieotronics iniature ceramic shear ICP, model 352A24) and a battery-powered ICP sensor signal conditioner (model 480E09) were used (see Figure 5-2). The acceleration signal was recorded using the microphone input of a computer.

In the study by van Aken (2013), a microphone was used to record the acoustic signal. Microphone and accelerometer are both devices which translate sound vibrations into electronic signals. In a microphone, the sound is first converted into a mechanical motion by the means of a

diaphragm. The motion of the diaphragm is then converted into an electric signal. In constrast, an accelerometer measures an acceleration, the rate of change of velocity. When an acclerometer experiences acceleration, the displacement of a mass on a spring is measured to give the acceleration. The accelerometer used in this experiement featured piezoelectric components to convert the mechanical motion into an electrical signal. Piezoelectric components such as lead zirconate titanate have the abilty to produce a voltage when they are subjected to pressure or shear.

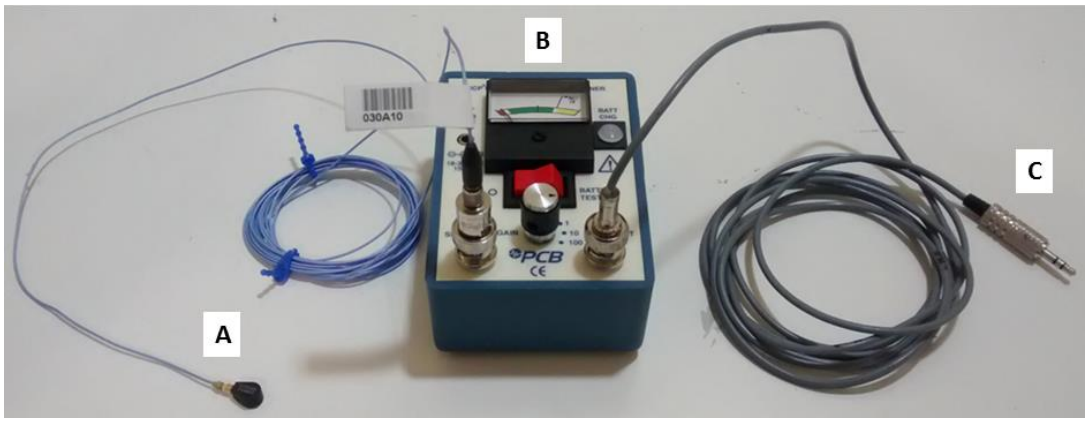



*Figure 5-2: Typical setup to connect to a personal computer via a 3.5 mm stereo plug (C) into the microphone input of a computer. A) PCB Piezotronics miniature ceramic shear ICP accelerometer with 10-ft detachable cable, B) PCB 480E09, 1-Channel, battery-powered.*

### *5.2.2 Method Development*

To gain an understanding of the sensitivity of the accelerometer and how best to measure vibrations originating from the mouth, preliminary one-subject based work was carried out. Firstly, it was determined where and how to attach the accelereometer best onto the skin. To do so, different positions (see Figure 5-3) were tested and breathing was recorded.

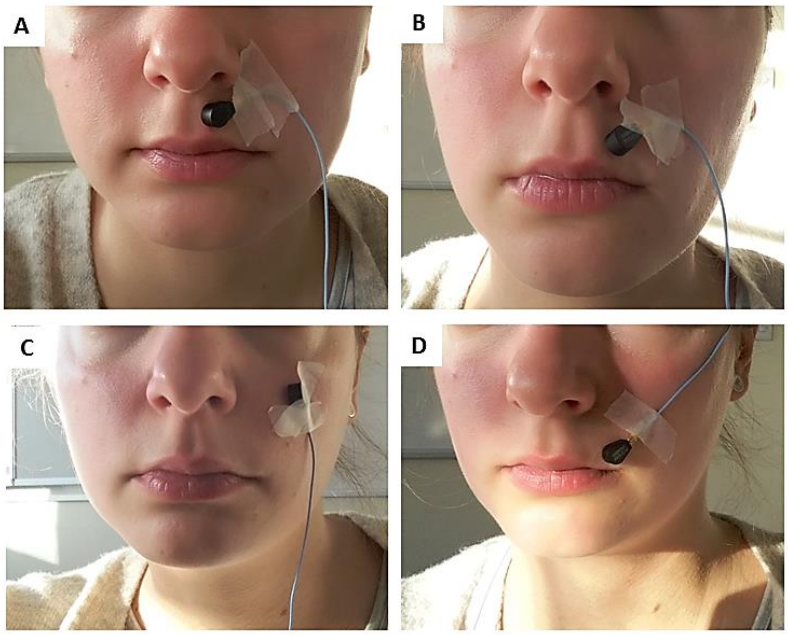



*Figure 5-3: Different set-up of accelerometer on the face from which sound was recorded to identify the best position to record the rubbing noise of the tongue.*

The images show that the accelerometer was attached using medical tape, however the medical tape was not strong enough to hold the accelerometer in place. This problem was solved by attaching the accelerometer on the medical tape using double-sided tape as in the illustration in Figure 5-4.

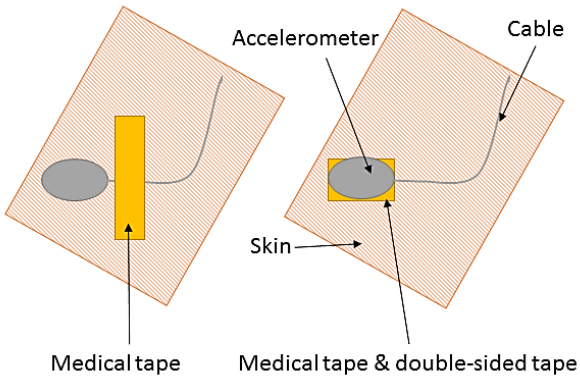



*Figure 5-4: Illustration of different attachment of the accelerometer. The attachment of the right side was used for the main study.*

Figure 5-5 shows the recorded signal caused by breathing for the four different positions of the accelerometer shown in Figure 5-3. Any signals shown in the figure are caused by breathing as the subject did not move

their tongue during the recording. Position A was the attachment of the accelerometer above the upper lip and under one nostril. As seen in Figure 5-5, the accelerometer picked up a strong signal caused by breathing which interfered with the rubbing and therefore this position was not suitable. In position B the accelerometer was moved away from the nostril to be in line with the outside of the nose. However, as can be seen in Figure 5-5 the accelerometer still recorded the breathing. For position C the accelerometer was attached to the cheek bone. It was thought that the vibrations inside the oral cavity could be transmitted through the bones and picked up by the cheek bone, but the signal recorded was noisy and unstable which might be due to the movement of the jaw bones. The final position, position D, was the attachment of the accelerometer on the skin above the corner of the mouth. In this position the accelerometer did not record any vibration caused by breathing which ensured that the background signal created by breathing would be minimal in the actual acoustic experiments. Thus, for recordings shown from Figure 5-6 onwards the accelerometer was attached in position D (see Figure 5-3D).

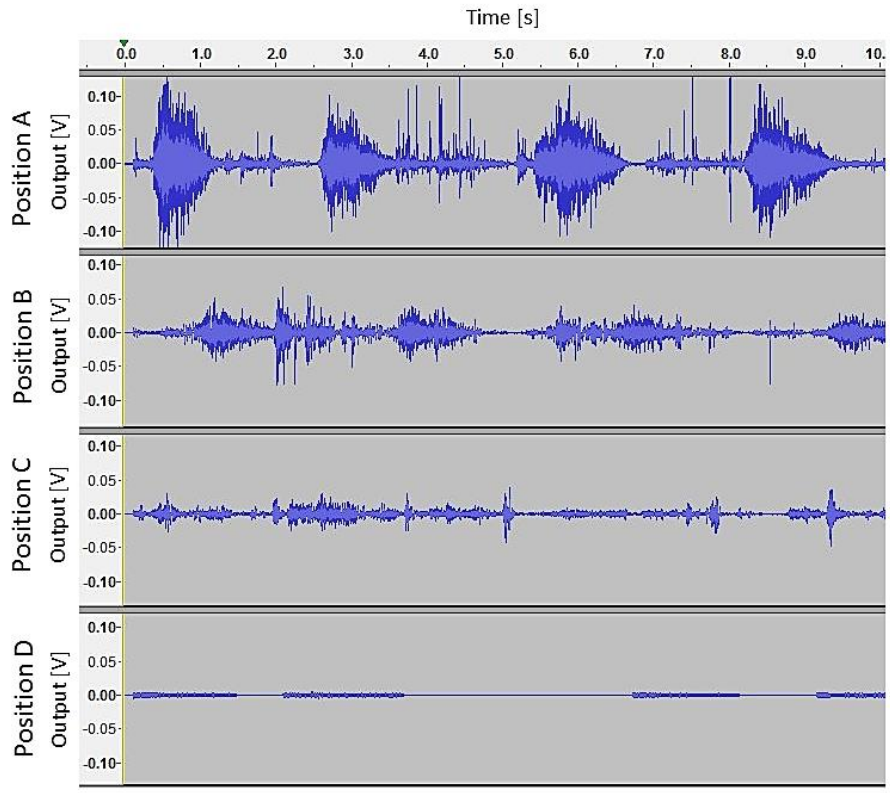



*Figure 5-5: Recorded breathing when accelerometer is attached in different positions. (A) above upper lip & under nostril, (B) above upper lip & not directly under nostril, (C) cheekbone, (D) above corner of mouth.*

Besides establishing which position of the accelerometer works best, possible artefacts were recorded. In Figure 5-6 examples of vibrations caused by moving the head are shown. At the beginning and end of each recording the subject hummed to indicate the start and finish of the data collection. The first recording shows the signal originating from vibrations when moving the head to one side and back to the centre whereas the second recording shows the signal originating from vibrations when moving the head up and down. The third recording shows the signal arising from vibrations when hair touches the cable and the head is moved. It was established that moving during the recording leads to significant artefacts which could overwrite the actual recording of rubs. Therefore, for future recordings subjects were instructed not to move during the recording.

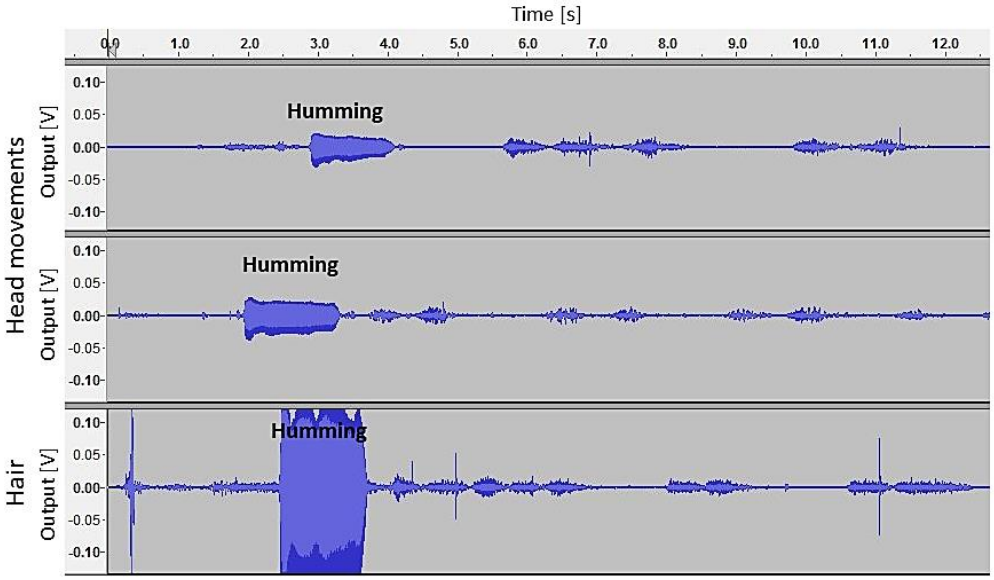



*Figure 5-6: Examples of artefacts caused by movements. First recording: movement of the head (to one side & back to the centre), second recording: movement of the head (down & up), third recording: hair touching the cable of the accelerometer & head movement.*

Further artefacts which cannot be avoided were caused by swallowing. The recording in Figure 5-7 shows an example of the vibrations measured when breathing and the vibrating measured by swallowing. Although the vibration signal of swallowing can interfere with rub signals, filters applied during the data analysis were able to correctly identify and remove the swallowing events.

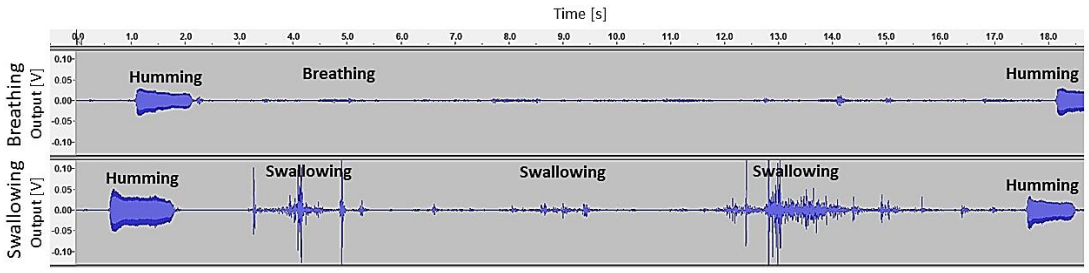



*Figure 5-7: Signal recorded when breathing (top recording) and swallowing three time (start at 3.2 s, 7.7 s, 11.8 s).*

In the next part of the method development, different protocols for rubbing the tongue across the palate were performed. An illustration of the protocols can be found in Figure 5-8 and is also described here. In Figure

5-9 the recorded acoustic signals are shown. For rub A and B, the tongue was moved from the front of the palate (close to the teeth) to approximately the middle of the palate and for rub B a higher tongue pressure was used than for rub A. Comparing these two recordings, it is clear that the tongue pressure plays a major role in the intensity of the recorded rubs. The vibration signals recorded from rubs with higher tongue pressure resulted in a clearer acoustic signal than the ones with lower tongue pressure. The recording of rub C (lower tongue pressure) shows rubs where the tongue was moved across the palate from the front to the middle of the palate and back to the front again. The vibration signals recorded were of lower intensity and with some high spikes and it was difficult to identify the beginning and end of a rub. The protocol for rubs D was the same as for rub C with a final rub from the front to the back at the end. Although the vibration signals from these rubs were clear, the intense rubbing of the tongue against the palate would lead to fatigue over the course of a session and also irritate the tongue, leading to a soar sensation which would affect mouthfeel perception. Therefore, protocol B was followed for the study with the subjects.

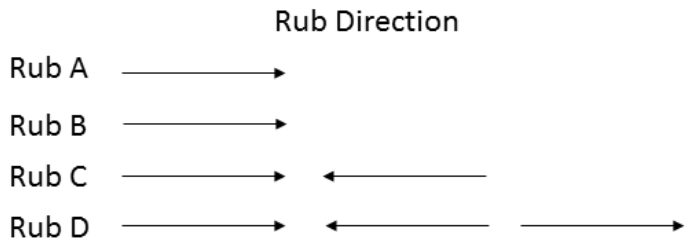



*Figure 5-8: Illustration of rub direction using for each rub set. An arrow pointing towards the right indicates a rub from the front of the palate to the back. An arrow points towards the left indicated a rub from the back of the palate to the front. Audio data can be found in Figure 5-9*.

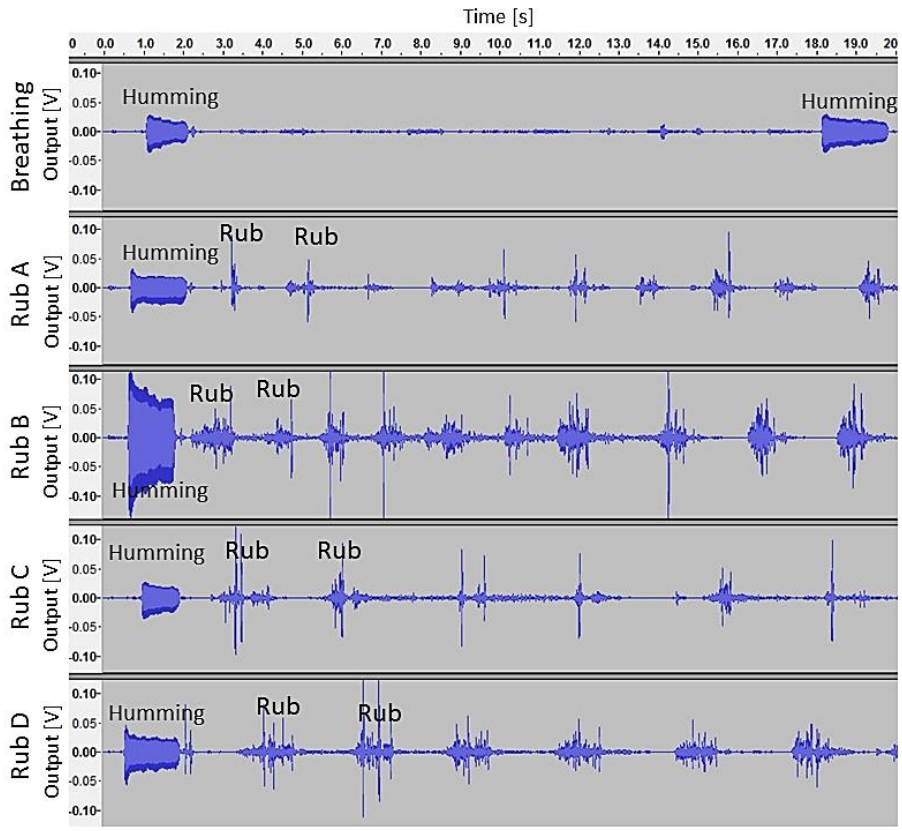



*Figure 5-9: Recording of breathing and different rubs. For each recording of rubs, the first two rub events are labelled. (A) Single rubs moving the tongue from front to back, (B) same as (A) with higher tongue pressure, (C) two rubs in one set, (D) three rubs in one set.*

### *5.2.3 Subjects and Samples*

Ethical approval for this study was granted by the Medical Ethics Committee of the University of Nottingham (reference number: I12072016 SoBS SSC). Three subjects that previously were part of the panel for the sensory studies conducted during this PhD research (Chapter 2 & 3) participated in this study. The subjects were inviated to take part in the study as they had a similar saliva flow rate (see Appendix 2) and were available for the proposed sessions. A difference in saliva flow could lead to a different dilution of the samples which might affect the recordings.

Each subject attended 6 sessions; in the first session they were familiarised with the set-up of the accelerometer and practised the rubbing protocol. In the remaining five sessions, different samples were evaluated.

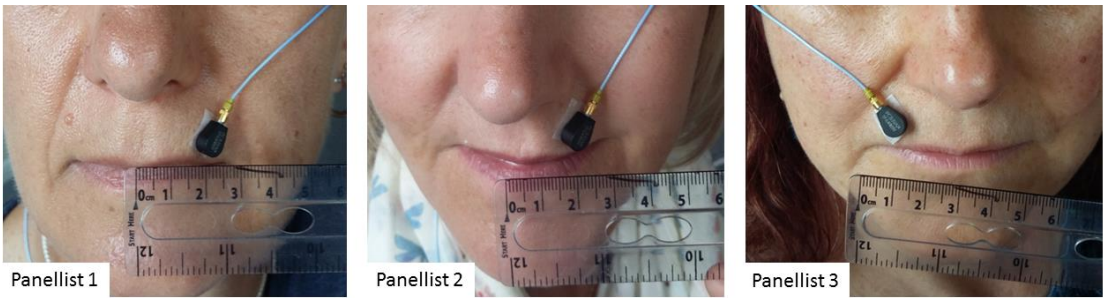


*Figure 5-10: Set-up of accelerometer for the different subjects who attended the study.*

At the beginning of each session, the accelerometer was attached to the skin of the subject using the photos in Figure 5-10 as a guide. After the attachment of the accelerometer, breathing was recorded to ensure that the signal did not interfere with the rubs recorded later on. Furthermore, the vibration noise of rubs prior to sample consumption was recorded as a baseline measurement.

A schematic illustration of the protocol followed during the sessions is displayed in Figure 5-11. Once the baseline measurement at the beginning of the session was carried out, the subject consumed the first sip (10 ml) of a sample, swirled the sample around the mouth for 5 s and then swallowed the sample. When the subject had the sample in the mouth, the recording was started. Once the sample was swallowed, the subject hummed to indicate the start of the actual data collection and started rubbing the tongue across the palate. After approximately one minute, the subject was instructed to stop the tongue rubs, which was marked with a

further hum. After that, the subject rated the perception of thickness, mouth coating, bitterness and astringency on an unstructured scale with anchor points (0 = low, 10 = high). A training for the evaluated attributes was not required since the subjects had experience in rating these attributes due to attending the earlier studies in this PhD research. After the perception was rated, the second sip of the same sample (10 ml) was consumed and the same protocol as for sip one was followed (see Figure 5-11). After the second sip, a third sip of the same sample (10 ml) was consumed and the same protocol as for sip 1 and 2 was followed. Finally, the subject had a 5 minute break during which crackers (Rakusen´s Limited, UK), honeydew melon (local retailer) and water (Evian, Danone Waters, France) were used for palate cleansing. Then a different sample was presented and the subject followed the same protocol for this new sample. In total, each subject evaluated 6 different samples per session (see Table 5-1) which were presented in a randomised order.

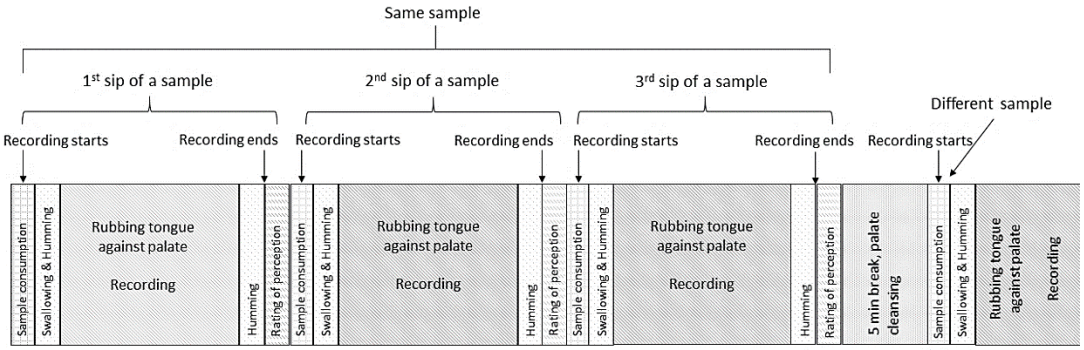



*Figure 5-11: Schematic of session protocol followed by the subjects. In one session 6 different samples were consumed.*

The samples listed in Table 5-1 were chosen to represent different mouthfeel attributes as indicated. Tannic acid and 1% tea were chosen as examples of astringent samples, whereas quinine was chosen to represent

a bitter stimulus. The same concentrations of tannic acid and quinine were also used during the Force plate experiment and the same suppliers were used (see section 4.2.3.). 0.1% tea and 10% fat was chosen to represent a mouth coating and thicker sample as was determined in the first sensory study (see section 2.3.4). The two tea samples were prepared as described in section 2.2.2. Single cream was chosen as a sample which exceeded the fat content of tea in order to investigate if a higher fat content and a higher viscosity are characterised by a different vibrational profile. It had a fat content of 19 % and was purchased from a local retailer. The samples containing tea were served at 50°C and the remaining samples were served at 5°C.

*Table 5-1: Samples evaluated by three subjects. 10 ml per sample were consumed per sip. In each session 3 sips were consumed and 5 sessions with the samples were performed by each subject.*

| Sample | Concentration | Mouthfeel Attribute |
|---|---|---|
| Water | | Thin |
| Tea | 1% | Thin, Astringent, (Bitter) |
| Low tea & fat | 0.1% tea, 10% fat | Thin, Mouth coating |
| Single cream | Undiluted, 19 % fat | Thick, Mouth coating |
| Tannic acid | 0.75 g/l | Thin, Astringent |
| Quinine | 15 mg/l | Thin, (Bitter) |

## 5.3 Data Analysis

The following section describes how the raw audio data was processed in order to characterise the acoustic signal. Each recording is analysed on a rub-to-rub basis, meaning that for each rub different parameters were calculated. However, this means that the beginning and end of each rub needed to be identified. In each recording approximately 10 rubs were recorded, resulting in approximately 900 rubs per subject (6 samples x 3 sips x 5 sessions). Therefore, it was not practical to identify the rub timings

manually and a categorisation tool was developed to identify the start and end of a rub.

### *5.3.1 Processing of the raw Audio Data*

The acoustic signal caused by vibrations was recorded using Audacity (version 2.1.2). The raw data was filtered in MATLAB into 9 different frequency bands; each covering one octave (see Table 5-2). The MATLAB scripts used for the data analysis were provided by Unilever. Since the files are user configurable, the author as the user applied and changed them in order to gain the best results. The filtered raw data in Figure 5-12 shows that with increasing frequency the signal changes. However, neighbouring bands produce similar signals. When listening to the filtered data it was observed that the recordings from band 1 and 2 contained very low rumbles whereas bands 4 to 6 contained clear rubs. In band 6 some noise which may be caused by air bubbles breaking was also heard. Bands 7 to 9 contained high pitch noise.

*Table 5-2: 9 Frequency bands used to filter the raw acoustic signal. Each frequency band covers an octave.*

| **Band No** | **Band Centre [Hz]** | **Band Bottom [Hz]** | **Band Top [Hz]** |
|---|---|---|---|
| 1 | 31.25 | 22.1 | 44.2 |
| 2 | 62.5 | 44.2 | 88.4 |
| 3 | 125 | 88.4 | 176.8 |
| 4 | 250 | 176.8 | 353.6 |
| 5 | 500 | 353.6 | 707.1 |
| 6 | 1000 | 707.1 | 1414.2 |
| 7 | 2000 | 1414.2 | 2828.4 |
| 8 | 4000 | 2828.4 | 5656.9 |
| 9 | 8000 | 5656.9 | 11313.7 |

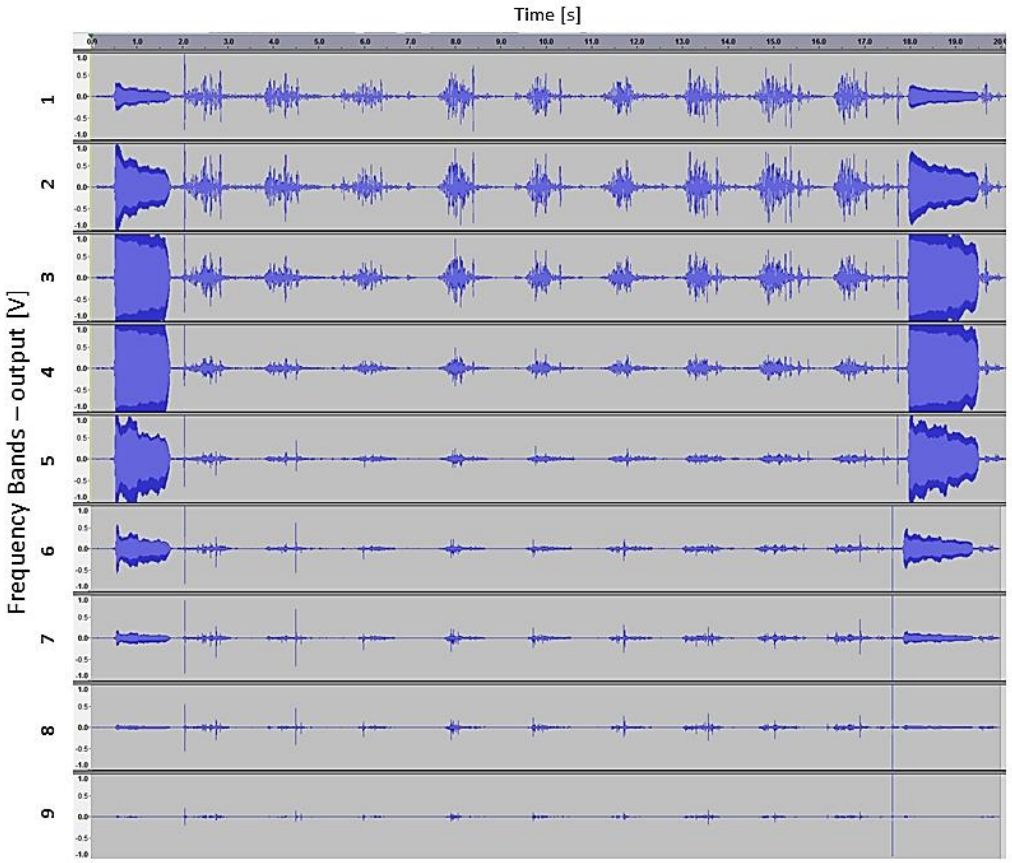



*Figure 5-12: Recorded data filtered into 9 frequency bands. These recording show the vibrations recorded after the consumption of water.*

Since it was observed that neighbouring frequency bands produce similar signals, it was decided to filter the audio signal into three bands: low, medium and high frequency with the low frequency band covering 16 – 64 Hz, the medium frequency band covering 65 – 500 Hz and the high frequency band covering 501 – 2000 Hz. The three bands are associated with the 9 bands the signal was previously filtered in. However, now the bands cover a slightly lower frequency and do not cover the highest frequency since it was observed that the high frequency data does not contain much information. Figure 5-13 shows an example of the data in the three frequency bands obtained after consuming water. The low band contains data of a very low rumbling. In the medium band rumbling noise can be heard as well as the breaking of air bubbles. The high band contains

mainly crackling noises caused by fluid bridges. With increasing frequency the signal intensity decreased, showing the low and medium frequencies contribute more to the vibration sound of the rubs than the high frequency sounds. The accelerometer is more sensitive to high frequency vibrations, however, this was not observed here. A possible explanation is that the flesh might block high frequency vibrations and therefore the low and medium frequency vibration are picked up stronger.

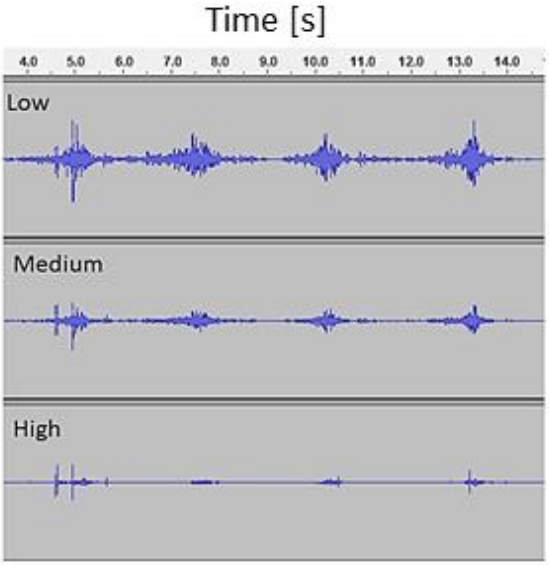



*Figure 5-13: 3 frequency bands for rubs recorded after water consumption. This section shows 4 rubs. Low frequency band = 16 - 64 Hz, Medium frequency band = 65 – 500 Hz, High frequency band = 501 – 2000 Hz*

In order to quantify the intensity of the vibration from the rub, the rub needed to be identified. After the recordings were filtered into three frequency bands, a Gaussian filter was applied to the low frequency band. Since the three frequency bands show the acoustic signal at the same time, it was not important which frequency band was used for the Gaussian filtering. The Gaussian filter was used to smoothen the signal and to ensure that the rubs which are automatically identified are the same ones that were recorded in the audio file. Figure 5-14 shows an example of two

different Gaussian filters which were applied to the same recording (preliminary data). In Figure 5-14A a Gaussian filter of 1 s was applied whereas in Figure 5-14B the Gaussian filter was 2 s. For a Gaussian filter of 2 s, it was found that not all rubs were correctly identified as can be seen when comparing the signals to the rub signals in the recorded audio file where more rubs can clearly be identified. However, a Gaussian filter of 1 s seemed to work better for this data and all rubs were correctly identified.

This was also checked for the data from the different subjects and it was found that a suitable Gaussian filter can be subject specific. For example for subject 1 a Gaussian filter of 2 s lead to a better identification of rubs in comparison to a Gaussian filter of 1 s.

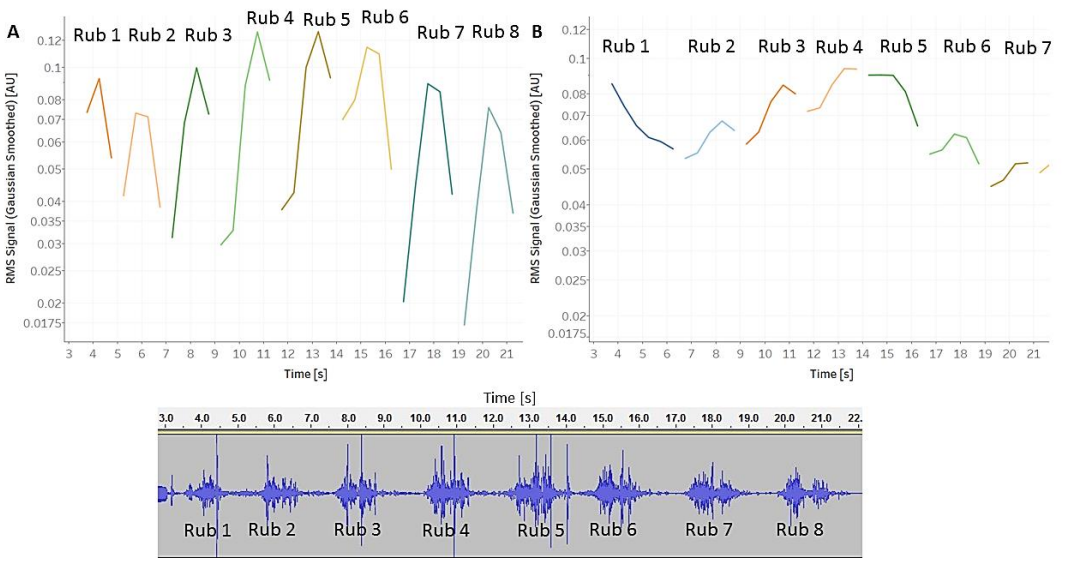



*Figure 5-14: Rubs in the low frequency band (A) with a Gaussian filter of 1s, (B) with a Gaussian filter of 2s. Rubs identified with the Gaussian filter need to match the rubs in the audio recording below. For this subject, a Gaussian filter of 1s identified rubs best.*

Once the Gaussian filter was optimised, the data was processed to identify rubs and find criteria which distinguish between rubs and artefacts. The two main artefacts are swallowing which can wrongly be identified as rub and the humming at the beginning and end of the recording. To distinguish between rubs and artefacts without listening to each of the numerous recordings, two different variables of each rub were calculated: the distinctiveness and the duration of a rub. The distinctiveness of a rub was calculated as the ratio between the highest Gaussian smoothed signal and the lowest Gaussian smoothed signal. The duration of a rub was calculated as the difference in time between the start and the finish of a rub.

Figure 5-15 shows an example of the rub durations of different rubs which were identified in one recording. Most rubs have a duration of approximately 4 s, however two rubs have a much longer duration (> 8 s). Comparing the timing of these two rubs to the raw data in the audio file shows that these two rubs are artefacts. The first long rub, as can be seen in the audio file, is caused by the humming and the second long rub is caused by swallowing. Although, two artefacts were identified, the humming at the end of the recording did not have a longer rub duration. This shows that rub duration alone is not a sufficient filter to distinguish between rubs and artefacts.

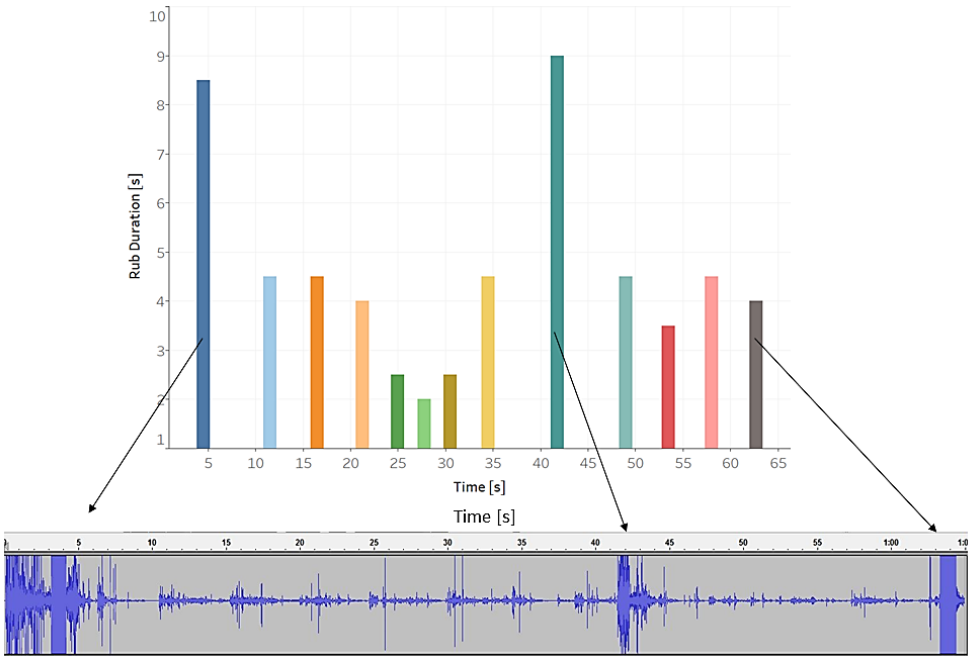



*Figure 5-15: Rub duration of different rubs over time (subject 1, sample: water). The first rub with a longer rub duration is due to humming and other rub at 42s is caused by swallowing. The rub at 62 s which are caused by humming did not have a longer rub duration than other rubs.*

The second parameter used to identify artefacts was the distinctiveness of a rub. Figure 5-16 shows the recording as presented in Figure 5-15 with the distinctiveness of different rubs plotted over time. Three rubs were identified to have a greater distinctiveness than the other rubs. These three rubs were identified as artefacts and matched to the recording. Using the distinctiveness of rubs as a filter to identify all three artefacts in this recording showed improved results.

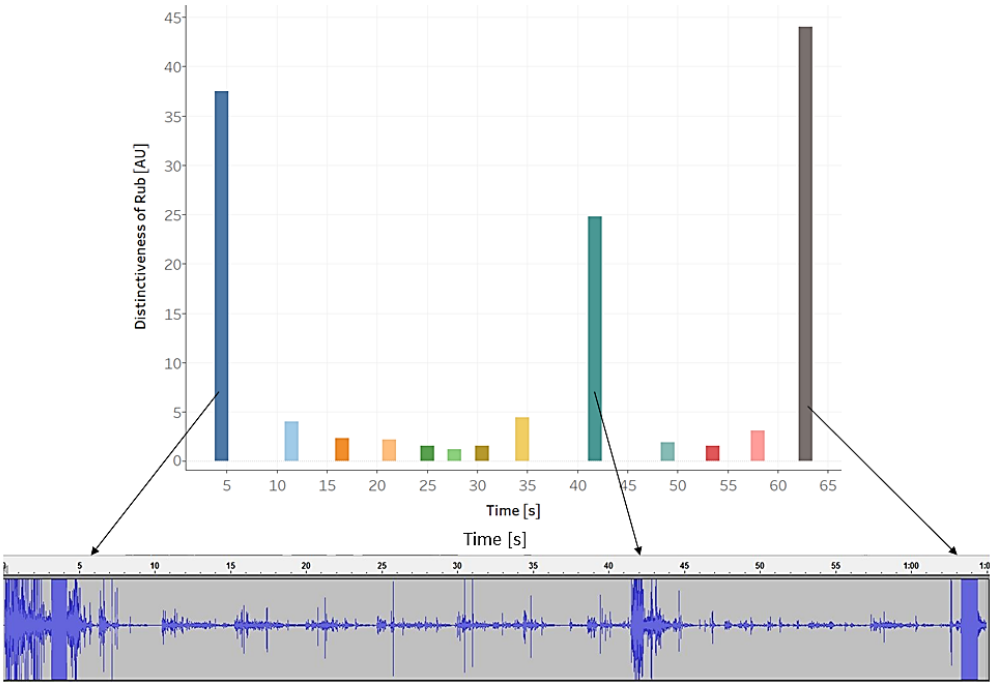



*Figure 5-16: Distinctiveness of different rubs over time (subject 1, sample: water). The recording of the bottom shows that the rubs at the beginning and end are caused by humming. The intense rub at 40 s is caused by swallowing.*

In the examples shown here, the rub distinctiveness was an improved measure to identify artefacts such as humming and swallowing. However, for other recordings it was found that the rub duration identified artefacts that the rub distinctiveness did not and therefore it was decided to use both criteria as filters.

The criteria needed to be checked for each subject since rubs were likely to have different characteristics for different subjects. A third criterion was introduced to ensure that the wrongly identified rubs at the beginning were correctly filtered out since it was observed that the first rub could have a short duration and low distinctiveness which would wrongly be identified as a rub. To filter out the first one or two rubs which were caused by humming, any rubs between 0 to 10 s of each recording were filtered out. For subject 1, it was found that a rub duration below or equal to 6 s and a distinctiveness below or equal to 6 AU filtered out artefacts. The

combination of these three criteria was a powerful tool to identify real rubs and artefacts. This ensured that only the data from real rubs were processed and analysed, ensuring that no false positive or negative results were obtained. The filters were checked by comparing the identified artefacts to the audio recordings to ensure they were working appropriately.

### *5.3.2 Variables and Result Plots*

Once it was ensured that artefacts were filtered out, different parameters were calculated to characterise the rubs. These parameters included the integral of band 1, band 2 and band 3, the ratio between band 3 and band 1, the ratio between band 2 and band 1, and the ratio between band 3 and band 2 as well as rub duration. The integrals of the different bands represented the area of each rub for a given frequency band and the median of these areas were plotted. The area of a rub gave an insight into the intensity of the rub. Besides characterising the intensity of the rub for different frequency bands, the radio between two frequency bands was calculated. The ratio between different frequency bands can be characteristic for a sample and may produce a "fingerprint" for different samples, independent to intensity. The rub duration was calculated as the time duration [s] from the start to the end of the rub.

For the plots shown in the result section of this chapter, median values were plotted. Each recording consisted of approximately 10 rubs for which the above listed rub parameters were calculated. The median of the rubs in one recording was calculated and plotted. The median was chosen since it is more robust to any outliers and variation. Furthermore, it was

observed that the rub duration of some rubs was shorter than the average in a recording. This could mean that one rub was wrongly split into two or three. By using the median, events like this will not affect the results to a great degree. The error bars in the results plots show the upper and lower quartiles. The quartiles are able to reveal any outlier which might be more difficult to identify when plotting the standard deviation.

### *5.3.3 Statistical Analysis*

In order to identify differences between samples, statistical tests were performed. The data used for the statistical analysis was the median data from different rubs. A 3-way ANOVA (samples, sips, and sessions) with interactions followed by Post-hoc test (Tukey) was performed using XLSTAT (version 19.01) to identify significant differences between samples and understand the reproducibility of the data. Significant differences between samples and between sessions were found, however, no significant differences between sips were identified. This showed that the consumption of 3 sips without palate cleansing between sips did not change the vibration measured. The significant differences between sessions is not surprising since the accelerometer might have been attached in a slightly different position and the tongue might have been rubbed in a different way. Notably, session 1 was found to have the lowest value for all variables across the different sessions. Therefore, an outlier test was performed (Grubbs`test for outliers, XLSTAT – v. 19.01). The results from Grubb`s outlier test showed that no outlier was identified and the data of all five sessions could be used to identify significant differences between samples. Furthermore, a 2-way ANOVA (samples, sessions) with interactions was

performed to identify significant differences in the mouthfeel perception of the samples evaluated by subject 1.

A Principal Component Analysis (PCA) was performed using XLSTAT (version 19.01) to analyse the overall structure of the data set, visualise correlations between measured parameters and visualise relationships between samples. PCA analyses use the associations between variables to create a set of new uncorrelated variables which explain the variation in the data set best. In this case a correlation PCA was carried out since not all variables could be plotted on the same scale, for example rub duration was on a different scale to the integrals. Therefore, it was important to standardise the data first before calculating the new principal components.

In order to understand how samples were related to sensory and instrumental data a multiple factor analysis (MFA) was carried out using XLSTAT (version 19.01). MFA is an extension of PCA and can be used to identify correlations and relationships between data from different sources, for example sensory and instrumental data. In essence, for each table a PCA was performed, the data was standardised and combined to run a global PCA. In this way, it could be identified which parameters were correlated and how they were related to different samples.

## 5.4 Results and Discussion

### *5.4.1 Ability of Subjects to produce Rubs*

Figure 5-17 shows three recordings of rubs after the consumption of one sip of tea by the three subjects engaged in this acoustic tribology study. The figure reveals that for the same sample great differences between subjects existed and that only the data from the first subject showed clear

and defined rubs. Numerous reasons can lead to the variance in the recorded data. It is possible that due to differences in the physiological structure of the oral cavity, the signals which were recorded varied between individuals. Furthermore, the protocol which was followed for this experiment might have been the cause for the different recordings. It seems that one subject could follow the protocol easily and produce rubs which show a good separation, however for the other subjects the rubs were less defined and separated. For future experiments, it might be useful to provide the subjects with a screen to see the audio signal whilst they perform the experiment.

The results discussed in the following sections are the results obtained from the data produced by subject 1 since the data from the other two subjects could not be processed as just explained.

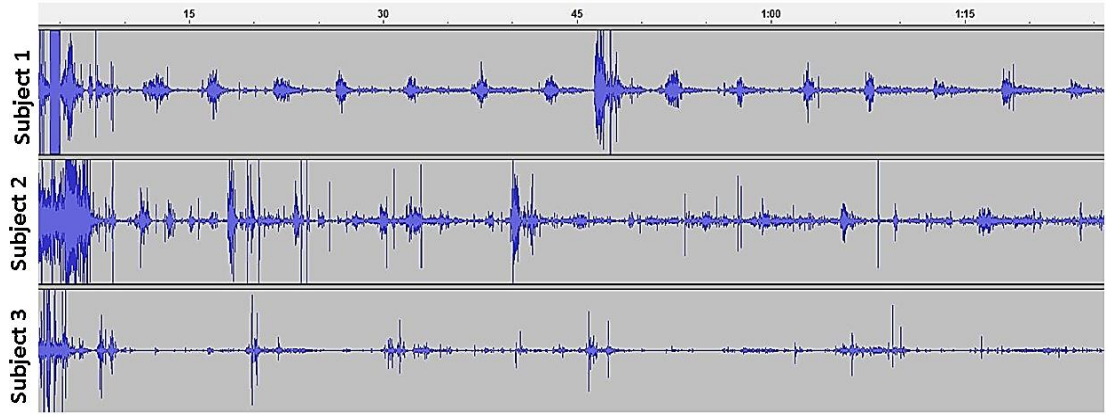



*Figure 5-17: Example of recording for three different subjects after the consumption of one sip of tea. Only the first recording shows clear rubs.*

### *5.4.2 Perception of Samples*

Table 5-3 shows the mean scores of thickness, mouth coating, bitterness and astringency for the 6 samples scored by subject 1. As expected, cream

was found to be the thickest and most mouth coating sample, followed by 0.1% tea 10% fat, which was somewhat thick and mouth coating. Single cream is known to have a viscosity of 50 mPa·s (Refina Ltd., 2018) which is approximately 50 times higher than the viscosity of 0.1% tea with 10% fat (viscosity = 1.1 mPa·s, see Table 2-8). The other samples were perceived to be not thick and not mouth coating. For bitterness, 1% tea was found to be the most bitter sample, followed by 0.1% tea 10% fat and tannic acid. Somewhat surprising is that quinine was not rated to be bitter. The detection threshold of quinine is 0.0083 mM (Keast & Roper, 2007) which is equivalent to 0.03 g/L. The concentration used in this study was with 0.15 g/L well above the average detection threshold, indicating that this particular subject had a high detection threshold for quinine.

*Table 5-3: Mean scores of sensory perception of different samples evaluated by subject 1. The mean and standard deviation (SD) were calculated from 15 replicates (3 sips of the same sample per session, 5 sessions). Samples sharing the same letter are not significantly different (p>0.05) as identified by ANOVA with Post Hoc Tukey.*

| Sample | Thick | | Mouth coating | | Bitter | | Astringent | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| **Cream** | $8.9^{A}$ | 0.3 | $8.7^{A}$ | 0.3 | $0^{C}$ | 0 | $0.1^{C}$ | 0.2 |
| **1% Tea** | $0^{C}$ | 0 | $0^{C}$ | 0 | $7.0^{A}$ | 0.8 | $6.8^{A}$ | 1.9 |
| **0.1% Tea 10% Fat** | $2.2^{B}$ | 1.7 | $2.7^{B}$ | 2.1 | $1.3^{B}$ | 0.9 | $2.9^{B}$ | 1.9 |
| **Quinine** | $0^{C}$ | 0 | $0^{C}$ | 0 | $0.5^{BC}$ | 0.6 | $0.1^{C}$ | 0.1 |
| **Tannic acid** | $0^{C}$ | 0 | $0^{C}$ | 0 | $1.3^{B}$ | 1.4 | $6.6^{A}$ | 1.6 |
| **Water** | $0^{C}$ | 0.1 | $0^{C}$ | 0 | $0^{C}$ | 0 | $0^{C}$ | 0 |

Tannic acid and 1% tea were perceived to have a similarly high level of astringency, followed by 0.1% tea 10% fat. The remaining samples were not perceived to be astringent. The scores from the rating validate that the samples selected for this study have different mouthfeel characteristics.

### *5.4.3 Acoustic Data*

The results of the 2-way ANOVA are included in Table 5-4. Significant differences between samples were found ($p<0.05$) as well as significant interactions between samples and sessions ($p<0.0001$) for all parameters except rub duration. In the following sections, significant differences between samples as identified by Post-hoc test are discussed in detail.

#### *5.4.3.1 Integrals for different Frequency Bands*

The integrals are calculated from the area of the acoustic signal and describe the intensity of the acoustic signal. In Table 5–4 the integrals for the different frequency bands and different samples are listed and significant differences between samples were found ($p<0.05$). For each recording, the area of a rub was calculated and the median of all rub integrals was taken. The means shown in Table 5–4 were calculated from the medians of 15 recordings of the same samples with 3 replicates (= sips) consumed in one session and 5 sessions performed by the subject. Figure 5-18 shows an example of the integrals for band 2 calculated for all sessions and samples and shows the variation within the data.

*Table 5-4: Means of the medians of 15 experiments. 3-way ANOVA (sample, sip, session) with Tukey Post Hoc found significant differences between samples and replicates. Samples sharing the same letter are not significantly different (p>0.05).*

| Samples | Rub Duration [s] | Integral (Band 1) | Integral (Band 2) | Integral (Band 3) | Ratio Band 3/Band 2 | Ratio Band 2/Band 1 | Ratio Band 3 /Band 1 |
|---|---|---|---|---|---|---|---|
| Tannic acid | $4.23^{AB}$ | $0.280^{A}$ | $0.072^{A}$ | $0.035^{A}$ | $0.46^{BC}$ | $0.25^{A}$ | $0.12^{B}$ |
| 1% Tea | $4.32^{A}$ | $0.271^{A}$ | $0.064^{AB}$ | $0.027^{AB}$ | $0.40^{CD}$ | $0.23^{B}$ | $0.09^{C}$ |
| Water | $3.80^{B}$ | $0.219^{C}$ | $0.050^{C}$ | $0.027^{A}$ | $0.63^{A}$ | $0.23^{AB}$ | $0.15^{A}$ |
| Quinine | $4.15^{AB}$ | $0.207^{BC}$ | $0.050^{BC}$ | $0.028^{AB}$ | $0.52^{B}$ | $0.24^{AB}$ | $0.13^{B}$ |
| 0.1% Tea 10% Fat | $4.12^{AB}$ | $0.237^{ABC}$ | $0.057^{ABC}$ | $0.031^{AB}$ | $0.50^{B}$ | $0.24^{AB}$ | $0.12^{B}$ |
| Cream | $4.05^{AB}$ | $0.241^{AB}$ | $0.058^{ABC}$ | $0.022^{B}$ | $0.36^{D}$ | $0.23^{B}$ | $0.08^{C}$ |

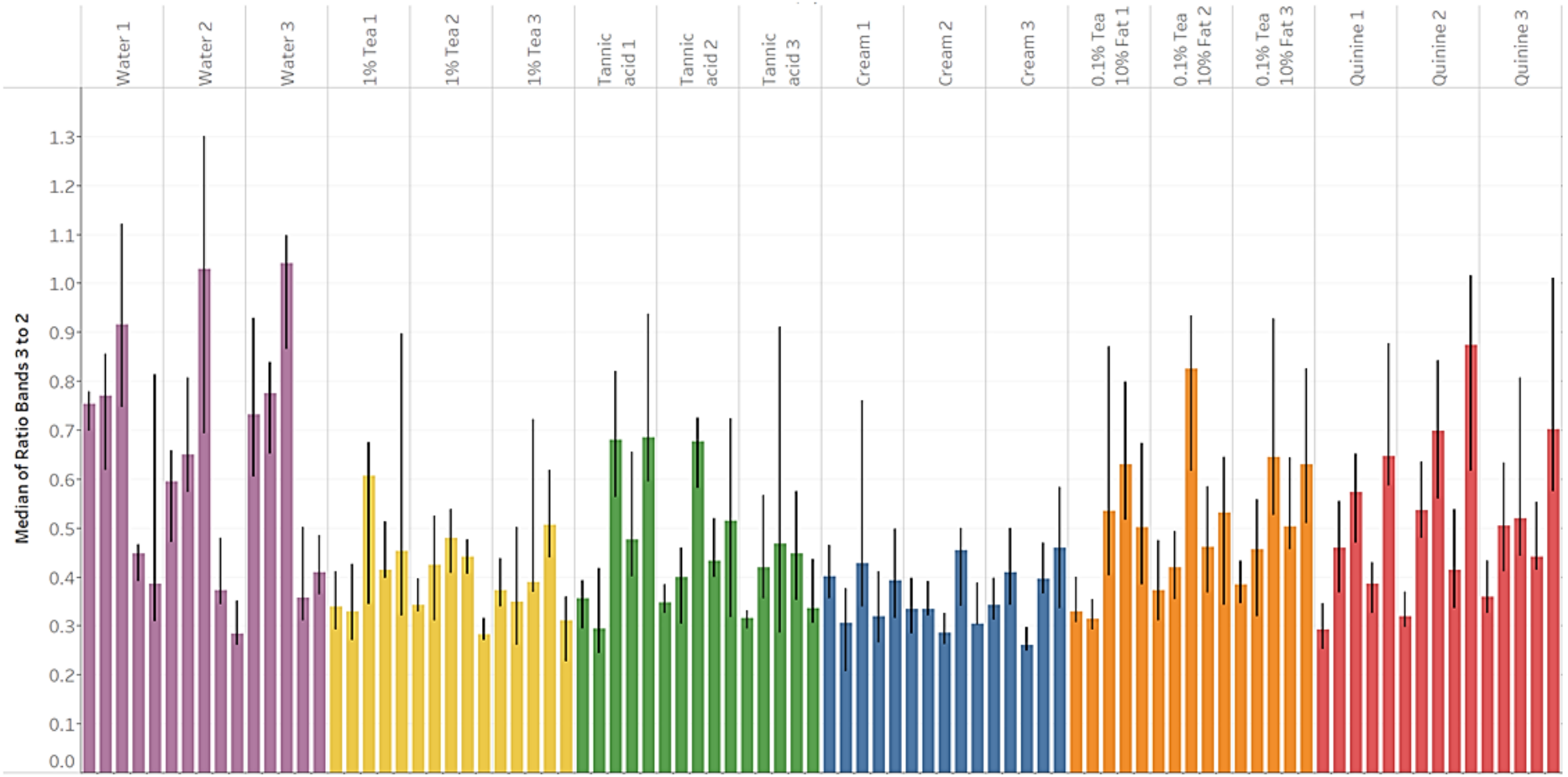



*Figure 5-18: Example of variation of integrals of band 2 calculated for all samples assessed by subject 1 in 5 sessions. Water 1 = median of rubs recorded after first sip of water was consumed. Water 2 = median of rubs recorded after second sip of water was consumed. Water 3 = median of rubs recorded after third sip of water was consumed.*

Band 1 covered the low frequencies and, as previously mentioned, listening to recordings of this band a distinct low rumble was identified. ANOVA identified significant differences between integrals of different samples in this frequency band (see Table 5-4 for Post-hoc groupings). Tannic acid and 1% tea had the greatest integral (rub area) which were significantly greater than the integrals calculated for water and quinine. This is an indication that astringent samples which are known to cause a rough sensation in the mouth result in a higher acoustic signal than samples which are not astringent. Polyphenols and salivary proteins interact, forming irregular shaped aggregates. When these are entrained between the tongue and the palate and rubbed across the palate they might cause more vibration and therefore leading to a louder low frequency sound than samples which do not form these aggregates. Cream and 0.1% tea 10% fat had integrals smaller than tannic acid and tea but greater than water and quinine. Cream had a significantly greater integral than water. Cream and 0.1% tea 10% fat contain fat droplets and also have a higher viscosity than the other samples. When fat droplets get entrained between the tongue and the palate they are known to lubricate both surfaces (de Hoog et al., 2006). This lubrication produces less vibrations and therefore a reduced noise. However, the cream was much thicker than the other samples, and therefore more force was required to disperse the cream in the mouth which might have resulted in the increased sound. It is worth noting that although significant differences between samples were identified, the integrals varied only between 0.280 and 0.207 AU corresponding to a difference of only 16% between the highest and lowest value.

When listening to the medium frequency band, clear rubs could be identified. For the medium frequency band, band 2, similar significant differences were observed as in band 1. However, the integrals in band 2 was smaller than in band 1. The integrals in band 2 ranged from 0.05 to 0.072 AU.

In the highest frequency band, band 3, high pitch sounds were heard which might be part of the rubs or due to air bubbles breaking. In band 3, only few significant differences were found. Tannic acid and 0.1% tea 10% fat had the greatest integrals in this band, however only tannic acid and water had a significantly different integral to cream. Cream had the lowest integral which might be due to its increased viscosity dampening the high frequency vibrations.

*5.4.3.2 Ratios between Frequency Bands*

Table 5-4 shows the results of the 2-way ANOVA including the significant differences between the ratios of different samples identified by Post-hoc tests. The ratio between two frequency bands gives an insight into which frequency band contributed more to the acoustic signal of a specific sample and varied between different samples. The ratio between band 3 and band 1 describes the ratio between high and low frequency signals measured from the rubbing. A high ratio therefore shows that the high frequency bands contributes more to the signal of the rub than the low frequency band. Water had the highest 3/1 ratio, showing that the high frequency contributed more to the acoustic signal than the low frequency band. Cream had the lowest 3/1 ratio showing that the rubs which were recorded after consuming cream contained a higher proportion of low frequency

signals than the ones recorded after the consumption of water. This might be because the thickness and mouth coating properties of cream damped high frequency vibrations.

The ratio 2/1 describes the contribution of medium and low frequency band to the acoustic signal. Tannic acid was found to have a high proportion of medium frequency sound. Tannic acid had the highest contribution of medium frequency signal to the acoustic signal which was significantly higher than for 1% tea and cream, which both had the lowest contribution of medium frequency data.

The ratio 3/2 describes the contribution of high and medium frequency band to the acoustic signal. Water had the highest contribution of high frequency signal to its acoustic signal which was significantly different ($p<0.05$) to the other samples. As for the ratios 3/1 and 2/1, 1% tea and cream had the lowest contribution of high frequency signal.

#### *5.4.3.3 Rub Duration*

Figure 5–19 shows the median of rub duration for one session including the rub duration of individual sips. The graph shows that the rub duration across one session was consistent, meaning that the subject was able to reproducibly rub the tongue across the palate at a similar speed. However, the results of the ANOVA show significant differences across sessions with the mean rub duration varying significantly between 2.7 to 4.8 s ($p<0.05$) (data not shown). The differences across sessions are most likely caused by the differences in tongue speed used to rub the tongue across the palate.

Despite the variation across sessions, significant differences ($p<0.05$) in rub duration between samples were identified (see Table 5–4). It was found that tannic acid and 1% tea had the longest rub duration with 4.2 and 4.3 s, respectively. The salivary proteins and astringent substances are known to interact, forming irregularly shaped aggregates (Nayak & Carpenter, 2008). These aggregates become entrained when the tongue is rubbed across the palate and due their irregular shape they could slow down the tongue, resulting in a longer rub duration. Water had the shortest rub duration which was significantly different from the rub duration of 1% tea ($p<0.05$). Water does not have a taste and should also not stimulate any other receptors in the mouth. It only dilutes the saliva in the mouth which might explain why water had the shortest rub duration.

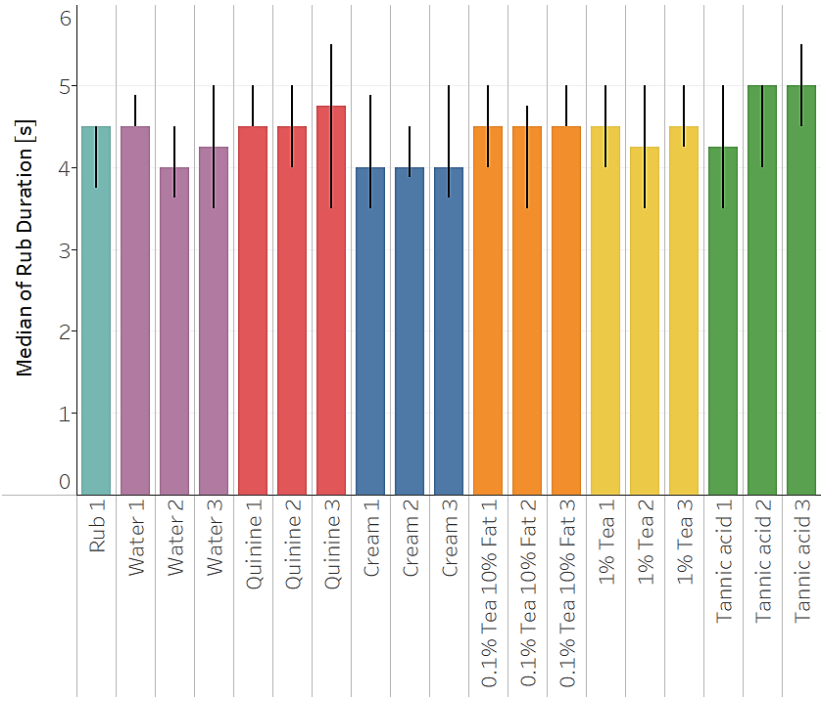



*Figure 5-19: Rub duration of all rubs recorded in 4th session by one subject. The bars for the median of rub duration calculated from all rubs of one recording. The error bars show the lower and upper quartile of the rub duration for each recording.*

### 5.4.4 Further Results

*5.4.4.1 Relationship between Samples and Acoustic Parameters*

A Principal Component Analysis (PCA) was carried out to visualise the relationship between the samples and the acoustic parameters: the integrals of the three frequency bands, rub duration and ratios between frequency bands. Figure 5–20 shows the bi-plot using the first and second principal component which explained the majority of the variation in the data set (81.30%).

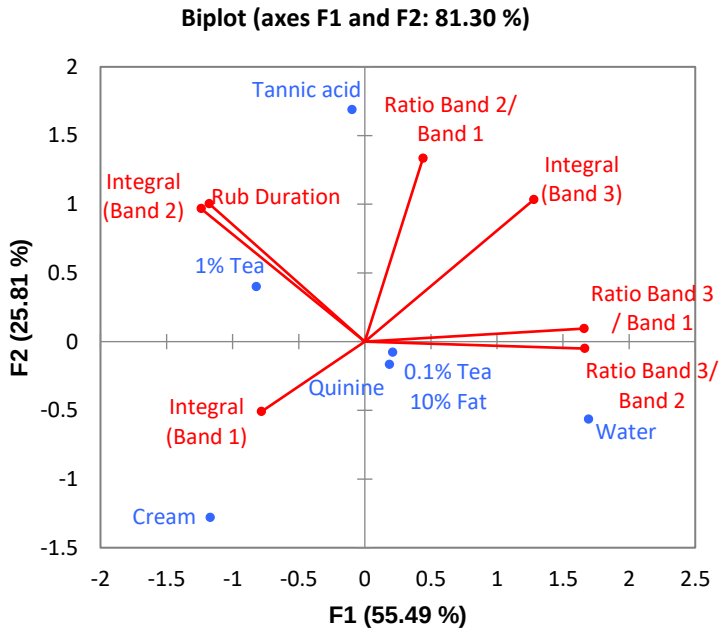



*Figure 5-20: Correlation Bi-plot of PCA showing how samples are related to acoustic parameters. Band 1 = low frequency data (16 – 64 Hz), band 2 = medium frequency data (65 – 500 Hz), band 3 = high frequency data (501 – 2000 Hz).*

Focusing on the acoustic parameters first, it was observed that the integral of band 1 was opposed to the integral of band 3. This means that there was a negative correlation between these two parameters and if a sample was observed to have a high integral for band 1 (low frequency), it had a low integral for band 3 (high frequency) and vice versa. Rub duration and integral of band 2 (medium frequency) were closely correlated to one

another suggesting that samples with a longer rub duration also had a higher contribution of medium frequency signal. The ratio between band 3 and 1 and the ratio between band 3 and 2 were positively correlated to each other and the ratio of band 2 and 1 was somewhat correlated to the integral of band 3.

Samples which were placed near each other were found to have similar acoustic parameters and samples placed far apart had different acoustic scores. It was found that the samples perceived as most astringent, tannic acid and 1% tea, were closely correlated and found to be mostly influenced by rub duration and the integral of medium frequencies (band 2). Cream could be found closely correlated to and driven by the integral of low frequencies (band 1). Cream was opposed to tannic acid and 1% tea. Water was the only sample which was somewhat correlated to the ratio of band 3 and 1 and the ratio of band 3 and 2. Quinine and 0.1% tea 10% fat were not associated to any of the acoustic parameters in particular.

#### *5.4.4.2 Relationship of Sensory Perception and Acoustic Parameters*

Multiple Factor Analysis (MFA) is a statistical tool that is based on PCA to analyse data of different origin e.g. instrumental and sensory data. In this case, the data from the acoustic measurements and the data from the sensory rating were analysed together to understand how they were correlated. The MFA plot is displayed in Figure 5-21.

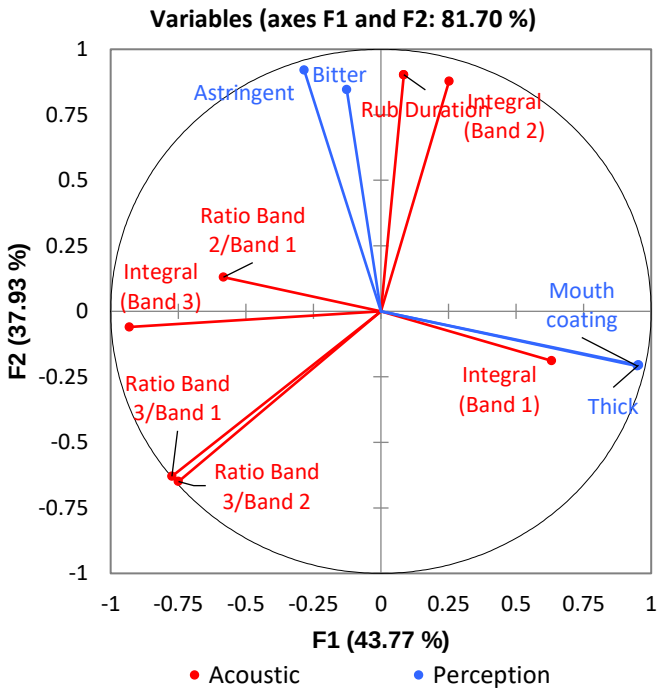



*Figure 5-21: Result of Multiple Factor Analysis. The plot shows correlations between sensory perception and acoustic parameters. Band 1 = low frequency data (16 – 64 Hz), band 2 = medium frequency data (65 – 500 Hz), band 3 = high frequency data (501 – 2000 Hz).*

As also observed in chapter 2 section 2.3.5, the fat-driven attributes mouth coating and thickness were closely correlated. Both attributes were also positively related to the low frequency integral. This suggests that samples which were mouth coating and thick produced a high component of low frequency vibrations when rubbed across the palate. The thick and mouth coating sample used in this study was single cream which also contained a high level of fat. When the cream was squeezed between the palate and the tongue, fat droplets cover the space between the two oral surfaces and might dampen any high frequency vibrations. This is also supported by the negative correlation with the integral of high frequencies.

Unsurprisingly, astringency and bitterness were highly correlated as observed in chapter 2. Both attributes were somewhat related to rub duration and the integral of medium frequencies. The rub duration of the

two astringent and bitter samples, tannic acid and 1% tea, was found to be longer than for other samples which explains this correlation.

The multiple factor analysis also revealed that no attribute was positively correlated to the integral of the high frequency band and any of the ratios between frequency bands. This might be an indication that the vibrations produced when rubbing the tongue across the palate are mainly medium or low frequency. The ratios between frequency bands do not give more insight into the correlation between acoustic parameters and mouthfeel perception and might therefore be negligible.

## 5.5 Conclusions

The aim of this study was to measure the acoustic signal when rubbing the tongue against the palate and link it to sample composition and mouthfeel perception. Acoustic tribology is a relatively unexplored area and therefore the results of this study are all exploratory.

The acoustic signal of vibrations was measured by attaching an accelerometer to the skin close to the mouth and it was possible to record an acoustic signal. Attaching the accelerometer outside the mouth is less invasive then the protocol used by van Aken (2013), who placed a microphone inside the mouth. However, although the protocol worked during preliminary data collection, in this study conducted with three subjects only data from one subject was clear enough to be analysed. Therefore, it is important to conduct further work to improve the protocol by for example providing a live feedback for the subjects so that they can observed what signal the rubbing is producing.

A significant advance of this study was the development of the sophisticated data analysis which was capable of identifying the beginning and end of each rub and to distinguish between rubs and artefacts caused by swallowing. Furthermore, for each rub characteristic parameters such as rub duration, area of the rub for different frequency bands as well as ratios between frequency bands were calculated.

For most of the acoustic parameters significant differences between samples were identified. The rubs after consuming a mouth coating and thick sample such as cream were mainly characterised by low frequency sounds. Tannic acid and 1% tea showed the longest rub duration and rubs were identified to have a high proportion of medium frequency signals. This shows that different acoustic signals can be linked to different mouthfeel attributes and might help to understand their mechanisms of perception further. Acoustic tribology is an exciting and promising technique which, once the method is sufficiently improved, could advance our understanding how acoustic signals are linked to mouthfeel. In order to do so, the sensitivity as well as reproducibility of the method need to be evaluated and a study with more subjects should be performed.

# 6 Overall Conclusions and Future Work

The overall aim of this research project was to investigate the mouthfeel attributes which are of importance to black tea beverages, and the impact of varying amounts of key ingredients such as tea, fat and sugar on these mouthfeel attributes. Although the mouthfeel of tea has previously been explored in other studies, they have mainly focused on astringency (Koch et al., 2012; Lee & Chambers, 2007; Wismer & Goonewardene, 2004). However, since milk and sugar are also commonly added to tea, other mouthfeel attributes were of interest in this research.

At the beginning of this research it was hypothesised that mouthfeel attributes are linked to compositional factors and the results of chapter 2 confirm this hypothesis showing tea- driven, fat-driven and sugar-driven attributes. Furthermore, it was hypothesised that instrumental measures can be used to predict mouthfeel perception. Overall the results of this research show that instrumental parameters can be correlated to certain mouthfeel attributes, however for complex attributes such as astringency, a correlation between instrumental and sensory data remains challenging.

Following the comprehensive literature review on tea presented in chapter 1, the first results chapter (chapter 2) presented findings based on a black tea model system. The black tea model system was developed to allow independent variation of tea, fat and sugar contents. The tea content in the model system used throughout this research varied between 0.1% to 1% which is equivalent to a total polyphenol range of 19.2 – 192 mg/g of dry matter. In commercially available black teas, the total polyphenol content varies between 80.5 – 134.9 mg/g of dry matter (Khokhar &

Magnusdottir, 2002), which shows that the medium tea level (0.55%) used here with a total polyphenol content of 106 mg/g of dry matter is representative of the amount of polyphenols consumed in commercially available tea. This highlights that the tea levels used in this research are comparable to teas on the market.

Using a QDA approach, a list of key mouthfeel attributes was developed. The mouthfeel lexicon included astringency, thickness, slipperiness and mouth coating; furthermore, bitterness and sweetness were included to prevent attribute dumping. Following the lexicon development, predictive and validated models were generated to reveal how tea, fat and sugar impact all the above-listed attributes. The knowledge of how mouthfeel can be manipulated by varying key ingredients is important as it can be used to formulate novel and healthier products without negatively impacting mouthfeel and consumer preference. The market of ready-to-go tea products is expanding rapidly and fundamental knowledge about the impact of ingredients such as fat and sugar is of paramount importance. The tea mouthfeel lexicon presented in this research was developed using instant black tea powder, sucrose and an oil-in-water emulsion as fat source. Since a wide variation of black tea exists, the investigation of different black teas could reveal if different mouthfeel attributes are elicited and if for example astringent sub-qualities become important. In this model system a liquid non-dairy creamer was used to add fat to tea, however, many ready-to-go tea products use instant creamers (usually spray-dried emulsions), which could impact on mouthfeel perception. Using a different fat source and emulsifier as well as changing droplet size

and viscosity could give more insight into how tea mouthfeel can be manipulated further.

The predictive models generated showed that bitterness and astringency were perceived and impacted by tea, fat and sugar concentration in a very similar fashion. Therefore, a study on the temporal perception of both attributes over multiple sips of tea was conducted and presented in chapter 3 of this thesis. The results gave a novel insight into the temporal perception of astringency and bitterness in black tea beverages and revealed that the temporal profiles of both attributes assessed over multiple sips of tea are distinctively different from each other. With increasing number of sips, the perception of astringency and bitterness increased. This is an interesting finding because most sensory evaluations of beverages are conducted after the consumption of one sip. This suggests that measuring the liking of tea after one sip for example could lead to different results compared to when measuring the liking after a whole cup of tea, since astringency and bitterness (which can decrease liking) build up in intensity. Furthermore, for the first time this study has revealed how additives such as fat and sugar impact the temporal perception of astringency and bitterness. Sugar was found to reduce perceived bitterness and astringency, when evaluated at one time point and over time. However, since the addition of sugar increases viscosity and sweetness simultaneously, it was not clear if the reduction of astringency and bitterness was due to increased viscosity or sweetness. The effect of sugar was decoupled by assessing tea with sweeteners and tea with thickener. The results indicated that the sweetness of sugar is key in reducing the temporal perception of astringency and bitterness, suggesting a cognitive

process such as cross-modal interaction and taste-taste interaction taking place. This is an important finding for the development of healthy beverages since it shows that unwanted attributes such as bitterness and astringency can be reduced by the addition of low-calorie sweeteners and not just sucrose. The addition of a thickener to tea which produced the same viscosity as 5% sucrose was found not to reduce bitterness and astringency. It was also observed that the perceived thickness of tea with thickener and tea with 5% sucrose was different, with the former being perceived as thinner than the latter. It could therefore of interest to conduct a sensory study where the perceived thickness of tea with thickener and tea with 5% sucrose is matched in order to investigate if matching the perceived thickness affects astringency and bitterness perception. It should also be considered to investigate the effect of other thickeners on astringency ad bitterness in order to confirm that this is not just a phenomenon specific to dextran.

After exploring the mouthfeel perception of black tea beverages, two instrumental methods were used to investigate if and how instrumental measurements can be correlated with mouthfeel perception. Using a force plate, the frictional and vibrational behaviour of different black tea beverages were assessed and presented in chapter 4 of this thesis. It was thought that using vibrational amplitude as well as the friction measurements would add further knowledge about the physical behaviour of the tea samples, however, friction coefficients and vibration amplitudes were found to distinguish between tea samples in the same manner. It was further observed in this study that the addition of fat had the greatest impact on friction coefficient and vibration amplitude of tea beverages,

thereby indicating that one of the mechanisms by which astringency is reduced by fat is through a physical reduction of the friction coefficient. Furthermore, due to the impact of fat on measured friction coefficient and vibration amplitude, a negative correlation between both instrumental parameters and mouthfeel attributes such as mouth coating, thick and slippery was observed. This shows that the force plate can be used to characterise tea beverages with different fat-driven mouthfeel attributes. The force plate also confirmed that sugar does not reduce astringency by a physical interaction since the friction coefficient and vibration amplitude were unaffected by the addition of sugar. This supports the findings of the Time-Intensity study which showed that sweetness alone is able to reduce astringency through cross-modal interactions. Although the measured friction coefficients and vibration amplitudes were well correlated with fat-driven attributes, the observed correlation between these instrumental parameters and astringency was minimal. This confirms, as other studies have (Rossetti et al., 2009; Vardhanabhuti et al., 2011) that it is difficult to use tribological measurements to predict astringency, and that sensory methods are still the best way to evaluate this complex mouthfeel attribute. There are two factors that could be considered to further improve the experimental set-up of the current study and improve its similarity to in-mouth scenario. Firstly, the mixing ratio between tea and saliva could be optimised in order to ensure a representative ratio between beverage and saliva as found in the mouth. Secondly, the Bioskin used in this study to represent oral surfaces is a textured surface which is rougher than the surfaces of the tongue. Furthermore, the Bioskin is not as flexible/elastic as the tongue and might represent the tissue of the hard palate better. The

fingertip which was used as the other surface is more elastic which might represent the tongue is a better way. However, it would be of value to investigate other possible materials which match the tongue and/or hard palate better, focusing on surface roughness, elasticity and hydrophobicity. An improved experimental set-up could improve correlations between analytical measures and sensory perception of astringency in tea.

Finally, "acoustic tribology" was used to record vibrations originating from in-mouth tongue rubs against the palate and the protocol development can be found in chapter 5. It was found that samples with different mouthfeel attributes were correlated to different acoustic parameters. For example, cream - a thick, mouth coating sample was well correlated with low frequency signals, whereas astringent samples such as tea and tannic acid were characterised by medium frequency signals. The results from this novel study showed that "acoustic tribology" is an interesting research area and that vibrations measured from the mouth can be used to better understand mouthfeel perception. More work is required in order to improve the technique to record vibrations from tongue rubs. A possible solution could be to provide the subjects with prompts when to rub the tongue against the palate and then to have a break, however this would influence the natural tongue movement and might lead to bias. The six samples assessed using acoustic tribology had very different mouthfeel characteristics and correlations between perception and certain acoustic parameters were found. However, this was expected since the samples were so different in terms of their composition. It is therefore important to assess samples with similar composition but different mouthfeel perception using the method presented in this study. This would confirm if this method

is capable of distinguishing between samples of similar composition as suggested by van Aken (2013).

In summary, the combination of sensory and instrumental methods has proven to be a powerful tool capable of enhancing our understanding of mouthfeel in black tea beverages. While mouthfeel remains a key sensory property of food and beverages which has received little attention to date, the results of this PhD have advanced our understanding of this important sensory property in tea and how it can be manipulated by additives.

# Appendix 1: Droplet size

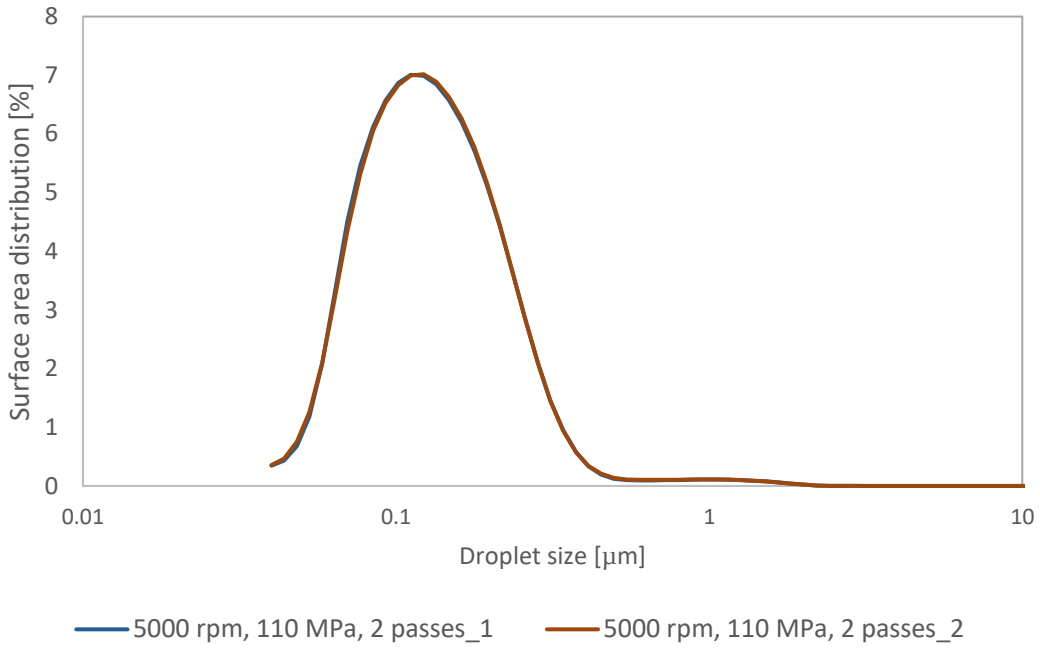
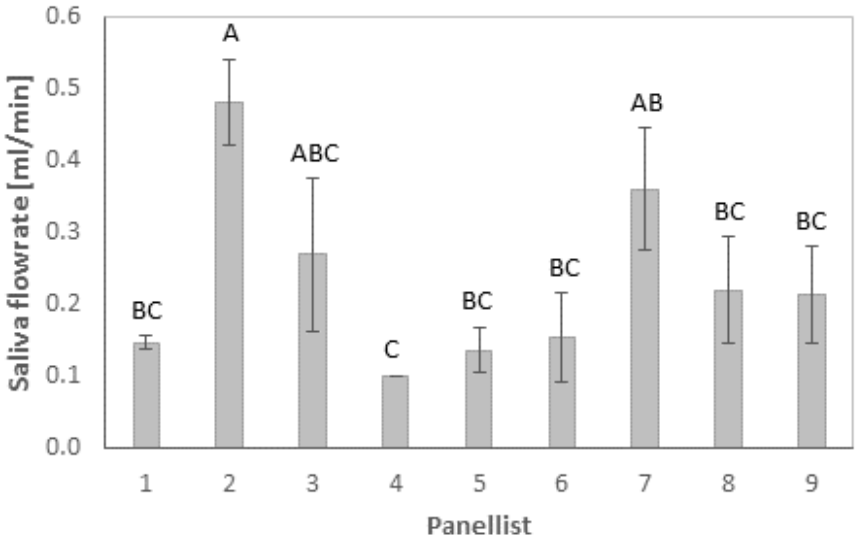



*Figure A2: Droplet size distribution of emulsions processed as described in chapter 2.2.1. Average droplet size ($D_{3,2}$) = 177 nm*

# Appendix 2: Saliva flow rate

The saliva flow rate was determined following the drooling method (Navazesh & Kumar, 2008). Saliva was collected over a period of 10 min on three separate occasions. The subjects were instructed to sit still in a relaxed and upright position. At the beginning of the trial, the subjects were asked to swallow and then collected saliva in a beaker.

The Figure A3 shows the mean data for each panellist calculated from 3 replicates. Panellists sharing the same letter were found not to have a significant different saliva flow ($p>0.05$). Panellists 1, 5 and 9 participated in the "acoustic tribology" study described in Chapter 5.



# Appendix 3: Achievements

**Oral:** 3rd Nursten Flavour Symposium, Northumbria (2015)

**Oral:** KTN Early Career Food Researchers Event, Birmingham (2015)

**Award:** Mars Petcare best oral presentation award at Early Career Food Researchers Event, Birmingham (2015)

**Oral:** IFST Young Scientist of the Year Competition, Nottingham (2016)

**Award:** Winner of IFST Young Scientist of the Year Competition (Region: Midlands) & Winner of Audience Price (2016)

**Poster:** Food Oral Processing Conference, Lausanne, Switzerland (2016)

**Poster:** Eurosense Symposium, Dijon, France (2016)

**Poster:** Cocoa, Coffee and Tea Conference, Turin, Italy (2017)

**Award:** Graduate School Travel Prize Award (2017)

**Oral:** IFST Sensory Science Conference, Nottingham (2017)

**Oral:** E3S Student Group Conference, Madrid (2017)

**Award:** SCI AJ Banks Student Travel Grant (2017

**Oral:** Seminar to Unilever Discovery Team, Colworth (2018)

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