The influence of food texture on satiety and satiation

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The candidate confirms that the work submitted is her own, except where work, which has formed part of jointly authored publications, has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others. Details of the jointly authored publications and contributions of each author are outlined on the next page.

Further details of the jointly authored publications in peer-reviewed journals, which chapters they appear in and the contributions of the candidate and the other authors are outlined below:

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Ecaterina Stribiţcaia: developed the search protocol, conducted the systematic review, collected, organized the data for the meta-analysis, interpreted the meta-analysis results, wrote the manuscript and replied to the comments of the reviewers. Designed the experimental protocol for the rest of the studies and conducted the measurements, collected the data, performed the data analysis and interpretation as well as drafted and edited the manuscript and replied to the comments from reviewers.

Evans, C.E.L.: ran the meta-analyses and helped with the interpretation of the meta-analysis results.

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Abstract

Addressing obesity issues by enhancing satiety in food in order to reduce energy intake and control appetite has been acknowledged as a promising nutritional strategy. Often food textural interventions have been used to generate satiety, specifically in short-term and preload study design. Although oral lubricity and oral coating are important aspects of oral processing which may influence oral residence time and satisfaction, their effect on satiety and satiation remain unclear. Therefore, the aim of this thesis was to investigate the effects of complex textural attributes of food on appetite ratings, food intake, salivary and blood biomarkers by developing non-calorific (model food: hydrogels) and calorific (protein beverages) preloads with different textural properties. A series of sensory, expected satiety and satiety trials involving collection of saliva and blood (n=393) were conducted in this PhD to understand the role of complex textural attributes such as lubricity and mouth coating in addition to viscosity. Initially, a set of non-calorific preloads expressed through model food such biopolymeric hydrogels have been developed and analyzed instrumentally (tribology, rheology, texture analysis) and sensorially (n=113). Based on the results, two types of hydrogels with: 1. the lowest and 2. the highest levels of lubricating properties were assessed for their impact on appetite ratings, food intake, salivary biomarkers and lubricating properties of human saliva in a pilot satiety trial (n=17); water acted as a control. Results showed that hunger decreased and fullness increased immediately and 10 min after consumption of high lubricating non-calorific hydrogels compared to control ($p<0.05$), however, no effect on food intake, salivary biomarkers and friction of coefficient of saliva were demonstrated. Further, we proposed to investigate the combinatorial effect of lubricity, oral coating and calories/ macronutrients. Therefore, preloads expressed through protein beverages (whey and casein) have been developed and instrumentally analyzed (tribology, viscosity and adsorption onto biomimetic surfaces, latter

emulating coating). First, using a video-based online survey (n=211), it was shown that calorific preloads differing in viscosity can generate expected satiety with high viscosity and medium viscosity protein beverages being visually perceived as being more satiating as compared to the low viscosity beverages ($p < 0.05$). Then the calorific preloads differing in their lubricating and coating properties were assessed in satiety trials (n=52). Results demonstrated that hunger decreased and fullness increased immediately and 30 min after consumption in the high coating beverages compared to control ($p<0.05$), therefore, suggesting that the combination of coating and calories have a prolonged effect on appetite ratings (n=37) compared to non-calorific preloads. In addition, fullness increased in high coating compared to low coating condition. There was a correlation between concentration of protein in saliva and appetite ratings; the higher the concentration of protein in saliva the lower the desire to eat ($r = - 0.963$; $p <0.05$) and prospective food consumption ratings ($r = - 0.980$; $p <0.05$). Human saliva was more lubricating after ingesting preload with high coating properties, and this may explain the results on appetite ratings. There was no effect of oral coating on blood biomarkers, suggesting that complex textural attributes in food having influence on oral processing might not have any effect on gut peptides (n=15). In summary, findings suggest that the oral lubricity and/or coating can have a subtle effect on appetite suppression, with such effect lasting longer when it is combined with macronutrients/energy load. This is the first work highlighting the effect of lubricity and coating on psychological and biochemical aspects of satiety. Further studies are necessary with larger textural contrast in lubricity and coating between preloads to understand if such textural intervention may trigger control on food intake.

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ANOVA Analysis Of Variance

BMI Body Mass Index

BSA bovine serum albumin

CaA Calcium Alginate

Cas Casein

CI Confidence Interval

CCK Cholecystokinin

DA sensory Descriptive Analysis

GLP-1 Glucagon-like Peptide

HC High Coating

HL High Lubricating

HV/LP; HV/HP high viscous/low protein; high viscous/high protein

HWP Heated Whey Protein

I^2 Degree of Heterpgeneity

κC Kappa-Carrageenan

LC Low Coating

MA Meta-Analysis

M/F Male/Female

MC Medium Coating

MRI Magnetic Resonance Imaging

MTM Mini-Traction Machine

MUC5B Mucin

MV/LP; MD/HP medium viscous/low protein; medium viscous/high protein

NA Not Applicable/Available

NaA Sodium Alginate

OAS Overall Appetite Score

PDMS Polydimethylsiloxane

PFC Prospective Food Consumption

PICOS Population, Intervention, Comparison, Outcome, and Setting

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analysis

PROSPERO International Prospective Register of Systematic Reviews

PYY Peptide YY

QDA Quantitative Descriptive Analysis

QCM-D Crystal Microbalance with Dissipation

LV/LP; LV/HP low viscous/low protein; low viscous/high protein

RE model Random Effects Model

SD Standard Deviation

SEM Standard Error of Mean

TA Texture Analysis

CATA Check-All-That-Apply

TDS Temporal Dominance of Sensations

TFEQ Three Factor Eating Questionnaire

UWP Unheated Whey Protein

VAS Visual Analogue Scales

WHO World Health Organization

List of nomenclature

n Number

U Entrainment speed

W_T Normal load

η Viscosity

μ Friction coefficient

μm micron/bead size

Chapter 1

General Introduction

1.1. Overall research background

It is largely agreed that the overconsumption of food has contributed to high prevalence of overweight and obese population globally (Tedstone, et al., 2018). Prevalence of obesity has increased dramatically over the last decade (WHO, 2021) and has been associated with chronic non-communicable diseases (McMillan, Sattar, Lean, & McArdle, 2006; Rexrode, et al., 1998; Steppan, et al., 2001) leading to significant morbidity and mortality consequences. Therefore, exerting some control over food consumption is a priority for weight management and the prevention of obesity. Achieving satiety and satiation through food design is one of the promising nutritional strategies. According to the theoretical framework of the 'Satiety cascade' (Blundell, et al., 2010) which provides the examination of food that impacts satiety and satiation, satiety is being associated with the process that inhibits further eating and suppression of hunger, while satiation is associated with the process that brings to end an eating episode. These two processes (satiety and satiation) are determined by several factors: from sensory and cognitive perspective (prior believes/experience, sensations and expectations of food), post-ingestive perspective (stomach emptying, gastrointestinal hormones known as satiety hormones too: cholecystokinin – CCK, glucagon-like peptide – GLP-1, peptide YY – PYY) to a post-absorptive perspective (related to liver and metabolites – release of glucose, insulin, amino acids) (Blundell, 2009; Blundell, et al., 2010; McCrickerd & Forde, 2015). This thesis focuses on early stages of satiety (from the first bite to post-ingestive stage) and it is measured for a period of time at certain time points through visual analogue scale (appetite

sensations), salivary and blood biomarkers as recommended by the literature (Blundell, et al., 2010).

Although satiation is conceptualised as a process that brings an eating episode to an end and measured through the measurements of *ad libitum* food consumption of a particular experimental meal (weight in grams or calories in kcal) (Blundell, et al., 2010) the satiation in this thesis, is measured as *ad libitum* intake as a consequence of prior ingestion. This measurement has been used previously in the literature where the preload happened to be novel as in this thesis (Larsen, Tang, Ferguson, & James, 2016; Tang, Larsen, Ferguson, & James, 2016, Krop, et al., 2019) knowing to have an influence on appetite and eating behaviour (Siddiqui, et al., 2022; Yeomans, Blundell, & Leshem, 2004).

One of the food design approaches to generate satiety and in turn reduce food intake is to consider 'food texture' manipulation. Textural/structural complexity of food is often defined by the degree of heterogeneity or inhomogeneity in a food, where the food or the intervention product includes some additive materials (*e.g.* hydrogels with sunflower/poppy seeds or alginate beads), which distinguishes it from a control product which has a homogenous texture lacking any inclusions (Larsen, et al., 2016; Tang, et al., 2016). The literature presents quite a good number of studies that assessed food texture on satiety from a simpler perspective such as form (liquid vs. solid) or viscosity (low viscous vs high viscous) (Campbell, Wagoner, & Foegeding, 2017; Dhillon, Running, Tucker, & Mattes, 2016). To date, a limited number of studies has investigated the effect of structural/textural complexity of food on satiety (Krop, Hetherington, Miquel, & Sarkar, 2019; Krop, et al., 2018; Larsen, et al., 2016; Tang, et al., 2016). Within the textural complexity, important constructs in the food textural manipulation such as *lubricating* (friction between oral surfaces) and *mouth-coating* (absorption onto oral surfaces) properties of food have been rarely studied for their impact on satiety and satiation. Only one study has investigated the effects of oral lubrication on satiety in a snack

trial setting and it was concluded that snack intake was reduced by 32% following consumption of a low chewing/high lubricating gel (Krop, et al., 2019). However, this study focused on external food texture manipulation only and this provides an opportunity for researchers to investigate further and to understand the full mechanism of how oral lubricity can influence satiety and satiation through the internal manipulation of food, such as the mixture of food varying in lubricating properties with human saliva (known to be a highly potent nature-engineered lubricant itself) (Xu, et al., 2020). Also, to date, there is no study in the literature investigating the role coating in the context of satiety.

The mechanisms, stated in the literature, by which food texture would impact appetite and food intake are explained by cognitive (satiety expectations), oro-sensory exposure time and post-ingestive (gastrointestinal hormones) factors. For instance, consumers may assess the satiety capacity of food, firstly, by visual cues using previous knowledge about the food to be eaten (knowing that solid food is more satiating that liquid one) (de Graaf 2012, Hogenkamp et al. 2011, McCrickerd et al. 2012). Oro-sensory exposure time can also explain the mechanism of food texture – it is known that solid foods/thick beverages need longer oral processing time as compared to liquid foods/thin beverages (Krop, et al., 2018). This may lead to an increased oro-sensory exposure time and appears to be essential in the perception of satiety or expected satiety (McCrickerd, et al., 2012). Accordingly, the learned experience or the learned association between the sensory attributes of food and the metabolic response of the food after ingestion may explain the way consumers perceive/anticipate the satiating capacity of the food they are consuming. Lastly and not least, another mechanism is through gastrointestinal (GI) peptides. Complex structural and textural characteristics of foods and their components have been shown to influence postprandial gastrointestinal (GI) function and metabolism (Juntunen, et al., 2002; Marciani, et al., 2007; Kong & Singh, 2008; Juvonen, et al., 2009). For instance, solid and viscous food have been shown to delay gastric emptying

(GE) and the subsequent absorption of nutrients in the upper GI tract (Kong & Singh, 2008; Juvonen, et al., 2009; Kristensen & Jensen, 2011). Previous studies have shown that the texture of food and structure modulates the postprandial release of GI hormones (Marciani, et al, 2007; Kong & Singh, 2008; Juvonen, et al., 2009; Tieken, et al., 2007) and may affect sensations of appetite at the same time (Mattes & Rothacker, 2001; Tieken, et al., 2007). Therefore, from the oral process perspective, the potential mechanism that would have an impact on satiety is that the longer time the food is chewed (longer oro-sensory exposure time) the faster increases in gut peptides release is observed (Miquel-Kergoat, Azais-Braesco, Burton-Freeman, & Hetherington, 2015).

In this thesis, the mechanism by which lubricity/mouth coating is believed to impact appetite and food intake is related to oro-sensory exposure time. The more lubricating the preload is the more coated the mouth would be, which would lead to longer oro-sensory exposure time, and in turn would increase the release of gut peptides, suppress appetite and reduce subsequent food intake. A key role considered to be central to the mechanistic pathway for lubricity to impact satiation and satiety processes in this thesis is saliva.

Salivary amylase helps in food digestion during oral processing by hydrolysing starch into maltose (Zakowski & Bruns, 1985) and it has been proposed that the concentration of salivary α-amylase may influence directly the hunger levels. For instance, in people with lower concentration of α-amylase, the digestion of carbohydrates will be slow, and this would lead to a presence of hunger for a longer period of time resulting in greater food intake before achieving satiety (Moreno-Padilla, Maldonado-Montero, Enguix-Armada, & Reyes del Paso, 2020). Beside this, saliva is known to be a lubricant itself. It is known that salivary proteins such as mucin (MUC5B) and other low molecular weight proteins contribute to the salivary composition and influence lubrication behaviour (Hopkins, et al., 2020; Humphrey & Williamson, 2001; Sarkar, Xu, & Lee, 2019; Sarkar, Ye, & Singh, 2017). However, how

consumption of a high lubricating foods affects the tribological properties of the human saliva and MUC5B or protein content and how such change (if any) in salivary lubrication affects satiety remains largely unknown. It can be hypothesized that eating a high lubricating food might increase the lubricating properties of saliva and keep the oral surfaces moistened and coated longer. This in turn may lead to appetite suppression and lower subsequent food intake. To date, no studies have reported effects of food texture on satiety and satiation while considering the tribology properties of saliva when consuming a high lubricating versus a low lubricating model food. It is therefore appropriate to examine the relationship between the lubricating behaviour (based on higher concentration of proteins or MUC5B or mechanically measured tribological properties) of saliva on consumption of lubricating preloads and its influence on appetite control and food intake.

Such fundamental knowledge is crucial to allow food researchers and industries to focus on the most appropriate aspects of textural and structural manipulations for rationally designing the next generation of satiety-enhancing foods with 'just-right texture'. Therefore, studying the precise effects of textural/structural complexity of food on appetite control and food intake is very relevant for designing foods with targeted satiety-enhancing properties, and ultimately to contribute to the nutritional management of the global pandemic of overweight and obesity.

Thesis Objective. In this thesis, the main objective was to develop further understanding on how oral lubricity/coating might have an impact on satiety and satiation. With that in mind, two type of preloads have been designed: non-calorific (polysaccharide) hydrogels and calorific (protein) beverages differing in their degree of lubricity and coating. A series of human trials have been conducted to investigate the effect of oral lubricity/coating expressed through the aforementioned preloads on appetite, expected satiety, subsequent food intake, salivary and blood biomarkers, and lubricating properties of saliva after ingesting the preloads. This PhD project is highly multidisciplinary involving 1. Food science – to design

and characterise the preloads from a sensory perspective; 2. Mechanical engineering – to characterise the preloads using material characterization techniques (rheology, tribology and absorption/coating); 3. Psychology – to design the satiety trials (human trials and online video-based survey); and 4. Biochemistry – to analyse salivary and blood biomarkers.

Thesis Hypothesis. The hypothesis behind this thesis is in two parts, firstly: that oral lubricity/coating expressed through high lubricating/coating preloads (the mixture of high lubricating preloads with saliva) can suppress appetite and reduce subsequent food intake, and secondly: that the effect on appetite will last longer in calorific lubricating/coating preloads versus non-calorific lubricating/coating preloads.

1.2. Rationale behind different techniques

This thesis is focused on designing preloads with different degrees of lubricity/coating, characterising them both instrumentally and sensorially and investigating their effect in terms of oral lubricity/coating on satiety and satiation conducted through a series of human trials. Beside external manipulation of the preloads (manipulating the level of lubricity), this thesis will highlight the importance of the internal manipulation the preloads undergo once they are oral processed, more specifically, the mixture of the preloads differing in their lubricity/coating with the lubricity of human saliva itself. Therefore, human saliva will be given an important key role in explaining the effect of oral lubricity/coating. In the following sections, all the instrumental techniques used in the PhD project to characterise the preloads, design the satiety trials and measurements used are described.

1.2.1. Rheology

Rheology is defined as the science of deformation and flow of matter under the applied forces (Rao, 2005). The rheological properties of food can be measured both at a small and large deformation scale.

1.2.2 Large deformation measurements

Large deformation measurements have been widely used in oral processing studies to describe best food texture in terms of the first bite. Texture Analyser is one of the common instrument used to measure the force needed to break down the food. Classical tests that are suited for solid foods are uniaxial compression tests, uniaxial extension tests, bending tests, wedge tests and wire cutting tests (van Vliet, 2013).

Uniaxial compression plate-to-plate is the most used technique to determine large deformation and fracture properties of foods. The material is compressed with a constant force. The applied force over time is measured, and a fracture stress and fracture strain can be used to describe the deformation behaviour. Usually compression tests are used to measure hardness or firmness of the product/food. Another test widely used to evaluate mechanical properties of food is the puncture test. During a puncture test, the force required to push a probe into the product/food is measured. Both the tests simulate the first bite of oral processing.

In this thesis, compression and puncture tests (to mimic first bite) to characterise the model foods (hydrogels) were used (**Chapter 3**). Compression plate to plate test is illustrated in **Figure 1.1** which shows the hydrogels before compression (a) and hydrogels after compression (b).

a b

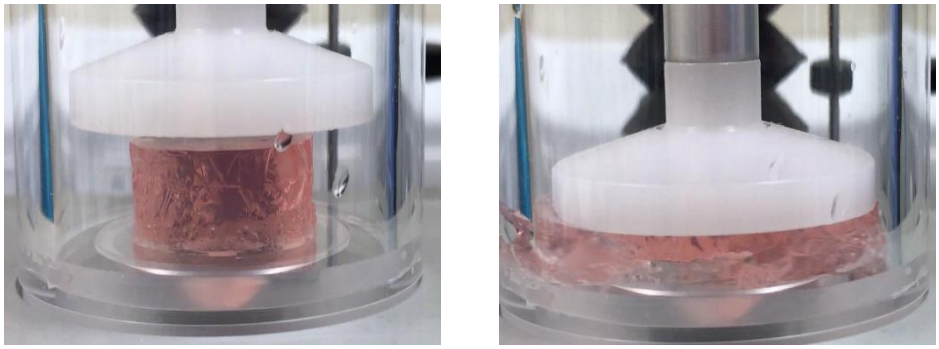

Figure 1.1. Compression plate to plate test (a) hydrogels before compression and (b) hydrogels after compression used large deformation measurements

1.2.3. Oral viscosity using small deformation

After the first bite, food is subject to a range of other mechanical deformation processes. As such, oral viscosity helps to explain how food flows and moves in the oral cavity. Usually, flow behaviour of foods is characterised by viscosity measurements and the product/food is subjected this time to shear-induced deformation. The literature describes two types of flowing behaviours or viscosity flow: Newtonian and non-Newtonian. The Newtonian fluids are simple fluids such as water, soft drinks, milk, whereas the non-Newtonian are yogurt, mayonnaise, ice cream, chocolate, butter, dough. The viscosity for the Newtonian fluids is constant and independent of the shear/stress rate applied to it, whilst that of the non-Newtonian fluids is dependent on the shear/stress rate applied to it, and to the duration of the shear and the temperature.

In this thesis, the apparent viscosity of simulated hydrogels samples (boli – food mixed with saliva and ready to be swallowed) (**Chapter 3**), and the whey and casein protein beverages, were monitored as a function of shear rate (**Chapters 5** and **6**). The flow curve of the products/material can be measured by using a well stress-controlled rheometer. The rheological measurements can be obtained by using different types of geometries: plate on plate (two parallel plates), con and plate or concentric cylinder (*i.e.* cup and bob). Due to the

heterogeneous nature of hydrogels bolus samples with gel particles, a plate on plate geometry was used where the gap could be widened so that the gel particles of various sizes in the different samples could be accommodated. The same plate on plate method was used to assess the flow curve of the protein beverages. It is commonly accepted that the shear rate in the mouth is 50 s^{-1} (van Vliet, 2013), therefore the apparent viscosity of the material used in this thesis will be discussed at this shear rate for comparison purposes, whilst the viscosity over a range of shear history will be discussed to understand the oral deformation of various samples.

1.2.4. Oral tribology

After the first bite and mastication that has been explained by texture analysis and rheological analyses, food undergoes the next steps of transformation, such as reduction in particle size, release of macronutrients, combination with saliva, formation of bolus ready to be swallowed and formation of oral coating in the mouth after swallowing the bolus (Chen, 2009; Stokes, Boehm, & Baier, 2013). The first two steps (mechanical and bulk rheology) of oral processing are well described in the literature (Prakash, Tan, & Chen, 2013). However, little is known about how food and food-saliva mixture interacts with the surfaces, which may affect their tribological performance (Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019a; Sarkar, Soltanahmadi, Chen, & Stokes, 2021). More importantly, how mouth coating properties of food influences perception and what happens to the oral cavity after food is ingested remains poorly understood (Sarkar & Krop, 2019b). Therefore, this can be investigated by studying the frictional responses of the food saliva mixture between the oral contact surfaces (Sarkar, et al., 2019a; Sarkar, et al., 2021; Selway & Stokes, 2013; Stokes, et al., 2013).

Tribology studies the friction, wear and lubrication of interacting surfaces in relative motions (Chen & Engelen, 2012a) (pp 260). Oral processing also involves interacting surfaces in relative motion such as tongue-palate, tongue-teeth, teeth-food, tongue-food etc. Generally speaking, while rheology is considered to be more important in the initial stages of oral

processing, tribology governs oral processing and subsequent mouthfeel with progress of oral processing time as the food is masticated and the oral surfaces interacts (Sarkar, et al., 2019a). Consequently, tribology becomes more dominant in the later stages of oral processing. In order, to quantify the tribological properties of food, an off-the-shelf machine that simulates the tongue-palate interaction is the mini traction machine (MTM).

Materials for these interacting surfaces range from nylon, rubber and modified polydimethylsiloxane (PDMS) to animal tissue such as pig tongue (Stokes, et al., 2013; van Vliet, van Aken, de Jongh, & Hamer, 2009). In order to measure tribological behaviour in conditions close to in-mouth oral processing, soft contact materials such as polydimethylsiloxane (PDMS) elastomers (see **Figure 1.2**) were used in this PhD project (Sarkar, et al., 2019a; Selway, et al., 2013). It is worth noting that the contact pressure generated using PDMS is two-orders of magnitude higher than that found in oral contacts (Sarkar, et al., 2019a). Nevertheless, this was the best proxy so far used in many laboratories and was employed during the starting phase of this PhD, although recent progress of softer elastomers and biomimetic tongue-like surfaces are being introduced in the field (Andablo-Reyes, et al., 2020).

Figure 1.2. Commercial PDMS ball and disc used for tribology measurements.

The MTM measures the frictional properties of lubricated and non-lubricated contacts under a wide range of rolling and sliding conditions. The ball is loaded against the face of the disc, and the ball and disc are turned independently to create a mixed rolling/sliding contact (see **Figure 1.3**). The frictional force between the ball and disc representing the palate and the tongue, the turning speed of the ball and the disc, the applied load and the lubricant and the pot temperature are recorded by the tribometer. The tests are performed at 37 °C to mimic body temperature.

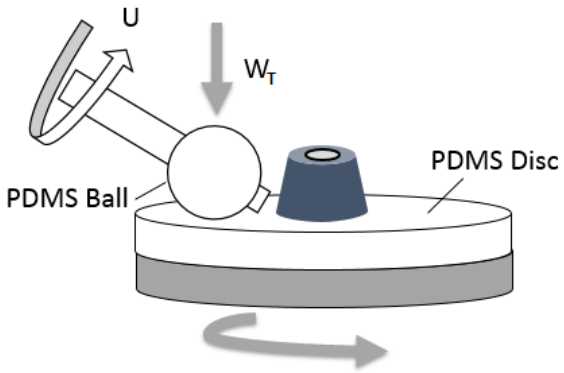

Figure 1.3. Schematic representation of the tribometer set-up. U = entrainment speed, W_T = normal load.

Using the aforementioned contact surfaces, the friction behaviour can be represented with the help of the Stribeck curve. In this curve, the coefficient of friction is plotted against a controlling parameter, such as the entrainment speed (viscosity multiplied by velocity divided by load). The Stribeck curve, shown in **Figure 1.4** can be divided into three distinctive regimes: the boundary, mixed and hydrodynamic lubrication regimes (Chen & Stokes, 2012b; Joyner, Pernell, & Daubert, 2014; Sarkar, et al., 2021; You & Sarkar, 2021b). The curve is read from right to left in the case of food tribology where speed as well as film thickness of food/ food-saliva mixtures in the mouth decreases from ingestion of food to swallowing, unlike mechanical engineering disciplines. In the hydrodynamic regime (which simulates the early stages of oral tribology), where the food sample clearly separates the two surfaces

(tongue and palate) from each other, the pressure is sustained by the sample and its rheological properties. With increasing sliding speeds, the friction will start to decrease, shifting to the mixed regime and giving food higher lubricating properties as compared to that in the hydrodynamic regime. Here, the pressure is partly borne by the food and partly by the surfaces in sliding contacts such as tongue and palate. In the boundary regime, a constant, relatively high friction can be observed at low sliding speeds giving food lower lubricating properties. In this phase, the film thickness is lowest and the pressure is fully borne by the surfaces in contact as the surfaces are minimally wetted by food/ saliva.

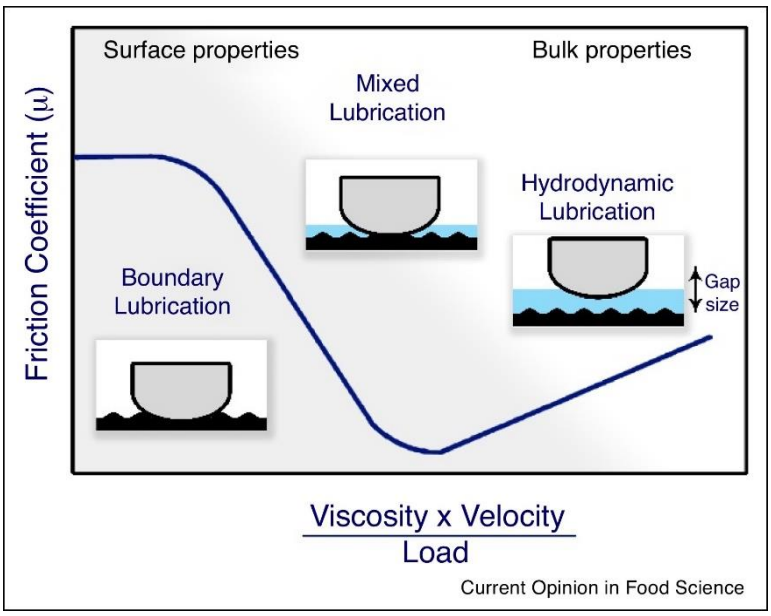

Figure 1.4. Typical Stribeck curve taken from de Rudge and et al. (Rudge, Scholten, & Dijksman, 2019).

In the hydrodynamic lubrication regimes, the friction coefficient largely depends on the bulk viscosity properties whilst, in the boundary regime the friction coefficient depends on the surface properties of the lubricant. In the mixed regime, both surface and bulk properties of the lubricant are important. In this thesis, the emphasis is placed on the frictional forces in the mixed and boundary regimes as they are expected to influence the sensory perception and therefore hypothesized to affect satiety (Sarkar, et al., 2019b) (**Chapters 3, 4** and **6**).

1.2.5. Oral/mouth – coating

Mouth coating can be defined as the residual food that sticks to the oral surfaces (tongue, palate, cheeks, teeth) after food ingestion and is known to play an important role in both the delivery of food components and mouth feel and after-feel perceptions (Fan, Shewan, Smyth, Yakubov, & Stokes, 2021; Repoux, et al., 2012). The instrumental techniques used to measure coating properties of foods/beverages, throughout the years, range from visual inspection (Kashket, van Houte, Lopez, & Stocks, 1991) and 'mouth rinse' (Pivk, Ulrih, Juillerat, & Raspor, 2008) to video fluoroscopy and Magnetic Resonance Imaging (MRI) (Buettner, Beer, Hannig, & Settles, 2001) and Infrared Reflectance (IR) (Prinz, Huntjens, & de Wijk, 2006). Recently, a new developed equipment that tries to measure to some degree the mouth-coating and/or adsorption onto surfaces behaviour of the products is the quartz crystal microbalance with dissipation (QCM-D) (Kew, Holmes, Stieger, & Sarkar, 2021; Zembyla, et al., 2021) and this is what we used to measure coating properties of the preloads in this thesis.

For the QCM-D analysis, usually PDMS-coated QCM-D sensors are designed to emulate oral surfaces (Kew, et al., 2021; Macakova, Yakubov, Plunkett, & Stokes, 2010; Stokes, Macakova, Chojnicka-Paszun, de Kruif, & de Jongh, 2011; Xu, et al., 2020; Zembyla, et al., 2021). In addition, QCM-D using these PDMS surfaces serve as better comparison to the tribology data, which are also performed using PDMS tribopairs. For the preparation of PDMS-coated QCM-D sensors, briefly, 100 μL of 0.5 wt% PDMS solution is placed on the substrate and is spin-coated at 5000 rpm speed for 60s. The real-time coating behavior of proteins is measured by QCM-D (E4 system, Q-Sense, Sweden), described in details elsewhere (Glumac, Ritzoulis, & Chen, 2019; Rodahl, Höök, Krozer, Brzezinski, & Kasemo, 1995; Xu, et al., 2020). QCM-D can simultaneously measure the shifts in frequency and dissipation at different overtones occurring during adsorption and provide wealthy information on the mass of the

adsorbing film corresponding to coating. This method has been used to measure the coating properties of the preloads in **Chapter 6**.

1.2.6. Sensory perception

Texture has a crucial role in sensory acceptance or rejection (Szczesniak, 2002) as well as in the recognition of food (Schiffman, 1977). Texture can be perceived by touch, sight and hearing (Jowitt, 1974) and through oral contact of food. Oral contact can occur through the lips, tongue, palate, cheeks and teeth; together all of these provide textural information (Chen, et al., 2012a).

Food texture is usually described by terms or atributes such as thick, thin, crunchy, soft, astrigent *etc*. (Chen, et al., 2012a). It can be measured applying different methods such as texture profile (Brandt, Skinner, & Coleman, 1963), Quantitative Descriptive Analysis (QDA) (Stone, 1974) and Spectrum Descriptive Analysis (Muñoz & Civille, 1998). However, these techniques are limited as they do not include measurements for the whole oral processing, from the first bite until swallowing.

In order to capture as much as possible the assessment of the food texture for the entire oral process, a combination of discriminative and descriptive sensory methods were used in this thesis in untrained participants. Untrained participants have been chosen for sensory evaluation, to be closer to simple consumers with the thought that its satiating capacity will be further investigated in untrained participants with the same rational (closer to simple consumers). For discriminative method a series of Triangle tests were used (Rainey, 1979) to select the samples that were distinguishable based on the size of inclusions used. For the descriptive method, rating tests were used with atributes and their description provided to panelists (Issanchou, Lesschaeve, & Köster, 1995). The selected sensory atributes in this thesis are the ones that express or describe best the oral tribology. The atributes were rated on an 100 mm unstructured line scale anchored from “not at all” to “extremly” (**Chapters 3 and 5**).

1.2.7. Saliva

Saliva is believed to be of high importance for the perception of food in the mouth. It plays a role in the breakdown of food (Engelen, et al., 2003), precepitation of proteins (Noble, 1995) and the lubrication of the oral tissue (Tabak, Levine, Mandel, & Ellison, 1982). Saliva consists of 99% of water and contains a wide number of organic and inorganic constituents that play a huge role in mastication and perception of food (Sarkar, Ye, & Singh, 2017). From the multitude of organic and inorganic components in the saliva, proteins, in particular mucins and α-amylase tend to affect oral processing most significantly (Engelen, et al., 2007; Sarkar, Xu, & Lee, 2019c; Torres, Andablo-Reyes, Murray, & Sarkar, 2018). Proteins are responsible for perception of astringency, viscosity and other mouthfeel attributes (Guinard & Mazzucchelli, 1996). Mucin (MUC5B) is believed to play a role in lubricating properties of saliva (van der Reijden, Veerman, & Nieuw Amerongen, 1993). On the other hand, α-amylase initiates starch digestion in the mouth , and sensorially, has been shown to influence the sensation of melting in semi-solids (Engelen, et al., 2003). Interestingly, little is known about the physical and physiological properties of saliva and its effect on satiety. Salivary components *i.e.* total proteins, mucins and α-amylase have been described and analysed in this thesis before and after ingesting the preloads at specific timepoints in relation to satiety and satiation (**Chapters 3 and 6**). For this purpose human saliva was collected during the clinical trials (registered at ClinicalTrials.gov as NCT04240795; NCT04868461).

Knowing that saliva plays a key role in the perception of food texture, an important aspect to consider while measuring the texture of food involves using saliva, *i.e.* mixing saliva with the food to be measured. The difficulty that arises is which type of saliva to be used, model or real? Due to the rapid physical changes that real saliva undergoes ex-vivo (it needs to be

analysed immediately), a model was used for instrumental analysis (rheology and tribology) (**Chapters 3 and 6**).

1.2.8. Satiety studies

Satiety is defined as being associated with the (post-prandial) inter-meal period, through the suppression of hunger and the inhibition of further eating whereas, satiation describes within-meal inhibition and can be said to determine meal size and bring a particular eating episode to an end (Blundell, 2010; Blundell, et al., 2010; Blundell, Rogers, & Hill, 1987). A conceptual framework that describes the impact of food on satiety and satiation can be seen in the "Satiety Cascade" (see **Figure 1.5**). This figure identifies a number of processes and factors that occur before and after the consumption of food.

It is meaningful to describe two stages of satiety cascade: early (short-term) and late (long-term). Each stage has key factors that explain its impact on satiety itself. The factors that can affect the early stage of satiety are sensory, cognitive and post-ingestive, while for the late stage of satiety, post-absortive factors have a stronger effect. This thesis, will focus on early stage of satiety as food textural manipulation and cosequently oral processing is expected to

influence particularly this stage; therefore, sensory, cognitive and post-ingestive factors will be discussed where appropriate (**Chapters 4, 5 and 6**).

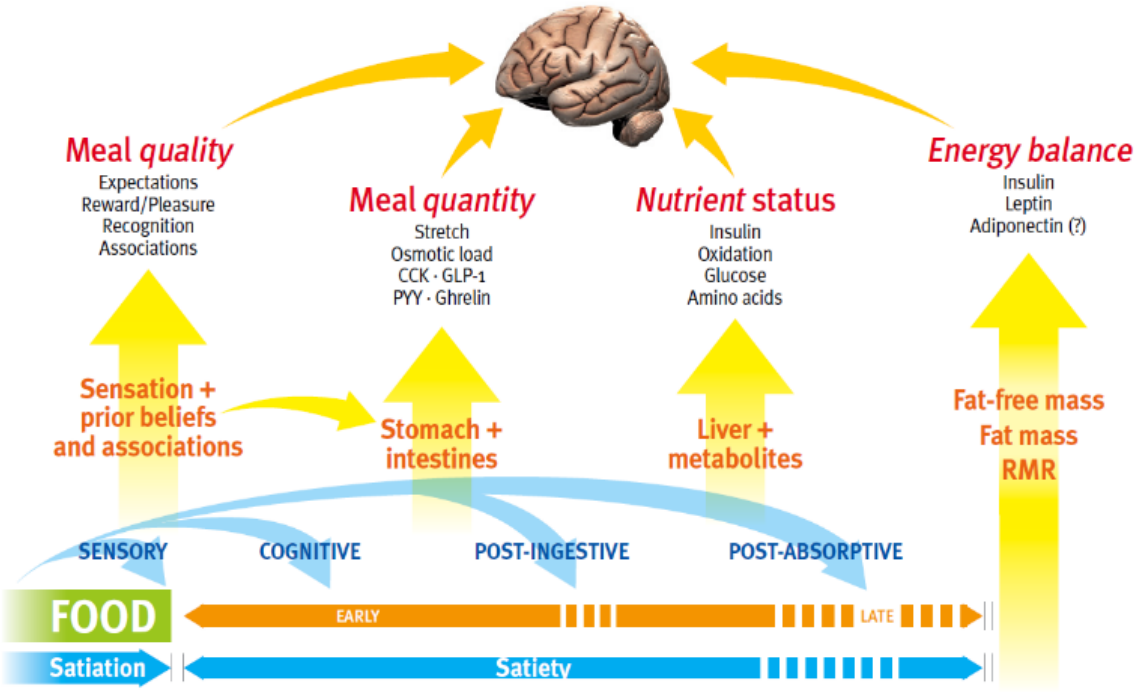

Figure 1.5. 'Satiety Cascade' adapted from Blundell et al. (Blundell, et al., 2010).

Another aspect of the "Satiety Cascade" to be consiered is expected satiety. Expected satiety is the extent to which foods/beverages are subjectively believed to confer satiety when they are compared on a calorie-for-calorie basis (Brunstrom, Collingwood, & Rogers, 2010). Here, factors such as sensory, cognitive and learned experience are employed to explain how this pheonomenon is taking place. It is believed that the learned experience or the learned association between the sensory attributes of food and the metabolic response of the food after ingestion may explain the way consumers perceive/anticipate the satiating capacity of the food they are consuming. In **Chapter 5** of this thesis, the influence of food texture and sensorial atributes on perceived/expected satiety will be discussed. This chapter was introduced and conducted as a result of Covid-19 pandemic restrictions where laboratory studies and in person

human trials were prohibited. Therefore, a novel approach for measuring expected satiety using video-based online survey was developed and will be discussed in **Chapter 5**.

One of the the most influential experimental study design to measure the short-term impact of food on satiety and satiation is the preload design studies described in (Blundell, et al., 2010). Usually, such studies are conducted using within subject repeated-measures design and participants are presented with prepared foods matched for taste, appearance and other organoleptic properties but differing in the varaible/property whose effect is to be studied – namely energy load, macronutrinets, or texture (for this thesis). In addition to the target, treatments/conditions, a control condition is required such as non-preload or placebo (Blundell, et al., 2010; Blundell, et al., 1987) to provide an appropritae comparison.

Satiety can be evaluated through psychological, behavioural and physiological procedures (Gibbons, Hopkins, Beaulieu, Oustric, & Blundell, 2019). Psychological measurements include perceived visual appetite ratings (such as hunger, fullness, desire to eat, prospective food consumption), whilst physiological measures mainly involve, changes in gastrointestinal biomarkers such as stomach dynamics or peptides hormones in blood, although changes in saliva may also be of significance (Gibbons, et al., 2019; Harthoorn, et al., 2007). Psychological measures include self-reported ratings and are usually measured on uni- and bipolar structured and unstructured lines, verbal categories and numerical scoring procedures (including magnitude estimation). However, the most common method used is the bipolar unstructured line of 100 mm also referred to as the 'visual analogue scale' (VAS) anchored by terms such as 'Not at all' to 'Extremely' as these have been shown to be valid and reliable for appetite research (Flint, Raben, Blundell, & Astrup, 2000; Stubbs, et al., 2000). In terms of behavioural measures, test meals are usually employed (Blundell, et al., 2010), involving the measurements of *ad libitum* food intake at a determined time after the preload.

For physiological measures, gut peptides analysis is frequently used. Ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY), amongst others, are thought to play a role in the episodic control of appetite and are known to fluctuate around meal times (Cummings & Overduin, 2007). These peptides are released from several sites throughout the gastrointestinal system. Ghrelin is released from the stomach and is often referred to as the 'hunger hormone' (Kojima & Kangawa, 2005). It is high during periods of fasting and decreases in response to food intake, therefore being regarded as orexigenic. CCK, GLP-1, and PYY are released from the small and large intestines and are considered satiety peptides (Murphy & Bloom, 2006). They are low during fasting and increase in response to food consumption and can therefore be referred to as anorexigenic.

As the thesis progressed, liking and wanting has been included as additional measurements knowing to affect satiety and satiaotion (Finlayson, King, & Blundell, 2007).

In this thesis preload, within-subject, single blinded studies have been conducted using 100 mm visual analogue scale (VAS), test meal (subsequent *ad libitum* food intake) along with the analysis of salivary proteins and gut peptides using salivary and blood biomarkers in healthy weight participants (**Chapters 4, 5** and **6**). Knowing that appetite control differ significantly between healthy and overweight or obese population (Hetherington, 1996; Molfino & Imbimo, 2022), and that lubricity/coating are new constructs of food texture (never studied before/no previous information to begin with) in the context of satiety, this thesis focused on demonstrating first an effect, if any, in healthy population with the potential to be investigated further in overweight and obese population.

1.3. Materials

In the next sections a rationale about the types of the selected preloads will be discussed.

1.3.1. Non-calorific preloads

In the first satiety trial, model food such as hydrogels were used. These materials are known to have much simplified structure therefore giving the opportunity to manipulate some aspects of texture which can be readily compared to real food (Funami, 2011; Krop, et al., 2019; Krop, Hetherington, Miquel, & Sarkar, 2020). To make sure that the texture/lubricity alone is assessed, indipendent of other cofactors (energy load/macronutrients) on satiety, the hydrogels were non-calorific.

Hydrogels can be prepared from different hydrocolloids – a heterogeneous group of hydrophilic long chain polymers that can form viscous dispersions and/or gels upon dispersion in water due to the presence of large number of hydroxyl (-OH) groups (Saha & Bhattacharya, 2010). The hydrogels most commonly used in research are alginate, carrageenan, agar, locust bean gum, xanthan, pectin, gelatine (Banerjee & Bhattacharya, 2012; Nishinari, Zhang, & Ikeda, 2000; Saha, et al., 2010) and there is now an extensive literature that has focused on tribological properties of these polysaccharides either on their own or their mixtures (Andablo-Reyes, et al., 2019; Huang, et al., 2021; Stokes, et al., 2011; Torres, et al., 2019; You, Murray, & Sarkar, 2021a; You, et al., 2021b; Zinoviadou, Janssen, & de Jongh, 2008). Combining different concentrations and mixture of the hydrocolloids, hydrogels with different rheological, tribological and sensorial textural properties can be obtained – harder, softer, smoother, pastier, chewier etc. (Hayakawa, et al., 2014; Krop, et al., 2019).

For the purposes of this PhD project, no fats or sugars were used in the food model systems and hydrocolloids were selected for their ability to form gels. As such, food grade *κ*-carrageenan and alginate were selected based on their non-thermo-irreversibility, *i.e.* no change in gel structure is expected when exposed to oral temperatures of 37 °C. Preparation, instrumental and sensorial analysis is discussed in **Chapter 3** of this thesis.

1.3.2. Calorific preloads

In order to check the combinatorial effect of lubricity and energy load/macronutrients, dietary protein beverages were used as preloads. Dietary protein such as whey and casein have been reported to be the most satiating compared to other macronutrients (Halton & Hu, 2004; Latner & Schwartz, 1999). Therefore, casein and whey protein beverages differing in their lubricating properties were used as pre-oads in the second and third satiety trial.

Casein has been experimentally evidenced as a 'slow' protein, while whey protein is considered as a 'fast' protein mainly on the basis of gastric emptying (Greco, et al., 2017; Luhovyy, Akhavan, & Anderson, 2007). Consequently, in humans, intake of whey results in a fast, but short and transient increase in plasma amino acids that peak in 40 min to 2 hours after its ingestion and returns to baseline values after 3 to 4 hours. In contrast, the intake of casein results in plasma amino acid concentrations that rise more slowly and are lower, but sustain a prolonged plateau lasting for at least 7 hours after its consumption (Boirie, et al., 1997; Dangin, Boirie, Guillet, & Beaufrère, 2002). Since there is a big difference in the release time of amino-acids of these two types of proteins that would lead to different responses on satiety, this PhD project aimed to investigate the immediate and short (up to 1 hour) combinatorial effect of lubricity and protein, therefore examining the first stages of satiety from an oral processing perspective. This appears to be the first occasion in which this experimental approach has been employed.

The reason for the use of two types of proteins was to achieve clear and different levels of lubricity. To maintain high protein contents in the preloads, protein isolates were used vs concentrated ones. The concentration, preparation and characterisation of the protein beverages are discussed in **Chapter 6**.

1.4. Outline of the thesis

Having presented the background and rationale of this thesis, the outline is now described in **Figure 1.6** This thesis starts with a systematic review and a meta-analysis, continues with instrumental and sensorial characterisation of the preloads followed by a series of systematically designed satiety trials. The chapter breakdown is discussed below:

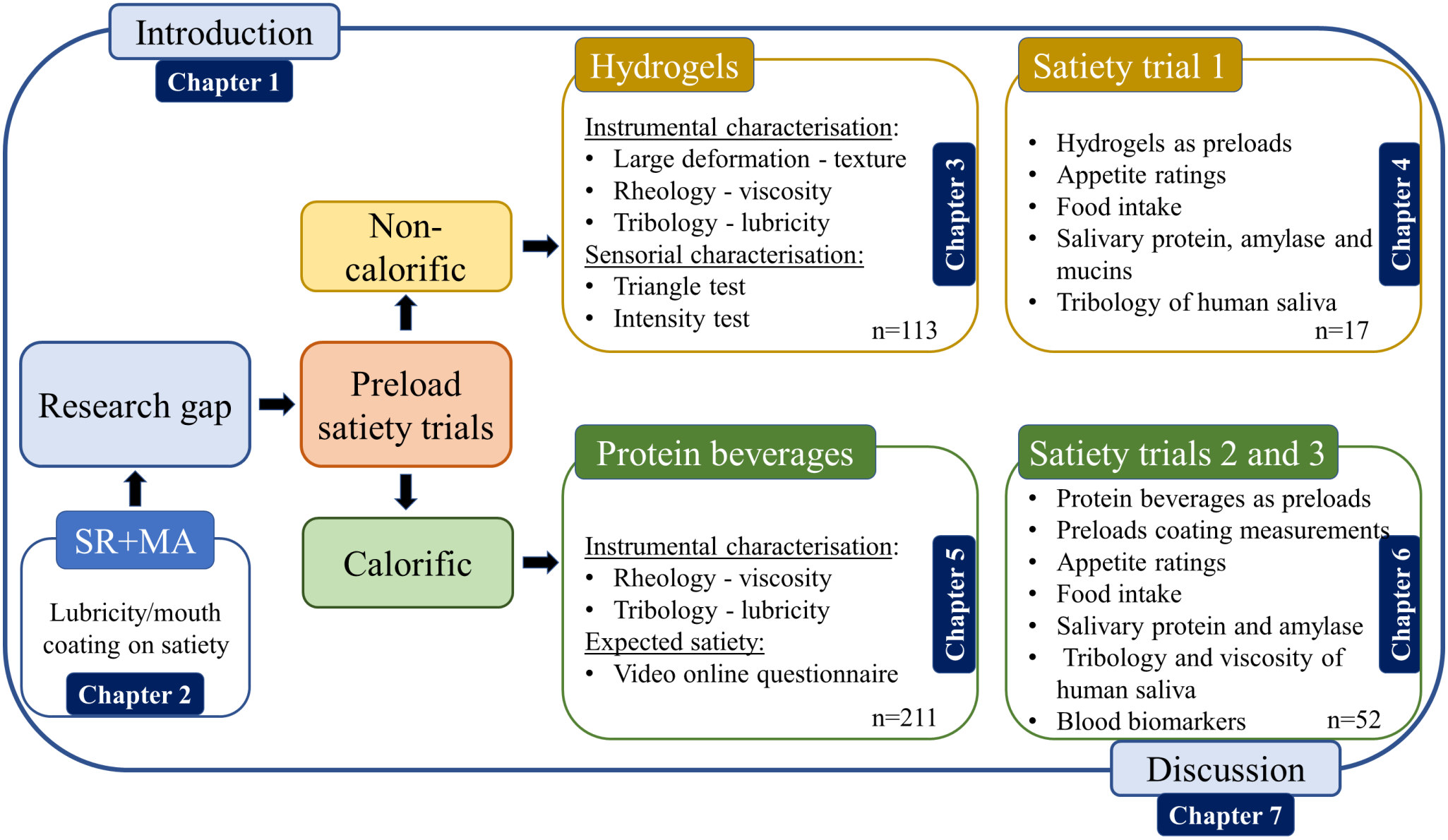

Figure 1.6. Schematic framework of this thesis.

Chapter 2 includes a comprehensive systematic review and a meta-analysis investigating the role of food texture on satiety. The aim of this chapter was to have a full understanding of current state-of-the-art science on the influence of food texture on appetite control, including appetite ratings, such as hunger, fullness, desire to eat, thirst, prospective food consumption (how much food participants thought they could eat), food intake, and gut peptides, such as ghrelin, GLP-1, PYY and CCK. The content of this chapter has been published in the peer-reviewed journal '*Scientific Reports'* and registered on PROSPERO (CRD42019128434).

Chapter 3 describes a variety of non-calorific/non-fat hydrogels being developed to achieve different levels of lubricity. The hydrogels were analysed both instrumentally (large deformation, rheology and tribology) and sensorially (triangle and intensity tests, n=113). Based on this analysis, hydrogels with clear lubricating properties were selected for further satiety trials. The content of this chapter has been published in the peer-reviewed journal '*Food Hydrocolloids'*.

Chapter 4 presents first satiety trial out of four trials of this thesis. In this chapter, selected hydrogels based on the previous chapter have been assessed for their satiating effect. More specifically, the effect of oral lubricity, expressed through non-calorific hydrogels differing in their lubricating properties, on appetite, food intake, salivary biomarkers and lubricating properties of human saliva will be discussed within this chapter. The results have been published in the peer-reviewed journal '*Appetite'*.

In **chapter 5** the second satiety trial is presented. The aim of this study was to examine the effect of food texture, more specifically the effect of different levels of viscosity, on perceived satiety through an online survey where the viscosity levels of protein-based beverages were visually perceived using a newly developed video-based demonstration. This study was developed as a consequence of the Covid-19 pandemic during which period access to the

laboratory and in-person human trials were restricted. The content of this chapter has been published in the peer-reviewed journal '*Food Quality and Preference'*.

Chapter 6 presents the third and fourth satiety trials. These two studies aimed to evaluate the effect of oral lubricity in combination with energy load/macronutrients using whey and casein protein beverages as preloads varying in their lubricating properties on appetite, food intake, salivary and blood biomarkers, and lubricating properties of human saliva.

Chapter 7 presents a general summary and discussion of the main findings of this PhD thesis. In addition, the implications of the results with recommendations for future research are included.

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Chapter 2

Food texture influences on satiety: Systematic review and meta-analysis[1]

Abstract

Obesity is one of the leading causes of preventable deaths. Development of satiety-enhancing foods is considered as a promising strategy to reduce food intake and promote weight management. Food texture may influence satiety through differences in appetite sensations, gastrointestinal peptide release and food intake, but the degree to which it does remains unclear. Herein, we report the first systematic review and meta-analyses on effects of food texture (form, viscosity, structural complexity) on satiety. Both solid and higher viscous food reduce hunger by -4.97 mm (95% confidence interval (CI): -8.13, -1.80) and -2.10 mm (95% CI: -4.38, 1.18), respectively compared to liquid and low viscous food. An effect of viscosity on fullness (95% CI: 5.20 (2.43, 7.97) and a moderate effect of the form of food (95% CI: -26.19 (-61.72, -9.35) on food intake were noted. Due to the large variation among studies, the results should be interpreted cautiously and modestly.

2.1. Introduction

Obesity is an escalating global epidemic that falls in the spectrum of malnutrition and is associated with substantial morbidity and mortality consequences. In addition to obesity-induced physical disabilities and psychological problems, excess weight dramatically increases

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a person's risk of developing chronic non-communicable diseases, such as cardiovascular diseases (Rexrode, et al., 1998), cancers (McMillan, Sattar, & McArdle, 2006) and diabetes (Steppan, et al., 2001). For the first time in the human history, the population with obesity (body mass index, BMI $\geq$ 30 kg/m^2) and overweight (BMI $\geq$ 25 kg/m^2) has surpassed that of the population with underweight (Collaboration, 2016) with current estimation of 1.9 billion adults with overweight globally, of which 650 million are obese (WHO, 2018). Medical treatment of obesity is currently limited to drug administration and bariatric surgery. The latter carries significant post-operative risks (Ionut, Burch, Youdim, & Bergman, 2013) and even after the surgery, sustained weight loss can only be achieved through well-designed nutritional interventions. Hence, there is an immense need for applying nutritional prevention strategies to change the current "obesogenic" food environment to become more "leanogenic (Chambers, McCrickerd, & Yeomans, 2015)".

Weight gain is described as an imbalance between the dietary energy intake and energy expenditure (Garrow, 1974). In other words, to maintain a healthy weight, it is required that the quantity of energy consumed matches the quantity of energy expended. Hence, one promising approach adopted by food scientists, nutritionists and psychologists has been to design or optimise food to achieve satiety (that suppresses appetite for longer periods after consumption) (Chambers, et al., 2015), because this directly leads to a reduction in dietary energy intake and at the same time reduces the impact of sensations of hunger on motivation.

One way to conceptualise appetite control is to consider the Satiety Cascade (Blundell, 2010; Blundell, Rogers, & Hill, 1987). 'Satiation' and 'satiety' are two distinct terms with the satiety cascade which are often erroneously used as synonyms when referring to different aspects of appetite control. Satiation describes within-meal inhibition and can be said to determine meal size and bring a particular eating episode to an end. On the other hand, satiety is known to be associated with the inter-meal period, through the suppression of hunger and

the inhibition of further eating. Satiety is most commonly measured through both subjective appetite ratings such as, hunger, fullness, desire to eat, prospective food consumption (how much people think they could eat) and thirst, whilst satiation can be measured through meal size – that is through food intake (Blundell, et al., 2009).

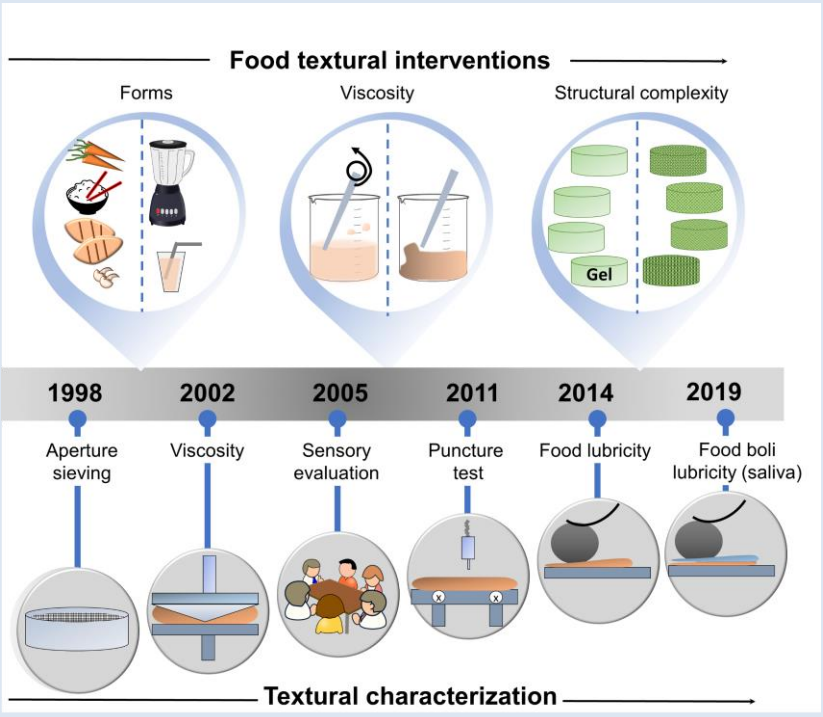
The current literature on satiety suggests that 'food texture' should be an important factor in the control of satiation, satiety, and daily caloric intake. Over the years, the strategy of using food textural manipulations has evolved enormously to the assessment of satiety (see **Box 1 and Box 1 Figure**). In addition, various gut peptides, such as ghrelin also known as 'hunger hormone' (Kojima & Kangawa, 2005), cholecystokinin (CCK), glucagon-like peptide (GLP-1), peptide YY (PYY) are considered to be involved in the regulation of appetite and satiety signalling (Cummings & Overduin, 2007). Ghrelin is known to increase during fasting and decrease after food intake whereas (Kojima, et al., 2005) GLP-1, CCK and PYY are reduced during fasting periods and released into the circulation after a meal (Murphy & Bloom, 2006). CCK is also believed to play a role in satiation by reducing food intake (Kissileff, Carretta, Geliebter, & Pi-Sunyer, 2003; Smith, Gibbs, & Kulkosky, 1982).

Considering the topical nature of this field, there have been excellent systematic reviews and meta-analyses on appetite control focusing mainly on the intrinsic aspects of eating such as, the effect of chewing (Miquel-Kergoat, Azais-Braesco, Burton-Freeman, & Hetherington, 2015), eating rate (Robinson, et al., 2014) or oral processing (Krop, et al., 2018), which involve physical and physiological aspects of eating and are closely related to an individual's behaviour. In addition, a meta-regression was conducted on the effects of the time

Box 1: History of food texture interventions in satiety trials.

The field of 'food texture-satiety' was initiated by manipulation of physical forms of food *i.e.* solid versus liquid or versus semi-solid. In the 1990s, this was achieved by using foods naturally available in different forms, such as whole vegetables and/or meat versus pureed vegetables and/or meat. The techniques used often included blending a solid food resulting in a pureed texture or other kitchen-based food processing techniques, such as boiling, chopping etc. (Mattes, 2005; Tournier & Louis-Sylvestre, 1991). Initially, for instrumental measurements of those texture generated, Santangelo et al. (1998) used a simple 4 mm^2 aperture sieve to clearly define which food was solid and which one was liquid. Later, the focus on textural intervention shifted to specifically altering the viscosity of food by using different dietary fibres (polysaccharides) to thicken, such as alginate (Solah, et al., 2010), locust bean gum (Camps, Mars, de Graaf, & Smeets, 2016), or guar-gum (Zhu, Hsu, & Hollis, 2013b) and terms used to describe those textures ranged from 'low viscosity' to 'high viscosity'. At the beginning of 2000, change in viscosity was measured for the first time for use in a satiety trial by Mattes and Rothacker (2001) using a spindle. The solid food texture was measured using puncture stress (Juvonen, et al., 2011) to determine firmness. Besides measurements by instruments, sensory evaluation of food determined by untrained (Labouré, van Wymelbeke, Fantino, & Nicolaidis, 2002) or trained panels (Solah, et al., 2010) allowed defining food texture in consumer terms, such as 'thin' or 'thick'. With the field evolving, the texture of food manipulations was more precisely measured in its viscosity and firmness using sophisticated rheological instruments.

A shift in focus occurred a decade later with more attention being given to the structural complexity of food, and to satiety studies using gel-based model foods with precise control over the texture; such gels avoid any emotional association with real food. For instance, Tang et al. (2016) and Larsen et al. (2016) were the first ones to use model foods i.e. hydrocolloid based gels with various inclusions to create different levels of textural complexity or in other words higher degree of heterogeneity and assess the relationships between the gels and satiety. Besides classical rheological measurements, McCrickerd et al. (2014) and Krop et al. (2019b) measured the lubricity of foods without or with simulated saliva (food boli i.e. food and simulated saliva mixture), respectively, using a Mini Traction Machine tribometer. Such differentiation in the lubricity of hydrogels was used for the first time by Krop et al. (2019b) to see their effects on snack intake.



Box 1. History of food texture interventions in satiety trials.

interval between preload and next meal on energy compensation with additional investigation on the effects of physical forms of the preload on energy compensation (Almiron-Roig, et al., 2013). The key finding was that the compensatory behaviour decreases faster over time after consumption of semi-solid and solid foods compared to that of liquid products, therefore, suggesting that semi-solids and solids have a greater satiating effect than that of liquids. Also, elegant narrative reviews on the effect of food forms *i.e.* physical state of food on appetite and energy balance (Dhillon, Running, Tucker, & Mattes, 2016) and impact of food texture and oral processing on satiation/ satiety (Campbell, Wagoner, & Foegeding, 2017) are available in the literature reflecting similar conclusions, that semi-solid and solid foods appear to have a stronger satiation response and elicit stronger energy compensation than their liquid counterparts. Along with the previous reviews on this subject, our systematic review adds specific information in regards to the inclusion of more sophisticated and advanced food-texture manipulations to affect satiation and satiety, which is more relevant for future product design and reformulation consideration. Moreover, this study includes the first meta-analysis to quantify the effects of food form and viscosity on hunger, fullness and subsequent food intake. Such details are crucial to allow food researchers and industries to focus on the most appropriate aspects of textural and structural manipulations for rationally designing the next generation foods with 'just-right texture'. Therefore, studying the precise effects of food texture on appetite control and food intake is very relevant in designing foods with targeted satiety-enhancing properties, and to contribute to the nutritional management of the global pandemic of overweight and obesity.

Here, we report the first systematic review and meta-analysis that aims to investigate the effect of food texture from an external perspective, *i.e.* how the manipulation of food, its physical state (texture and structure) can impact satiety. The objectives were to understand the influence of food texture on appetite control, including appetite ratings, such as hunger,

fullness, desire to eat, thirst, prospective food consumption (how much food participants thought they could eat), food intake, and gut peptides, such as ghrelin, GLP-1, PYY and CCK. We hypothesize that higher textural characteristics (solid form, higher viscosity, higher lubricity, higher degree of heterogeneity, etc.) would lead to greater suppression of appetite and reduced food intake. In this systematic review, the term 'form of food' refers to the physical state of food *i.e.* liquid, solid, semi-solid throughout the entire manuscript.

2.2. Methods and materials

This review was registered on the International Prospective Register of Systematic Reviews (PROSPERO) using the registration number: CRD42019128434.

2.2.1. Eligibility criteria

Participants. Studies with healthy adults (≥ 18 years old) with a normal weight (BMI=18.5-24.99 kg/m²) were included. Studies involving unhealthy, obese population (obesity is considered a medical condition)(WHO, 2018) or involving patients suffering from other medical conditions, children (< 18 years old) and elderly (> 60 years old) population were excluded.

Interventions. Interventions included any study that manipulated the food texture externally *i.e.* ranging from varying food forms to its complexity (see **Table 2.1**). Only those studies with a fixed-portion preload design *i.e.* studies where participants were given a fixed amount of pre-load followed by collection of appetite ratings and/or food intake measurements at a certain interval period of time were included. Any study that involved manipulation of the intrinsic behaviours such as chewing, eating rate have been excluded (de Wijk, Zijlstra, Mars, de Graaf, & Prinz, 2008). Studies that investigated the effects of fibre/fiber or fibre dose and its physiological effects other than manipulation of texture (Juvonen, et al., 2011; Kehlet, Pagter, Aaslyng, & Raben, 2017) or effects of sugar (Gadah, Kyle, Smith, Brunstrom, & Rogers,

2016), studies that compared only high energy density with low energy density with ambiguous reference to the texture were excluded (Hogenkamp, Mars, Stafleu, & de Graaf, 2010). Also, studies that failed to make the link between food texture and appetite control or food intake or gut peptides, were excluded, for instance studies which assessed expected satiety (McCrickerd, Lensing, & Yeomans, 2015). Studies that measured food intake following an *ad libitum* experimental intervention were excluded too (Bolhuis, Forde, Cheng, Xu, & de Graaf, 2014; Lasschuijt, et al., 2017; McCrickerd, et al., 2014; Pritchard, Davidson, Jones, & Bannerman, 2014). Likewise, studies that included any cognitive manipulation (Cassady, Considine, & Mattes, 2012), a free-living intervention or partial laboratory intervention designs (Hovard, et al., 2015) were excluded to reduce heterogenity in study design. A detailed information on the search terms is given in **Supplementary Table A.1**.

Table 2.1. Food texture parameters of the interventions/preloads as described across studies

Parameters	Comparison factors	
Form	Liquid	Solid/semi-solid
Viscosity	Low viscous/ thin	High viscous/ thick
Lubricity	Low lubricity	High lubricity
Homogeneity	Homogeneous	Heterogeneous
Structural complexity	Low complexity	High complexity
Aeration	Non-aerated	Aerated

2.2.2. Meta-analysis

Articles were assessed for eligibility for inclusion in a meta-analysis. All outcomes were assessed for suitability for pooled analysis. A minimum of 3 studies were needed for each meta-analysis. Studies with no reported measure of variation such as standard deviation or standard error were excluded. If data were insufficient to allow inclusion in the meta-analysis, authors were contacted for retrieving the information (Evans, Christian, Cleghorn, Greenwood, & Cade, 2012). Appetite is usually measured on a 100 mm visual analogue scale (VAS) (Mattes, 2015). Where 9, 10 or 13 point scales were used to measure appetite rating, these scales were converted into a 100 point scale, so that the appetite ratings were comparable

(Krop, et al., 2018). Food intake is measured in either weight (g) or energy (kcal or kJ). The given values were converted to kcal to allow comparison across the studies. For appetite ratings, available data from the medium follow up period (60 min after preload consumption) were extracted for synthesis in meta-analyses. Where meta-analysis was possible, mean differences were calculated to account for variable outcome measures for each comparison, using the generic inverse variance method, in a random-effect meta-analysis model (Evans, et al., 2012). Stata15 software was used for all analysis. Heterogeneity was assessed using the I^2 statistic, where I^2 values of < 50% were considered as acceptable levels of heterogeneity. Funnel plots were presented to assess small study publication bias. Where such data pooling was not possible, findings were narratively synthesised and reported according to the outcomes (Evans, et al., 2012).

Note, in the section 3.5 on Meta-analysis, P values in the text refers to the effect size of food texture on the outcome, while P values on the figures refer to the degree of heterogeneity (I^2).

2.3. Results

The literature search yielded 29 studies that met the inclusion criteria of this systematic review. All studies measured subjective appetite ratings such as hunger, fullness, desire to eat and/or prospective consumption (*i.e.* how much food participants thought they could eat). Of these, 19 measured subsequent food intake and eight measured gut peptide responses.

2.3.1. Study selection

The study selection was conducted in several phases following the checklist and flowchart of the PRISMA (Preferred Reporting for Systematic Reviews and Meta-Analyses) guidelines (Moher, Liberati, Tetzlaff, Altman, & PRISMA, 2009) as shown in **Figure 2.1**. Initially, a total number of 8530 articles were identified using literature search in the afore-mentioned six electronic databases.

After removing the duplicates (2602), the remaining 5928 titles were screened by the first author (ES) based on their relevance to this review. Firstly, 5661 studies were excluded based on the PICOS (Population, Intervention, Comparison, Outcome, and Setting) criteria *i.e.* articles involving animal studies (55), or clinical studies involving patients and/or children or elderly population (141) were excluded. Additionally, articles not addressing the topic of interest were excluded (5465).

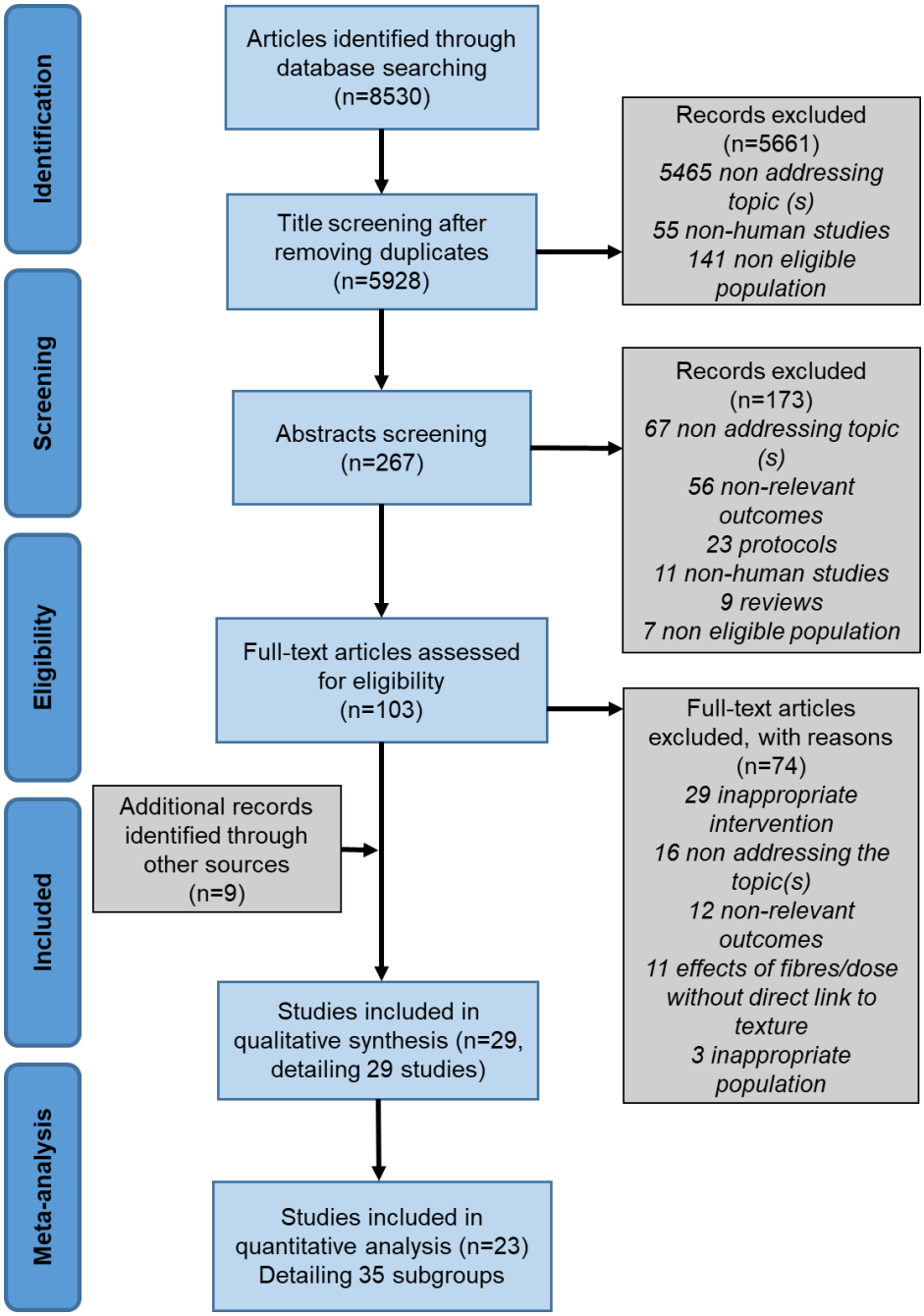


Figure 2.1. PRISMA flow-chart of the study selection procedure.

The articles were taken to the next phase where 267 abstracts were screened by ES and AS, resulting in the exclusion of an additional 173 articles (67 articles had no relevance to the topic (s) of systematic review, 56 had non-relevant outcome measures, 23 were new or validation of existing protocols, 11 were non-human studies with additional 7 being non-eligible population and 9 were reviews without any original data). A total of 103 full-text articles, including 9 articles that have been identified through supplementary approaches (*e.g.* manual searches of reference list of pre-screened articles) were equally divided and screened independently by ES, AS and CG. After a mutual agreement, articles with inappropriate interventions and designs (*e.g.* eating rate, chewing, free-living design, that included any cognitive manipulation, effect of sugar and fats on satiety and appetite) (n = 29) were excluded. In addition, studies not addressing the topic of interest (n = 16) or having non-relevant outcomes (n = 12) were not considered. Articles where the effects of fibres or dosage of fibre were studied without any direct relevance to textural manipulation (n = 11), articles with no full-text (n = 3) or non-relevant population (n = 3) were also eliminated. To sum it up, a total of 29 articles were included for qualitative synthesis.

2.3.2. Study characteristics

Relevant information such as study design, participant gender, type of intervention on texture manipulation, methods of analysing/measuring the texture of food as well as study outcome on appetite ratings, gut peptides and food intake was extracted from the 29 included studies (**Table 2.2**).

Study design. Many included studies adopted a within-subject design, with the exception of three which used a between-subject design (Krop, et al., 2019b; Mourao, Bressan, Campbell, & Mattes, 2007; Yeomans, McCrickerd, Brunstrom, & Chambers, 2014).

Participants. A total of 817 participants were included in the qualitative synthesis with age ranging from 18 to 50 years (mean age 24.7 years), with the exception of two studies not reporting the participants' age (Larsen, et al., 2016; Tournier, et al., 1991). Ideally, studies should have an equal ratio of men and women, however, in five studies more women were included than men (Hogenkamp, Mars, Stafleu, & de Graaf, 2012a; Hogenkamp, Stafleu, Mars, & de Graaf, 2012b; Krop, et al., 2019b; Mattes, 2005; Melnikov, et al., 2014). On the other hand, a number of studies included more men than women (Dong, et al., 2016; Marciani, et al., 2012; Tang, et al., 2016). Moreover, in twelve studies men only were included (Camps, et al., 2016; Juvonen, et al., 2011; Labouré, et al., 2002; Martens, Lemmens, Born, & Westerterp-Plantenga, 2011; Martens, Lemmens, Born, & Westerterp-Plantenga, 2012; Santangelo, et al., 1998; Wanders, et al., 2014; Yeomans, et al., 2014; Yeomans, Re, Wickham, Lundholm, & Chambers, 2016; Zhu, Hsu, & Hollis, 2013a; Zhu, et al., 2013b; Zijlstra, et al., 2009). No study included only females and two studies did not mention gender ratio (Larsen, et al., 2016; Solah, et al., 2010). Only five studies had an equal male/female ratio (Clegg, Ranawana, Shafat, & Henry, 2013; Flood-Obbagy & Rolls, 2009; Flood & Rolls, 2007; Mourao, et al., 2007; Tsuchiya, Almiron-Roig, Lluch, Guyonnet, & Drewnowski, 2006). All studies selected participants within a healthy BMI range. Mourao et al. (2007) included both lean and obese participants. However, for this systematic review, the results of lean subjects only were included. In most studies, participants with dietary restrictions or dramatic weight change were specifically excluded as well as those who reported high levels of dietary restraint (11 out of 29) as assessed by either the Dutch Eating Behaviour Questionnaire (DEBQ) or the Three Factor Eating Questionnaire were excluded. Only one study was double-blinded (Dong, et al., 2016) and 14 studies used cover stories to distract participants from the real purpose of the study. In only twelve of the studies, a power calculation was used to determine the number of participants needed to find a significance difference (Camps, et al., 2016; Dong, et al., 2016;

Flood-Obbagy, et al., 2009; Flood, et al., 2007; Hogenkamp, et al., 2012a; Marciani, et al., 2012; Martens, et al., 2011; Martens, et al., 2012; Melnikov, et al., 2014; Mourao, et al., 2007; Tsuchiya, et al., 2006; Wanders, et al., 2014).

Intervention. In 16 studies (Clegg, et al., 2013; Dong, et al., 2016; Flood-Obbagy, et al., 2009; Flood, et al., 2007; Hogenkamp, et al., 2012a; Hogenkamp, et al., 2012b; Labouré, et al., 2002; Marciani, et al., 2012; Martens, et al., 2011; Martens, et al., 2012; Mattes, 2005; Mourao, et al., 2007; Tournier, et al., 1991; Tsuchiya, et al., 2006; Zhu, et al., 2013a; Zijlstra, et al., 2009), manipulations of food forms that were included consisted of liquid *vs* solid or liquid *vs* semi-solid or semi-solid *vs* solid, and included chunky and pureed food. Food consisted mainly of vegetables, fruit, meat and beverage (fruit juices) and texture was manipulated by blending the food. Eight studies (Camps, et al., 2016; Juvonen, et al., 2011; Juvonen, et al., 2009; Solah, et al., 2010; Wanders, et al., 2014; Yeomans, et al., 2014; Yeomans, et al., 2016; Zhu, et al., 2013a) investigated the effect of viscosity, such as low viscosity/ (sensorially termed as 'thin') *vs* high viscosity/ (sensorially termed as 'thick'), and the texture was manipulated by adding fibres such as, starch, tara-gum, locust-beam gum, alginate, guar-gum, casein and pectin to food products, such as milk products or fruit juices. Two studies (Larsen, et al., 2016; Tang, et al., 2016) examined the effect of structural complexity, such as low complexity *vs* high complexity, and the intervention consisted of model foods *i.e.* hydrogels enclosing various layers and particulate inclusions such as poppy and sunflower seeds. One study (Santangelo, et al., 1998) looked at the homogenization of food, one at the aeration of food incorporating N_2O into a liquid drink (Melnikov, et al., 2014) and one study assessed the effect of gels with different lubricity (low *vs* medium *vs* high lubricity) using *κ*-carrageenan and alginate to manipulate the texture (Krop, et al., 2019b).

Food texture measurements. Nineteen studies measured food texture instrumentally, of which 14 assessed viscosity (Camps, et al., 2016; Dong, et al., 2016; Flood, et al., 2007; Juvonen, et

al., 2011; Juvonen, et al., 2009; Krop, et al., 2019b; Marciani, et al., 2012; Mattes, 2005; Solah, et al., 2010; Wanders, et al., 2014; Yeomans, et al., 2016; Zhu, et al., 2013a; Zhu, et al., 2013b; Zijlstra, et al., 2009), two measured lubricity indirectly by measuring friction coefficients (Krop, et al., 2019b; Yeomans, et al., 2016), one measured foam volume as a function of time (Melnikov, et al., 2014) and one used aperture sieving (Santangelo, et al., 1998). Eleven studies assessed food texture using sensory evaluation, of which three studies have used a trained panel (11, 29, 33 panellists) (Hogenkamp, et al., 2012a; Krop, et al., 2019b; Solah, et al., 2010), four studies untrained (20, 20, 24, 32 panellists) (Larsen, et al., 2016; Tang, et al., 2016; Yeomans, et al., 2014; Zijlstra, et al., 2009), and in two studies it is unclear whether it was a trained or untrained panel (20 panellists) (Camps, et al., 2016; Hogenkamp, et al., 2012b). Two studies did not publish or did not show the data (Labouré, et al., 2002; Tsuchiya, et al., 2006). The sensory evaluation was carried out by using Quantitative Descriptive Analysis (QDA) (Krop, et al., 2019b; Larsen, et al., 2016), modified Texture Profile (TP) and Temporal Dominance of Sensations (TDS) (Larsen, et al., 2016).

Additional information with regards to objective textural manipulation that is characterized by instrumental and sensorial techniques and information on weight and energy density of the intervention, and time to next meal can be found in **Supplementary Table A.2.**

Table 2.2. Characteristics of studies included in this systematic review

Reference	**Participants**		**Study design**	**Food form/texture manipulation**		**Food/texture measurements**	**Outcomes measurements**					
	n	***Gender M/F***		***Type of food***	***Type of manipulation***		***Appetite method***	***Effect appetite***	***Food intake method***	***Effect food intake***	***Gut peptides method***	***Effect gut peptides***
Camps, Mars, de Graaf and Smeets (2016)	15	15/0	Randomized, cross-over, within-subjects design, sample size power calculation	**Thick vs thin** Shakes	**Fibre added** Locust bean gum	Viscosity Sensory	VAS-100mm	Fullness ↑ in thick condition compared to thin one	Ad libitum	No difference in food intake between thick and thin conditions	N/A	N/A
Clegg, Ranawana, Shafat and Henry (2013)	12	6/6	Randomized, cross-over, within-participants, non-blind design	**Solid vs. chunky vs smooth** Rice, vegetable and chicken	**Blending** Half ingredients blended + rice added All ingredients blended together	N/A	VAS-100mm	Fullness ↑ in smooth condition compared to solid one	N/A	N/A	N/A	N/A
Dong, Sargent, Chatzidiakou, Saunders, Harkness (2016)	24	17/7	Randomized, cross-over, within-subjects, double-blind design, sample size power calculation	**Liquid vs semi-solid vs solid** Oranges	**Whole + fibre added** Orange juice Orange juice with orange pomace fibre added Whole oranges chopped	Viscosity	VAS-100mm	Fullness ↑ in semi-solid and solid condition compared to liquid one	N/A	N/A	N/A	N/A
Flood and Rolls (2007)	60	30/30	Randomized, cross-over, within-subjects design, sample size power calculation	**Solid vs chunky vs chunky-purred vs purred** Broth and vegetables	**Blending** Ingredients combined into a chunky soup All ingredients blended together	Viscosity	VAS-100mm	No difference in appetite ratings between preloads	Ad libitum	No difference in food intake between conditions	N/A	N/A

Flood-Obbagy and Rolls (2009)	58	30/28	Randomized, cross-over, within-subjects design, sample size power calculation	**Solid vs semi-solid vs liquid** Apples	**Blending + pectin added** Slices, pureed and apple juice with fibre	N/A	VAS-100mm	Hunger ↓ and fullness ↑ in solid and semi-solid condition compared to liquid one	Ad libitum	Food intake ↓ after solid and semi-solid consumption compared to liquid	N/A	N/A
Hogenkamp, Stafleu, Mars and de Graaf (2012b)	27	9/18	Randomized, cross-over, within-subjects design	**Liquid vs semi-solid** Gelatine	**Fibre added** Starch	Sensory evaluation (n=20)	10-point scale	Fullness ↑ in semi-solid condition compared to liquid one	Ad libitum	No difference in food intake in regard to texture	N/A	N/A
Hogenkamp, Mars, Stafleu and de Graaf (2012a)	53	12/41	Randomized, cross-over, within-subjects design, sample size power calculation	**Liquid vs semi-solid** Milk-based products	**Fibre added** Starch	Sensory evaluation (trained, n=29)	VAS-100mm	Hunger ↓ and fullness ↑ in semi-solid condition compared to liquid one	N/A	N/A	N/A	N/A
Juvonen, Purhonen, Salmenkallio-Marttila, Lahteenmaki et al. (2009)	20	4/16	Randomized, cross-over, within-subject, single-blind, design	**Low viscous vs high viscous** Oat bran beverages	**Fibre added** Beta-glucanase enzyme	Viscosity	VAS-100 mm	Satiety ↑ in low viscous condition compared to high viscous one	Ad libitum Food records	No difference in food intake between conditions after ad libitum meal Food intake ↑ after low viscosity condition when energy intake of ad libitum and during the rest of the day was combined	Ghrelin, CCK, GLP-1 and PPY	CCK, GLP-1, PYY ↑ and ghrelin ↓ in low viscous condition compared to high viscous one

Juvonen, Karhunen, Vuori, Lille, Karhu, Jurado-Acosta, Laaksonen et al. (2011)	8	8/0	Randomized, cross-over, within-subject design	**High viscous vs low viscous** Milk protein based	**Fibre added** Casein and transglutaminase-treated casein	Puncture test (firmness) Viscosity	VAS-100 mm	Fullness ↑ in gel condition compared to low and high viscous ones	N/A	N/A	GLP-1 and PYY	CCK↑ in high and low viscous condition compared to rigid gel
Krop, Hetherington, Miquel and Sarkar (2019b)	55	16/39	Randomized, between-subject design	**High lubricity vs low lubricity** Hydrogels	**Gelling agents** *k*-carrageenan and sodium alginate added	Compression test Viscosity Friction Lubrication Sensory evaluation (trained, n=11)	VAS-100mm	No difference in appetite ratings between preloads	Ad libitum	Food intake ↓ after high lubricating gel consumption compared to medium and low lubricating ones	N/A	N/A
Laboure, van Wymelbeke, Fantino and Nicolaidis (2002)	12	12/0	Cross-over, within-subject design Randomization unclear	*Product 1* **Semi-solid vs liquid** Vegetables with beef *Product 2* **Solid vs liquid** Rusk	*Product 1* **Blending** *Product 2* **Toasted or dissolved** in unskimmed chocolate milk	Sensory evaluation (unpublished results)	VAS-100mm	No difference in appetite ratings between preloads	Ad libitum	No difference in food intake between conditions	N/A	N/A
Larsen, Tang, Ferguson and James (2016)	26	N/A	Randomized, cross-over, within-subjects design	**High complexity vs low complexity** Gelatine agar gels	**Fibre added** Gelatine-agar + ground poppy and sunflower seeds	Sensory evaluation (untrained, n=20)	VAS-10cm	No difference in appetite ratings between preloads	Ad libitum	Food intake ↓ after high complex gel condition	N/A	N/A
Marciani, Hall, Pritchard, Cox, Totman, Lad et al. (2012)	22	13/9	Randomized, cross-over, within-subjects design, sample size power calculation	**Solid vs liquid** Chicken and vegetables	**Blending** All ingredients blended together	Viscosity	VAS 1 to 10	Hunger ↓ in soup condition compared to solid-liquid one	N/A	N/A	N/A	N/A
Martens, Lemmens, Born and Westerterp-Plantenga (2012)	10	10/0	Randomized, cross-over, within-subject, design, sample size power calculation	**Solid vs liquid** Peaches	**Blending** Whole peeled peached or blended	N/A	VAS-100mm	No difference in appetite ratings between preloads	N/A	N/A	Ghrelin	No difference in ghrelin between conditions

Martens, Lemmens, Born and Westerterp-Plantenga (2011)	10	10/0	Randomized, cross-over, within-subjects design, sample size power calculation	**Solid vs liquid** Chicken	**Blending** Whole steamed chicken or blended	N/A	VAS-100mm	Hunger ↓ in solid condition compared to liquid one	N/A	N/A	Ghrelin	No difference in ghrelin between conditions
Mattes (2005)	31	13/18	Cross-over, within-subject design Randomization unclear	**Solid vs soup** Apple Chicken breast Peanuts	**Blending** Whole ingredients or blended	Viscosity	13-point bipolar category	Hunger ↓ in beverage compared to soup and solid conditions Fullness ↑ in soup and solid conditions compared to beverage one	Food records Unclear if it was served ad libitum or fixed	Energy intake ↓ after soups consumption compared to sild one after 24 hours	N/A	N/A
Melnikov, Stoyanov, Kovacs, Arnaudov, de Groot, Schuring et al. (2014)	24	3/21	Randomized, cross-over, within-subjects design, sample size power calculation	**Liquid vs aerated** Liquid drink	**Aerated** N2O incorporated	Stability	VAS-100mm	Hunger ↓ and fullness ↑ in aerated condition compared to non-aerated one	N/A	N/A	N/A	N/A
Mourao, Bressan, Campbell and Mattes (2007)	60	30/30	Between-subjects design, sample size power calculation Randomization unclear	**Beverage vs solid** Cheese, watermelon fruit and coconut meat	**No texture manipulation** Whole or bought juice	N/A	VAS-100mm	No difference in appetite ratings between preloads	Food records	Food intake ↓ after solid consumption compared to liquid one	N/A	N/A
Santangelo, Peracchi, Conte, Fraquelli and Porrini (1998)	8	8/0	Randomized, cross-over, within-subject design	**Solid-liquid vs homogenized** Vegetables, cheese, croutons and olive oil	**Blending** Whole ingredients or homogenized	Aperture sieve	100-mm fixed point scale	Satiety ↑ in homogeneous condition compared to solid one	N/A	N/A	CCK	No difference in CCK between conditions
Solah, Kerr, Adikara, Meng, Binns, Zhu et al. (2010)	33	N/A	Randomized, cross-over, within-subject, single-blinded design	**High viscosity vs low viscosity** Water based drinks	**Fibre added** Alginate and protein in water	Viscosity Sensory evaluation (trained, n=33)	VAS-100mm	Hunger ↓ in high viscous condition compared to low viscous one	N/A	N/A	N/A	N/A

Tang, Larsen, Ferguson and James (2016)	38	22/16	Randomized, cross-over, single-blind, design	**Low complexity vs medium complexity vs high complexity** Gelatine agar gels	**Fibre added** Gelatine-agar + ground poppy and sunflower seeds	Puncture stress Sensory	VAS-100mm	Hunger ↓ and fullness ↑ in high complex gels compared to low complex ones	Ad libitum	Food intake ↓ after high complex gels consumption compared to low complex ones	N/A	N/A
Tournier and Louis-Sylvestre (Tournier, et al., 1991)	13	7/6	N/A	**Liquid vs solid** Vegetables and tomato juice	**Blending + fibre** All ingredients mashed and added gelatine	N/A	100-mm lines	No difference in appetite ratings between preloads	Ad libitum Food records	No difference in food intake between conditions	N/A	N/A
Tsuchiya, Almiron-Roig, Lluch, Guyonnet and Drewnowski (2006)	32	16/16	Randomized, cross-over, within-subjects design, sample size power calculation	**Semi-solid vs liquid vs beverage** Peaches	**Blending** Peach pieces in yogurt, the same yogurt homogenized	Sensory evaluation No data shown	9-point category scale	Fullness ↑ in semi-solid and liquid condition compared to beverage one	Ad libitum	No difference in food intake between conditions	N/A	N/A
Wanders, Feskens, Jonathan, Schols, de Graaf and Mars (2014)	29	29/0	Randomized, cross-over, within-subjects, single-blind design, sample size power calculation	**Gels vs capsules vs liquids** Mixture of soft cheese, milk, apple juice and strawberry syrup	**Fibre added** Pectin	Viscosity	VAS-100mm	Hunger ↓ and fullness ↑ in gel condition compared to capsules and liquid ones Fullness ↑ in capsules condition compared to liquid one	Ad libitum	Energy intake ↓ after capsules consumption compared to liquid condition	N/A	N/A
Yeomans, Wickham, Lundholm and Chambers (2016)	23	23/0	Counterbalanced, within-subjects, design	**Thin (**low sensory) **vs thick** (enhanced sensory) Fruit yogurt beverages	**Fibre added** Tara-gum added	Viscosity and lubrication (stated elsewhere)	VAS-100mm	Hunger ↓ in thick condition compared to thin one	Ad libitum	No difference in food intake in regard to texture	CCK	No difference in CCK between conditions regards to texture

Yeomans, McCrickerd, Brunstrom and Chambers (2014)	48	48/0	Randomized, between-subjects design	**Thin (**low sensory) **vs thick** (enhanced sensory) Mango and peach yogurt beverages	**Fibre added** Tara-gum added	Sensory evaluation (untrained, n=24)	VAS-100mm	Hunger ↓ and fullness ↑ in thick condition compared to thin one	Ad libitum	No difference in food intake in regard to texture	N/A	N/A
Zhu, Hsu and Hollis (2013b)	15	15/0	Randomized, cross-over, within-subjects design	**Standard viscosity vs high viscosity** Chocolate pudding	**Fibre added** Guar-gum added	Viscosity	VAS-100mm	Hunger ↓ and fullness ↑ in high viscous condition compared to low viscous one	Ad libitum	No difference in food intake between conditions	N/A	N/A
Zhu, Hsu and Hollis (2013a)	19	19/0	Randomized, cross-over, within-subjects design	**Liquid-solid vs liquid** Vegetables	**Blending** Whole pieces of vegetables in chicken broth or all blended	Viscosity	VAS-100mm	Fullness ↑ after liquid condition compared to solid one	Ad libitum	No difference in food intake between conditions	CCK Ghrelin	CCK ↑ in liquid condition compared to solid one No difference in ghrelin between conditions
Zijlstra, Mars, de Wijk, Westerterp-Plantenga, Holst and de Graaf (2009)	32	12/20	Randomized, within-subjects cross-over design	**Liquid vs semi-solid** Milk based products	**Fibre added** Starch added	Viscosity Sensory	10-point category scale	Fullness ↑ in semi-solid condition compared to liquid one	Ad libitum	No difference in food intake between conditions	CCK and GLP-1	No difference in CCK between conditions

Appetite ratings method. All studies used 100 mm visual analogue scale (VAS) or categorical rating scales to assess appetite ratings. The majority of studies that assessed appetite control measured hunger (n=27), fullness (n=25), and desire to eat ratings (n=21). Two studies (Juvonen, et al., 2009; Santangelo, et al., 1998) referred only generally to satiety instead of specifying exactly which appetite ratings were being measured.

Food intake measurements. Subsequent food intake was measured using ad libitum meal consumption after the intervention in most of the studies (n=17), but two studies used a food record method (Mattes, 2005; Mourao, et al., 2007).

Gut peptides. From the limited number of studies that measured gut peptides (n=8) using blood plasma samples drawn at baseline and different time points after the intervention, two measured CCK alone (Santangelo, et al., 1998; Yeomans, et al., 2016), two measured ghrelin alone (Martens, et al., 2011; Martens, et al., 2012) and four studies measured more than one gut peptides GLP-1 and PYY (Juvonen, et al., 2011), GLP-1 and CCK (Zijlstra, et al., 2009), CCK and ghrelin (Zhu, et al., 2013a), ghrelin, CCK, GLP-1 and PYY (Juvonen, et al., 2009). The gut peptides were mainly assayed using commercial plate-based immunoassay test kits.

2.3.3. Quality assessment

To assess the quality of studies (n=29) included in this systematic review, Cochrane's tool of risk of bias was used (Higgins, et al., 2011) with regards to random sequence generation, allocation concealment and blinding of participants and personnel and it is reported in **Supplementary Table A.3**. One study (Dong, et al., 2016) reported on all three criteria (random sequence generation, allocation concealment and blinding of participants and personnel), and therefore was included in the low risk-of-bias category. Twenty-five studies reported on one or two criteria and were considered as in the medium risk-of-bias category.

And three trials (Labouré, et al., 2002; Mattes, 2005; Tournier, et al., 1991) did not report clearly on the assessment criteria, therefore were judged within the high risk-of-bias category.

2.4. Narrative synthesis

2.4.1. Effect of food texture on appetite control

Of the total studies that measured appetite control (n=29), 16 found a significant effect of food texture on reducing hunger and increasing fullness ratings. The textural manipulation within these studies ranged from the manipulation of solid-like characteristics to viscosity and to the design of well-characterized model gels with structural complexity (**Table 2.2**). For instance, it was noticed that the consumption of solid and/or semi-solid food more strongly suppressed appetite ratings as compared to ratings of liquid food. Flood-Obbagy and Rolls (2009) found that whole apples led to decreased hunger ratings and increased fullness when compared with their liquid counterparts (*i.e.* apple sauce and juice). These authors argued that the effect of food on satiety was due to the structural form of food itself and the larger volume in case of whole fruit as compared to the liquid versions, even when matched for energy content and weight. Interestingly, these findings were not associated with the amount of fibre as the fibre content was similar across liquid and solid conditions. Similar findings by Hogenkamp et al. (2012a) indicated that hunger decreased, and fullness increased in the semi-solid condition compared to the liquid condition. They found that the semi-solid product (comparable with firm pudding) suppressed appetite greater than the liquid product (comparable with very thin custard). The authors related their findings to the triggering of the early stages of the satiety cascade (Blundell, et al., 1987) through cognitive factors and sensory attributes such as visual and oral cues; whereas food forms might not affect the later processes in satiety cascade that are postulated to be governed by post-ingestive and post-absorptive factors (Hogenkamp, et al., 2012a).

Foods with high viscosity also appeared to play a key role in appetite suppression compared to food with low viscosity (Solah, et al., 2010; Yeomans, et al., 2014; Yeomans, et al., 2016; Zhu, et al., 2013b). Aiming to determine the effect of viscosity on satiety, Solah et al. (2010) used low and high viscous alginate-based breakfast drinks, on 33 subjects. It was found that hunger was lower after participants consumed the high viscous alginate drink as compared to those who consumed low viscous ones. The authors speculated that such findings were related to the gastric distention as a result of the ingested gel-forming fibre, although they did not measure the rheological properties of these foods in the gastric situation. In a rather long-term (7 non-consecutive days over a month) study, Yeomans et al. (2014) investigated low (thin) and high (thick) viscous drinks with both low and high energy content, respectively. They found that initially, appetite was suppressed after consuming high viscous foods as compared with those who consumed low viscous foods, corroborating the afore-mentioned effect of viscosity on satiety. They related their findings to a slower gastric emptying rate in the high viscous food. However, after repeated consumption of the drinks with seven non-consecutive days over a month, there were no noticeable differences in satiety between the low and high viscous conditions (Yeomans, et al., 2014). Expected satiation was higher for both high energy drinks and lower for both low energy drinks irrespective of the viscosity of the foods. This suggests that in a repeated consumption setting, the effect of viscosity can be negligible.

It is noteworthy that some of the authors relate their findings of increased satiety after consuming high viscous foods to a slower gastric emptying rate, which should be interpreted with some caution. For instance, Camps et al. (2016) directly measured the effect of viscosity on gastric emptying in their study using magnetic resonance imaging (MRI) abdominal scans and found that not only the viscosity of food but also the energy load led to a slow gastric emptying. The preloads in their case were four shakes differing in viscosity (low and high

viscosity) measured in perceived thickness using 100-mm VAS scale and also differing in energy content (low/100 kcal and high/500 kcal) consumed within 2 minutes. The increase in the energy load led to slower gastric emptying over time; it only significantly slowed the emptying under the low-energy-load conduction. Therefore, they suggested that viscosity loses its reducing effect on hunger if energy load is increased to a meal size of 500 kcal indicating that viscosity may not always affect the later parts of satiety cascade through delayed gastric emptying route, but contributes to the early parts of satiety cascade via mouth feel and oral residence time.

In addition to form and viscosity, textural complexity has also shown some significant effects on appetite control. However, the term textural complexity is rather poorly defined in the literature. Often it refers to the degree of heterogeneity or inhomogeneity in a food where the pre-load includes some inclusions, which distinguishes it from a control; the latter having a homogenous texture *i.e.* without inclusions. This research domain of studying the effects of *so-called* textural complexity on satiety is still in its early infancy. Tang et al. (2016) conducted the first trial on textural complexity (of the preload) (**Box1 Figure**) and demonstrated that hunger ratings decreased when model food gels with higher complexity (*i.e.* gels layered with particulate inclusions) were served. The authors noticed that higher inhomogeneity in the gels with particle inclusions led to a decrease in hunger and desire to eat, and an increase in fullness ratings, suggesting that levels of textural complexity may have an impact on post-ingestion or post-absorption processes leading to a slowing effect on feelings of hunger.

The technique of aeration, (*i.e.* incorporation of bubbles in a food) has been also used as a textural manipulation and been shown to have an influence on satiety. Melnikov et al. (2014) found that hunger was lower, and fullness was higher in aerated drinks as compared to the non-aerated counterparts. Although these drinks differed in energy content (low/high energy non-aerated and low/high energy aerated), they demonstrated that such aeration

independent of energy content was a promising textural manipulation to suppress appetite. The authors attributed the findings to the effect of the air bubbles on gastric volume leading to the feelings of fullness.

In thirteen studies out of the 29 studies, food texture was reported to have no effect on appetite ratings. This disparity in the results may be associated with the methodology employed. For instance, in several studies (Larsen, et al., 2016; Martens, et al., 2012; Mourao, et al., 2007) participants were instructed to eat their usual breakfast at home. Therefore, the appetite level before the preload was not controlled and this might have influenced the appetite rating results. Furthermore, some studies did not conceal the purpose of the study from the participants (Labouré, et al., 2002; Tournier, et al., 1991). Thus, participants' responses might have been biased and could have led to less reliable results (Athanassoulis & Wilson, 2009). Moreover, Mourao et al. (2007) firstly served an *ad libitum* meal to participants and then immediately the preload with different textural attributes. As such, the time interval between *ad libitum* intake and preload may have accounted for variation in outcomes (Blundell, et al., 2010). All these factors may explain the disparities with regards to the effects of food texture on subjective appetite ratings.

2.4.2. Effect of food texture on gut peptides

Out of the limited number of studies (n=8) that included gut peptides measurements; only two (Juvonen, et al., 2009; Zhu, et al., 2013a) studies found an effect of food texture. Contrary to our expectations, Juvonen et al. (Juvonen, et al., 2009) found that CCK, GLP-1, PYY increased and ghrelin decreased in low viscous condition compared to high viscous one. The authors speculate that after consuming a high viscous drink, viscosity of the product may delay and prevent the close interaction between the nutrients and gastrointestinal mucosa required for efficient stimulation of enteroendocrine cells and peptide release. The same results were found in regard to food form. Zhu et al. (2013a) found that liquid food (pureed liquid-solid soup)

resulted in a higher postprandial response of CCK comparing with solid food (whole pieces of vegetables in a chicken broth). They related it to the capacity of CCK to be secreted in the duodenum in response to the presence of nutrients. As such, they suggest that the increase in the surface area of the nutrients due to the smaller particle sizes resulted from the pureeing could stimulate secretion of CCK more potently.

The rest of the studies found no significant effect of food texture (form, viscosity or complexity) on triggering relevant gut peptides. This may be due to the type of macronutrients used in such intervention. For example, intervention in Martens' et al. (2011) study was high in protein and it is known that proteins are less effective in suppressing ghrelin (Cummings, et al., 2007). Therefore, one may argue that the effect of food texture is only restricted to early stages of satiety cascade rather than later stages, where the type and content of macronutrient might play a decisive role. However, such interpretations might be misleading owing to the limited number of studies in this field. Also, in the majority of studies conducted so far, the biomarkers were limited to one gut peptide, such as CKK (Santangelo, et al., 1998; Yeomans, et al., 2016; Zhu, et al., 2013a) or ghrelin (Martens, et al., 2011; Martens, et al., 2012), which provides a selective impression of the effects on gut peptides. Measuring more than one gut peptide could provide richer data and wider understanding of the relationship between food texture and gut peptides, which has yet to be fully evaluated (Athanassoulis, et al., 2009).

2.4.3. Effect of food texture on energy intake

Seven out of the total 29 studies found a significant effect of texture on food intake. Food form, such as solid, appeared to play a role in the subsequent food/energy intake. For example, in the study by Flood and Rolls (2009), 58 participants consumed apple segments (solid food) on one day and then apple sauce (liquid food) made from the same batch of apples used in the whole fruit conditions on another day. The preload was controlled for the energy density and consumed within 10 minutes and the *ad libitum* meal was served after a total of 15 minutes. As

a result, they found that apple pieces reduced total energy intake at lunch as compared to the apple sauce, therefore suggesting that consuming whole fruits before a meal can enhance satiety and reduce subsequent food intake. Mourao et al. (2007) also confirmed such findings where participants consumed less energy after ingesting solid food form (cheese/watermelon fruit/coconut meat) as compared to the beverage form (milk/watermelon juice/coconut milk). However, it is worth noting that they had a different experimental approach in contrast to the rest of the studies in this systematic review. First, an *ad libitum* meal was served and then followed by a fixed preload consisting of solid and beverage form with one predominant macronutrient (milk-protein, watermelon-carbohydrate and coconut-fat). The time between *ad libitum* meal and the preload was not stated; it is only clear that it was served at lunch time. Food records were kept on each test day (for 24 h) to determine energy intake. Despite this different approach, it was demonstrated that solid food led to a lower subsequent energy intake compared with liquid food counterparts. Consequently, this study supports an independent effect of texture on energy intake.

In terms of viscosity, it has been found that higher viscous food can also lead to a reduced subsequent energy intake. This was noted in Juvonen's et al. (2009) study, where participants consumed two identical, isoenergetic and isovolumic oat bran beverages that differed only in their viscosity (low, <250 mPa; high, >3000 mPas) which was measured instrumentally. Authors reported that the beverage with high-viscosity led to a lower energy intake compared to the low-viscous beverage when energy consumption during the meal consumed *ad libitum* and during the rest of the test day was combined. Although authors attribute their findings to a slower gastric emptying rate, they did not measure it directly, nor was the effect of viscosity on mouth feel or oral residence time affecting early stages of satiety cascade investigated.

Even with a limited number of studies, textural complexity has been demonstrated to have a clear impact on subsequent food intake. For instance, in the studies of Tang et al. (2016) and Larsen et al. (2016), gels mixed with poppy and sunflower seeds reduced subsequent food intake independently of the oral transit time and energy density, suggesting a sole impact of food texture on food intake.

Interestingly, Krop et al. (2019b) also showed a clear effect of texture on reducing subsequent snack intake by using hydrogels (having no energy content or micronutrients) that differed in their textural complexity in terms of their lubricating properties, which was measured both instrumentally and sensorially (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019a). These authors related their findings to hydrating and mouth-coating effects after ingesting the high lubricating carrageenan-alginate hydrogels that in turn led to a lower snack intake. Moreover, they demonstrated that it was not the intrinsic chewing properties of hydrogels but the externally manipulated lubricity of those gel boli *i.e.* gel and simulated saliva mixture that influenced the snack intake. All these reports suggest that there is a growing interest in assessing food texture from a textural complexity perspective. This means introducing heterogeneity such as tribological/ lubrication alternation in food to have enhanced satiety and satiation consequences. This strategy needs attention in future satiety trials as well as longer-term repeated exposure studies.

The energy density of the preload across the studies varied from zero kcal (Krop, et al., 2019b) or a modest energy density 40 kcal (Larsen, et al., 2016; Tang, et al., 2016) up to a higher value of – 600-700 kcal (Santangelo, et al., 1998; Tournier, et al., 1991) (see the **Supplementary Table A.2**). It is noteworthy that the lower the energy density of the preload, the shorter the time interval between the intervention (preload) and the next meal (*ad libitum* meal). Some of these studies showed an effect of texture on appetite ratings and food intake, with food higher in heterogeneity leading to a suppression of appetite and reduction in

subsequent food intake (Larsen, et al., 2016; Tang, et al., 2016). Also, gels with no calories but high in their lubrication properties showed a reduction in snack intake (Krop, et al., 2019b). Contrary to those textures with zero (or modest levels of) calories, those textures high in calories tended to have a larger time gap between the intervention (preload) and the next meal. An interesting pattern observed across these studies employing high calorie-dense studies, is that an effect of texture on appetite ratings was found but no effect on food intake (Tsuchiya, et al., 2006; Zhu, et al., 2013a; Zhu, et al., 2013b). Therefore, in addition to the high energy density of the preload, it appears that time allowed between the preload and the next meal is an important methodological parameter.

2.5. Meta-analysis

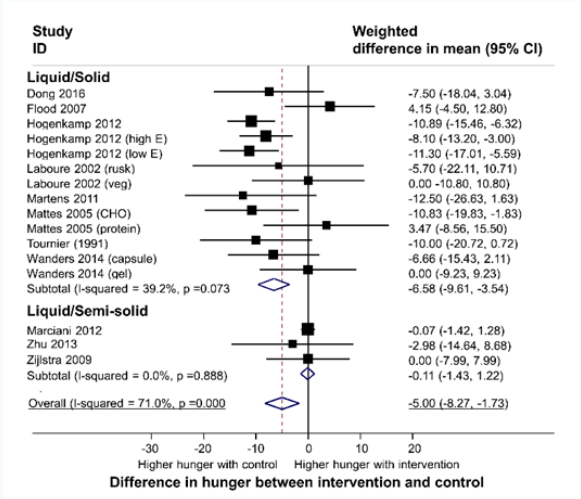
A total of 23 articles were included in the meta-analysis. Two articles were excluded as data on a number of outcomes were missing (Mourao, et al., 2007; Santangelo, et al., 1998). Meta-analysis on structural complexity (Larsen, et al., 2016; Tang, et al., 2016), lubrication (Krop, et al., 2019b), aeration (Melnikov, et al., 2014) and gut peptides could not be performed due to limited number of studies addressed this issue, and therefore a further four articles were excluded. Finally, meta-analysis was performed on the effect of form and viscosity of food on three outcomes: hunger, fullness and food intake. Data from 22 within-subjects and 1 between-subjects trials reporting comparable outcome measures were synthesised in the meta-analyses. These articles were expanded into 35 groups as some studies provided more than one comparison group. In most of the studies (n=18), appetite was measured on 100 mm visual analogue scale (VAS).

Meta-analyses presenting combined estimates and levels of heterogeneity were carried out on studies investigating form (total of 20 subgroups, 651 participants) and viscosity (total of 15 subgroups, 281 participants) for the three outcomes hunger, fullness and food intake (see data included in the meta-analysis in **Supplementary Tables A.4a-c**). There was an

insufficient number of studies to carry out meta-analyses for the ones investigating complexity (n=2) (Larsen, et al., 2016; Tang, et al., 2016), lubrication (n=1) (Krop, et al., 2019b), aeration (n=1) (Melnikov, et al., 2014) (total of 4 studies, 103 participants) and gut peptides (total of 8 studies, 130 participants (*e.g.* 3 studies assessed GLP-1 with available data on 2 studies (Juvonen, et al., 2011; Zijlstra, et al., 2009), and 2 studies assessed PYY with available data on 1 study only (Juvonen, et al., 2011)).

Hunger. A meta-analysis of 556 participants, from 16 subgroups based on food form (13 comparing solid with liquid food and 3 comparing semi-solid with liquid food) revealed an overall significant decrease in hunger with the intervention (solid or semi-solid) group of -5.00 mm (95% confidence interval (CI) -8.27 to -1.73, p= 0.003, I^2=71%). There was a significant decrease in hunger with solid food of -6.58 units (95% CI -9.61 to -3.54, p<0.001, I^2=39%) however no difference in hunger was seen for comparisons of semi-solid with liquid food (see **Figure 2.2a**). A meta-analysis of 191 participants from 11 subgroups based on viscosity revealed a borderline significant decrease in hunger with higher viscosity food of -2.10 mm (95%CI -4.38 to 0.18, p=0.071, I^2=59%) (see **Figure 2.2b**).

a



b

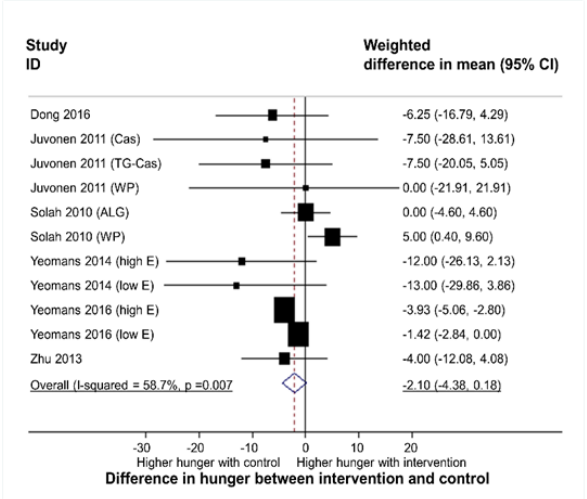


Figure 2.2. Meta-analysis of effect of food texture on hunger ratings. Pooled estimate of the differences in hunger ratings between intervention and control by food form (liquid/solid; liquid/semi-solid) (a) and viscosity (low/high viscous) (b), respectively. Available data from the medium follow-up period (60 minutes after intervention/control) was used for synthesis. The bottom horizontal line denotes 95% CIs. The diamond indicates the overall estimated effect. ID represents identification.

Fullness. A meta-analysis of 263 participants, from 12 subgroups based on form (9 comparing solid with liquid food and 3 comparing semi-solid with liquid food) revealed no overall difference in fullness between the intervention (solid or semi-solid) group and control group (-0.75 units, 95% CI -3.93 to 2.43, p= 0.644, I^2=91%). There was no difference in fullness between groups for either of the two subgroups (see **Figure 2.3a**).

A meta-analysis of 155 participants from 11 subgroups based on viscosity revealed an overall significant increase in fullness for higher viscosity food of 5.20 mm (95%CI 2.43 to 7.97, p<0.001, I^2=76%) (see **Figure 2.3b**).

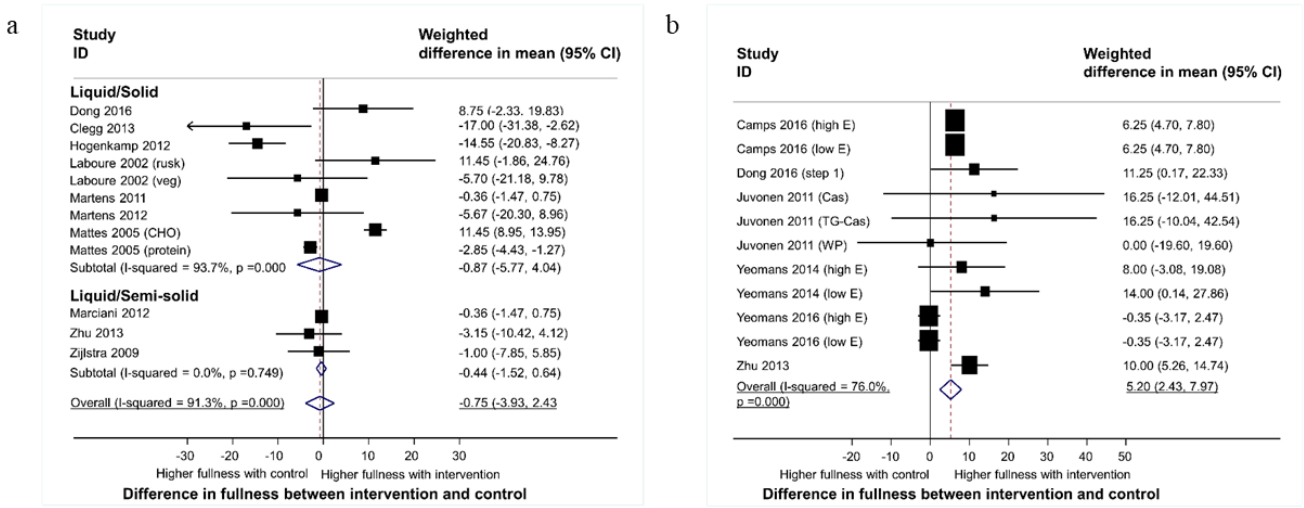


Figure 2.3. Meta-analysis of effect of food texture on fullness ratings. Pooled estimate of the differences in fullness ratings between intervention and control by food form (liquid/solid; liquid/semi-solid) (a) and viscosity (low/high viscous) (b). Available data from the medium follow-up period (60 minutes after intervention/control) was used for synthesis. The bottom horizontal line denotes 95% CIs. The diamond indicates the overall estimated effect. ID represents the identification.

Food intake. A meta-analysis of 458 participants, from 12 subgroups based on form (9 comparing solid with liquid food and 3 comparing semi-solid with liquid food) revealed no overall difference in food intake with the intervention (solid or semi-solid) group compared with the control group (-26.2kcal, 95% CI -61.7 to 9.4kcal, p= 0.149, I^2=0%) (see **Figure 2.4a**).

There was a borderline significant reduction in food intake for studies comparing solid with liquid food of -55.5kcal (95% CI -111.1 to -0.1kcal, p=0.05, I^2=0%) however no difference in food intake was seen for comparisons of semi-solid and liquid food. A meta-analysis of 191 participants from 9 subgroups based on viscosity revealed a non-significant decrease in food intake with higher viscosity food of -66.7kcal (95%CI -144.2 to 10.9kcal, p=0.092, I^2=84%) (see **Figure 2.4b**). Funnel plots (see **Supplementary Figures A.1a-c**) reveal that there was some evidence of asymmetry and therefore a small publication bias may be present, particularly for the meta-analyses for hunger.

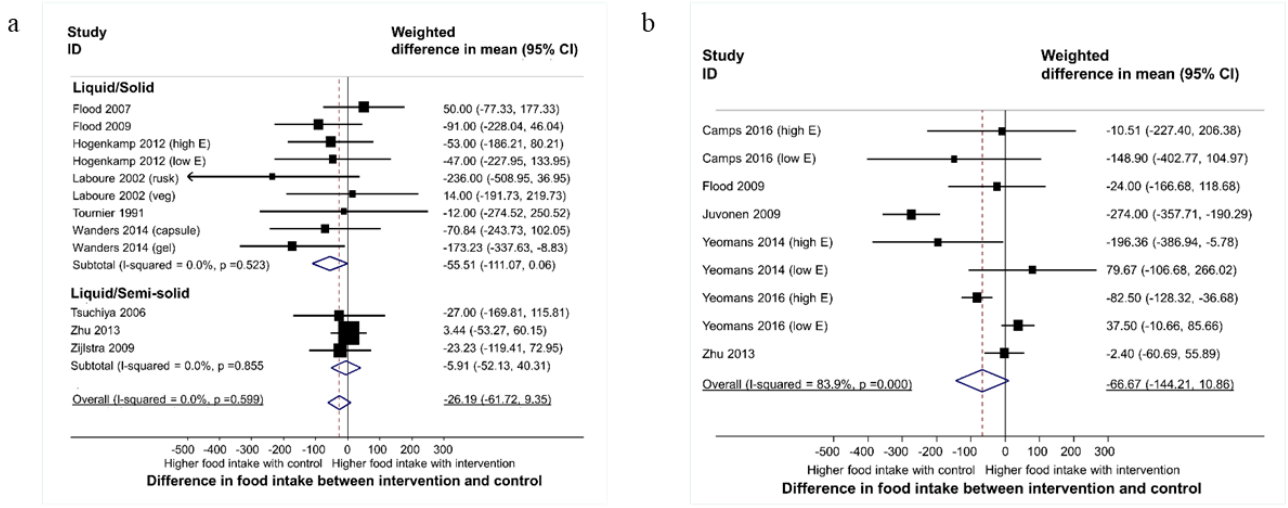


Figure 2.4. Meta-analysis on effect of food texture on food intake. Pooled estimate of the differences in food intake between intervention and control by food form (liquid/solid; liquid/semi-solid) (a) and viscosity (low/high viscous) (b). Available data from the medium follow-up (60 minutes after intervention/control) was used for synthesis. The bottom horizontal line denotes 95% CIs. The diamond indicates the overall estimated effect. ID represents the identification.

2.6. Discussion

In this comprehensive systematic review and meta-analysis, we investigated the effects of food texture on appetite, gut peptides and food intake. The hypothesis tested was that food with higher textural characteristics (solid form, higher viscosity, higher lubricity, higher degree of heterogeneity, *etc.*) would lead to a greater suppression of appetite and reduced food intake. In fact, the qualitative synthesis showed that in half of the studies included in this systematic review food texture such as, solid form (Dong, et al., 2016; Flood-Obbagy, et al., 2009; Hogenkamp, et al., 2012a; Hogenkamp, et al., 2012b; Martens, et al., 2011; Mattes, 2005; Mourao, et al., 2007; Tsuchiya, et al., 2006; Zijlstra, et al., 2009), higher viscosity (Camps, et al., 2016; Juvonen, et al., 2011; Solah, et al., 2010; Wanders, et al., 2014; Yeomans, et al., 2014; Yeomans, et al., 2016; Zhu, et al., 2013b), higher lubricity (Krop, et al., 2019b), higher degree of complexity/heterogeneity (Larsen, et al., 2016; Tang, et al., 2016) and aerated

(Melnikov, et al., 2014) food was reported to suppress appetite and reduce food intake. Likewise, the quantitative analysis (meta-analysis) clearly indicated a significant decrease in hunger with solid food compared to liquid food. Also, a significant increase was noted in fullness with high viscous food compared to low viscous food. However, no effect of food form on fullness was observed. Food form showed a borderline significant decrease in food intake with solid food having the main effect.

The main explanation for the varying outcomes could be the methodology applied across the studies which was supported by a moderate to a high heterogeneity of studies in the meta-analysis. Within the preload study designs that were included in the current article, attention should be paid to the following factors that were shown to play an important role in satiety and satiation research: macronutrient composition of the preload, time lapse between preload and test meal, and test meal composition (Blundell, et al., 2010).

Considerable data supports the idea that the macronutrient composition, energy density, physical structure and sensory qualities of food plays an important role in satiety and satiation. There appears to be a hierarchy (protein>carbohydrate>fat) in the extent to which macronutrients can impact satiety and satiation (Johnson & Vickers, 1993; Stubbs, Prentice, & James, 1997). For instance, it has been demonstrated that eating a high-protein and high-carbohydrate preload can lead to a decrease in hunger ratings and reduced food intake in comparison with eating high-fat preload (Johnson, et al., 1993). As such, it is worth noting that interventions across the studies included in this systematic review and meta-analysis differed hugely in terms of macronutrient composition. For example, in some studies the preload food was higher in fat and carbohydrate (Flood, et al., 2007; Labouré, et al., 2002) compared to protein which may be a reason for finding no effect on appetite and food intake. In contrast, where the preload was high in protein (Martens, et al., 2011), a significant suppression of appetite ratings was observed. Moreover, it is important to highlight that a recent development

in the food science community is the ability to create products such as hydrogel-based that do not contain any calories. As these gels are novel products, they are also free from any prior learning or expected postprandial satisfaction that could influence participants. These hydrogels have been proven to have an impact on satiety (Tang, et al., 2016) and satiation (Krop, et al., 2019b) suggesting there is an effect of food texture alone, independent of calories and macronutrients composition.

An important factor that may also explain variation in outcomes, may be the timing between preload and test meal. It has been argued that the longer the time interval between preload and test meal the lower the effect of preload manipulation (Rolls, et al., 1991). Accordingly, the range of intervals between preload and test meal differed substantially across the studies included in this systematic review: from 10 to 180 minutes. Studies with a shorter time interval (10-15 min) between preload and *ad libitum* food intake showed an effect of food texture on subsequent food intake (Krop, et al., 2019b; Larsen, et al., 2016; Tang, et al., 2016). In contrast, those studies with a longer time interval, such as Camps et al. (2016), Tsuchiya et al. (2006), Yeoman et al. (2014; 2016) (90 min) and Tournier et al. (1991) (180 min) found no effect on food intake.

As such, it can be deduced that the effects of texture might be more prominent in studies tracking changes in appetite and food intake over a shorter period following the intervention. In addition, the energy density of the preload is a key factor that should not be discounted when designing satiety trials on food texture. For instance, the lower the energy density of the preload, the shorter the interval between the intervention and next meal should be in order to detect an effect of food texture on satiation as observed by Tang et al. (2016), Larsen et al. (2016), Krop et al. (2019b) (see **Supplementary Table A.2**). Therefore, the different time intervals between preload and *ad libitum* test meal, and a difference in energy densities of the

preload can lead to a modification of outcomes, which might confound the effect of texture itself.

The test meals in the studies were served either as a buffet-style (participants could choose from a large variety of foods) or as a single course (food choice was controlled). It has been noticed that in studies where the test meal was served in a buffet style (Hogenkamp, et al., 2012b; Labouré, et al., 2002; Tsuchiya, et al., 2006), there was no effect on subsequent food intake. Choosing from a variety of foods can delay satiation, stimulate more interest in different foods offered and encourage increased food intake (Hetherington, Foster, Newman, Anderson, & Norton, 2006) leading to the same level of intake on both conditions (*e.g.* solid and liquid conditions). In contrast, in studies that served test meal as a single course (Juvonen, et al., 2009; Krop, et al., 2019b; Larsen, et al., 2016; Tang, et al., 2016), the effect of texture on subsequent food intake has been shown as more prominent. Therefore, providing a single course meal in satiety studies may have scientific merit although it might be far from real-life setting.

It was also noticeable that some studies with a larger sample size (Mattes, 2005; Solah, et al., 2010; Yeomans, et al., 2016) showed less effect of food texture on hunger and fullness in our meta-analysis. Although, it is not possible to confirm the reasons why this is the case we can only speculate it could be due to considerable heterogeneity across the studies. For instance, one of the reasons could be the selection criteria of the participants. Even though, we saw no substantial differences from the information reported in individual studies there may be other important but unreported factors contributing to this heterogeneity. Furthermore, studies with larger sample sizes often have larger variation in the selected participant pool than in smaller studies (Yusuf, Held, Teo, & Toretsky, 1990) which could potentially reduce the precision of the pooled effects of food texture on appetite ratings but at the same time may produce results that are more generalizable to other settings.

Although the meta-analysis showed a clear but modest effect of texture on hunger, fullness and food intake, the exact mechanism behind such effects remains elusive. Extrinsically-introduced food textural manipulations such as those covered in this meta-analysis might have triggered alterations in oral processing behaviour, eating rate or other psychological and physiological processing in the body. However, at this stage, to point out one single mechanism underlying the effect of texture on satiety and satiation would be premature and could be misleading. A limited number of studies have also included physiological measurements such as gut peptides with the hypothesis that textural manipulation can trigger hormonal release influencing later parts of the Satiety Cascade (Blundell, 2010; Blundell, et al., 1987). However, with only eight studies that measured gut peptides, of which five failed to show any effect of texture, it is hard to support one mechanism over another. Therefore, more studies are needed especially incorporating physiological measurements in order to understand the whole spectrum of mechanisms underlying an effect of food texture on satiety/satiation.

2.7. Future strategies

Employing food textural manipulations such as increasing viscosity, lubricating properties and the degree of heterogeneity appear to be able to trigger effects on satiation and satiety. However, information about the physiological mechanism underlying these effects have not been revealed by an examination of the current literature. Unfortunately, many studies in this area were of poor-quality experimental design with no or limited control conditions, a lack of the concealment of the study purpose to participants and a failure to register the protocol before starting the study; thus, raising questions about the transparency and reporting of the study results. Future research should apply a framework to standardize procedures such as suggested by Blundell et al. (2010) in order to have more consistent results and to justify a claim for an effect of food on subjective aspects of appetite and food intake.

Also, the recent development of food colloidal approaches to create products/hydrogels with no calories and macronutrients was noted. It is, therefore, crucial to carry out more studies involving these types of well-characterized model foods and see how they may affect satiety and food intake. To date, only one study (Krop, et al., 2019b) has looked at the lubricating capacity of food using hydrogels with no calories which clearly showed the effect of texture alone; eliminating the influence of energy content. As such, a clear gap in knowledge of the influence of food with higher textural characteristics, such as lubrication, aeration, mechanical contrast, and variability in measures of appetite, gut peptide and food intake is identified through this systematic review and meta-analysis.

There are limited number of studies that have assessed gut peptides (ghrelin, GLP-1, PPY, and CCK) in relation to food texture to date. Apart from the measurement of gut peptides, no study has used saliva biomarkers, such as α-amylase and salivary PYY to show the relationship between these biomarkers and subjective appetite ratings. Therefore, it would be of great value to assess appetite through both objective and subjective measurements to examine possible correlations between the two.

Besides these aspects, there are other cofactors that are linked to food texture and hard to control, affecting further its effect on satiety and satiation. To name, pleasantness, palatability, acceptability, taste and flavour are some of the cofactors that should be taken into account when designing future satiety studies. In addition, effects of interactions between these factors such as taste and texture, texture and eating rate *etc*. on satiety can be important experiments that need future attention.

Also, measuring the texture of the food/preload both instrumentally and by sensory procedures, can increase the quality of study design and give more accurate and robust results. This would help to objectively understand the degree of sensorial distinction/ instrumental difference needed between the intervention and the control to have an effect on satiety. For

instance, the higher viscous food should have at least 10-100 factor higher viscosity than the control at orally relevant shear rate (*i.e.* 50 s^{-1}) to see some effects of viscosity on satiety. Therefore, objectively characterizing the pre-loads in the study by both instrumental and sensory terms is important to have a significant effect of texture on satiety.

Furthermore, having a control condition, such as water or placebo condition, will make sure that the effects seen are due to the intervention (preload) and not to some other factors. Also, time to the next meal is crucial. Studies with a low energy density intervention should reduce the time between intervention and the next meal. Also, double-blind study designs should be considered to reduce the biases. Finally, intervention studies with repeated exposure to novel food with higher textural characteristics and less energy density are needed to clearly understand their physiological and psychological consequences, which will eventually help to create the next-generation of satiety- and satiation-enhancing foods.

The next chapters of this thesis will focus on studying the influences of oral lubricity/coating on satiation and satiety. The instrumental fracture, lubricating and sensorial properties of different hydrocolloid gels were measured (**Chapter 3**) and a number of non-calorific preloads expressed through hydrogels with varying lubricating properties were selected for further study. Then, the effect of the selected non-calorific preloads/hydrogels varying in lubricating properties on appetite ratings, food intake, lubricity of saliva and salivary biomarkers was investigated (**Chapter 4**). Due to pandemic restrictions, a video online questionnaire has been developed to assess the perceived/expected satiety of different levels of viscosity and its feasibility was examined (**Chapter 5**). And then finally, the effects of calorific preloads expressed through protein beverages differing in their lubricating and coating properties on the appetite ratings, food intake, lubricating and viscosity properties of saliva, salivary and blood biomarkers were examined (**Chapter 6**).

2.8. Reference

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Chapter 3

Tribology and rheology of bead-layered hydrogels: influence of bead size on sensory perception[2]

Abstract

The aim of this study was to understand the influence of the size of soft beads embedded in layered hydrogels on mechanical performance as well as sensory discrimination and perception. Layered hydrogels were designed using a monolayer of calcium alginate (CaA) beads of small, medium and large size (diameter of 805, 1413 or 1725 μm, respectively) sandwiched in between layers of *kappa*-carrageenan (*κ*C) gel matrix, with controls created using pure *κ*C hydrogels and *κ*C+sodium alginate (NaA) mixed gels. Large deformation rheology of the hydrogels followed by apparent viscosity as well as tribological properties of the hydrogel boli (after homogenising with simulated saliva) were analysed. Sensory discrimination tests (n=113) and intensity ratings (n=60) were conducted with untrained panellists. Bead size did not have an influence on the rheological properties of the layered hydrogels and hydrogel boli, respectively ($p > 0.05$). However, the lubrication behaviour of the layered hydrogel boli was influenced by bead size, with gels containing large-sized beads showing highest lubrication in both boundary and mixed regimes ($p < 0.05$). Although panellists were able to discriminate non-layered gels from bead-layered counterparts based on textural attributes, such as "hard", "chewy" and "pasty", they could not distinguish between small and large-sized bead-layered gels in contrast to the oral tribology results. The low

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modulus of the beads appeared to be the limiting factor to detect differences based on bead sizes in this study. Findings on instrumental characterization and consumer perception of bead-layered hydrogels can have important implications for development of novel food texture.

3.1. Introduction

Foods in general are heterogeneous composite structures with particles of various sizes, shapes and viscoelastic moduli embedded in complex polysaccharide and protein networks. Examples of such composite foods may range from the conventional use of freeze-dried fruit pieces in porridge and yoghurt, and starch granules in custards to the more recent usage of flavoured gelatine pearls in confectionery, pieces of cookies in ice creams and seeds/nuts inclusion in cheese, *etc*. Indeed such interesting inclusions of particles are increasingly enabling novel texture creations and triggering hedonic escalation of these palatable foods. In addition to creating new hedonic textural experiences, there is an increasing body of evidence showing texturally complex foods containing inclusions can influence oral processing behaviour in human subjects (Krop, Hetherington, Miquel, & Sarkar, 2019c; Santagiuliana, Christaki, Piqueras-Fiszman, Scholten, & Stieger, 2018a; Santagiuliana, Piqueras-Fiszman, van der Linden, Stieger, & Scholten, 2018b). These complex foods containing particle inclusions have shown the ability to reduce food intake and so offer a contributory element toward the global obesity challenge (Krop, Hetherington, Miquel, & Sarkar, 2019b; Krop, et al., 2018; Larsen, Tang, Ferguson, & James, 2016; Tang, Larsen, Ferguson, & James, 2016). In addition, manipulating texture by incorporation of soft gel particles in model foods such as hydrogels has been shown to increase the oral residence time during oral processing experiments in elderly population cohorts, without necessarily altering the large deformation properties of the gels (Laguna, Hetherington, Chen, Artigas, & Sarkar, 2016a; Sarkar, 2019). Thus, research into model and real foods with textural complexity particularly with embedded inclusions will

increase the understanding of sensory and functional relationships during oral processing and contribute toward the two key global challenges of obesity and healthy ageing.

Conventionally, hard particles in gel networks have been used to study the effect on oral sensation, where even 10 µm-sized alumina particles have been shown to cause sensory 'grittiness' (Utz, 1986). Interestingly, in another study 230 µm-sized spherical deformable polystyrene particles were perceived to be smaller than that 80 µm-sized irregular, hard silica particles (Engelen, van der Bilt, Schipper, & Bosman, 2005). Both modulus and shape of the particles are highly relevant in textural perception, such that sharp-faceted hard particles tend to have a lower threshold to be perceived easily than the relatively soft spherical particles (Tyle, 1993). Such findings have later been shown to be related to friction, with spherical particles having significantly lower friction than particles with sharp facets (de Wijk & Prinz, 2005; Liu, Tian, Stieger, van der Linden, & van de Velde, 2016; Sarkar, Kanti, Gulotta, Murray, & Zhang, 2017). In addition, the matrix in which the particles are dispersed can also play a key role in oral sensation, for example, depending on the variety of cheese in which cellulose particles were embedded, the threshold size of microcrystalline cellulose ranged from 52 to 86 µm (Santagiuliana, et al., 2019).

Recently, the focal point in studying textural complexity has shifted from studying the inclusion of hard particles to soft polymeric gel particles. For instance, Laguna and Sarkar (2016b) demonstrated that the inclusion of soft calcium alginate beads can generate structural defects in carrageenan-based hydrogels affecting oral processing behaviours, such as the number of chews and swallowing time by using a range of large deformation rheological characterizations and sensory analyses using a trained panel. Krop, Hetherington, Holmes, Miquel, and Sarkar (2019a) further highlighted that the presence of 1000 µm-sized calcium alginate gel beads can not only affect the bulk rheological properties, but also influence the tribological properties of the hydrogel boli, with the latter affecting lubrication-related sensory

attributes such as “pasty”, “smooth” and “melting” as compared to the non-beaded mixed sodium alginate-carrageenan gels. In another recent work, Santagiuliana, et al. (2018a) studied the impact of the particle size of *κ*-carrageenan gel beads varying in size (0.8-4.2 mm) on the large deformation properties and sensory perception of proteins gels and soups, however tribological properties were not investigated in this study. Noteworthy, in these studies by Krop, et al. (2019a) and Santagiuliana, et al. (2018a) the beads were uniformly dispersed throughout the hydrogel matrix and it was not possible to identify whether the beads had been displaced from the hydrogels matrix during oral processing or if they were still associated even if they were ‘inactive fillers”.

Hence, it is important to understand how the instrumental and sensorial response of these hydrogels with polymeric gel particle inclusions might alter if the gel particles were present as “layers” rather than being incorporated homogeneously in the matrix. Although effect of bi-layer food gels has been investigated by Santagiuliana, et al. (2018b) and Devezeaux de Lavergne, Van de Velde, van Boekel, & Steiger (2015) on the textural perception and sensory profile, to our knowledge, layered hydrogels incorporating soft polymer beads has not been reported in literature and a fundamental understanding of the rheological, tribological and sensory profiles of such complex hydrogels and the effect of size of the embedded soft beads remains to be elucidated.

Soft tribology analyses determining the lubrication and friction of oral surfaces in relative motion using polymeric substrates is progressively becoming a useful mechanical technique to understand the physical mechanism behind perceived texture (Pradal & Stokes, 2016; Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019a; Stokes, Boehm, & Baier, 2013). The use of model saliva (also referred to as artificial saliva) in previous literature by Sarkar, Goh, and Singh (2009) has been observed to further strengthen the correlation with sensory attributes, however only few studies have used this to simulate realistic oral processing

conditions (Sarkar & Krop, 2019b). Hence, it is crucial to combine traditional rheological measurements with tribological analyses to comprehend the mechanical phenomena behind sensory perception.

Therefore, the aim of this study was to investigate textural complexity in terms of the size of soft polymeric beads in layered hydrogels using instrumental characterization with and without simulated oral processing and identify whether such textural properties attributed to different bead sizes can then be sensorially discriminated and perceived by consumers (untrained panellists). We hypothesize that consumers will be able to discriminate layered from non-layered hydrogels and also distinguish samples based on bead size, which can be explained by the intensity ratings of the textural attributes and the tribological behaviour of the samples.

3.2. Materials and methods

3.2.1. Materials

Food grade kappa-carrageenan (*κ*C), sodium alginate (NaA) and watermelon flavouring were purchased from Special Ingredients Ltd (Chesterfield, UK). Watermelon food colouring was purchased from AmeriColor Corp. (Placentia, California USA). Stevia granulated sweetener was purchased from a local supermarket (Leeds, UK). Food grade potassium chloride (KCl) was purchased from Minerals Water Ltd. (Purfleet, UK) and calcium chloride dehydrate ($CaCl_2.2H_2O$) was obtained from VWR Chemicals (Leuven, Belgium). All chemicals used to prepare the model saliva: sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), potassium citrate ($K_3C_6H_5O_7.H_2O$), uric acid sodium salt, urea ($C_5H_3N_4O_3Na$), lactic acid sodium salt ($C_3H_5O_3Na$) and porcine gastric mucin Type II, were purchased from Sigma-Aldrich (Dorset, UK). All materials were used without further purification. Distilled water was used to prepare both the hydrogels and model saliva.

3.2.2. Preparation of hydrogels

The composition of the hydrogels used in this study is shown in **Table 3.1**. A batch of 200 mL of each of the gel samples was prepared in a bottle and transferred into a petri-dish to a height of 10 mm (150 g gel in each of the petri-dishes, diameter: 140 mm), and then kept at 4 °C overnight to set. The hydrogels were prepared in at least three replicates and cut from the petri-dish using a circular-cutter (diameter of 25 mm and 5.6 g in weight) or a heart-shaped cutter (largest dimensions of 15 mm horizontally and 14 mm vertically, respectively and 1.7 g weight). The heart shape was used for the sensory evaluations to increase sample acceptability as determined during pilot testing with these model gels.

Table 3.1. Composition of the hydrogels

Hydrogel samples[3]	*κ*C (wt%)	NaA (wt%)	CaA beads (wt%)	Water (wt%)
2*κ*C	2.00			96.50
1.67*κ*C+0.33NaA	1.67	0.33		96.50
1.67*κ*C+0.33CaA$_{Small}$	1.67		0.33	96.50
1.67*κ*C+0.33CaA$_{Medium}$	1.67		0.33	96.50
1.67*κ*C+0.33CaA$_{Large}$	1.67		0.33	96.50

[3] All hydrogels also contained 0.5 wt% watermelon flavouring, 0.5 wt% diluted colouring and 0.5 wt% sweetener. The CaA beads were prepared from a 1 wt% NaA solution and the final total hydrocolloid composition was achieved by layering of 2.5 wt% *κ*C solution and 1 wt% CaA beads (2:1).

3.2.3. Kappa-carrageenan (κC) hydrogels

Firstly, 0.02 M KCl solution was prepared and 0.5 wt% of the sweetener was added and stirred until it was completely dissolved. Appropriate quantities of *κ*C (see Table 1) were added to the sweetened KCl solution and mixed for 30 min on a magnetic stirring plate. Once the *κ*C was hydrated, the polymer solution was heated to 90 °C in a shaking water bath at 80 rpm (OLS26, Aqua Pro, Grant Instruments, Royston, UK), for at least 60 min until it was completely dissolved. Then 0.5 wt% of the watermelon flavouring and 0.5 wt% of the diluted food colouring (2 wt%) was mixed into the *κ*C gel mixture, before being allowed to set in the petri-dishes at 4 °C overnight.

3.2.4. Kappa-carrageenan + sodium alginate (κC+NaA) hydrogels

Mixed *κ*C+NaA hydrogels were prepared using appropriate quantities of *κ*C and NaA in 0.02 M KCl solution containing sweetener (as described above). The mixture was stirred for 30 min until it was fully hydrated. The polymer solution was then heated to 90 °C in a shaking water bath at 80 rpm, for at least 60 min until it was completely dissolved. The mixed gelling solution was then removed from the water bath, and 0.5 wt% the flavouring and colouring was mixed into the *κ*C+NaA solution before being allowed to set in the petri-dishes at 4 °C overnight.

3.2.5. Layered kappa-carrageenan + calcium alginate hydrogels (κC+CaA)

The *κ*C+CaA layered hydrogels consisted of CaA beads of different sizes, these beads were incorporated as a sandwiched layer between two distinct layers of *κ*C gel. The CaA beads were prepared by extruding 1 wt% NaA solution through a vibrating nozzle of either 150, 300 or 450 μm (Buchi Encapsulator B-390®, Buchi UK Ltd, Chadderton, UK) into a beaker containing

0.01 M $CaCl_2$ under constant stirring at 300 rpm to prevent aggregation of the CaA beads. A frequency of 500 Hz and electrode setting of 1500 V were used, and, depending on nozzle size, the air pressure varied from 250 to 750 mbar to achieve a constant flow of the NaA droplets. After stirring for 20 min, the CaA beads were collected by filtration (Whatman™ filter paper, Grade 1, 185 mm diameter, Buckinghamshire, UK). The beads were then washed three times using distilled water to remove any residual $CaCl_2$. The layered hydrogels were prepared by pouring 50 g of 2.5 wt% κC solution containing the sweetener, flavouring and colouring (prepared as mentioned above) into a petri-dish and leaving to set for approximately 5 min. Then, a layer of 50 g of CaA beads of processing size of 150, 300 or 450 μm was added. Finally, another layer of 50 g of κC solution was transferred onto the top of the layer of beads, sandwiching the layer of beads between the two κC layers and resulting in a total biopolymer concentration of 2 wt%. The petri-dish was left to set at 4 °C overnight.

3.2.6. Optical microscopy

The prepared CaA beads were analysed using a microscope (Nikon SMZ-2T, Japan) to determine the actual size of the beads generated by the Buchi Encapsulator B-390®. The CaA beads were carefully placed on a glass slide and 0.1 mL of distilled water was added on top of the beads to avoid dehydration and shrinkage of the beads. Using a magnification of 4×, images showing the full outline of the spherical individual beads were captured. This was repeated for approximately 75 individual beads from each nozzle size. The ImageJ software (version 1.48r, National Institute of Health, Bethesda, USA) was used to determine the diameter of the different beads and the mean bead size was calculated for the different processing nozzles.

3.2.7. Large-strain compression

Both uniaxial single compression tests and puncture tests were carried out on each of the five hydrogels using a TA-TX2 Texture Analyser (Stable Micro System Ltd., Surrey, UK) attached with a 50 kg load cell. A cylindrical 59 mm platen probe was used for the compression tests (Stable Micro Systems Ltd., Surrey, UK), which were performed at room temperature (22 °C) at a deformation level of 80 % strain and a constant speed of 1 mm s^{-1}. Gel samples were cut from each of the petri dish using the circular cutter (25 mm diameter). At least six repetitions were performed for each of the hydrogel cut-outs from at least two different preparation days. The force-distance curves obtained from each test were recorded using the Exponent software (TEE32, v6.1.9.0, Stable Micro Systems Ltd., Surrey, UK). The data from the force-distance curves were converted into stress-strain curves, and the maximum peak of the curve was used to determine the gel's fracture point.

For the puncture tests, a Volodkevitch Bite Jaw probe (Stable Micro Systems Ltd., Surrey, UK) was used, with the same parameter settings. The fracture force (N) of the hydrogels was determined directly from the peak of the obtained force-distance curves. At least six repetitions were performed for each of the hydrogels from at least two different preparation days.

3.2.8. Preparation of artificial saliva

The model saliva was prepared following the composition previously described by Sarkar, et al. (2009), except ammonium nitrate was excluded. Briefly, to prepare 1 L of model saliva, 1.59 g L^{-1} NaCl, 0.64 g L^{-1} KH_2PO_4, 0.20 g L^{-1} KCl, 0.31 g L^{-1} $K_3C_6H_5O_7.H_2O$, 0.02 g L^{-1} $C_5H_3N_4O_3Na$, 0.20 g L^{-1} H_2NCONH_2, 0.15 g L^{-1} $C_3H_5O_3Na$ and 3.00 g L^{-1} porcine gastric mucin type II were dissolved in distilled water. After adjusting the pH to 7.0 using 1 M NaOH, the volume was made up to 1 L using a volumetric flask. Porcine gastric mucin was used to

prepare the model saliva to have a comparison with previous studies (Krop, et al., 2019a; Torres, et al., 2019) and due to the ability of this mucin to simulate the rheological properties of human saliva. It is noteworthy, however, that bovine submaxillary mucin is the optimal source of commercially available mucin for lubricating properties (Sarkar, Xu, & Lee, 2019c), and therefore this is a limitation of the current study. In addition, α-amylase was not included in the model saliva formulation as starch was not used in any of the hydrogels tested and therefore, the role of α-amylase was considered to be negligible as seen in previous literature dealing with non-starch polysaccharides (Torres, et al., 2019).

3.2.9. Preparation of simulated hydrogel boli

For the viscosity and tribology measurements, hydrogel boli were generated to simulate oral processing by mixing the various hydrogels or CaA beads alone with model saliva at 4:1 w/w ratio based on previous literature (Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015). About 160 g of hydrogels (twenty-eight circular hydrogel cut outs) were mixed together in a food blender (Andrew James UK Ltd., Bowburn, UK) with 40 g of simulated saliva for 20 s at the lowest speed setting (speed 1) (Krop, et al., 2019a).

3.2.10. Apparent viscosity

The apparent viscosity of the hydrogel boli in the presence of model saliva was measured with a rheometer (Kinexus Ultra+, Malvern Instruments Ltd, Worcestershire, UK) using a plate-plate geometry (diameter 60 mm). For the 2κC boli a gap size of 1 mm was used, whereas for the other gel boli samples the gap size was 0.5 mm, adjusting for the hydrogels' bead sizes once broken down. The samples were sealed off with a thin layer of silicone oil to prevent evaporation. Flow curves were obtained for all hydrogel boli after simulated oral processing at shear rates ranging from 0.01 to 1000 s^{-1} at 37 °C. A minimum of three replicates were measured for each hydrogel sample.

3.2.11. Tribology

Soft tribology measurements of MilliQ water, 1 wt% NaA solution, CaA beads alone, model saliva, CaA beads boli (*i.e.* CaA beads + model saliva) and hydrogel boli (*i.e.* hydrogels + model saliva) were carried out using a MTM2 Mini-Traction Machine (PCS Instruments, UK). Polydimethylsiloxane (PDMS) ball (diameter of 19 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) and disc (diameter of 46 mm, thickness of 4 mm) were used for the measurements (surface roughness of PDMS tribopairs, R_a < 50 nm). Approximately 30 g of sample was loaded onto the pot equipped with the PDMS disc; the ball was lowered onto the disc and then the pot was covered with a lid. The entrainment speed was decreased from 0.3 to 0.001 m s^{-1}, and the friction coefficients were recorded at slide-roll-ratio of 50 % at 2 N load with a Hertzian contact pressure of ~200 kPa (Sarkar, et al., 2019a). The temperature was set and maintained at 37 °C, to imitate the temperature at which oral processing occurs. A minimum of three repetitions were carried out for each sample.

In order to understand the role of the bulk rheological properties on tribology, Stribeck curves were plotted showing the evolution of friction coefficient as a function of the product of the entrainment speed component (U) and the shear rate viscosity (η) at 1000 s^{-1} of the simulated hydrogel boli of 2κC, 1.67κC+0.33CaA_{Small} and 1.67κC+0.33CaA_{Large} with MilliQ water as a reference.

3.2.12. Discriminative and sensory ratings tests

Two types of sensory tests were carried out: a discriminative test and a descriptive test. The sensory trials were approved by the Faculty Research Ethics Committee of the University of Leeds (MEEC-16-046) and all participants were required to read a participant information sheet and sign a consent form before taking part in the study. At the end of the test, the participants were reimbursed for their participation.

A total of 113 untrained panellists, 42 males and 71 females (ranging 20-55 years, mean age 28.8 ± 7.8 years), participated in triangle tests involving the κC+NaA and 150, 300 and 450 µm layered κC+CaA hydrogels. In each triangle, the participant received a set of three hydrogels, two identical and one that was different. A randomisation order was generated using the CompuSense software (v5.0, Ontario, Canada). The samples were placed in small, clear plastic sampling cups, labelled with a random three-digit codes. Participants were seated in individual sensory booths, set up with red lighting to avoid panellists being able to visually distinguish between the hydrogel samples before consuming them. Participants were asked to collect the samples from the cup using a teaspoon, to chew each sample as they would normally do, going from left to right in the presented order of samples, and then to identify the anomalous sample. Answers to each triangle test were recorded on a paper questionnaire. Participants were encouraged to sip water and consume cracker (Jacob's Cream Crackers, Jacob's Bakery, Leicestershire, UK) between each triangle test, to cleanse the oral palate and ensure accurate sensory perception in each of the triangle tests.

Besides the discriminative test, 60 participants (24 males, 36 females, mean age 30.4 ± 8.0 years) took part in a rating test where they were asked to rate the mixed and bead-layered hydrogels compared to a reference sample for six different texture attributes (see **Table 3.2**) that were determined to be relevant based on a previous study using similar types of κC-based hydrogels (Krop, et al., 2019a). The attribute 'gritty' was included based on previous literature dealing with different sizes of hydrogel beads (Santagiuliana, et al., 2018a). The four samples were presented in randomised order in a balanced block design. The intensities of the attributes were rated on an unstructured line scale of 100 mm, anchored from 'not at all' (0 mm) to 'extremely' (100 mm), as compared to 2κC (the reference sample) of which the intensity scores were provided (see **Table 3.2**). The reference sample was tasted first and provided again to the participants at any time during the sensory evaluations upon request.

The scores for the reference sample were already filled out on the rating scales for all the attributes (see **Supplementary Figure B.1**). Participants were asked to place the whole sample in their mouth and chew, after which they were presented with two options – either to swallow the sample or to expectorate the samples in the provided cups if they felt inclined to do so. Between the samples, panellists were instructed to rinse their mouth with some water and to consume cracker to cleanse their palate. Data was recorded using CompuSense software (v5.0, Ontario, Canada) and exported for analysis.

Table 3.2. Texture attributes used in the sensory ratings tests with a short description. In between brackets the score for the 2κC reference sample are shown

Texture attributes	Definitions
Hard	The force needed to compress the sample between the tongue and the palate (100 mm).
Chewy	The amount of chews needed to break down the sample to be ready for swallowing (100 mm).
Smooth	Absence of abrasiveness/resistance of the products' surface as perceived by the tongue or palate (100 mm).
Slippery	The ease in which the sample slides through the mouth during chewing and slips away from the teeth (100 mm).
Pasty	The sensation of the presence of wet/soft (immiscible) solids in the mouth *i.e.* muddy (0 mm).
Gritty	The presence of small hard particles that stick to the teeth/palate *i.e.* presence of residues (0 mm).

3.2.13. Statistical analysis

Mean values and standard deviations (SD) were calculated using Microsoft® Excel (Microsoft Office Professional Plus 2016, Microsoft Corporation), and data was plotted using the software Origin® (OriginPro 2018, OriginLab Corporation, Northampton, USA). Differences between measured bead size, puncture force and compression fracture strain of hydrogels, coefficients of friction of hydrogel boli, beads boli and beads alone in the boundary and mixed lubrication regimes were determined by analysis of variance (one-way ANOVA). Least significant differences were calculated by Bonferroni's post-hoc tests. Statistical significance was set at $\alpha < 0.05$ level.

For the sensory discrimination test, the significance was determined for each triangle test separately due to the variation in total number of participants that completed each test. For the sensory intensity ratings, the panel performance was checked by evaluating the variance as well as identification of any potential outliers in the data. To check for differences in the sensory intensity ratings between hydrogels, non-parametric repeated measure Friedman tests were applied with post-hoc pairwise multiple comparison tests according to (Nemenyi, 1963). To check for correlations between sensory attributes, Pearson correlations were computed. All statistical analyses were done using SPSS (IBM® SPPS® Statistics, v25, SPSS Inc, Chicago, USA) or R version 3.5. (Team, 2018), and the significance level was set at $\alpha < 0.05$.

3.3. Results and discussion

3.3.1. Characteristics of the CaA beads

CaA beads of varying sizes were prepared using the three different vibrating nozzles with processing sizes of 150, 300 and 450 µm in order to generate textural complexity in the hydrogel matrix. High-resolution optical microscopy images confirmed that the encapsulator

was producing spherical beads, as desired (see **Figure 3.1**). Qualitatively, comparing beads shown in the optical micrographs in **Figures 3.1ai-ci**, one can easily appreciate that there is a clear difference in the diameter of the beads depending on the type of nozzle (150-450 μm diameter) used. For instance, **Figure 3.1ai** shows four entire beads, while **Figure 3.1bi** shows only one of them and in **Figure 3.1ci**, even a single bead could not be fitted within the microscopic view.

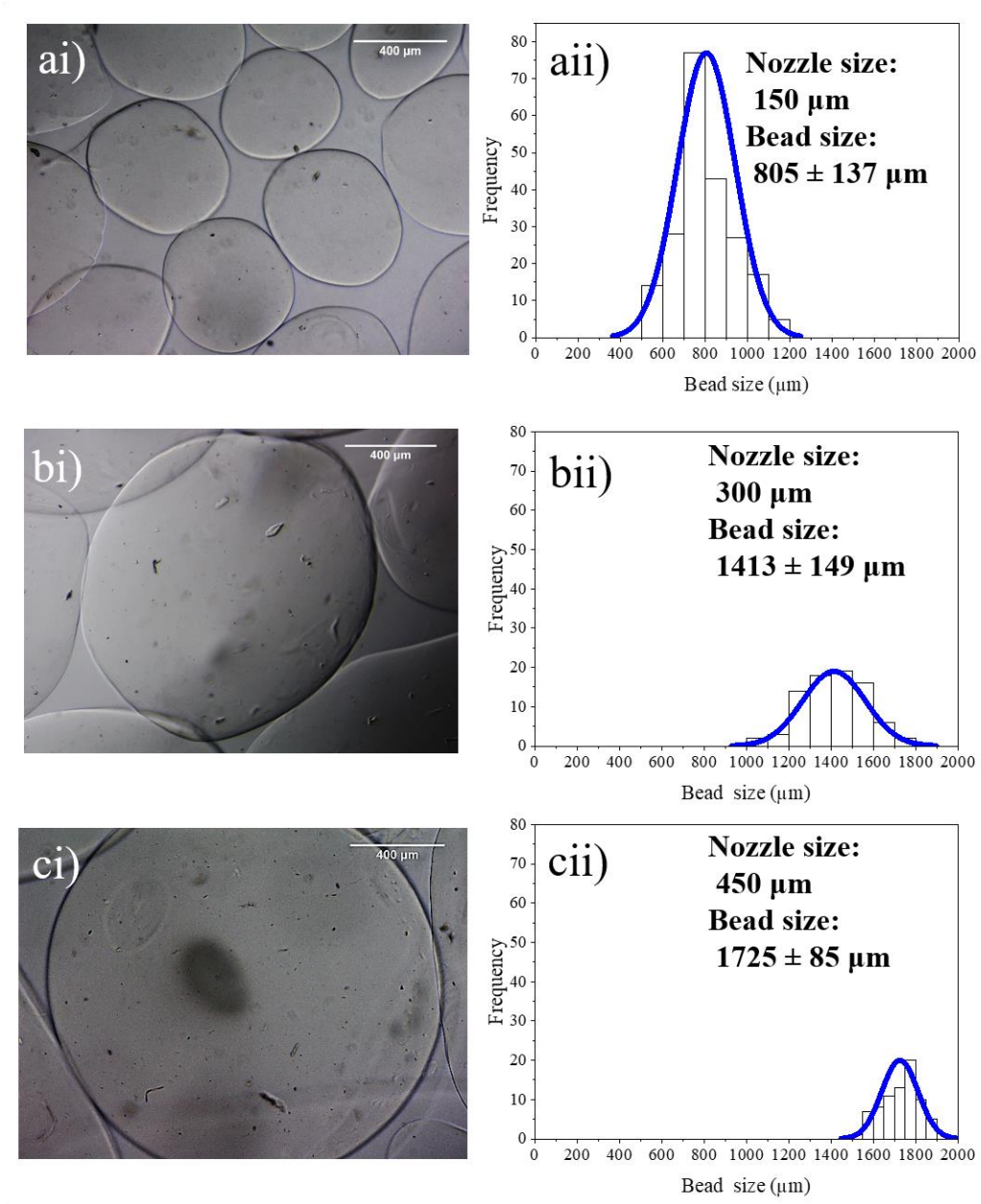

Figure 3.1. Optical micrographs (i) and histogram with log-normal fitting (blue solid line) of the bead size distribution (ii) of 1 wt% CaA beads synthesised using the Buchi Encapsulator® with 150 µm (a), 300 µm (b), and 450 µm (c) sized nozzle, producing small, medium and large beads, respectively. Based on the microscopy images of at least 75 beads, the actual mean diameter of the beads was assessed using ImageJ. Each bead size differed significantly from each other: $p < 0.05$. Scale bars represent 400 µm.

Quantitatively, the size distribution of at least 75 individual beads for the three groups of CaA beads are displayed in histograms (**Figure 3.1aii-cii**). The average diameter calculated from the images obtained through microscopy were notably different from the nozzle diameter. The 150 µm nozzle produced small beads with an average diameter of 805 µm (**Figure 3.1aii**), the

300 µm nozzle formed medium-sized beads with an average diameter of 1413 µm (**Figure 3.1bii**), and the 450 µm nozzle produced large beads with an average diameter of 1725 µm (**Figure 3.1cii**). Although distinctively different in size from each other ($p < 0.05$), the beads were considerably larger than expected. Such increase in size could be explained by the fact that the beads were rather soft with elastic modulus ranging from 0.1-1.0 kPa (Mahdi, Diryak, Kontogiorgos, Morris, & Smith, 2016) and thus, these beads can be hypothesized to become flat 'pancake'-shaped when interacting with the glass slide during microscopy analysis. One might also expect such beads to be flattened in the mouth when such beads interact with the tongue surface with modulus of the tongue/palate (2.5 kPa) and oral contact pressure (30-70 kPa) being higher than the modulus of the beads (Sarkar, et al., 2019a). Another possible reason for the increase in size is that these beads might have been swollen in the presence of buffer increasing their volume, which can also occur *in vivo* upon interaction with human saliva.

3.3.2 Tribological behaviour of the CaA beads

It is likely that during oral processing of the layered hydrogels the CaA beads might be expelled from the hydrogel matrix. Hence, the tribological properties of the CaA beads on their own were first characterised by plotting the friction coefficient values against the entrainment speeds. **Figure 3.2** shows the friction coefficient curves for each of the different CaA beads, 1 wt% NaA solution and MilliQ water and a general trend in reduction of friction coefficients in the direction of the applied entrainment speed ramp. The friction coefficient curve for the NaA solution serves as a control for the beads to understand if the friction behaviour is dominated by the bursting of the CaA beads or if the beads remain relatively intact in the contact zone where the PDMS contact pressure in the tribometer (~200 kPa) can be expected to be two orders of magnitude higher than that of the alginate gel beads (Sarkar, et al., 2019a). Irrespective of the size of the beads, all the CaA bead curves as well as NaA solution curve

demonstrated boundary and mixed lubrication regimes because the speeds were varied in the orally relevant speed range of 0.001 to 0.300 m s^{-1} (**Figure 3.2**), and therefore a hydrodynamic regime was not expected in this low speed range.

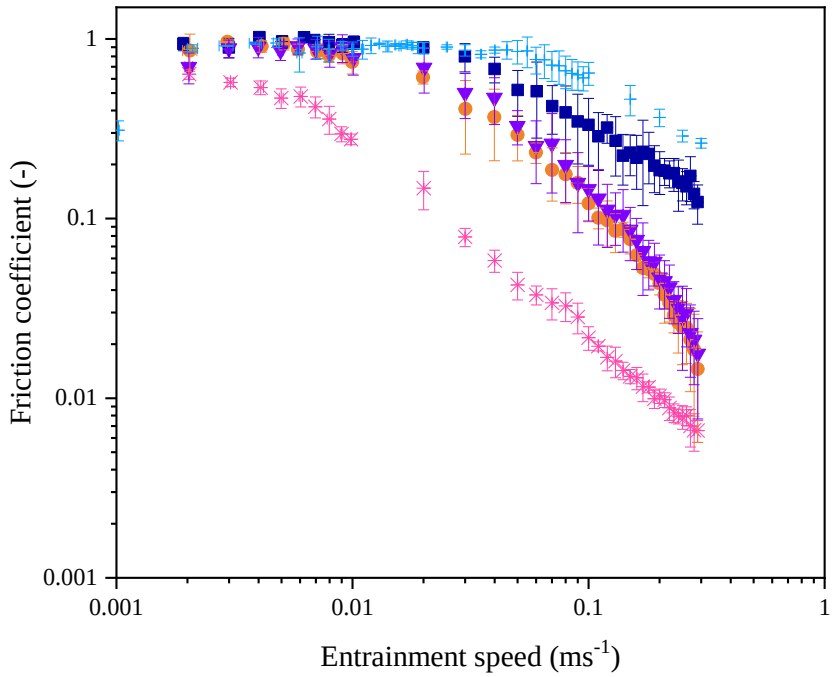

Figure 3.2. Mean friction coefficient of MilliQ water (-), 1 wt% NaA solution (×) and CaA beads - small (■), medium (●) and large (▼) as a function of entrainment speed at 37 °C, respectively. The mean was calculated based on at least three replicates. Error bars show the standard deviation.

In the boundary regime, where the entrainment speed is at its lowest (≤ 0.01 m s^{-1}), the friction coefficient values for all the CaA beads of three different sizes appeared to be very similar ($p > 0.05$) (see **Supplementary Table B.1a** for statistics). The micron-sized CaA beads were too large in size to enter the contact region and reduce the friction coefficients between the PDMS ball and disc, where the contact radius is generally expected to allow only a few molecules to nanometer thick layers of lubricating materials. This is further evidenced by the CaA beads having a similar boundary friction profile to MilliQ water (**Figure 3.2**), and thus the beads were possibly excluded from the hydrophobic PDMS-PDMS contact region until the curves shifted from boundary to a mixed lubrication regimes allowing the CaA beads to be

entrained. Such behaviour has also been seen previously in starch-based hydrophilic microgel particles, where microgels of ≥ 30 μm were unable to enter the contact zone in the boundary regime (Torres, Tena, Murray, & Sarkar, 2017) or in case of agarose fluid gels where particles were not entrained in PDMS-PDMS contact unless the critical sliding speed was reached (Gabriele, Spyropoulos, & Norton, 2010). It can be noted that the friction coefficients of the NaA solution were significantly lower than those of CaA beads irrespective of the entrainment speeds (**Supplementary Table B.1a**). This suggests that although hydrophilic NaA might not be expected to be adsorbed to hydrophobic PDMS surface, NaA with a radius of gyration of ~50 nm may be able to enter the contact and provide some boundary lubrication properties (Strand, Bøe, Dalberg, Sikkeland, & Smidsrød, 1982).

The friction coefficient of the CaA beads and NaA solution decreased with increasing entrainment speed at > 0.01 m s^{-1} to 0.3 m s^{-1} (**Figure 3.2**), indicating that all these samples at these conditions were in the mixed lubrication regime of the friction curves. As can be expected from the difference in the nanometric sized layers of NaA solution and the hundreds of micron-sized CaA beads, NaA solution expectedly accelerated the onset of the mixed lubrication regime (< 0.01 m s^{-1}) as compared to that of the bead dispersions. In the mixed regime, particularly ≥ 0.1 m s^{-1}, the CaA beads (medium and large) showed significantly lower friction coefficients as compared to that of the small CaA beads ($p < 0.05$) (**Supplementary Table B.1a**). The trend of friction coefficient values with increasing bead sizes was small > medium ≈ large, *i.e.* higher lubricating properties correlated with larger bead sizes.

It is worth noting that the Young's modulus of gel beads may scale inversely with the particle volume *i.e.* cubed function of the particle diameter (Hashmi & Dufresne, 2009). The CaA beads have a very low modulus (≤ 1.0 kPa) (Mahdi, et al., 2016), and consequently, the large-sized CaA beads can be anticipated to have an order of magnitude lower modulus as

compared to that of the small-sized beads $\left(\left(\text{Diameter}_{\text{CaA}_{\text{small}}}\right)^3 \Big/ \left(\text{Diameter}_{\text{CaA}_{\text{large}}}\right)^3\right) \approx 0.1$ (see **Figure 3.1** for mean bead size diameter). It is thus possible that the medium and large beads were able to entrain, easily deform in shape and flatten to fit in the contact zone between the ball and disc and were capable of efficiently separating the tribo-surfaces, as has been observed in a previous study using micron-sized starch-based microgels (Torres, et al., 2017). Another possibility is that due to their extremely low moduli, the large and medium-sized CaA beads were compressed to the extent that they released the alginate solution consequently reducing the friction. However, the NaA solution and CaA beads had significant differences in their friction coefficient values at 0.05 m s^{-1} entrainment speeds ($p < 0.05$), which indicates that the beads were not completely destroyed during the tribological shear. It is only when the speed was increased to 0.1 m s^{-1}, no further statistical difference between NaA solution and CaA beads could be noted ($p > 0.05$) (**Supplementary Table B.1a**). Therefore, at such high entrainment speeds, the possibility of the beads bursting and leaching out of the alginate solution from the beads cannot be completely ignored. Another possibility is that the gap between the contact surfaces was sufficiently high in such high entrainment speeds to allow all beads into the gap without compression, reducing their overall influence on the friction behaviour compared to the NaA solution.

3.3.3 Mechanical characteristics of the hydrogels and the simulated boli

The hydrogels were characterized independently using large deformation rheological tests (puncture and compression test) to mimic the first bite and chewing aspects, while the simulated hydrogel boli were analysed using apparent viscosity measurements and soft tribology analyses to emulate the later part of oral processing (Chen & Stokes, 2012; Krop, et al., 2019a; Sarkar, et al., 2019b; Stokes, et al., 2013).

3.3.4. Textural properties of the hydrogels

The average fracture force and fracture stress of the hydrogels prepared with varying levels of textural complexity (1.67κC+0.33NaA and the three layered 1.67κC+0.33CaA beaded gels) were obtained by puncture tests and compression tests, respectively, and compared to 2κC. It is noteworthy that all hydrogels were composed of the same total biopolymer concentration for comparison purposes (**Table 3.1**). The puncture test was performed with a Volodkevitch tooth probe to mimic the first bite-related oral processing properties. **Figure 3.3a** shows a clear difference between the average fracture force for the homogeneous 2κC gel and the four heterogeneous hydrogels that have either NaA or CAA beads of various sizes incorporated in a layered structure. The 2κC gel has a considerably higher mean puncture force of 4.56 N (± 0.28), compared to the remaining four heterogeneous hydrogels in this study ($p < 0.05$). The κC+NaA hydrogel demonstrates the lowest puncture force of 1.29 N (± 0.10), whilst all the three layered κC+CaA beaded hydrogels with small, medium and large beads have similar average puncture forces of ~ 1.4 N, respectively ($p > 0.05$). It is noteworthy that the presence of CaA beads appears to have no impact on the puncture force of the heterogeneous hydrogels as compared to that of the NaA-containing κC hydrogel, which lacks beads ($p > 0.01$).

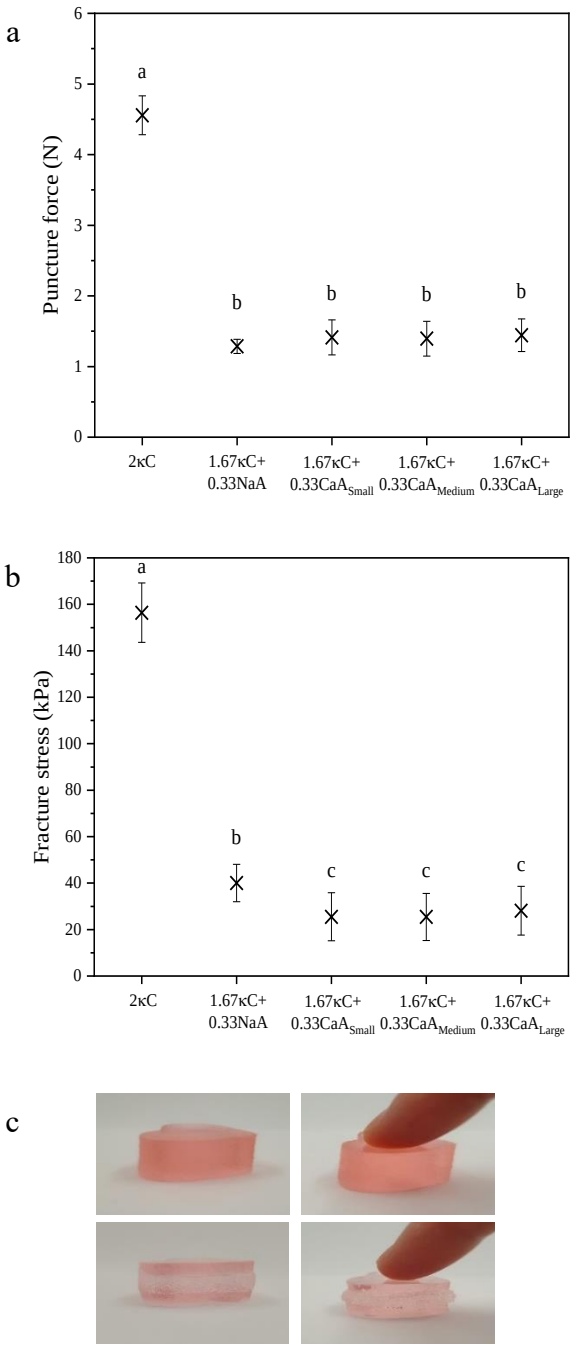

Figure 3.3. Mean puncture force (a) and fracture stress (b) obtained using uniaxial puncture and compression tests, respectively, of hydrogels 2κC, 1.67κC+0.33NaA and layered hydrogels 1.67κC+0.33CaA with small, medium and large bead size; and visual images (c) of 1.67*κ*C+0.33NaA (top: without and with pressing with a finger), and layered 1.67*κ*C+0.33CaA hydrogel (bottom: without and with pressing with a finger). Mean was calculated based on at least four measurements performed on three different days. Error bars indicate the standard deviation.

The compression test, however, in which the sample cut-out is uniaxially compressed with a probe that fully covers the sample, showed a difference between the heterogeneous non-layered *κ*C+NaA and *κ*C+CaA bead layered hydrogels (see **Figure 3.3b**). Noteworthy, the embedding

of soft beads to reduce the fracture properties of a semi-solid gel matrix has been also reported elsewhere (Santagiuliana, et al., 2018a). Similar to the results of the puncture test, the fracture stress of 2κC was highest (156.39 kPa ± 12.80) whilst the κC+NaA and three κC+CaA bead-layered hydrogels had nearly five-times lower average fracture stress ranging from 25-40 kPa, respectively ($p < 0.05$). The puncture force and fracture stress of the 2κC hydrogels were in line with previous studies (Krop, et al., 2019a; Krop, et al., 2019c; Laguna, et al., 2016b). Interestingly, unlike the puncture test, the compression test was able to distinguish between the CaA-bead layered hydrogels and the non-beaded mixed gels (κC+NaA) with the addition of the CaA beads resulting in a significantly lower fracture stress as compared to that of the κC+NaA mixed gel ($p < 0.05$). Although one might expect that the fracture stress of the layered and non-layered gels to be similar based on puncture tests results (**Figure 3.3a**), the fracture stress was significantly lower in the CaA beaded-samples (**Figure 3.3b**), most likely due to the expulsion of the beads during the compression (see **Figure 3.3c**). This is in stark contrast to the behaviour observed by Krop, et al. (2019a), where κC hydrogels containing CaA beads had a higher fracture stress as compared to the κC+NaA gel of similar total hydrocolloid concentration. This discrepancy might be attributed to the layered structure used in the current gel preparations as opposed to the procedure of dispersing the gel beads on top of κC gels used in the previous study.

In summary, addition of NaA or CaA beads to a κC matrix produces softer hydrogels compared to that of homogeneous κC hydrogel, as shown by their lower puncture forces and fracture stresses. These observations are in line with previous findings by Laguna, et al. (2016b), due to segregating interactions between NaA/CaA beads and κC. In the case of κC+NaA hydrogels, addition of NaA, a linear anionic polysaccharide (Braccini & Perez, 2001), interferes with the crosslinking of the κC helices and weakens the overall structure. In the case of the κC+CaA beaded hydrogels, the beads most likely acted as “inactive fillers” or in other

words “holes” that were not efficiently bound to the κC matrix (Laguna, et al., 2016b), thus reducing both the fracture stress and puncture force in the resultant κC+CaA hydrogels (**Figure 3.3a-b**). Such segregating interactions consequently resulted in local phase separation between the beads and κC layers, as opposed to a single continuous κC gel phase. Instead of the beads binding to the κC hydrogel and improving its resistance to deformation, the lack of interactions between the CaA beads and κC led to the interruption of the κC gel structure and a decrease in its ability to resist the deformation (Ching, Bansal, & Bhandari, 2016). It is worth noting that the texture analyses alone were not able to distinguish the layered hydrogels containing CaA beads of small, medium and large sizes from each other ($p > 0.05$) (**Figure 3.3a-b**). Due to this absence of differences seen in the puncture force and compression fracture stress of the hydrogels with different bead sizes, it appears that the CaA beads acted as inactive fillers within the κC gel matrix, independent of the bead size, as can be observed in the visual images in Figure 3c, which is expected to be due to the limited interaction between the beads and the κC matrix (Liu, Chan, & Li, 2015).

3.3.5. Frictional behaviour of the simulated boli

Friction coefficients of the hydrogel boli and CaA bead boli (without being incorporated into the κC matrix) of different sizes are shown in **Figures 3.4a** and **3.4b**, respectively. Model saliva acted as the control (**Figure 3.4a-b**), generating the highest friction coefficients as compared to all other bolus samples in the mixed regime ($p < 0.05$) (see **Supplementary Table B.1** for statistics). Whilst the 2κC, 1.67κC+0.33NaA and 1.67κC+0.33CaA hydrogels with small and medium beads produced very similar mean friction coefficients in the boundary regime ($p > 0.05$, **Supplementary Table B.1b**), the κC+CaA bolus with large beads yielded the lowest friction coefficients in both boundary as well as mixed lubrication regime (at 0.1 m s^{-1} speed) (**Figure 3.2a**, see **Supplementary Table B.1b-c** for statistics). It is worth noting that in

contrast to all other friction curves showing both boundary and mixed lubrication regimes, $1.67\kappa C+0.33CaA_{Large}$ hydrogel boli showed only mixed lubrication regime (**Figure 3.4a**). No sign of a boundary lubrication regime was observed for this sample even at very low entrainment speeds (< 0.005 m s^{-1}) highlighting that this sample could somehow create sufficient surface separation even at such low speeds by the hydrodynamic pressure of the bolus beads (**Figure 3.4a**).

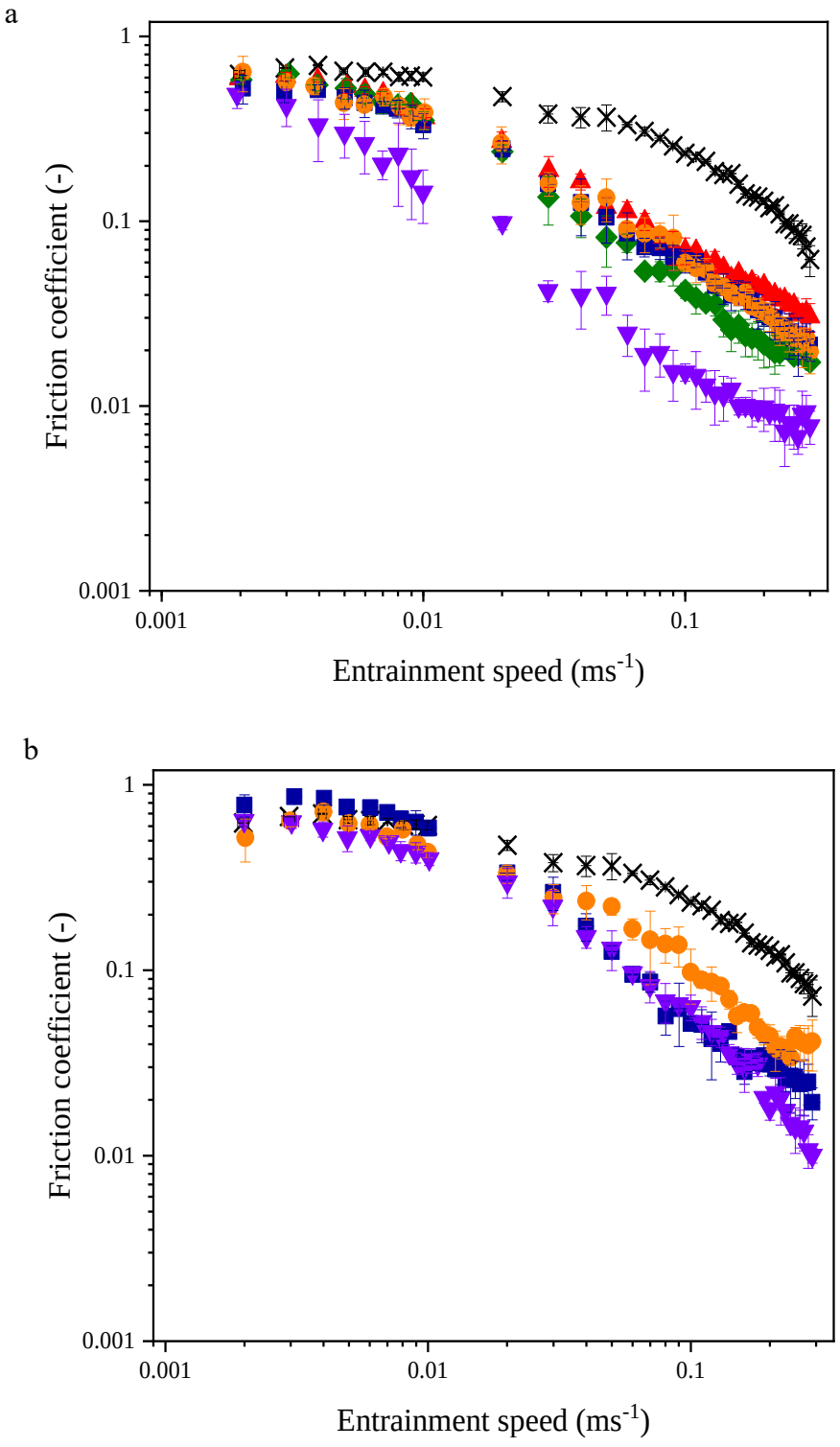

Figure 3.4. Mean friction coefficient of the simulated hydrogel boli (a) and simulated beads boli (b) with 2κC (▲), 1.67κC+0.33NaA (♦) and 1.67κC+0.33CaA with small (■), medium (●), and large beads (▼) as a function of entrainment at 37 °C, respectively. The mean was calculated based on at least three replicates. The curve for model saliva (x) was added to both graphs for reference. Error bars show the standard deviation.

In **Figure 3.4b**, in the boundary regime, the friction coefficients for medium and large-sized CaA beads appeared to be comparable to that of the model saliva ($p > 0.05$). Interestingly, the friction coefficient of small-sized CaA beads was higher than that of the medium- and large-sized beads ($p < 0.05$) (**Supplementary Table B.1c**). This behaviour of high friction coefficient values can be expected based on Figure 2, which suggests that the CaA beads were not capable of providing boundary lubrication as they were excluded from the contact region either as beads or as bead boli (**Figure 3.4b**). In the mixed regime, however, the variation between the CaA bead boli and model saliva was more apparent ($p < 0.05$). Interestingly, the boli of the CaA beads did not show any significant difference in their lubrication properties based on size ($p > 0.05$). Compared to the beads on their own without model saliva (**Figure 3.2**), the incorporation of model saliva to form the simulated bolus appeared to be detrimental to the lubricating properties of all the CaA beads leading to higher friction coefficients between the PDMS ball and disc in the mixed regimes in the latter (**Figure 3.4b**). One might argue that the beads might have been destroyed subjected to the tribological shear. As can be seen from the optical microscopy images of the bead boli (see **Supplementary Figures B.2a and B.2b** for CaA_{Small} bead boli and CaA_{Large} bead boli, respectively), the CaA beads were resilient to dissolution in the model saliva, beads were deformed but still clearly discernible in the samples even after subjecting them to the tribological shear.

3.3.6. Apparent viscosity and Stribeck curve of the hydrogel boli

Figure 3.5a shows the apparent viscosity of the hydrogel boli beads after simulated oral processing in the presence of model saliva. It can be seen that the viscosity of all the boli decreased upon increasing the shear rates, which is a recognized signature of a shear thinning behaviour. Similar values were also found for the hydrogel boli in our previous study (Krop, et al., 2019a). Interestingly, similar to the fracture stress behaviour of the hydrogels (**Figure 3.3b**), the apparent viscosity was similar for the beaded hydrogel boli at orally relevant shear

rates of 50 s^{-1} ($p > 0.05$) but the values for the layered hydrogel boli were significantly smaller than the apparent viscosities of non-layered mixed NaA-based hydrogel boli (see **Supplementary Table B.1d** for statistics).

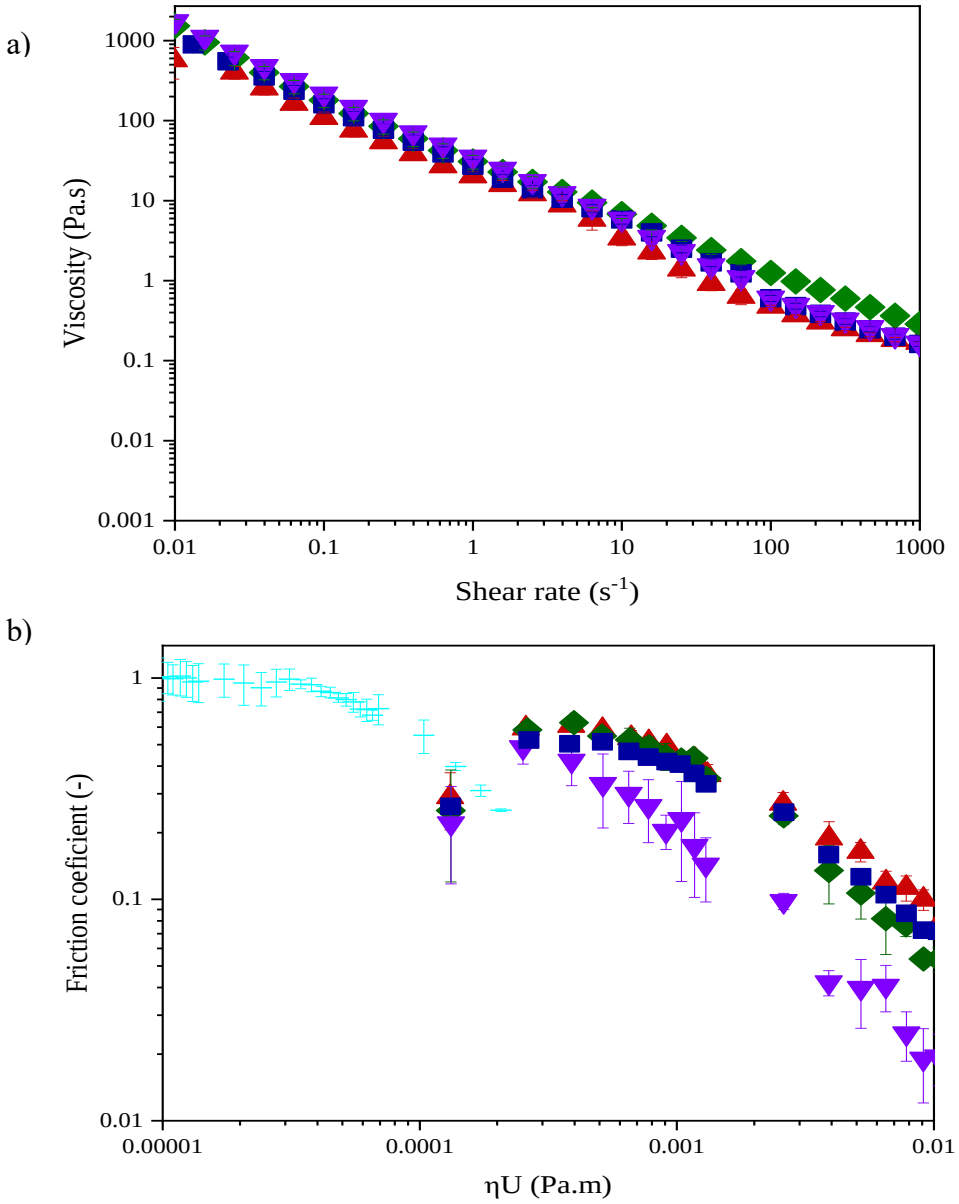

Figure 3.5. Viscosity (a) as a function of shear rate at 37 °C and Stribeck master curve (b) as a function of the product of the entrainment speed component (U) and viscosity (η) of the simulated hydrogel boli of 2*κ*C (▲), 1.67*κ*C+0.33NaA (♦) and 1.67*κ*C+0.33CaA with small (■) and large beads (▼), respectively. The curve for MilliQ water (-) was added for reference in (b). The mean was calculated based on at least three replicates. Error bars show the standard deviation.

In order to understand the role of the bulk rheological properties on tribology, we calculated the Sommerfeld number to correct for differences in the viscosity of the boli samples. **Figure 3.5b** shows the friction coefficient as function of the product of viscosity (η) and entrainment speed component (U) of the simulated hydrogel boli of 2κC, 1.67κC+0.33NaA, 1.67κC+0.33CaA$_{Small}$ and 1.67κC+0.33CaA$_{Large}$. The highest shear rate explored in the rheological measurements in this study is 1000 s^{-1} (**Figure 3.5a**). Unfortunately, current procedures available to measure higher shear rate viscosities rely on using narrow gaps (~50 μm) (Davies & Stokes, 2008), which is not suitable for this study owing to the large size of the beads (above 100 μm) contained in the boli. Hence, to plot the Stribeck curves, the high shear rate viscosity value of the 2κC hydrogel boli was used, where the start of the high shear rate plateau was evident (~0.17 Pa.s, **Figure 3.5a**).

As it can be seen from Figure 5b, to arrive at similar friction coefficients of μ ~ 0.5, the ηU component dominating the tribological performance of the boli samples had to be at least one order of magnitude higher in comparison to that of MilliQ water. Although, the second Newtonian plateau viscosity of dispersions of small particles like microgels with hydrodynamic radius around 100 nm (Andablo-Reyes, et al., 2019), has been found to dominate the lubrication performance, the large particle size of the beads studied here (in comparison to tribological gaps in the order of hundreds of nanometers to few microns) limits the material entering the gap until the speed is increased. It is also worth noting that 2κC, 1.67κC+0.33NaA and 1.67κC+0.33CaA$_{Small}$ gel boli showed overlapping trends in the Stribeck analysis (**Figure 3.5b**) confirming the role of viscosity in the lubrication phenomena. On the other hand, 1.67κC+0.33CaA$_{Large}$ gel boli continued to demonstrate significantly lower friction coefficient in the 0.001-0.01 Pa m regime as compared to the non-layered and 1.67κC+0.33CaA$_{Small}$ boli (**Figure 3.5b**), indicating that such difference might be perceived in *in vivo* oral conditions.

3.3.7. Sensory discrimination and perception of texturally complex hydrogels

The results from each of the triangle tests carried out are shown in **Table 3.3**, indicating the number of panellists that successfully identified the anomalous gel sample in a set of three (where two were the same and one was different). For a total number of 53 panellists, a minimum of 30 correct answers are needed to establish a significance of $p < 0.001$ (Meilgaard, Civille, & Carr, 2006). When panellists were asked to distinguish between κC+NaA and κC+CaA hydrogels, layered with small, medium or large beads, a high number of correct answers can be observed. In other words, participants were able to correctly identify the different sample at a significant level of $p < 0.001$. However, this was not the case with respect to the two layered hydrogel samples *i.e.* layered 1.67κC+0.33CaA$_{Small}$ versus 1.67κC+0.33CaA$_{Large}$. It was evidently more difficult to distinguish between these two samples with beads, where only 21 participants could identify the odd sample (**Table 3.3**). Thus, it can be statistically inferred that participants were not able to distinguish between hydrogels layered with small compared to those with large CaA beads, the former being half the size as compared to the large-sized beads (**Figure 3.1ai-ci**).

Table 3.3. Number of correct responses for sensory discrimination test for hydrogels

Hydrogels	Total number of responses	Number of correct responses	Significant difference
1.67κC+0.33NaA vs 1.67κC+0.33CaA$_{Small}$	53	51	$p < 0.001$
1.67κC+0.33NaA vs 1.67κC+0.33CaA$_{Medium}$	53	46	$p < 0.001$
1.67κC+0.33NaA vs 1.67κC+0.33CaA$_{Large}$	52	45	$p < 0.001$
1.67κC+0.33CaA$_{Small}$ vs 1.67κC+0.33CaA$_{Large}$	60	21	$p > 0.05$

It is now well recognized that the sensory detection of particles depends not only on the size of the particles but also on the particle type, shape, concentration, matrix properties *etc.* (Engelen, et al., 2005; Imai, Hatae, & Shimada, 1995). As discussed previously, the modulus of the beads (≤ 1.0 kPa) (Mahdi, et al., 2016) embedded within the κC matrix appeared to be the governing factor: the modulus was extremely low to detect differences between the bead-layered hydrogels based on bead sizes. Also, depending upon the size of the beads, the number of beads within the layered hydrogels would differ. For instance, the number of beads in the 1.67κC+0.33CaA$_{Large}$ layered hydrogels was calculated to be 208 (see diameter in **Figure 3.1cii**) in the 0.56 g bead layer of a single hydrogel cut-out under the assumption thet the density of alginate is 1 g cm^{-3}. This is approximately one order of magnitude lower than the number of small in the 1.67κC+0.33CaA$_{Small}$ hydrogel cut-out (2050 beads, see diameter in diameter **Figure 3.1aii**). Hence, it is also possible that this number of soft beads was not sufficient to identify the difference between these bead-layered hydrogels.

Figure 3.6 shows the intensity ratings (see **Supplementary Figure B.3** for gel-wise sensory attributes) and **Table 3.4** shows the Pearson's correlation in order to check for inter-relationships between the sensory textural attributes. The comparison of the samples with respect to each attribute is described as follows:

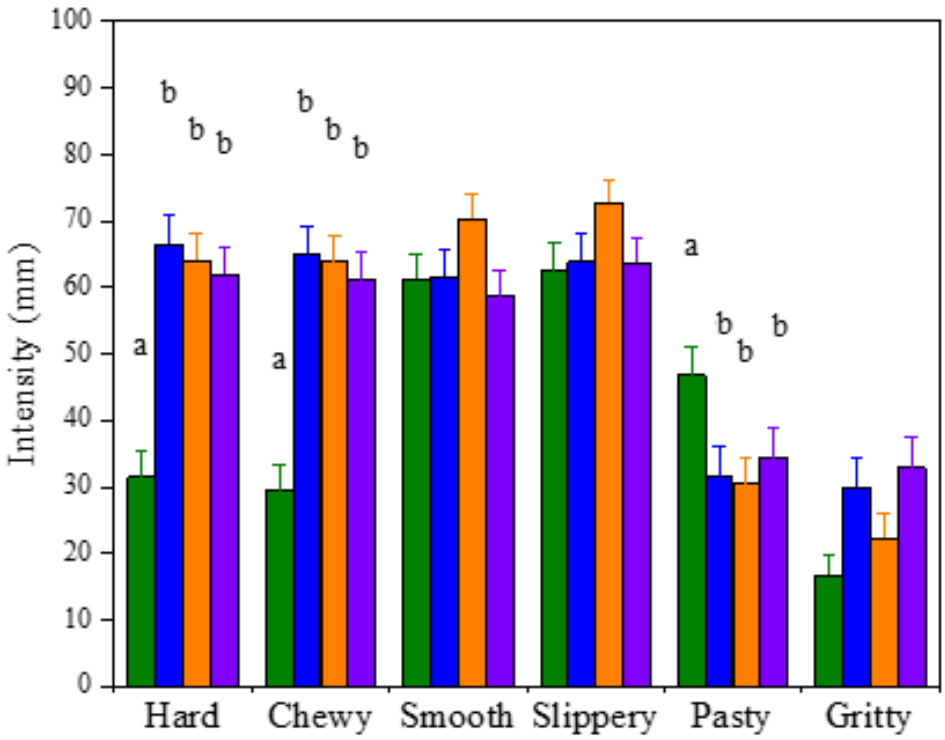

Figure 3.6. The intensity ratings of the sensory attributes for 1.67κC+0.33NaA (■), 1.67κC+0.33CaA$_{Small}$ (■), 1.67κC+0.33CaA$_{Medium}$ (■) and 1.67κC+0.33CaA$_{Large}$ (■) with respect to the reference sample (2κC). Data points represent the mean intensity ratings of untrained panellists (n=60). Error bars indicate the standard error of mean and different lowercase letters represent a statistically significant difference ($p < 0.05$).

Table 3.4. Pearson's correlations between sensory attributes of the hydrogels. Green colour indicates positive and red colour a negative correlation with *p < 0.05* in light colours and *p < 0.01* in the darker shade

	Hard	Chewy	Smooth	Slippery	Pasty	Gritty
Hard	1	.854**	.420**	.381**	-.268**	0.032
Chewy	0.854**	1	.412**	.432**	-.210**	0.051
Smooth	0.420**	0.412**	1	.719**	-.155*	-.238**
Slippery	0.381**	0.432**	0.719**	1	-0.065	-.197**
Pasty	-0.268**	-0.210**	-0.155*	-0.065	1	.487**
Gritty	0.032	0.051	-0.238**	-0.197**	0.487**	1

Hard. As can be seen in Figure 6, the mixed non-layered 1.67*κ*C+0.33NaA was rated to be less hard than the layered hydrogels (1.67*κ*C+0.33CaA - small, medium and large) ($p < 0.05$). However, the bead-layered samples of different sizes were perceived by the untrained panellists as having the same level of hardness ($p > 0.05$).

Chewy. With regards to chewiness, similar results to the hardness can be noted, which is corroborated by a high correlation between the attributes "chewy" and "hard" attributes (**Table 3.4**). The *κ*C+NaA hydrogel was perceived significantly less chewy than the layered samples ($p < 0.05$) (**Figure 3.6**).

Consistent with a study by Larsen, Tang, Ferguson, Morgenstern, and James (2016), where the more complex gels (heterogeneous) were perceived as harder and chewier, the layered gels (with higher degree of complexity) were rated with a high score on hardness and chewiness. This might be attributed to the fact that participants were most likely rating the top and bottom layers of the *κ*C+CaA gels (with small, medium and large beads size), comprising of 2.5 wt% *κ*C, which were hard and chewy in comparison to the *κ*C+NaA hydrogels.

Smooth and Slippery. Participants identified 'smooth' samples as 'slippery' and vice versa as can be inferred from the high correlation coefficients between these two attributes (**Table 3.4**). There was no significant difference between the layered and non-layered samples (**Figure 3.6**) ($p > 0.05$). It can be observed that the layered gel with CaA beads of medium size was scored slightly higher than the rest of the samples, however this was not significantly different.

Pasty. In terms of pastiness, 1.67κC+0.33NaA was perceived to be significantly pastier than the layered 1.67κC+0.33CaA$_{Medium}$ (**Figure 3.6)**. Interestingly, the attribute 'pasty' was found to be inversely correlated with hard, chewy ($p < 0.05$) and 'smooth' ($p < 0.01$) (**Table 3.4**). Consequently, the bead-layered gels were perceived to less pasty than the non-layered hydrogels ($p < 0.05$).

Gritty. It is noteworthy that 'gritty' was highly correlated with the 'pasty attribute' and thus negatively correlated with 'smooth' and 'slippery' (**Table 3.4**). With regards to grittiness, even the κC+NaA mixed hydrogel was perceived as gritty (**Supplementary Figure B.3**) with respect to the reference (2κC) and it can be noted that there was no significant difference between non-layered and bead-layered samples ($p > 0.05$) (**Figure 3.6**). Similar to a study by Santagiuliana, et al. (2018a), it can be suggested that increased particle size can cause heterogeneous sensations in the perception of food.

Overall, it can be concluded that participants were able to distinguish easily between layered and non-layered hydrogels, but not within the layered gels with small bead size when compared to that of the large bead size.

3.3.8. Explanation of sensory characteristics of hydrogels using instrumental characteristics

There is increasing evidence now on relating instrumental textural measurements to sensory perception, yet most of these studies are carried out with trained panellists (Pradal, et al., 2016;

Prakash, Tan, & Chen, 2013; Sarkar, et al., 2019b; Shewan, Pradal, & Stokes, 2019). In this study, compression tests (**Figure 3.3b**) revealed that mixed hydrogels (*κ*C+NaA) required significantly more force to be broken down as compared to the layered gels, which is exactly opposite to what was revealed by the untrained participants with layered gels being perceived to be significantly 'harder' and 'chewier' as compared to the mixed gels (**Figure 3.6**). Therefore, this indicates that humans may perceive or evaluate food texture, particularly in case of layered hydrogels, differently compared to large deformation measurements. Of more importance here is that the role of saliva during *in vivo* oral processing of the samples that cannot be ignored, and saliva was not included in the compression test. In addition, the missing link between uniaxial compression and perceived texture could be due to the heterogeneous structure of the model foods used in this study, unlike the previously studied homogenous gels (Çakır, et al., 2012; Devezeaux de Lavergne, et al., 2015; Krop, et al., 2019a), where the relation between uniaxial compression tests and sensory perception is well recognized. More specifically, current results might be linked to the top and bottom *κ*C layers in the *κ*C+CaA bead-layered hydrogels that were perceived to be harder by the participants than the *κ*C+NaA hydrogels during the first compression between tongue and palate.

Noteworthy is that although 'smooth' and 'slippery' can be important lubrication-related attributes that can distinguish fat-based samples (Kokini, Kadane, & Cussler, 1977; Upadhyay & Chen, 2019), they can be difficult to understand in non-fat hydrogel samples (**Figure 3.6** and **Supplementary Figure B.3**) and thus were not a differentiating factor between current samples, which was also observed even with a trained panel in our previous study with hydrogels (Krop, et al., 2019a). Interestingly, both **Figures 3.2** and **3.4a** show high lubricity behaviour for the large-sized beads irrespective of them being on their own or as simulated hydrogel boli in the mixed regime. One should then expect that the high lubricating capacity of the large beads would result in lower perceived grittiness, which is not the case as shown in

Figure 3.6. These results might be attributed to the much higher contact pressures existing in a tribometer with PDMS tribopairs as compared to the tongue-palate contact pressures (Sarkar, et al., 2019a). The *in vitro* tribological experiments might have allowed squeezing out the alginate solution to create a 'hydrating layer' separating the surfaces as discussed in previous sections (**Figure 3.2**, **Supplementary Table B.1**). In contrast, in a real oral processing scenario during *in vivo* sensory evaluation by consumers, the pressures might be reasonably low: proving insufficient to break these beads down to a hydrating biopolymeric layer. Consequently, these large beads were perceived as 'particles' described by higher perceived 'gritty' intensity.

3.4. Conclusions

In this study, instrumental methods were used to quantify differences in textural complexities of layered hydrogels for the first time by incorporating a monolayer of soft beads of different sizes in the gel network and we aimed to determine whether such instrumental differences (if any) could then be sensorially perceived by untrained panellists. In this study, neither fracture stress of the hydrogels nor apparent viscosity of the hydrogel boli at orally relevant shear rate could statistically distinguish the layered hydrogels based on bead size. On the other hand, soft tribology analysis of beads as well as the hydrogel boli containing beads could successfully discriminate the large-sized beads from the smaller-sized beads in the mixed lubrication regime. Although textural differences between the mixed (NaA) and the three CaA beaded carrageenan hydrogels were sensorially perceived, participants were unable to distinguish the beaded samples in the present study based on bead size, which can be attributed to the low modulus of the beads used in these layered hydrogels. Overall, this study has important implications for generating novel texture by incorporating soft beads as a layer in hydrogels, where the presence of soft beads can generate distinguishing textural features versus non-beaded hydrogels that can be perceived by consumers.

Based on the instrumental and sensorial analysis of the hydrogels, two of them that showed a distinctive different lubricating properties have been selected for a satiety trial that will be discussed in the next **Chapter 4**. The next chapter will discuss the effect of the oral lubricity expressed through non-calorific hydrogels varying in their lubricating properties on appetite control, food intake, lubricating properties of saliva and salivary biomarkers.

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Chapter 4

Effects of oral lubrication on satiety, satiation and salivary biomarkers in model foods: A pilot study[4]

Abstract

With a dramatic increase in overweight and population with obesity over the last decades, there is an imminent need to tackle this issue using novel strategies. Addressing obesity issues by generating satiety in food to reduce energy intake has been one of those prominent strategies and often textural interventions have been used to generate satiety, specifically in short-term trials. This study aimed to investigate the role of preloads varying in their oral lubrication properties on appetite sensations, food intake, salivary friction and concentration of salivary biomarkers (proteins, α-amylase and mucins) in collected human saliva (n=17 healthy participants). The preloads were model foods (flavored hydrogels) either high or low in their lubricating properties, assessed both by instrumental and sensorial measurements. The results showed that hunger and desire to eat decreased immediately after preload and remained decreased for 10 and 20 min, respectively, after preload in the high lubricating condition compared to control (all $p < 0.05$). Fullness increased immediately after preload and remained increased for 10 and 20 min, respectively, after preload in high lubricating condition compared to control ($p < 0.05$). However, after controlling the values for baseline, such significant effect of the intervention does not exist anymore. Only the effect of time is observed. Therefore, the

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results should be interpreted with caution. Oral lubrication showed a brief effect on appetite sensations (data not controlled for baseline) but no effect on food intake and salivary biomarkers. Salivary lubrication correlated with feeling of fullness. Considering the large time-interval (30 min) between preload and next meal in this study, it is worthwhile investigating the immediate effects of oral lubrication on appetite control, food intake and salivary biomarkers.

4.1. Introduction

It is well known that the prevalence of obesity has increased dramatically over the last decade (WHO, 2018) and has been associated with chronic non-communicable diseases (McMillan, Sattar, & McArdle, 2006; Rexrode, et al., 1998; Steppan, et al., 2001) that could have significant morbidity and mortality consequences. It is widely agreed that the overconsumption of food (above energy needs for body size) contributes to the high prevalence of obesity (Public Health England, 2018). Therefore, exerting some control over food consumption is a priority for weight management and the prevention of obesity and therefore, achieving satiety and satiation through food textural design is one of the promising nutritional strategies.

Satiation describes within-meal inhibition and can be said to determine meal size and bring a particular eating episode to an end, whereas satiety is known to be associated with the inter-meal period, through the suppression of hunger and the inhibition of further eating (Blundell, 2010; Blundell, et al., 2010; Blundell, Rogers, & Hill, 1987). Satiety can be evaluated through psychological, behavioural and physiological procedures (Gibbons, Hopkins, Beaulieu, Oustric, & Blundell, 2019). Psychological measurements include perceived visual appetite ratings (such as hunger, fullness, desire to eat, prospective food consumption), whilst physiological measures mainly involve, changes in gastrointestinal biomarkers such as stomach dynamics or peptide hormones in blood, although changes in saliva may also be of significance (Gibbons, et al., 2019; Harthoorn, et al., 2007).

One promising approach to gain satiety and in turn reduce food intake is to consider ‘food texture’ manipulation during designing of the food application. A recent systematic review and meta-analysis has revealed that foods with higher textural characteristics (solid, high viscous, high lubricity and heterogeneous) have an effect on both satiation and satiety by suppressing appetite and reducing food intake (Stribiţcaia, Evans, Gibbons, Blundell, & Sarkar, 2020a). In recent years, there has been increased interests from researchers in understanding the role of structural/ textural complexity of food, specifically through the development of model foods such as hydrogels in generating satiety. The construct of food structural/ textural complexity offers quite a new concept in oral processing and satiety research field (Krop, Hetherington, Miquel, & Sarkar, 2019a; Krop, et al., 2018; Larsen, Tang, Ferguson, & James, 2016; Tang, Larsen, Ferguson, & James, 2016). Textural/ structural complexity of food is often defined by the degree of heterogeneity or inhomogeneity in a food, where the food or the intervention product includes some additive materials (*e.g.* hydrogels with sunflower/poppy seeds or alginate beads), which distinguishes it from a control product which has a homogenous texture lacking any inclusions (Stribiţcaia, et al., 2020a). To date, a limited number of studies has investigated the effect of structural/ textural complexity of food on satiety, however, these studies suggest that a higher structural complexity of food may lead to a reduced subsequent food intake and suppressed appetite (Krop, et al., 2019a; Larsen, et al., 2016; Tang, et al., 2016)

Among the textural complexity, effect of oral lubrication on satiety has thus far attracted very limited attention in literature. Krop et al. (2019a) studied the effects of oral lubrication on satiety in a snack trial setting and it was concluded that snack intake was reduced by 32% following consumption of a low chewing/ high lubricating gel. The mechanism by which lubrication influence food intake is hypothesized to be associated with mouth coating and thereby extending the oro-sensory exposure time leading eventually to a significant reduction in food intake. In other words, high lubricating gels coated oral surfaces better as compared to

gels with low lubricating properties, which resulted in reduced food intake in a previous proof of concept snack trial (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019b; Krop, et al., 2019a). However, the exact mechanisms of oral lubrication on both physiological and psychological aspects of eating remains elusive in literature. Therefore, the mechanism by which lubrication plays a role in food intake and appetite control remains to be investigated in formal experimental trials.

An innovative way to explain the link between food texture and satiety and satiation is to consider salivary biomarkers, which are important contributors to oral lubrication. Some initial observations have shown an association between salivary biomarkers (*e.g.* α-amylase) and perceived satiety and subsequent food intake (Harthoorn, 2008; Harthoorn, et al., 2007). For instance, it has been found that the level of α-amylase increased significantly after a starch based custard preload and *ad libitum* meal (Harthoorn, 2008). Salivary amylase helps in food digestion during oral processing by hydrolysing starch into maltose (Zakowski & Bruns, 1985) and it has been proposed that the concentration of salivary α-amylase may influence directly the hunger levels. For instance, in people with lower concentration of α-amylase, the digestion of carbohydrates will be slow, and this would lead to a presence of hunger for a longer period of time resulting in greater food intake before achieving satiety (Moreno-Padilla, Maldonado-Montero, Enguix-Armada, & Reyes del Paso, 2020). In addition, a link between macronutrient composition of foods and saliva characteristics has been reported. For example, intake of fatty food was reflected in a fatty acid profile of the collected saliva (Actis, Perovic, Defago, Beccacece, & Eynard, 2005). Likewise, carbohydrate intake showed an antioxidant capacity and increased amylase activity in the collected saliva (Méjean, et al., 2015). Also, the secretion of α-amylase has been reported to be dependent on the diet (Perry, et al., 2007). Taken together, these studies suggest that the composition of saliva is dependent on the type of food consumed. However, in these studies the focus was on food macronutrient composition with no direct or

indirect link to lubricity of food. Consequently, there is lack of studies showing the independent effects of oral lubricity, and specifically any studies on model food such as hydrogels on satiety, whilst controlling for the macronutrient or energy composition of food.

Interestingly, it is known that salivary proteins such as mucin (MUC5B) and other low molecular weight proteins contribute to the salivary composition and influence lubrication behaviour (Hopkins, et al., 2020; Humphrey & Williamson, 2001; Sarkar, Kanti, Gulotta, Murray, & Zhang, 2017; Sarkar, Xu, & Lee, 2019; Sarkar, Ye, & Singh, 2017b). However, how consumption of a high lubricating gel affects the tribological properties of the human saliva and MUC5B or protein content and how such change (if any) in salivary lubrication affects satiety remains largely unknown. It can be hypothesized that eating a high lubricating food might increase the lubricating properties of saliva and keep the oral surfaces moistened and coated longer. This in turn may lead to appetite suppression and lower subsequent food intake. To date, no studies have reported effects of food texture on satiety and satiation while considering the tribology properties of saliva when consuming a high lubricating versus a low lubricating model food. It is therefore appropriate to examine the relationship between the lubricating behaviour (based on higher concentration of proteins or MUC5B or mechanically measured tribological properties) of saliva on consumption of lubricating preloads and its influence on appetite control and food intake.

Therefore, the aim of the present study was to examine the effects of hydrogels as model foods varying in their oral lubrication properties on satiety, satiation as well as concentration of salivary biomarkers and frictional properties of collected saliva. These effects were evaluated through appetite ratings, subsequent food intake, measurement of frictional properties of saliva and measurement of concentration of salivary biomarkers at specific time points before and after the ingestion of preloads. The main objectives were to: 1) examine whether a systematic model food design with higher lubricity would lower subsequent food

intake and suppress appetite in a meal-trial; 2) understand the changes in lubricity of saliva after ingesting hydrogels with different oral lubrication properties; and 3) investigate the effect of hydrogel lubricity on salivary biomarkers, such as MUC5B, proteins and α-amylase. It is hypothesised that ingesting hydrogels possessing higher lubrication properties would lead to 1) a lower energy intake and suppressed appetite ratings; 2) higher levels of lubrication properties of the saliva; and 3) higher concentration in certain salivary biomarkers such as MUC5B and protein and a potential correlation between the salivary biomarkers, salivary lubricating properties, food intake as well as perceived appetite ratings. The strategic objective is to demonstrate whether or not structural/ textural complexity in terms of oral lubricity of food can affect both psychological and physiological aspects of eating behaviour in a meal setting.

4.2. Methods

4.2.1. Participants

We recruited healthy male and female participants between 18 and 55 years old. The participants were recruited from staffs and students of the University of Leeds. Subjects were excluded if they were smokers, had oral infections/diseases/ problems in chewing and swallowing, had a chronic or acute health condition that could affect the ability to sense, eat, digest or absorb food, were using prescribed or non-prescribed medication that could interfere with their ability to sense, eat, digest or absorb food; were pregnant or lactating, had a food allergy or intolerance, were on a special diet or taking protein/ fibre supplements, were not able to tolerate food gels, had underweight (BMI <18.5 kg/m^2), overweight or obesity (BMI $\geq$ 25 kg/m^2), had blood-borne diseases. The study was approved by University of Leeds Faculty Research Committee (MEEC 18-049). Sample size was calculated with GPower3.1. As the manipulation of this study was novel food, there was not enough information in the literature

in terms of the expected size effect. Therefore, the power analysis was a priori one, and it has been done to determine the number of participants needed for a small effect size (f=0.25) across all four outcomes. As such, according to GPower calculation, 24 participants are required to identify a small effect size (f = 0.25, α = 0.05 and 1-β = 0.80) across 3 groups (low lubricating, high lubricating and control) with 4 outcome (appetite ratings, food intake, salivary biomarkers and lubricity of saliva), with outcomes varying from 3 to 5 measurements. However, due to a UK lockdown related to the COVID-19 pandemic, data were collected and analysed for 17 participants.

4.2.2. Design

The study (registered at ClinicalTrials.gov as NCT04240795) was an acute, single-blinded, randomized, counterbalanced, within-subject designed cross-over trial. Recruitment poster were placed across the University of Leeds campus and emails were sent to students and staffs. Further, interested participants were emailed an information package (participant information sheet, eligible criteria, Three Factor Eating Questionnaire (TFEQ) and the link to online health screening questionnaire) in which they were informed that the aim of the study was to investigate the acceptance, pleasantness and taste perception of food gels with different textural attributes. At the end of the study, participants were debriefed and informed about the exact purpose of the study. The study took place at the University of Leeds, UK, School of Food Science and Nutrition, between November 2019 and end of March 2020. Participants gave their written informed consent before taking part in the study (ethics approved by University of Leeds (MEEC-16-046)) and received shopping vouchers of £20 value as compensation for their time.

4.2.3. Session procedure

Before taking part into the study, participants were first screened for eligibility criteria using an online health screening questionnaire. They were also tested for restrained eating using TFEQ. A total of 34 participants was screened, of which 17 were included in the study for data analysis (13 did not meet the inclusion criteria, 2 withdrew from the study and 2 did not finish the study due to the COVID-19 associated lockdown). Each participant was asked to come to the laboratory on three different occasions with 3-7 days of wash out period in between each session. Participants were instructed to fast for 11 h (from 10.00 pm) and to refrain from drinking except water for 24 h before each session. Alcohol was prohibited. Each session lasted 3.5-4 h. Participants were required to come to the laboratory at 8.45 am.

In the first session, weight and height were measured. Body weight was measured to the nearest 0.1 kg after voiding (Seca 763, Seca Birmingham, UK) and height was measured to the nearest 0.5 cm using a portable stadiometer (Seca Portable height measure, Leicester, UK). A schematic overview of the study protocol is presented in **Figure 4.1a**. Participants provided baseline appetite ratings on a 100-mm visual analogue scale (VAS). After that, at 9.00 am, they were given a fixed amount of breakfast which consisted of muesli (Neal's Yard Muesli Base), raisins (Neal's Yard Raisins), sultanas (Neal's Yard Sultanas), honey (Sainsbury's Runny Honey) with yogurt (Yeo Valley Natural Yoghurt) purchased from a local supermarket and 150 g of water. The total allocation was 250 kcal for females and 350 kcal for males in order to standardise the appetite levels for all the participants before consuming the preloads (flavoured hydrogels). Participants were required to eat all of the breakfast. Participants then rated their appetite on visual analogue rating scales (VAS hunger, fullness, desire to eat, prospective food consumption and thirst) at every 30 minutes for the next 2.5 h and whole unstimulated saliva was collected at 3 time points, 90 min after breakfast, pre- and post-preload.

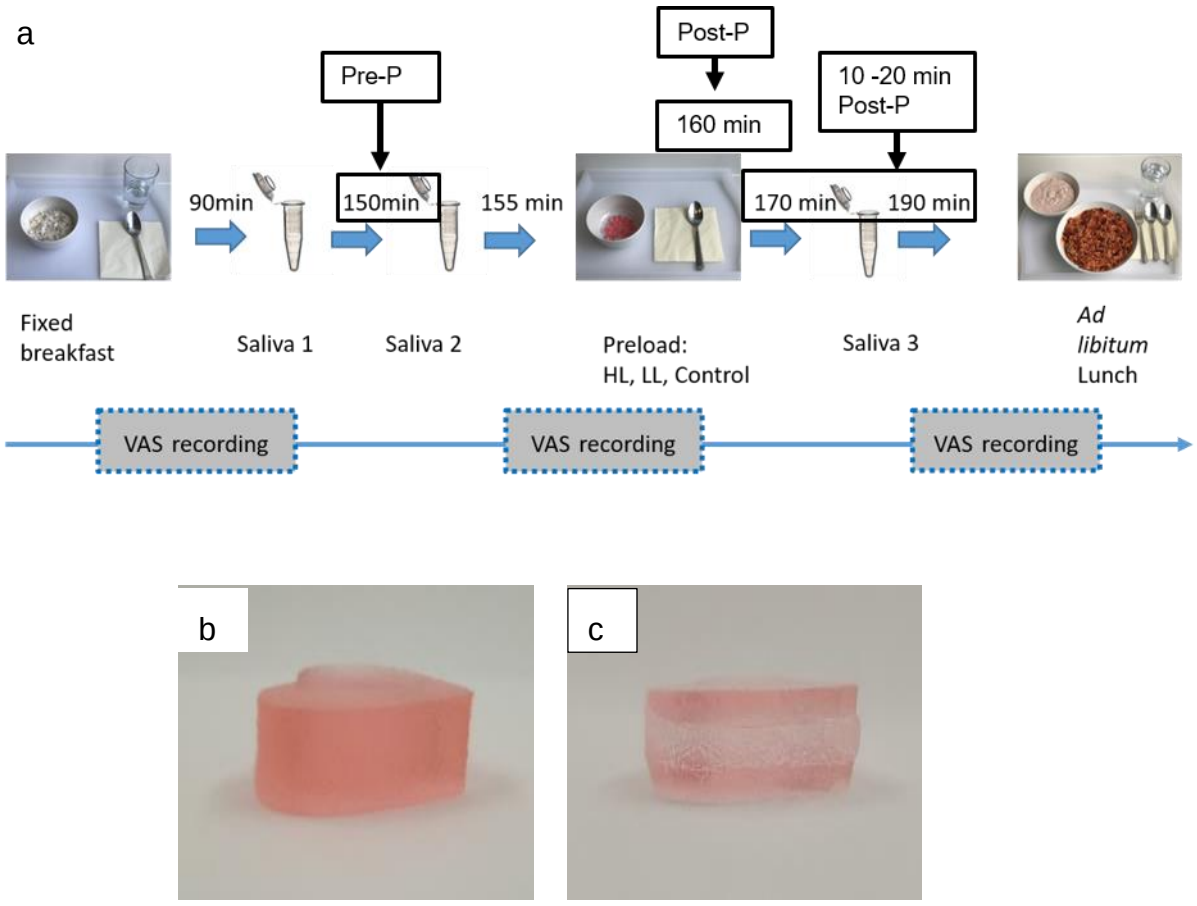

Figure 4.1. Overview (a) of the study protocol. First, fasting ratings were taken on a visual analogue (VAS) scale (mm), then a fixed breakfast was provided, and then appetite was rated on a VAS scale (mm) over eleven time points in total. Whole unstimulated saliva was collected on three time points (Saliva 1-Saliva 3). *Ad libitum* lunch was given to the participants 190 min after breakfast or 30 min after the preloads. Preloads represent watermelon-flavoured hydrogels cut in heart shape, (b) *κ*C+NaA - pasty/ high lubricity – HL, and (c) *κ*C + CaA – hard / low lubricity – LL and control was flavoured water. Pre-P=pre-preload, Post-P=post-preload, 10 min Post-P=10 min post-preload, 20 min Post-P=20 min post-preload.

After that, they were given the preload: hydrogels differing in lubricity, or water (control). After consuming the preload, appetite ratings were recorded by the participants at three time points (every 10 min for a duration of 30 min). Whole unstimulated saliva was collected after breakfast, immediately after consuming the hydrogels as well as after 10 min of resting period. An *ad libitum* lunch was offered 30 min after ingesting the preload followed by the final appetite ratings. The *ad libitum* lunch consisted of vegetarian chilli (Stagg Low Fat Vegetable Chilli, manufactured by Danish Crown Ltd. in Manchester, UK) and rice (Microwave Rice Basmati, manufactured by Sainsbury's Supermarkets Ltd. in London, UK) and strawberry yogurt (Yeo Valley, manufactured by Yeo Valley in Blagdon, UK) purchased from a local supermarket and *ad libitum* water. Participants were provided with 770.4 kcal/ 845 grams of chilli and 778.2kcal/ 525 grams of yoghurt. They were asked to eat until a comfortable level of fullness.

4.2.4. Preload characterisation

The preload consisted of watermelon-flavoured hydrogels (see **Figures 4.1b** and **4.1c**) that were selected based on their difference in textural attributes (measured sensorially) and oral lubrication properties (measured instrumentally) as described previously (Stribiţcaia, Krop, Lewin, Holmes, & Sarkar, 2020b). Briefly, the hydrogels were cut in heart shape and each participant received a total amount of 30 g of each hydrogel or control (water) on different testing days. The difference in sensorial and oral lubrication attributes was achieved by mixing the same gelling agents but structuring differently. One type of hydrogel contained a mixture of κ-carrageenan (κC) and sodium alginate (NaA) (see **Figure 4.1b**), whilst the other hydrogel was layered containing κC and alginate, with the latter in the form of calcium alginate-based spherical beads (CaA) of 1800 μm and consisted of three layers: top and bottom layers were pure κC and the middle layer contained CaA beads (see **Figure 4.1c**).

The concentration was the same for both hydrogels: κC + NaA hydrogel (1.67 wt% κC and 0.33 wt% NaA) and κC + CaA hydrogel (1.67 wt% κC and 0.33 wt% CaA). Based on instrumental (see **Figure 4.2a** for tribological *i.e.* friction measurement) and sensorial analyses (see **Figure 4.2b**), the κC + NaA hydrogel was characterised as pasty (**Figure 4.2b**) and was high in oral lubrication properties (**Figure 4.2a**) (*i.e.* low in friction), and this hydrogel is referred to as high lubricating hydrogel (HL) hereafter. On the other hand, the κC + CaA hydrogel was characterised as sensorially hard (**Figure 4.2b**) and the inclusion (CaA beads) resulted in high frictional properties (**Figure 4.2a**) and consequently low in oral lubrication properties, therefore is referred to as low lubricating hydrogel (LL), hereafter. The instrumental characteristics of the hydrogels were determined by performing tribology analysis using a Mini Traction Machine (MTM2) tribometer (PC Instruments, London, UK), and the ratings of the sensorial attributes were obtained by performing intensity ratings with 60 untrained participants, details have been provided previously (Stribiţcaia, et al., 2020b). The hydrogels were flavoured with food-grade watermelon aroma (Special Ingredients Ltd, Chesterfield, UK), coloured with food-grade watermelon food colouring (AmeriColor Corp., Placentia, California USA) and sweetened with stevia granulated sweetener from a local supermarket (Leeds, UK) to increase acceptability of these model foods by the consumers without addition of any calorific sugar. Water was provided to the participants as a control. The water also contained the watermelon flavour, colour and sweetness to match the flavour profile and intensity of sweetness of the hydrogels

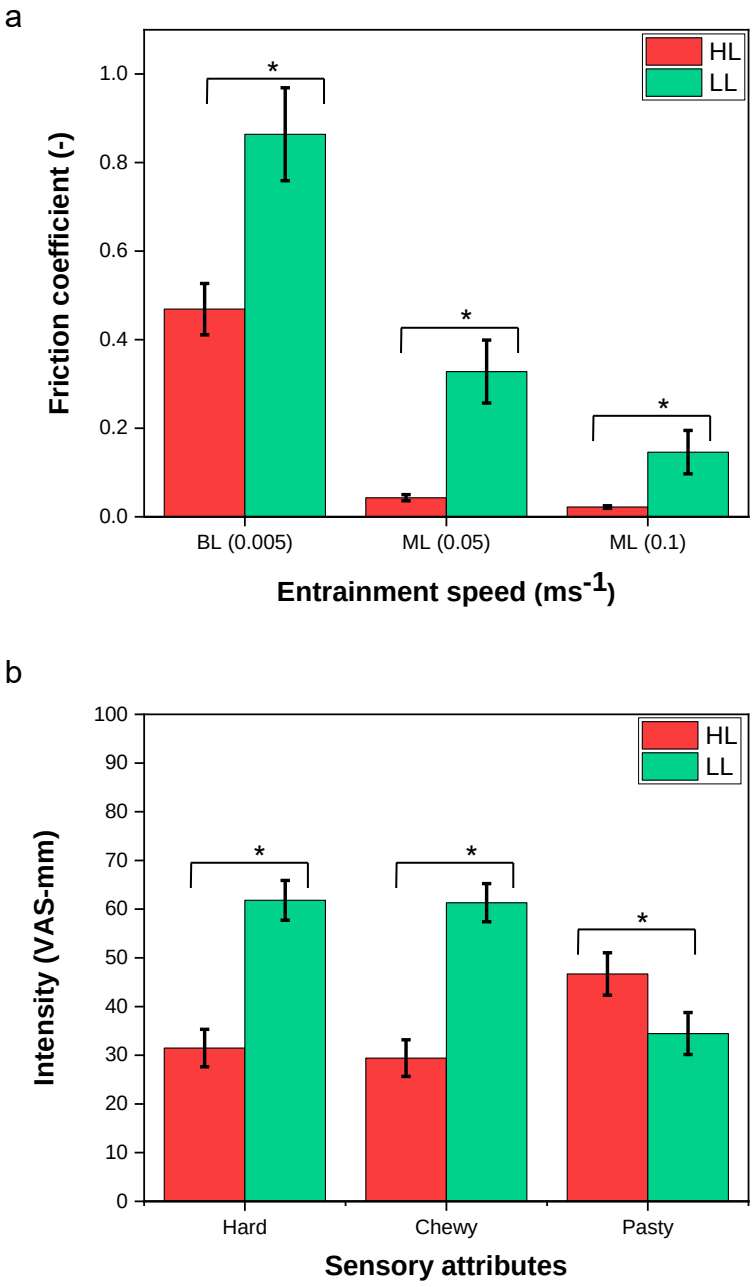

Figure 4.2. Instrumental lubricity analysis (a) where data is expressed as friction coefficients at boundary (0.005 m s-1 speed) and mixed (0.05 m s-1; 0.1 m s-1 at speed) lubrication regimes for the HL (high lubricating) and LL (low lubricating) hydrogels, respectively at various speeds; and sensory analysis (b) including three attributes: hardness, chewiness and pastiness for both HL (high lubricating) and LL (low lubricating) hydrogels. Error bars represent standard error of means (SEMs). The asterisks (*) denote a significant difference between the samples. A lower friction coefficient represents higher lubrication properties of the hydrogels. BL = boundary regime, ML = mixed regime.

4.2.5. Study measures

4.2.5.1. Appetite ratings

Participants rated their appetite at eleven different time points using a 100-mm VAS scale, which has been shown to be valid and reliable for appetite research (Flint, Raben, Blundell, & Astrup, 2000; Stubbs, et al., 2000). The scales anchored from 'not at all' to 'extremely' were administered at: -5, 0, 30, 60, 90, 120, 150, 160, 170, 180, 210 min on each testing day (see **Figure 4.1a**). The participants rated hunger, fullness, desire to eat, prospective food consumption and thirst. Additional scales contained questions concerning nausea and the mood – contentedness and mental alertness. In addition, participants rated the palatability and the acceptability of the hydrogels and control. The time point of 150 min is referred to as 'pre-preload', 160 min to as 'post-preload', 170 min to as '10 min after preload' and 180 min to as '20 min after preload' throughout the text.

4.2.5.2. Energy intake

Ad libitum foods and beverages were accurately weighed (to the nearest 0.1g) prior to being served to participants, and were re-weighed after the participants finished eating in order to determine the amount of food and beverage consumed by each participant. Energy intake (EI) at each meal was calculated. For completeness in reporting, first, the energy intake was calculated (the number of grams of carbohydrate, protein and fat was multiplied by 3.75, 4 and 9, respectively) for rice and vegetable alone and this was referred to as 'main course' and then for yogurt alone referred to as 'dessert' throughout the text. Then, a total EI was calculated for both rice with vegetable and yogurt, and is referred to as 'combined' meal.

4.2.5.3. Tribology of human saliva

As illustrated in **Figure 4.1a**, saliva was collected at three time points. Participants were

asked to spit into a pre-cooled tube till they felt comfortable. The collected saliva from each participants at three different time points were centrifuged for 5 min at at 4000 × *g* and the precipitate containing cell debris was discarded. Approximately, 3 mL of the supernatant was made up to the volume to 10 mL using pre-chilled 20 mM phosphate buffer (pH 7) (*i.e.* 16 vol% unstimulated whole human saliva) (Hopkins, et al., 2020) and was stored at 4 ºC for tribology analysis within the same day using ball-on-disc tribological set up in a Mini Traction Machine (MTM2, PCS Instruments, London, UK) and three separate aliquots (250 µL each) were stored at −20 °C until further use for total protein, α-amylase and MUC5B assays, respectively.

Tribology was performed to determine the lubrication properties of saliva after breakfast, before and after preload (Saliva 1-Saliva 3, respectively). Commercially available polydimethylsiloxane (PDMS) ball (diameter of 4 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) and disc (diameter of 46 mm, thickness of 4 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) were used as surfaces to mimic palate and tongue, respectively for the oral tribology measurements (surface roughness of the PDMS tribopairs, R_a < 50 nm). The saliva supernatant (9 mL) was loaded into the minipot equipped with the PDMS ball and disc, where these tribopairs were rotated at different speeds to create a relative motion between the surface of the ball and the disc, resulting in a slide-to-roll ratio (SRR) of 50 %, and the temperature was maintained at 37 °C, simulating oral procedures. The entrainment speed was calculated as the average velocity of the two contacting surfaces (*i.e.* ball and disc) and reduced from 300 to 1 mm/s to simulate tongue movement, and friction forces were measured at a load of 2 N with a maximum of 343 kPa of Hertzian contact pressure (Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019). Friction forces in presence of saliva collected at different time points and after consuming preloads or controls were compared at boundary (BL, speed of 0.05 m s^{-1}) and mixed (ML,

speed of 0.5 m s^{-1}, 0.1 m s^{-1}) lubrication regimes (Stribiţcaia, et al., 2020b).

4.2.5.4. Biochemical assays of salivary biomarkers

Supernatants (*i.e.* 50 vol% unstimulated whole human saliva) collected in 250 µL aliquots were assayed for total protein using Pierce BCA Protein Assay Kit (Pierce, Fisher Scientific, Loughborough, UK) and the results were compared to a standard curve generated using bovine serum albumin (BSA). Salivary mucin (MUC5B) was analyzed using human MUC-5B ELISA Kit (OKEH02841, Aviva Systems Biology, Insight Biotechnology, Wembley, UK). Salimetrics α-amylase kit (Stratech, Ely, UK) was used to measure salivary α-amylase enzyme activity. The biochemical assays were run in duplicate and absorbance values recorded using Tecan Spark 10 M microplate reader (Tecan, Reading, UK). Results were expressed as Units/mg protein for amylase, ng/ mg protein for MUC5B and µg/mL for protein.

4.3. Statistical analysis

Data are presented as mean and standard deviations (SDs) in the text and tables, and means and SEMs in the figures. All statistical analyses were performed using SPSS (IBM® SPSS® Statistics, v25, SPSS Inc, Chicago, USA). Differences between conditions were tested by repeated measures ANOVA for appetite ratings at relevant time points, overall appetite ratings, food intake, salivary biomarkers, and lubricating capacity of human saliva after ingesting the preloads. The differences in palatability of the preload, nausea, mental alertness and content mood were also assessed by repeated measures ANOVA. 3 × 5 level factorial repeated measure ANOVA was used to examine the main effect of the intervention condition (LL, HL, Control), time (pre-preload, post-preload, 10 min, 20 min after preload and after lunch) and condition*time interaction on appetite ratings. Analysis of appetite ratings were also compared

after controlling for baseline ratings using the analysis of difference from baseline. As the food in this study was novel, there is sufficient uncertainty about the immediate post-gel experience to make conclusion based on analysis controlled for baseline only. Therefore, appetite results from both with and without controlled for baseline analysis are reported. Where the assumption of sphericity had been violated, indicated by Mauchly's test, Greenhouse-Greisser corrected tests are reported. Statistical significant differences were calculated by Bonferroni corrected post-hoc t-tests and was set at $\alpha < 0.05$ level. Pearson correlations were performed to assess the relationship between appetite ratings, food intake, tribology of saliva and concentration of biomarkers (protein, *α-amylase)*. Data for appetite ratings, overall appetite scores and food intake were analysed for all 17 participants. For salivary biomarkers data were analysed for α-amylase and protein on all 17 participants, however, for mucin data were analysed on 9 participants due to the negative values or values out of standard range on the rest of the participants. To check the outliers, the Explore function in SPSS was used, with the IQR (interquartile range) multiplier approach (Tukey, 1977). Where the values from the end of the box plot were more than 3 IQR's (also, labelled as 'extreme') and where denoted with an asterisk (*), then the data were treated as outliers and excluded further from analysis (Hoaglin & Iglewicz, 1987). For tribology, due to insufficient remaining saliva (priority has been given to salivary biomarkers analysis), there were complete data for 7 participants only. After removing the outliers (n=3), the salivary tribological analysis was completed on 4 participants only. Therefore Pearson's correlation was also analysed on data from 4 participants. Data were plotted using the software Origin® (OriginPro 2018; OriginLab Corporation, Northampton MA, USA).

4.4. Results

4.4.1. Participants' characteristics

A total of 17 participants (6 males/ 11females) completed the study, see the characteristics in **Table 4.1**. The age of the participants ranged from 20 to 29 years. Their BMI was 22.6 ± 2.9 kg/m², with 15 participants in the healthy range and two with overweight (both 26.7 kg/m²); all 17 were included in the analysis. The TFEQ analysis revealed that 4 participants had a high restraint score (between 14-18).

Table 4.1. Participant's characteristics[1].

Characteristics	Values	Units
Male/ female	6/ 11	
Age	25.4 ± 2.7	years
Weight	63.9 ± 11.3	kg
Height	1.67 ± 0.06	m
BMI	22.6 ± 2.9	kg/m²
TFEQ Restrain	10.9 ± 4.5	
TFEQ Disinhibition	9.2 ± 4.5	
TFEQ Hunger	7.9 ± 6	

[1]Values are means ± SDs (n=17). TFEQ, Three Factors Eating Questionnaire; BMI, Body Mass Index.

4.4.2. Appetite ratings

Descriptive data for appetite ratings between preloads at relevant time points (fasting, pre-/post-preload, 10, 20 min after preload and after lunch) are given in **Table 4.2**. There was no significant difference between groups for fasting for all appetite ratings and for pre-preload time, whereas an increased fullness was noticed in HL (high lubricating) versus LL (low lubricating condition) and vs Control (see **Table 4.2**). Palatability was measured on a 100-mm VAS scale immediately after preload in terms of texture, sweetness and flavour. The only difference noted was between LL and Control in terms of sweetness ($p < 0.005$) where Control was perceived sweeter than LL. In terms of texture and flavour, there was no significant

difference between preloads (see **Supplementary Table C.1**). In the following sections, we focussed on the appetite rating differences.

Table 4.2. Appetite ratings (mm) at relevant time points for subjects eating HL (high lubricating), LL (low lubricating) or Control preloads, n=17 (means ± SD).

Appetite ratings	HL	LL	Control	p-value[1]
Hunger				
Fasting	66 ± 23	66 ± 21	64 ± 22	n.s.
Pre-preload	58 ± 21	57 ± 16	60 ± 21	n.s.
Post-preload	42 ± 22^{a}	49 ± 21^{ab}	59 ± 20^{b}	p=0.014
10 min after	47± 27^{a}	48± 23^{ab}	59 ± 22^{b}	p=0.009
20 min after	59 ± 20	62 ± 19	67 ± 18	n.s.
After lunch	3 ± 3	3 ± 5	2 ± 3	n.s.
Fullness				
Fasting	20 ± 19	17 ± 18	16 ± 15	n.s.
Pre-preload	37 ± 23^{a}	29 ± 18^{b}	30 ± 24^{bc}	*p < 0.05*
Post-preload	49 ± 28^{a}	39 ± 24^{ab}	33 ± 24^{b}	*p = 0.004*
10 min after	43 ± 26^{a}	40 ± 24^{ab}	30 ± 22^{b}	*p = 0.004*
20 min after	36 ± 22^{a}	27 ± 16^{ab}	28± 23^{b}	*p = 0.039*
After lunch	90 ± 8	87 ± 20	84 ± 30	n.s.
Desire to eat				
Fasting	69 ± 24	63 ± 24	67 ± 25	n.s.
Pre-preload	61 ± 21	53 ± 20	63 ± 20	n.s.
Post-preload	45 ± 28	48 ± 27	53 ± 23	n.s.
10 min after	50 ± 27^{a}	52 ± 24^{ab}	60 ± 22^{b}	*p = 0.030*
20 min after	59 ± 25	62 ± 22	67 ± 20	n.s.
After lunch	5 ± 7	6 ± 9	5 ± 8	n.s.
Prospective food consumption				
Fasting	67 ± 20	66 ± 16	64 ± 18	n.s.
Pre-preload	60 ± 21	55 ± 19	63 ± 21	n.s.
Post-preload	50 ± 22	48 ± 22	58 ± 22	n.s.
10 min after	53 ± 23^{a}	52 ± 21^{ab}	64 ± 23^{c}	*p < 0.05*
20 min after	60 ± 22	60 ± 21	67 ± 19	n.s.
After lunch	7 ± 8	6 ± 7	9 ± 9	n.s.
Thirst				
Fasting	72 ± 22	65 ± 20	70 ± 22	n.s.
Pre-preload	62 ± 25	61 ± 28	63 ± 23	n.s.
Post-preload	54 ± 27	54 ± 27	50 ± 24	n.s.
10 min after	53 ± 27	50 ± 30	53 ± 23	n.s.
20 min after	61 ± 30	61 ± 33	59 ± 26	n.s.
After lunch	16 ± 21	15 ± 21	18 ± 22	n.s.

[1]A statistical significant difference (*p<0.05*) between the interventions (preloads) is denoted by different letters in superscripts. A non-significant difference (*p > 0.05*) between the interventions is denoted by the letters n.s.

Hunger

There was no main effect of intervention F(2,32) = 1.83 ($p = 0.18$) but there was a main effect of time F(2.17, 34.82) = 94.02 ($p = 0.000$) and intervention*time interaction on hunger F(8,128) = 2.13 ($p = 0.024$) (**Table 4.2**). A post-hoc pairwise comparison test revealed that hunger significantly decreased in HL condition versus Control ($p < 0.05$) and was significantly lower post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, the key effect was confirmed. There was a main effect of intervention*time interaction on hunger F(8,128) = 2.13 ($p = 0.024$). However, the effect of intervention alone does not exist anymore. Only the effect of time is noted. A post-hoc pairwise comparison test revealed that hunger significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Fullness

There was an effect of intervention F(2,32) = 8.01 ($p = 0.002$) and time F(1.73, 27.76) = 53.77 ($p = 0.000$), but no effect of intervention*time interaction on fullness F(8,128) = 1.53 ($p > 0.05$) (**Table 4.2**). A post-hoc pairwise comparison test showed that fullness significantly increased in HL condition versus Control ($p < 0.05$) and was significantly higher post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, an effect of time on fullness F(1.73, 27.76)=53.77 ($p=0.000$) was noticed. A post-hoc pairwise comparison test showed that that fullness significantly increased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Desire to eat

For desire to eat, there was an effect of time only F(2.37, 37.92) = 78.53 ($p = 0.000$), and no effect of intervention F(2,32) = 2.18 ($p > 0.05$) or intervention*time interaction F(4.41, 70.54)

=1.51 (*p>0.05*) (**Table 4.2**). Post-hoc pairwise comparison test revealed that desire to eat was significantly lower post-preload, 10 min after preload and *ad libitum* lunch (*p < 0.05*) compared to pre-preload and 20 min after preload (*p > 0.05*). After controlling for baseline ratings, an effect of time on desire to eat F(2.37, 37.92)=78.53 (*p=0.000*) was observed. A post-hoc pairwise comparison test showed that desire to eat ratings significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch (*p < 0.05*) compared to pre-preload and 20 min after preload (*p > 0.05*).

Prospective food consumption

For prospective food consumption there was an effect of intervention F(2,32) = 4.55 (*p = 0.018*) and an effect of time F(1.69, 27.13) = 91.72 (*p = 0.000*), but no effect of intervention*time interaction F(8,128) = 1.11 (*p > 0.05*) (**Table 4.2**). Post-hoc pairwise comparison test showed that prospective food consumption significantly decreased in HL condition compared to Control one (*p < 0.05*) and was significantly lower post-preload, 10 min after preload and *ad libitum* lunch (*p < 0.05*) compared to pre-preload and 20 min after preload (*p > 0.05*). After controlling for baseline ratings, an effect of time on prospective food consumption F(1.69, 27.13) = 91.72 (*p=0.000*) was seen. A post-hoc pairwise comparison test showed that it prospective food consumption ratings significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch (*p < 0.05*) compared to pre-preload and 20 min after preload (*p > 0.05*).

Thirst

Thirst had the same pattern as desire to eat, there was an effect of time only F(4,64) = 41.93 (*p = 0.000*), and no effect of intervention F(2,32) = 0.014 (*p > 0.05*) or intervention*time interaction F(8,128) = 0.328 (*p > 0.05*) (**Table 4.2**). Post-hoc pairwise comparison test revealed that thirst significantly decreased only after *ad libitum* lunch (p<0.05) compared to pre-preload, post-preload, 10 and 20 min after preload (*p > 0.05*). After controlling for baseline ratings,

again, an effect of time on thirst F(4,64) = 41.93 (*p=0.000*) was noticed. A post-hoc pairwise comparison test showed that thirst significantly decreased only after *ad libitum* lunch (*p < 0.05*) compared to pre-preload, post-preload, 10 and 20 min after preload (*p=0.000*).

To check if there was any significant difference in overall appetite suppression (OAS) the following equation was used:

$$\text{OAS} = \frac{Hunger + PFC + (100 - Fullness)}{3} \quad (1)$$

There was a significant difference (see **Figure 4.4**) immediately after preload (post-preload) between HL (48 ± 21) and Control (61 ± 20) conditions (*p = 0.05*), 10 min after preload between HL (52 ± 22) and Control (64 ± 21) conditions (*p = 0.000*), and between LL (53 ± 18) and Control (64 ± 21) (*p = 0.016*). Also, at 20 min after preload, there has been noted a significant difference between HL (61 ± 20) and Control (69 ± 18) conditions (*p = 0.039*). These results corroborate with the ones discussed above with respect to **Table 4.2**. There was no significant difference of AUC between groups for all appetite ratings (see **Supplementary Table C.2**).

After controlling for baseline ratings, the overall appetite scores showed an effect of time F(1.69,27.14) = 100.07, *p=0.00* and an effect of intervention*time interaction F(8,128) = 2.38, *p=0.02*. A post-hoc pairwise comparison test revealed that overall appetite scores significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch (*p < 0.05*) compared to pre-preload and 20 min after preload (*p > 0.05*).

Appetite ratings for all the eleven time points (from fasting until after *ad libitum* food intake (after lunch), including breakfast as well) are illustrated in **Figure 4.3**. A clear pattern can be noted where hunger (**Figure 4.3a**), desire to eat (**Figure 4.3c**), prospective food consumption (**Figure 4.3d**) and thirst (**Figure 4.3e**) decreased immediately after breakfast, increased before preload (almost 2.5 h after breakfast), decreased immediately after the

intervention and returned to the baseline/ fasting level 20 min after preload. As expected, a contrasting pattern could be noted for fullness too (**Figure 4.3b**), where it (fullness) increased immediately after breakfast, decreased before preload (almost 2.5 h after breakfast), increased immediately after intervention and finally returned to baseline levels 20 min after preload.

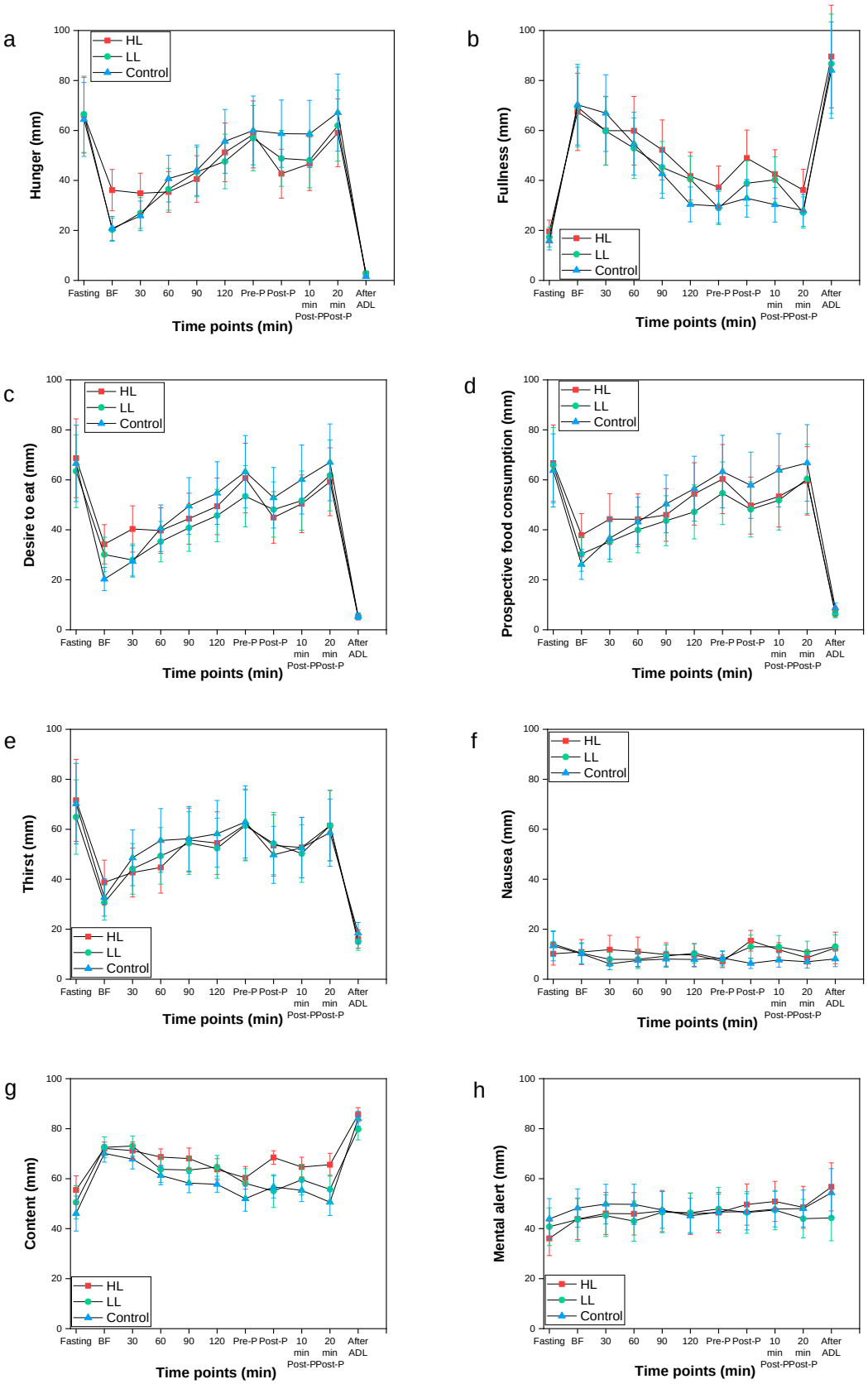

Figure 4.3. Ratings (mm) for (a) hunger, (b) fullness, (c) desire to eat, (d) prospective food consumption (PFC) (e) thirst, (f) nausea, (g) content, and (h) mental alert (h) over time, from fasting and breakfast (BF) until after ad libitum lunch (After ADL) including the relevant time points: pre-preload (Pre-P), post-preload (Post-P), 10 min after preload (10 min Post-P), 20 min after preload (20 min Post-P) during HL (high lubricating), LL (low lubricating) and Control conditions. Values are means and SEMs.

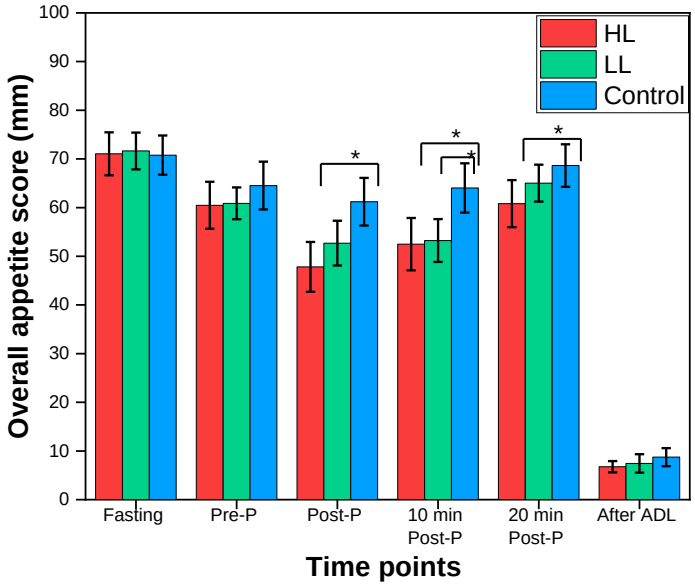

Figure 4.4. Overall appetite suppression (mm) for fasting, pre-preload (Pre-P), post-preload (Post-P), 10 min Post-preload (10 min Post-P), 20 min Post-preload (20 min Post-P) and after ad libitum lunch (After ADL) for HL (high lubricating), LL (low lubricating) and and Control conditions. Values are means and SEMs. The asterisks (*) denote a significant difference between conditions.

Due to the novelty of the model foods used in the current study as preloads (hydrogels), we assessed for the feelings of nausea, as well as for the mood of participants after ingesting the preloads. Therefore, three more measurements were taken (on a 100 mm VAS scale): nausea (**Figure 4.3f**), content (how content participants felt at each time point during each study session) (**Figure 4.3g**) and mental alert (how mentally alert participants felt at each time point during each study session) (**Figure 4.3h**). There was no significant main effect of intervention/preload, time point or intervention*time interaction in terms of nausea and mental alertness. However, a significant effect of intervention $F(1.44, 23.08) = 5.621$ ($p = 0.017$), time $F(1.21, 19.40) = 17.91$ ($p = 0.000$) and no effect of intervention*time interaction $F(3.53, 56.54) = 2.254$ ($p = 0.082$) was noted for contentedness (how content participants felt at each time point during each study session). Post-hoc pairwise comparison test revealed that participants felt more content after eating HL preload compared to LL and Control ($p = 0.027$) immediately after preload, 10 and 20 min thereafter ($p < 0.05$). It is unknown why this occurred. One

explanation could be that participants liked more HL preload in comparison with LL and Control, however, there was no significant difference in preloads palatability (see **Supplementary Table S1**).

4.4.3. Energy intake

For *ad libitum* energy intake at lunch (see **Figure 4.5**), there was no statistical difference for main course, dessert, and combined meal between interventions – HL, LL and Control. Therefore, the total amount of food participants consumed was almost the same in all three conditions. The same was noticed for water, no significant difference between groups in the water intake.

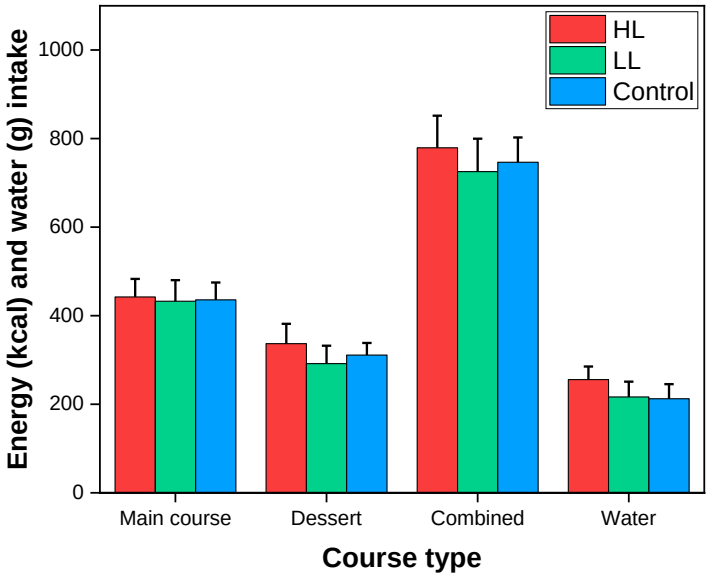

Figure 4.5. Energy intake (kcal) and water intake (g) for HL (high lubricating), LL (low lubricating) and Control conditions. Values are means and SEMs.

4.4.4. Lubrication properties of collected saliva

To check if there were differences in lubrication properties of saliva between conditions before and after the intervention, tribological measurements were performed on the collected saliva. There was no significant difference in the lubrication properties of saliva expressed through friction of coefficient between interventions/preloads (HL, LL and Control) before preload (see **Figure 4.6a**) and after preload (see **Figure 4.6b**). See **Supplementary Table C.3a and C.3b** for the descriptive data showing no difference in the lubrication properties after consuming HL, LL or Control (after breakfast data were excluded from analysis due to its irrelevance to the study).

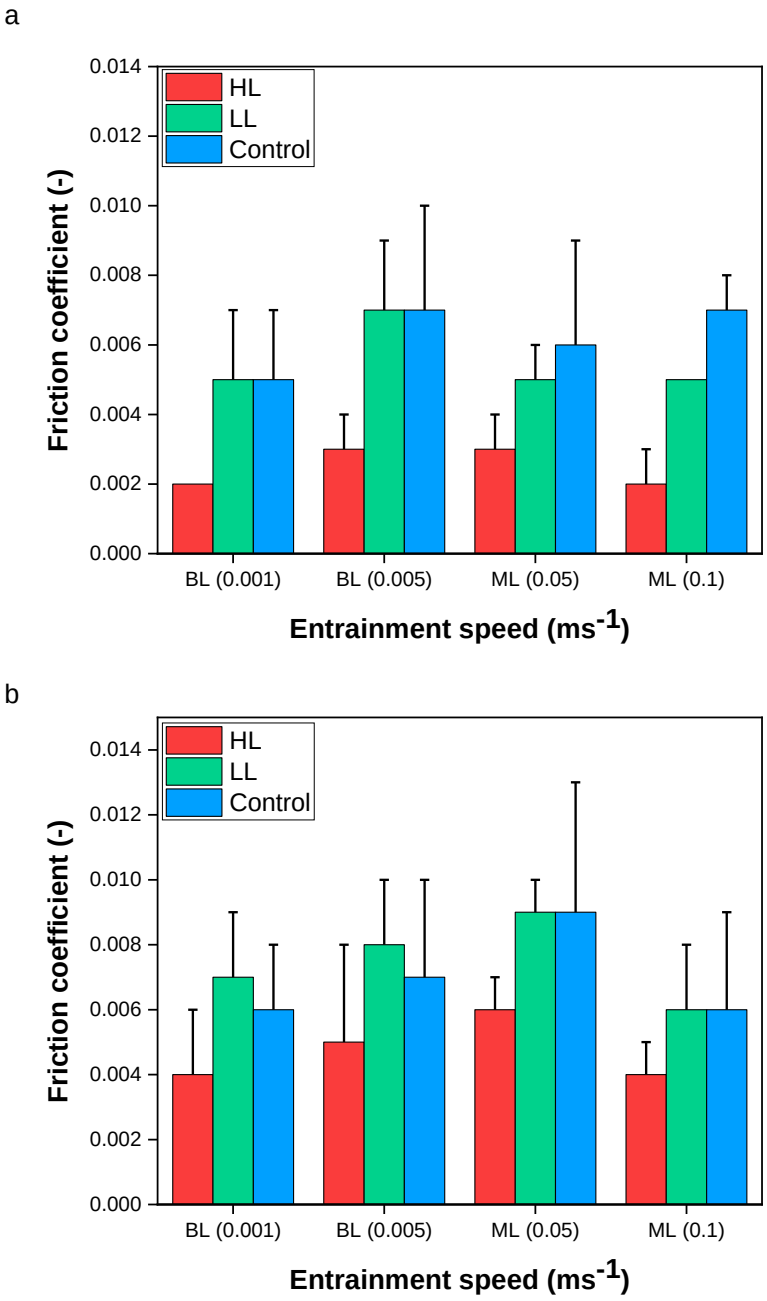

Figure 4.6. Friction coefficient of saliva before preload (a) and after preload (b) in all three conditions of HL (high lubricating), LL (low lubricating) and Control at boundary (0.001 m s-1; 0.005 m s-1 speed) and mixed (0.05 m s-1; 0.1 m s-1 speed) lubrication regimes, n=4 (after removing outliers). Values are mean and error bars of means (SEMs). BL = boundary lubrication regime, ML = mixed lubrication regime. A lower friction coefficient represents higher lubrication performance of saliva.

4.4.5. Salivary biomarkers

A total concentration of protein (see **Figure 4.7a**), α-amylase (see **Figure 4.7b**) and MUC5B (see **Figure 4.7c**) were assessed for each condition at two time points: before and after preload

(after breakfast data were excluded from analysis due to its irrelevance to this study). There was no significant differences in any salivary biomarkers (protein, α-amylase and MUC5B) between interventions (see **Figure 4.7a-c**). Surprisingly, an increase in total MUC5B was noted in LL compared to HL and Control condition after intervention, however this was not statistically significant (see **Figure 4.7c**). For total protein, there was no effect of time $F(1,16) = 4.21$ ($p = 0.057$), intervention $F(2,32) = 0.623$ ($p = 0.543$) or intervention*time interaction $F(2,23) = 0.751$ ($p = 0.480$). The same was noted for α-amylase and MUC5B. There was no effect of time $F(1,16) = 0.550$ ($p = 0.469$), intervention $F(2,32) = 2.46$ ($p = 0.101$) or intervention*time interaction on salivary α-amylase concentration $F(1.42, 22.78) = 2.40$ ($p = 0.126$). Also, there was no effect of time $F(1,8) = 0.356$ ($p = 0.567$), intervention $F(1.03, 8.28) = 2.12$ ($p = 0.182$) or intervention*time interaction on salivary MUC5B concentration $F(1.01, 8.12) = 1.45$ ($p = 0.263$). These data suggest no effect of consuming non-calorific model-food differing in its lubrication properties on salivary biomarkers such as α-amylase, protein and MUC5B.

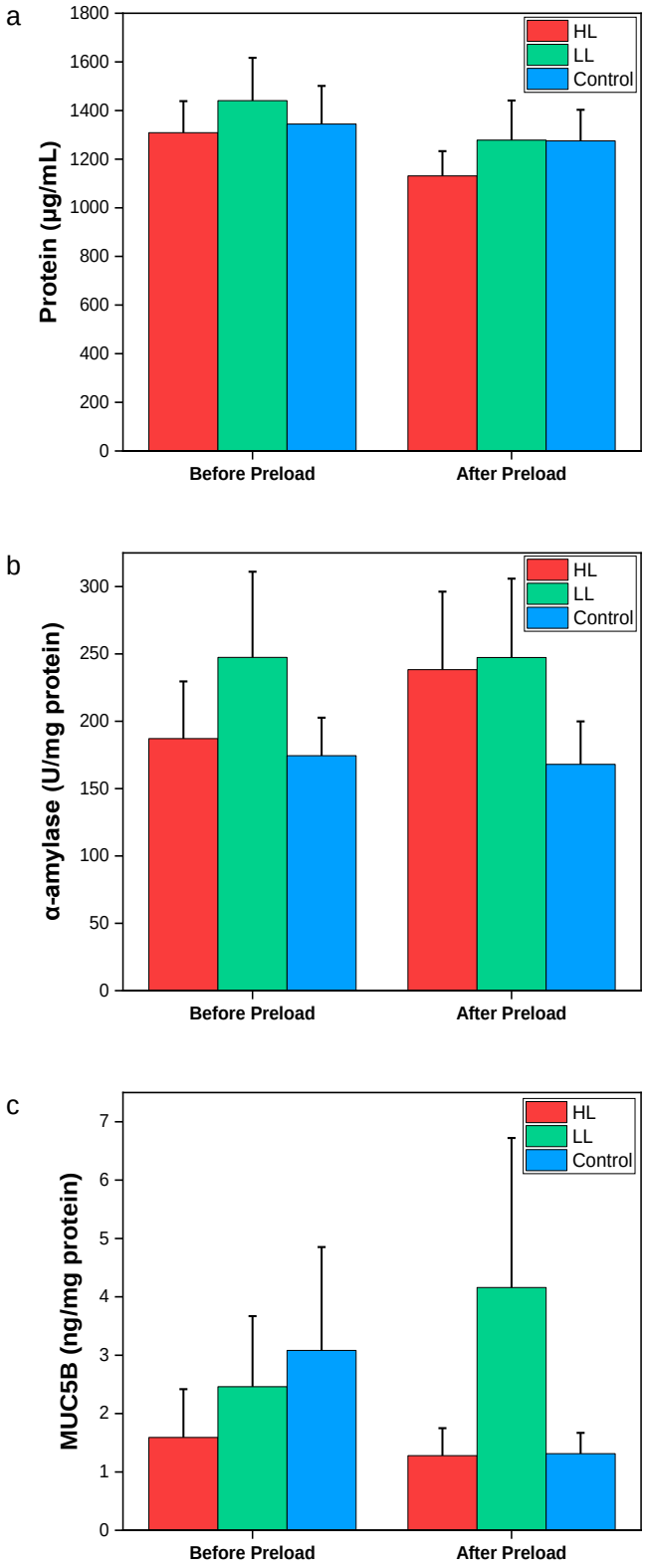

Figure 4.7. Total protein (µg/mL) (n=17) (a), α-amylase (U/mg protein) (n=17) (b), and MUC5B (ng/mg protein) (n=9) (c) in saliva for HL (high lubricating), LL (low lubricating) and Control conditions before preload and after preload. Values are means and error bars represent standard error of means (SEMs).

4.4.6. Pearson's correlation

To examine whether the changes after preload in appetite ratings, energy intake and tribological properties of saliva were related to salivary biomarkers, we performed Pearson's correlation between the aforementioned parameters for all interventions (HL, LL and Control) (see **Supplementary Table C.4**). Statistical association was noted between dessert (yogurt) and *protein* activity (r = 0.985; *p = 0.025*). Also, there was a statistical association between friction coefficient (tribology) of saliva and fullness (r = -0.991; p = 0.009, r = -0.995; p = 0.005) meaning that the lower the friction coefficient (which means higher lubricating properties of saliva), the higher the feeling of fullness which in line with our hypothesis. In the rest, there was no relation between appetite ratings, energy intake, tribological properties of saliva and salivary biomarkers (see **Supplementary Table C.4**). The results of the correlation need to be interpreted carefully as it is based on 4 participants only and this may explain the high correlation coefficients.

4.5. Discussion

In this article, we investigated the effect of oral lubricity on appetite control, food intake and salivary biomarkers using model foods *i.e.* hydrogels varying in their lubricating properties. Additionally, we explored the lubrication properties of human saliva after eating the hydrogels, as well as the relation between oral lubricity and appetite, food intake and salivary biomarkers. With regard to appetite ratings, an effect of HL (high lubricating hydrogels) on reducing hunger, desire to eat and prospective food consumption as well as increase in fullness was observed as compared to Control (water) immediately after ingestion, and 10 and 20 min thereafter. Although HL lowered appetite ratings such as hunger, desire to eat and prospective food consumption as well as increased the fullness ratings as compared to LL, difference

between HL (high lubricating) and LL (low lubricating) hydrogels on appetite was not significant. These findings suggest there was no effect of high lubricity versus low lubricity conditions on subjective appetite sensations in this study, however, there was an effect of HL (high lubricating hydrogels) condition compared to the Control. This is the first study to show an effect of oral lubricity on appetite sensations on a meal setting.

In a previous study by Krop et al. (2019a) employing hydrogels differing in their lubricating properties on appetite ratings in a snack trial there was reported no difference in appetite ratings. A potential explanation of inconsistency in outcomes between these two studies could be the study design in terms of appetite measurements. Krop et al. (2019a) measured appetite at lesser number of time points than our study. For instance, they rated the appetite before, immediately after preload and after *ad libitum* snack, whereas in the current study appetite was rated on two more time points after preload. Therefore, we showed the dynamic of appetite over a period of 30 min after ingesting the preloads differing in their lubricating properties, and a significant suppression of appetite in HL condition compared to Control was noted. It is also noteworthy that appetite sensations returned to their initial level after 20 min after ingesting the preload. This suggests that the lubricity may have a brief effect on appetite sensations. However, one should be careful in interpreting these results as in addition to lubricity the preloads could have been confounded by a variety of other factors (taste, palatability, different oral processing - solid vs liquid, expected satiety etc.).

It is worth pointing that the energy intake was similar in all the three conditions HL, LL and Control. These findings are not in agreement with other studies dealing with textural complexity (Krop, et al., 2019a; Larsen, et al., 2016; Tang, et al., 2016). For instance, Krop et al. (2019a) demonstrated that the snack intake was lowered in high lubricating hydrogels as compared to low lubricating hydrogels. To explain the inconsistency in results, the following factors should be taken into account. Firstly, literature shows that the longer the time between

the intervention and the next meal, the weaker is the effect of preload on subsequent food intake (Blundell, et al., 2010; Rolls, et al., 1991; Stribiţcaia, et al., 2020a). Secondly, the energy density of the preload plays a role too. Studies that had the preload with a low energy density had a shorter time interval between intervention and next meal (Krop, et al., 2019a; Larsen, et al., 2016; Stribiţcaia, et al., 2020a; Tang, et al., 2016). As such, the preload in our study was free of energy density and macronutrients, and the time between the preload and *ad libitum* next meal was of 30 min. Whereas other studies with a reduced energy density of the preload had a reduced time to the next meal *i.e.* 10 min after preload (Tang, et al., 2016) or immediately after preload (Krop, et al., 2019a). Thus, the long-time interval between the preload and *ad libitum* lunch in our study may have diminished the effect of lubricity on food intake.

Interestingly, appetite ratings returned to the baseline 20 min after the intervention, which means that the appetite sensations were the same in all three conditions before serving the *ad libitum* lunch. This also may explain the lack of significant differences between conditions regarding food intake that was associated with the time interval between the intervention and the *ad libitum* lunch. Therefore, we can infer that effect of lubricity of hydrogels on food intake is time dependent *i.e.* the 30 min time between the preload and the next meal in this study might be too long to show an effect on food intake. As such, it may imply a very short time effect or an immediate effect on the subsequent food intake as it was seen in a similar recent study (Krop, et al., 2019a). Thus, for future research addressing the role of lubricity on subsequent food intake, the time between preload and next *ad libitum* meal should be short or even immediately after preload. Also, it would be of high interest to investigate the effect of oral lubricity on satiation. Therefore an *ad libitum* intake design of the model food differing in their lubricating properties would add better understanding on this matter.

With regard to lubricating properties of saliva after ingesting the preload, we could not detect any significant differences between interventions *i.e.* saliva had the same level of lubricity regardless the preload varying in lubricity. In other words, this means that the lubricating properties of the hydrogels did not translate into physiologically detectable increase or decrease in lubrication property of the saliva after consumption of the preload. It is known that lubricating properties of saliva depend on the presence of salivary proteins such as mucins (Aguirre, et al., 1989; Hahn Berg, Lindh, & Arnebrant, 2004), statherins (Douglas, et al., 1991; Hahn Berg, et al., 2004), α-amylase (Aguirre, et al., 1989) and others. In fact, no differences between the interventions were observed regarding the presence of proteins, α-amylase and mucin in saliva. Therefore, this may explain the lack of significant difference in lubricating properties of saliva after preload. Likewise, this suggests a potential correlation between the presence of salivary biomarkers and lubricating properties of saliva as was observed by (Hopkins, et al., 2020). An important factor to consider while interpreting lubricating properties of saliva, is the inter-individual variation. It is worth noting that the variations among individuals were very large irrespective of the conditions and time to detect any noticeable difference.

In terms of salivary biomarkers, there was no significant differences in protein, α-amylase and MUC5B concentration in saliva between interventions. Interestingly, a trend could be noted where the total concentration of protein in saliva seemed to slightly decrease after preload compared to before preload in all three conditions (HL, LL and Control). One might argue that this is linked to the fact that although unstimulated saliva was collected, it was stimulated enough by the preload resulting in lowering in protein concentration (Al-Manei, Almotairy, Bostanci, Kumar, & Grigoriadis, 2020). Although not significant, an increase of total α-amylase concentration in saliva in HL and LL condition compared to Control was noted, which might suggest that there could be some association between external lubricity of preloads

and the α-amylase activity. However, these results must be interpreted with caution, as we did not detect any significant statistical difference. It is known that α-amylase secretion is initiated more in the presence of starch or after ingesting starch-based food (Froehlich, Pangborn, & Whitaker, 1987) and α-amylase is often used as an objective measure of satiety in starch based food, such as starch-based custard (Harthoorn, 2008).

The total MUC5B concentration slightly decreased after preload in HL and Control condition, but increased in LL condition, though not significant. It might be linked to the fact that the hydrogels (HL) was lubricating enough that it did not require intrinsic lubrication salivary mucins. However, interpreting such data in lack of statistical significance can be challenging as MUC5B levels might be affected by the degree of stimulation by the hydrogels, age of the participants and time of the day of the intervention *etc.* (Helmerhorst & Oppenheim, 2007; Mariscal, et al., 2019). Overall, it can be inferred that subtle changes in lubricity of samples might not alter the biochemical components of saliva. Factors such as macronutrients, energy density of the food (Harthoorn, 2008) might play an important role in the physiological aspect of satiety and satiation, and thus are worthwhile to explore in conjunction with lubricity in future research**.**

4.5.1. Limitations and strengths

A limitation of this study is the sample size, it was smaller than planned due to pandemic, which influenced the results. Measuring saliva after breakfast did not give us any relevant information, therefore, future studies should focus on two or three time points of saliva collection after preload with one immediately after consuming the preload. Also, as there was a change in statistical analysis (controlling baseline values of the appetite ratings and removing the outliers) that has not been initially planned, is another limitation of this study. Nevertheless, a clear strength of this study is the measurements of saliva in terms of tribological aspects and biomarkers. This is first study that has attempted to link food texture (from an oral lubrication

perspective) to satiety together with salivary biomarkers, as well as lubricating properties of saliva, which presents a feasible approach to connect psychological aspects of appetite to physiological aspects of salivary properties. Also, using a within-subject (each of the participants acts as their own control) design gives a strong edge to the current study as recommended by the literature (Gibbons, et al., 2019).

4.6. Conclusion

In summary, when data are not controlled for baseline, model food (hydrogels) with higher lubricating properties showed to suppress appetite ratings compared to water, and such effect is brief. However, after controlling the data for baseline, the effect of intervention does not exist anymore. Therefore, the results should be interpreted with caution. No effect of lubricity on food intake and salivary biomarkers was found, which might be associated with the subtle change in lubrication between the preloads or the long time between the intervention and the measurement. Therefore, future research should reduce the time between preload and next *ad libitum* meal in order to demonstrate the immediate effect of lubricity on satiety and satiation. In addition, studies should also employ energy density and macronutrients/real food as opposed to non-calorific hydrogels to understand the combinatorial effect of calorie and lubricity to be closer to real food and test the effects on satiety.

Based on the findings of this chapter, we aimed to expand further on understanding the role of oral lubrication on satiety and satiation by developing calorific preloads, therefore investigating the combinatorial effect lubricity with macronutrients/energy load. As this was not possible, at that stage, due to Covid-19 pandemic lockdown (no human trial allowed), we designed a study that would be of relevance to the purpose of this PhD project. Therefore, protein beverages with different texture, more specifically, with different levels of viscosity were developed. The effect of different levels of viscosity, on perceived satiety through an

online survey where the viscosity levels of protein-based beverages were visually perceived using a newly developed video-based demonstration will be discussed in the next chapter.

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Chapter 5

Video based online questionnaire: the influence of viscosity on perceived satiety[5]

Abstract

Food texture seems to offer a promising strategy for the control of expected satiety, satiety, satiation and daily caloric intake. The aim of this study was to examine the effect of food texture, more specifically the effect of different levels of viscosity, on perceived satiety through an online survey where the viscosity levels of protein-based beverages were visually perceived using a newly developed video-based demonstration. Whey protein beverages were prepared with viscosities being manipulated using xanthan gum and their viscosity and tribological properties were measured instrumentally. Subjects (n=211) watched beverages being poured in videos streamed online and were instructed to imagine drinking them. The results showed that instrumentally measured HV (high viscous) and MV (medium viscous) beverages were visually perceived by the participants as being more satiating immediately and 2 h later after the imagined drinking event as compared to LV (low viscous) beverages ($p<0.05$). Also, sensory attributes such as visually perceived smoothness, thickness, creaminess and watery were shown to be important factors in the perception of satiety (the creamier or thicker the beverage the higher the perceived satiety scores). Therefore, a video-based online demonstration is a highly feasible and convenient tool to measure the effect of food texture on perceived/expected satiety that can be useful in Covid-19 pandemic situation, latter necessitates

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online participation in many situations. More importantly, key role of food/beverage texture expressed through visual cues alone, may open new avenues of informing consumers about the degree of the perceived satiety/fullness even before the product is consumed.

5.1. Introduction

Obesity is recognised as a major risk to the health of people across the world, and the problem is increasing dramatically (Deitel, 2003). The prevalence of obesity has nearly tripled over the last decades (WHO, 2018). Moreover, the overconsumption of foods is seen as one of the major determinants of obesity. Consequently, there has been a growing interest among scientists and food industries to design satiety-enhancing foods/beverages that would facilitate appetite control and would lead to a lower food intake in order to address global obesity crisis (Blundell, 2010; Chambers, McCrickerd, & Yeomans, 2015; Halford & Harrold, 2012).

Among the many features of food that influence eating and therefore affect satiety, food texture seems to be a promising strategy in the control of satiety, satiation and daily caloric intake (Stribiţcaia, Evans, Gibbons, Blundell, & Sarkar, 2020a). Satiation is the process believed to lead to the termination of eating, while satiety is the process that leads to the inhibition of the further eating during the inter-meal period (Blundell, et al., 2010). Recently a systematic review and a meta-analysis showed that texture of food may play a role in appetite control and the amount of food people eat, revealing that solid and high viscous foods/beverages can suppress appetite and reduce food intake to a greater degree when compared to liquid and low viscous foods/beverages (Stribiţcaia, et al., 2020a).

Moreover, it has been shown that food texture can also have an effect on expected satiety indicating that subtle manipulation of texture can increase expectations where thick drinks showed a greater expected satiety compared to thin drinks (McCrickerd, Chambers, Brunstrom, & Yeomans, 2012). Expected satiety is the extent to which foods/beverages are expected to confer satiety when they are compared on a calorie-for-calorie basis and has been

studied along with portion/plate size, energy density, macronutrients, labelling, food texture and other factors (Brunstrom, Collingwood, & Rogers, 2010; Chambers, Ells, & Yeomans, 2013; Crum, Corbin, Brownell, & Salovey, 2011; Nguyen & Varela, 2021; Nguyen, Wahlgren, Almli, & Varela, 2017), while expected satiation can be expressed through immediate fullness (Brunstrom, 2011; McCrickerd, Lensing, Yeomans, 2015). Considering texture, the literature indicates an independent effect on expected satiety. For instance, Hogenkamp et al. (2011) showed that texture rather than flavour determines expected satiety, where solid and semi-solid foods were perceived as being more satiating than liquid and semi-liquid foods. In addition, McCrikered et al. (2012) reported an effect of texture on expected satiety independently of energy load; thicker drinks (more viscous) were perceived by participants as being more filling than thinner drinks (less viscous). As such, the strong effect of texture alone on expected satiety was notable. Another important factor known to affect consumers judgement about food is labelling. The literature demonstrates an effect of the food labels on portion size (Brown, Rollo, de Vlieger, Collins, & Bucher, 2018), expected liking (Ekelund, Fernqvist, & Tjärnemo, 2007; Johansen, Næs, Øyaas, & Hersleth, 2010) and expected sensory characteristics of food (McGuinness, et al., 2022; McCrickerd, Tang, & Forde, 2020). Therefore, it is worth investigating to what extent label of food nutrition alongside texture would impact expeted satiety/satiation.

The mechanism by which food texture may influence expected satiety is that, from a cognitive perspective consumers may ‘feel’ that solid foods or thick beverages are more likely to be filling than liquid foods or thin beverages. In other words, consumers perceive that solid foods/thick beverages will contain more energy compared to liquid foods/thin beverages independent of their actual calories (de Graaf, 2012). Moreover, the perception of the role of food texture on satiety and satiation may be influenced through oro-sensory exposure time. It is known that solid foods/thick beverages need longer oral processing time as compared to

liquid foods/thin beverages (Krop, et al., 2018). This may lead to an increased oro-sensory exposure and appears to be essential in the perception of satiety or expected satiety (McCrickerd, et al., 2012). Accordingly, the learned experience or the learned association between the sensory attributes of food and the metabolic response of the food after ingestion may explain the way consumers perceive/anticipate the satiating capacity of the food they are consuming.

Interestingly, the literature on food texture and expected satiety contains studies where participants are given the product to taste it and are then asked to evaluate its filling properties or its expected satiety using various forms of questionnaires (Hogenkamp, et al., 2011; McCrickerd, et al., 2012). Such studies are invariably laboratory-based. There has been some online work/survey on expected satiety in relation to macronutrient composition and energy load of the products/food (Buckland, Stubbs, & Finlayson, 2015), where perceived satiety was associated with lower energy density, lower fat and higher protein. However, less is known about the effect of food texture on expected satiety when assessed indirectly through online surveys using visual cues.

Recently, online surveys have become recognised as an efficient tool, and have been used to adjust and adapt the research to the current Covid-19 related pandemic situation; and to gather data in a faster, easier and more sustainable way (Bayudan-Dacuycuy, Orbeta Jr, Serafica, & Baje, 2020; Berg, Furrer, Harmon, Rani, & Silberman, 2018). In this context, an online survey clearly cannot directly measure a person's response to the taste or textural differences between foods. However, an interesting question arises about whether the effects of texture can be evaluated when foods are presently visually in a screen-based survey when the visual perception of texture of a beverage can be demonstrated using a video-recording. In such a situation, would visual cues alone be enough to convey the texture of a food to influence the feeling of perceived fullness?

A further factor to consider is whether food texture conveyed through such video-recording based visual cues influences food reward which incorporates the dimensions of "liking" and "wanting" (Finlayson, King, & Blundell, 2007). According to the definitions of Berridge, liking refers to the palatability (pleasure of eating a given food) and wanting refers to the disposition to eat (Berridge, 1996; Berridge, 2007). It is known that food with higher palatability can lead to a greater food intake (Spiegel, Shrager, & Stellar, 1989). Moreover, seeing the preferred food can increase hunger (Hill, Magson, & Blundell, 1984) suggesting that the palatability of food may have an effect on anticipated stimulation of appetite. However, little is known in regard to "liking" and "wanting" from a textural perspective of food and expected satiety. Therefore, liking and wanting was measured in this planned online video-based survey.

It is also known that sensory attributes can influence the expected satiety (Forde, Almiron-Roig, & Brunstrom, 2015). For instance, manipulating the thickness level in beverages can lead to different sensory perception in terms of smoothness and creaminess (Camps, Mars, de Graaf, & Smeets, 2016). It was shown that the more viscous the beverage was, the participants perceived them as being smoother and creamier. Therefore, it was important to investigate if such differences in sensory attributes such as smoothness (i.e. (absence of lumps), creaminess can be also observed or detected, to some extent, in video-based online survey. Furthermore, it was worth investigating whether there could be any relationship between such sensory attributes and expected satiety, in other words if sensory attributes can influence the perceived/expected satiety to some extent.

Understanding if food texture can have an impact on the way consumers perceive its filling/satiating value (before they consume the product/food) could be important to enable them to choose more filling/satiating food that would contribute to the overall control of consumption. In turn, this would inform the food industry sector on the development of satiety

enhancing foods/beverages. Therefore, the aim of this study was to assess the effect of food texture, more specifically the effect of different levels of viscosity on perceived/expected satiety or on ratings of fullness through an online survey where the viscosity levels were demonstrated using video recording of samples. In other words, the impression of viscosity of the foods was conveyed by means of a video of beverages, varying in thickness/viscosity, being poured from one container to another. The beverages were prepared using whey protein with viscosity being manipulated using xanthan gum and their viscosities and tribological properties were measured instrumentally. Also, we investigated if there is a relationship between liking and wanting of beverages differing in their texture/viscosity, and perceived satiety or perceived fullness. The relationships among other visually perceived sensory attributes, such as smoothness, watery, creaminess and perceived satiety/fullness were also assessed. As a secondary aim, we also investigated whether there was any relationship between instrumentally measured parameters and visually perceived texture/ sensory attributes. In summary, this investigation employed a highly feasible yet simple method of an online survey with video recordings of food samples to assess the impact of the perception of food texture (viscosity) observed in the screen on perceived satiety and on elements of food reward and can be a highly feasible remote sensory testing approach in current pandemic situation.

5.2. Materials and methods

5.2.1. Participants

Participants (n=245) were recruited through University email distribution lists, social network platforms and Prolific online participant recruitment platform. Adults >18 years old possessing basic level of English skills (reading/ writing) could take part in the survey. From the total number of the participants who entered the survey, 87.92% (n=211, 57.1% females (121)) completed the entire survey. Of the whole sample who completed the survey (n=211), 37.7%

(n=80) were employed full-time, 36.3% (n=77) were students, 12.7% (n=27) were employed half-time, 8.9% (n=19) were unemployed, 2.4% (n=5) were housewife/ househusband, 0.5% (n=1) were retired, 0.5% (n=1) were unable to work due to health disability and 0.5% (n=1) preferred not to declare their employability status. Participants were aged 18-64 years (average 28.95 ± 9.34) with a BMI calculated from self-reported height and weight that ranged between 17.44-52.66 kg/m² (average 25.01 ± 6.68).

5.2.2. Beverages preparation and characteristics

All the beverages were designed and prepared in the Food Science and Nutrition School Pilot Plant at the University of Leeds. The beverages were made from whey protein isolate powder – 15 g per 100 mL water. The viscosity of the beverages was manipulated by adding xanthan-gum (see **Table 5.1** for the recipe of the beverages). The beverages had three levels of viscosity: low viscous (no xanthan-gum added), medium viscous (0.5 g xanthan-gum per 100g of solution) and high viscous (1 g xanthan-gum per 100 g of solution). A total of 200 mL of protein beverage was prepared for each condition. Whey protein isolate was purchased from MYPROTEIN (Manchester, UK). The xanthan gum was purchased from Special Ingredients (Special Ingredients Ltd, Chesterfield, UK). The whey protein powder was dissolved in distilled water and left to stir on a magnetic stirring plate for 2 h until a complete hydration was obtained. Afterwards, xanthan gum was added to the protein solution and the solution was left to stir for 2 h. Finally, the beverages were blended for 1 min with a hand blender (Braun, Germany). Immediately after preparation, short videos of each beverage were recorded.

Table 5.1. Recipe of the beverages

	LV[a]	MV[b]	HV[c]
Whey Protein (g)	30	30	30
Water (g)	170	169.5	169
Xanthan-gum (g)	0	0.5	1
Total (g)	**200**	**200**	**200**

[a]Low viscous
[b]Medium viscous
[c]High viscous

5.2.3. Videos of the beverages

Each beverage was placed on a mini portable photo studio box (Bodhi200, UK) and short videos were taken of each beverage using a video camera (mobile phone camera). Each video shows the beverages being poured from one container into another (**Figure 5.1a-f** - screenshot of the videos). For the full videos see **Supplementary Table D.1**. A total of 200 mL of each protein solution (low, medium and high viscous) was poured into a transparent glass, where the viscosities were measured instrumentally. On average, each video lasted 12 s. In each video, a label about the protein content was added: high and low. As such, participants saw 6 short videos containing beverages differing in their viscosity (3 levels – low, medium and viscous) and protein content (2 levels – low and high). Hereafter, the beverages are referred throughout the article as: LVLP (low viscous/ low protein), LVHP (low viscous/ high protein), MVLP (medium viscous/ low protein), MVHP (medium viscous/ high protein), HVLP (high viscous/ low protein) and HVHP (high viscous/ high protein). Note, the protein content was not changed in the actually prepared beverages. As this study presented visual cues, the protein content was indicated only using the labels. There was no actual differential manipulation of protein content (all samples contained a standard 30 g whey protein). The label manipulation was included to

test any possible effect of a perceived protein difference on the ratings of visually perceived satiety.

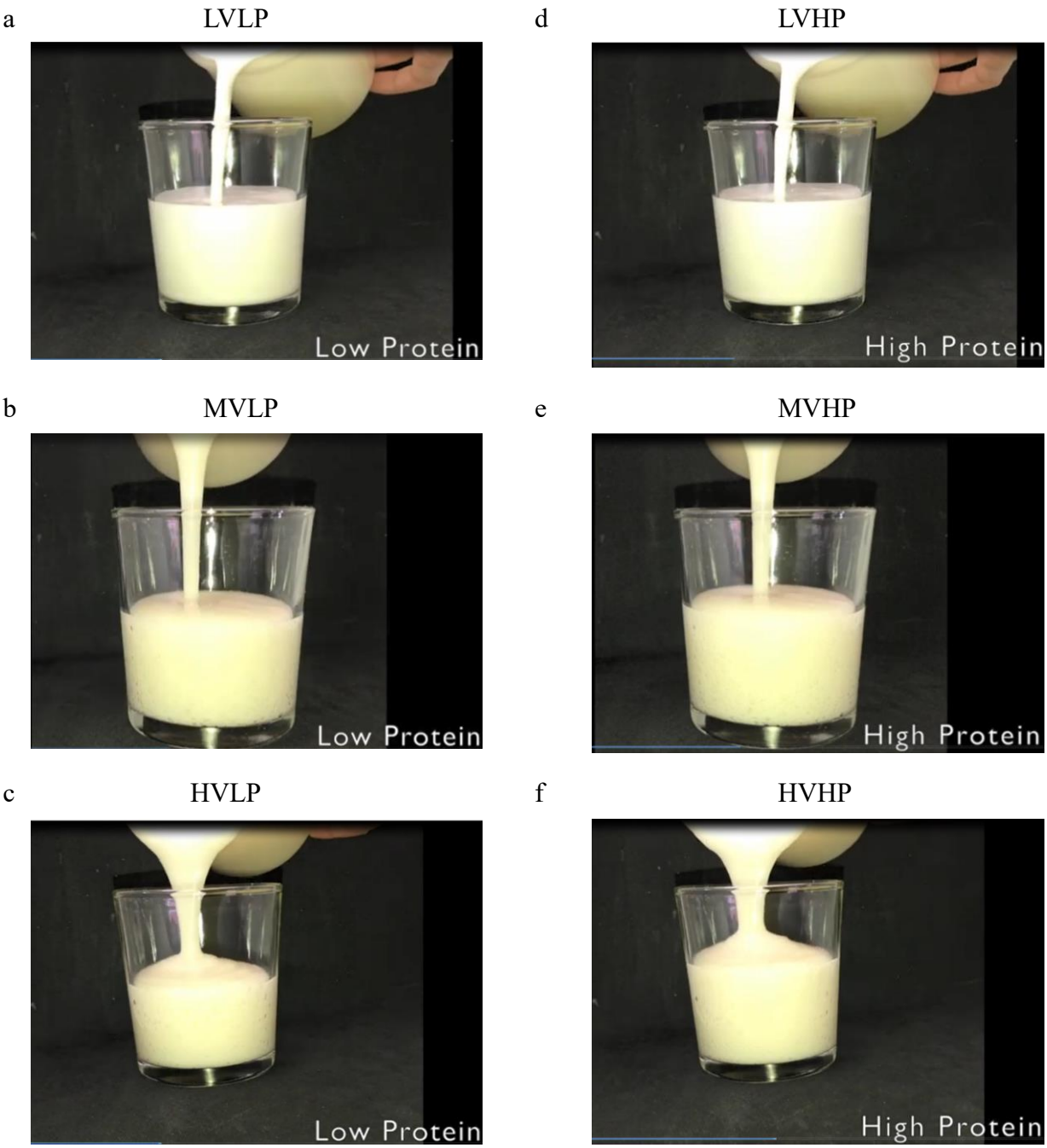

Figure 5.1. Images showing the beverages being poured from one container into another (transparent glass). In total there were six beverages: a) LVLP; b) MVLP; c) HVLP; d) LVHP; e) MVHP and f) HVHP. The images are screenshots of the videos. For the full videos see Supplementary Table S1.

5.2.4. Apparent viscosity and lubricity of the beverages

The apparent viscosity of the beverages was measured with a rheometer (Kinexus Ultra+, Malvern Instruments Ltd, Worcestershire, UK) using a plate-plate geometry (diameter 60 mm) with a gap size of 0.5 mm. The samples were sealed off with a thin layer of silicone oil to prevent evaporation. Flow curves were obtained for all beverages after simulated oral processing at shear rates ranging from 0.01 to 1000 s^{-1} at 37 °C. A minimum of three replicates were measured for each beverage sample.

Although it is very difficult or almost impossible to assess lubricity visually, an instrumental analysis of frictional coefficients was performed. It is known that lubricity of food/ beverages can be translated into sensory attributes that can be perceived by consuming the food such as smoothness, pastiness or creaminess that can also influence satiety (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019a; Krop, Hetherington, Miquel, & Sarkar, 2019b; Sarkar & Krop, 2019; Sarkar, Soltanahmadi, Chen, & Stokes, 2021; Stribițcaia, et al., 2021; Stribiţcaia, Krop, Lewin, Holmes, & Sarkar, 2020b). A soft tribology measurement was carried out to measure the lubricating properties of the beverages and a relation (if any) between these (instrumental and visually perceived sensory attributes) was examined. Lubricity of the beverages was measured using a MTM2 Mini-Traction Machine (PCS Instruments, UK). Polydimethylsiloxane (PDMS) ball (diameter of 19 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) and disc (diameter of 46 mm, thickness of 4 mm) were used for the measurements (surface roughness of PDMS tribopairs, R_a < 50 nm). Approximately 30 g of the protein beverages of different viscosities was loaded onto the pot equipped with the PDMS disc; the ball was lowered onto the disc and then the pot was covered with a lid. The entrainment speed was decreased from 0.3 to 0.001 m s^{-1}, and the friction coefficients were recorded at slide-roll-ratio of 50 % at 2 N load with a Hertzian contact pressure of ~200 kPa

(Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019). The temperature was set and maintained at 37 °C, to imitate the temperature at which oral processing occurs. A minimum of three repetitions were carried out for each sample.

5.2.5. Measure of perceived satiety, liking, wanting and sensory attributes

Participants rated visually perceived satiety/fullness, liking, wanting, sensory attributes of the beverages (smoothness, thickness, creaminess, watery) and initial appetite sensations (before rating the perceived satiety of the beverages) using a visual analogue scale (VAS) of 100 mm anchoring from 'Not at all' to 'Extremely', which has been shown to be valid and reliable for appetite research (Flint, Raben, Blundell, & Astrup, 2000; Stubbs, et al., 2000), including expected satiety (Forde, et al., 2015). Participants were asked to rate perceived satiety immediately after observing the pouring of the protein beverages in the video and 2 h later. Participants were instructed to imagine how full they would be immediately after drinking the beverages and 2 h later. **Table 5.2** shows the questions showed to the participants in the video-based online questionnaire used to assess all the subjective attributes mentioned above.

Table 5.2. Subjective attributes and questions assessed in the online survey.

Subjective attributes	Questions
a) Immediately perceived expected satiety/ fullness	a) How full do you think you will be immediately after eating this portion of food?
b) Perceived expected satiety/ fullness 2 h after	b) How full do you think you will be 2 hours after eating this portion of food?
Smoothness	How smooth do you think this drink is?
Thickness	How thick (viscous) do you think this drink is?
Watery	How watery do you think this drink is?
Creaminess	How creamy do you think this drink is?
Liking	How pleasant does this drink typically taste?
Wanting	How much do you want to consume this drink right now?

5.2.6. Procedure

After receiving the invitation to take part into the online survey, participants clicked on a link that directed them to the on line survey (Qualtrics XM Platform, USA, www.qualtrics.com). The experimental protocol of this study was approved by the University of Leeds, Faculty Research Ethics Committee (AREA 20-133, June 2021). Firstly, participants were provided with a participant information sheet with details about the survey and then informed consent was obtained before participants could proceed further. Participants then indicated their age, gender, employment status, self-reported their height and weight, and rated their initial appetite (hunger, fullness, desire to eat, prospective food consumption and thirst) on a VAS scale of 100 mm anchored from 'Not at all' to 'Extremely'. After this, participants were presented with the first video showing the beverage being gradually poured into a transparent glass (see **Figure 1.5** for screenshot and **Supplementary Table D.1** for full videos). Participants were asked to

watch each video carefully once or twice and answer several questions (see **Table 5.2** for the questions) related to the video they had just watched. In total, there were 6 videos showing different textures (3 levels – low viscous, medium viscous and high viscous) and labels of protein content (2 levels – low protein and high protein). Each video followed by questions was presented on a separate page. After completing the survey, participants entered in to a prize draw to win 1 × £50, 2 × £20 and 3 × £30. Participants who were recruited through Prolific platform have been remunerated according to the platform suggestion - £7.5/ h. Between 15 and 25 min were needed to complete this video-based online survey.

5.3. Statistical analysis

Data are presented as mean and standard deviations (SDs) in the text and tables, and as means and standard errors of means (SEMs) in the figures. All statistical analyses were performed using SPSS (IBM® SPSS® Statistics, v26, SPSS Inc, Chicago, USA). Differences between conditions were tested by repeated measures ANOVA for perceived satiety/ fullness. The differences in sensory attributes: smoothness, thickness, watery, creaminess, and liking and wanting of the protein beverages, were also assessed by repeated measures ANOVA. 3 × 2 level factorial repeated measure ANOVA was used to examine the main effect of the texture/viscosity (LV, MV, HV), protein content (LP, HP) and texture*protein content interaction on perceived satiety/fullness ratings. Where the assumption of sphericity had been violated, indicated by Mauchly's test, Greenhouse-Greisser corrected tests are reported. Statistical significant differences were calculated by Bonferroni corrected post-hoc t-tests and was set at $\alpha < 0.05$ level. Pearson correlations were performed to assess the relationship between perceived satiety/fullness ratings and sensory attributes and liking and wanting. Relationship between initial hunger state/rating and the perceived/expected satiety was

assessed. In addition, the relationship between instrumental analysis and visually perceived sensory attributes were evaluated.

5.4. Results

5.4.1. Instrumental characteristic of the beverages and the relationship with visually perceived sensory attributes

Figure 5.2a shows the apparent viscosity of the beverages. It can be seen that the level of viscosity differed significantly between the beverages at orally relevant shear rate of 50 s^{-1} with HV (high viscous) having the highest mean: 321 mPa.s; followed by MV (medium viscous): 102 mPa.s and LV (low viscous): 15 mPa.s. In other words, addition of xanthan gum had a marked effect on increasing the viscosity of the whey protein beverages (Philips & Williams, 2000). Both HV and MV had a classic shear-thinning behaviour but LV had a Newtonian behaviour (**Supplementary Figure D.1a**). The difference in viscosity between the beverages was also obvious from the video demonstrations (see **Supplementary Table D.1**).

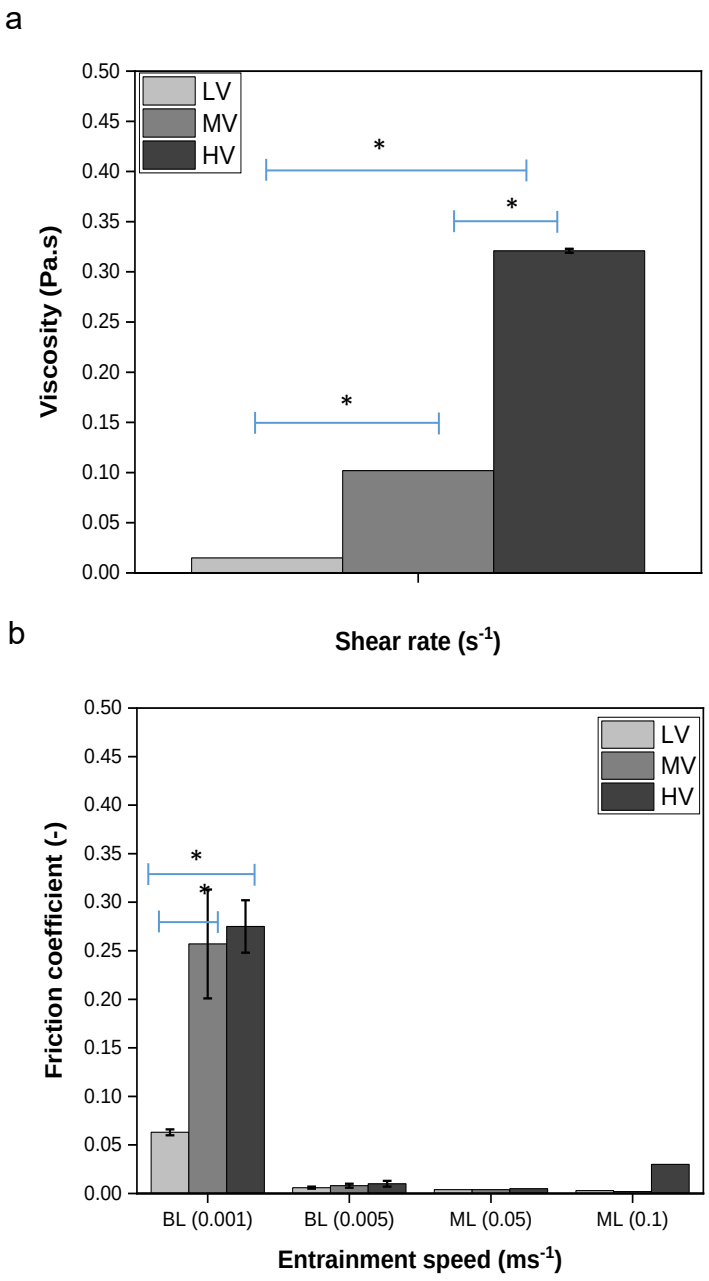

Figure 5.2. Instrumental viscosity (a) as a function of shear rate (50 s-1) and lubricity analysis (b) where data is expressed as friction coefficients at boundary (0.001; 0.005 m s-1 speed) and mixed (0.05 m s-1; 0.1 m s-1 at speed) lubrication regimes for the HV (high viscous), MV (medium viscous) and LV (low viscous) beverages, respectively at various speeds. Error bars represent standard error of means (SEMs). Significant differences between the beverages are shown by the blue lines with asterisks above each line. A lower friction coefficient represents higher lubrication properties of the beverages. BL = boundary regime, ML = mixed regime.

In terms of the lubricity of the beverages (**Figure 5.2b**), a significant difference between the friction coefficient of HV and LV, and between MV and LV beverages was observed in the boundary lubrication regime only (BL 0.001 m s^{-1}) (see **Supplementary Figure D.1b** for the friction coefficient versus entrainment speed curves). This means that the LV beverage containing no xanthan gum was the most lubricating as compared to the MV and HV ones (the lower the friction of coefficient the higher the lubricating properties of food/beverages) in the BL owing to the surface properties of whey protein, which has been previously reported (Kew, Holmes, Stieger, & Sarkar, 2021; Zembyla, et al., 2021).

A statistical relationship (see **Table 5.3**) between visually perceived smoothness and friction coefficient in boundary regime (BL 0.001 and BL 0.005; r=-0.909, $p<0.05$; r=-0.999, $p<0.001$ respectively) was noted. This means that the lower the friction coefficient (which means higher lubricating properties of the beverages), the higher the perceived of smoothness; and this suggests that 'smoothness' can be an important lubricating-related attribute (Kokini, Kadane, & Cussler, 1977; Upadhyay & Chen, 2019). More importantly, this suggests visually perceived smoothness inversely correlates with friction coefficient, which is similar to that obtained using taste-based perception of smoothness reported previously (Upadhyay, et al., 2019). In addition, there was a positive relationship between smoothness and watery ($r = .930$, $p<0.001$) and an inverse relationship between smoothness and thickness ($r = -.932$, $p<0.001$), which was not expected. Creaminess (r=-0.953, $p<0.001$) and thickness (r=-0.996, $p<0.001$) were found to inversely correlated with watery, which appears to be logical.

Table 5.3. Pearson's correlations between perceived sensory attributes (smoothness, thickness, creaminess and wateriness) and instrumental viscosity analysis as a function of shear rate (50 s-1) and lubricity analysis where data is expressed as friction coefficient at boundary (0.001; 0.005 m s^{-1} speed) and mixed (0.05 m s^{-1}; 0.1 m s^{-1} at speed) lubrication regimes for the HV (high viscous), MV (medium viscous) and LV (low viscous) beverages. Green colour indicates positive and orange colour a negative correlation with *p < 0.05* in light colours and *p < 0.01* in the darker shades.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	Smoothness	***Thickness***	***Creaminess***	***Wateriness***	***Viscosity at 50 s^{-1}shear rate***	***Lubr. 0.1 m s^{-1}***	***Lubr. 0.05 m s^{-1}***	***Lubr. 0.005 m s^{-1}***	***Lubr. 0.001 m s^{-1}***	***Liking***	***Wanting***
1	1										
2	-.932**	1									
3	-0.789	.957**	1								
4	.930**	-.996**	-.953**	1							
5	-0.781	0.718	0.592	-0.690	1						
6	0.416	-0.405	-0.367	0.446	0.242	1					
7	-0.579	0.526	0.424	-0.489	.961**	0.500	1				
8	-.909*	.841*	0.701	-.822*	.970**	0.000	.866*	1			
9	-.999**	.933**	0.791	-.934**	0.771	-0.432	0.565	.902*	1		
10	0.725	-0.443	-0.184	0.438	-0.706	0.091	-0.605	-0.751	-0.716	1	
11	-0.085	0.325	0.473	-0.317	-0.283	-0.540	-0.406	-0.157	0.092	0.537	1

5.4.2. Effect of protein beverages differing in texture on perceived satiety (immediate and 2 h later)

The effect of protein beverages differing in viscosity and protein label content on perceived satiety is shown in **Figure 5.3a** (see **Supplementary Table D.2a** for means and SDs values). There was an effect of viscosity ($F(2,420) = 240.06$, $p<0.001$), no effect of protein label content ($F(1,210) = 2,53$, $p=0.113$), and there was an interaction between texture*protein label content on perceived satiety/fullness immediately after drinking ($F(2,420) = 4.922$, $p=0.008$). The pairwise comparison tests revealed that in the low protein label content condition immediate perceived satiety/fullness was significantly higher in HV compared to LV ($p<0.05$) and in MV compared to LV ($p<0.05$). The same pattern was noted in high protein content, where perceived satiety/fullness was significantly higher in HV compared to LV ($p<0.05$) and in MV compared to LV ($p<0.05$). Also, here perceived satiety/fullness was significantly higher in HV compared to MV ($p<0.05$).

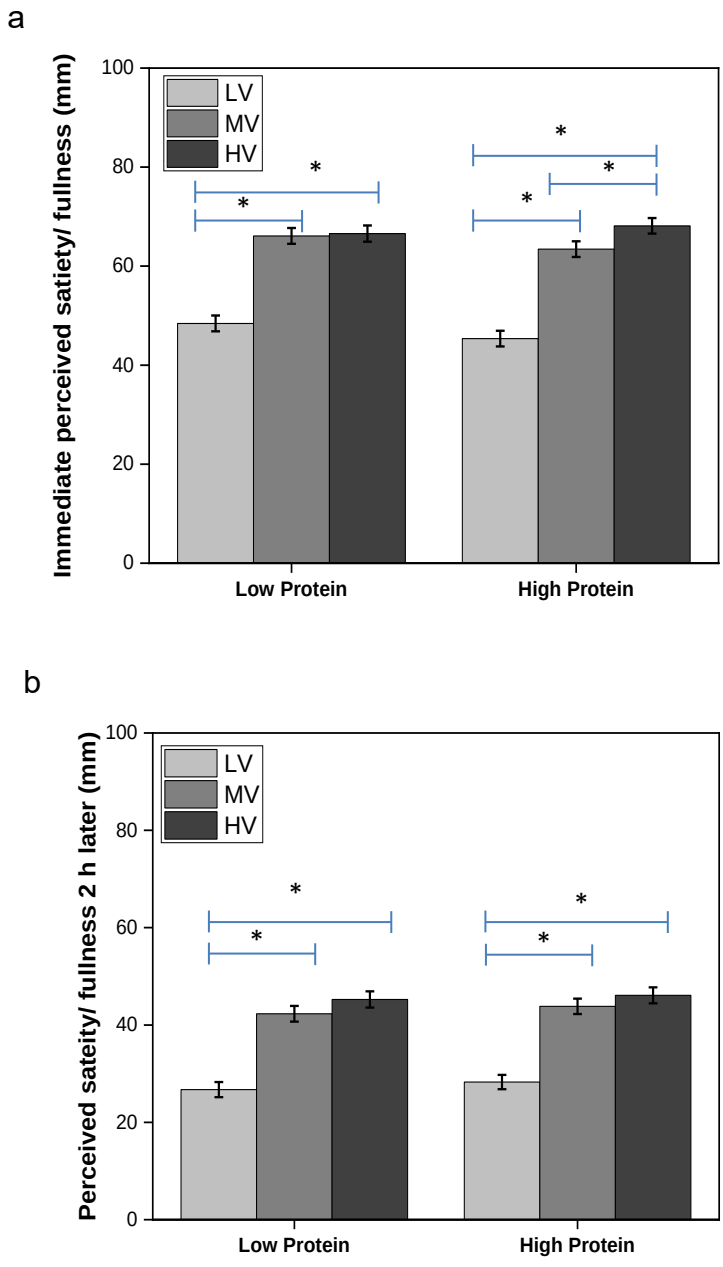

Figure 5.3. Mean and standard error of means (±SEM) of immediate perceived satiety/fullness (a) and 2h later (b) of the protein beverages in the low and high protein conditions between low viscous (LV – grey), medium viscous (MV – light grey) and high viscous (HV – dark grey). Significant differences between the beverages are shown by the blue lines with asterisks above each line.

The effect of protein beverages differing in viscosity and protein label content on perceived satiety after 2h is shown in **Figure 4.3b** (see **Supplementary Table D.2b** for means and SDs values). There was an effect of viscosity ($F(2,420) = 177.379$, $p<0.001$), no effect of protein content ($F(1,210) = 1.384$, $p=0.241$), and no effect of interaction texture*protein label content on perceived satiety/fullness 2 h later ($F(2,420) = 0.154$, $p=0.857$). The pairwise comparison tests revealed that in the low protein label content condition, the perceived satiety/fullness 2 h later was significantly higher in HV compared to LV ($p<0.05$) and in MV compared to LV ($p<0.05$). Also, here perceived satiety/fullness was significantly higher in HV compared to MV ($p<0.05$). In the high protein content, perceived satiety/fullness was significantly higher in HV compared to LV ($p<0.05$) and in MV compare to LV ($p<0.05$).

5.4.3. Visually perceived sensory evaluation, and liking and wanting of the beverages

The means and SDs values of the visually perceived textural attributes, liking and wanting of the beverages are shown in **Table 5.4**.

Table 5.4. Means and SDs for visually perceived sensory attributes, liking and wanting ratings for the beverages.

	Low protein			High protein		
	LV	**MV**	**HV**	**LV**	**MV**	**HV**
Smoothness	81.27 ± 19.76 [a]	53.46 ± 26.71 [b]	50.22 ± 26.65 [b]	80.79 ± 19.90 [a]	54.32 ± 24.42 [b]	51.29 ± 25.18 [c]
Thickness	48.33 ± 24.46 [a]	76.57 ± 17.59 [b]	77.27 ± 19.39 [b]	25.94 ± 23.04 [a]	69.41 ± 18.44 [b]	75.18 ± 17.21 [c]
Creaminess	60.08 ± 23.70 [a]	69.81 ± 21.67 [b]	69.32 ± 23.32 [b]	38.14 ± 23.44 [a]	63.91 ± 20.59 [b]	66.13 ± 22.46 [b]
Watery	47.59 ± 25.74 [a]	22.37 ± 18.21 [b]	22.43 ± 19.27 [b]	69.27 ± 25.39 [a]	24.92 ± 17.20 [b]	22.72 ± 17.60 [b]
Liking	54.19 ± 25.94 [a]	50.70 ± 28.12 [a]	48.07 ± 29.02 [b]	50.07 ± 25.36	49.16 ± 27.79	48.92 ± 29.03
Wanting	38.54 ± 29.97	39.04 ± 31.07	37.88 ± 31.61	37.78 ± 27.50	38.10 ± 29.49	38.11 ± 30.58

A statistical significance ($p>0.05$) between conditions is denoted by different letters in superscripts (^{abc}).

Smoothness. There was an effect of viscosity ($F(2,416) = 295.275$, $p<0.001$), no effect of protein label content ($F(1,208) = 0.376$, $p=0.540$), and no interaction between texture*protein label content on the perception of smoothness ($F(2,416) = 0.204$, $p=0.816$). The pairwise comparison tests revealed that in the low protein content condition perceived smoothness was significantly higher in LV compared to MV and HV ($p<0.05$). In the high protein condition, again, perceived smoothness was higher in LV compared to MV and HV ($p<0.05$). Also, here perceived smoothness was higher in MV compared to HV ($p<0.05$).

Thickness. There was an effect of viscosity ($F(2,420) = 477.113$, $p<0.001$), an effect of protein label content ($F(1,210) = 121.528$, $p<0.001$), and there was an interaction between texture*protein label content on the perception of thickness ($F(2,420) = 54.104$, $p<0.001$). The pairwise comparison tests revealed that in the low protein content condition perceived thickness was significantly higher in MV and HV compared to LV ($p<0.05$). In the high protein condition, again, perceived thickness was higher in HV compared to MV and LV ($p<0.05$). Also, here perceived thickness was higher in MV compared to HV ($p<0.05$).

Creaminess. There was an effect of viscosity ($F(2,420) = 114.439$, $p<0.001$), an effect of protein label content ($F(1,210) = 108.394$, $p<0.001$), and there was an interaction between texture*protein label content on the perception of creaminess ($F(2,420) = 54.81$, $p<0.001$). The pairwise comparison tests revealed that in the low protein label content condition perceived creaminess was significantly higher in MV and HV compared to LV ($p<0.05$). In the high protein condition perceived creaminess was higher again in HV compared to MV and LV ($p<0.05$).

Watery. There was an effect of viscosity ($F(2,416) = 429.867$, $p<0.001$), an effect of protein label content ($F(1,208) = 68.902$, $p<0.001$), and there was an interaction between texture*protein label content on the perception of wateriness ($F(2.416) = 71.228$, $p<0.001$).

The pairwise comparison tests revealed that in the low protein label content condition perceived wateriness was significantly higher in LV compared to MV and HV ($p<0.05$). In the high protein condition, again, perceived wateriness was higher again in LV compared to MV and HV ($p<0.05$).

Liking. There was an effect of viscosity ($F(2,420) = 4.194$, $p=0.016$), but no effect of protein label content ($F(1,210) = 3.173$, $p=0.076$), and an interaction between texture*protein label content on liking of the beverages ($F(2.420) = 5.275$, $p=0.005$). The pairwise comparison tests revealed that in the low protein label content condition liking was significantly higher in LV and MV compared to HV ($p<0.05$). In the high protein condition, there was no significant difference between beverages ($p>0.05$).

Wanting. There was no effect of viscosity ($F(2,412) = 0.096$, $p=0.908$), or effect of protein label content ($F(1,206) = 0.005$, $p=0.943$), or interaction between texture*protein label content on wanting of the beverages ($F(2,412) = 0.218$, $p=0.804$). The pairwise comparison tests revealed that neither in the low protein label content nor in the high protein label content condition was there any significantly difference in wanting between the beverages ($p>0.05$).

5.4.4. Relationship between visually perceived sensory attributes, liking, wanting, initial hunger and perceived satiety/fullness.

It is important to understand the relationship (if any) between the visually perceived sensory attributes (*e.g.* smoothness, creaminess, thickness and watery), liking and wanting with the perceived satiety. There was a positive relationship between thickness and immediate perceived satiety, and perceived satiety 2 h later in all conditions: LVLP, MVLP, HVLP, LVHP, MVHP and HVHP, ($p=0.01$) (see **Supplementary Table D.3 – a, b, c, d, e, and f**). Also, a positive relationship was noted between creaminess and immediately perceived satiety in HVLP, LVHP and MVHP conditions, ($p<0.005$), (see **Supplementary Table D.3 – c, d and e**). A negative relationship could be noted between smoothness and immediate perceived

satiety, and perceived satiety 2 h later in HVLP and LVHP conditions ($p<0.05$), (see **Supplementary Table D.3 – c and d**). Also, a negative relationship was noted between wateriness and immediate perceived satiety, and perceived satiety 2 h later, ($p<0.05$) across all five conditions: MVLP, HVLP, LVHP, MVHP and HVHP, (see **Supplementary Table D.3 – b, c, d, e and f**).

Liking, wanting and perceived satiety/fullness. There was a positive relationship between liking and immediate perceived satiety in LVHP only, ($p<0.05$), (see **Supplementary Table D.3 – d**). In terms of wanting, there was a positive relationship between wanting and perceived satiety 2 h later in LVLP condition, ($p<0.05$) and between wanting and immediate perceived satiety and perceived satiety 2 h later in LVHP condition, ($p<0.05$), (see **Supplementary Table D.3 – d**).

Initial hunger and perceived satiety. To check if the initial state of hunger might have impacted the perceived/expected satiety scores, we performed a Pearson's correlation. There was no relationship between initial hunger level and immediate perceived satiety and/or perceived satiety 2 h later in any of the conditions, (see **Supplementary Table D.3 – a, b, c, d, e and f**).

5.5. Discussion

In this study, we investigated the role of visually distinct different levels of viscosity (LV, MV and HV) of whey protein beverages without/ with addition of xanthan gum along with a label of different protein content (low and high) on immediate and 2 h later perceived satiety/fullness using a video-based remote online survey for the first time. It was instrumentally verified that the protein beverages were indeed significantly different from each other in viscosity at orally relevant shear rates due to the addition of xanthan gum (Philips, et al., 2000). To understand if lubricity can be a confounding factor, the friction coefficients were measured. It was found that the friction coefficients of LV in the boundary lubrication regime was significantly lower than

those of MV or HV due to surface interaction of whey protein with hydrophobic surfaces in absence of xanthan gum (Kew, et al., 2021; Zembyla, et al., 2021). In addition to the effect of viscosity of perceived satiety, we also investigated the relationship (if any) between visually perceived sensory attributes, liking, wanting and perceived satiety.

There was a clear effect of visually perceived texture/viscosity on perceived satiety/ fullness. It appeared that MV (medium viscous) and HV (high viscous) beverages were perceived as being more filling/satiating compared to LV (low viscous) beverages immediately after imaging drinking and 2 h later. Interestingly, although in this study we used a video online method to assess the role of texture/viscosity on perceived/expected satiety, the results are similar to the laboratory studies, where a strong effect of texture was noted on expected satiety (Hogenkamp, et al., 2011; McCrickerd, et al., 2012). Moreover, as previous studies showed, when texture is assessed in combination with other characteristics/factors, such as flavour, creaminess or energy content (Hogenkamp, et al., 2011; McCrickerd, et al., 2012), texture appears to have a strong and independent effect on expected satiety. Similar effects were noted in the current study where there was an effect of texture/viscosity on perceived satiety irrespective of the protein label content (low and high). As such, on one hand it emphasises once again the strong effect of the texture on perceived/expected satiety, however, on the other hand it may suggest that the other factors, such as protein label content in the current study may not be important factors for perceived/expected satiety when they are assessed/presented along with texture of food/beverage.

In terms of the perceived sensory attributes of the beverages, there were effects both of texture and protein content, except for smoothness. Participants perceived the LV beverage smoother than MV and HV, regardless the protein label content. This is in close agreement with the instrumental characterized friction coefficient results where LV was found to be most lubricious and strong inverse relationship existed between smoothness and friction coefficient.

Similar smoothness-tribology relationships have been noted in previous study where smoothness was measured in laboratory studies using participants tasting the samples. For thickness, it was noted that participants perceived the MV and HV beverages as being thicker compared to LV one, which is in agreement with the instrumental rheological measurements. This again highlights a clear promise of video-based online assessment of textural perception which has received rare attention in literature (Upadhyay, et al., 2019). Interestingly, LV beverage was perceived as being thicker in the low protein condition compared to the high protein condition. The same pattern was seen for the creaminess, where both the MV and HV beverages were perceived as being creamier than LV; and LV beverage was perceived creamier in the low protein compared to the high protein condition. For wateriness, participants perceived the LV beverage more watery compared to MV and HV ones. Interestingly again, the LV beverage was perceived more watery in the high protein vs low protein condition. It is hard to explain why LV beverage was perceived thicker and creamier in the low protein compared to the high protein condition, and more watery in the high protein condition. One may expect to see the vice versa, as has been shown previously in the literature, where beverages high in their protein content have been perceived as being more viscous than low protein beverages by consumers (Legarová & Kouřimská, 2010). This pattern of events is quite difficult to interpret, but it suggests that a perception of a protein label content can exert different effects according to the presence of other sensory features. A likely explanation of the discrepancy in results of the current study, could be that sensory attributes have been assessed based on visual cues rather than tried/tasted by consumers.

In terms of the relationship between visually perceived sensory attributes and perceived satiety, in line with our expectations, the thicker or the creamier the beverages gave rise to the highest scores for the perceived satiety/fullness. Likewise, as expected, the attribute watery led participants to perceive the beverages to be less satiating/filling. Such relationships, where the

sensory attributes or food texture contribute to the perception of the expected satiety/fullness have been previously noted in the literature. For instance, Forde et al. (2013) showed that the more solid the food is (hotdogs, burgers, stakes) the greater the expected satiation or the more filling the food is, compared to semi-solid ones (mashed vegetables). The same was noted in Hogenkamp et al. (2010) study where higher thickness in both yogurts and soups predicted higher expected satiation.

Interestingly, the results of the current study derived from a visually presented online demonstration, indicated the key role of texture expressed through visual cues only. This indicated that participants may have an intuitive/ learned knowledge that foods/beverages that have higher sensory intensity (thicker, creamier) have a higher satiating effect in contrast to foods/beverages with less sensory intensity (watery) (Forde, et al., 2013). And this intuitive/ learned knowledge/experience may be related to the oro-sensory exposure time (de Graaf, 2012) – the longer the oro-sensory exposure time is the greater the expected satiety/fullness will be. Although participants in the current study did not taste the beverages, the results suggest that they might have used their previous learnt experience to assess the satiating properties of the beverages based on the videos.

With respect to liking, it was noted that LV and MV beverages were liked more compared to HV but only in the low protein content condition. In terms of wanting, there was no difference irrespective of texture or protein label content. It is important to mention that the beverages in the current study differed in their viscosity significantly, showed both by the instrumental analysis (rheology) and by visual cues. Therefore, it is not a surprise that LV and MV were liked more compared to HV, and we tend to believe that this could be due to the fact that HV beverages were too viscous to be liked.

A positive relationship between liking and immediate perceived satiety/fullness in the LVHP condition (the more the beverage was liked the more filling or satiating it was perceived

to be immediately after drinking) was noted. Additionally, a positive relationship between wanting and perceived satiety/fullness 2h later was noted in the LVLP condition (the more the beverages was wanted the greater would be the perceived satiety 2 h later); and between wanting and both immediate and 2 h later perceived satiety/fullness in LVHP condition. Interestingly, studies that used more or less the same methodology *i.e.* pictures to assess the expected satiety of different products found no relation between liking/palatability and expected satiety (Brunstrom & Shakeshaft, 2009; Pilgrim & Kamen, 1963) contrary to the current study. It is known that the preferred food can increase hunger and such it can be suggested that the palatability of food may have an effect on anticipated stimulation of the appetite (Hill, et al., 1984). Therefore, in a perfect scenario of the appetite/ satiety research, one would expect to see no differences in palatability of the products (as the products are control for palatability so that this does not affect the desired outcome). However, we need to take into account that we did not measure appetite ratings before and after each video and it is hard to know if the relationships between liking and perceived satiety seen in the current study may have been mediated by the hunger state after seeing the videos. As such, the findings of this study may suggest that someone may select food based on palatability and the expectation that this food or beverage would be more satiating compared to some less palatable food.

5.5.1. Strengths and limitations

One of the main strengths of the current study is showing that a video online demonstration could be a potential tool to assess the role of food texture on perceived/expected satiety. Of course this approach still needs to be validated. Reproducible results have been reported in the literature, where by using picture images of standard food, consumers were able to discriminate between differences in how filling or satiating foods are expected to be (Brunstrom, Shakeshaft, & Scott-Samuel, 2008) and this gives confidence for a further investigation of this method.

Also, the idea of collecting data quicker and in larger samples compared to laboratory methods should be acknowledged.

However, there are some limitations to recognise in such kind of research. Firstly, it should be taken into account that the findings are based only on videos (visual cues), as such it cannot be assumed that the same findings would be found in situation where participants taste the product. Validation requires simultaneous and parallel testing with visual and taste conditions. When based on visual cues only, it can be difficult for consumers/participants to detect subtle differences in texture, such as lubricity. It is certain that texture experienced in the mouth will generate a distinct pattern of sensations from the purely visual experience. This is particularly with respect to smoothness, creaminess which are extremely hard to understand by visual cues, and thus the results and empirical correlations to instrumental data should be read with caution. Secondly, the fact that the beverages were presented as being poured from one container to another (not packed or in a bottle) also could have affected the findings (Laguna, et al., 2020). We wanted to exclude as many confounding factors as possible and wanted make sure that we show the flow of the beverage only, that it is visible enough to participants. We therefore excluded use of bottles, which might have influenced their decision in the survey. However, on the other side it might be seen as a downfall/ limitation of the study as consumers are more familiar seeing food/ beverage packed in bottles and poured from a bottle to a glass rather than poured from one container to another, and this might have influenced to results to some extent. Thirdly, with only 3 levels of variation across the samples (low, medium and high viscous), it makes difficult to have enough variability in the sensory attributes to interpret its effect on expected satiety. Therefore, the results, especially on correlation must be interpreted with caution. Also, the fact that participants have not been randomized to the conditions (videos/conditions have been randomized on how they will appear on the screen but not randomized for each participants separately), all participants saw

the same order of the videos, could have had an impact on results. Finally, there were many other factors that were not accounted for and could have also impacted the results of the current study. To mention some, health status such as eating disorders, diabetes, social and culture differences, time of the day and familiarity with the food/ beverages could have contributed to the results (Forde, et al., 2015; Heatherton & Polivy, 2013; Irvine, Brunstrom, Gee, & Rogers, 2013; Kristensen, 2000).

5.6. Conclusions

Although it needs to be validated, a video based online demonstration showed a highly feasible method to assess the role of food/beverage texture perceived particularly viscosity on expected satiety. In addition, sensory attributes such as smoothness, thickness and creaminess were shown to be important characteristics of perceived satiety for the beverages in this study. Nevertheless, one should be cautious interpreting these results as all the textural attributes in this study have been assessed online based on observing the visual behaviour using videos and thus the perception can be different when consuming these beverages in real life particularly with respect to smoothness and creaminess. When presented along some other factors, a perception of high or low protein label content appears to have a weak and unpredictable effect on expected satiety. Thus, this study demonstrates an excellent remote sensory tool for understanding the effect of viscosity on perceived satiety that can be highly useful in the current Covid-19 pandemic situation where in person laboratory visits are highly restricted in many countries. However, it is worth recommending that this is not a tool to replace tasting for sensory evaluation of food products as textural properties of food are multidimensional. Although viscosity was perceived visually in this study, not all textural properties such as smoothness, creaminess, astringency *etc.* can be assessed just by visual observations and need tasting evaluation by consumers.

To highlight, this chapter was not initially planned, it was developed as a consequence of the Covid-19 pandemic lockdown, where the access to the laboratory was restricted. Instead of this chapter, initially we planned to do a similar chapter as Chapter 3, to analyse the preloads both instrumentally and sensorially and select the ones that are in line with the purpose of this thesis for further satiety trial. However, as this was not possible, the calorific preloads in the next chapter have been assessed instrumentally only. The next chapter will focus on the effect of calorific preloads, expressed through protein beverages differing in their lubricating and coating properties on appetite ratings, food intake, lubricating proprieties of pooled saliva, salivary and blood biomarkers.

5.7. References

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Chapter 6

Towards understanding the effect of oral coating and lubricity on satiety and satiation: a randomized controlled trial using protein beverages[6]

Abstract

Often food textural interventions have been used to generate satiety, specifically in short-term and preload study design. Although oral lubricity and coating are important aspects of textural perception, which may influence oral residence time, their effects on satiety remain unclear.

We investigated the effects of complex textural attributes of foods, such as lubricity and coating, on appetite ratings, food intake, salivary and blood biomarkers. Preloads expressed through protein beverages (whey and casein) have been developed and instrumentally analyzed (tribology, viscosity and adsorption onto biomimetic surfaces, latter emulating oral coating). Then the calorific preloads differing in their coating properties (low coating, medium coating and high coating) were assessed in two cross-over satiety trials (n=52). Hunger decreased and fullness increased immediately and 30 min after consumption in the high coating beverages compared to control ($p<0.05$), suggesting that the combination of coating and calories have a prolonged effect on appetite ratings (n=37) compared to non-calorific preloads. In addition, fullness increased in high coating compared to low coating condition. There was a correlation between concentration of protein in saliva and appetite ratings; the higher the concentration of

[6] Stribițcaia, E., Gibbons, C., Finlayson, G., You, K.M., Araiza-Calahorra, A., S. Hafiz, M.S., Ellis, L.R., Boesch, C., Sier, J., Blundell, J., and Sarkar, A. Towards understanding the effect of oral coating and lubricity on satiety and satiation: a randomized control trial using protein beverages (Submitted to American Journal of Clinical Nutrition)

protein in saliva the lower the desire to eat ($r = -0.963$; $p < 0.05$) and prospective food consumption ratings ($r = -0.980$; $p < 0.05$). Human saliva was more lubricating after ingesting preload with high coating properties, thus explaining the results on appetite ratings. There was no effect of oral coating on blood biomarkers, suggesting that complex textural attributes having influence on oral processing might not have any effect on the later parts of the satiety cascade (n=15). Oral lubricity and/or coating can have a subtle effect on appetite suppression, with such effect lasting longer when it is combined with macronutrients/energy load. This study has been registered on ClinicalTrials.gov as NCT04868461.

6.1. Introduction

With the world facing a dramatic increase in obesity over the last decades and more so with current COVID-19 pandemic (Grosso, 2021; Hepatology, 2021; WHO, 2021), from the multitude of the strategies that seems to address it, food texture is postulated to be capable of making a meaningful contribution to satiety and consequentlty weight management (Stribiţcaia, Evans, Gibbons, Blundell, & Sarkar, 2020a; Stribiţcaia, et al., 2021). Food texture has been shown to have a significant but short-term effect on the control of satiety, satiation (Stribiţcaia, et al., 2020a; Stribiţcaia, et al., 2021) and daily caloric intake (Forde & de Graaf, 2022). Although the current food design paradigm focuses on viscosity manipulation, important constructs in the food textural manipulation such as the *lubricating* and in particular *mouth-coating* properties of food have been rarely studied for their impact on satiety and satiation.

Recently, food varying in lubricating properties has been shown to have an effect on subjective appetite sensations (Stribiţcaia, et al., 2021) and snack intake (Krop, Hetherington, Miquel, & Sarkar, 2019b). The mechanism by which lubrication influences food intake is often hypothesized to be associated with mouth coating thereby extending the oro-sensory exposure time leading eventually to a significant reduction in food intake, better appetite control and release of gastrointestinal peptides (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019a; Krop, et al., 2019b; Stribiţcaia, et al., 2021), the longer time the food is chewed (longer oro-sensory exposure time) the faster increases in gut peptides release is observed (Miquel-Kergoat, Azais-Braesco, Burton-Freeman, & Hetherington, 2015). In other words, high lubricating gels were postulated to coat oral surfaces better when compared to gels with low lubricating properties, resulting in reduced food intake in a previous proof-of-concept snack trial (Krop, et al., 2019b). However, to date, oral coating has never been quantified in this context and remains

to be studied in relation to satiety. Although, instrumental tribological analysis provides quantification of oral lubricity (Stribiţcaia, Krop, Lewin, Holmes, & Sarkar, 2020b), it does not give quantification of real-time oral coating. Hence, a new technique *i.e.* quartz crystal-microbalance with dissipation monitoring (QCM-D) that measures the actual coating behavior of the food products using oral-mimicking surfaces has been employed in the current study for the first time and used as a manipulation tool to understand the effect on satiety (Kew, Holmes, Stieger, & Sarkar, 2021; Zembyla, et al., 2021).

In the case of lubricity of food, an association has been found in the literature between fullness and intrinsic oral lubricating properties of saliva as a result of ingesting the preloads/ non-calorific hydrogels varying in their lubricating properties: the more lubricating the saliva, the higher were the ratings of fullness (Stribiţcaia, et al., 2021). However, it is noteworthy that only non-calorific foods such as hydrogels have been used to test the efficacy of lubricity on satiety, which has been modest so far (Stribiţcaia, et al., 2021). Therefore, it is of considerable interest to understand the combinatorial effect of food calories and textural manipulaion *i.e.* mouth coating in a more realistic food material, and to test its effects on satiety.

Dietary proteins such as whey and casein have been reported to have a greater satiating effect as compared to other macronutrients (Halton & Hu, 2004; Latner & Schwartz, 1999). From a food texture perspective, viscosity had an effect on appetite response irrespective of the protein type (Juvonen, et al., 2011). However, the way oral coating properties of proteins may affect satiety remains elusive. In addition to appetite ratings, objective food intake measurements, saliva characterization, various gut peptides, such as ghrelin (Kojima & Kangawa, 2005), glucagon-like peptide (GLP-1) and peptide YY (PYY) known as short-term gut peptides and are considered to be involved in the regulation of appetite and early satiety signalling (Cummings & Overduin, 2007) were measured. Our systematic review and meta-analysis have shown that influence of food texture on gut peptides has rarely been studied

(Stribiţcaia, et al., 2020a), only two studies were noted evidencing that low viscous/ liquid food led to a decrease in ghrelin and increase in GLP-1 and PYY levels (Juvonen, et al., 2011; Zhu, Hsu, & Hollis, 2013).

In the current study, we questioned whether oral coating has an effect on early stages of satiety (from first the bite to post-ingestive stage), from an oral processing perspective. Whey and casein protein beverages differing in their coating properties, achieved *via* suitable processing were investigated for their satiating effect in two concurrent studies. Study 1 evaluated the effect of three levels of mouth coating: high coating (HC), medium coating (MC) and low coating (LC), together with a control (water), on appetite, food intake, salivary biomarkers and oral lubricity of saliva post ingestion. Study 2 evaluated the effect of two levels of coating: LC and MC using only whey protein focusing on gut peptides with higher quantities of preload. We hypothesized that higher mouth coating will result in higher satiety, however the influence of lubricity in such mouth coating cannot be fully ignored and is thus measured and discussed simultaneously.

6.2. Methods

6.2.1. Participants

Participants in both studies were healthy, 18-55 years old women and men with a BMI of 18.5 – 27.9 kg/m². The subjects were recruited from students and staff of the University of Leeds, UK. Participants were excluded if they were: smokers, had any oral infections/ diseases/ problems in chewing and swallowing, had chronic or acute health conditions that may affect the ability to sense, eat, digest or absorb food. Subjects using prescribed or non-prescribed medication that may interfere with the ability to sense, eat, digest or absorb food were excluded. Pregnant or lactating subjects, or subjects having a food allergy or intolerance were excluded. Also, subjects, who were on a special diet or were taking protein/ fibre supplements, or who could not tolerate protein beverages or had dairy allergies, had a BMI <18.5 kg/m² or >28 kg/m², or having blood-born diseases were excluded. The studies were approved by University of Leeds MaPS and Engineering joint Faculty Research Committee (MEEC 16-046, November 2020).

A total of 37 participants (13 males and 24 females) completed study 1, see the characteristics in **Table 6.1a**. The age of the participants ranged from 18 to 47 years and the BMI ranged from 19.3 to 27.8 kg/m^2. The Three Factor Eating Questionnaire (TFEQ) analysis revealed that 11 participants had a high restraint score (between 13 and 18). A total of 15 participants (10 males and 5 females) completed study 2, see the characteristics in **Table 6.1b**. The age of the participants ranged from 18 to 33 years and the BMI ranged from 19.65 to 28.3 kg/m^2. The TFEQ analysis revealed that 4 participants had a high restraint score (between 13 and 17).

Table 6.1. Participants' characteristics[a]

Characteristics	Values
a) Study 1	
Male/Female	13/24
Age (years)	26.51 ± 6.18
Weight (kg)	67.3 ± 10.34
Height (m)	1.69 ± 0.08
BMI (kg/m²)	23.47 ± 2.26
TFEQ Restraint	9.9 ± 4
TFEQ Disinhibition	7.1 ± 3.6
TFEQ Hunger	5.9 ± 3.2
b) Study 2	
Male/Female	5/10
Age (years)	26 ± 3.7
Weight (kg)	69.9 ± 11.1
Height (m)	1.7 ± 0.09
BMI (kg/m²)	23.9 ± 3.7
TFEQ Restraint	8.5 ± 4.7
TFEQ Disinhibition	5.7 ± 3.9
TFEQ Hunger	3.9 ± 2.5

[a] Values are means ± SDs. TFEQ, Three Factors Eating Questionnaire; BMI, Body Mass Index.

Sample size was calculated with G*Power version 3.1.9.3 (Heinrich-Heine-Universität Düsseldorf). The power analysis was a priori one, and was done to determine the number of participants needed for a small effect size ($f = 0.25$) across all four outcomes (as the manipulation of this study involved novel parameter *i.e.* coating properties of the beverages, there was not enough information in the literature in terms of the expected size effect). As such, according to G*Power calculation, 24 participants are required to identify a small effect size ($f = 0.25$, $\alpha = 0.05$ and $1\text{-}\beta = 0.80$) across 4 groups (high coating, medium coating, low coating and control) with 4 outcome (appetite ratings, food intake, salivary biomarkers and lubricity of saliva), with outcomes varying from 3 to 5 measurements. We targeted to recruit 40 participants to account for any dropouts. The second study was a pilot one due to restricted time and resources of the project, therefore we targeted for 15 participants.

6.2.2. Design

Both of the studies were acute, randomized, counterbalanced, cross-over, within-subject and single-blinded, registered at ClinicalTrials.gov as NCT04868461. Participants in both studies were not told the exact aim of the study, instead they were informed that the aim of the study was to investigate the acceptance, pleasantness and taste perception of protein beverages. At the end of the studies, participants were verbally debriefed and the real purpose of the studies was revealed. The studies took place at the University of Leeds, UK, School of Food Science and Nutrition Human trial unit: April – October 2021 for study 1, and February – April 2022 for study 2. Subjects gave their written informed consent before taking part in either of the studies and received £30 for the first study and £100 for the second study as a compensation for their time.

6.2.3. Session procedure

Before taking part in the studies, subjects were first screened for eligibility using an online health screening questionnaire. They were also tested for eating restraint using the Three Factor Eating Questionnaire (TFEQ) to further check of any possible influence on the subsequent *ad libitum* food intake.

Study 1. A total of 66 subjects were screened, of which 37 were included in the study and further analysis (26 did not meet the inclusion criteria, 3 withdrew from the study). Each participant was asked to come to the laboratory on four different occasions with a 7 day washout period in between each session. Participants were instructed to fast for 11 h (10.00 pm onwards) and to refrain from drinking (except water) for 24 h before each session. Alcohol consumption was prohibited. Each session lasted for 1.5 h. Participants were asked to come to the laboratory at 8.40 am.

In the first session, weight and height were measured. Body weight was measured to the nearest 0.1 kg after voiding (Seca 763, Seca Birmingham, UK) and height was measured to the nearest 0.5 cm using a portable stadiometer (Seca Portable height measure, Leicester, UK). Participants then provided baseline appetite ratings on a 100-mm visual analogue scale (VAS), and a first sample of whole mouth saliva was collected. After that, at 9.00 am they were given the preload – protein beverages differing in their mouth coating properties or water (control). Immediately after the preload, participants rated their appetite, and the second sample of saliva was collected. Appetite was rated at every 10 min intervals for a duration of 30 min. Before the *ad libitum* breakfast (30 min after preload), the last sample of saliva was collected, and after consuming the breakfast participants completed the last appetite ratings. In total, appetite was rated at 6 time points: -10 min, 0 min, 10 min, 20 min, 30 min and 50 min. Saliva was collected at 3 time points: before preload, immediately after preload and 30 min after preload. Also, oro-sensory exposure time for the preload and salivary flow rate at each time point of collection was measured. The *ad libitum* breakfast consisted of cereals (Wholegrain Malties and Wholegrain Brown Flakes, produced by Sainsbury's Supermarkets Ltd, London, UK), milk (Semi Skimmed Milk, produced in UK) along with water and tea or coffee (participants' choice). Participants were provided with 1125.3 kcal/ 350 g of Wholegrain Malties (cereals), 990.7 kcal/ 330 g of Wholegrain Brown Flakes (cereals) and 486 kcal/ 1000 g of milk. They were asked to eat to a comfortable level of fullness and were told that more food could be provided if they wanted more. A schematic overview of the study protocol is presented in **Figure 6.1a**.

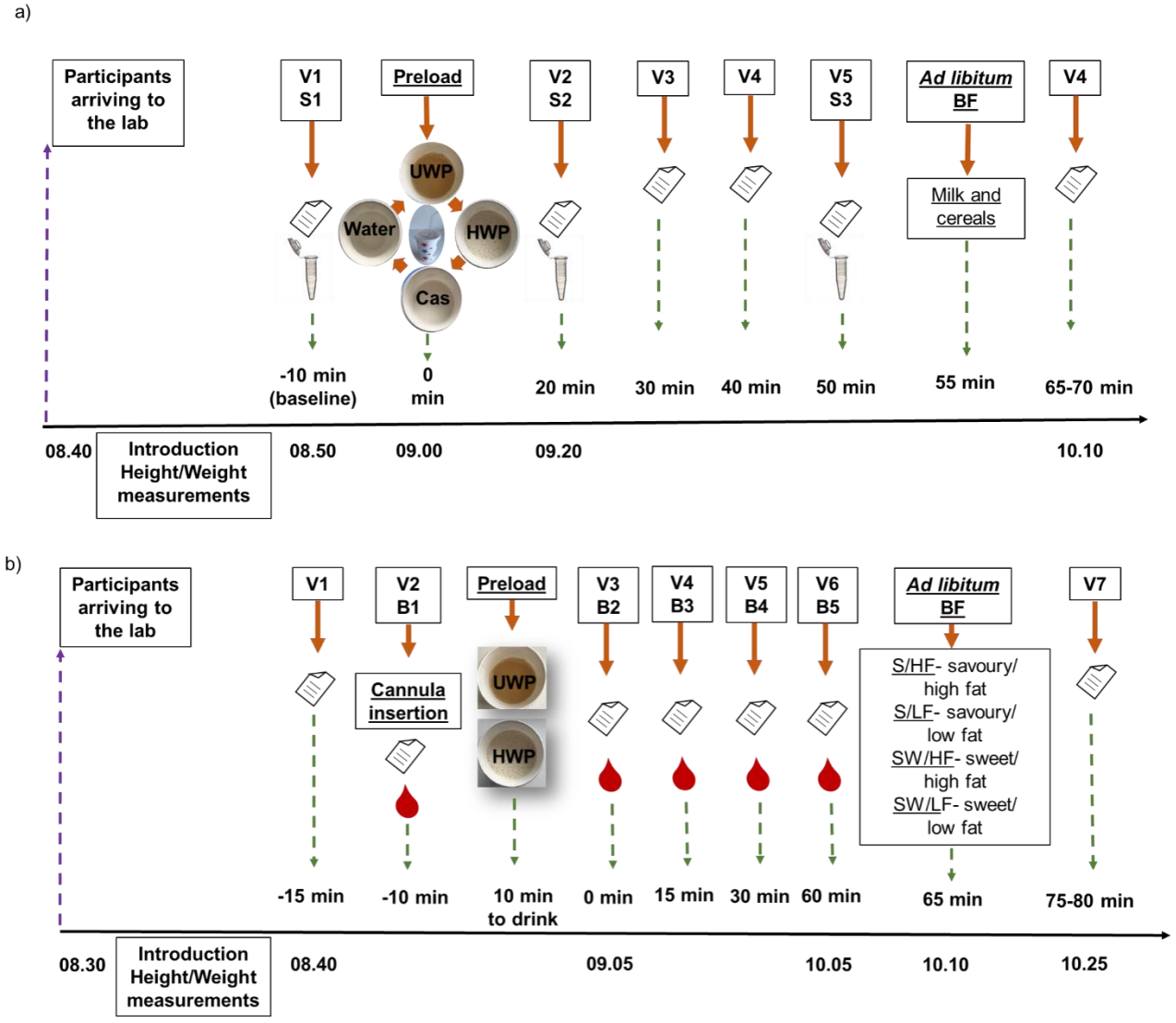

Figure 6.1. Overview of the study protocols. S*tudy 1* - VAS (visual analogue scales) are represented by letter V, 6 in total (V1-V6). Collection of saliva is represented by letter S, 3 in total, for each visit (S1-S3). Preloads were banana-flavoured sweetened protein beverages containing 15 g protein per 100 g water (read the pictures of the preload/ beverages in clockwise direction) – UWP (unheated whey protein beverage), HWP (heated whey protein beverage), Cas (casein beverage) and control (water) served in a cup with the lid on and a straw (see the picture in the middle of the beverages). BF represents breakfast – *Ad libitum* BF. Each visit lasted for around 1.5 h; b) *Study 2* - VAS (visual analogue scales) are represented by letter V, 7 in total (V1-V7). Collection of blood is represented by letter B, 5 in total, for each visit (B1-B5). Preload were banana-flavoured sweetened protein beverages containing 15 g protein per 100 g water – UWP (unheated whey protein beverage) and HWP (heated whey protein beverage) served in a cup with the lid on and a straw (see the picture in the middle of the beverages in study 1). BF represents breakfast – *Ad libitum* BF. Each visit lasted for around 1h 45 min.

Study 2. A schematic overview of the study protocol is presented in **Figure 6.2b**. A total of 45 subjects were screened, of which 15 were included in the study and further analysis (26 did not meet the inclusion criteria, 4 withdrew from the study). Each participant was asked to come to the laboratory on two different occasions with 7 days washout between sessions. Participants were instructed to fast for 11 h (10.00 pm onwards) and to refrain from drinking except water for 24 h before each session. Alcohol conumsption was prohibited. Each session lasted 1 h 45 min. Participants were asked to come to laboratory at 8.40 am.

Similar to study 1, in the first session, weight and height were measured. Participants then provided baseline appetite ratings (-15 min) on a 100-mm visual analogue scale (VAS), after which a cannula was inserted in their forearm. Five minutes later, another VAS was provided by the participants and this was used to check for any effect of cannula insertion on the appetite responses. Immediately following this, a fasting (-10 min) blood sample (pre-preload) was collected and the preload (whey protein beverages of varying coating properties) was given to participants to drink. Participants were instructed to drink the beverage within 10 min and a stopwatch was placed in front of them with a 10 min count down time. After finishing the preload, the third VAS was given to participants and second blood sample (post-preload) was collected (0 min). After this, VAS and blood were collected every 15 min for a duration of 30 min. The next VAS and blood was collected after a further 30 min had elapsed (at 60 min). The last VAS was collected after the *ad libitum* breakfast. In total, appetite was rated at 7 time points: -15 min, -10 min (after cannula insertion), 0 min, 15 min, 30 min, 60 min and after *ad libitum* breakfast. Blood was collected at 5 time points: -10 min (pre-preload/fasting), 0 min (post-preload), 15 min, 30 min and 60 min. The *ad libitum* breakfast consisted of: 1. savoury/high fat food (S/HF) – plain bagel (New York Bakery Co., produced by Waitrose and Partners Meanwood, Leeds, UK) with cream cheese (Philadelphia Original Soft Cheese, produced by Waitrose and Partners Meanwood, Leeds, UK); 2. savoury/low fat food (S/LF) –

crackers (Jacob's Crackers, produced by Waitrose and Partners Meanwood, Leeds, UK) with cottage cheese (Morrisons Low Fat Cottage Cheese, produced by Wm Morrisons Supermarkets PLC, Bradford, UK); 3. sweet/high fat food (SW/HF) – chocolate and butter pastries (Morisons Chocolate and Butter Brioche Rolls, produced by Wm Morrisons Supermarkets PLC, Bradford, UK); 4. sweet/low fat food (SW/LF) – apples and pineapples (Morrisons Pink Lady Apples and Pineapples, produced by Wm Morrisons Supermarkets PLC, Bradford, UK). The breakfast was served along with water, milk and tea or coffee (at participants' choice). Participants were provided with 282 kcal/ 141 g of bagel and cream cheese (S/HF), 194 kcal/112 g of crackers and cottage cheese (S/LF), 621 kcal/180 g chocolate and butter brioches (SW/HF), 151 kcal/ 350 g of fruits (SW/LF), and 243 kcal/ 500g of milk. In total, participants were provided 1491 kcal for breakfast. They were asked to eat to a comfortable level of fullness and were told that more food could be provided if they wanted.

6.2.4. Preload preparation and instrumental measurements

Study 1. Four preloads were tested in this study: whey protein solution (unheated, UWP), whey protein solution (heated, HWP– heating was used to achieve different levels of mouth coating), casein solution (Cas), and water which acted as a control. Whey protein isolate and casein were purchased from MYPROTEIN (Manchester, UK). The powders were bought unflavoured, and were subsequently flavoured using banana essence in our laboratory. The flavour was purchased from Special Ingredients (Special Ingredients Ltd, Chesterfield, UK). The beverages were sweetened by adding small amount of stevia granulated non-nutritive sweetener purchased from a local supermarket (Leeds, UK). On average, a minimum of 10 g of whey/casein protein per 100 g of water is required to detect an effect on satiety (Abou-Samra, Keersmaekers, Brienza, Mukherjee, & Macé, 2011). Consequently, each protein beverage in our study contained 30 g of protein powder to a total of 200 mL water, *i.e.* 15 g per 100 g water (see **Table 6.2** for beverages recipe). The control was 200 mL water which contained the

sweetener and banana flavour in an appropriate proportion to match the taste and flavour of the protein beverages based on a small pilot trial. The whey and casein protein powders were dissolved in distilled water and were left to stir on a magnetic stirring plate for 2 h until a complete hydration was obtained. For the heated whey protein beverage, the protein solution was heated at 80 °C for 8.5 min in a water bath at 80 rmp (OLS26, Aqua Pro, Grant Instruments, Royston, UK). Before serving it to the participants, the HWP beverage was blended for 30 sec with a hand blender (Braun, Germany) and served at room temperature similar to the other beverages or water.

Table 6.2. Recipe of preloads – Study 1.

	UWP[a]	**HWP[b]**	**Cas[c]**	**Control (Water)**
Protein (g)	30	30	30	-
Water (g)	169	169	169	197.9
Flavour –banana (mL)[d]	0.5	0.5	0.5	2
Stevia sweetener (g)	0.5	0.5	0.5	0.1
Total (g)	200	200	200	200

[a]UWP (unheated whey protein)
[b]HWP (heated whey protein)
[c]Cas (Casein)
[d]Firstly, 0.5 g of banana flavour was diluted in 50 g of water, and then 2 mL of the diluted solution was added to the control (water).

The protein beverages and the control (water) were poured into opaque cups. Each cup had a lid on and the participant drank the preload through a straw. Each participant received a total amount of 200 mL of each protein beverages or control (water) on different testing days. The preloads were prepared a day prior to each test day and kept in the fridge overnight at 4°C and served to the participants at room temperature. All the preloads, except water contained around 105 kcal (see **Table 6.3** for nutritional composition).

Table 6.3. Nutritional information of the preloads – Study 1.

Food item	Weight (g)	Energy (kcal)	Protein (g)	Carbohydrate (g)	Sugar (g)	Fat (g)
UWP[a]/ HWP[b]	30	119.7	27	0.75	0.75	0.09
Cas[c]	30	105	24.6	1.41	1.38	0.21
Control (Water)	200	-	-	-	-	-

[a]UWP (unheated whey protein)
[b]HWP (heated whey protein)
[c]Cas (Casein)

Study 2. Two preloads were tested in this study: whey protein solution (unheated, UWP) and whey protein solution (heated, HWP). In order to exclude any effect of protein type and focus on texture solely, the whey protein beverages have been chosen. The ingredients were identical to those used in the study 1, with the same preparation method. However, the amount (kcal) of the beverages in this study was doubled to account for blood collection. The gut peptides need higher calories load to see an increase/decrease (Gibbons, et al., 2013). Each protein beverage contained 60 g of protein powder to a total of 400 mL water, *i.e.* 15 g per 100 g water (see **Table 6.4** for beverages recipe and **Table 6.5** for macronutrient composition of the beverages).

Table 6.4. Recipe of preloads – Study 2.

	UWP[a]	HWP[b]
Protein (g)	60	60
Water (g)	338	338
Flavour –banana (mL)	1	1
Stevia sweetener (g)	1	1
Total (g)	400	400

[a]UWP (unheated whey protein)
[b]HWP (heated whey protein)

Table 6.5. Table 5. Nutritional information of the preloads – Study 2.

Food item	Weight (g)	Energy (kcal)	Protein (g)	Carbohydrate (g)	Sugar (g)	Fat (g)
UWP[a]/ HWP[b]	60	239.4	57	1.5	1.5	0.18

[a]UWP (unheated whey protein)
[b]HWP (heated whey protein)

Viscosity, lubricity and mouth coating of the preloads were measured using rheometer, tribometer and QCM-D, respectively. The apparent viscosity of the beverages was measured with a rheometer (Kinexus Ultra+, Malvern Instruments Ltd, Worcestershire, UK) using a plate-plate geometry (diameter 60 mm) with a gap size of 0.5 mm. Flow curves were obtained for all of the beverages after simulated oral processing at shear rates ranging from 0.01 to 1000 s^{-1} at 37 °C. For lubricity of the preloads or the saliva, commercially available polydimethylsiloxane (PDMS) ball (diameter of 4 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) and disc (diameter of 46 mm, thickness of 4 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) were used as surfaces to mimic oral surfaces for the oral tribology measurements (surface roughness of the PDMS tribopairs, R_a < 50 nm). For the mouth coating analyses, PDMS-coated QCM-D (Quartz crystal microbalance with dissipation) sensors were designed to emulate oral surfaces (Kew, et al., 2021; Macakova, Yakubov, Plunkett, & Stokes, 2010; Stokes, Macakova, Chojnicka-Paszun, de Kruif, & de Jongh, 2011; Xu, et al., 2020; Zembyla, et al., 2021). For the preparation of PDMS-coated QCM-D sensors, briefly, 100 μL of 0.5 wt% PDMS solution was placed on the substrate and was spin-coated at 5,000 rpm speed for 60 s. QCM-D can simultaneously measure the shifts in frequency and dissipation at different overtones occurring during adsorption and provide wealthy information on the mass of the adsorbing film corresponding to coating. All the protein solutions (Cas, UWP and HWP) were supplied into QCM-D chamber containing the PDMS

sensors by a peristaltic pump with a flow rate of 100 µL/min at 25 °C. The first step was to inject water until a stable baseline was observed. Subsequently, for the adsorption of protein (0.1 mg/mL) solutions on PDMS surfaces, solutions were injected into the system for two hours, allowing the system to equilibrate, followed by rinsing in water for 30 min. The data were fitted using the Voigt model for viscoelastic solids (namely, "Smartfit Model") by Dfind software (Q-Sense, Sweden) to obtain the mass of the hydrated protein layers, in order words oral coating. For improved visualization only the 5th overtone has been used in graphs (see frequency shifts in **Supplementary Figure E.1a** and dissipation shifts in **Supplementary Figure E.1b**) plots. A minimum of three replicates were measured for each beverage sample for all three instrumental analysis – viscosity, lubricity and coating and a detailed method and protocol for all three measurements are decsribed in our prevoius studies (Kew, et al., 2021; Stribiţcaia, et al., 2021; Stribiţcaia, et al., 2020b; Zembyla, et al., 2021).

6.2.5. Appetite ratings

Study 1. Participants rated their appetite at 6 time points using a 100-mm VAS scale, which has been shown to be valid and reliable scale used for appetite research (Flint, Raben, Blundell, & Astrup, 2000; Stubbs, et al., 2000); the scale anchor points ranged from 'not at all' to 'extremely'. Time points were: -10 min, 0 min, 10 min, 20 min, 30 min and 50 min on each testing day. Rating scales included hunger, fullness, desire to eat, prospective food consumption (how much food they could consume) and thirst. Ratings were also performed for mood - contentment, mental alertness and nausea. In addition, participants rated wanting and liking, as well as palatability and acceptability of the preloads (including control) in terms of texture, flavor and sweetness. The time point of -10 min will be referred to 'before preload' and 0 min to 'after preload' throughout this article. *Study 2*. Participants rated their appetite at 7 time points using the same 100-mm VAS as in study 1. The time points were:

-15 min, -10 min (after cannula insertion), 0 min, 15 min, 30 min, 60 min and after *ad libitum* breakfast. Blood was collected at 5 time points: -10 min (pre-preload/fasting), 0 min (post-preload), 15 min, 30 min and 60 min. The time point of -10 min will be referred to 'before preload' and 0 min to 'after preload' throughout this article.

6.2.6. Energy intake

For both studies, *ad libitum* foods and beverages were weighed (to the nearest 0.1g) prior to being served to the participants, and were re-weighed after the participant had finished eating to determine the amount of food and beverage actually consumed by each participant. For completeness in reporting, the food intake was initially calculated in grams and the weights of carbohydrate, protein and fat were converted to energy using appropriate factors (3.75, 4 and 9).

6.2.7. Oro-sensory exposure time and salivary flow rate- Study 1

Oro-sensory exposure time of the preloads was measured using a countdown timer (Fisher Scientific Ltd, UK). On average, the time for these beverages to be drunk can vary between 5 to15 min (Abou-Samra, et al., 2011; Nilsson, Holst, & Björck, 2007). Participants were instructed to press 'Start' on the timer when they began to drink the preload (at their first sip) and press 'Stop' when they finished drinking; they were instructed the procedure would not last more than 15 min.

Salivary flow rate was measured every time saliva was collected (at three time points on each visit) before and after preload and 30 min after preload. The same countdown timer (Fisher Scientific Ltd, UK) was used starting from 5 min. Again, participants were instructed to 'Start' the timer when they first started spitting into the tube and 'Stop' when they finished (at ≈ 2mL of saliva); they were told the procedure should not take more than 5 min.

6.2.8. Lubrication properties and viscosity of human saliva – Study 1

As illustrated in **Figure 6.1a**, saliva was collected at three time points. Participants were asked to spit 2 mL of saliva into a pre-cooled tube. The collected saliva from each participant at three different time points was pre-processed according to previously reported method (Hopkins, et al., 2020; Stribițcaia, et al., 2021). Briefly, the samples were centrifuged for 5 min at 4,000 *g* and the precipitate containing cell debris was discarded. Approximately, 2 mL of the supernatant was made up to 4 mL volume using pre-chilled 20 mM phosphate buffer (pH 7) (*i.e.* 16 vol% unstimulated whole human saliva) (Hopkins, et al., 2020) and was stored at -80 ºC until analysis of total protein, α-amylase and MUC5B, respectively. Tribology and rheology was performed to determine the lubrication and viscosity properties of pooled saliva before, after preload and 30 min after preload (immediately before the *ad libitum* breakfast). Friction forces in the presence of saliva collected at different time points, and after consuming preloads or controls, were compared at boundary (BL, speed of 0.005 m s^{-1}) and mixed (ML, speed of 0.05 m s^{-1}, 0.1 m s^{-1}) lubrication regimes (Stribiţcaia, Krop, Lewin, Holmes, & Sarkar, 2020b). The viscosity values were compared at orally relevant shear rates *i.e.* 50 s^{-1} shear rate.

6.2.9. Biochemical assays of salivary biomarkers - Study 1

Supernatants (*i.e.* 50 vol% unstimulated whole human saliva) collected in 250 µL aliquots were assayed for total protein using Pierce BCA Protein Assay Kit (Pierce, Fisher Scientific, Loughborough, UK) and the results were compared to a standard curve generated using bovine serum albumin (BSA). Salivary mucin (MUC5B) was analyzed using human MUC-5B ELISA Kit (OKEH02841, Aviva Systems Biology, Insight Biotechnology, Wembley, UK). Salimetrics α-amylase kit (Stratech, Ely, UK) was used to measure salivary α-amylase enzyme activity. The biochemical assays were run in duplicate and absorbance values recorded using

Tecan Spark 10 M microplate reader (Tecan, Reading, UK). Results were expressed as Units/mg protein for amylase, ng/ mg protein for MUC5B and μg/mL for protein.

6.2.10. Biochemical assays of gut peptides - Study 2

Blood samples were collected using cannulation by two trained personnels. A total of 25 mL (5 mL on each time point – 5 time points per session) of blood was collected on each visit (50 mL for whole study). Out of the 5 mL blood, 3 mL were placed in pre-cooled gut peptides tubes and 2 mL in pre-cooled glucose tubes. Immediately after collection, blood was centrifuged at 1,500 *g* for 10 min at 4 °C. Afterwards, 250 μL of plasma (for each appetite biomarker/ gut peptide and glucose) was placed in a 2 mL Eppendorf tube and was stored at -80°C until biochemical analysis.

The plasma samples were analysed by BIOIATRIKI Central Lab (Athens, Greece). The analysed appetite biomarkers were total ghrelin, GLP-1 (glucagon-like peprides) and PPY (peptide tyrosine tyrosine). Total ghrelin was analysed using RayBio® Human Ghrelin ELISA kit (RayBiotech, Norcross GA, USA) (Cat. No.ELH-GHRL-1). GLP-1 was analysed using RayBio® Human GLP-1 ELISA kit (RayBiotech, Norcross GA, USA) (Cat. No.ELH-GLP137-1), and PYY was analysed using RayBio® Human PYY ELISA kit (RayBiotech, Norcross GA, USA) (Cat. No.ELH-PYY-1). The plasma level of glucose was determined by using Hexokinase test (enzymatic ultra-violet) (ROCHE, Basel, Switzerland) using a HITACHI cobas 800c system/701 analyser.

The protocol was the same for all gut peptides and glucose analysis. The assays employed an antibody specific for human GHRL/Ghrelin, GLP-1 and PYY coated on a 96-well plate. Standards and samples were pipetted into the wells and GHRL/Ghrelin, GLP-1 and PYY present in a sample were bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human GHRL/Ghrelin, GLP-1 and PYY antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted

to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color develops in proportion to the amount of GHRL/Ghrelin, GLP-1 and PYY bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm.

6.3. Statistical analysis

Data are presented as mean and standard deviations (SDs) in the text and tables, and means and SEMs in the figures. All statistical analyses were performed using SPSS (IBM® SPSS® Statistics, v25, SPSS Inc, Chicago, USA). Differences between conditions were tested by repeated measures ANOVA for appetite ratings at each time point. Overall appetite ratings, food intake, salivary and blood biomarkers, and lubricating capacity of human saliva were measured after ingesting the preloads. The differences in palatability nausea, mental alertness and content mood after ingesting the preloads were also assessed by repeated measures ANOVA. In Study 1, a 4 × 5 level factorial repeated measures ANOVA was used to examine the main effect on appetite ratings of the intervention condition (Control, HC, MC, LC), time (post-preload, 10 min, 20 min, 30 min after preload and after ad libitum breakfast) and condition*time interaction. In study 2, to check if cannula insertion affected the appetite ratings, there were 2 baseline time points – one before cannula insertion and one after. After comparing the means between these 2 time points, using paired t-test, the first one was selected for further analysis since there was no significant difference between them. Therefore, in study 2, a 2 × 5 level factorial repeated measures ANOVA was used to examine the main effect of the intervention condition (MC, LC), time (post-preload, 15 min, 30 min, 60 min after preload and after ad libitum breakfast) and condition*time interaction on appetite ratings. Analysis of appetite ratings and blood biomarkers were also compared after controlling for baseline ratings using the analysis of difference from baseline. As the textural manipulation of

food (protein beverages) in this study was quite subtle and oral coating is used as a construct for the first time, there is uncertainty about the immediate post-preload experience to make conclusion based on analysis controlled for baseline only. Therefore, appetite results from both with and without controlled for baseline analysis are reported and discussed. Where the assumption of sphericity had been violated, indicated by Mauchly's test, Greenhouse-Greisser corrected tests are reported. Significant differences were calculated by Bonferroni corrected post-hoc t-tests and was set at $\alpha < 0.05$ level. Pearson correlations were performed to assess the relationship between appetite ratings, food intake, and concentration of salivary biomarkers (protein, *α-amylase) for Study* 1. Data were plotted using the software Origin® (OriginPro 2018; OriginLab Corporation, Northampton MA, USA).

6.4. Results

6.4.1. Preload characteristics

Figure 6.2a shows that the most viscous beverage was casein (Cas), followed by heated whey protein (HWP) and unheated whey protein (UWP). For lubricity (expressed as friction coefficient at relevant entrainment speed), as shown in **Figure 6.2b**, the opposite trends were observed to viscosity, the most lubricating beverage was HWP followed by UWP and the least lubricating was Cas.

As shown in **Figure 6.2c** it can be seen that the adsorbed mass is higher for casein (Cas), followed by heated whey protein (HWP) and unheated whey protein (UWP). In other words, Cas has a high mouth coating behaviour followed by HWP with medium mouth coating behaviour and UWP has a low coating behaviour. Summarising the textural measurements the beverages presented the following properties: Cas (casein) – high viscous/low lubricating/high coating, UWP (unheated whey protein) – low viscous/medium lubricating/low coating and HWP (heated whey protein) – medium viscous/high lubricating/medium coating. Taking into account the coating perspectives, henceforth the pre-loads will be called as HC – high coating, MC – medium coating and LC – low coating.

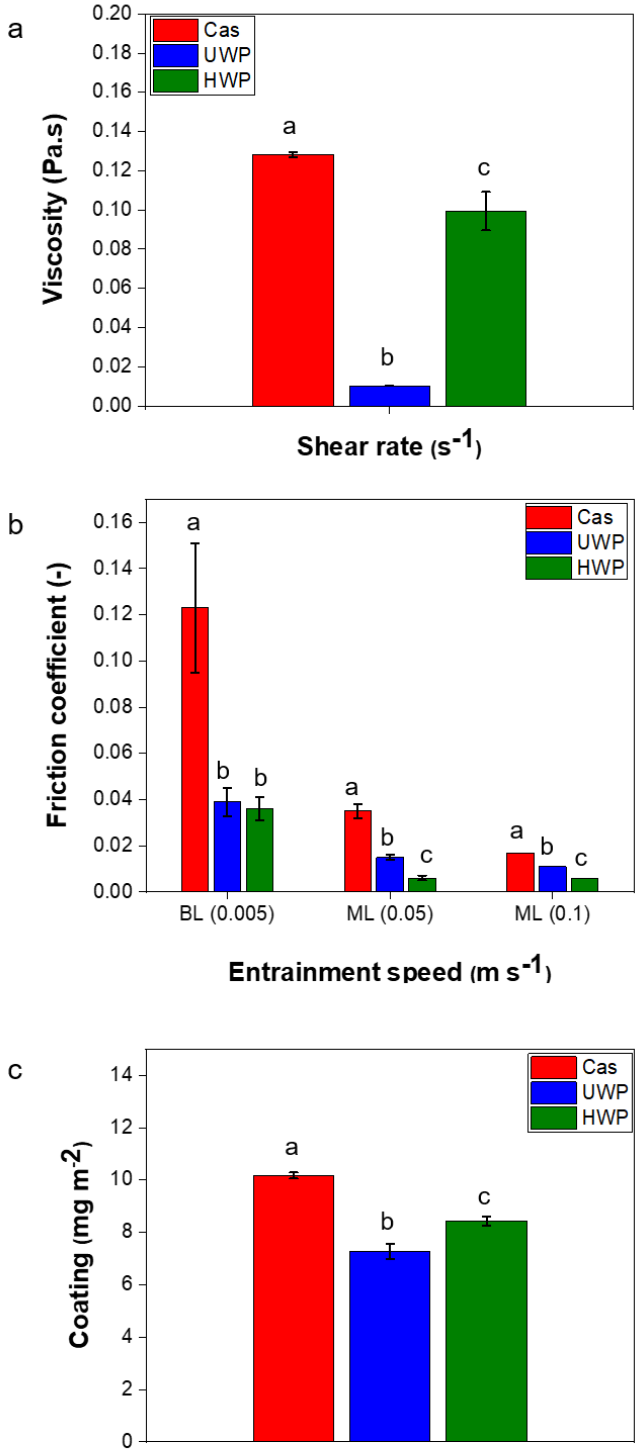

Figure 6.2. Viscosity (a) as a function of orally-relevant shear rate of 50 s^{-1}, friction coefficient (b) at boundary (0.005 m s^{-1} speed) and mixed (0.05; 0.1 m s^{-1} speed) lubrication regimes and (c) coating expressed through adsorbed mass per unit area of the beverages included in the study (Cas – Casein, unheated whey protein – UWP and heated whey protein – HWP). Values are means and error bars represent standard error of means (SEMs). Different letters denote a significant difference between beverages ($p < 0.05$). BL = boundary lubrication regime, ML = mixed lubrication regime. A lower friction coefficient represents higher lubrication performance of the beverages. All measurements were carried out at 37° C.

6.4.2. Appetite ratings

Figures 6.3 and **6.4** show the appetite ratings over time in study 1 and study 2 respectively. Both figures indicate a decrease in hunger (**Figures 6.3a** and **6.4a**), desire to eat (**Figures 6.3c** and **6.4c**), prospective food consumption (**Figure 6.3d** and **6.4d**) and thirst (**Figure 6.3e** and **6.4e**) immediately post-preload and with a slightly increase 10 min post-preload and reaching the baseline ratings at 30 min post-preload in study 1 and at 60 min post-preload in study 2. Opposite can be seen for fullness (**Figure 6.3b** and **6.4b**) in both figures (for both studies) where fullness increased immediately post-preload and with a slightly decrease 10 min post-preload and reaching the baseline ratings at 30 min post-preload in study 1 and at 60 min post-preload in study 2. We also assessed for the feelings of nausea, as well as for the mood of participants (mental alertness and content) after ingesting the preloads, in both studies (see means and SDs for nausea, content and mental alertness in **Supplementary Table E.1** - study 1 and **Supplementary Table E.2** - study 2). A plateau-like pattern for nausea in (**Figures 6.3f** and **6.4f**), content (**Figure 6.3g** and **6.4g**) and mental alert (**Figure 6.3h** and **6.4h**) was observed, with a slightly increase in content and mental alertness after the study finished – after *ad libitum* breakfast (ADDB – for both studies). However, for content there was a significant difference between conditions at three time points: pre-preload, 30 min and 60 min post-preload in study 2. Participants were more content in MC compared to LC conditions *($p < .05$)* in study 2 *(*see **Supplementary Table E.2**).

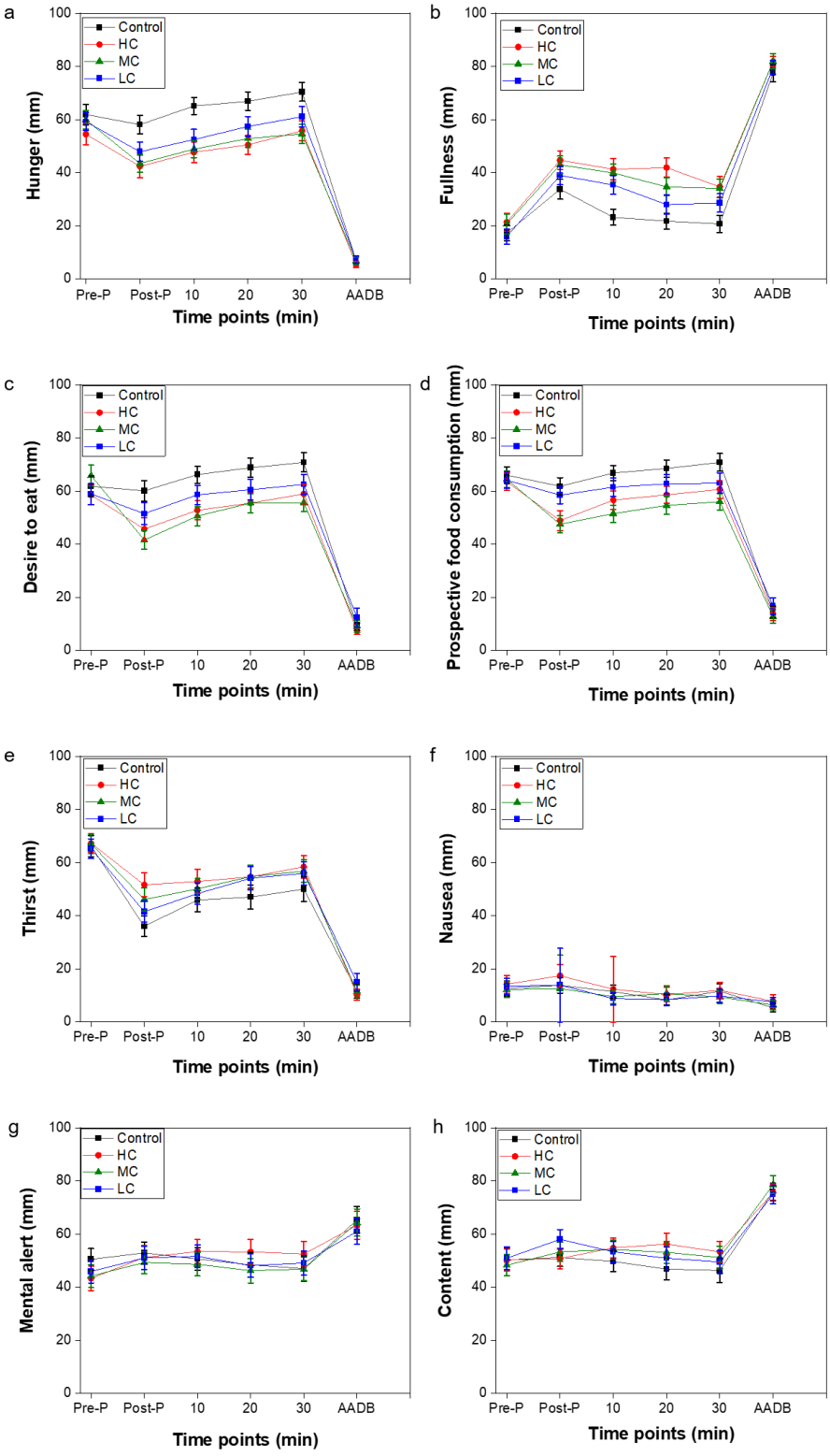

Figure 6.3. Study 1. Ratings (mm) for (a) hunger, (b) fullness, (c) desire to eat, (d) prospective food consumption (PFC) (e) thirst, (f) nausea, (g) mental alert, and (h) content over time: pre-preload (Pre-P), post-preload (Post-P), 10 min, 20 min, 30 min and after *ad libitum* breakfast (AADB) in Control, HC (high coating), MC (medium coating) and LC (low coating) conditions for Study 1. Values are means and SEMs (n=37).

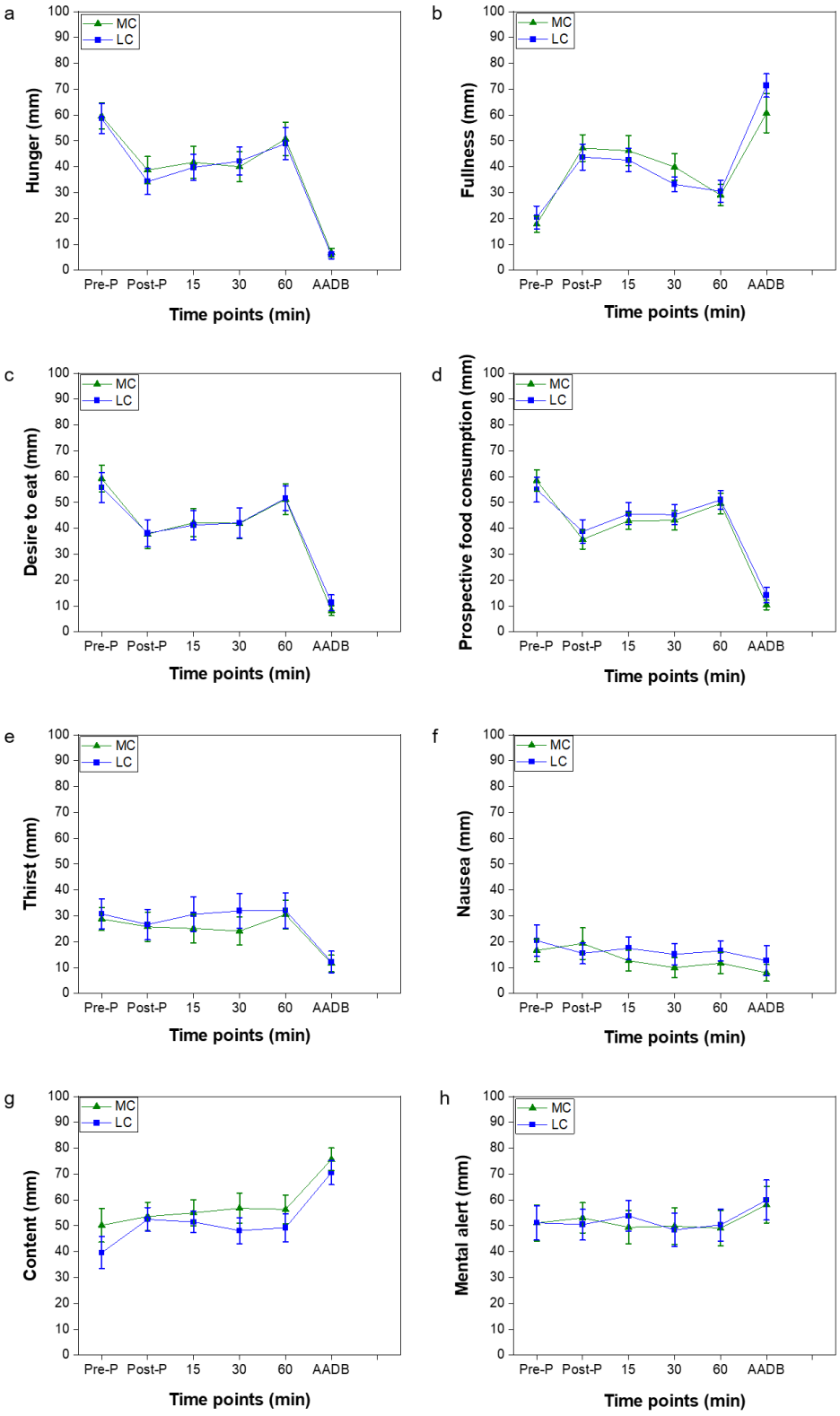

Figure 6.4. Study 2. Ratings (mm) for (a) hunger, (b) fullness, (c) desire to eat, (d) prospective food consumption (PFC) (e) thirst, (f) nausea, (g) mental alert, and (h) content over time: pre-preload (Pre-P), post-preload (Post-P), 15 min, 30 min, 60 min and after *ad libitum* breakfast (AADB) in MC (medium coating) and LC (low coating) conditions. Values are means and SEMs (n=15).

For study 1, there was a significant effect of condition (hunger: $F(3, 108) = 12.61$; fullness: $F(3, 108) = 14.17$; desire to eat: $F(3, 108) = 7.32$; prospective food consumption: $F(3, 108) = 10.78$; and thirst: $F(3, 108) = 2.69$), all at $p < .001$; a significant effect of time (hunger: $F(5, 180) = 125.71$; fullness: $F(5, 180) = 130.84$; desire to eat: $F(5, 180) = 119.77$; prospective food consumption: $F(5, 180) = 130.70$ and thirst: $F(5, 180) = 113.80$), all at $p < .001$; and a significant effect of condition*time interaction (hunger: $F(15, 540) = 3.90$; fullness: $F(15, 540) = 2.70$; desire to eat: $F(15, 540) = 4.99$; prospective food consumption: $F(15, 540) = 4.13$; and thirst: $F(15, 540) = 2.43$), all at $p < .001$. A post-hoc pairwise comparison tests revealed that there was a significant difference between all three protein beverages - HC, MC and LC and Control (water): hunger, desire to eat, prospective food consumption and thirst significantly decreased immediately post-preload maintaining its effect until 30 min post-preload after ingesting HC, MC and LC preloads compared to Control ($p < .05$). Fullness significantly increased in HC, MC and LC compared to Control immediately post-preload maintaining its effect until 30 min post-preload ($p < .05$). There could be observed a sporadic effect of the condition between three protein beverages on some of the appetite sensations: participants felt significantly fuller in HC compared to LC 20 min post-preload ($p < .05$), and felt they could eat significantly less (prospective food consumption) in MC and HC compared to LC immediately post-preload ($p < .05$), and significantly less in MC compared to LC 10 min post-preload ($p < .05$).

After controlling for baseline ratings, main effects of condition, time and condition*time interaction across all appetite ratings were confirmed, with the exception of thirst (no effect of condition anymore). Effect of condition: hunger - $F(3, 108) = 4.38$; fullness - $F(3, 108) = 4.62$; desire to eat - $F(3, 108) = 10.49$; prospective food consumption - $F(3, 108) = 7.29$ (all $p < .05$) and thirst - $F(3, 108) = 1.78$ ($p > .05$). Effect of time: hunger - $F(4, 144) = 167.99$; fullness - $F(4, 144) = 139.40$; desire to eat - $F(4, 144) = 158.75$; prospective food

consumption - $F(4, 144) = 146.45$ and thirst - $F(4, 144) = 111.49$ (all $p < .05$). Effect of condition*time interaction: hunger - $F(12, 432) = 3.75$; fullness - $F(12, 432) = 2.25$; desire to eat - $F(12, 432) = 3.17$; prospective food consumption - $F(12, 432) = 3.47$ and thirst - $F(12, 432) = 2.65$. A post-hoc pairwise comparison tests revealed that there was a significant difference between all three protein beverages - HC, MC and LC and Control (water): hunger, desire to eat, prospective food consumption and thirst significantly decreased immediately post-preload maintaining its effect until 30 min post-preload after ingesting HC, MC and LC preloads compared to Control ($p < .05$). Fullness significantly increased in HC, MC and LC compared to Control immediately post-preload maintaining its effect until 30 min post-preload ($p < .05$). Appetite ratings means and SDs are given in **Supplementary Table E.3**, study 1.

In study 2, there was an effect of time only (hunger - $F(5, 70) = 35.165$; fullness - $F(5, 70) = 26.824$; desire to eat - $F(5, 70) = 38.521$; prospective food consumption - $F(5, 70) = 41.333$ and thirst - $F(5, 70) = 6.700$) all $p < .05$. There was no effect of condition (hunger - $F(1, 14) = 0.591$; fullness - $F(1, 14) = 0.003$; desire to eat - $F(1, 14) = 0.001$; prospective food consumption - $F(1, 14) = 0.301$ and thirst - $F(1, 14) = 0.693$ all $p > .05$ or condition*time interaction (hunger - $F(5, 70) = 0.659$; fullness - $F(5, 70) = 1.627$; desire to eat - $F(5, 70) = 0.408$; prospective food consumption - $F(5, 70) = 1.041$ and thirst - $F(5, 70) = 0.436$, all $p > .05$. A post-hoc pairwise comparison test revealed that hunger, desire to eat, prospective food consumption and thirst significantly decreased immediately post-preload and was maintained up to 60 min post-preload ($p < .05$). The opposite was observed for fullness where it significantly increased immediately post-preload and was maintained up to 60 min post-preload ($p < .05$). However, all the appetite sensations had the same levels irrespective of the condition *i.e.* participants reported the same levels of appetite ratings in both MC and LC conditions ($p > .05$).

After controlling for baseline ratings, the same effect of time was noticed, with no effect of condition or condition*time interaction. Effect of time: hunger - $F(4, 56) = 32.863$; fullness - $F(4, 56) = 18.580$; desire to eat - $F(4, 56) = 45.441$; prospective food consumption - $F(4, 56) = 53.850$ and thirst - $F(4, 56) = 8.389$, all $p > .05$. Effect of condition: hunger - $F(1, 14) = 0.016$; fullness - $F(1, 14) = 0.016$; desire to eat - $F(1, 14) = 0.616$; prospective food consumption - $F(1, 14) = 5.092$ and thirst - $F(1, 14) = 0.037$, all $p > .05$. Effect of condition*time interaction: hunger - $F(4, 56) = 1.027$; fullness - $F(4, 56) = 2.144$; desire to eat - $F(4, 56) = 0.265$; prospective food consumption - $F(4, 56) = 0.122$ and thirst - $F(4, 56) = 0.715$, all $p > .05$. A post-hoc pairwise comparison test revealed that hunger, desire to eat, prospective food consumption significantly decreased immediately post-preload and was maintained up to 30 min post-preload ($p < .05$). Thirst significantly decreased only after *ad libitum* breakfast ($p < .05$). The opposite was noticed for fullness where it significantly increased immediately post-preload and 15 min post-preload ($p < .05$). There were no significant difference between conditions, *i.e*. participants reported the same levels of appetite sensations irrespective of the conditions MC and LC ($p > .05$). Appetite ratings means and SDs are given in **Supplementary Table E.4**, study 2.

In terms of the area under the curve (AUC), for study 1, (**Supplementary Table E.5**), for all appetite ratings it was significantly higher in Control compared to the rest of the conditions: HC, MC and LC ($p < .05$). In study 2, in terms of AUC there was no significant difference between conditions for all appetite ratings (**Supplementary Table E.6**).

There was no significant difference in palatability in terms of texture, sweetness and flavour, likewise on liking and wanting ($p > .05$) between the conditions in both studies (see **Supplementary Table E.7** for study 1 and **Supplementary Table E.8** for study 2).

6.4.3. Energy intake

For *ad libitum* energy intake at breakfast, there was no statistical difference between the conditions for both studies: Control, HC, MC and LC, $F(3, 108) = 2.139$, $p > .05$ (**Figure 6.5a**); MC and LC, $F(1, 14) = 0.679$, $p > .05$ (**Figure 6.5b**). Therefore, the total amount of food participants consumed was almost the same in all conditions in both studies. The same was observed for water; no significant difference between groups in the water intake in both studies. However, there was a significant difference between the type of breakfast participants ate in study 2 (**Figure 6.5c**). Participants opted for SW/LF compared to the rest S/LF, S/HF and SW/HF ($p < .05$).

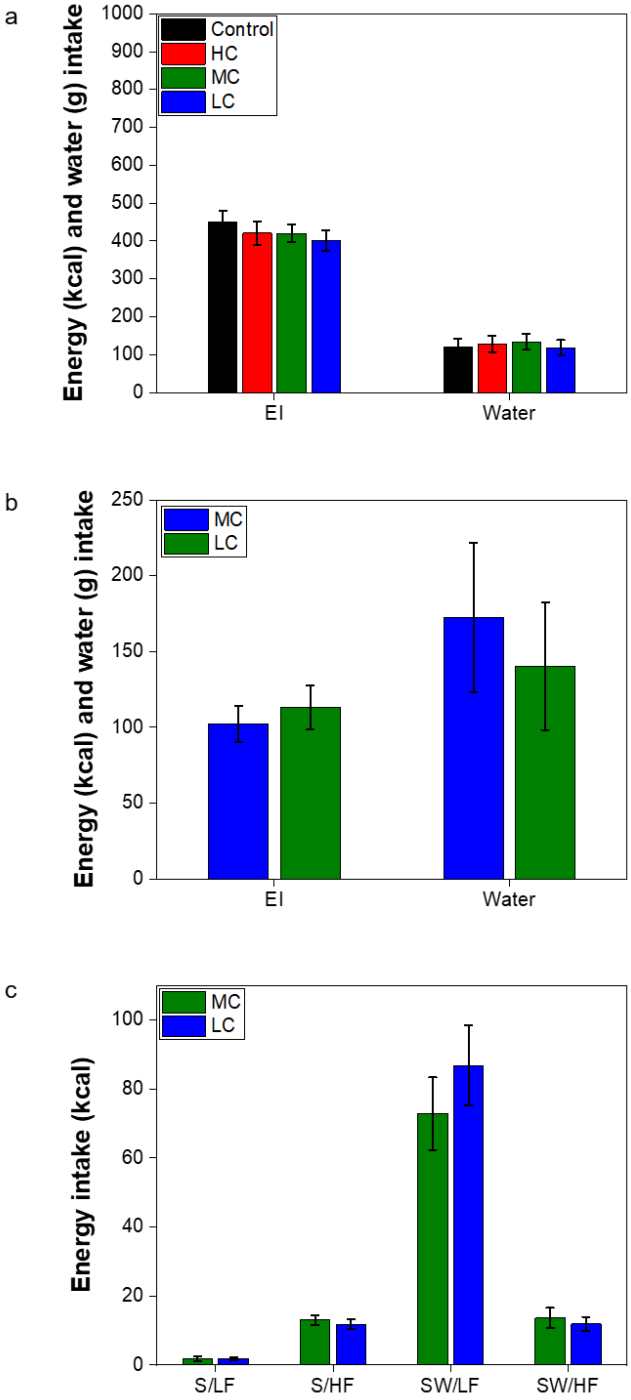

Figure 6.5. Energy intake (kcal) and water intake (g) for Study 1 (a) and Study 2 (b) and energy intake depending on breakfast type for Study 2 (c). Values are means and SEMs.

6.4.4. Oro-sensory exposure time and salivary flow rate- Study 1

The oro-sensory exposure time and salivary flow rate has been assessed for each condition. In terms of oro-sensory exposure time, it was significantly longer in HC and MC compared to Control and LC (p < .05) (**Figure 6.6a**). For the salivary flow, there was no significant difference between conditions at any tie point: pre-preload, post-preload and 30 min post-preload (p > .05) (**Figure 6.6b**).

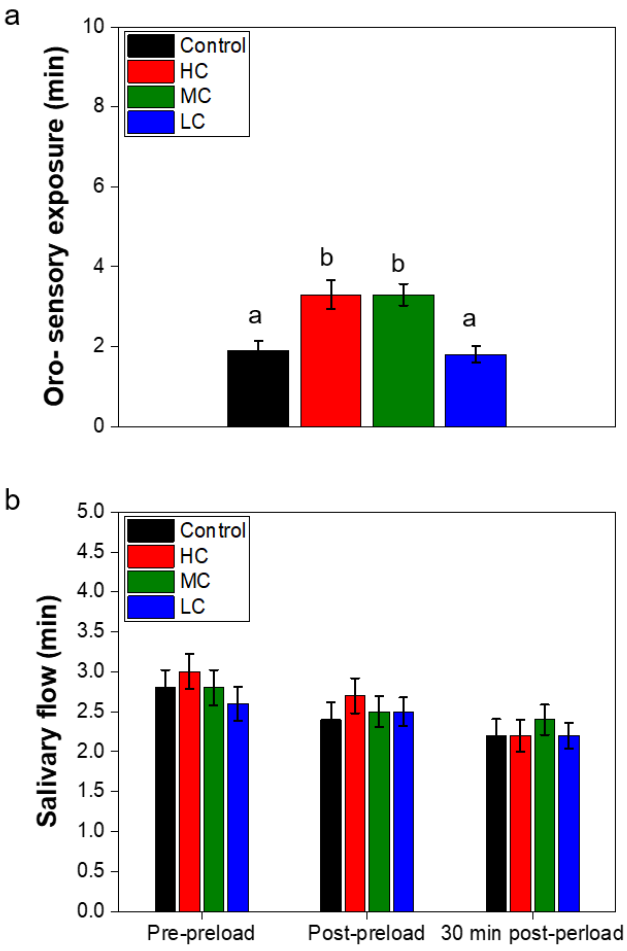

Figure 6.6. Oro-sensory-exposure time (min) (a) and salivary flow (min) (b) between conditions Control, HC (high coating), MC (medium coating) and LC (low coating) for Study 1. Values are means and error bars represent standard error of means (SEMs). Different letters indicate significant differences (p < .05), (n=37).

6.4.5. Lubricating and viscosity properties of saliva

To check if there were differences in lubrication properties of saliva between conditions before, after the intervention and 30 min after intervention, tribological measurements were performed on the collected pooled saliva. There was no significant difference in the lubrication properties of saliva expressed through friction of coefficient between conditions (Control, HC, MC and LC) before preload (**Figure 6.7a**) which means that the baseline conditions were similar. However, there was a significant difference in the lubrication properties of saliva between conditions after preload (**Figure 6.7b**). Saliva showed to be more lubricious in HC and Control compared to MC and LC ($p < .05$); and in Control compared to HC ($p < .05$) in boundary regime (BL 0.005); more lubricating in Control and HC compared to MC and LC ($p < .05$) in mixed regimes (ML 0.05 and ML 0.1). After 30 min post-preload (**Figure 6.7c**), saliva was more lubricious in LC compared to Control ($p > .05$) in mixed regime (ML 0.1). Also, viscosity of saliva was measured and there was no significant differences in its level of viscsoity between the conditions across all time points: before, after and 30 min after preload ($p < .05$) (see **Figure 6.7d**).

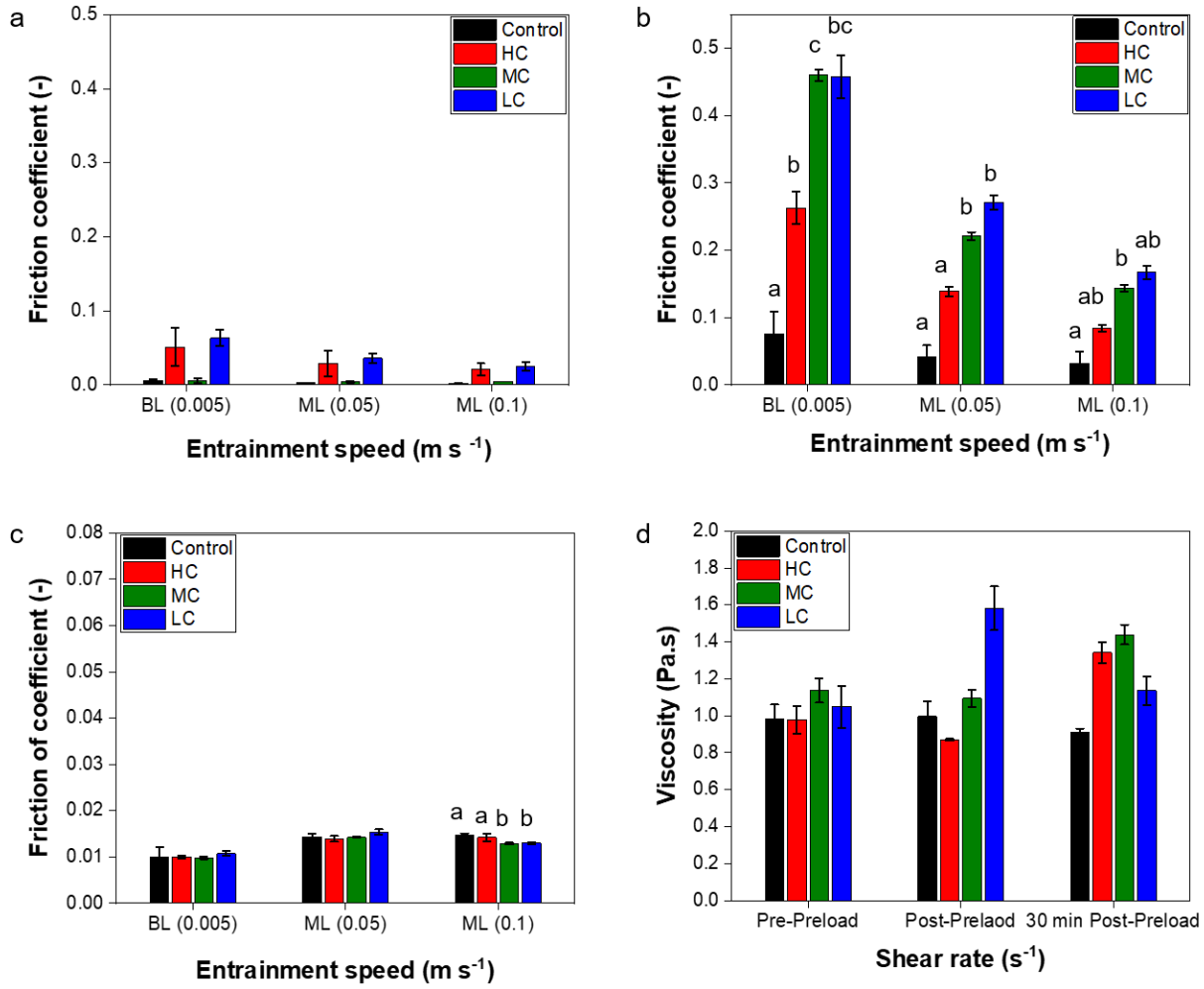

Figure 6.7. Friction coefficient of saliva before preload (a), after preload (b) and 30 min after preload (c) at boundary (0.005 m s^{-1} speed) and mixed (0.05 m s^{-1}; 0.1 m s^{-1} speed) lubrication regimes and viscosity (d) of saliva as a function of orally-relevant shear rate of 50 s^{-1}, in all four conditions of Control, HC (high coating), MC (medium coating) and LC (low coating), n = 37 for Study 1. Values are mean and error bars of means (SEMs). BL = boundary lubrication regime, ML = mixed lubrication regime. A lower friction coefficient represents higher lubrication performance of saliva. Different letters indicate significant differences ($p < .05$).

6.4.6. Salivary biomarkers

The total concentration of protein (**Figure 6.8a**) and α-amylase (see **Figure 6.8b**) were assessed for each condition at three time points: pre-preload, post-preload and 30 min post-preload. For both protein and α-amylase activity at baseline/pre-preload there was no significant difference between conditions. Post-preload, for the protein activity, there was significant differences between all conditions, with the highest activity in LC followed by MC, HC and Control with the lowest protein activity ($p < .05$). No significant difference was noted 30 min post-preload in protein activity between conditions. For the α-amylase activity, there was a significant difference immediately post-preload only between HC condition compared to Control one ($p < .05$). The same was seen 30 min post-preload with a significant difference in α-amylase between HC condition compared to Control one (**Figure 6.8b**). For total protein, there was an effect of time $F(2, 72) = 44.753$, $p = .001$, condition $F(3, 108) = 40.033$, $p = .001$ and condition*time interaction $F(6, 216) = 53.412$, $p = .001$. The same was noted for α-amylase. There was an effect of time $F(2, 70) = 16.416$, $p = .001$, condition $F(3, 105) = 3.910$, $p = .011$ and condition*time interaction on salivary α-amylase concentration $F(6, 210) = 3.595$, $p = .002$. Further, mucin (MUC5B) content was determined in saliva samples, however out of 37 saliva samples, MUC5B was only found in 4 samples, which could be due to insuficient time of preload/beverages interacting with saliva). Therefore, these results cannot be treated as robust and have been included in Supplemenatry file for the record (see **Supplementary Figure E.2**).

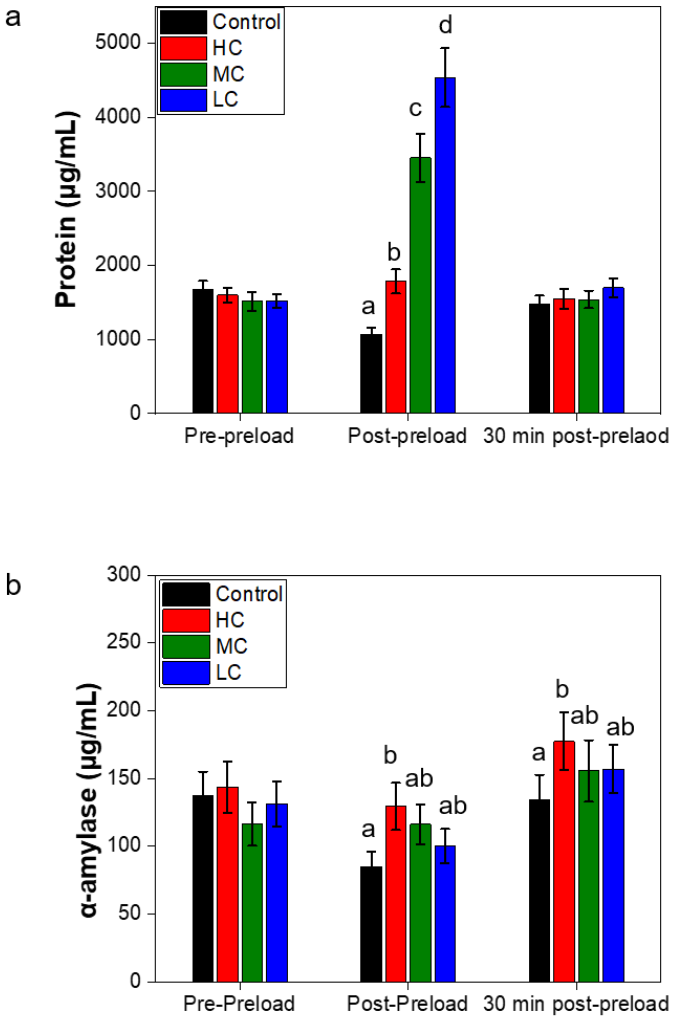

Figure 6.8. Total protein (µg/mL) (n = 37) (a) and α-amylase (µg/mL) (n = 37) (b) in saliva for Control, HC (high coating), MC (medium coating) and LC (low coating) conditions for Pre-preload, Post- preload and 30 min post-preload for Study 1. Values are means and error bars represent standard error of means (SEMs). Different letters indicate significant differences (*p* < *.05*), (n=37).

6.4.7. Blood biomarkers/gut peptides

There was no difference in the fasting levels between MC and HC conditions for glucose, total ghrelin and PYY (all p > .05) as shown in **Table 6.6**. However, fasting levels between conditions significantly differed for GLP-1 (p < .05), resons for this are not clear, but may be related to the high variation.

Table 6.6. Absolute fasting levels of glucose, total ghrelin, GLP-1 and PYY before consumption of preloads. Data are mean and SDs, (n=15).

Fasting levels	Medium Coating (MC)	High Coating (HC)	P value
Glucose, mg/dL	92.33 ± 5.72	89.2 ±8.77	0.236
Total ghrelin, pg/mL	884.2 ± 809	596.73 ± 483.42	0.158
GLP-1, pg/mL	25.63 ± 10.91	32.12 ± 19.88	0.029
PYY, ngL	674.87 ± 196.67	680.27 ± 310.73	0.958

Although we calculated both absolute and controlled for baseline data, we will focus on the controlled for baseline results in this section (results for absolute data can be seen in **Supplementary Table E.9**). Therefore, after controlling for baseline, there was no main effect of condition for glucose $F(1, 14) = .165$ and all gut peptides: total ghrelin $F(1, 14) = 0.209$, GLP-1 $F(1, 14) = 1.776$ and PYY $F(1, 14) = 0.204$ (all $p > .05$). There was a main effect of time for glucose, with this getting significantly decreased 30 and 60 min after preload $F(3, 42) = 39.336$, $p = .001$. For the rest of the gut peptides there was no main effect of time: total ghrelin $F(3, 42) = 1.785$, GLP-1 $F(3, 42) = 0.719$, PYY $F(3, 42) = 1.999$ (all $p > .05$). There was a significant effect of condition*time interaction for PYY only $F(3, 42) = 3.674$, $p = .019$. For the rest there was no condition*time interaction effect: glucose $F(3, 42) = 0.349$, total ghrelin $F(3, 42) = 0.383$ and GLP-1 $F(3, 42) = 1.994$ (all $p > .05$) (see **Figure 6.9a-d** for glucose and all gut peptides).

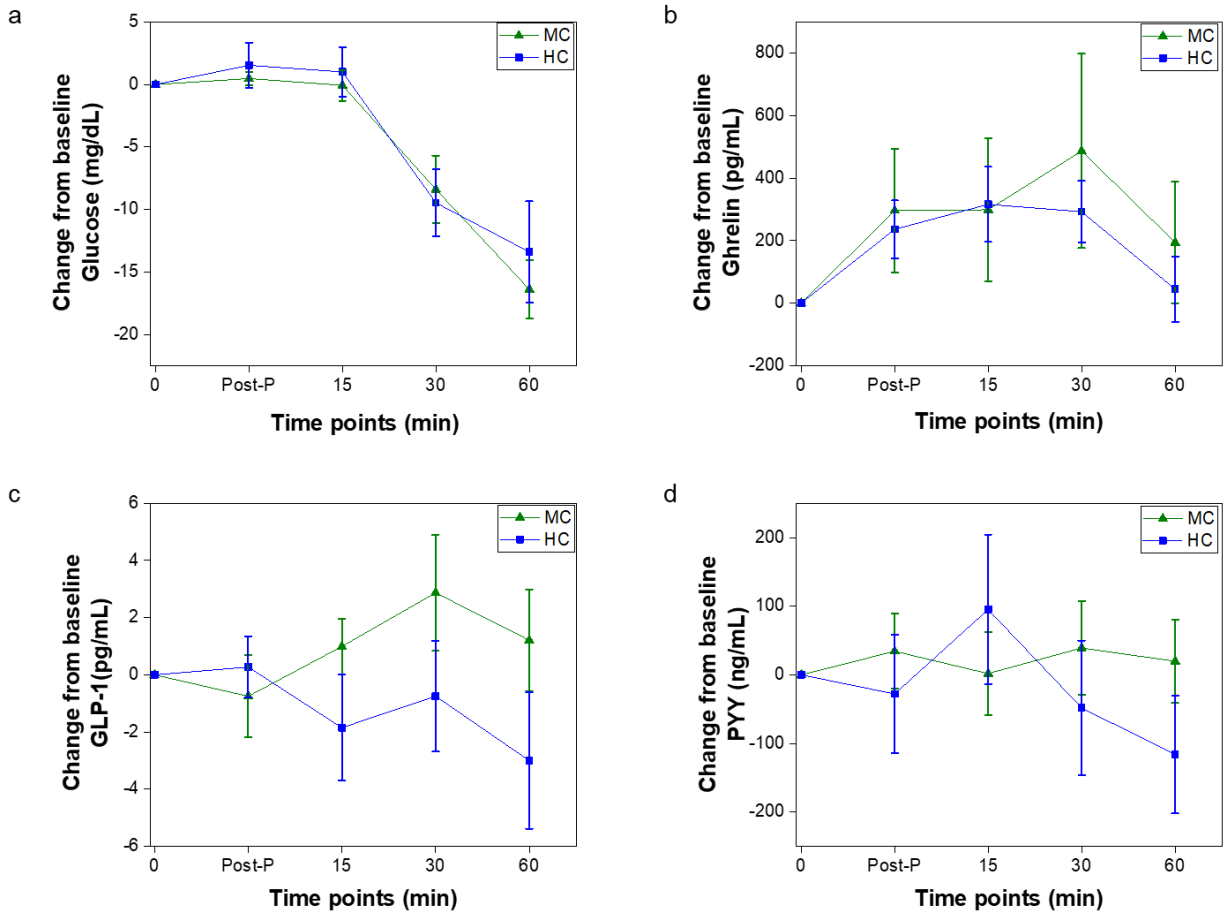

Figure 6.9. Postprandial profial of glucose (a), total ghrelin (b), GLP-1 (c) and PYY (d) after ingesting the preloads differing in their coating properties (MC and HC) for Study 2. Data are represented as means and SEM of the means, (n=15).

6.4.8. Pearson's correlation

To examine whether the changes in study 1, in appetite ratings and energy intake immediately post-preload were related to tribological/viscosity properties of saliva, salivary biomarkers and oro-sensory time exposure, we performed Pearson's correlation between the aforementioned parameters for all interventions (Control, HC, MC and LC) (**Supplementary Table E.10**). Statistical associations were noted between: protein activity and prospective food consumption (PFC) - r = - .980, p < .05, protein activity and desire to eat - r = - .963, p < 0.05 meaning that the higher concentration of protein in saliva the lower the desire to eat and the

prospective food consumption ratings. Another statistical associations were noted between friction coefficient (tribology) of saliva and hunger - r = - .980, p < .05, fullness -r = .951, p < .05, r = .990, p < .01, desire to eat - r = - .976, p < .05, r = - .993 and r = - .999; p < .001 and prospective consumption - r = - .983, r = - .968 and r = - .968, p < .05. These mean that the lower the friction coefficient (which means higher lubricating properties of saliva), the higher the feeling of hunger, desire to eat, prospective consumption and the lower the feeling of fullness. This is against our expectations and hypothesis. Another surprising significant association was between protein activity and friction of coefficient (tribology) -r = .973, p < .05, r = .978, p < .05 meaning the higher friction of coefficient (which means lower lubricating properties of saliva) the higher the salivary protein activity. Again, it is against our expectations. The correlation was not possible on individual data as some measurements, such as lubricity of saliva was based on pooled saliva not for each individual alone. Therefore, correlation on means was more appropriate and this might explain the high coefficients, which needs to be treated with caution.

6.5. Discussion

In the current study we investigated the effect of mouth-coating and lubricity on appetite control, food intake, salivary biomarkers (Study 1) and gut peptides (Study 2) using texture-manipulated protein beverages as preloads. In order to achive different texture properties of the preloads, whey and casein protein beverages were subjected to heat treatment method. It is known that casein is a ‘slow’ protein, while whey protein is considered as a ‘fast’ protein mainly on the basis of gastric emptying (Greco, et al., 2017; Luhovyy, Akhavan, & Anderson, 2007). Consequently, intake of whey results in a fast, but short and transient increase in plasma amino acids that peak in 40 min to 2 hours after its ingestion and returns to baseline values after 3 to 4 hours. In contrast, the intake of casein results in plasma amino acid

concentrations that rise more slowly and are lower, but sustain a prolonged plateau lasting for at least 7 hours after its consumption (Boirie, et al., 1997; Dangin, Boirie, Guillet, & Beaufrère, 2002). Since there is a big difference in the release time of amino-acids of these two types of proteins that would lead to different responses on satiety, this study aimed to investigate the immediate and short term (up to 1 hour) effect, therefore examining the first stages of satiety from an oral processing perspective, trying to eliminate, as much as possible, any effect of the protein type itself, particularly its gastric emptying effects. This appears to be the first occasion in which this experimental approach has been employed. Additionally, we explored the lubricating properties of human saliva after ingesting these preloads. To understand if the time of the preloads in the mouth affected in any way the results we also investigated the oro-sensory exposure time and salivary flow.

With reference to the appetite ratings, in study 1, an effect of protein intake versus Control (water) irrespective of mouth coating (HC, MC, LC) properties in reducing hunger, desire to eat, prospective food consumption and increasing fullness was observed immediately after ingestion, which continued 30 min after. Interestingly, in study 1, a sporadic effect of coating was noticed where fullness increased in HC condition vs LC 20 min after preload, meaning that participants felt fuller after ingesting beverages with high coating properties compared to low coating. Also, a decrease in prospective food consumption ratings (how much participants felt they could eat after the preloads) was noted, where participants felt eating less after HC and MC immediately as well as after preload intake compared to LC. As such, we could see a clear effect of protein intake vs Control which is well-reported in literature (Giezenaar, et al., 2017; Hutchison, et al., 2015) and a much more sporadic effect of HC (high coating) vs LC (low coating) on appetite sensations. This sporadic effect of coating could be explained by several factors. Firstly, the oro-sensory exposure time of the preloads was higher in HC and MC vs LC. Therefore, the more time the beverages spent in the mouth the higher

feelings of fullness participants experienced in HC and MC compared to LC. Secondly, saliva was more lubricating in HC and MC vs LC which can explain the mechanism behind it, where lubrication is believed to be associated with mouth coating thereby extending the oro-sensory exposure time leading eventually to a better appetite control (Krop, et al., 2019a; Krop, et al., 2019b; Stribițcaia, et al., 2021). Thirdly, there was a negative association between protein activity in saliva and prospective food consumption and desire to eat, meaning that the higher the protein activity in saliva the lower was the desire to eat and the less feelings of prospective food consumption. However, one should be cautions drawing conclusion from these results as the effect of coating was sporadic and was not consistent across all appetite sensation and at all time points.

With respect to appetite ratings in study 2, there was no difference between the conditions MC and LC. It is not a surprise as this comes in accordance with the results of study 1 where there also was no difference between MC and LC. Although in the study 1 the level of coating and lubricity in the preloads was clearer (lubricity – low, medium and high; coating – low, medium and high), in order to exclude any effect of the protein type (Casein – high coating/low lubricating, Heated Whey protein – medium coating/high lubricating, Unheated Whey protein – low coating/medium lubricating) the two whey protein beverages -Heated Whey protein – MC (medium coating) and Unheated Whey protein – LC (low coating) were selected for study 2. As such, it appears that a subtle change in texture (lubricity/coating) properties of the preloads does not influence the appetite ratings which corroborates with previous studies (Krop, et al., 2019b; Stribițcaia, et al., 2021). This is on the contrary to studies where manipualtion of the preloads texture is stronger such as liquid vs solid, low viscous vs high viscous or low viscous vs gels (Juvonen, et al., 2011; Stribiţcaia, et al., 2020a).

We also tried to understand the effect of texture in combination with macronutrients/energy load on appetite. From this perspective, it could be seen that the effect lasted up to 30 min. In

our previous study (Stribițcaia, et al., 2021) where the texture (lubricity) was expressed through non-calorific preloads (hydrogels) the effect on appetite ratings was immediate and very short (10 min) compared to the current study where effect of coating was combined with macronutrients/energy load expressed through protein beverages preloads and lasted up to 30 min. A combinatorial effect of texture and macronutrients/energy has been demonstrated. However, one should be careful in interpreting these results as in addition to lubricity the preloads could have been confounded by a variety of other factors (taste, palatability, different oral processing, expected satiety etc.).

Regarding energy intake, there was no effect of oral coating in both studies. In study 1, the energy intake was similar in all conditions – HC, MC, LC and Control. A previous study in the literature, reporetd an immediate effect of food texture (oral lubricity) on snack intake, with this being lower in high lubricating condition compared to low lubricating one (Krop, et al., 2019b). It may suggest that the effect of texture (coating/lubricity) could be immediate regardless of the presence or absence of macronutrients/energy load in the preloads. Moreover, a high score on restrain across participants (11 out of 37 in study 1) could also explain the lack of effect of texture on energy intake.

In study 2 we changed the content of *ad libitum* breakfast because of two main reasons – 1) to exclude any learned experience on energy intake from the previous study (half of the participants were from study 1, and 2) to investigate the effect of oral coating on the preference of chosen food (S/LF, S/HF, SW/LF and SW/HF). Despite this, the energy intake has been similar in both MC and LC conditions. Moreover, it was noted that participants chose SW/LF compared to the rest (S/LF, S/HF and SW/HF) irrespective of the condition (MC and/or LC). This could suggest that paricipants deliberately opted for more heathy choices irrespective of study conditions (texture manipulation). Therefore, it can imply that the changes in the texture of the preloads (manipulation of coating) in both studies, were too subtle to trigger a

physiological (body signals) response in relation to food intake (participants to eat significantly more or less depending on the conditions).

To add to the understanig of the mechanism behind coating and appetite, we measured glucose and satiety peptides such as total ghrelin, GLP-1, PYY. While the trend in glucose levels with a plateau up to 15 min and a sharp decrease after 15 min up to 60 min was in aligment with the literature (Bowen, Noakes, & Clifton, 2006; Hutchison, et al., 2015; Stribiţcaia, et al., 2020a), there was no significant difference in glucose levels between the conditions MC and HC. Our findings come in agreement with previous works on texture that reports no differences in glucose levels, althgough the differences in texture (in the previous reported studies) were clearer, such as solid versus liquid were clearer, such as solid versus liquid (Martens, Lemmens, Born, & Westerterp-Plantenga, 2012; Martens, Lemmens, Born, & Westerterp-Plantenga, 2011) compared to this study – medium coating versus high coating.

The same has been noted for total ghrelin, GLP-1 and PYY with no effect of condition/texture and time. This does not come as a surprise as previous studies showed no effect of texture on ghrelin (Martens, et al., 2012; Martens, et al., 2011; Zhu, et al., 2013), GLP-1 and PYY (Juvonen, et al., 2011; Zijlstra, et al., 2009). Recent studies have suggested that gut peptides such as GLP-1 and/or PYY can be released in proportion to the energy load and macronutrients (Adrian, et al., 1985; Degen, et al., 2005; Juvonen, et al., 2011), indicating that the higher the energy and fat load of the test meal/preload is, the more GLP-1 and PYY is released. Therefore, meals that have only protein with low or/and equal energy load may explain to some extent the lack of differences in the results of the gut peptides in the current study. For instance, an effect of food texture on ghrelin, GLP-1 and PYY has been shown in preload starting with 300 kcal (Juvonen, et al., 2009; Zhu, et al., 2013), while in the current study the preloads were of 239 kcal. Therefore, it may be suggested that the preloads in our study did not have enough kcal load to elicit reduction in ghrelin and release of GLP-1 and

PYY. Likewise, it can be suggested that texture alone is not enough to trigger a physiological/ gut hormonal response. It seems that oral processing has a limited or no effect on blood hormonal response when the manipulation of food texture is subtle, based on one macronutrient only and has a reduced amount of kcal.

This brings us to the question, does texture (coating) influence satiety and satiation? There was a clear effect of protein beverages vs Control and a sporadic effect of coating on some appetite sensations at certain time points which was also observed with a consequent increase in salivary lubricity. However, at this stage it is unclear and premature to give a robust answer to this question. Nevertheless, there is certainly room for more research into the area especially in relation of the interaction between saliva and food with high coating properties. The strength of this study is showing the importance of saliva in underpinning the mechanism of oral lubricity/coating in the context of satiety. When it interacts with food high in coating properties, strikingly it becomes more lubricating which might helped to coat oral surfaces better and for longer time and in turn led to higher rating in fullness and lower ratings in desire to eat and prospective food consumption in this study. Thus, this study offers a novel textural construct of oral coating along with consequent changes in salivary lubricity in the context of satiety.

However, one should interpret the results with caution as the effect of coating was not consistent across all appetite sensations and at all-time points, a sporadic effect as mentioned above. Future research should investigate whether the effects of coating are observed in a repeated exposure and long-term design. In addition, future research should aim at creating preloads with a higher degree of difference in coating properties between preloads and examine its effect on satiety. At last, investigating the effect of oral coating on satiety and satiation in an *ad libitum* intake design would add valuable information to the mechanism proposed above.

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Chapter 7

General discussion

7.1. Summary of the main thesis results

Understanding food texture, in particular from its lubricating and coating properties and how texture transforms on mixing with saliva while consumers perceiving it sensorially and eventually how it impacts satiety and satiation can help both scientists and food industries to design satiety-enhancing foods/ beverages. Such designed foods/ beverages may facilitate appetite control and would lead to a lower food intake in order to contribute to global overweight and obesity crisis. The aim of this thesis was therefore to understand the influence of food texture, more specifically from an oral tribological and mouth coating perspective on satiety and satiation. For this, model food and beverages differing in their lubricating/ coating properties have been created and a series number of preload satiety trials have been conducted to investigate its effect on appetite and subsequent food intake. To understand better the mechanism, salivary and blood biomarkers have been measured as well. A special attention has been given to the changes in properties of human saliva upon ingestion, as saliva is known to play a crucial role in lubricating the mouth intrinsically. A summary of the chapters in this thesis and their outcomes is shown in **Figure 7.1**, from the development of the non-calorific and calorific preloads/foods to their physical properties/ sensory characterisation and their lubricating/coating effect on short-term satiety.

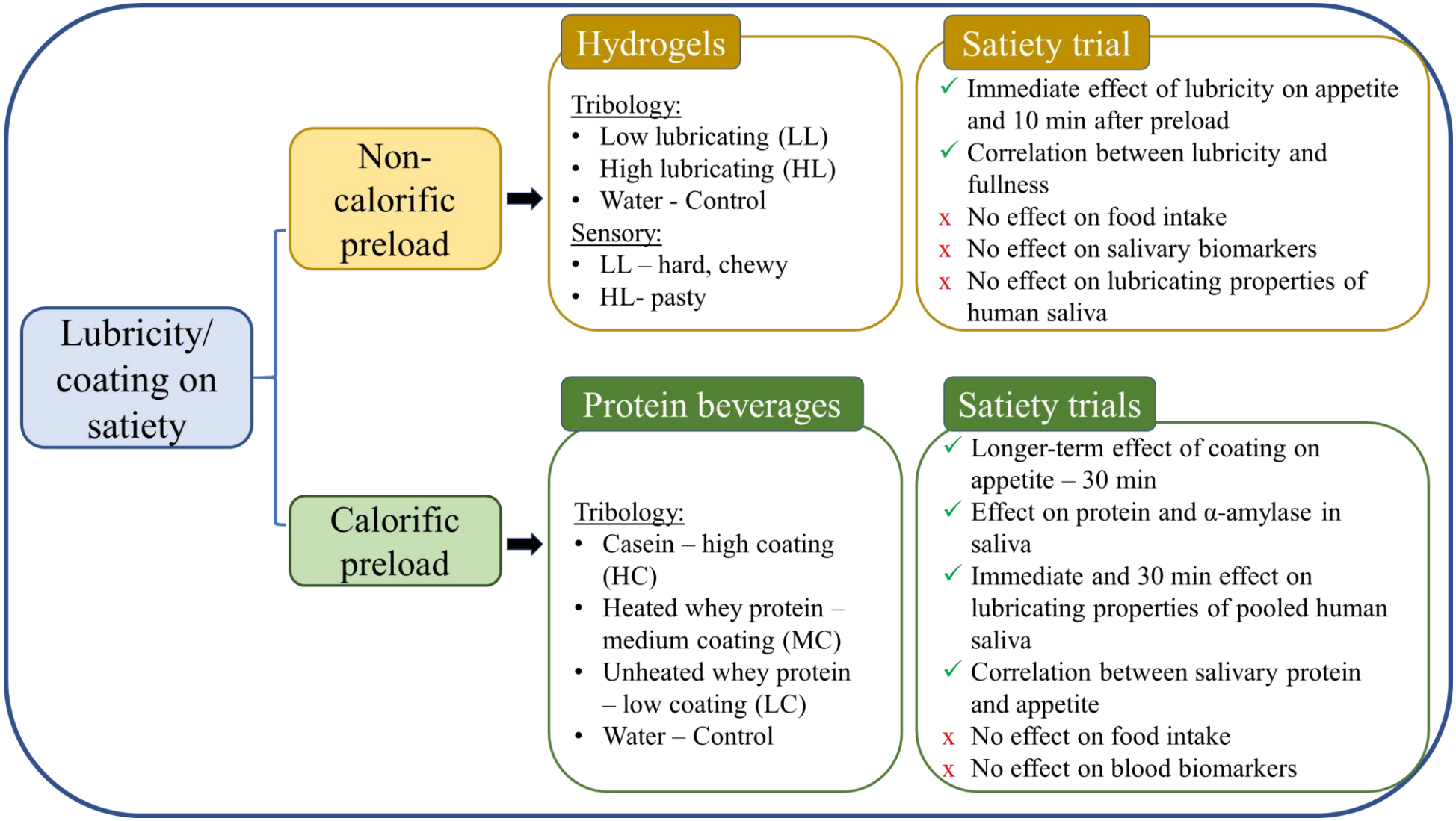

Figure 7.1. The summary of the chapters in this thesis.

Based on the perceived knowledge gap in the literature on the effects of food texture aspects on satiety using systematic review and meta-analysis (**Chapter 2**), this PhD project has advanced the knowledge base by moving beyond the simple aspects of food texture (form and/or viscosity) to more complex ones, such as lubricity and mouth coating. In order to test the effect of oral lubrication, a set of non-calorific model foods such as hydrogels have been developed and their lubricating degree was analysed both instrumentally and sensorially (**Chapter 3**). After selecting two non-calorific hydrogels with differences in their lubricating properties, first satiety trial was designed (**Chapter 4**). It was hypothesised that oral lubrication will suppress appetite and reduce food intake. The hypothesis was partially supported showing that oral lubrication immediately suppressed appetite (hunger decreased and fullness increased), and it lasted for 10 min after the ingestion of the preloads, but did not have an effect on food intake.

Based on this, the next preloads were developed, but with calorific *i.e.* macronutrient-containing hydrogels. Protein beverages have been developed and their lubricating and rheological properties have been analysed instrumentally. Due to Covid-19 pandemic (where the access to the laboratory was prohibited), a study trial was designed and involved a novel way to assess the effect of food texture on expected satiety remotely (**Chapter 5**). In this study, the effect of texture (viscosity) on perceived/expected satiety was assessed through a video online questionnaire where short videos of protein beverages being poured from one container into another was shown to participants. It has been shown that an online video survey can be a reliable method to assess food texture on expected satiety remotely.

Finally, the in person satiety trial was conducted with the protein beverages where in addition to lubricity, we added a new quantitative dimension in textural analyses *i.e.* coating properties of the preloads (**Chapter 6**). The combinatorial effect of lubrication/coating and macronutrients was investigated on satiety and satiation in two more studies (**Chapter 6**). In

this chapter we tried to identify the mechanism underlying the oral lubrication/ coating in the context of satiety. The hypothesis was that combining mouth coating with macronutrients/energy load will suppress appetite for longer and will reduce subsequent food intake. The hypothesis was partially supported, showing that the effect of mouth coating (in combination with macronutrients/energy load) suppressed appetite for longer and the effect lasted for 30 min, as compared to 10 min in the first study (**Chapter 4**). Fullness increased, desire to eat and prospective food consumption decreased in high coating condition compared to low coating condition (this was a sporadic effect, not consistent across all appetite sensations and all time points). However, there was no effect on food intake. Also, in this study it has been shown the role the human saliva plays in explaining the effect of oral lubrication/coating on satiety and satiation (**Chapter 6, Study 1**). The interaction of the preloads with higher degree of coating and human saliva made saliva more lubricious, coating the mouth better, leading to a longer oro-sensory exposure time, and in turn, had an effect on some of the appetite sensations. In addition, a correlation was observed between salivary biomarkers and appetite ratings, the higher the concentration of the proteins in saliva, the lower the desire to eat and prospective food consumption ratings. To understand further the effect of oral lubrication/ coating, another satiety trial was carried out, this time involving gut peptides (**Chapter 6, Study 2**). After controlling for baseline, there was no effect of oral lubricity/ coating on glucose, ghrelin, GLP1 and PYY.

This discussion chapter reflects on the novelty of the key findings obtained in this thesis and highlights the major study parameters. Finally, the implications of the current findings and recommendations for future work are discussed.

7.2. Novelty of this thesis

The novelty of this thesis lays in the understanding and explaining the mechanism of oral lubricity and coating in the context of satiety. Although, it is in infancy stage, we managed

to show that lubricity/ coating can suppress appetite over control, and the effect can last relatively longer when it is combined with calories/ macronutrients. We demonstrated the crucial role saliva plays in explaining this mechanism. Saliva appeared to be more lubricating after ingesting preloads higher in coating properties. Also, the higher concentration of the proteins in saliva, the lower was the desire to eat. As such, the mechanism could be explained as follow, the more lubricating the saliva is, the more coated the mouth is which would lead to a longer oro-sensory exposure time and in turn would suppress appetite and reduce food intake. With this, we showed the importance of calories/macronutrients and saliva on oral lubricity in the context of satiety and satiation.

7.2.1. Non-calorific versus calorific preloads

This thesis concerns lubricity expressed through non-calorific and calorific hydrocolloids preloads. In our first satiety trial we wanted to eliminate cofactors such as macronutrients/energy load, and assessed the sole effect of lubricity. Therefore, we developed model foods such as hydrogels that did not contain any energy load or macronutrients. Similar kinds of model foods have been previously employed in the literature in relation to satiety and satiation (Larsen, Tang, Ferguson, & James, 2016; Tang, Larsen, Ferguson, & James, 2016) with only one study addressing directly the lubricity aspect in a snack trial (Krop, Hetherington, Miquel, & Sarkar, 2019). We used the same ingredients as Krop et al. (Krop et al., 2019) for the hydrogels: kappa-carrageenan (*κ*C), sodium alginate (NaA) and calcium alginate beads (CaA). The difference in the hydrogels between this thesis and the previous work (Krop et al., 2019) is the different concentration of the material and the processing *i.e.* the way in which the beads and the layers were structured. Krop et al. (Krop et al., 2019) used *κ*C hydrogels alone (3 wt%), *κ*C (1.5 wt%) with NaA (0.5 wt%) and *κ*C (2.4 wt%) with CaA beads of 300 μm (0.2 wt%). The latter had two layers, one layer of CaA beads with one layer of *κ*C on top. In the current thesis, we used *κ*C (1.67 wt %) with NaA (0.33 wt%) mixture hydrogels and *κ*C (1.67

wt%) with CaA beads of 400 μm (0.33%). And the latter had three layers, first of κC, second of beads and the third one of κC again. By using different concentration and different structure/ layering of the hydrogels compared to the previous study (Krop et al., 2019) we achieved a higher degree of lubricity difference in our hydrogels both instrumentally and sensorially for the sensory trial.

Certain hydrocolloids such as agar, gelatine, xanthan gum, pectin were not considered for further investigation in this thesis as they are known to possess specific characteristics unsuitable for the purposes of this thesis. For instance, gelatine can show a melting behaviour at orally relevant temperatures. This melting behaviour would further complicate the sensory experience, particularly when comparisons are made with systems that do not melt at body temperatures (Devezeaux de Lavergne, Strijbosch, Van den Broek, Van de Velde, & Stieger, 2016). Moreover, based on a series of preliminary studies, other hydrocolloid systems were excluded as they did not form stable gels that would be pleasant to eat. Although, we managed to create hydrogels higher in lubricity properties compared to other studies in the literature (Krop et al., 2019), we demonstrated the effect of oral lubricity on appetite ratings but not on subsequent food intake. On the other hand, Krop et al. (Krop et al., 2019) demonstrated an effect of oral lubricity on snack intake with participants eating 32% in high lubricating conditions compared to low lubricating one but failed to demonstrate any effect on appetite ratings. Hence, we believe that the differences in lubricity between the hydrogels in this thesis although being one order of magnitude was still too subtle to trigger an effect on food intake in a test meal. Based on this, there is a potential for future studies to consider higher levels of lubricity *e.g.* two orders of magnitude between the hydrogels and assess its effect on food intake in a test meal.

Another aspect to consider when assessing the effect of lubricity on satiety and satiation is the combination with macronutrients/calories. In the first trial, we assessed the effect of

lubricity alone taking out from the equation any effect of energy load or macronutrient. Showing the effect alone of the lubricity, the next step was to see the combinatorial effect of lubricity/coating with energy load/macronutrients. Proteins such as whey and casein have been selected for the preloads for the next satiety trials known to have a greater satiating capacity compared to other macronutrients (Halton & Hu, 2004; Latner & Schwartz, 1999). Whey and casein beverages have been used largely in the satiety trials (Bowen, Noakes, Trenerry, & Clifton, 2006; Giezenaar et al., 2017; Hall, Millward, Long, & Morgan, 2003; Juvonen et al., 2011), however, to date, none assessed the effect from an oral lubrication perspective as has been done in this thesis. In this thesis, the protein preloads were used as beverages like aforementioned studies where the texture was manipulated by heat treatment, *i.e.* heating it gradually at different temperature (70°, 80°, 90°) and time (5, 6, 7, 8, 8,5 min) until the desired level of lubricity has been obtained. Ideally, it would have been more appropriate if the preloads were hydrogels as in the first satiety trial, or at least to match the solid-like texture of the hydrogels. Unfortunately, having no access to the laboratory during the pandemic for nearly a year and restricted time for this PhD project, we opted for beverage preloads.

There has been an attempt to create beverages as preloads of alternative proteins such as plant proteins for sustainability reasons. We developed beverages of pea, lupine and potato protein (Kew, Holmes, Stieger, & Sarkar, 2021; Zembyla et al., 2021). However, due to two main and important reasons they have not been considered into further investigation for this thesis: 1). the lubricating behaviour of these plant proteins compared to animal one (whey and casein) was limited (Kew et al., 2021); 2). the large amount of the beverage to be consumed by the participants particularly for study 2 involving blood collection for quantification of gut peptides (200 g in Study 1 and 400 g in Study 2 in **Chapter 6**) was sensorially unacceptable. There are indeed strategies to make them pleasant, for instance using citric acid (Abou-Samra, Keersmaekers, Brienza, Mukherjee, & Macé, 2011). However, this was not possible for this

PhD project as this would have affected salivary flow and also the material properties of the collected saliva in **Chapter 4** and **6**.

It would be worth to consider solid-like preloads instead of beverages ones for future studies. For instance, one should consider creating model food of whey and/or casein protein such as solid gels similar to that designed using hydrocolloids in **Chapter 4**. From an oral processing perspective, this is crucial as oro-sensory time exposure plays a key role in satiety and satiation (Krop et al., 2018; Miquel-Kergoat, Azais-Braesco, Burton-Freeman, & Hetherington, 2015). The more time the food spends in the mouth (chewed) the more satiating/filling consumers feel. Therefore, it would be interesting to investigate the effect of oral lubricity in relation with oro-sensory exposure time in solid-like model foods as compared to beverages. Prolonged chewing and subsequent particles generated from model gel-like foods could also lead to a better mouth coating and consequently to a better appetite control. Moreover, it would be paramount to assess oral lubricity/coating expressed through alternative proteins for sustainability reasons. Although, plant protein presents a challenge in terms of lubricity compared to animal one, it is worth investigating ways to increase the lubricity behaviour of the alternative proteins close to the degree of the animal ones and assess its effect on satiety and satiation.

Another important thing to consider is to report and give more attention to the effect sizes across the outcomes which would have informed additionally in regards to the practical significance of the intervention and for a better/more appropriate power calculation for the next studies. Just to give an example between high lubricating and control conditions, looking back through the results, a nine point change in appetite sensations would be enough to have a medium effect size (d = 0.40), or 55 g change in food intake for a small effect size (d = 0.14) or 0.0042 units for friction coefficient (for lubricity of saliva) for a medium effect size (d = 0.5). As lubricity and mouth coating are new constructs/characteristics of food texture, there

was no literature in terms of the effect sizes expected to be elicited by the subtle differences in texture, we did a prior power analysis to determine the number of participants needed for a small effect size (f = 0.25).

7.2.2. The role of human saliva

Saliva has an important role in food oral processing, for example in the breakdown of food (Engelen et al., 2003) and also it acts as a lubricant of the oral tissues (Sarkar, Xu, & Lee, 2019; Tabak, Levine, Mandel, & Ellison, 1982). Based on this, we attributed an important role to saliva in order to explain and understand better oral lubricity and its effect on satiety.

It is known that lubricating properties of human saliva derives mainly from two major structurally and functionally distinct mucins: MUC5B and MUC7 (Park, Chung, Kim, Chung, & Kho, 2007; Perez-Vilar & Hill, 1999) as well as low molecular weight cationic species (Xu et al., 2020). In this PhD project, we collected and analysed human saliva, both in terms of lubricating behaviour and salivary biomarkers. To our knowledge, this is first time where human saliva has been measured for its tribological properties in a satiety context. In the first satiety trial (**Chapter 3**), lubricating properties of saliva after preloads was analysed for each individual. Due to many outliers in the first study, we decided to pool saliva in the next trial (**Chapter 6**). Future studies should consider to analyse human saliva immediately after it is collected. In this thesis, after collection, saliva has been stored at -80°C until the last participant (when the entire study finished). It is known that even one freeze-thaw cycle of human saliva can alter the results (Fan, Shewan, Smyth, Yakubov, & Stokes, 2021; Sarkar et al., 2019), therefore it is suggested that human saliva should be analysed immediately or within 30 min of collection, which would require additional human and financial resources.

Challenges were met while analysing salivary biomarkers, especially MUC5B. In the first trial (**Chapter 4**), out of 17 samples we could detect mucin activity (MUC5B) in 9 samples. In second study (**Chapter 6**) out of 37 samples we could detect mucin activity

(MUC5B) in 4 samples only. This might be linked to the freeze-thaw cycle and precipitation of mucin as discussed before. In addition, robust method of quantification of mucin should be developed and employed in future studies. Besides this, a key salivary biomarker that could affect satiety is the peptide YY (PYY). Peptide YY (PYY), a well-characterized molecular mediator of satiation, is released mostly by L-endocrine cells in the distal gut epithelia in response to the amount of calories ingested (Zolotukhin, 2013). Recently, it has been found in saliva as well, and was linked to satiety, both in animal and human studies, with latter being limited in the literature (Acosta et al., 2011). PYY_{3-36} was detected in saliva samples of healthy human male volunteers those who fasted overnight. Interestingly, 30 min after consumption of a 450 kcal meal the concentration of PYY_{3-36} increased significantly suggesting a possible association between feeding and the concentration of PYY_{3-36} in saliva (Acosta et al., 2011). Due to the technical challenge where the detection of this peptide (PYY) is difficult (rapid degradation), the analysis of the salivary PYY was not possible during this PhD project. Therefore, future studies should consider the quantification of salivary PYY in regards to satiety.

7.3. Future research

Following consideration are suggested for future research in the area:

1. Formulation perspective:

Although, there was a significant difference in terms of texture between the preloads, it was not enough to trigger an effect on food intake. Therefore, a change in food/ preload formulation should be considered. For instance, addition of oil or fats to modify the model food could be more helpful as fat is known to be a good lubricant as well as can add significant energy density (Chojnicka-Paszun, De Jongh, & De Kruif, 2012; Wang, Zhu, Ji, & Chen, 2021). Moreover, it is important to see the effect of lubricity or coating in real

foods. In this thesis we assessed lubricity and coating in model food such as hydrogels or in a one type macronutrient beverages (protein). Therefore, assessing model food versus real meal would add valuable information to the food texture and satiety field.

2. Analytical perspective:

The next generation of equipment to measure the lubricity of the products should be developed to be more representative to the mouth surfaces (tongue and palate). The surfaces to mimic the tongue and the palate in this thesis were silicon PDMS ball and disk (see **Chapter 6**). However, a more representative equipment would be a tongue-palate-like surface and this has been in development recently – a 3D biomimetic tongue-emulating surfaces (Andablo-Reyes et al., 2020). This will give more accurate measurements and closer to real oral surfaces and help in identifying and screening products with lubricating properties. Nevertheless, aforementioned analysis should be backed up with sensory analysis as well. Although we did some basic sensory tests (discriminative and intensity rating with untrained panellists), use of tools such as Rate-All-That-Apply (RATA) (Oppermann, De Graaf, Scholten, Stieger, & Piqueras-Fiszman, 2017) would be useful to clearly understand the sensorially perceived differences in lubricity between the preloads.

Although we measured mucin in saliva (MUCB5), the quantification suffered from severe challenges. Therefore, a robust protocol needs to be developed for human saliva collection and analysis. Previous literature showed a link between salivary PYY and satiety (see above). Therefore, future study should include salivary PYY collection by developing a robust and reliable protocol.

3. Satiety trial:

This was the first time when lubricity and coating has been measured in a satiety context. Although the preloads varied in their lubricating and coating properties in this

thesis, it was not measured as separate parameters. Therefore, a 2 × 2 (high/ low lubricating and high/ low coating) study design would be more appropriate to achieve richer results. It would be also interesting to perform both expected and real satiety trial with the same participants to identify associations (if any) between expected and actual satiety

An acute short-term study design was used in this thesis, however, repeated-measures and long-term study designs would deepen the understanding of lubricity and coating on satiety. Also, it would be worth using *ad libitum* intake design of the products themselves (model or actual food differing in lubricating properties). It will give researchers the understanding of oral lubrication/coating on satiation itself. Moreover, the type of the participants play a key role in terms of the results. In this thesis, we recruited healthy adults, therefore broader categories of participants, such as overweight, with obesity and/other non-communicable diseases or participants with different age groups, knowing to differ significantlly in terms of appetite control and eating behavior (Blundell, Finlayson, Gibbons, Caudwell, & Hopkins, 2015; Blundell, Lawton, & Hill, 1993), could bring new understanding to how lubricity and oral coating may have satiety consequences. For instance, it has been shown that the level of hunger in population with obesity is 1.35 points (on a 7 point scale) higher compared to normal weight population (Slyper, Shenker, & Israel, 2021). Therefore, based on the results of this thesis, where a nine points (on 100 mm scale) change in appetite sensations are enough to detect a medium size in healthy weight population, someone should consider a higher value for future reasesrch planning in overweight and obese population. However, at this stage, the results of this thesis can be only inrepreted in normal weight population.

7.4. Conclusion

In summary, this thesis showed a clear effect of oral lubricity/coating versus control on appetite ratings. We also, demonstrated that combining lubricity/coating with macronutrients/energy load can prolong the effect on appetite ratings compared to non-calorific lubrication. For the first time, human saliva have been analysed and linked to the mechanism of oral lubrication/coating in a satiety context. Therefore, we showed a correlation between lubricity of saliva and fullness, the higher the lubricating properties of saliva the higher the fullness ratings. Moreover, a correlation has been found between salivary proteins and appetite ratings: the higher the concentration of proteins in saliva the lower the desire to eat and prospective food consumption ratings. Although, oral lubricity/coating is in its infancy stage it offers promises and opportunity for further exploration as a possible strategy for food design for weight management to tackle overweight and global obesity.

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Appendix A

Supplementary information for Chapter 2

Supplementary Table A.1. Searching terms used across the databases (this example was used in MEDLINE Ovid database 1946-2019) for the current systematic review.

Food texture: **#1** Food texture.sh. or food texture.ti. or food characteristics.sh. or food characteristics.ti. or food properties.sh. or food properties.ti. or Viscosity.sh. or viscosity.ti. or Semi-solid.sh. or semi-solid.ti. or Liquid.sh. or liquid.ti. or Rheology.sh. or rheology.ti. or Lubrication.sh. or lubrication.ti.	
Appetite: **#2** appetite regulation.ti. or appetite control.ti. or appetite.sh. or appetite.ti. or desire to eat.ti. or prospective food consumption.ti. or satiation.sh. or satiation.ti. or hunger.sh. or hunger.ti. or fullness.ti. or thirst.ti.	and
Food intake: **#3** Food intake.ti. or energy intake.ti. or food behaviour.ti. or food behaviour.ti. or eating behavio*r	and
Gut hormones: **#4** Gut hormones.ti. or gut peptides.ti. or acylated ghrelin.ti. or appetite-related hormones.ti. or appetite-related peptides.ti. or episodic hormones.ti. or episodic peptides.ti. or satiety hormones.ti. or gastrointestinal hormones.sh. or gastrointestinal hormones.ti. or cholecystokinin.ti. or GLP-1.ti. or ghrelin.ti. or PYY.ti. or amylase.ti. or biomarkers.sh. or biomarkers.ti.	and

Supplementary Table A.2. Characteristics of the preloads across the studies included in the systematic review. Measurements units for instrumental and sensorial characteristics are given as in original articles. This table includes also time until next meal (min) and time frame of appetite ratings.

Reference	Instrumental quantification	Sensory quantification	Volume/weight	Energy density (kcal)	Time until next meal (min)	Time frame of appetite ratings
Camps et al. 2016	**Viscosity (at 50 s^{-1} shear rate):** Thin 100 kcal = 2.9 mPa.s Thin 500 kcal = 11.26 mPa.s Thick 100 kcal = 319 mPa.s Thick 500 kcal = 5897 mPa.s	**Thickness (VAS scale):** Thin 100 kcal= 14.3±2.6 mm Thin 500 kcal=30.8±4.2 mm Thick 500 kcal=72.7±4.1mm Thick 500 kcal=53.3±4.9 mm	No	Thin/thick = 100 kcal Thin/thick = 500 kcal	90	Every 10 min for a total of 90 min
Clegg et al. 2012	No	No	274 g	No	N/A	Every 15 min for 1 h Every 30 min for 3 h
Dong et al. 2016	**Viscosity (at 65 s^{-1}shear rate):** Liquid = 33±3 cP Semi-solid = 790±12 cP Solid = 8000±970 cP	No	240 mL	Liquid = 45 kcal/100 g Semi-solid = 55 kcal/100 g Solid = 36 kcal/100 g	N/A	Every 15 min for 2 h
Flood et al. 2007	**Viscosity (at 10 s^{-1} shear rate):** Chunky = 0.7 cps Chunky-pureed = 55 cps Pureed = 235 cps	No	Women = 350 mL Men = 475 mL	Women = 115 kcal Men = 156.75 kcal	15	On three time points: before, after preload, and after *ad libitum* lunch
Flood et al. 2009	No	No	266 g	125 kcal	15	On three time points: before, after preload, and after *ad libitum* lunch
Hogenkamp et al. 2012	No	**Thickness (VAS scale)**: LE liquid= 11±15 mm LE semi-solid = 67±19 mm **Firmness:** LE liquid= 10±18 mm LE semi-solid= 64±21 mm **Thickness**: HE liquid = 15±20 mm HE semi-solid = 90±12 mm **Firmness**: HE liquid = 12±19 mm	Women = 273-330 g Men = 354-418 g	**Low energy** (liquid and semi-solid) = 30 kcal/100g **High energy** (liquid and semi-solid) = 130 kcal/100g	Immediately after preload	On three time points: before, after preload, and after *ad libitum* lunch

		HE semi-solid = 87±17 mm				
Hogenkamp et al. 2012	No	**Thickness (VAS scale):** Liquid = 15±14 mm Semi-solid= 85±13 mm	Women = 468 g Men = 594 g	Liquid = 96 kcal/100g Semi-solid = 98 kcal/100g	N/A	Every 30 min for 3h
Juvonen et al. 2011	**Viscosity (at 50 s^{-1} shear rate):** Low viscous = 0.00 Pa s High viscous = 0.32 Pa s **Firmness (puncture test):** Low viscous = 0 mN High viscous = 0 mN Solid = 5900 mN	No	400 g	230 kcal	N/A	Every 15 min for 1h, Hourly for 3h
Juvonen et al. 2009	**Viscosity (at 50s^{-1} shear rate):** Low viscous = <250 mPas High viscous = >3000 mPas	No	300 mL	300 kcal	180	Every 15 min for 1h, Hourly for 3h
Krop et al. 2019	**Fracture stress**: Hard/low lubricating= 218 kPa Soft/high lubricating = 27 kPa **Lubrication properties (coefficient of friction (μ) at 50 mm s^{-1} speed)**: Hard/low lubricating = 0.26; Soft/high lubricating = 0.01	**Chewiness (VAS scale)**: Hard/low lubricating= 77±7mm Soft/high lubricating= 3±2 mm	Women= 25 g Men = 30 g	0 kcal	Immediately after preload	On three time points: before, after preload, and after *ad libitum* lunch
Laboure et al 2002	No	Unpublished results	**Food 1** = 591 g **Food 2** = 350 g	Type 1= 499 kcal Type 2= 499 kcal	Approximately after 5-6 h after preload	Every 30 min for 4 h
Larsen et al. 2016	No	**Chewiness rate (chews/s):** Low complex=1.35±4 s High complex=1.40±4s	32 g	48 kcal	10 min	Before, after preload and immediately after lunch and 3 h after lunch
Marciani et al. 2012	**Viscosity (at 50 s^{-1} shear rate):**	No	200 g	240 kcal	N/A	Every 45 min for 3 h

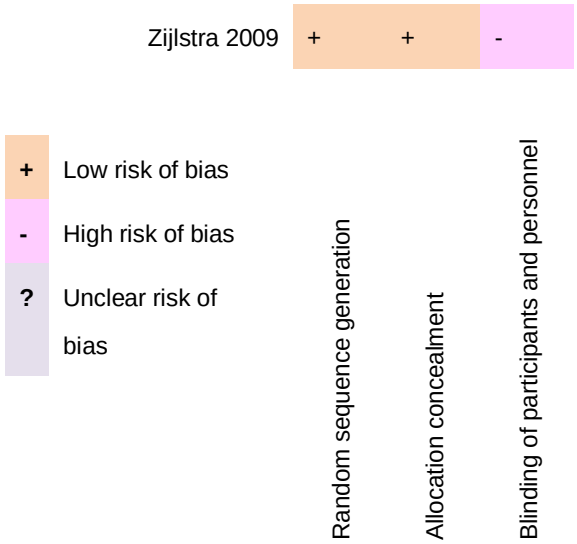
	of liquid = 509 mPa s					
Martens et al. 2012	No	No	Subject specific	Subject specific	N/A	Every 10 min for 1.5 h Every 15 min for another 1.5 h
Martens et al. 2011	No	No	Subject specific	Subject specific	N/A	Every 10 min for 1.5 h Every 15 min for another 1.5 h
Mattes 2005	**Viscosity (at 60 rmp):** *Food 1* Liquid = 11 cps *Food 2* Liquid = 45 cps **Hardness in terms of solid content (g):** *Food 1* Solid = 770 g *Food 2* Solid=257 g	No	**Food 1** = 652 g **Food 2**: Liquid = 670 g Solid= 199 g	300 kcal	Food records	Every 15 min for 1.5 h
Melnikov et al. 2014	**Oral stability:** Aerated = 18% reduction in upon simulated mastication Non-aerated = 68% reduction in upon simulated mastication	No	**Version 1** Non-aerated = 162 mL Aerated = 500 mL **Version 2** Non-aerated = 325 mL Aerated = 1,000 mL	Version 1 = 95 kcal Version 2 = 190 kcal	N/A	Every 30 min for 3 h
Mourao et al. 2007	No	No	**Food 1** = 400 g **Food 2:** Liquid = 79 g Solid = 35 g	Food 1 and Food 2 = 125 kcal	Food records	Before and after preload Every hour before leaving the laboratory
Santangelo et al. 1998	**Aperture sieve:** Homogenized = 15% retained on the sieve Solid = 75% retained on the sieve	No	660 g	614 kcal	N/A	Every 15 min for 1h, Every 30 min 1.5 h
Solah et al. 2010	**Viscosity:** Low viscous = 23.7 cP High viscous = 27.1 cP	No data	250 g	199 kcal	N/A	Before, after preload and every 30 min for 4h

Tang et al. 2016	**Puncture (length of curves):** Low complex = 425.7±4.9 mm High complex = 429.7±35.9 mm	**Chewiness (100 mm scale):** Low complex = 3.13±2.35 mm High complex = 5.24±2.14mm **Hardens:** Low complex =2.68±2.21mm High complex =7.10±2.12 mm	30 g	40 kcal	10 min	Before, after preload and immediately after lunch and 3 h after lunch
Tournier et al. 1991	No	No	538.8 g	679 kcal	180 min and food records	Before, after preload and immediately after lunch and 3 h after lunch
Tsuchiya et al. 2006	No	No data shown	400 mL	200 kcal	90 min	Before, after preload and every 10 min for 2 h
Wanders et al. 2014	**Viscosity (oral conditions at 100 s^{-1} shear rate):** Gels = 1.1 mPa.s **Viscosity (gastric conditions at 100 s^{-1} shear rate):** Gels=0.8 mPa.s Capsules=0.8 mPa.s Liquid = 0.8 mPa.s	No	425 g	366 kcal	180 min	Before, after preload and every 15 min for 3 h
Yeomans et al. 2014	No	**Thickness (VAS scale):** Low sensory= 61.3±3.0 mm High sensory = 68.3±2.6 mm **Creaminess (VAS scale):** Low sensory = 64.2±3.1 mm High sensory = 70.5±2.3	320 g	Type 1 = 77 kcal Type 2 = 325 kcal	90 min	Before and after preload

Yeomans et al. 2016	**Viscosity (at 50 s^{-1} shear rate):** Thin = 29.97 mPas Thick = 333.33 mPas **Lubrication properties (coefficient of friction (μ) at 50 mm s^{-1} speed):** Thin = μ = 0.15 Thick = μ = 0.09	**Thickness (VAS scale):** *Product 1* Thin = 44.4±3.0 mm Thick = 60.9±2.7 mm *Product 2* Thin = 48.3±3.0 mm Thick = 64.1±2.8 mm **Creaminess (VAS scale):** *Product 1* Thin = 53.5±3.3 mm Thick = 60.9±3.2 mm *Product 2* Thin = 56.1±3.1 mm Thick = 64 ±3.2 mm	300 mL	Type 1 = 77 kcal Type 2 = 325 kcal	90 min	Before, after preload, every 30 min for 1.5 h and after lunch
Zhu et al. 2013	**Viscosity (at 30 RPM):** Standard viscosity = 7,142 cP High viscosity = 57,142 cP	No	350 g	404 kcal	180 min	Before, after preload, every 15 min for 1.5 h and hourly for 2 h
Zhu et al. 2013	**Viscosity (at 59 RPM shear rate):** Liquid-solid = 10.5 cP Liquid = 396.7 cP	No	763 g	278.7 kcal	181 min	Before, after preload, every 15 min for 1.5 h and hourly for 2 h
Zijlstra et al. 2009	**Viscosity (at 50 s^{-1} share rate):** Liquid = 0.09 Pas Semi-solid = 2.9 Pas	**Thickness (10 point scale):** Liquid = 3.4±1.4 cm Semi-solid = 6.0± 0.9 cm	Women = 400 g Men = 500 g	Women = 388 kcal Men 485 kcal	90 min	Before, after and every 30 min for 1.5 h

Supplementary Table A.3. Quality assessment of studies using Cochrane tool.

Camps 2016	+	+	-
Clegg 2013	+	-	-
Dong 2016	+	+	+
Flood 2007	+	-	-
Flood-Obbagy 2009	+	-	-
Hogenkamp 2012	+	-	-
Hogenkamp 2012	+	-	-
Juvonen 2009	+	-	-
Juvonen 2011	+	-	?
Krop 2019	+	+	-
Laboure 2002	?	-	?
Larsen 2016	+	?	?
Marciani 2012	+	?	?
Martens 2012	+	?	-
Martens 2011	+	?	-
Mattes 2005	?	?	-
Melnikov 2014	+	?	?
Mourao 2007	-	?	-
Santangelo 1998	+	?	?
Solah 2010	+	?	-
Tang 2016	+	?	-
Tournier 1991	-	-	-
Tsuchiya 2006	+	+	?
Wanders 2014	+	+	-
Yeomans 2016	+	+	?
Yeomans 2014	+	?	-
Zhu 2013	+	?	?
Zhu 2013	+	?	?


Zijlstra 2009
+
+
-
+
Low risk of bias
-
High risk of bias
?
Unclear risk of bias
Random sequence generation
Allocation concealment
Blinding of participants and personnel

Supplementary Table A.4a. Participants data of studies included in the meta-analysis.

Authors	Category	C[1]	I[2]	Male	Female	Mean Age ± SD	Mean BMI ± SD
Camps 2016 (low E)[7]	Viscosity	15	15	15	0	22 ± 2	22.6 ± 1.6
Camps 2016 (high E)[7]	Viscosity	15	15	15	0	23 ± 2	22.6 ± 1.7
Glegg 2013	Form	12	12	6	6	28.7 ± 5.9	23.5 ± 2.9
Dong 2016	Viscosity	24	24	17	7	42 ± 16.16	23 ± 2.1
Dong 2016	Form	24	24	17	7	42 ± 16.16	23 ± 2.1
Flood 2007	Form	60	60	30	30	26.15 ± 3.87	24 ± 2.32
Flood 2009	Form	58	58	30	28	26.95 ± 4.18	24 ± 2.88
Flood 2009	Viscosity	58	58	30	28	26.95 ± 4.18	24 ± 2.88
Hogenkamp 2012 (low E)[7]	Form	81[3]	78[4]	27	54	21 ± 2.4	22.2 ± 1.6
Hogenkamp 2012 (high E)[7]	Form	81[3]	81	27	54	21 ± 2.4	22.2 ± 1.6
Hogenkamp 2012	Form	48	48	9	39	21 ± 2.9	21.8 ± 2
Juvonen 2009	Viscosity	20	20	4	16	22.6 ± 3.13	21.6 ± 1.34
Juvonen 2011 (Cas)[7]	Viscosity	8	8	8	0	24 ± 2.31	23.3 ± 1.41
Juvonen 2011 (TG-Cas)[7]	Viscosity	8	8	8	0	24 ± 2.31	23.3 ± 1.41
Juvonen 2011 (WP)[7]	Viscosity	8	8	8	0	24 ± 2.31	23.3 ± 1.41
Laboure 2002 (veg.)[7]	Form	12	12	12	0	21.5 ± 2.07	22.28 ± 1.93
Laboure 2002 (rsuk)	Form	12	12	12	0	21.5 ± 2.07	22.28 ± 1.93
Marciani 2012	Form	22	22	13	9	29 ± 4.22	21.1 ± 3.75
Martens 2011	Form	10	10	10	0	21.1 ± 3.9	22.4 ± 1.2
Martens 2012	Form	10	10	10	0	21.1 ± 4.11	22.4 ± 1.26
Mattes 2005 (CHO)[7]	Form	31	31	13	18	23.7 ± 5	23 ± 3.9
Mattes 2005 (protein)	Form	31	31	13	18	23.7 ± 5	23 ± 3.9
Solah 2010 (ALG)[7]	Viscosity	33	33	16	17	21.2 ± 1.8	22.7 ± 1.81
Solah 2010 (WP)[7]	Viscosity	33	33	16	17	21.2 ± 1.8	22.7 ± 1.81
Tournier 1991	Form	13	13	7	6		
Tsuchiya 2006	Form	32	32	16	16	27.1 ± 4.7	22.9 ± 1.9
Wanders 2014 (capsule)	Form	29	29	29	0	21 ± 2	21.9 ± 2.8
Wanders 2014 (gel)	Form	29	29	29	0	21 ± 2	21.9 ± 2.8
Yeomans 2014 (low E)[7]	Viscosity	12[5]	12	12	0	20.4 ±1.73	23.65 ± 2.77
Yeomans 2014 (high E)[7]	Viscosity	12[5]	12	12	0	22.2 ±1.38	24.1 ± 3.46
Yeomans 2016 (low E)[7]	Viscosity	22	22	22	0	31[6]	24
Yeomans 2016 (high E)[7]	Viscosity	22	22	22	0	31[6]	24
Zhu 2013	Viscosity	15	15	15	0	27 ± 2	24.2 ± 2.32
Zhu 2013	Form	19	19	19	0	28 ± 2	24.2 ± 2.61
Zijlstra 2009	Form	32	32	12	20	22 ± 2	21.9 ± 2.2

[1] Control/comparison

[2] Intervention

[3] Participants had the preload 3 times a day, 27*3=81

[4] Data on 2 participants missing

[5] Between-participants study design

[6] Data missing on standard deviation

[7] **Abbreviations**:
low E = low energy
high E= high energy
Cas = casein
TG-Cas = transglutaminate treated casein
WP = whey protein
veg. = vegetables
CHO = carbohydrates
ALG = alginate

Supplementary Table A.4b. Meta-analysis data on appetite ratings (hunger and fullness).

Authors	Category	C[1]	I[2]	C[1] Mean Hunger	C[1] SD Hunger	I[2] Mean Hunger	I[2] SD Hunger	C[1] Mean Fullness	C[1] SD Fullness	I[2] Mean Fullness	I[2] SD Fullness
Camps 2016 (low E)	Viscosity	15	15					38.75	2.75	45	1.37
Camps 2016 (high E)	Viscosity	15	15					42.5	1.37	48.75	2.75
Glegg 2013	Form	12	12					57	13.32	40	21.64
Dong 2016	Viscosity	24	24	43.75	17.63	37.5	19.59	28.75	19.59	40	19.59
Dong 2016	Form	24	24	43.75	17.63	36.25	19.59	28.75	19.59	37.5	19.59
Flood 2007	Form	60	60	31.25	24.16	35.4	24.16				
Hogenkamp 2012 (low E)	Form	81[3]	78[4]	57.3	17.3	46	19.4				
Hogenkamp 2012 (high E)	Form	81	81	63.3	16.4	55.2	16.7				
Hogenkamp 2012	Form	48	48	29.07	11.43	18.18	11.43	78.18	11.43	63.63	19.05
Juvonen 2011 (WP)	Viscosity	8	8	32.5	28.28	32.5	14.14	37.5	22.62	37.5	16.97
Juvonen 2011 (Cas)	Viscosity	8	8	32.5	28.28	25	11.31	37.5	22.62	53.75	33.94
Juvonen 2011 (TG-Cas)	Viscosity	8	8	32.5	14.14	25	11.31	37.5	16.97	53.75	33.94
Laboure 2002 (veg.)	Form	12	12	20	14.97	20	11.84	71.4	11.84	65.7	24.66
Laboure 2002 (rusk)	Form	12	12	32.82	24.66	27.12	15.27	51.4	19.74	62.85	12.81
Marciani 2012	Form	22	22					3.71		3.35	
Martens 2011	Form	10	10	32.5	18.97	20	12.64	65	31.62	77.5	15.81
Martens 2012	Form	10	10					76.66	10.65	70.99	21.06
Mattes 2005 (CHO)	Form	31	31	36.53	12.69	25.7	22.21	68.55	3.17	80	6.34
Mattes 2005 (protein)	Form	31	31	36.53	12.69	40	31.73	68.55	3.17	65.7	3.17
Solah 2010 (ALG)	Viscosity	33	33	16.65	9.53	16.65	9.53				
Solah 2010 (WP)	Viscosity	33	33	11.65	9.53	16.65	9.53				
Tournier 1991	Form	13	13	20	18.02	10	8				
Wanders 2014 (capsule)	Form	29	29	34.98	17.93	28.32	16.1				
Wanders 2014 (gel)	Form	29	29	34.98	17.93	34.98	17.93				
Yeomans 2014 (low E)	Viscosity	12[4]	12	-8		-21		14		28	
Yeomans 2014 (high E)	Viscosity	12[4]	12	-16		-28		18		26	

Yeomans 2016 (low E)	Viscosity	19	19	2.13		0.71		1.77		1.42	
Yeomans 2016 (high E)	Viscosity	19	19	-2.13		-6.06		0		-13.62	
Zhu et al. 2013	Viscosity	15	15	30	15.49	26	3.87	50	5.8	60	7.35
Zhu et al. 2013	Form	19	19	26.49		23.51		30		26.85	
Zijlstra et al. 2009	Form	32	32	52.5	15	52.5	17.5	51	11.25	50	16.25

[1] Control/comparison

[2] Intervention

[3] Participants had the preload 3 times a day, 27*3=81

[4] Data on 2 participants missing

[5] Between-participants study design

Supplementary Table A.4c. Meta-analysis data on food intake.

Authors	Category	C[1]	I[2]	C[1] Food intake Mean	C[1] Food intake SD	I[2] Food intake Mean	I[2] Food intake SD
Camps 2016 (low E)	Viscosity	15	15	625.71	365.43	476.81	343.69
Camps (high E)	Viscosity	15	15	541.58	283.46	531.07	321.46
Flood 2007	Form	60	60	654	340	704	371
Flood 2009	Form	58	58	800	373	709	380
Flood 2009	Viscosity	58	58	890	388	866	396
Hogenkamp 2012 (low E)	Form	81[3]	78[4]	1767	581	1720	583
Hogenkamp 2012 (high E)	Form	81	81	1549	427	1496	438
Juvonen 2009	Viscosity	20	20	2007	154	1733	113
Laboure 2002 (veg.)	Form	12	12	777	301	791	204
Laboure 2002 (rusk)	Form	12	12	940	301	704	377
Tournier 1991	Form	13	13	781	259.29	769	407.42
Tsuchiya 2006	Form	32	32	803	299.81	776	282.84
Wanders 2014 (capsule)	Form	29	29	1128.84	332.51	1058	339.24
Wanders 2014 (gel)	Form	29	29	1128.84	332.51	955.61	305.74
Yeomans 2014 (low E)	Viscosity	12[5]	12	942.74	266.77	1022.41	193.15
Yeomans 2014 (high E)	Viscosity	12[5]	12	873.69	275.95	677.33	193.15
Yeomans 2016 (low E)	Viscosity	22	22	1337.5	75	1375	87.5
Yeomans 2016 (high E)	Viscosity	22	22	1282.5	75	1200	80
Zhu 2013	Viscosity	15	15	791.2	80	788.8	82.88
Zhu 2013	Form	19	15	878.96	80.32	882.4	88
Zijlstra 2009	Form	32	32	394.92	212.94	371.69	178.1

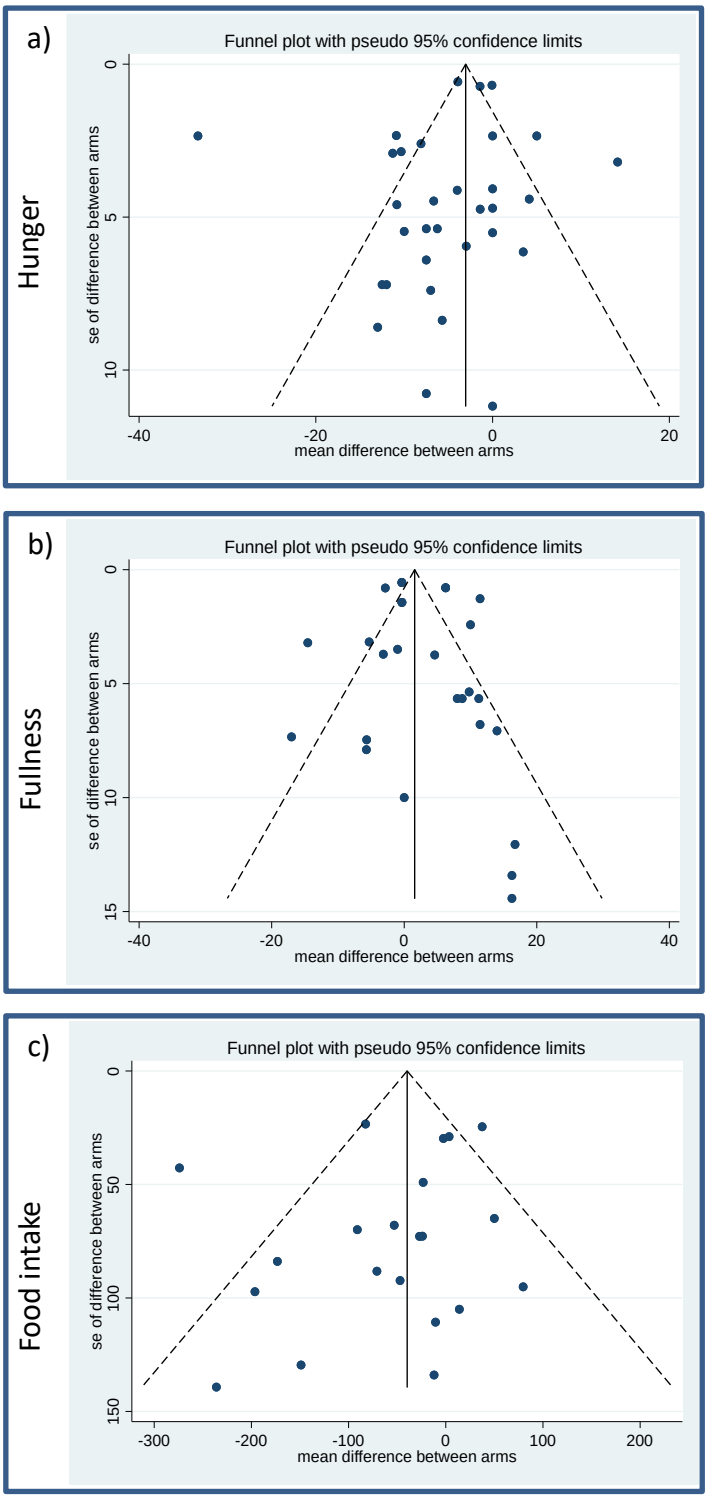
[1] Control/comparison

[2] Intervention

[3] Participants had the preload 3 times a day, 27*3=81

[4] Data on 2 participants missing

[5] Between-participants study design



Supplementary Figure A.2. Funnel plots of food texture effects on hunger (a), fullness (b) and food intake (c) with a 95% confidential interval (CI). The data (blue dots) indicate each study included in meta-analysis. The more symmetric the plots are, the less is the publication bias of the studies.

Appendix B

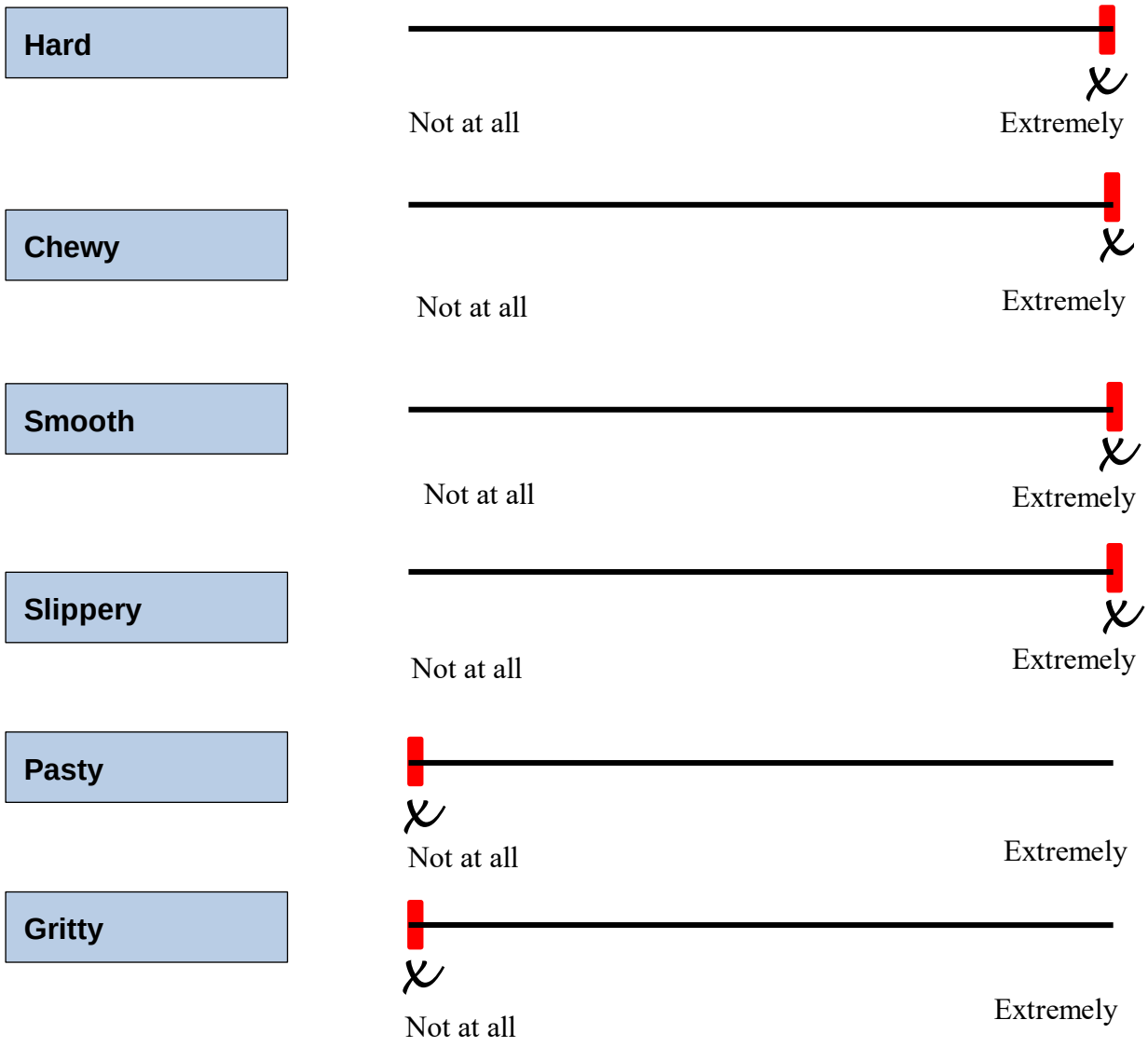
Supplementary information for Chapter 3

Supplementary Table B.1. Mean and standard deviation (SD) of the friction coefficients of beads alone (a), hydrogel boli (b), beads boli (c), in the boundary and mixed regimes and (d) apparent viscosity of hydrogel boli at 50 s^{-1} shear rate. The samples were prepared using simulated oral processing in the presence of artificial saliva, and compared to artificial saliva as a control measure. Different lower case letters in the same column indicate a statistically significant difference ($p < 0.05$).

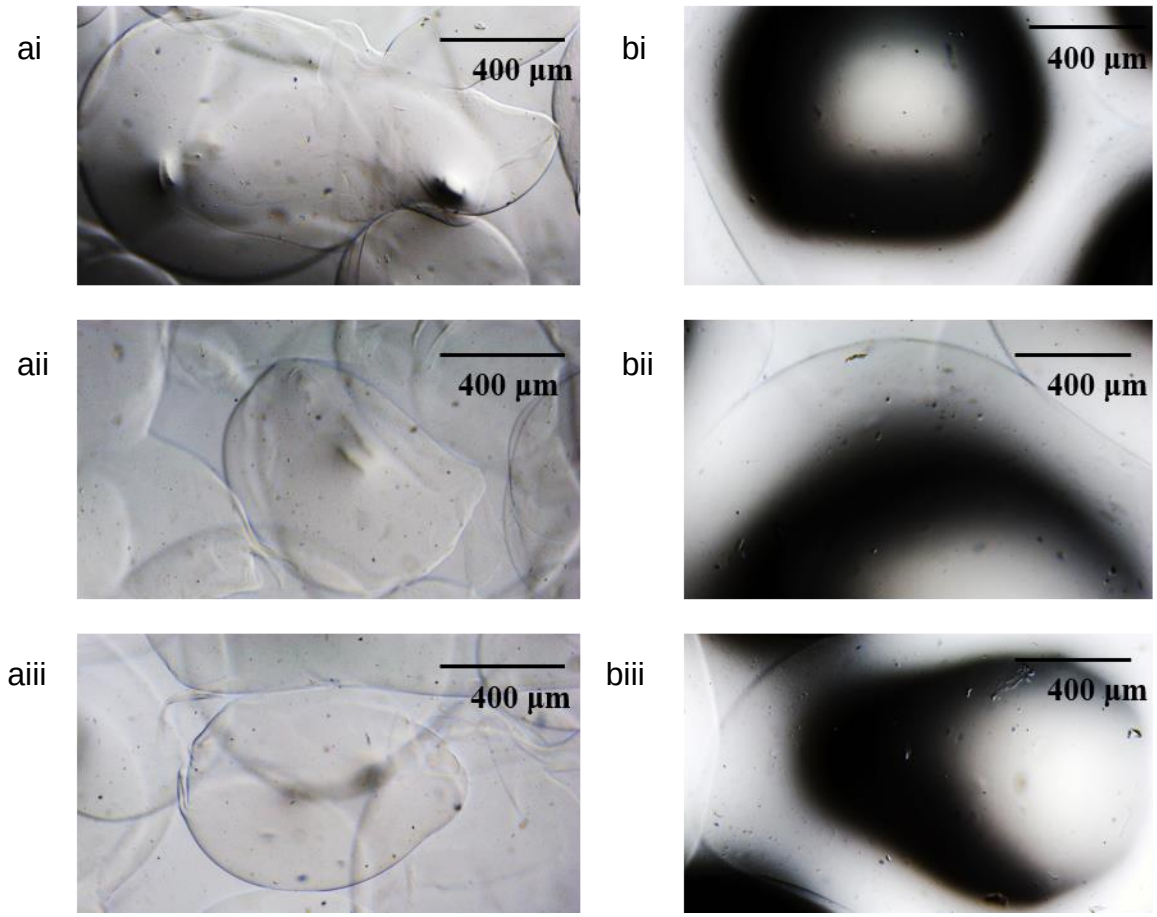
(a)	**Coefficient of friction of the CaA beads alone**					
	Boundary lubrication regime (0.005 m s^{-1})		Mixed lubrication regime (0.05 m s^{-1})		Mixed lubrication regime (0.1 m s^{-1})	
	Mean	SD	Mean	SD	Mean	SD
CaA_{Small}	0.967^{b}	0.076	0.520^{b}	0.149	0.333^{b}	0.135
CaA_{Medium}	0.942^{b}	0.073	0.292^{b}	0.083	0.121^{a}	0.025
CaA_{Large}	0.864^{b}	0.105	0.328^{b}	0.071	0.146^{a}	0.049
NaA solution	0.469^{a}	0.058	0.043^{a}	0.007	0.022^{a}	0.003
(b)	**Coefficient of friction of the hydrogel boli**					
	Boundary lubrication regime (0.005 m s^{-1})		Mixed lubrication regime (0.05 m s^{-1})		Mixed lubrication regime (0.1 m s^{-1})	
	Mean	SD	Mean	SD	Mean	SD
2κC	0.536^{bc}	0.044	0.119^{ab}	0.015	0.070^{c}	0.011
1.67κC+0.33NaA	0.526^{bc}	0.067	0.082^{ab}	0.025	0.042^{b}	0.004
1.67κC+0.33CaA_{Small}	0.465^{b}	0.061	0.105^{ab}	0.029	0.061^{c}	0.009
1.67κC+0.33CaA_{Medium}	0.439^{ab}	0.083	0.134^{b}	0.035	0.059^{bc}	0.005
1.67κC+0.33CaA_{Large}	0.300^{a}	0.080	0.041^{a}	0.010	0.015^{a}	0.002
Artificial saliva	0.649^{c}	0.026	0.367^{c}	0.059	0.233^{d}	0.005
(c)	**Coefficient of friction of the CaA beads boli**					
	Boundary lubrication regime (0.005 m s^{-1})		Mixed lubrication regime (0.05 m s^{-1})		Mixed lubrication regime (0.1 m s^{-1})	
	Mean	SD	Mean	SD	Mean	SD
CaA_{Small}	0.759^{b}	0.037	0.127^{a}	0.009	0.052^{a}	0.005

CaA_{Medium}	0.620[a]	0.019	0.221[a]	0.023	0.098[a]	0.032
CaA_{Large}	0.519[a]	0.083	0.131[a]	0.032	0.064[a]	0.010
Artificial saliva	0.649[ab]	0.026	0.367[b]	0.059	0.233[b]	0.005

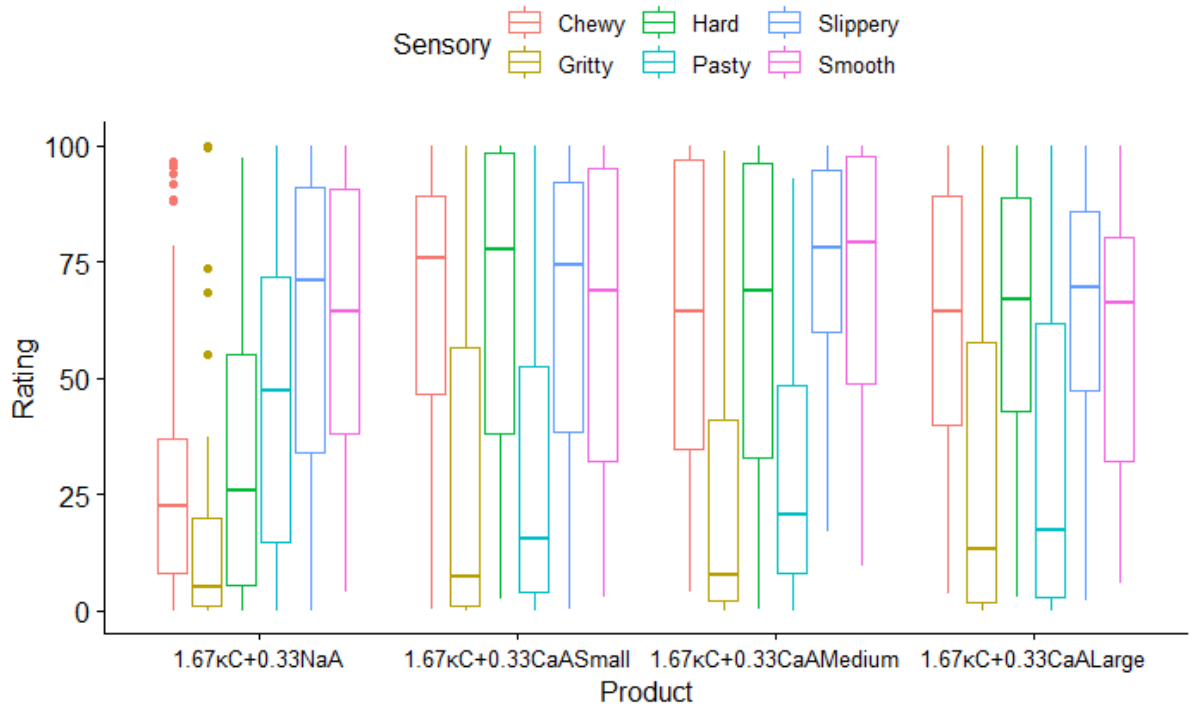
(d)	**Apparent viscosity of the hydrogel boli**	
	Shear rate (50 s^{-1})	
	Mean	SD
2*κ*C	0.886[c]	0.172
1.67*κ*C+0.33NaA	1.929[a]	0.212
1.67*κ*C+0.33CaA_{Small}	1.479[b]	0.159
1.67*κ*C+0.33CaA_{Large}	1.399[b]	0.113



Supplementary Figure B.1. Scoring sheet used for the sensory intensity rating test including the six different texture-related attributes. The attributes are written in the left-side blue boxes. The unstructured scales (0-100 mm) are represented by horizontal black lines. Short vertical red lines with a symbol of "x" represent the scores for the reference samples (2κC hydrogel): the beginning of the line represents the 0 mm score and the end of the line represents the 100 mm score.



Supplementary Figure B.2. Optical microscopy images (i-iii representing different regions of the same sample slide) of boli (*i.e.* CaA beads + model saliva) of small beads (CaA_{small}) (a) and large beads (CaA_{large}) (b) after subjecting to tribological measurements.



Supplementary Figure B.3. Boxplot of the intensity ratings of the sensory attributes for κC+NaA, κC+CaA$_{Small}$, κC+CaA$_{Medium}$ and κC+CaA$_{Large}$ hydrogels with respect to the reference sample (2κC). Data points represent the mean intensity ratings of untrained panellists (n=60). Median, interquartile range are indicated with outliers identified as dots.

Appendix C

Supplementary information for Chapter 4

Supplementary Table C.1. Palatability of the preloads (HL, LL and Control) measured on a 100-mm VAS scale (n=17).

	HL	LL	Control	p-value[1]
Texture	37±25	43±32	56±27	n.s.
Sweetness	25±22	15±17[a]	41±23[b]	$p < 0.005$
Flavour	26±22	25±24	41±28	n.s.

[1]The lower-case letters (subscripts) denote a significant difference between preloads ($p < 0.05$). Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table C.2. Area under the curve (AUC) for appetite ratings (mm) over time for subjects eating HL (high lubricating), LL (low lubricating) or Control preloads, n=17 (mean + SEM).

	HL	LL	Control	p-value[1]
Hunger	8944 ± 874	8548 ± 893	9253 ± 756	n.s.
Fullness	11369 ± 921	10423 ± 881	10160 ± 828	n.s.
Desire to eat	9418 ± 1055	8562 ± 1007	9505 ± 794	n.s.
PFC	10029 ± 961	9069 ± 839	10164 ± 853	n.s.
Thirst	10556 ± 1179	10441 ± 1244	11037 ± 1135	n.s.

[1] Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table C.3. Coefficient of friction[1] of saliva in all three conditions (HL, LL and Control) before preload (a) and after preload (b) at two boundary lubricating regime (0.001 ms^{-1}; 0.005 ms^{-1}) and two mixed lubricating regime (0.05 ms^{-1}; 0.1 ms^{-1}), n=4 (mean + SD).

a	Coefficient of friction of saliva before preload							
	Boundary lubricating regime				Mixed lubricating regime			
	0.001 m s^{-1}		0.005 m s^{-1}		0.05 m s^{-1}		0.1 m s^{-1}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HL	0.002	0.000	0.003	0.001	0.003	0.001	0.002	0.001
LL	0.005	0.002	0.007	0.002	0.005	0.001	0.005	0.000
Control	0.005	0.002	0.007	0.003	0.006	0.003	0.007	0.001

b	Coefficient of friction of saliva after preload							
	Boundary lubricating regime				Mixed lubricating regime			
	0.001 ms^{-1}		0.005 m s^{-1}		0.05 m s^{-1}		0.1 ms^{-1}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HL	0.004	0.002	0.005	0.003	0.006	0.001	0.004	0.001
LL	0.007	0.002	0.008	0.002	0.009	0.001	0.006	0.002
Control	0.006	0.002	0.007	0.003	0.009	0.004	0.006	0.003

[1]No significant differences were found for friction coefficient for saliva at any entrainment speed between conditions neither before preload or after preload (HL, LL, and Control).

Supplementary Table C.4. Pearson's correlations between appetite ratings, energy intake, tribology of saliva and salivary biomarkers, after preload (n=4, MUC5B was not included due to insufficient data). Green colour indicates positive and orange colour a negative correlation with $p < 0.05$ in light colours and $p < 0.01$ in the darker shades.

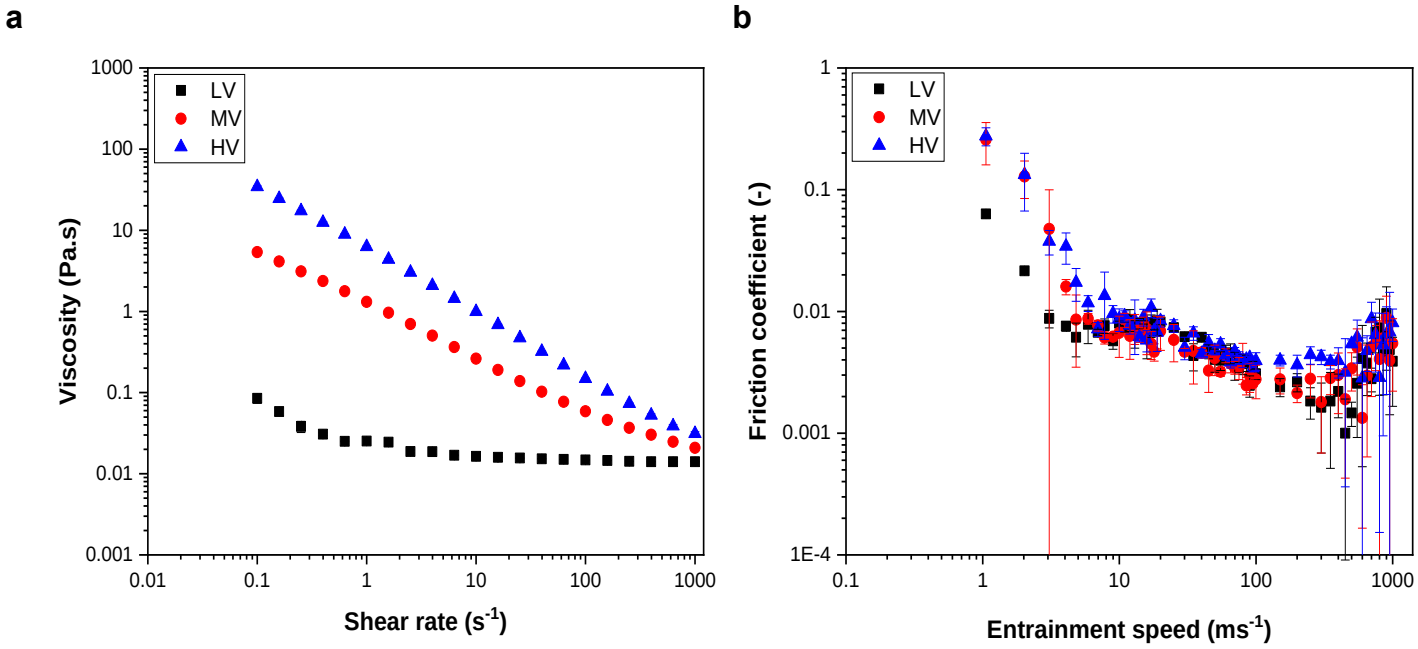
		Appetite ratings					Energy and water intake				Tribology of saliva							Salivary biomarkers	
		Hunger	Fullnes	Desire to eat	Pros. Consum.	Thirst	Main	Desert	Combined	Water	ES300	ES200	ES100	ES50	ES10	ES5	ES1	Protein	Amylase
Appetite ratings	Hunger	1																	
	Fullnes	-0.922	1																
	Desire to eat	.967*	-.988*	1															
	Pros. Consum.	.987*	-0.949	.986*	1														
	Thirst	0.131	-0.494	0.379	0.257	1													
Energy intake and water	Main	-0.755	0.727	-0.717	-0.673	-0.021	1												
	Dessert	0.607	-0.633	0.673	0.714	0.447	0.030	1											
	Comined	0.291	-0.325	0.365	0.419	0.405	0.384	0.934	1										
	Water	0.674	-0.908	0.836	0.738	0.792	-0.585	0.530	0.281	1									
Tribology of saliva	ES300	0.565	-0.800	0.706	0.581	0.670	-0.747	0.168	-0.112	0.922	1								
	ES200	0.812	-.966*	0.918	0.839	0.609	-0.761	0.477	0.169	.966*	0.927	1							
	ES100	0.881	-.991**	.962*	0.903	0.534	-0.771	0.540	0.223	0.937	0.873	.991**	1						
	ES50	0.894	-.995**	.971*	0.918	0.524	-0.760	0.568	0.253	0.931	0.854	.986*	.999**	1					
	ES10	.955*	-.995**	.998**	.975*	0.415	-0.735	0.647	0.335	0.863	0.747	0.940	.976*	.984*	1				
	ES5	0.474	-0.778	0.678	0.557	0.908	-0.433	0.450	0.261	.970*	0.904	0.880	0.824	0.813	0.714	1			
	ES1	0.574	-0.835	0.741	0.620	0.782	-0.639	0.329	0.076	.975*	.980*	0.944	0.893	0.878	0.779	.968*	1		
Salivary biomarkers	Protein	0.709	-0.758	0.785	0.808	0.507	-0.132	.985*	0.862	0.660	0.330	0.623	0.679	0.703	0.766	0.568	0.476	1	
	Amylase	-0.202	-0.193	0.047	-0.106	0.903	0.042	0.025	0.038	0.586	0.609	0.393	0.278	0.255	0.097	0.766	0.667	0.086	1

Appendix D

Supplementary information for Chapter 5

Supplementary Table D.1. Videos of the beverages.

	Low protein	High protein
Low Viscous	 LVLP.mp4	 LVHP.mp4
Medium Viscous	 MVLP.mp4	 MVHP.mp4
High Viscous	 HVLP.mp4	 HVHP.mp4



Supplementary Figure D.1. Apparent viscosity as a function of shear rate (a) and lubricity analysis (b) where data is expressed as friction coefficients at different entrainment speed for the HV (high viscous), MV (medium viscous) and LV (low viscous) beverages. Data are presented as means and SDs.

Supplementary Table D.2. Means and SDs of the immediate perceived satiety (a) and perceived satiety 2 h later (b) for both low and high protein content and different textures – LV (low viscous), MV (medium viscous) and HV (high viscous|)[a].

a	Immediate perceived satiety		b	Perceived satiety 2 h later	
	Low protein	*High protein*		*Low protein*	*High protein*
LV	48.43 ± 23.32 [a]	45.36 ± 23.17 [a]	***LV***	26.72 ± 22.59 [a]	28.28 ± 21.39 [a]
MV	66.1 ± 23.16 [b]	63.44 ± 23.14 [b]	***MV***	42.31 ± 23.50 [b]	43.84 ± 23.00 [b]
HV	66.58 ± 23.91 [b]	68.14 ± 22.86 [c]	***HV***	45.25 ± 24.59 [c]	46.09 ± 23.88 [b]

[a] A statistical significant difference ($p<0.05$) between the beverages is denoted by different letters in superscripts ([abc]).

Supplementary Table D.3. Pearson's correlations between initial hunger (Hunger0) and immediate perceived satiety (FullNow) and perceived satiety 2 h later (Full2h), between sensory attributes (smooth, thick, watery and creamy), wanting and liking in LVLP (a), MVLP (b), HVLP (c), LVHP (d), MVHP (e), and HVHP (f) conditions. Green colour indicates positive and orange colour a negative correlation with $p < 0.05$ in light colours and $p < 0.01$ in the darker shades.

a **LVLP**

	1	2	3	4	5	6	7	8	9
1 Hunger0	1								
2 LVLPFullNow	-0.017	1							
3 LVLPFull2h	-0.085	.480**	1						
4 LVLPSmooth	0.029	0.088	-0.049	1					
5 LVLPThick	0.050	.279**	.210**	-0.123	1				
6 LVLPWatery	0.043	-0.009	-0.103	.301**	-.517**				
7 LVLPCreamy	.150*	0.108	0.097	.144*	.373**	-.154*			
8 LVLPWant	.281**	0.083	.138*	.220**	.176*	-0.082	.285**	1	
9 LVLPLike	0.072	0.116	0.063	.274**	0.101	0.072	.246**	.615**	1

b **MVLP**

	1	2	3	4	5	6	7	8	9
1 Hunger0	1								
2 MVLPFullNow	0.019	1							
3 MVLPFull2h	-0.036	.651**	1						
4 MVLPSmooth	.175*	-0.036	-0.123	1					
5 MVLPThick	-0.013	.422**	.229**	-0.015	1				
6 MVLPWatery	0.018	-.161*	-.139*	.338**	-.297**	1			
7 MVLPCreamy	-0.013	0.115	0.079	.194**	.291**	0.007	1		
8 MVLPWant	.319**	-0.036	-0.007	.490**	-0.090	.190**	.222**	1	
9 MVLPLike	0.090	-0.064	-0.054	.498**	-0.025	.174*	.263**	.782**	1

c **HVLP**

	1	2	3	4	5	6	7	8	9
1 Hunger0	1								
2 HVLPFullNow	0.112	1							
3 HVLPFull2h	-0.016	.638**	1						
4 HVLPSmooth	.174*	-.135*	-.205**	1					
5 HVLPThick	0.051	.463**	.333**	-.297**	1				

6	HVLPWatery	-0.041	-.227**	-.183**	.423**	-.408**	1			
7	HVLPCreamy	-0.042	.180**	0.115	0.065	.301**	-.176*	1		
8	HVLPWant	.325**	-0.068	-0.027	.496**	-0.103	.163*	.250**	1	
9	HVLPLike	.180**	-0.050	-0.076	.503**	-0.069	0.100	.291**	.819**	1

d) LVHP

		1	2	3	4	5	6	7	8	9
1	Hunger0	1								
2	LVHPFullNow	0.072	1							
3	LVHPFull2h	-0.070	.643**	1						
4	LVHPSmooth	-0.054	-.142*	-.161*	1					
5	LVHPThick	0.117	.348**	.274**	-.254**	1				
6	LVHPWatery	-0.095	-.240**	-.224**	.304**	-.552**	1			
7	LVHPCreamy	.144*	.176*	0.125	-0.043	.388**	-.290**	1		
8	LVHPWant	0.118	.162*	.217**	0.029	0.108	-0.023	.246**	1	
9	LVHPLike	0.074	.176*	0.121	.146*	0.087	-0.017	.265**	.698**	1

e MVHP

		1	2	3	4	5	6	7	8	9
1	Hunger0	1								
2	MVHPFullNow	0.053	1							
3	MVHPFull2h	-0.025	.588**	1						
4	MVHPSmooth	.137*	-0.035	-0.101	1					
5	MVHPThick	0.007	.374**	.341**	-0.006	1				
6	MVHPWatery	-0.003	-.224**	-.183**	.252**	-.337**	1			
7	MVHPCreamy	0.024	.153*	0.103	.223**	.307**	-.145*	1		
8	MVHPWant	.237**	0.032	0.090	.520**	0.045	0.093	.309**	1	
9	MVHPLike	.146*	0.056	-0.007	.529**	0.116	0.049	.392**	.806**	1

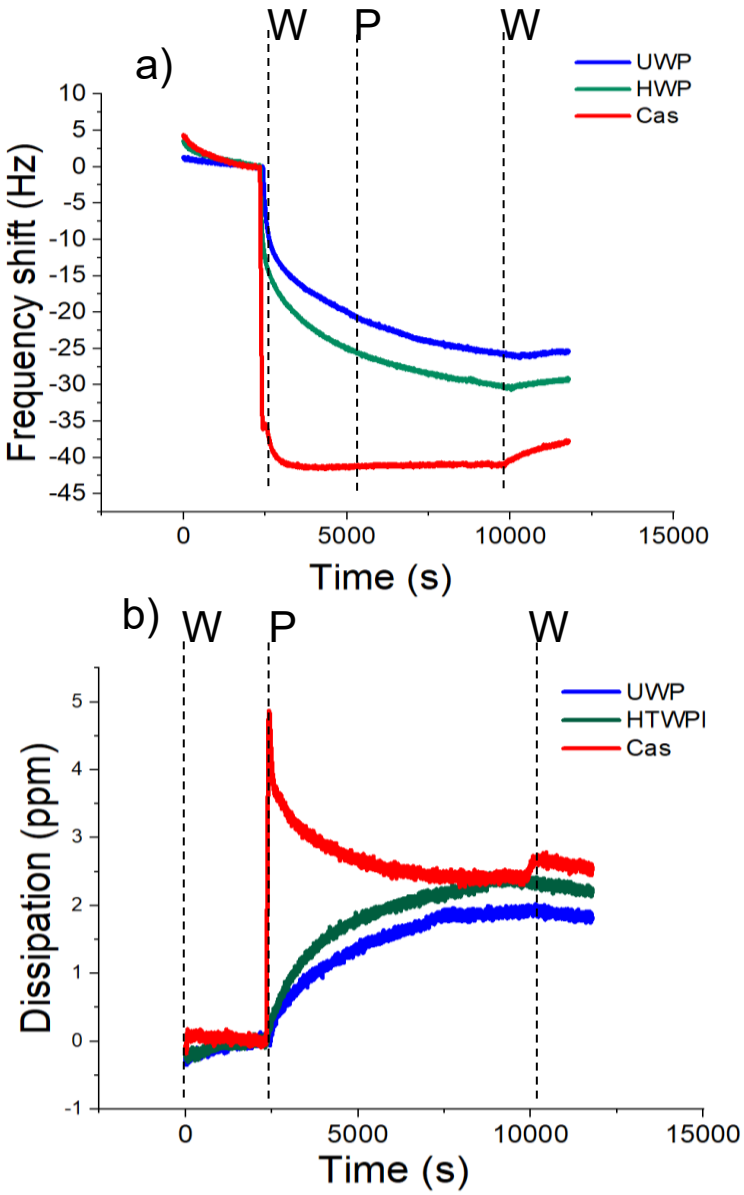
Supplementary Table D.3 (continuation).

HVHP

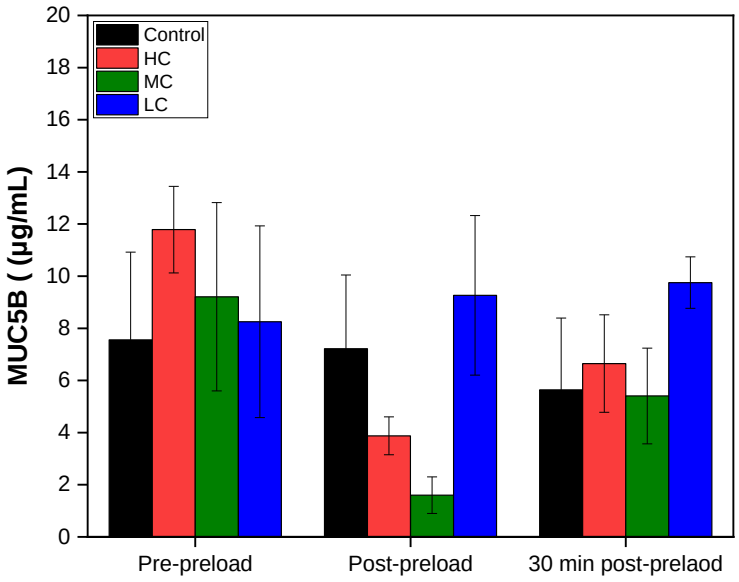
f		1	2	3	4	5	6	7	8	9
1	Hunger0	1								
2	HVHPFullNow	0.078	1							
3	HVHPFull2h	-0.041	.593**	1						
4	HVHPSmooth	.148*	-0.050	-0.057	1					
5	HVHPThick	0.021	.304**	.258**	0.053	1				
6	HVHPWatery	-0.077	-.249**	-.139*	.202**	-.289**	1			
7	HVHPCreamy	-0.100	0.079	0.080	.190**	.271**	-0.055	1		
8	HVHPWant	.232**	0.014	0.069	.460**	-0.009	0.019	.295**	1	
9	HVHPLike	.161*	0.003	-0.006	.493**	0.072	0.000	.398**	.808**	1

Appendix E

Supplementary information for Chapter 6



Supplementary Figure E.1. Mean frequency shift (a) and mean dissipation (both frequency and mean data of 5th overtone) (b) of the proteins (n=3) adsorbed on bare polydimethylsiloxane (PDMS) coated sensors, acquired by quartz crystal microbalance with dissipation monitoring (QCM-D). At bare PDMS surfaces measurements were taken in presence of 0.1 mg /mL^{-1} protein dissolved in water. In the plots, steps W, P and W refers to rising and achieving baseline, protein addition and post-rinsing to check for any desoprion, respectively.



Supplementary Figure E.2. Total MUC5B (µg/mL) (n = 4) in saliva for Control, HC (high coating), MC (medium coating) and LC (low coating) conditions Pre-preload, Post- preload and 30 min post-preload. Values are means and error bars represent standard error of means (SEMs).

Supplementary Table E.1. Mood ratings (mm) over time for subjects having Control, HC, MC and LC preloads, n=37 (mean + SDs) for Study 1.

Mood ratings	Control	HC	MC	LC	p-value[1]
Nausea					
Pre-preload	12.5± 17	14.2 ± 19.9	12.1 ± 18.1	13.4 ± 18.5	n.s.
Post-preload	13.7 ± 17.8	17.3 ± 26.3	12.6 ± 17.8	13.9 ± 18.8	n.s.
10 min after	11.4 ± 15	12.2 ± 17.5	9.4 ± 15	8.8 ± 13.9	n.s.
20 min after	8.3 ± 12.2	10.3 ± 16.4	10.7 ± 16.8	8.4 ± 14.4	n.s.
30 after	11.5 ± 17.2	11.8 ± 19	9.4 ± 14.4	9.7 ± 14.7	n.s.
After ad libitum breakfast	5.2 ± 9.5	7.7 ± 14.6	6.3 ± 11.3	7.4 ± 10.3	n.s.
Mental alert					
Pre-preload	50.5 ± 24.4	43.3 ± 27.9	44.2 ± 26.1	45.8 ± 26.5	n.s.
Post-preload	52.8 ± 25	51.1 ± 27.4	49.3 ± 24.8	51 ± 26.8	n.s.
10 min after	50.5 ± 26	53.3 ± 27.4	48.6 ± 26.5	51.6 ± 26.1	n.s.
20 min after	48.3 ± 28.1	53.3 ± 28.1	46.2 ± 28.2	48 ± 26.5	n.s.
30 after	47 ± 29.3	52.4 ± 28	46.7 ± 27	49 ± 27.3	n.s.
After ad libitum breakfast	65.4 ± 29.6	63.3 ± 30.7	64.3 ± 30.1	61 ± 29.1	n.s.
Content					
Pre-preload	50.2 ± 25.8	50.1 ± 25.3	48.2 ± 25.2	50.9 ± 25.8	n.s.
Post-preload	51.1 ± 20.8	50.8 ± 23.6	53.2 ± 21.9	57.9 ± 22.2	n.s.
10 min after	49.7 ± 23.1	54.7 ± 23.1	54.1 ± 21.9	53.2 ± 24.3	n.s.
20 min after	46.7 ± 25.1	56.2 ± 24.8	53.1 ± 24.7	50.9 ± 25.3	n.s.
30 after	46.3 ± 27.7	53.2 ± 23.5	51.1 ± 25.3	49.5 ± 25.9	n.s.
After ad libitum breakfast	76 ± 19.7	75.5 ± 19.2	78.5 ± 20	74.8 ± 21.4	n.s.

[1] Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table E.2. Mood ratings (mm) over time for subjects having ML (medium lubricating) and HL (high lubricating) preloads, n=15 (mean + SDs) for Study 2.

Mood ratings	MC	LC	p-value[1]
Nausea			
Pre-preload	16.6 ± 16.8	20.4 ± 23.3	n.s.
Post-preload	19.3 ± 23.8	15.5 ± 15.8	n.s.
15 min after	12.6 ± 15.3	17.4 ± 17.3	n.s.
30 min after	10 ± 15.1	15.1 ± 15.6	n.s.
60 after	11.7 ± 16.2	16.4 ± 15.1	n.s.
After ad libitum breakfast	8 ± 12.6	12.6 ± 22.7	n.s.
Mental alert			
Pre-preload	51 ± 26.8	51.1 ± 25.8	n.s.
Post-preload	53 ± 22.7	50.4 ± 23	n.s.
15 min after	49.4 ± 25.2	53.8 ± 22.9	n.s.
30 min after	49.8 ± 27.3	48.4 ± 24.9	n.s.
60 after	49 ± 26.4	50.3 ± 23.9	n.s.
After ad libitum breakfast	58.1 ± 27.3	59.9 ± 30	n.s.
Content			
Pre-preload	50.2 ± 25.1 [b]	39.6 ± 24 [a]	n.s.
Post-preload	53.6 ± 20.9	52.4 ± 17.6	n.s.
15 min after	55 ± 19.7	51.4 ± 16.2	n.s.
30 min after	56.8 ± 22.5 [b]	48.1 ± 19.5 [a]	*p<0.05*
60 after	56.3 ± 21.8 [b]	49.2 ± 20.6 [a]	*p<0.05*
After ad libitum breakfast	75.6 ± 17	70.5 ± 17.8	n.s.

[1]The lower-case letters (subscripts) denote a significant difference between preloads ($p < 0.05$). Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table E.3. Appetite ratings (mm) at all-time points for participants ingesting Control, HC (high coating), MC (medium coating) and LC (low coating) preloads, n = 37 (means ± SD) for Study 1.

Appetite ratings[a]	Control	HC	MC	LC
Hunger (mm)				
Pre-preload (fasting)	61.9±24.2	54.4±25.3	59.9±22.2	59.2±20.1
Post-preload	58.1±21.3[a]	42.4±25.7[b]	43.5±20[b]	47.9±22.3[ab]
10 min after	65.2±19.2[a]	47.7±23.9[b]	48.9±19.4[b]	52.5±23[b]
20 min after	66.9±21.6[a]	50.5±22[b]	52.9±20.6[b]	57.5±22.4[ab]
30 after	70.4±21.5[a]	55.8±23.3[b]	54.5±21.6[b]	61.1±22.9[b]
After ad libitum breakfast	7.1±8.6	5.5±7.1	6.8±9.4	7.3±8.7
Fullness (mm)				
Pre-preload (fasting)	17.3±17.6	21.4±20.1	20.7±22.7	16.1±17.1
Post-preload	33.8±22.8[a]	44.7±21[b]	43.1±21[ab]	39.1±21.4[ab]
10 min after	23.3±18.1[a]	41.3±24.7[b]	39.9±20.3[b]	35.4±22[b]
20 min after	21.8±18.7[a]	40.9±22.2[c]	34.7±20.7[bc]	28±21.9[ab]
30 after	20.7±19.5[a]	34.7±23.8[b]	34.2±21.2[b]	28.6±21.6[ab]
After ad libitum breakfast	77.5±19.7	81.9±11.5	81.9±16.8	79.6±16
Desire to eat (mm)				
Pre-preload (fasting)	61.8±24.3	58.5±22.4	65.8±24.9	58.9±24
Post-preload	60.1±23.8[a]	45.7±25.8[b]	41.6±21.9[b]	51.5±25.5[ab]
10 min after	66.2±19.6[a]	52.8±21.3[b]	50.6±21.8[b]	58.6±22.1[ab]
20 min after	68.8±22.4[a]	55.5±23[b]	55.5±21.7[b]	60.5±24[ab]
30 after	70.8±21.7[a]	58.9±23.1[b]	55.6±20[b]	62.5±22.3[ab]
After ad libitum breakfast	9.1±8.5	7.4±8.8	9.1±13.5	12.4±21.9
Prospective food consumption (mm)				
Pre-preload (fasting)	66.1±18.3	63.5±19.4	64.5±18.6	64.1±18.8
Post-preload	61.8±19.4[a]	48.9±22.5[b]	47.5±19.3[b]	58.5±20[a]
10 min after	66.8±17.3[a]	56.6±21.5[bc]	51.5±19.8[b]	61.5±20.6[ac]
20 min after	68.5±19.8[a]	58.6±20.2[b]	54.6±20.6[b]	62.7±22.1[ab]
30 after	70.8±19.9[a]	60.6±20.2[b]	56±19[b]	63±22.9[ab]
After ad libitum breakfast	14.4±13.1	13.8±14.8	12.8±15.7	16.7±18.2
Thirst (mm)				
Pre-preload (fasting)	66.2±24.3	67.3±21.4	67±22.4	65.2±22.4
Post-preload	36±23.8[a]	51.6±27.5[c]	46.1±25.9[bc]	41.5±24.4[ab]
10 min after	45.9±27.4	52.9±27.1	50.1±23.6	48.3±27.4
20 min after	47.1±27.3	54.6±26.3	54.7±26.3	54.1±26.7
30 after	50±27.8	58.4±26.5	56.8±26.3	55.9±26.1
After ad libitum breakfast	11.5±16	10.2±12	11.5±14.8	14.9±20.8

[a]A statistical significant difference ($p < .05$) between the conditions (preloads) is denoted by different letters in superscripts.

Supplementary Table E.4. Appetite ratings (mm) at all-time points for participants ingesting MC (medium coating) and LC (low coating) preloads, n = 15 (means ± SDs) for Study 2.

Appetite ratings	MC	LC
Hunger (mm)		
Pre-preload	60 ± 19.4	58.7 ± 22.4
Post-preload	38.7 ± 20.4	34.3 ± 19.4
15 min after	41.7 ± 24	39.7 ± 19.3
30 min after	40 ± 22.3	42 ± 21
60 min after	50.7 ± 25.2	48.9 ± 24.1
After ad libitum breakfast	6.8 ± 6.4	5.7 ± 5.3
Fullness (mm)		
Pre-preload	18 ± 12.1	20.4 ± 17.1
Post-preload	47.2 ± 20	43.7 ± 19.6
15 min after	46.2 ± 22.5	42.6 ± 17.7
30 min after	39.9 ± 19.9	33.2 ± 11
60 after	29.1 ± 15.7	30.5 ± 16.6
After ad libitum breakfast	60.7 ± 29.3	71.4 ± 17.5
Desire to eat (mm)		
Pre-preload	59.3 ± 20.2	55.8 ± 22.5
Post-preload	37.8 ± 21.6	38.1 ± 20.2
15 min after	42.2 ± 21.2	41.3 ± 22.3
30 min after	41.9 ± 23.2	42.1 ± 22.2
60 after	51.3 ± 23	51.7 ± 18.6
After ad libitum breakfast	8.2 ± 7.2	11.3 ± 11.6
Prospective food consumption (mm)		
Pre-preload	58.5 ± 16.1	55 ± 18.8
Post-preload	35.7 ± 14.3	38.8 ± 17.5
15 min after	42.9 ± 12.3	45.6 ± 16.5
30 min after	43.1 ± 14.4	45.3 ± 15.2
60 after	49.6 ± 15.5	51 ± 14.3
After ad libitum breakfast	10.4 ± 7.1	14.2 ± 11.7
Thirst (mm)		
Pre-preload	28.8 ± 17.2	30.7 ± 22.4
Post-preload	25.8 ± 21.8	26.7 ± 22.7
15 min after	25.1 ± 21.3	30.6 ± 26
30 min after	24.1 ± 21	31.9 ± 25.7
60 after	30.5 ± 21.2	32.1 ± 26.4
After ad libitum breakfast	11.6 ± 12.4	12 ± 16.1

Supplementary Table E.5. Area under the curve (AUC) for appetite ratings (mm) over time for subjects having Control, HC, MC and LC preloads, n=37 (mean + SDs) for Study 1.

Appetite Ratings	Control	HC	MC	LC	p-values
Hunger	2845.4 ± 755.76 [a]	2175.13 ± 912.20 [b]	2226.62 ± 799.42 [b]	2425.74 ± 871.79 [b]	*p< .05*
Fullness	1587.29 ± 749.12 [a]	2259.32 ± 848.94 [b]	2162.83 ± 806.63 [bc]	1921.08 ± 813.90 [c]	*p< .05*
Desire to eat	2907.63 ± 829.34 [a]	2364.32 ± 865.28 [b]	2301.01 ± 839.83 [b]	2599.18 ± 964.13 [ab]	*p< .05*
PFC	2974.25 ±752.32 [a]	2511.95 ± 805.57 [bc]	2373.91 ± 794.98 [c]	2753.64 ± 860.85 [ab]	*p< .05*
Thirst	2076.68 ±1124.55	2436.35 ± 1085.50	2357.90 ± 1029.18	2308.78 ± 1087.54	n.s.

[1]The lower-case letters (superscripts) denote a significant difference between preloads ($p < 0.05$). Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table E.6. Area under the curve (AUC) for appetite ratings (mm) over time for subjects having MC and LC preloads, n=15 (mean + SDs) for Study 2.

Appetite Ratings	ML	HL	p-values[1]
Hunger	3398.6 ± 1646.6	3315.5 ± 1513.1	n.s.
Fullness	3442 ± 1296.9	3351.6 ± 985.9	n.s.
Desire to eat	3466.3 ± 1628.3	3492.1 ± 1512.9	n.s.
PFC	3460.5 ± 959	3645 ± 1104.1	n.s.
Thirst	2129.3 ± 1542.9	2446.3 ± 1846.9	n.s.

[1] Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table E7. Palatability of the preloads (Control, HC, MC and LC) measured on a 100-mm VAS scale (n=37) (means ± SDs) for Study 1.

	Control	HC	MC	LC	p-value[1]
Texture	47.5 ± 26.7	51.1 ± 25	54.4 ± 26.7	56.3 ± 25.7	n.s.
Flavour	34.2 ± 25.9	28.8 ± 23.6	33.2 ± 23.8	40.4 ± 28	n.s.
Sweetness	16.1 ± 21.8	18.2 ± 17	20.6 ± 20.8	22.9 ± 18.6	n.s.
Liking	35.5 ± 23.2	36 ± 23.5	36.1 ± 28.1	42.5 ± 27.9	n.s.
Wanting	35.6 ± 26.8	35.7 ± 24.8	34.9 ± 27.7	39.7 ± 28.6	n.s.

[1] Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table E.8. Palatability of the preloads (MC and LC) measured on a 100-mm VAS scale (n=15) (means ± SDs) for Study 2.

	MC	LC	p-value[1]
Texture	49.4 ± 27.9	58.2 ± 20.6	n.s.
Flavour	47.1 ± 23.6	39 ± 25.2	n.s.
Sweetness	25 ± 22.7	21.9 ± 20.4	n.s.
Liking	44.4 ± 24.9	44 ± 25.1	n.s.
Wanting	41.7 ± 24.7	38.7 ± 20.6	n.s.

[1] Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table E.9. Analysis on absolute data on glucose and gut peptides (ghrelin, GLP-1 and PYY) for Study 2.

There was no main effect of condition for glucose $F(1, 14) = 1.575$, for GLP-1 $F(1, 14) = 4.173$, $p = .060$ and for PYY $F(1,14) = 0.845$ (all $p > .05$). However, there was a main condition effect for total ghrelin $F(1, 14) = 16.256$, $p = 0.001$ with higher levels in MC condition compared to HC. A post-hoc pairwise comparison test showed that total ghrelin was significantly higher in MC condition compared to HC immediately after preload ($p = .14$), 15 min after ($p = .051$ – bordeline significance), 30 min after ($p = .054$ – borderline significance) and 60 after preload ($p = .015$). GLP-1 levels were significantly different in HC condition compared to MC at 15 min after ($p = .040$). And PYY levels were significantly higher in MC compared to HC at 60 min ($p = .021$). There was a main effect of time for glucose $F(4,56) = 35.672$, $p = .001$ and total ghrelin $F(4, 56) = 3.055$, $p = .024$ but not for GLP-1 $F(4, 56) = 0.288$ or PYY $F(4, 56) = 0.376$ (all $p > .05$). There was no significant effect of condition*time interaction for glucose $F(4, 56) = 0.317$, total ghrelin $F(4, 56) = 0.324$, GLP-1 $F(4, 56) = 1.924$ and PYY $F(4, 56) = 1.830$ (all $p > .05$).

Supplementary Table E.10. Pearson's correlations between appetite ratings, energy intake, lubricity (ES0.1, ES0.05 and ES0.005 represents the friction coefficients at mixed (0.1 m s^{-1},0.05 m s^{-1}) and boundary (0.005 m s^{-1}) regimes respectively, viscosity of saliva (at 50 s^{-1} shear rate), oro-sensory exposure time/salivary flow and salivary biomarkers, after preload (n=37) for Study 1. Green colour indicates positive and orange colour a negative correlation with $p < 0.05$ in light colours and $p < 0.01$ in the darker shades.

		Appetite ratings					Energy and water intake		Salivary biomarkers		Lubricity			Oro-sensory exposure time and saliva flow		Viscosity		
		Hunger	Fulness	DE	PFC	Thirst	EI	WI	Protein	Amylase	ES0.1	ES0.05	ES0.005	OSET	SFlow	SR50	SR10	SR1
Appetite ratings	Hunger	1																
	Fulness	-.985*	1															
	DesireToEat	.951*	-0.939	1														
	PFC	0.898	-0.938	.956*	1													
Energy and water inatke	EI	0.895	-0.836	.960*	0.839	-0.695	1											
	WI	-0.470	0.477	-0.176	-0.172	0.592	-0.077	1										
Salivary biomarkers	Protein	-0.853	0.876	-.963*	-.980*	0.794	-0.886	-0.003	1									
	Amylase	-0.591	0.489	-0.366	-0.176	0.459	-0.443	0.766	0.104	1								
Lubricity	ES0.1	-0.943	.951*	-.993**	-.983*	0.878	-0.922	0.185	.978*	0.301	1							
	ES0.05	-0.944	0.940	-.999**	-.968*	0.852	-0.948	0.164	.973*	0.330	.997**	1						
	ES0.005	-.980*	.990**	-.976*	-.968*	0.941	-0.890	0.351	0.932	0.417	.985*	.978*	1					
Oro-sensory exposure time and saliva flow	OSET	-0.415	0.374	-0.116	-0.033	0.449	-0.096	.959*	-0.112	0.888	0.093	0.092	0.255	1				
	Sflow	-0.380	0.228	-0.226	0.041	0.130	-0.410	0.454	-0.036	0.909	0.127	0.182	0.196	0.672	1			
Viscosity	SR50	-0.473	0.503	-0.718	-0.758	0.388	-0.683	-0.512	0.857	-0.351	0.736	0.736	0.612	-0.605	-0.333	1		
	SR10	-0.546	0.532	-0.778	-0.742	0.377	-0.804	-0.482	0.861	-0.167	0.767	0.785	0.646	-0.511	-0.094	.968*	1	
	SR1	-0.081	-0.085	-0.160	0.120	-0.287	-0.430	-0.320	0.027	0.358	0.048	0.123	-0.015	-0.063	0.697	0.111	0.341	1