The influence of protein type, protein concentration, and mucin on oral lubrication properties in relation to mouthfeel

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I confirm that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. My contribution and the other authors to this work has been explicitly indicated below. I confirm that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Abstract

This thesis aims to enhance understanding of the sensory properties of protein fortified foods during oral processing by studying flow and lubrication behaviours. During oral processing, saliva plays an important role through mixing with samples, dilution and varying degree of interaction with proteins. However, the role of saliva in sensory perception of protein-based foods is not yet fully understood. A systematic review screening 36,604 articles narrowing down to 33 articles highlighted that although oral processing and salivary interactions of dairy proteins particularly at low protein concentrations are relatively well understood, little is known about plant proteins, particularly at higher concentrations. As a result, this thesis aimed to gain a deeper understanding of the role of different protein types, both in the presence and absence of model saliva, during oral processing. To accomplish this, the research methodology chosen used a diverse range of techniques combining instrumental analysis looking at lubrication properties with human sensory evaluations. Two plant proteins, pea protein concentrate and soy protein isolate, were compared to two dairy proteins, whey protein isolate and sodium caseinate with the addition of a control, skimmed milk powder. Systems were analysed in a range of protein concentrations (5wt%-20wt%) and in different media of varying complexity, from aqueous solutions to oil-in-water emulsions to finally a model food. In general, plant proteins demonstrated poor adsorption, solubility and lubrication properties. This was shown to impair their sensorial mouthfeel (measured using Quantitative Descriptive Analysis (QDA®) when implemented in a model food system. Increasing protein concentration improved lubrication for dairy but not for plant proteins. Although the friction was decreased when proteins were in an emulsion system owing to oil-induced lubrication, still the high friction coefficients of plant proteins were apparent. Overall, the addition of model saliva to proteins had little effect on lubrication, with protein still dominating the frictional dissipation with disparate degree of influence on adsorption behaviour in plant versus dairy proteins. In summary, simpler systems with plant protein in bulk solutions can

provide mechanistic insights about lubrication and adsorption behaviour, which are often challenging to pinpoint when they are present in model food systems. Overall, food manufacturers may require careful formulation engineering strategies to mitigate lubrication-mediated sensory differences when transitioning towards plant proteins to achieve sustainable formulation in the future.

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List of Abbreviations

AFM	Atomic force microscopy
ANOVA	Analysis of variance
ATR-FTIR	Attenuated total reflection–Fourier transform infrared
BAA	Bicinchoninic acid assay
BSA	Bovine serum albumin
BSM	Bovine submaxillary mucin
CD	Circular dichroism
CLSM	Confocal laser scanning microscopy
DB	Detection bias
d_H	Hydrodynamic diameter
DI	Deionized
DLS	Dynamic light scattering
EHL	Elastohydrodynamic lubrication regime
F	Female
FG	Focus group
FP	Flash profiling
g	Grams
h	Hours
HEPES	4-(2-hydrozyethyl)-1-piperazineethanesulfanoic acid
HS	Human saliva
Hz	Hertz
IPA-	Isopropyl alcohol
soft-EHL	iso-viscous elastic hydrodynamic lubrication regime
JBI	Joanna Briggs Institute
LC-MS	Shotgun Liquid Chromatography-Mass Spectrometry
LD	Laser diffraction
LS	Light scattering
LTSEM	Low-temperature scanning electron microscopy
M	Male
MilliQ-	Millipore MilliQ water
mPas	Millipascal- seconds

MRs	Mechanoreceptors
MS	Model saliva
MTM	Mini-traction-machine
n	Number of
NA	Not applicable
NaCas	Sodium caseinate
NaCL	Sodium chloride
NaOH	Sodium hydroxide
ND	Not disclosed
NMR	Nuclear magnetic resonance
O/W	Oil in water emulsion
ODT	Orthonasal detection threshold
OP	Oral processing
PAGE	Native-Polyacrylamide Gel Electrophoresis
PB	Performance Bias
PDMS	Polydimethylsiloxane
PGM	Porcine gastric mucin
pH	Potential of hydrogen
pI	Isoelectric point
PICO	Population, Intervention, Comparison and Outcome
PNA	Protein non-associated
PPc	Pea protein concentrate
PPi	Pea protein isolate
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRPs	Proline-rich proteins
QCM-D	Quartz crystal microbalance with dissipation
QDA®	Quantitative Descriptive Analysis
RI	Refractive index
rpm-	Repetition per minute
RQ	Reporting quality
s	Seconds
SB	Selection Bias
SD	Standard Deviation

SDS	Sodium dodecyl sulphate
SDS-PAGE:	Sodium dodecyl sulphate polyacrylamide gel electrophoresis,
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SMP	Skimmed milk powder
SPI	Soy protein isolate
SRR	Sliding to rolling ratio
SWHS	Stimulated whole human saliva
TCTA	Temporal check-all-that-apply
TDS	Temporal Dominance of Sensations
TI	Time-Intensity
TPA	Texture Profile Analysis
TRCs	Neuroepithelial taste receptor cells
U	Entrainment speed
UPHLC	Ultra-high-performance liquid chromatography-diode array detector-tandem mass spectrometry
UV	Ultra violet
UWHS:	Unstimulated whole human saliva
W/O	Water in oil emulsion
WP	Whey protein
WPI	Whey protein concentrate
WPH	Whey protein hydrolysate
WPI	Whey protein isolate
ZP	Zeta potential
β-lg	βeta-lactoglobulin
VAS	Visual Analogue Scale

List of Accepted Conference Abstracts

Oral Presentations:

Frances Brown, Alan Mackie, Qi He, Jochen Pfeifer, Anwesha Sarkar (2022). Surface Absorption and Tribology of Plant Proteins compared to Whey Protein Isolate, *5th Food Structure and Functionality Symposium*, Cork, Ireland, 18th-21st September 2022.

Frances Brown, Alan Mackie, Qi He, Jochen Pfeifer, Siavash Soltanahmadi, Anwesha Sarkar (2023). Oral Frictional Properties of Plant and Dairy Proteins- the role of saliva, *77th Society of Tribologists and Lubrication Engineers Annual Meeting & Exhibition*, Longbeach California, United States, 21st-25th May, 2023.

Poster Presentations:

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Frances Brown, Alan Mackie, Qi He, Jochen Pfeifer, Anwesha Sarkar (2022). Comparison of the Frictional Properties of Plant and Dairy proteins. *36th European Federation of Food Science and Technology,* Dublin, Ireland, 7th-9th November 2022.

List of Publications

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The contribution of the candidate and the other authors to this work has been explicitly indicated below. Chapter 2: 'Protein–saliva interactions: A systematic review' is published in the journal Food and Function, 2021. The contributors listed are Frances Brown, Professor Anwesha Sarkar, Professor Alan Mackie, Dr Qi He and Alison Branch. Frances brown conducted the literature search, critical evaluation of evidence and synthesis of evidence. Both Professor Sarkar and Mackie contributed ideas to the design and organization of the work. All authors edited and reviewed the manuscript.

Chapter 1. Introduction

1.1. Objectives

Dietary protein is a highly sought-after macronutrient in humans that serves as the main nitrogen source, while amino acids play a fundamental role as building blocks for body tissues (Qin et al., 2022). As such, proteins play a vital role in maintaining muscle health, which is particularly relevant for older adults (Deer and Volpi, 2015). Protein also has been shown to have the ability to reduce hunger, ad libitum calorie intake and fat mass more effectively than carbohydrates and fat (Weigle et al., 2005). Hence, it is unsurprising that protein-fortified foods and beverages are increasingly popular (Mills et al., 2018). Consumption of plant-based protein is a growing trend (Qin et al., 2022, Hertzler et al., 2020) caused partly by increasing awareness of environmental sustainability and ethical and welfare concerns over the treatment of animals (Hertzler et al., 2020). However, a concerning issue in fortifying foods with all types of protein is the impact on palatability (Mills et al., 2018). High protein foods are associated with unpleasant dry after tastes (Stokes et al., 2013), specifically astringency (Carter et al., 2020). Therefore, understanding texture/mouthfeel of protein can provide important insights for food engineers in designing protein-based foods, which are accepted by the consumer.

Oral processing is a dynamic and complex procedure which involves the breakdown of food structure into smaller particles and mixing with saliva to create a bolus capable of being safely swallowed (Chen and Stokes, 2012). During this process, food properties undergo a significant transition. Initially when food enters the mouth sensory properties are dominated by rheology, but during later stages this shifts to tribology dominated. By analysing properties related to both rheology and tribology we can better understand mouthfeel (Stokes et al., 2013).

The use of instrumental instead of sensory panels to predict sensory perception is most common as they tend to be cheaper, more reproducible, and quicker (He, 2014, Sarkar and Krop, 2019). However, results from these methods need to be validated through sensory analysis (de Lavergne et al., 2015).

The primary objective of this research was to gain a better understanding of how protein type influences sensory food properties during oral processing. Given the complex nature of oral processing, this project adopts a multidisciplinary approach, involving food science, sensory science, and mechanical engineering. Three main research areas are identified: (1) the material and texture properties of aqueous solutions with and without model saliva; (2) the material and texture properties of high protein emulsions; and (3) the material and texture properties of model food systems in relation to sensory analysis.

By comprehensively studying a range of instrumental characteristics, the aim is to uncover the specific role of protein, both in isolation and relation to other food components. The research endeavours to identify the material properties in protein-rich systems that can predict sensory perception, thereby aiding in the design of protein fortified foods. The hypothesis being tested is that protein type will result in different material properties due to inherent structural and functional differences between proteins. Consequently, the material properties will further affect the sensory characteristics of the proteins.

1.2. Fundamentals of mouthfeel

To understand mouthfeel and texture, an exploration of the physiology underling mouthfeel is essential. Subsequently, methodologies that have previously been used to understand mouthfeel are introduced.

1.2.2. Physiology of mouthfeel

Astringency is typically defined as the "complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins" (ASTM, 1989). Similar attributes of dryness and roughness are commonly thought of as synonymous with astringency (Carter et al.,2020). Astringency can occur when consuming many foods and has been extensively studied in polyphenol products such as tea, fruit and wine (Liu et al., 2022). Astringency when associated with dairy products has been considered a 'textural defect' (Norton et al., 2021, Lemieux and Simard,1994). Both dairy protein, such as whey and plant-derived protein beverages, for example those fortified with soy and pea, are described as astringent (Norton et al., 2021, Bajec and Pickering, 2008, Carter et al.,2020). The mechanisms behind protein producing astringency are not currently fully understood. However, it is suggested that the mechanisms behind polyphenol astringency may also apply to protein (Carter et al., 2020). These mechanisms include association between salivary proteins and polyphenols which is thought to produce complexes which may aggregate or precipitate: both are thought to cause a loss in lubricity (Carter et al., 2020).

Astringency is thought to be an oral tactile rather than a taste sensation and is therefore perceived when mechanoreceptors are stimulated (Liu et al., 2022). When consuming food/beverages, the first contact with the oral cavity occurs through the lips and teeth. Subsequently the food/beverage will be moved inside the mouth and coat oral surfaces. Oral surfaces include the palate, cheeks, and tongue. The somatosensory system processes a variety of sensory information including touch and pressure to detect astringency (Carter et al., 2020). Astringency is also thought to be induced by changes in friction and lubrication (Carter et al., 2020). Sensory perception within this is influenced by the various mechanoreceptors present at these points (Bajec and Pickering, 2008, van Aken,2010).

There are neuroepithelial taste receptor cells (TRCs) and mechanoreceptors (MRs) which are both believed to play critical roles in the determination of astringent sensations. TRCs, otherwise known as taste buds, are exposed to the oral cavity through pores. Whereas MRs, respond to both static and dynamic force applications (Bajec and Pickering, 2008). The different papillae within the mouth are both TRCs and MRs. The non-taste papillae (MRs), specifically filiform papillae which are hair like structures, are thought to be responsible for mouthfeel perception (van Aken, 2010). The detection of astringent thresholds is known to vary between individuals (Zhou et al.,2021). It is proposed that this variation may in part be due to different oral physiology (Zhou et al., 2021). For instance, the number of receptors or fungiform papillae density. Typically, a higher fungiform papillae density is related to greater fat taste sensitivity (Zhou et al., 2021, Andreeva et al.,2023). For astringency, it is to date unclear if there is a relationship between fungiform papillae density and heightened sensory astringency or not (Zhou et al., 2021). Further research is required to establish if a relationship between fungiform papillae density research and astringency exists (Zhou et al., 2021).

Astringency is just one aspect of the overall sensory experience when consuming protein fortified food. To gain a full understanding of protein mouthfeel, it is vital to explore broader aspects beyond astringency and the overall wider mouthfeel sensations (de Lavergne et al., 2017). Mouthfeel encompasses the tactile sensations experienced in the mouth when consuming food or beverages, distinct from the sensation of taste. Among these mouthfeel sensations is texture, which relates to the tactile perception of a surface, encompassing sensations like ‘roughness’. Mouthfeel and texture are vital components in predicting consumer acceptability (de Lavergne et al., 2017). Texture specifically is a predictor of food rejection and aversion (Drewnowski,1997).

Texture and mouthfeel are dependent on the transformation a food undergoes (de Lavergne et al., 2017). This process involves solid food being reducing to

small particle sizes, mixing with saliva and formation of a saliva/ food bolus. As mentioned earlier, the mouth is a sensitive organ with many mechanoreceptors which respond to tactile stimuli. It is suggested that mouthfeel and texture are perceived by the tongue, palate and soft tissues within the mouth (Norton et al., 2021). How an individual masticates (chews) influences the texture and therefore sensitivity and oral reception.

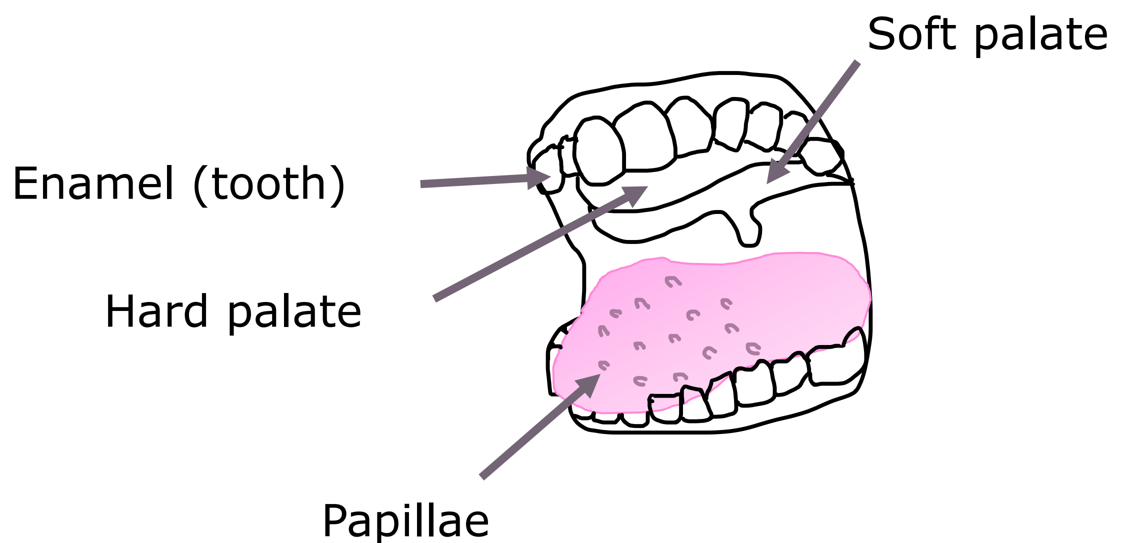


Figure 1. The oral surfaces

1.2.2. Role of saliva

Saliva plays many key roles in our eating experience with an unavoidable presence in the mouth that therefore has major impacts on oral sensations (Agorastos et al., 2023). Fundamentally, saliva prevents feelings of wear and is vital in the formation of a bolus capable of being safely swallowed (Agorastos et al., 2023). On a molecular level, saliva contributes to alterations in viscosity, changes in lubrication and friction, enzymatic breakdown, formation of aggregates and precipitates and destabilization of colloidal systems (Mosca and Chen, 2017). All these roles have the ability to significantly alter the texture and mouthfeel perception. Aside from the focus of this thesis, mouthfeel, saliva also plays important roles in taste through binding to aroma compounds and as the

fluid medium in which tastants are transported as well as dispersed to the receptors (Mosca and Chen, 2017).

Saliva's characteristics play important roles in its function. Saliva's wettability characteristics are due to its low surface tension from its active components (Mosca and Chen, 2017). This allows for efficient mixing as food is wetted fast and tastants are quickly dispersed (Mosca and Chen, 2017). Without saliva, oral surfaces are hydrophobic due to saliva's wettability (Sarkar et al., 2019b). Having higher viscosity than water as well as being very elastic helps saliva coat and lubricate the oral surfaces, as well as helping to form a cohesive food bolus (Mosca and Chen, 2017).

1.2.3. Saliva Composition

The viscosity and elasticity of saliva are due to saliva's unique composition. Human saliva comprises of predominantly water (99.5%) but also contains proteins, enzymes and inorganic substances (Sarkar et al., 2019b). Salivary proteins can be divided into major classes including proline-rich proteins (PRPs), statherin, cystatins, P-B peptides, histatins and mucins. Statherin, which is abundant in tyrosine residues and phosphorylated at certain serine sites, has roles in oral lubrication (García-Estévez et al., 2018). In contrast, cystatins primarily serve to protect the oral cavity by inhibiting bacterial and viral cysteine proteases. Histatins are minor components with antifungal properties (Sarkar et al., 2019b, García-Estévez et al., 2018). Mucins play vital roles in lubrication, these are high molecular weight glycoproteins which account for nearly 20% of total salivary proteins (García-Estévez et al., 2018, Gibbins and Carpenter, 2013). Mucins are amphillic whereby they possess both hydrophillic glycated regions as well as hydrophobic un-glycated regions (Çelebioğlu et al.,2019). These are 'abundant with bound water' so help maintain the lubrication of the oral surfaces (Laguna et al., 2017, Hand and Frank, 2014). The properties of mucins

include low solubility, high elasticity, adhesive and highly viscous (Gibbins and Carpenter, 2013). It should be noted that the salivary proteins which are responsible for lubrication (e.g. mucins, proline-rich-proteins) also play important roles in forming the salivary pellicle (Agorastos et al., 2023).

The submandibular and sublingual glands produce the two salivary mucins, MUC5B and MUC7 (Gibbins and Carpenter, 2013). There are important differences between the two types of mucins in relation to their properties and therefore lubrication function. MUC5B makes up a large component of the salivary pellicle which is essential for maintenance of the oral mucosa (Gibbins and Carpenter, 2013). The pellicle can be described as the proteinaceous film which is adsorbed to all oral surfaces. The pellicle acts as a barrier which protects enamel from dissolution as well as lubricating the mouth (Sarkar et al., 2019b, Agorastos et al., 2023). This occurs partially due to MUC5B's selective binding of hydroxyapatite to the enamel surfaces. MUC5B also has gel-forming properties to help serve as a protective barrier in preventing pathogens (Gibbins and Carpenter, 2013). MUC7 is lower in molecular weight compared to MUC5B, and is non-gel forming (Sarkar et al., 2019b).

Human saliva is described as a typical colloidal system (Glantz, 1997). Glantz subsequently produced a four-stage model of saliva to describe its structure. It first consists of a continuous phase, which is water and electrolytes that buffer the medium. Following this phase there is a scaffold-like structured network comprised of the highly glycated mucins. Thirdly, there are different salivary structures such as water-soluble proteins, salivary micelles and/or salivary globular structures within this network. The final stage is the dispersed water droplets which are comprised of insoluble lipid material, bacterial and epithelial cells (Sarkar et al., 2019b).

1.2.4. Saliva flow

After food is sensed in the mouth, neural reflexes will posit the release of more saliva (Mosca and Chen, 2017). The properties of saliva vary through the gland from which it is secreted, which depends on whether it is stimulated or not (García-Estévez et al., 2018). Saliva primarily originates from the secretions of three major salivary glands, which are parotid, submandibular, and sublingual glands along with minor salivary glands and gingival crevicular fluid (García-Estévez et al., 2018). Saliva can be stimulated from several different mechanisms, each eliciting different saliva compositions and flow rates. Stimulation mechanisms are categorized into mechanical (e.g. chewing), gustatory (e.g sour) or olfactory (e.g. smell). Unsurprisingly, resting saliva flow rates are the lowest at approximately 0.4mL per minute, followed by 0.5mL per minute for odour stimulation, then 1.1mL per minute for chewing stimulation and with gustatory (via citric acid) are increased to 2.3mL per minute. It should be reiterated that large variation occurs between these values (Mosca and Chen, 2017). Further, the formation and maintenance of the salivary pellicle is influenced by the flow rate. Slower rates are advantageous as they prevent adhesion with oral mucus. Alternatively, the pellicle thickness is decreased with quicker rates (Zeng et al., 2019). Importantly, the amount of saliva required to form a bolus for swallowing will depend on food properties as, for example, bread will require much more saliva than a liquid food so increasing taste intensity (Mosca and Chen, 2017).

1.2.5. Human saliva's use in research

Using human saliva in studies posits many challenges due to its high instability and individual variability (Schipper et al., 2007). Instability is partially due to saliva's rapid decay from bacterial metabolism. Examples of variation between individuals include the amount of saliva produced as well as its underlying

chemical composition that are dependent on the flow rate, circadian rhythm, type and size of salivary gland, duration and type of stimulus, diet, drugs, age and gender, blood type as well as physiological status. Usually, saliva has a pH of around 6.2-7.4, with pH being higher with increased secretion (Schipper et al., 2007). To complicate it further, saliva flow rate is also variable between individuals. In healthy individuals, unstimulated whole saliva typically ranges from 0.3-0.4mL/ minute (Dawes,1996). This can vary with factors such as hydration of an individual, age, gland size and even posture (lying or standing). Stimulated saliva, which is secreted in response to masticatory or gustatory stimulation, also has large variation. On average it is suggested to be around 7mL/minute and can also vary with a wide range of factors including gland size and smoking status (Dawes,1996).

1.2.6. Artificial saliva

Due to the aforementioned difficulties in using human saliva, artificial saliva is commonly used in laboratory studies and has lower variability and better reproducibility than human saliva (Mosca and Chen, 2017). Artificial saliva using mucin, the main lubricating protein in human saliva, is accepted as physiologically relevant for oral lubrication research (Sarkar et al., 2019b). There are two common types of model saliva, porcine gastric mucin (PGM) and bovine submaxillary mucin (BSM). To make model saliva, mucin is combined with salivary salts (Sarkar et al., 2019b). This method has shown to produce similar pH, viscosity and lubrication properties to human saliva (Mystkowska et al., 2018). However, as other salivary proteins are not present the properties of model saliva will not be identical to those of human. For instance, the complex architecture will not be replicated nor will interactions with any other proteins other than mucin (Sarkar et al., 2019b).

1.3. Previous measurement approaches and method rationale

A variety of different methods and experimental approaches have been used to try to understand astringency/mouthfeel and texture (Pires et al., 2020, see **Figure 2.**). Mouthfeel involves an intricate series of processes whereby food/beverages undergo a series of transformations. These include being sheared and mixed with model saliva, and during this process is when sensations of texture occur (Soltanahmadi et al., 2022, de Lavergne et al.,2015, Carter and Drake, 2021, Sarkar and Krop, 2019). As such, it should be no surprise that trying to effectively capture all aspects of this process is complex. Research focusses on a combination of material properties and human studies to try to address this complexity. Looking at material properties is advantageous for food manufacturers as it can provide some quantitative aspect to focus on which may be able to predict mouthfeel (Carter et al., 2020). However, it can be complicated to work out if the material attributes actually predict mouthfeel or not. Therefore, human studies are required to validate the material findings (de Lavergne et al., 2017, de Lavergne et al., 2015). However, sensory studies have their own set of problems, mainly relating to the subjectivity of defining sensory responses. How we eat and perceive texture will differ between individuals either due to subjective preference or ability to sense different textures (Jeltema et al., 2020, Jeltema et al., 2016). Also, the rate of saliva secretion varies between individuals as does how they chew and consume food which undoubtedly influences the saliva/ food bolus and, secondly, can influence perception of texture (Dawes,1996).

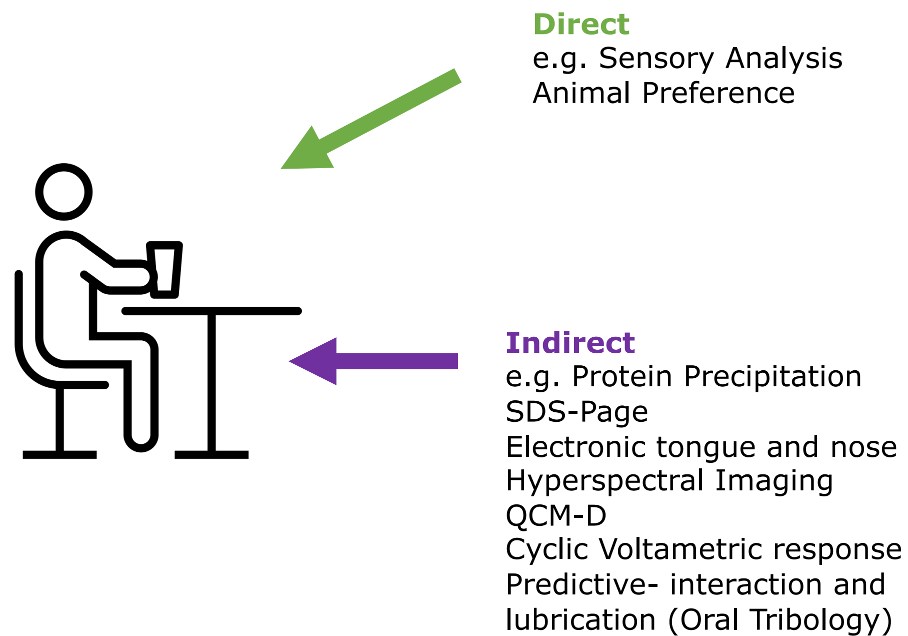


Figure 2. Schematic of different methods used to detect astringent perceptions adapted from (Pires et al., 2020).

1.3.1.Texture analysis

Early instrumental methods to analyse mouthfeel and texture relied on rudimentary mechanical and sensory approaches. These methods include Texture Profile Analysis (TPA) which measures the force required to compress, shear or puncture a sample. This method is related to attributes of hardness, cohesiveness, adhesiveness, springiness and chewiness (Nishinari et al., 2013). TPA involves a uniaxial compression test, which detects changes in stress and strain as the sample is subjected to vertical compression without lateral pressure. TPA was developed in 1963 by the General Foods company (Friedman et al., 1963). The movement of the plunger was developed to simulate the human jaw and chewing in a deformation test. The initial methodology has been subsequently adapted, with (Bourne, 2002) adapting the TPA to include a uniaxial compression machine. Notably, it is recommended that the surface area of the

TPA should be equal or larger to that of the food sample, with the relative deformation at 90% or more (Nishinari et al., 2019). Absolute deformation is the overall deformation experienced by a structural element, whereas relative deformation is the deformation relative to a reference line which helps compare deformations (Heuret and Lallemand, 2005). Results from TPA usually plot stress vs strain or time, resulting in a TPA curve. TPA is predominantly used for solid food samples, as the sensitivity of compression measurements is questioned for liquid samples (Nishinari et al., 2019, Rosenthal, 2010). In solid foods, such as plant-protein meats, TPA can compare compression and elongation forces which is useful for comparison to real meat (McClements et al., 2021). However, it should be noted that TPA analysis is suggested to have poor sensitivity to fully capture the material movement as the human oral cavity is complex with viscoelastic properties playing pivotal roles (Funami and Nakauma, 2022).

1.3.2. Physio-chemical characteristics

How astringent compounds interact with saliva is thought to be critical in understanding astringency, as this can alter rheological and lubrication properties such as negative impairment of the salivary pellicle and shrinkage of tissues. Previous research focuses on understanding the interactions between salivary proteins and food components (Pires et al., 2020). Looking at interactions between saliva and whey protein, which is the most commonly studied (Brown et al., 2021), changes in turbidity have been investigated. Observed changes in turbidity are associated with aggregation between saliva and astringent compounds (Carter et al., 2020). It is hypothesized, for whey protein, that interactions between whey and saliva are of electrostatic origin as saliva has negatively charged groups (Çelebioğlu et al., 2019). Therefore, understanding protein's charge in relation to saliva can provide insight into the origins of possible interactions.

Microscopy is used to analyse the microstructure of food and/or oral surfaces (Stieger and van de Velde, 2013). As a food is consumed, its structure will be changed as it is transformed during mastication to a bolus capable of being swallowed safely. By looking at the microstructure of food-saliva bolus, we can understand how mouthfeel during this process arises (Stieger and van de Velde, 2013). When looking at after-feel sensations, attenuated total reflection Fourier transform infrared spectroscopy (ATR FT-IR) can provide complimentary research to sensory analysis by measuring clearance of proteins and carbohydrates compared to fat in dressings. The greater adherence of proteins and carbohydrates is linked to sticky after feels (ae Jongh and Janssen, 2007). In addition, microscopy is used to look at how particles interact with saliva through turbidity (Vlădescu et al., 2021, Laguna et al., 2017).

1.3.3. Rheology-Tribology transition

Sensory perception is thought to be better understood from a rheology to tribology transition. This means, at early stages of eating sensory perception is thought to be governed primarily through rheological attributes. However as eating progresses, there is a transition to being predominantly governed by tribological attributes (Stokes et al., 2013, see **Figure 3.**). It is known that the organoleptic properties of food are governed by the constantly changing status of food into a saliva-bolus capable of being swallowed. We can divide eating into different stages. The first bite and initial chewing are governed by mechanics. Following this there is granulation, where the food is ground, and its size reduced. Food particles may rub against oral surfaces leading to abrasive or dry sensations. In addition, liquid may be released from the food in this stage. Next a bolus is formed, where particles become increasingly dispersed in saliva ready for swallowing. After swallowing, we have the residue, which can give way to after feel sensations such as dryness (Stokes et al., 2013).

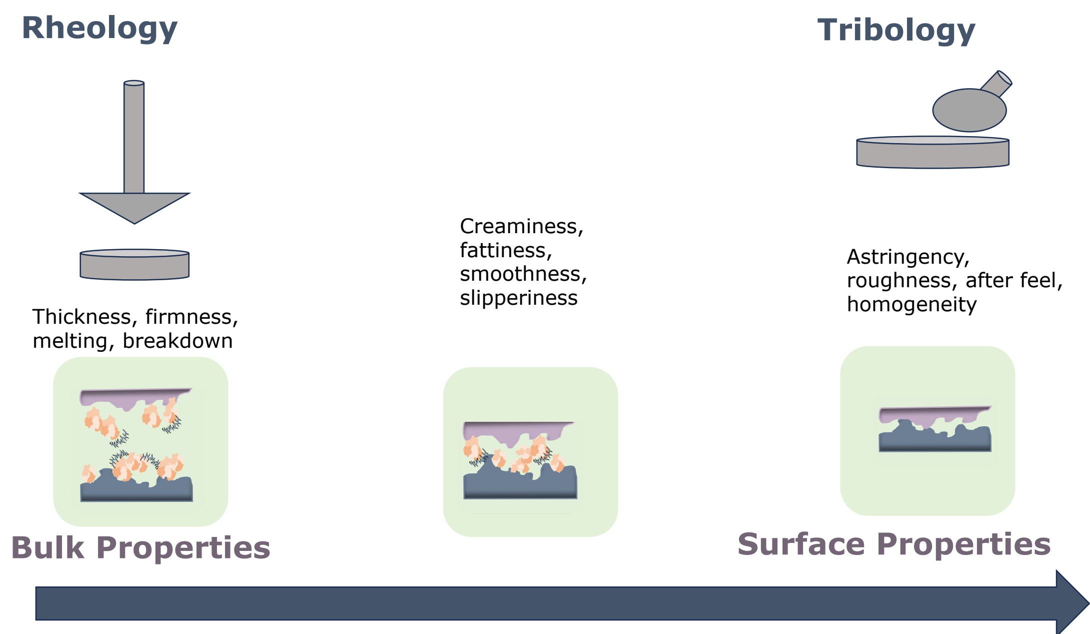


Figure 3. Rheology tribology transition and mouthfeel adapted from (Stokes et al., 2013)

1.3.4. Rheology

The definition of rheology is the study of flow and deformation of material (Barnes et al., 1989). The rheological properties of materials are determined by their response to flow and deformation when subjected to an applied stress. Rheological properties are important for food engineering as they can impact not just mouthfeel but also processing and product stability (Selway and Stokes, 2014). As texture arises from integration of food within the mouth and subsequent deformation, it is suggested to be synonymous with rheology (Selway and Stokes, 2014). Viscosity is perhaps the most common rheological property looked at when relating to sensory perception (Lv et al., 2017, Chen and Stokes, 2012). A standard shear rate of $50s^{-1}$ is commonly accepted as most appropriate for oral conditions (Wood, 1968). Foods are commonly characterised by their structure as either soft foods or hard foods. This is inherently linked to the energy required to orally process foods, with soft foods requiring lower mastication (Theocharidou

et al., 2021). For example, the national dysphagia diet (Force and Association, 2002) has said thin foods have a viscosity rating of 0-50mPas, a nectar-like (51-350mPas), honey-like (351-1750mPas) and pudding-like (>1750mPas), based on a shear rate of $50s^{-1}$ at 25°C (Funami and Nakauma, 2022).

Sensory studies aiming to capture texture commonly report rheology-based mouthfeel terms such as ‘thick’, ‘thin’ and ‘viscous’. Despite this, relationships correlating sensory data with rheology data have been elusive (Selway and Stokes, 2014). It is proposed that sensation and perception of food viscosity are intricately influenced by two fundamental factors. The first is the flow of the material (Lv et al., 2017). The most common flow behaviour for foods is non-Newtonian behaviour where the viscosity is dependent on shear rate. Non-Newtonian products are preferred because they have reduced likelihood of destabilization and phase separation, therefore improving shelf-life (see **Figure 4.**). A lot of food exists in the form of an emulsion, for example ice cream and mayonnaise, which can have many phases, such as oil dispersed in water (Selway and Stokes, 2014). Yield stress is an important factor in these materials, this being the minimum amount of stress (or force) applied to the material before it starts to deform or flow (Lv et al., 2017). Yield stress may provide important information for cohesiveness of a saliva-food bolus (Funami and Nakauma, 2022).

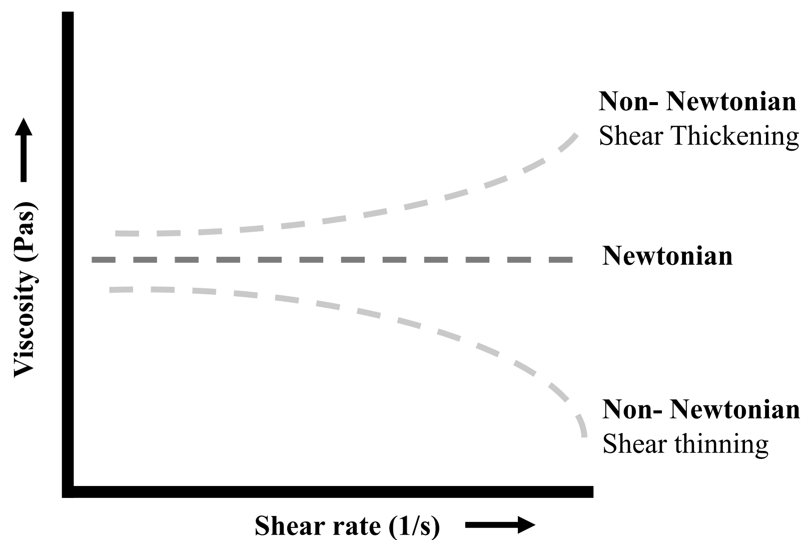


Figure 4. Typical viscosity responses to increasing shear

The second fundamental factor is the type of deformation. A range of different shear-deformations exist during eating which makes it difficult to accurately predict oral viscosity (Lv et al., 2017). Extensional deformation is where fluid particles undergo expansion or compression with perpendicular stress. Contrastingly, shear deformation is the deformation of a fluid when stress is applied parallel to its surface. Shear deformation causes fluid particles to slide over one another while maintaining similar interparticle distances (Lv et al., 2017). Both shear and extensional forces occur during oral processing, as such measuring both can provide insight into sensory texture understanding (Theocharidou et al., 2021, Lv et al., 2017, Chen and Stokes, 2012). During oral processing there are fundamental movements of downward bolus flow and upwards and downwards motions of the tongue against the palate (Theocharidou et al., 2021). As both motions are primarily driven by extensional forces, it is suggested as crucial to also understand these in relation to mouthfeel. This is cohobated with the oral system having greater sensitivity to variations in

extensional viscosity compared to shear viscosity (Lv et al., 2017, Theocharidou et al., 2021).

1.3.5. Tribology

More recently, oral friction has been looked at using tribology. Tribology was originally developed for quantifying friction in engineering applications and has subsequently been applied to oral applications (Andablo-Reyes et al., 2020). In general, tribology is the analysis between two surfaces in relative motion of wear, friction, and lubrication (Shewan et al., 2020, Andablo-Reyes et al., 2020). Tribology aims to encompass the deformation of food between numerous interacting surfaces such as tongue-palate, tongue-teeth, tongue-food (Funami and Nakauma, 2022). Specifically, during the latter stages of oral processing, tribology is thought to be dominant in mouthfeel (Funami and Nakauma, 2022).

It must be noted that tribology is a system property dependent on many factors rather than a physical property of the fluid (Shewan et al., 2020). Thus, it is important to note the conditions of measurement, e.g. the measurement system, surfaces and lubricant (Shewan et al., 2020). As it is a system property, relating tribological results to sensory data is difficult. The friction coefficient depends on the interplay between food and oral properties which results in tribological changes. Therefore, properties of the food, oral cavity and food/oral cavity interacting all need to be considered (Shewan et al., 2020). These properties include size, shape of particles which can affect the rheological flow of a colloidal system (Shewan et al., 2020)

Oral tribology results are typically plotted as the coefficient of friction against speed. The curve relates the friction coefficient to the ratio of friction force to the load which enables the assessment of lubrication properties of food (Paul et al., 2022, Prakash et al., 2013). The curve can subsequently be interpreted in

different regimes (see **Figure 6.**). Regarding oral processing, the regimes are typically read right to left, starting at higher speeds with thicker films. The high-speed regime is called the hydrodynamic regime and it can be characterized by the fluid being fully entrained between two surfaces (Shewan et al., 2020). Friction in this regime is dependent on fluid dynamics and predominantly dictated by the viscosity of the fluid and elastic modulus of the fluid (Shewan et al., 2020). As the surfaces in oral tribology encompass at least one deformable surface, the tongue, this regime is often called the iso-viscous elastic hydrodynamic lubrication regime (soft-EHL) (Upadhyay and Chen, 2019b). In the soft-EHL, the tongue is a highly elastic body which rolls and slides in a lubricated setting against a non-conforming contact. The tongue will rub against oral surfaces/food and the pressure of this will deform the surface instead of impacting fluid rheology (Upadhyay and Chen, 2019b). With a reduction in speed there is in turn a reduction in hydrodynamic lift. Henceforth, fluid thickness reduces to a similar height to surface roughness. Here fluid dynamics such as wetting, and material properties also govern friction. This regime is called the mixed lubrication regime. The final regime, the boundary, is characterized by further reduction in entrainment speed to a point where the load is supported by surface, which may include a boundary layer (Shewan et al., 2020). The fluid properties, surface roughness and elasticity, wettability and film formation all impact on the transition between regimes (Upadhyay and Chen, 2019b). For after-feel and roughness sensations, it is this regime which is most important (Upadhyay and Chen, 2019b). The fluid properties, surface roughness and elasticity, wettability and film formation all impact on the transition between regimes (Upadhyay and Chen, 2019b).

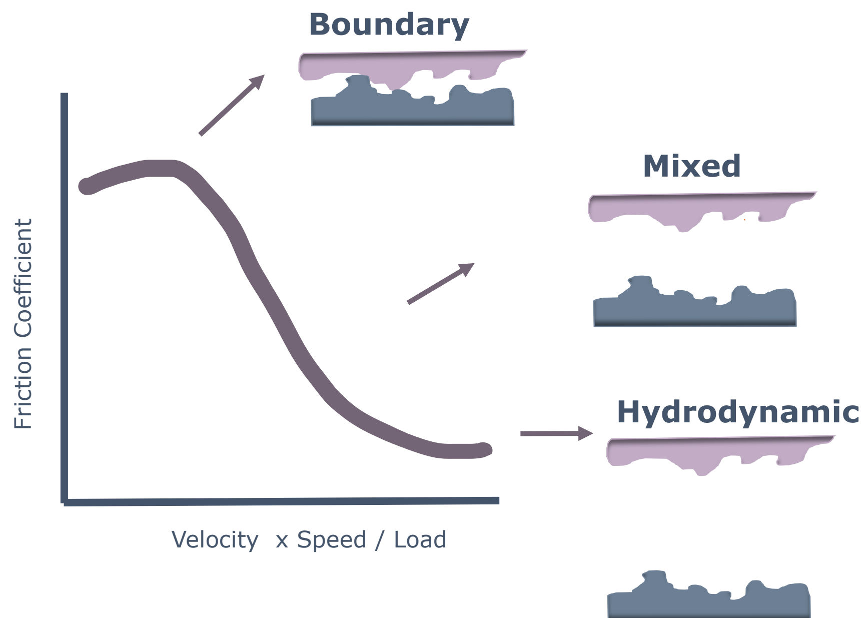


Figure 5. Example of a Stribeck curve

As tribology was originally developed measuring dry friction and linking to the oral cavity is inherently difficult, a range of different measurement methods exist. There is large variation between devised measurements with different speeds, materials and characteristics of interacting surfaces (Paul et al., 2022). One of the most abundant instruments is the mini-traction-machine (MTM). This method uses ball-on-disc apparatus which has the capabilities to measure both rolling and sliding friction (Sarkar and Krop,2019)(see **Figure 5.a.**). The ball and disc can be different material, traditionally they were steel but advancements have been made so they are commonly made from polydimethylsiloxane (PDMS). Using PDMS is preferable over steel as this material has more similar viscoelastic properties to the tongue and oral palate. Rheometers are also used for tribology when a tribo-cell accessory is attached (see **Figure 5.b.**). Commonly, the attachment involves a non-rotating spherical ball on three plates, which can measure sliding but not rolling friction. Advancements have also seen the replacement of the plates from steel to PDMS although the 'ball' is typically a glass probe (Sarkar and Krop, 2019, Paul et al., 2022). In addition, more in house tribometers have been created by individual research groups. These innovative

set ups involve PDMs disc set up on a modified texture analyser (Sarkar and Krop, 2019) as well as the use of a pigs tongue with an optical tribometer cell (Dresselhuis et al., 2008a).

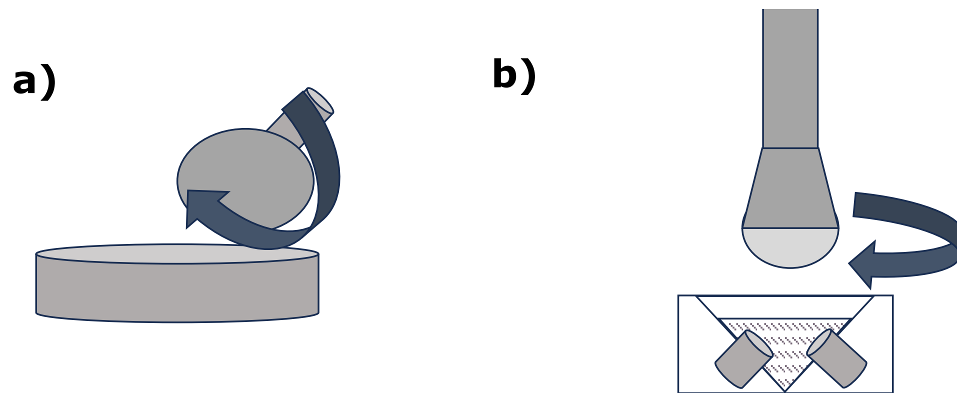


Figure 6. Examples of different tribometer set ups, displayed are ball and disc (a) and ball and pins (b).

Expanding this further, recently advancements were made through the use of a three-dimensional biomimetic tongue to understand friction (Andablo-Reyes et al.,2020). The tongue was created through moulds of human tongues to create a surface which shares similarities with the human tongue. Similarities include topography, deformability, and wettability- which have been proposed as likely responsible for reducing friction and potential enhancing the perception of a more life-like measurement of lubrication perception. The tongue mould is then mounted onto the surface of a cone and plate rheometer. (Andablo-Reyes et al.,2020). It has subsequently been used in the analysis of whey-protein microgels to provide insights of the friction arising from fluid trapped between papillae as well as the papillae themselves (Soltanahmadi et al.,2022). However, it has not yet been looked at closely with astringency and/or sensory analysis but has the potential to provide useful insights.

Finally, when discussing tribology, the influence of saliva should be noted. Saliva as discussed earlier, is a complex biological material composing of a multilayer

with smaller molecular weight proteins and a layer of larger weight glycoproteins otherwise known as mucins (Shewan et al., 2020). How the salivary film and the load interacts can lead to deformation or disruption of the salivary film, resulting in increased friction coefficient. Examples of deformation can be hydration of salivary films or protein aggregation- both of which may be induced by the presence of food compounds. When load is applied, mechanical deposition of salivary film can occur and friction will increase (Shewan et al., 2020). Therefore, to fully understand oral-tribology the inclusion of saliva is essential (Sarkar and Krop, 2019).

1.3.6. QCM-D

Recent advances in tribology understanding have benefited from adsorption techniques like the quartz crystal microbalance with dissipation (QCM-D). QCM-D is a real-time technique used for studying macromolecular adsorptions. The method can simultaneously detect alterations in resonance frequency and energy dissipation of a quartz crystal as materials adsorb onto it (Dixon, 2008, Zembyla et al., 2021). Further analysis of changes can help determine the mass/thickness of the adsorbed layer as well as the layer's viscoelastic properties. QCM-D is used to determine possible causes of astringency as well as investigate polyphenol and peptide interactions (Pires et al., 2020). The quartz crystals have also been coated with PDMS, allowing for comparison with tribology measurements also using PDMS and providing insights into lubricative ability of the film (Dixon, 2008, Zembyla et al., 2021). It is also used in conjunction with saliva to provide further insights into characteristics behind how a film may develop. This is particularly relevant for the boundary regime friction, where thin-film properties and surface asperities dominate friction measurements (Zembyla et al., 2021). Previous research has shown correlations between adsorbed mass and boundary friction (Stokes et al., 2011). However, fundamental differences in measurement conditions can affect this relationship. QCM-D uses very low flow

rates of materials whereas tribology involves a loading force which can disrupt the adsorbed film (Wang et al., 2021c).

1.3.7. Sensory

Texture of food products are often characterised by three different types of tests, discrimination tests, acceptance/linking tests, and descriptive analysis. Texture profiling is a classification technique where sensory descriptions are given and then classified into groups according to their common physical meaning (de Lavergne et al., 2017). Examples of physical meaning groups are mechanical, geometrical, or fat/moisture. By using an intensity scale, it allows differentiation between different samples.

Descriptive sensory, such as qualitative descriptive analysis (QDA) uses a more thorough method aiming to detect qualitative and quantitative sensory analysis using a trained panel (Pires et al., 2020). The method involves three phases, the first is selection of a descriptive analysis panel, the second is the panellists are trained and finally samples are evaluated. The selection of panellists aims to ensure they are suitable and will assess samples accurately with rigour. Training is given to set a common language and scale amongst panellists to try minimize between subject variability (Pires et al., 2020). During this stage a standardized set of sensory attributes are developed (de Lavergne et al., 2015). Advantages of the QDA include its repeatability due to extensive training as well as reduction of hedonic judgements (Pires et al., 2020, de Lavergne et al., 2015)

As oral processing is a dynamic process, numerous methods have been proposed, which try to quantify sensory response dynamically (Cosson et al., 2020). Time-Intensity (TI) sensory evaluation is a method which incorporates temporal factors into perceived sensations using a traditional scaling method (Pires et al., 2020). This allows insight into when sensations occur and fade away

(Pires et al., 2020, Lee and Vickers, 2010). Time intensity methods are particularly important for sensations like astringency and bitterness which often have incrementally increasing intensity which can be enhanced through repeated exposure (Cosson et al., 2020, Vidal et al., 2016). However, it is suggested that time intensity lacks sensitivity. Measuring astringency alone may not fully capture all sensations which are experienced simultaneously (Vidal et al., 2016).

A technique which allows for multiple sensations is Temporal Dominance of Sensations (TDS). This is a relatively new sensory method and considers the multidimensional aspect of sensory perception through analysis of the dominant attribute over time (Pineau et al., 2009, Schlich, 2017). In addition, the approach involves developing a list of attributes, which compared to QDA is typically a lot smaller (de Lavergne et al., 2017). The list of attributes is presented to assessors, who are asked to perceive as dominant at each moment of evaluation, therefore capturing different attributes intensities at a given time (Vidal et al., 2016). The sensation which captures the most attention at a certain time is described as the dominant sensation (de Lavergne et al., 2017). TDS is used to study astringency in wine (Vidal et al., 2016, Medel-Marabolí et al., 2017). The authors suggested TDS as an appropriate technique due to the added dynamic testing when astringency is thought to occur dynamically, as an after feel; therefore TDS may be particularly relevant. Limited studies appear to have used TDS with protein astringency in beverages, although it is becoming increasingly popular. One study did look at pea protein isolates and through TDS different mouthfeel and texture attributes were analysed dynamically, with off tastes such as beaniness and bitterness decreasing as eating progressed. Whereas fattiness typically build up as eating progresses (Cosson et al., 2020).

Similarly, another technique which has recently been developed is the ‘temporal check-all-that-apply (TCTA). This method involves panellists selecting attributes they consider appropriate to describe their oral sensation at a specific time. To

account for temporal changes, the questionnaires are provided at different stages of mastication (de Lavergne et al., 2017).

To effectively measure astringency through sensory panels it is suggested that the panel should be trained (Carter et al., 2020). Trained panels are preferential as untrained may not to be able to accurately and sensitively detect subtle changes. This sensitivity is important to enable the panel to accurately detect astringency from other tastes such as bitter. Training should provide reference solutions with a scale to allow participants to differentiate between samples (Carter et al., 2020). Further, as mentioned previously, saliva flow is thought to be indicative of clearance of astringent compounds, whereby after exposure it increases. Therefore, when analysing astringency through sensory methods, sufficient oral clearance time between measurements is essential to allow for clearance of the astringent compound and/or returning of neutral pH (Carter et al., 2020).

1.4. Rational behind proteins chosen

1.4.1. Whey Protein

Whey protein isolate (WPI) was selected due to its well-established research history at lower concentrations (Brown et al., 2021). Consequently, a substantial body of existing knowledge is available for reference, allowing for result validation and consistency checks. WPI served as an effective 'control' against which the less familiar proteins were compared. Whey is produced as a liquid by-product of the dairy industry during the production of coagulated dairy products, such as cheese (de Castro et al., 2017). The solid fraction of this liquid contains lactose, proteins, lipids and minerals. Approximately 20% of protein in bovine milk are whey proteins. Whey protein has many beneficial nutritional advantages including

containing the essential amino acids leucine, valine, isoleucine and cysteine. The main proteins within whey are beta-lactoglobulin, glycomacropeptide, alpha-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase and proteose peptone (de Castro et al., 2017). Whey proteins are globular proteins (Purwanti et al., 2010, Marinova et al., 2009) and can be characterized by the presence of disulphide bridges, tertiary structure and globular molecular configuration (Marinova et al., 2009). Overall, whey protein isolate typically contains at least 90% protein (Sert et al., 2021).

Whey protein has desirable physicochemical characteristics meaning it is incorporated into a wide range of food products. Whey protein is known to have good solubility over a range of pH at around 90%, although decreases at pH5 (Foley and O'Connell, 1990). In addition, whey was shown to have food emulsifying, thickening, gelation, foaming and water binding properties. Its use is extended into new-product development including hydrogels, nanoparticles, and edible films (de Castro et al., 2017).

1.4.2. Sodium Caseinate

Milk proteins can be classified into two groups according to structure, as whey proteins which have globular structures (Marinova et al., 2009), or as caseins which have different structures depending on subtype (Phadungath, 2005). As the biological function of milk is to supply nutrients to offspring, milk proteins form complexes with large amounts of calcium phosphate known as casein micelles (Phadungath, 2005). Within these casein micelles, there are four main types of caseins, α_{s1}-, α_{s2}-, β- and κ-casein (O'Regan and Mulvihill, 2009).

Native casein micelles are commonly processed into either micellar casein or sodium caseinate to be used within the food industry. Micellar casein undergoes less processing than sodium caseinate (NaCas) and is obtained through

separation of milk components. Micellar casein has slow-digesting properties and forms gel-like structures (San Martin-González et al., 2009). Meanwhile sodium caseinate is derived from acidification of casein and then neutralization by NaOH which removes the colloidal calcium phosphate (HadjSadok et al., 2008). This results in increased solubility which was over 90% between pH5-10 in aqueous solutions (Foley and O'Connell, 1990). These improved amphiphilic properties are desirable for emulsions as it means NaCa's can rapidly adsorb to the oil-water interface leading to its wide use as an emulsifier (O'Regan and Mulvihill, 2009, Liao et al., 2022). Specifically, foam stability has been compared to whey protein concentrate, and NaCas was able to form denser and thicker films improving stabilization (Marinova et al., 2009). Therefore, due to improved solubility, sodium caseinate specifically was chosen for investigation within this thesis (**Chapter 3**). Nutritionally, sodium caseinate is shown to have high digestibility, and also high contents of the essential amino acids, Phenylalanine and Tyrosine (Sindayikengera and Xia, 2006).

1.4.3. Soy Protein

Transitioning to plant-based proteins, an area identified in the systematic review as an area which had limited studies and therefore understanding (Brown et al., 2021), led to the selection of two distinct legume proteins. Considering plant proteins, it is important to acknowledge they are usually storage proteins (e.g. globulins) and are typically more flexible than dairy proteins (Day et al., 2022). In addition, plant proteins commonly exhibit lower solubility in comparison to dairy proteins, potentially influencing their capacity to absorb at interfaces (Nishinari et al., 2014).

Soy Protein was chosen due to its popularity as a dairy-protein alternative due to its high nutritional profile and functionality (Sui et al., 2021, Qin et al., 2022). Soy proteins are derived from soybeans (a type of legume) which are historically

prominent in Asia. Following this, soy proteins have seen substantial growth in Western nations, with soy protein having the highest level of industrial production in plant proteins (Qin et al., 2022). This popularity is due to soy proteins functionality, elevated protein content and potential health advantages (Sui et al., 2021, Qin et al., 2022). The functionality properties are critical for food preparation, processing and storage (Sui et al., 2021). Specifically isolated soy proteins have been suggested as most versatile soy proteins as they can emulsify fat and bind to water (Singh et al., 2008). This allows the integration of oil by the soy protein, which can enhance sensory characteristics like moisture while preserving the texture contributed by other components. Isolates are engineered to improve functional performance and, therefore mimic the characteristics of animal-based proteins, with some soy protein isolates having good gel-like consistency whilst others have higher viscosity or creaminess. Therefore, allowing the application of soy protein in a wide range of foods (Singh et al., 2008)

Soy protein isolate is the remainder from the process of extracting oil at low temperatures. Soybean proteins can be divided into either storage globulins or albumins by acidification to pH4.5-4.8. The major soybean storage proteins are present in the acid precipitable fraction. The remaining portion includes minor globulin y-conglycinin, and contaminating proteins, including whey proteins, constituting approximately 9-15.3% of overall soybean protein content (Nishinari et al., 2014). The storage proteins, globulins, are insoluble in water but dissolve in weak solutions of neutral salts. Between pH's of 4 to 5 soy globulins solubility is at its lowest. With heat, globulins further decrease in solubility (Nishinari et al., 2014).

Nutritionally, soy protein is acknowledged as a high-quality protein source which is suitable for individuals of all ages. It has a well-balanced composition of essential amino acids. Despite notably high lysine content, the levels of sulphur-containing amino acids are comparatively low (Millward, 1999). This makes soy protein a valuable addition to foods which do not have certain essential amino

acids, such as rice and wheat, increasing their overall nutritional value (Sui et al., 2021). Soy has subsequently been suggested to play a crucial role in diets for both vegetarian, vegans and those with milk-protein allergies (Sui et al., 2021).

1.4.4. Pea Protein

Pea protein was chosen for investigation in this thesis because it has a high nutritional value, widespread availability, and is known to have good cost-effectiveness (Lam et al., 2018). Pea protein additionally possesses an advantage over soy in terms of allergen concerns (Boye et al., 2010). However previous research has shown that substituting milk with pea protein in a gradual manner adversely affects sensory appeal (Omrani Khiabanian et al., 2020). The exact underlying physical mechanisms behind this phenomenon are still predominantly unexplored and therefore require investigation (Vlădescu et al., 2023).

Depending on pea source, growing conditions, and maturity of harvest, field pea contains between 23.1-30.9% protein (Boye et al., 2010, Lam et al., 2018). Pea proteins, like soy proteins, are primarily composed of the storage proteins, globulins, and albumins. Albumins constitute approximately 10-20% of total protein content in seed, whereas globulins constitute approximately 70-80% (Lam et al., 2018).

Pea protein is linked to promising functional properties meaning it is popular with food manufacturers. These include gelation, which is often used in production of dairy substitute beverages, emulsification and foaming (Shanthakumar et al., 2022). Specifically, heat-treated, pea proteins have been reported to form weaker and less elastic gels compared to soy protein (Shand et al., 2007). At neutral pH pea protein isolate has demonstrated poor solubility (Taherian et al., 2011), which hinders its functional use (Lam and Nickerson, 2013).

From a nutritional perspective, pea-derived products have been shown to have elevated levels of the essential amino acids arginine, valine, and methionine, while showing lower concentrations of the non-essential amino acids glutamic acid and cysteine, this is in contrast to soybeans (Dahl et al., 2012). Peas are also known to be rich in lysine but falls within the marginally sufficient to deficient range for methionine. The *in vitro* digestibility of raw protein is hindered by the presence of protease inhibitors, such as Bowman-Birk Inhibitor (BBI). BBI is naturally found in peas and is shown to impact the activity of proteases including trypsin and chymotrypsin (Dahl et al., 2012). Despite this, previous evidence has suggested pea protein has greater digestibility than soybeans and various other legume proteins, due to high lysine content and reduced oligosaccharides (Boye et al., 2010).

1.4.5. Skimmed milk powder

Skimmed milk powder (SMP) was used in later chapters as a control. SMP is created by converting milk to milk powder. Unlike whole milk powder, SMP is made from skimmed milk from which the fat is removed. The separation of fat from milk is commonly achieved through centrifugation. Dairy powders have advantages in terms of stability and convenience as well as for handling, processing and product formulations (Sharma et al., 2012). From a nutritional perspective SMP is commonly chosen for its minimal fat content compared to whole milk powder (0.8g/100g) content. Its protein content is around 36g/100g, with the remainder comprising of carbohydrates such as lactose, vitamins and minerals. In addition, as it is derived from milk it contains all essential amino acids (Sert et al., 2021).

The specific functional properties of SMP include emulsifying properties, low water activity, stickiness, ease of rehydration, foaming and flow-ability. Therefore, it is no-surprise that SMP is widely used in formulated foods. In addition, SMP can be tailored to fulfil specific functional requirements. For example, it can be heat-stabilized to endure processes like sterilization or high heat treatments, particularly useful for foods intended to be heated to high temperatures and consumed. (Sharma et al., 2012).

1.4.6. Rationale for different systems chosen

Proteins were analysed in bulk dispersions first to serve as a baseline for the subsequent research. This approach allows for a controlled and simplified environment where the influence of other ingredients and processing is minimized. This enabled a focus on the direct impact of proteins on mouthfeel perceptions.

Complexity of the systems is gradually increased, with proteins next looked at in emulsions. Emulsions were chosen as they are more representative of a model food system as they mimic composition of many food products with many common foods existing as emulsions. In addition, within the food sector there has been a shift in consumer preference towards clean-label, sustainable products which has prompted changes in formulation demands (Kim et al., 2020, Zhang et al., 2022). The integration of natural biopolymers, such as proteins, into emulsions has gained significant traction among researchers, aligning the need for versatile formulations to meet evolving consumer expectations (Zhang et al., 2022). The animal-based proteins whey and casein, are the most commonly used natural emulsifiers in foods such as ice cream, butter and cheese (Kim et al., 2020). However, the demand for plant-based proteins as emulsifiers is on the rise

(Zhang et al., 2022). Therefore, understanding how plant-based proteins act as emulsifiers compared to dairy proteins is of current interest and importance.

A confectionary filling is adopted as the model food system as they have relatively simple formulations, making them suitable for studying the fundamental interactions and behaviours of ingredients in a controlled environment. As the fillings had a low amount of ingredients (6), which allowed the effect of adding protein to be observed more clearly. Additionally, they were created without using sophisticated processing, again allowing effects to be observed more clearly without the complexity introduced by extensive processing steps. In addition, using a recognizable product enhances the relatability of findings and can also impact sensory ratings. Research has demonstrated that when dealing with unfamiliar food/beverages, even trained panels can misperceive attributes as more or less intense than they actually are (Kim et al., 2020). Therefore, the choice of a familiar product for our study ensures accurate perceptions.

1.5. Structure of thesis

This thesis starts with a systematic review of protein-saliva interactions and continues with experimental studies on protein in different systems in relation to mouthfeel.

Chapter 2: The second chapter provides the context for this thesis by presenting a systematic review (SR) of the literature. The aim of this chapter is to set the scene for subsequent research by identifying gaps in knowledge for the thesis to target, avoid redundancy ensuring investigation was novel, and to critically evaluate methods and quality of previous research to refine the methodological approach. The content of this chapter is published in the peer-reviewed journal *Food & Function.*

Chapter 3: Chapter three selected four proteins (two dairy and two legume) based on SR findings. The proteins were characterised in detail using different instrumental methods. Proteins were dispersed in simple aqueous solutions at high concentration (10-20wt%) and mixed with model saliva. This chapter aimed to fundamentally understand the protein properties which drive lubrication with and without mucin.

Chapter 4: In this chapter three proteins from Chapter 3 were selected, in addition to a control (Skimmed Milk Powder) to be used in model food systems. Protein was added to the dispersed phase of oil-in-water emulsions. The effects of type of protein on emulsion performance and material property in relation to mouthfeel were analysed.

Chapter 5: This chapter aimed to bring different systems together and combined instrumental and sensory analysis. The same proteins (and control) used in

Chapter 4 were integrated into a real-food system. The complexity of the system was further increased with added ingredients and processing steps. To compare, additional bulk solutions containing just the protein in aqueous solutions were looked at. Material properties of bulk solutions and food systems were analysed in conjunction with sensory analysis.

Chapter 6: Finally, the conclusions of this research are summarized in Chapter 6. As protein type and concentration was consistent between Chapter 4 and 5, the varying protein mediums from aqueous solution, emulsion to food matrix could be compared. Limitations of the studies as well as suggestions for further work are discussed in this chapter. Importantly, a summary of the overall findings and their implications for food manufacturing are given.

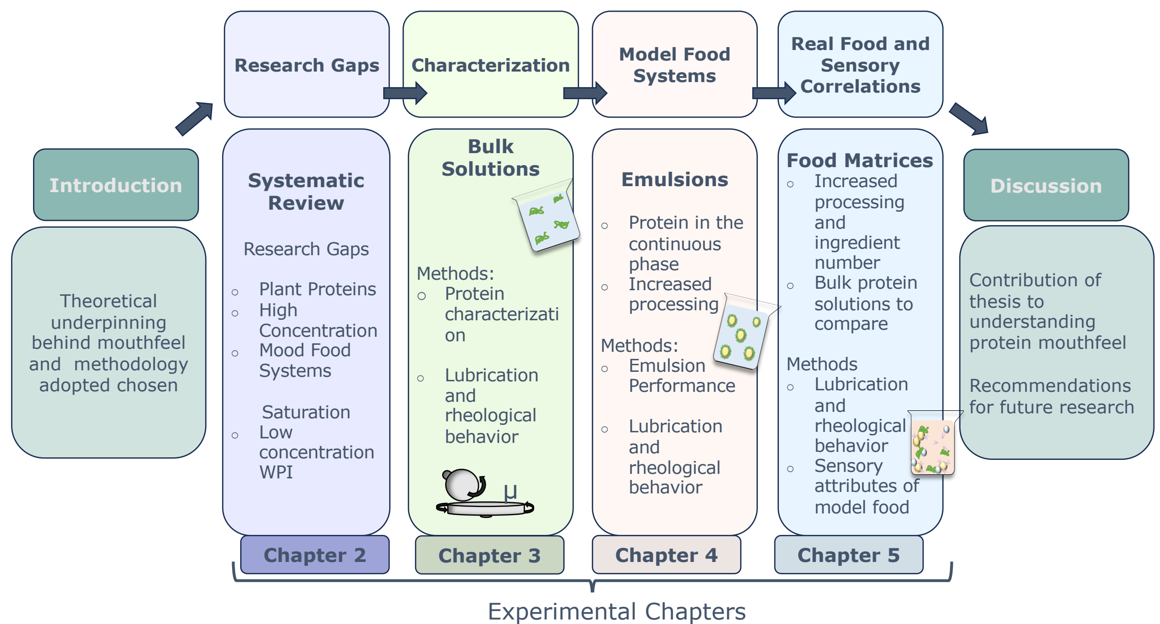


Figure 7. Schematic overview of the experimental approach employed in this thesis and the associated research chapters.

Chapter 2: Protein-saliva interactions: a systematic review

Abstract

Food industries are challenged to reformulate foods and beverages with higher protein contents to lower fat and sugar content. However, increasing protein concentration can reduce sensory acceptability due to astringency perception. Since the properties of food-saliva mixtures govern mouthfeel perception, understanding how saliva and protein interact is key to guide development of future protein-rich reformulations with optimal sensory attributes. Hence, this systematic review investigated protein-saliva interaction using both model and real human saliva, including a quality assessment. A literature search of five databases (Medline, Pubmed, Embase, Scopus and Web of Science) was undertaken covering the last 20 years, yielding 36,604 articles. Using pre-defined criteria, this was reduced to a set of 33 articles with bulk protein solutions (n=17), protein-stabilized emulsions (n=13) and protein-rich food systems (n=4). Interaction of dairy proteins, lysozyme and gelatine with model or human saliva dominated the literature. The pH was shown to have a strong effect on electrostatic interaction of proteins with negatively-charged salivary mucins, with greater interactions occurring below the isoelectric point of proteins. The effect of protein concentration was unclear due to the limited range of concentrations being studied. Most studies employed a 1:1 w/w protein: saliva ratio, which is not representative of true oral conditions. The interaction between protein and saliva appears to affect mouthfeel through aggregation and increased friction. The searches identified a gap in research on plant proteins. Accurate simulation of *in vivo* oral conditions should clarify understanding of protein-saliva interaction and its influence on sensory perception.

2.1. Introduction

The mouthfeel and subsequent sensory perception a food evokes undoubtedly govern consumer acceptance and prospective consumption (Silletti et al., 2007b). Food industries are under increasing pressure to reformulate foods and beverages to reduce fat and sugar while still maintaining desirable mouthfeel in order to address pressing global obesity challenges. However, both changes in formulations to reduce fat have been shown to result in reduced acceptability in texture and mouthfeel, which affects overall palatability (Stokes et al., 2013, Sarkar and Krop, 2019, Pradal and Stokes, 2016). For example, in ice-cream, when 6% fat was replaced with whey protein, there is a reduction in sensory scores for both smoothness and overall acceptability as compared to the full-fat counterpart (Yilsay et al., 2006). Protein is commonly used to modify texture and replace fat or used as bulking agent to replace sugar; but often with undesirable textural changes such as grittiness and chalkiness (Joyner Melito et al., 2014). Thus, understanding the physical mechanism behind mouthfeel is of paramount importance when re-designing food formulation with proteins.

Although rather underestimated, a critical component of mouthfeel results from the interaction of food components with saliva. For the purpose of this review, mouthfeel includes sensory perception and after feel. Saliva is an inherent bio-lubricant, that coats all surfaces within the mouth and therefore it is implicated in all stages of food processing (Carpenter, 2012, Schipper et al., 2007, Mosca and Chen, 2017). Saliva is primarily responsible for providing lubrication in the mouth preventing wear and also interacts with food and beverages. These interactions have previously been shown to impact mouthfeel. For example, the astringency in tea and wine have often been linked to the interaction of polyphenols (a key component in tea and wine) with salivary proline-rich proteins (PRP's) as well as salivary mucins (Gibbins and Carpenter, 2013, Laguna Cruañes and Sarkar, 2017, Upadhyay et al., 2016). Although some dietary protein alone is shown to elicit astringency, the mechanisms behind such astringent perception are not so

well understood (Carpenter, 2012). Therefore, a mechanistic understanding of the interaction of saliva with dietary proteins to understand those perception is important for reformulating food with higher protein content, this has received rare attention in literature to date.

Unstimulated whole human saliva is known for its high stretchability- a property aiding lubrication, coating and food bolus formation, subsequently enabling swallowing (Mao et al., 2013). Saliva wets and helps to cluster food particles and limits the friction between oral surfaces (Mosca and Chen, 2017, Xu et al., 2020a). It is a non-Newtonian fluid and that exhibits a shear thinning behaviour (Sarkar et al., 2019b). Saliva is a complex mixture, composed of predominantly water (99.5%) in addition to various proteins (0.3%), inorganic ions and trace substances (0.2%) (Schipper et al., 2007). It is the protein and ionic components of saliva which distinguish its properties from water (Carpenter, 2012). For instance, the proteins contained within the saliva are suggested as responsible for saliva's lubricating qualities. In particular, mucins (MUC5B), statherin, Proline-rich glycoproteins, acidic protein-rich proteins and positively-charged proteins such as lactoferrin have been suggested to have primary roles in the lubricating performance of saliva (Schipper et al., 2007, Hahn Berg et al., 2004, Xu et al., 2020b, Yakubov et al., 2015).

Specifically, self-assembly of high molecular weight, negatively-charged mucins together with small molecular weight positively-charged proteins such as lactoferrin is recently proposed to be the main cause of salivary lubrication. In this case, mucin aids in viscous lubrication and the lactoferrin aids in boundary lubrication (Xu et al., 2019). During oral processing saliva mixes with food to form a bolus. This bolus is formed to increase the ease of swallowing (Wada et al., 2017). The subsequent perception of foods or beverages texture will depend on the transforming status of food-salivary film coating (Stokes et al., 2013, Sarkar et al., 2019b, Chen and Stokes, 2012) from a 'rheology-dominant' to a 'tribology-dominant' phase. It is postulated that rheology attributes which are based on how material flows and if/how it responds to stress initially dominate mouthfeel

characteristics such as sensory thickness. However, as mastication and oral processing proceeds, tribological properties tend to dominate mouthfeel (Çelebioğlu et al., 2019, Pradal and Stokes, 2016, Krop et al., 2019, Sarkar and Krop, 2019). Tribology is the study of friction and lubrication for interacting surfaces in relative motion. Therefore, within oral processing, it elucidates how the tongue and palate interact with food and saliva coating the oral surfaces.

Although there is extensive research on salivary interactions with food, a detailed review of how saliva interacts with dietary proteins is a necessary undertaking. Therefore, this review aims to combine current fundamental understanding of protein-saliva interaction in order to aid the increasing demand for the design of high protein formulations with pleasurable mouthfeel.

To the best of our knowledge, there is no systematic review of protein-saliva interactions, although a narrative review exists (Çelebioğlu et al., 2019). Systematic reviews originate from the field of medical science, where they were created to help refine the mass of research being produced in quick succession with often contradictory findings. They have now become a well-established high-quality method for assessing research and uncovering gaps in the literature and are used in a variety of fields including nursing, crime, transport, policy and social research (Tranfield et al., 2003). Systematic reviews are beginning to become popular within food science and have been conducted on a range of aspects, for example, the impact of food structure on appetite and satiety (Stribiţcaia et al., 2020), consumer acceptance of reformulated products (Jaenke et al., 2017), as well as tribology-sensory relationship (Sarkar and Krop, 2019). The prior research used to inform a non-systematic review may be random, therefore, is at risk of selection bias with important articles omitted. Whereas systematic reviews use a developed search strategy which is stated to allow readers to replicate the search or evaluate and judge the search approach with greater transparency. Additionally, systematic reviews search a number of sources aiming to collate all of the currently available and relevant evidence. Grey literature areas such as reference lists may additionally be searched to increase rigour. For data analysis,

systematic reviews utilise a precise method to appraise and summarize findings in addition to assessment of the quality of included research. By doing so, it provides a clearer synthesis of evidence and can indicate the strength and accuracy of the present research. Systematic reviews are particularly beneficial for identifying research gaps as well as areas of saturation, which do not require further investigation. Moreover, methodology can be critical to highlighting concerns and providing recommendations for methodological development. Although an elegant narrative review exists (Çelebioğlu et al., 2019) on protein-saliva interactions and summarizes relevant electrostatic, hydrophobic interactions and hydrogen bonding between some dietary proteins and salivary proteins, using a systematic approach may yield a more critical overview of the field. In addition, a systematic review would help to understand the type of experimental techniques and conditions used to report those interactions. Consequently, with this *first* systematic review on protein-saliva interactions, we aim to examine the key interactions between saliva and salivary components with food proteins focussing on protein type, protein concentration, pH, processing of protein, saliva type and saliva-protein ratios to inspire future research in this field. To examine the field effectively, proteins as bulk solution as well as protein in lipid emulsions and food systems are covered.

2.2. Methods

2.2.1. Study identification

The systematic review aimed to summarise and synthesize evidence on saliva and protein interactions. The search strategy used synonyms of saliva as well as various salivary components, including mucins from bovine and porcine sources. In addition, protein as well as different types of proteins were added. Thirdly, terms used in relation to mouthfeel or instrumental characteristics with commonly

used techniques for analyses of protein-saliva interactions was included. Although instrumental characterization such as rheology and tribology may not measure mouthfeel, they can give indirect indications about mouthfeel, and thus were included (Sarkar et al., 2019a, Upadhyay et al., 2016).

The search terms are included below:
(saliva* OR amylase OR bovine-submaxillary OR BSM OR parotid OR porcine gastric OR proline-rich-protein* OR PRP OR PGM OR proline OR statherin OR stimulated OR unstimulated OR MUC5B OR MUC7) AND (protein OR casein* OR gelatin OR lactoferrin OR lupin OR pea OR potato OR soy OR whey OR dairy OR food OR gluten OR lysozyme OR milk OR plant OR protein OR skimmed-milk) AND (astringen* OR boli OR dry* OR friction OR lubric* OR mouth* OR mouthfeel OR oral processing OR perception OR SDS-Page OR sensor* OR sensory analysis OR surface* OR tribol* OR turbidity OR rheol*) AND (interact*).

The literature searching was an iterative process with search terms modified based on the search results. The developed search strategy was tested by checking if key studies identified in a previous review came up. In addition, the titles were screened to identify any new search terms. Based on this, the search terms ‘milk’ and ‘skimmed-milk’ were added. Additionally, as the initial search yielded an extremely large number of results (3,000,000+), interact* was included to reduce the breadth of the results based on the literature search. The following four databases were searched; Medline or PubMed, Embase, Scopus and Web of Science. In adherence to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, hand searches of reference lists in articles included for full text screening were undertaken.

2.2.2. Study selection

Articles were eligible if they were published in the last 20 years (between 2000 and 10.07.2020). As understanding mouthfeel is arguably a new area of development, with methodologies such as tribology and surface analysis only just emerging, the last 20 years was chosen. In addition, searching more recent literature is more likely to reflect consumer dietary preferences and habits which have evolved over time. Articles were only included if they were published in English. The first author (FB) performed the screening of potentially relevant studies based on title and abstract. Articles were independently checked by co-author (AS). Following the screening, full-text papers were evaluated using defined selection criteria by the first author and checked independently again by co-author (AS). Uncertainties regarding inclusion and exclusion were resolved involving discussion with another co-author (AM).

For paper inclusion, the following criteria were chosen based on the PICO (Population, Intervention, Comparison and Outcome) inclusion criteria. ***Population.*** Only human studies were included. Live animal studies were excluded, only studies using commercially animal saliva were included. For example, studies where protein was fed to animals to understand the influence of oral processing were excluded. However, bovine-submaxillary mucin and porcine gastric mucin studies were included as these are well-established sources of mucin for preparing model saliva (Sarkar et al., 2019b). Furthermore, studies involving unhealthy (with oral or other diseases and conditions), elderly or children participants were excluded. This was because salivary property can be affected by disease, which may also alter how it interacts with dietary protein (Sánchez et al., 2011, Liu and Duan, 2012). Furthermore, only adults from ages (18-64 in accordance with UK Office for National Statistic's age range) with no children or elderly were included, as salivary quantity and quality is shown to change with age (Xu et al., 2019). ***Intervention.*** Only studies in which the specific effect of protein was considered were included. This includes some

studies using bulk solutions which were designed such that the effects of protein could be isolated. Studies with complex designs that do not allow the specific effect of protein to be identified were excluded. ***Comparison.*** If saliva (or related synonym) was not included, then studies were omitted. ***Outcome.*** Articles were excluded if they were published as opinions, reviews, theoretical studies with no measurable outcomes.

The filtering process is shown in the PRISMA diagram in **Figure 8**. Initially, a total number of 36,604 articles were identified using literature by searching the four electronic databases mentioned in the method section.

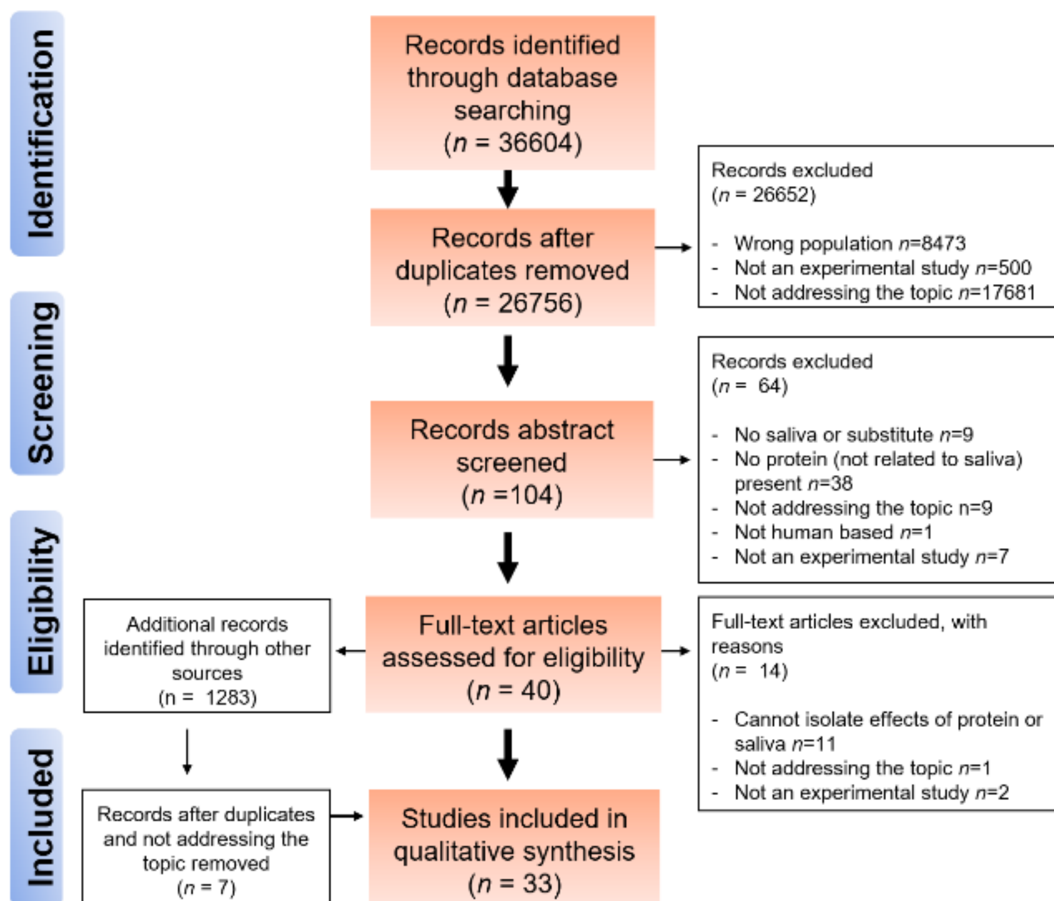


Figure 8. PRISMA flowchart of the study selection procedure.

As can be seen from **Figure 8.**, 26, 652 studies were excluded based on the PICOS criteria. After removing duplicates, articles involving excluded population *i.e.* animal studies, or clinical studies involving patients, older adults and/or children (n=8,472) were excluded. Additionally, articles not addressing the topic of interest were excluded (17,681) or non-experimental studies were excluded (500).

The resulting 104 articles were then taken to the abstract screening stage where abstracts were screened by FB and AS. This resulted in the exclusion of an additional 64 articles (57 articles had no relevance to the topic (s) of the systematic review *i.e.* involving no dietary protein or using saliva, 56 had non-relevant outcome measures, 23 were new or validation of existing protocols, 1 was a non-human study with an additional 7 being non-eligible because of a lack of any original experimental work. Forty full-text articles, including 7 additional articles that were identified through supplementary approaches (*e.g.* manual searches of reference list of pre-screened articles) were screened independently by FB and AS. By mutual agreement, articles with inappropriate interventions and designs (*e.g.* cannot separate based on protein or salivary interaction) further 14 articles were excluded. Finally, 33 articles were included in the qualitative synthesis.

2.2.3. Study characteristics and data extraction

For each study, study characteristics data were extracted (author, year of publication), protein type (concentration and pH), saliva type (model or human, if model saliva: the type of mucin, if human saliva: stimulated or unstimulated, number of human donors), the ratio of saliva: protein mixture, methods used, and the main findings.

2.2.4. Assessment of risk of bias and reporting quality

Despite the method of systematic reviewing being created to assess research quality and reporting of potential bias; for previous systematic reviews that analysed *in vitro* methods were shown to have a lack of quality and risk of bias reporting. For example, a systematic review of *in vitro* studies reporting of quality found only 19 out of 65 systematic reviews included a risk of bias for each individual study and assessed studies quality (Elshafay et al., 2019). A range of tools exist to analyse study quality for systematic reviews for example; Cochrane risk of bias tool for randomised trials (Higgins et al., 2011), Robins-1 tool for non-randomised studies of interventions (Hinneburg, 2017) and the JBI checklist for prevalence studies or the JBI checklist for qualitative research (Joanna Briggs Institute (Porritt et al., 2014). However, at present, there is no standard tool for assessing the quality and risk of bias employing *in vitro* studies (Elshafay et al., 2019, Golbach et al., 2016).

Hence, for the present systematic review, a bias tool was developed based on a tool previously used for calcium homeostasis and low-frequency magnetic and electric field exposure (Golbach et al., 2016). The bias tool assesses reporting quality, performance bias, selection bias and detection bias. Industry funding was not considered here to be as bias as this review is about understanding interactions rather than focusing on any health claims. The tool is shown in **Supplementary Table A.1.** and **Supplementary Table A.2.** is comprised of 15 items, for each of which articles were marked if they reported or not. If the article clearly disclosed the item (yes) 2 points were awarded, if they somewhat disclosed or it was ambiguous/ not directly reported 1 point was awarded, and 0 points were awarded if no attempt was made. As some of the items did not apply to each study (*i.e.* human saliva description when no human saliva was used), the item was not included in overall score for that study. The weighted percentage

total was calculated by equally weighting each score between the reporting quality, performance bias, selection bias and detection bias sections (*i.e.* 4×25%).

2.3. Results

Within the studies published on protein-saliva interaction between 2005 and 2020, 33 studies were identified which met the inclusion/ exclusion criteria **from** a total of 36,604 articles, as shown in **Figure 8.** As shown in the demographics (**Figure 9.**),17 analysed bulk protein solutions, 12 protein-stabilized emulsions, 5 protein-rich food systems with 1 incorporating both emulsion and bulk solution. In addition, a variety of methods were used to analyse different responses to possible interactions as demonstrated in **Figure 9**. The majority of research has focussed on rheology, zeta-potential, turbidity and sensory analysis. More recently tribology has been used (first seen in 2011) specifically for protein-human saliva interaction (Vardhanabhuti et al., 2011). The earliest studies identified in the present search were published in 2005 and used the techniques of particle size (Vingerhoeds et al., 2005) and sensory analysis (Sano et al., 2005). Seven bulk solution studies included links to *in vivo* methods by including sensory analysis, whereas only two emulsion studies included sensory analysis.

Study characteristics with quality assessment scores are shown in **Table 1.** and **Table 2.** involving bulk solution, and emulsions and food systems, respectively. Emulsions are an important system to study as they contribute to a large proportion of food formulations. Understanding how emulsions behave in oral conditions is critical in the manipulation of the physical and sensorial attributes of colloidal systems (Sarkar and Singh, 2012), hence a separate table is allocated to include interaction of protein-stabilized emulsions with saliva along with protein-rich food systems. All studies shown in **Table 1.** and **Table 2.** used animal-based protein, with the majority of studies focusing on dairy proteins. Of these, 18 studies investigated whey protein forms isolate or concentrate (WPI or WPC),12 investigated the whey protein derivative *i.e.* β-lactoglobulin (β-lg), three

investigated lactoferrin, three investigated sodium caseinate, two investigated casein. In addition, three investigated gelatine and six lysozyme (**Figure 9.**). Whey protein and β-lg are known for being globular glycoproteins whereas casein has a random coil structure and had different behaviour in presence of saliva (**Table 1.**). Five out of six studies using lysozyme investigated it in emulsion systems (**Table 2.**), which is a globular positively-charged protein at neutral pH. Gelatine which is a hydrophilic protein, with a high molecular weight is made by the thermal denaturation of collagen and has been used to measure interaction with saliva both in bulk phase as well as in emulsified form (**Table 1.** and **Table 2.**). All five studies using food matrices (**Figure 9.**) in formulating model foods and beverages or yoghurts, investigated whey protein either as WPI or WPC (**Table 2.**). In addition, these studies were more recent, published between 2010 to 2017.

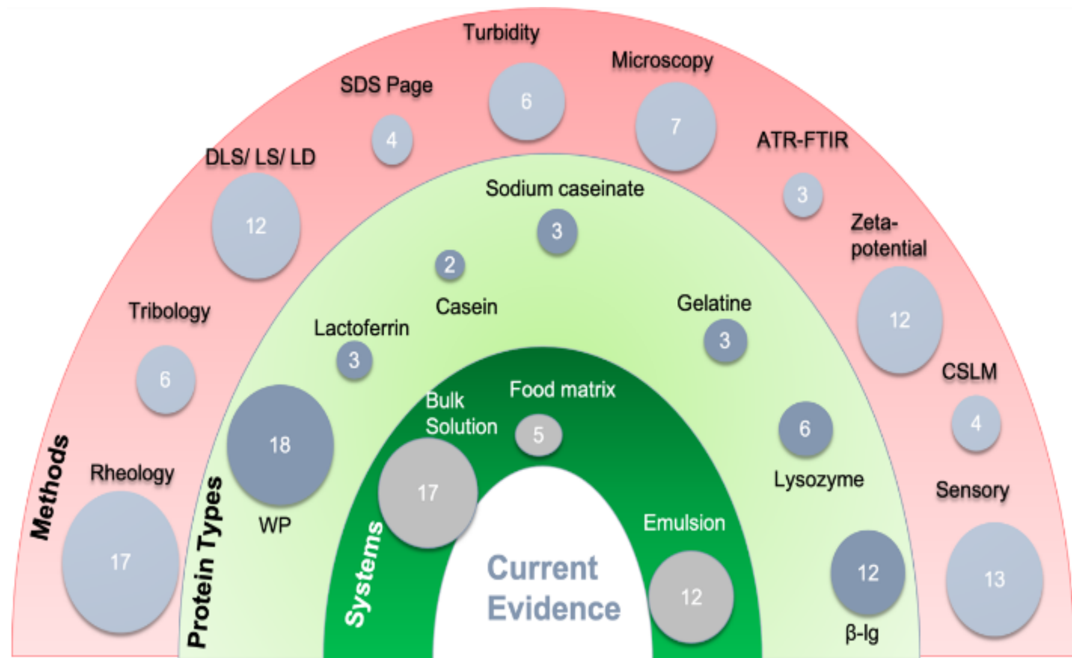


Figure 9. Demographics of study characteristics. Numbers reflect the number of studies using each method, protein type or system. (ATR-FTIR= Attenuated total reflection–Fourier transform infrared Spectroscopy, *β*-lg= beta-lactoglobulin, CLSM= Confocal laser scanning microscopy, LD= laser diffraction, LS= light scattering, SDS PAGE= sodium dodecyl sulphate polyacrylamide gel electrophoresis, WP= whey protein).

Figure 9. Demographics of study characteristics.

The demographics for the type of saliva were similar, with 23 using real human saliva and 16 using model saliva whereas 6 using both types of saliva (**Table 1. and Table 2.**) with limited number of studies using bovine submaxillary mucin (BSM) as the mucin source in case of model saliva. The quantitative assessment of each individual study's bias was conducted (see **Supplementary Table A. 2.**). Collectively the average percentage was 84%, within general reporting quality and performance bias scores the lowest and detection bias the highest (see **Table 1.** and **Table 2.**).

2.4. Discussion

Overall, all proteins identified had some indications of interacting with either model or human saliva. Shifting pH to around the isoelectric point (pI) of proteins indicated most proteins identified electrostatically interacted with mucin. Interactions observed however varied by protein type. Specifically, when casein was analysed in a bulk solution/ aqueous suspension, there was no indication of an interaction with mucin (Withers et al., 2013) (**Table 1.**). However, aggregation was found when casein was analysed in an emulsified system (Vingerhoeds et al., 2005) (**Table 2.**). Despite lysozyme eliciting aggregating in presence of saliva, the parameters driving interactions are not well-established. Lysozyme has only been investigated at a limited range of conditions, although pH dependence of interactions with saliva does suggest electrostatic interactions are involved (Silletti et al., 2007b, Silletti et al., 2010, Vingerhoeds et al., 2009). For whey protein, interactions with saliva (model or real) appeared to be predominately electrostatically driven. However, in different conditions they can be entropically and enthalpically driven too (Ye et al., 2011, Ahmad et al., 2020a, Andrewes et al., 2011, Beecher et al., 2008, Hsein et al., 2015, Kelly et al., 2010, Lee and Vickers, 2008, Sano et al., 2005, Wang et al., 2016) (**Table 1.**). Zeta-potential analysis again indicated that whey protein, *β*-lg, gelatine, and lactoferrin electrostatically interact with mucin (Çelebioğlu et al., 2016, Çelebioğlu et al., 2015, Celebioglu et al., 2017, Vardhanabhuti et al., 2010). However, gelatine and WPI have also been shown to interact with mucin via non-electrostatic mechanisms (**Table 1.**) (Ahmad et al., 2020b). Evidence supported entropically and enthalpically driven aggregation with formation of hydrogen bonds or hydrophobic interactions even at neutral pH where both whey protein, gelatine and mucins are negatively charged (Ahmad et al., 2020a, Ahmad et al., 2020b).

In the following sections, the effects of protein type (at neutral pH), variation of pH, protein concentration, saliva type, protein-saliva mixing ratio and heat treatments of proteins are discussed. Throughout the discussion, the focus was

on the proteins in bulk phase but included examples from emulsions. Examples from emulsions were included when interactions varied from bulk solutions. Interactions are shown schematically in **Figure 10.** and **Figure 11.**

2.4.1. Protein type

WPI. At neutral pH, limited changes have been observed for WPI and mucin mixtures (**Table 1.**). The zeta-potential of WPI, mucin (PGM) and WPI + mucin (1:1 w/w) was -38 mV, -15 mV and -28 mV, respectively (Ahmad et al., 2020a). The authors theorized this happens because mucin contains positively charged patches despite its negative charge, which attract to WPI's negatively charged acidic amino acids at neutral pH (Ahmad et al., 2020a). However, this is unlikely as the negative charge of WPI is so high (-38mV). Temperature-dependent fluorescence spectroscopy and the Benesi-Hildebrand equation used to assess the thermodynamic stability of interactions revealed that WPI-mucin interactions and phase separation at pH7.0 could not be explained by electrostatics. In fact, both endothermic with spontaneous binding and hydrophobic association appeared to influence interactions with mucin (Ahmad et al., 2020a). Hydrophobic interactions with non-glycated terminal peptide regions also cannot be ignored (Ahmad et al., 2020a) (**Table 1.**), which is likely if local charge repulsion is low. At neutral pH there was an increase in the viscosity of the whey protein-saliva mixture. This was hypothesized to originate from increased energy dissipation due to phase separation of WPI+mucin colloidal particles increasing the viscosity (Ahmad et al., 2020a). However, in whey protein-stabilised emulsions (**Table 2.**) viscosity was only minimally affected by the addition of human saliva at near-neutral pH (6.7) (Vingerhoeds et al., 2009). Equivocal results were found for turbidity, with only small changes reported by one study for WPI-stabilized emulsion + mucin interaction at neutral pH (Beecher et al., 2008) versus no changes in another study (Hsein et al., 2015). Tongue retention analysis found

β-lg, the main protein in WPI, retained on the tongue after oral processing of WPI emulsions (Vingerhoeds et al., 2009). Separately, at pH6.7 when parotid saliva containing no mucin was used, WPI reversibly aggregated highlighting the importance of non-mucinous salivary proteins in such aggregation (Vingerhoeds et al., 2005).

***β*-Lg.** As previously mentioned β-lg is the main fraction of WPI and it is therefore unsurprising results were similar to WPI when interacting with saliva. No model salivary interaction could be detected using nuclear magnetic resonance (NMR) at neutral pH (Çelebioğlu et al., 2015) (**Table 1.**). As previously discussed with WPI, this is likely due to repulsion between β-lg and mucin due both having net negative charges (Ye et al., 2011), (Celebioglu et al., 2017). Further, frictional behaviour was dominated by salivary proteins (bovine submaxillary mucin, BSM) (Çelebioğlu et al., 2016) rather than by β-lg. With regards to emulsions, at neutral pH, β-lg showed reversible flocculation with model (PGM) and human saliva (Silletti et al., 2007b). (Sarkar et al., 2009) **(Figure 10.** and **Table 2.**). Nevertheless, when parotid saliva was used β-lg aggregated, although it was again completely reversible at pH 6.7 (Vingerhoeds et al., 2005). Further rheological analysis showed limited changes in viscosity at this neutral pH (Silletti et al., 2008) in presence of unstimulated saliva, which has lower mucin concentration. This indicates mucin was primarily responsible for observed interactions with β-lg at neutral pH (Sarkar et al., 2009).

Lactoferrin. Interestingly, results for lactoferrin were less clear compared to β-lg and WPI. For example, bulk solution results were not definitive. When combined with unstimulated human saliva at pH6.8 in a 1:1 w/w mixing ratio, zeta-potential values and particle size were almost identical to lactoferrin alone (**Table 1.**). SDS-PAGE further showed mixtures of heated lactoferrin, and human saliva were found to be predominantly lactoferrin (Ye et al., 2011). Of the two studies including sensory analyses, one reported little or no astringency, whereas the other showed intense astringency in lactoferrin solutions, although precipitation in mixtures of lactoferrin and saliva was limited (Vardhanabhuti et al., 2010). This

may suggest that electrostatic interactions between lactoferrin and human saliva may not be sole factor governing astringency of lactoferrin. On the other hand, when lactoferrin was analysed in emulsions (pH 6.8 using model PGM saliva, 1%w/w lactoferrin) there are clear signs of interactions with pronounced bridging flocculation (**Table 2.**) (Sarkar et al., 2009). Since lactoferrin has an isoelectric point of around 8.5, the attractive interaction between lactoferrin-stabilized droplets and anionic mucins as schematically shown in **Figure 10.a.** led to aggregation. The zeta-potential went from +27mV to -27mV, when the mucin concentration was raised from 0.1 to 1.5 wt%. Zeta-potential measurements were screened when salivary salts (no mucin) were combined with emulsions (Ye et al., 2011). This indicates that besides electrostatic binding with mucin, charge screening effects by salts present in model saliva also caused aggregation in lactoferrin stabilized emulsion droplets **Figure 10.b.** In addition, mucin coverage was greater in lactoferrin stabilized emulsion (compared to β-lg) which was further hypothesized to be because of electrostatic interaction (Sarkar et al., 2009).

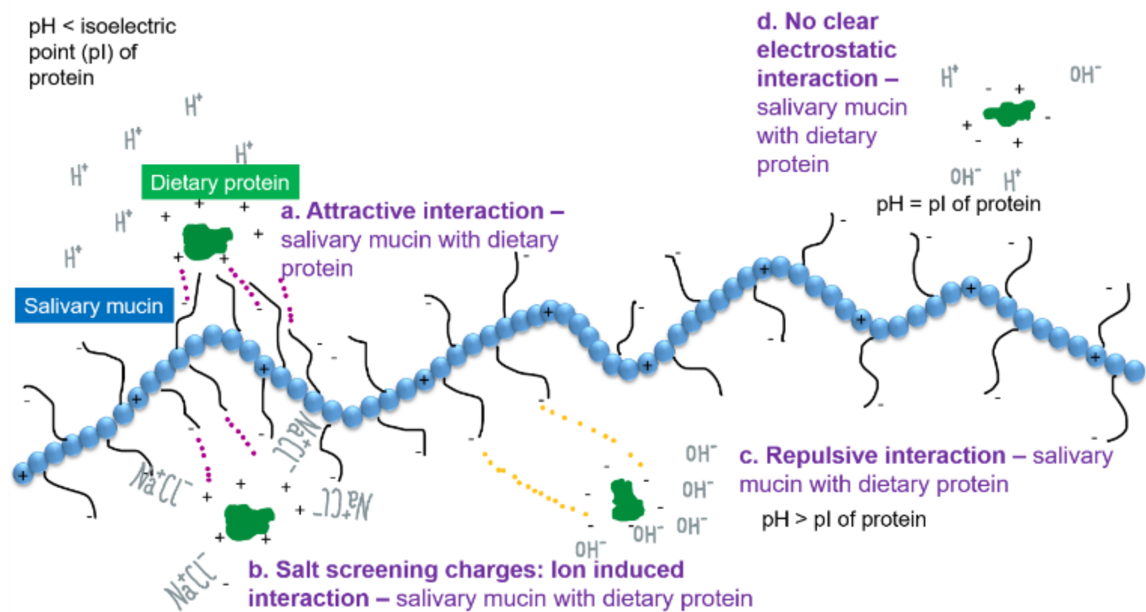


Figure 10. Schematic illustration of plausible mechanisms of interaction between mucin or salivary salts and dietary proteins. a) electrostatic attraction b) salt-induced aggregation c) electrostatic repulsion, and d) non-electrostatic interactions.

Caseins/ sodium caseinate. At neutral pH both sodium caseinate and PGM repelled each other as shown through low absorbance using light microscopy (Ritzoulis et al., 2012) **(Table 1**). In an emulsion format, sodium caseinate was found to have a highly negative zeta-potential which was reduced when combined with model saliva containing PGM (Koukoura et al., 2019) (**Table 2**). However, this article did not report at which pH the study was conducted and it is only assumed it occurred at neutral pH. Moreover, no flocculation or change in droplet size were seen in the presence of model saliva (PGM). Another study showed flocculation started to decrease at neutral pH with reduced droplet size, again with model saliva (PGM) (Ritzoulis et al., 2012). This is expected as the charge of both sodium caseinate and mucins were negative which increases repulsion and thus stability (**Figure 10.c.**). Interestingly, when using β-casein-stabilized

emulsions, no aggregation was found at pH6.7 when mixed with parotid saliva (not containing mucin) (Vingerhoeds et al., 2005). Further droplet size measured by laser diffraction appeared unchanged by the presence of whole saliva as well as parotid saliva. Similarly, no interaction was reported for bulk solution analysis between β-casein and mucin at pH7.4(Withers et al., 2013) most likely due to the repulsive interactions (**Figure 10.c.**).

Gelatine. Moving to non-dairy proteins, at neutral pH, phase separation of colloidal particles and aggregation was observed between gelatine and model saliva (PGM) (Ahmad et al., 2020b) (**Table 1.**). Interactions between gelatine and model saliva do not appear to be electrostatically driven at neutral pH. At neutral pH, both gelatin and mucin, the main component of model saliva, carry strong negative charges, therefore it is unlikely any electrostatic interaction would have taken place due to the obvious repulsive forces. Alternatively, it was postulated that interactions at neutral pH between gelatin and mucin (PGM) were caused by hydrogen bonds and other polar attractions, and/or induced dipole (hydrophobic) interactions (**Figure 11.b.**). This conclusion was derived from assessing the thermodynamic stability of interactions by using the Benesi-Hildebrand method. This showed mucin and gelatin binding was stronger at pH7.0 than pH3.0, with direct interactions occurring between gelatin and mucin. Collectively, although an increase in aggregation is observed at pH7.0, it is unknown if this translates into mouthfeel differences due to lack of evidence (Ahmad et al., 2020b).

Lysozyme. The majority of studies which investigated lysozyme investigated it only in relation to neutral pH which is below its isoelectric point (pI>10) (**Figure 10.a.**). At neutral pH lysozyme appeared to flocculate with human saliva (Silletti et al., 2007a, Silletti et al., 2008, Silletti et al., 2007b, Silletti et al., 2010) (**Table 1.**). SDS-PAGE analysis showed the lysozyme stabilised emulsion upon mixing with saliva had a lysozyme band and two mucin bands (MUC5B and MUC7) which authors proposed indicated interactions took place (Silletti et al., 2010). Separately, flocculation was shown to be reversible under dilution and shear which is indicative of weak interactions (Silletti et al., 2007a, Silletti et al., 2008)

(**Table 2.**). In addition, tongue retention analysis found lysozyme-stabilized emulsions to be retained on the tongue with less clearance compared to whey protein-stabilized emulsions. Further sensory analysis showed lysozyme was associated with dryness, roughness, astringency and raw tongue (Vingerhoeds et al., 2009). However, the majority of lysozyme studies were emulsion-based. Lysozyme was only investigated in a single bulk solution study with no studies using food systems. Overall results showed that lysozyme interacting with saliva most likely produces astringency (Biegler et al., 2016).

It is noteworthy that there has not been a single study performed to investigate the interaction of plant protein with saliva within the search date of this systematic review. A recent study on the interaction of pea proteins with BSM (published outside the inclusion dates) (Zembyla et al., 2021) shows that the adsorption capacity of pea protein to a hydrophobic surface is reduced in the presence of BSM due to electrostatic repulsion between pea protein and BSM. Nevertheless, the extent and kinetics of adsorption of pea protein has been found to be significantly higher than WPI on BSM-coated surfaces. This suggests pea protein might give rise to astringency perception due to more binding to BSM-coated surface compared to that of WPI, however no sensory evaluation was conducted in this study (Zembyla et al., 2021). Thus, understanding the interaction of pea protein with saliva and salivary proteins seems to be a key knowledge gap. Particularly in view of the growing interest in sustainability and designing plant-based food formulations.

2.4.2 pH

In the following section the effect of changing pH is discussed. Since electrostatics appears to be the key mechanism driving protein-saliva interaction as schematically shown in **Figure 10**. pH is an important factor that determines the attractive or repulsive nature of such interactions in presence of real or model saliva. The isoelectric points (pI) of proteins is referred to in **Table 1.** and **Table**

2. When the pH, was around the pI of whey proteins (**Table 1.**), turbidity increased (pH4.6-5.2) indicating interactions between WPI and human saliva (Kelly et al., 2010). Alternatively, as this is around the isoelectric point of WPI, hydrophobic interactions of WPI self-aggregation may have dominated (**Figure 10.d.**). However, some attractive interactions between negatively-charged mucins and some positively-charged patches of WPI at the pI cannot be neglected (Ahmad et al., 2020a). Regarding emulsion systems when pH was lowered to pH3.0 *i.e.* below the pI of WPI (**Figure 10.a.**), flocculation was no longer reversible. The saliva-induced flocs were also larger and densely packed (**Table 2.**) thus clearly dominated by electrostatics. Moving on, pH was also varied in food matrixes using whey-based yoghurt (Morell et al., 2017, Morell et al., 2015) (**Table 2.**). The pH of the whey-based yoghurts was set between 4.5-4.6 and when model saliva (PGM) was added, friction reduced compared to yoghurt alone (Morell et al., 2015). Importantly, sensory analysis was linked as the whey-based yoghurts were described as 'rough, gritty, and astringent' at these pH values (Morell et al., 2017). Further, a comparison of whey protein to milk-based yoghurts was made. The milk protein yoghurt was rated as creamy and thick whereas whey protein yoghurts were rated as grainy, lumpy and thick (Morell et al., 2015). Similarly, whey-based sports drinks formulated at pH2.6-3.4 were again rated very astringent in sensory analysis (Childs and Drake, 2010). Increased turbidity at pH3.4 was correlated with higher sensory astringency scores (Beecher et al., 2008). Interestingly, another study which also varied pH but also processing method reported no correlation between pH and astringency ratings for WPI beverages. However, whey protein concentrate and whey protein hydrolysate beverages had increased astringency with lower pH (Wang et al., 2016). As a whole these results highlight the importance of electrostatic attraction between positively charged whey protein at low pH (pH< pI) and anionic salivary mucins driving such astringency (**Figure 10.a.**).

Adjusting pH in β-lg, mirrored the results found for WPI which was expected as β-lg is the main component of WPI (Ye et al., 2011) (**Table 1.**). Zeta-potential

analysis indicated β-lg was positively charged (+21mV) at pH4.3 and thus attracted to the negatively-charged saliva and eliciting pronounced flocculation (Silletti et al., 2007b) (**Figure 10.a.**). At pH5.0, β-lg is near the isoelectric point (Kelly et al., 2010, Ye et al., 2011) and electrostatic repulsion will be minimal. Accordingly, at pH5.0, attractive hydrophobic interactions led to β-lg aggregation and network formation (Silletti et al., 2007b) (**Figure 10.d.**), which overshadowed any β-lg-mucin interaction (Celebioglu et al., 2017). Tribology showed that at pH5.0, a reduction in lubrication in β-lg was observed compared to that at pH3.5 and pH7.4 (Çelebioğlu et al., 2016) and an increase in friction (Vardhanabhuti et al., 2011) compared to pH3.5 (**Table 1.**). This is expected as β-lg aggregates were particulate in nature and were incapable of forming a continuous load bearing film at the tribo-contact surface as opposed to β-lg films or β-lg+mucin films. Sensory analysis showed that astringency increased as the pH was lowered, also observed in WPI (Vardhanabhuti et al., 2010, Ye et al., 2011, Çelebioğlu et al., 2015).

The results for lactoferrin do not appear to be affected by pH. As food-relevant pH are below the pI of lactoferrin and thus electrostatic interactions with salivary mucins remain irrespective of pH. Lactoferrin was shown to be astringent in all pH conditions (pH3.5-7.0) and interactions were predominantly electrostatic in origin as discussed previously (Vardhanabhuti et al., 2010). Human saliva combined with lactoferrin between pH2.0 to pH7.0 had a net positive charge which was very similar to lactoferrin alone (Ye et al., 2011) (**Table 1.**). Particle size increased when pH was lowered to 2.0, and between pH3.0 to pH8.3 particle size was small, but no precipitation of lactoferrin + human saliva was found in any pH condition (Ye et al., 2011). Moreover, lactoferrin was investigated in an emulsion system but only at a single pH (pH 6.8) (**Table 2.**), so could not give further indications of the effect of pH (Sarkar et al., 2009).

When sodium caseinate was analysed at a range of pH (1.0-7.0), interactions were again postulated to be electrostatic. This was demonstrated through zeta-potential and microscopy analysis which showed electrostatic interactions at

pH3.0 eliciting bridging flocculation, whereas depletion flocculation was observed at pH5.0 (**Table 2.**). Both pH3.0 and 5.0 led to irreversible flocculation (Ritzoulis et al., 2012). At pH1.0, 75% of PGM was found in the serum with the remaining 25% observed to be bound to the droplet surface, which were hypothesized to be because of interactions with interfacial sodium caseinate (Ritzoulis et al., 2012). In addition, fast flocculation driven creaming occurred at pH3.0 whereas at pH7.0 creaming was limited and emulsions were stable (Ritzoulis et al., 2012) (see **Figure 10.c.**).

With non-dairy proteins, the pH of solutions was shown to also affect gelatine's interaction with PGM (Ahmad et al., 2020b). Viscosity varied with pH, with the Trouton ratio (Tr) (ratio of extensional to shear viscosity) being relatively low at pH 3.0 (Tr= 200 for mucin and gelatine at a 6:4 ratio), and significantly higher at pH7.0 (Tr=1400)(Ahmad et al., 2020b). As Trouton ratio followed the same trend as extensional viscosity it was suggested the importance of binding regimes between gelatine and mucin for the extensional viscosity and hence on Trouton ratio. Fluorescence spectroscopy indicated binding between mucin and gelatine at both pH3.0 and 7.0(Ahmad et al., 2020b). Mucin (PGM) has a small net charge at pH3.0 that was suggested not to be large enough to attract positively-charged gelatine, although electrostatic attraction cannot be ignored (**Figure 11.a.**) (Ahmad et al., 2020b). As mentioned previously, it was postulated that interactions at neutral pH were caused by hydrogen bonds and hydrophobic interactions between gelatine and mucin (**Figure 11.b.**). At pH3.0, the interactions were weak (Ahmad et al., 2020b), and sensory analysis for gelatine at pH3.5 showed no astringency (Sano et al., 2005). When gelatine was investigated in emulsion format despite the study not specifically analysing differences in pH (**Table 2.**), the data at pH5.0 and 7.0 results do not appear to differ significantly. For instance, gelatine had a positive charge at pH5.0 (+10mV) and pH7.0 (+7mV). The oil droplet size was similar 1-2µm and when mixed with human saliva increased to over 25µm. Consistency *indicates the viscosity of a fluid. When K is below 1 the fluid tends to be shear thinning, and above 1 shear thickening. Consistency* increased in both conditions when saliva was added from

0.01 to 0.17K (Pa·s^n) for single droplet pH5.0 vs 0.005 to 0.25Pa.s for pH7.0. Friction in both pH5 and pH7 also decreased slightly in the boundary regime (Fuhrmann et al., 2019). Although the origin of interactions was not investigated, when comparing these emulsion result to results from bulk solution, the lack of difference between pH5.0 and 7.0 in case of gelatine+saliva is surprising.

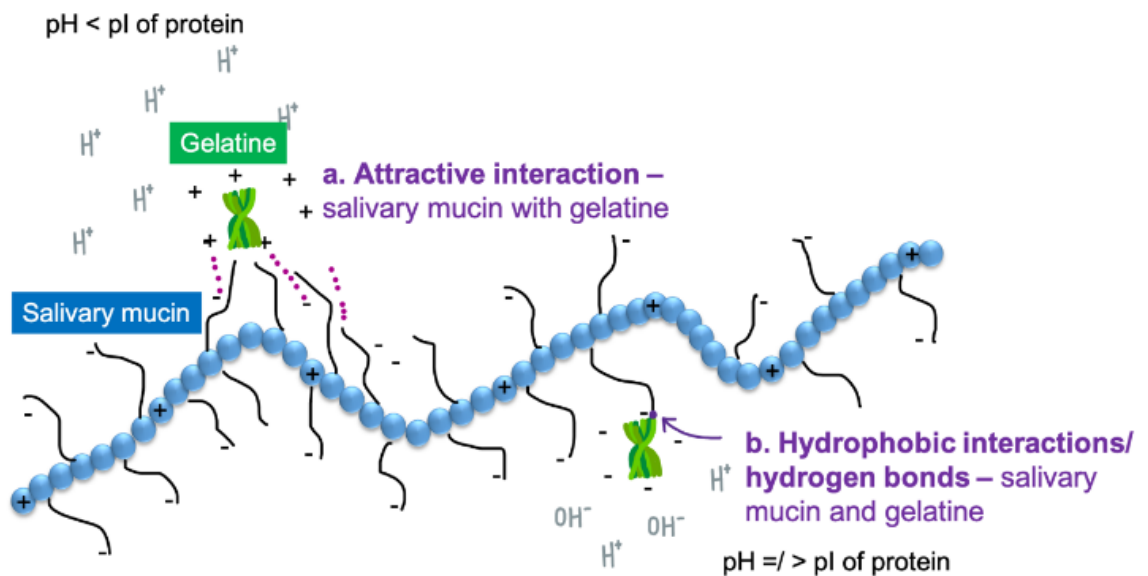


Figure 11. Schematic illustration of plausible interactions between gelatine and saliva, a) electrostatic interaction at low pH, and b) formation of hydrophobic interactions/ hydrogen bonds at neutral pH.

Finally, for lysozyme, when pH was lowered to 3.0, pronounced flocculation between unstimulated saliva and lysozyme stabilized emulsions were apparent (Silletti et al., 2007a). Additionally, in low pH conditions flocs were larger and more densely packed. Contrastingly, at neutral pH flocs were homogeneously dispersed. Viscosity was also shown to be increased at lower pH. However, only one study using lysozyme varied the pH and only used two conditions pH3.0 and pH6.7 (Silletti et al., 2007a), the remaining studies all used neutral pH. Therefore, the full impact of pH remains unknown and further research in a wider range of

pH may provide useful insights. Additionally, sensory analysis should be included to see if potential physicochemical and rheological changes observed as a function of pH translate into mouthfeel differences.

2.4.3. Protein concentration

When thinking about formulating food with high protein content, it is important to understand how increased protein concentration affects oral perception. The only studies which investigated the effect of protein concentration used either whey protein or the whey protein component *β*-lg. Collectively, protein-saliva interactions appear to be a function of protein concentration in addition to pH (Kelly et al., 2010). For example, two studies using bulk solution's and measuring turbidity reported a delayed time to reach maximum turbidity when protein concentration of WPI was increased from 0.5%w/w to 10%w/w (Andrewes et al., 2011, Kelly et al., 2010) (**Table 1.**). This was hypothesized to arise from more saliva being required to interact with the greater amounts of protein consumed (Andrewes et al., 2011).

Interestingly, different results were found for different types of whey protein (*i.e.* native versus denatured) (**Table 1.**). Increasing protein concentration (0.25 to 10.8%w/w) increased aggregate size and turbidity for denatured whey proteins (heated at 80°C) at pH6.8. Whereas the effect for native whey protein (rehydrated into deionized water) was much smaller. In addition, the viscosity increased in the denatured samples. It was hypothesized to be due to more opportunity for covalent bonding due to increased unfolding of chains inhibiting interpenetration of polymers and higher free thiol availability (Hsein et al., 2015). Alternatively, as heating proteins increases surface hydrophobicity this can also drive aggregation (Deng et al., 2019). Friction in polydimethylsiloxane tribopairs in presence of protein was found to be unaffected by concentration (0.5-4%w/w), indicating that protein concentration in this low range did not affect the loss of lubrication of human saliva (Kelly et al., 2010). However, similar to turbidity, a significant

difference in friction coefficient was observed at higher concentration (10%w/w) (Vardhanabhuti et al., 2011). By using a single sliding speed, entrainment of protein solutions at higher concentrations leads to an effective separation of contact bodies and therefore can contribute to lowering of friction. However, in real-life situations, it is known that frictional conditions are dynamic in the mouth occurring at various speeds depending upon the type of food consumed during oral processing. Also swallowing will impact the amount of protein solutions retained on the tongue surfaces. Therefore, the frictional reduction due to higher concentration of protein at higher entrainment speed might not translate into sensory responses (Vardhanabhuti et al., 2011).

Linking these concentration effects to sensory mouthfeel produced equivocal results. For example, Kelly et al. reported no effect of concentration on time-intensity sensory astringency analysis (Kelly et al., 2010). Thereby suggesting the mechanism of astringency might not always be lubrication failure-linked. In another study, greater astringency was reported with higher concentrations up to 4%w/w, after this point, a plateau was observed (Vardhanabhuti et al., 2011, Vardhanabhuti et al., 2010, Kelly et al., 2010). When protein concentration was varied in more complex food systems (**Table 2.**), it was found that increasing whey protein concentration (0 to 6%w/w) reduced consumer acceptance. Similarly, the sports drink used was rated increasingly astringent (Childs and Drake, 2010). As only limited studies appeared to include a sensory link when analysing concentration, further evidence is needed to fully confirm protein concentration effects.

In addition to the individual effects of protein concentration, the effects of concentration alongside pH was also investigated in few studies. This is because increasing protein concentration increases the amount of acid required to shift the overall pH due to the buffering capacity of protein (Kelly et al., 2010). When increasing protein concentration was investigated concurrently with pH, particularly in low pH conditions (pH2.6) and when protein concentration was raised, the maximum intensity of astringency was reduced. Conversely, at pH4.2,

when the protein concentration was increased, the maximum intensity of astringency was increased. Collectively indicating pH affects the relationship between concentration and astringency (Kelly et al., 2010). In other words, the amount of protein governs the buffering capacity of the solution dictating the magnitude of saliva-protein interactions via electrostatics. Furthermore, the saliva flow rate was shown to be raised with increasing protein concentration (0.5 to 10%w/w). With increasing saliva flow, there will be a quicker rate of clearance for the astringent compounds (Andrewes et al., 2011). To summarize, concentration effects appear to be range specific (0.5-4%w/w); further analysis is needed to confirm and see if the same differences also apply to a wider range of protein. In addition, a clear gap exists in the literature for higher concentrations of proteins (>10%w/w) where viscosity and elastohydrodynamic lubrication will play a key role in driving sensorial perception. Such as when translating this to food formulation where at least 20% of the energy value of the food provided by protein is required to make a content claim of “high protein”.

2.4.4. Heat treatment of proteins

Few studies openly varied the preparation of the protein solution and the studies which did, used whey protein or its derivative β-lg. For instance, one study included denatured whey heated to 80°C for 40 minutes. This was compared to whey protein samples that were re-hydrated in de-ionized water. Increased turbidity independent of pH and enhanced bio adhesion was recorded for denatured whey protein compared to native whey protein at pH6.8 (Hsein et al., 2015). Similarly, β-lg was processed by heating to 90°C for 10 minutes and immediately cooled in ice and compared to an unheated control but little difference was found in zeta-potential and SDS-page between the two conditions although turbidity was affected suggesting that interactions were hydrophobic in nature.

The heated sample had two peaks in particle size, which also drove an increase in turbidity at both pH3.4 and pH 5.0, whereas the unheated sample only had a peak at pH3.4 - which is suggestive of a complexation between human saliva and β-lg (Ye et al., 2011). Sensory ratings of astringency were similar despite these differences, therefore although heat treatment may affect turbidity, it may not translate into mouthfeel differences. In a study that used model food systems heating was used to vary the viscosity of the fluid and elicited distinctly different textures. Although the effect of heating was not looked at in isolation, rheological thickness and descriptive sensory viscosity were highly correlated with fluid (heating time 5 minutes) and semisolid treatments (heating time 15 or 30 minutes) (Campbell et al., 2017).

Moreover, a recent study on plant proteins and whey protein again (published outside the inclusion dates) used heat treatment. From this, it was shown that heat treatment increased viscosity of both pea protein and whey protein isolate solutions. This in turn reduced friction in mixed and elastohydrodynamic lubrication regimes but the boundary regime (where astringency is thought to arise) was unaffected (Zembyla et al., 2021). Furthermore, pea protein isolate was not affected by heat treatment whereas WPI had significant structural changes (Zembyla et al., 2021). In general heat treatment of milk is a critical process used by the dairy industry. It is used to prolong stability and enhance quality through lowering microbial load or manipulating functionality of dairy proteins and strengthening the organoleptic properties (Raikos, 2010). Therefore, further research on protein treatment effects, which can fundamentally change protein properties, is important for understanding the parameters protein related mouthfeel may operate.

2.4.5. Saliva type

There were a number of different saliva types used in the selected studies, with 17 using exclusively human saliva, 11 using exclusively model and 6 using both model and human saliva (**Table 1.** and **Table 2.**). A previous review comparing model saliva and human saliva studies concluded model saliva cannot fully replicate the physicochemical and biophysical properties of human saliva (Sarkar et al., 2019b). It is not yet possible to fully simulate the intricate architecture that dictates the properties of human saliva. However, very recently has lubrication performance has been able to be simulated (Xu et al., 2020a). Differences in behaviour are especially apparent when using more advanced methods to understand of how saliva and proteins interact. There will have been further variation in the studies that used human saliva, as human saliva is inherently variable. It is known to vary from different salivary gland, gender, age, diet, type of stimulations, circadian rhythm *etc* (Sarkar et al., 2019b). The issues associated with these variables are discussed in the limitations section.

The two main model saliva types are mucin based (PGM and BSM). Out of the 17 studies, which used model saliva (either exclusively or with human), 13 used PGM, 4 used BSM, 1 used a commercially available artificial saliva substitute and one of these studies using both PGM and BSM. Although mucin is often cited as being responsible for saliva's lubrication properties, it cannot replicate the lubrication performance of whole human saliva and its biophysical properties (Sarkar et al., 2019b). For instance, other salivary components such as proline-rich-proteins have been proposed to play a role in the development of astringency for other astringents (such as polyphenols) (Baxter et al., 1997). The role of low molecular weight protein, such as lactoferrin has also been recently acknowledged (Xu et al., 2020a) as tending to bind mucin to itself and to the surface.

When model saliva containing PGM was compared against human saliva, results in terms of turbidity where similar. Following this, model saliva without mucins but still containing saliva salts was used (Andrewes et al., 2011). This model saliva with no mucin present did not increase turbidity. Similarly, a separate study used parotid saliva which contains salivary salts but no mucin (Larsen and Pearce, 2003) found no aggregation measured by light microscopy (Vingerhoeds et al., 2005). Collectively this indicates mucin is a key component of saliva which drives turbidity and aggregation (Andrewes et al., 2011) (**Table 1.**). Another study again comparing both model (BSM) and human saliva used more advanced methods by analysing lubrication (Biegler et al., 2016). Although human and model saliva (BSM) produced similar friction coefficients, when the protein (lysozyme) was added incrementally, model and human saliva friction results differed (**Table 1.**). Human saliva showed an increase in friction coefficient as lysozyme was added. Conversely, the increase in friction for BSM: protein mixes reached its peak when lysozyme was first added and then plateaued. Thus, model saliva may not fully represent oral conditions when protein is added continuously- like what happens in *in-vivo* conditions. Furthermore, human saliva was more reproducible and had less variation between the repetitions (Biegler et al., 2016). Thus, further work may be warranted to repeat studies using model saliva alone in methods beyond turbidity with human saliva to check for correlations.

The type of mucin has been investigated in relation to surface adsorption and lubricity (Çelebioğlu et al., 2019). Both mucins have similarity in composition such as being heavily glycosylated in the central region and both absorb onto hydrophobic surfaces likely due to hydrophobic interactions (Sarkar et al., 2019b). Results comparing BSM and PGM showed PGM had greater absorbed mass (onto a PDMS surface) (Çelebioğlu et al., 2019). Lubrication was also different, with BSM having greater lubrication and elasticity (Çelebioğlu et al., 2019). Similar results were shown by Lee and Vickers (Lee and Vickers, 2008) who showed BSM to be more efficient in reducing frictional forces than PGM using atomic force microscopy (AFM). The differences in PGM/ BSM lubrication have been proposed to be related to the observed differences in adsorption

(Sarkar et al., 2019b). Moreover, it has been suggested BSM has greater pH sensitivity (Sarkar et al., 2019b). This may have impacted results as a proposed mechanism of interactions especially with whey proteins is electrostatic which is influenced by pH. Looking at the two emulsion studies which used both human and model saliva, no discourse in results were found between the two types (Vingerhoeds et al., 2005, Hu et al., 2019) as both these studies focussed on bulk interactions between saliva and protein (**Table 2.**).

Besides model saliva, there are large number of studies that employed real human saliva to understand interaction with dietary proteins. The use of stimulated versus unstimulated tends to affect results as the latter has higher protein and consequently mucin concentration. One study used both stimulated and unstimulated saliva with β-lg and lysozyme-stabilized emulsions (**Table 2.**). It was found that the unstimulated mixed with β-lg had different elastic properties compared to that of the stimulated saliva (Silletti et al., 2008).

There also appears to be a possible contribution from non-mucin components. Another component of saliva which can contribute to interactions but is at present understudied is the role of salivary salts. Salivary salts alone (with no mucin present) were found to elicit aggregation in lactoferrin stabilized emulsions but not for β-lg (Sarkar et al., 2009) (**Table 2.**). It was suggested the salivary salts screened the positive patches on lactoferrin molecules which reduces the overall positive charge (**Figure 10.b.**). Salt was also varied in another study using WPI-based food systems (Campbell et al., 2017). The study varied salt concentration from 0-30mM NaCl to change the degree of aggregation. Higher salt conditions lead to greater protein aggregation and aggregate size which in turn affected turbidity. Although the study incorporated sensory analysis, you could not isolate the effect of salt in the results (Campbell et al., 2017). Therefore, it would be useful to explore if salt-induced aggregation has similar sensory mouthfeel effects as electrostatic in terms of astringency perception.

2.4.6. Protein: saliva mixing ratio

Saliva to protein mixing ratio is another key parameter in understanding interactions of protein. Noticeably, the mixing ratio is directly related to the earlier discussed parameter of protein concentration however this section will also detail saliva's contribution. The majority of studies of bulk solutions used a 1:1w/w ratio (Kelly et al., 2010, Beecher et al., 2008, Biegler et al., 2016, Ye et al., 2011, Vardhanabhuti et al., 2010, Celebioglu et al., 2017, Çelebioğlu et al., 2015, Çelebioğlu et al., 2016) (**Table 1.**) and for emulsion studies (Silletti et al., 2007b, Silletti et al., 2008, Silletti et al., 2010, Vingerhoeds et al., 2005, Koukoura et al., 2019, Ritzoulis et al., 2012, Dresselhuis et al., 2008b, Madadlou et al., 2018, Fuhrmann et al., 2019) (**Table 2.**), irrespective of the type of saliva employed. However, using this ratio has been suggested not to fully simulate real oral conditions, studies revealed that saliva mixes with food in more of a 1:4w/w ratio (Devezeaux de Lavergne et al., 2015, Stribiţcaia et al., 2020). Of course, this depends upon the food type and the moisture content of the food. A limited number of studies have varied mucin: protein concentration; and in those that did, it was difficult to isolate the effects of this versus other confounding effects (*e.g.* pH, concentration). One study which did alter saliva: protein ratio reported differences in zeta-potential between protein: mucin ratios (**Table 1.**). For example, when mucin (PGM) and WPI were mixed at 1:1 w/w ratio, zeta-potential was -28mV, whereas, at 2:8w/w, the zeta-potential changed to -24mV indicating a monotonic dependence on the mixing ratio (Ahmad et al., 2020a). In terms of rheology, viscosity was increased by almost 20-folds from WPI alone to 2:8 WPI: mucin mixtures. Mucin alone was the most viscous sample, and when mixed at 1:1 w/w indicated little change in viscosity between 6:4, 5:5 and 4:6 w/w WPI: mucin mixing ratios (**Table 1.**). It was suggested the higher viscosity is a result of increased energy dissipation during flow because of phase separation by the colloidal particles of WPI+mucin (Ahmad et al., 2020a) and would match the rheology dominated high molecular weight of mucin (Haward et al., 2011).

Similarly, when mixtures with different gelatine: mucin ratios were tested at pH3.0, the Trouton ratio (ratio of extensional viscosity to shear viscosity) varied. Trouton ratios for mucin alone are c.100 and 239 for gelatine. As the ratio of mucin:gelatine decreases from 2:8, 4:6 and 8:2, Trouton ratio values rise from 100, to 200 and finally to 500-700, respectively (Ahmad et al., 2020b). The increase in Trouton ratio is a consequence of the assembly of mixtures and reflects increasing extensional viscosity thus, aggregation of gelatine-mucin appeared to reduce filament drainage which increased extensional viscosity (Ahmad et al., 2020b). The human threshold has greater sensitivity in discriminating extensional over shear viscosity (Lv et al., 2017) and these results indicate the self-assembly of mucin: gelatine mixtures (which is reflected by the Trouton ratio, as explained above) will govern how thick foods combined with saliva flow. However, a key question to raise here is how much the mucin concentration varies in case of unstimulated or stimulated human saliva. Will that change depending upon the type or concentration of protein consumed? This definitely needs further exploration to clearly understand if the stimulation of salivary flow and mucin release is related to the protein being consumed.

Besides the ratio of saliva itself, the way of adding saliva i.e. static versus dynamic can play an important role in its interaction with proteins. One elegant study varied the saliva: protein ratio by adapting how saliva was added to the protein to simulate the dynamics of oral processing (Andrewes et al., 2011) (**Table 1**). The study divided oral processing into two stages. The initial stage utilized a continuous flow method *via* a peristaltic pump delivering a continuous flow (1 mLmin^{-1}) of model saliva, which was gently stirred. Then WPI (5 or 10mL) was poured on top of the container containing the model saliva to simulate sipping of a beverage, and afterwards to simulate swallowing, the solution was drained. Results of the continuous flow model revealed minimal changes in turbidity initially. Aggregates formed after a short period of time as the pH increased towards WPI's isoelectric point, increasing the turbidity. When protein concentration was varied (1-10w/w%), there are little change in turbidity. During this stage, there was not enough saliva to significantly alter the pH from the

isoelectric point of the whey proteins. The second stage of stepwise mixing (Andrewes et al., 2011) (**Table 1.**) aimed to simulate residue and clearance. This method relied on two main assumptions. Firstly, that 1mL of liquid (saliva + beverage residues) constantly covers the mouth; and secondly, that 250mL of saliva + beverage is swallowed every 15 seconds. During stepwise mixing, turbidity further increased rapidly before a plateauing indicating maximum point was reached. The addition of more saliva caused the turbidity to quickly decline as residual whey proteins were diluted. When protein concentration was increased from 5 to 10mL, the same trends occurred however maximum turbidity occurred later. Collectively, the amount of saliva affected both turbidity build up and turbidity decline through clearance. This emphasises the importance of using methods like these which simulate the dynamic *in vivo* conditions where saliva is added more continuously or in sequential steps rather than all at once. Although results further understanding of how astringency may develop temporally, the research was limited by the methods used. Only turbidity was evaluated, and the study did not account for the possible formation of aggregates and friction increase which may contribute to astringency (Andrewes et al., 2011).

2.5. Limitations

Disclosing limitations are an integral part of transparent reporting and crucial for developing scientific discourse, allowing readers to interpret each study accurately. Disclosing limitations helps to rationally develop future studies with accurate methodology, which can target addressing the disclosed limitations for future work (Puhan et al., 2012), as well as communicating relevance (Ross and Bibler Zaidi, 2019). Limitations are inherent within research (Ross and Bibler Zaidi, 2019) and within the studies analysed, for example the use of model saliva reduces variability seen when using human saliva (Sarkar et al., 2019b), but it will have limited applicability when comparing results to the mouthfeel. It has been suggested for scholarly inquiry that disclosing limitations is also an ethical element, and that by not reporting limitations each study included will have a risk

of bias (Ross and Bibler Zaidi, 2019) consequently it would be recommended to include limitations in future research. Similarly, the present review will have its own limitations. The review only included articles published in English, therefore relevant articles published in different languages may have been omitted. However, when scanning reference lists no addition articles published not in English were found.

Looking at the quantitative bias assessment conducted (**Supplementary Table A.2.**), there are a range of scores from 49% to 100%. There is high variance between studies and a standard deviation of 13% across all studies. The scores did not appear to change over time and the lowest three scores recorded were in the date range 2008-2017(Campbell et al., 2017, Silletti et al., 2010, Silletti et al., 2008) whereas the highest two were published in 2009 and 2020 (Ahmad et al., 2020a, Sarkar et al., 2009). The two categories where scores were especially low were reporting quality (average 79%) and publication bias (average 79%). Whereas selection and detection bias had averages of 86% and 91% respectively. Specifically, the 23 studies using human saliva had high risk of bias by poor reporting quality. For example, 10 studies did not report age or sex, 5 studies reported one but not the other and 8 studies reported both. In addition, for health status, 7 studies reported health status and how it was obtained, 4 studies did not report health status and 9 studies reported ‘healthy’ but did not explain how. Ambiguity was again the problem for publication bias. For temperature control 7 studies reported exact temperature, 4 studies did not mention of temperature and 22 out of 33 studies used ambiguous terms such as ‘room temperature’. Thus, study quality especially when using human saliva was low, and in general study quality does not appear to have improved with advancing techniques. Therefore, a more conscious effort to consider areas of bias should be undertaken in future research.

2.6. Conclusions

To sum it all up, dairy protein interactions with saliva are dominated by electrostatics, and in low charge scenarios by hydrophobic interactions; which was also concluded in the similar narrative review (Çelebioğlu et al., 2019). As electrostatics tends to drive the interactions, a strong influence of pH for whey protein and *β*-lactoglobulin is observed, with enhanced interactions being found at a pH below the isoelectric point of proteins. Due to a lack of studies using sensory analysis, it cannot be concluded whether electrostatic interactions always translate into sensorial differences for proteins other than whey protein. Food system analysis was only conducted on whey protein. It showed increased turbidity, lower viscosity and higher friction in whey vs control conditions which translated into increased astringency with increased electrostatic interactions. At neutral pH both whey protein and gelatine interacted non-electrostatically. However, further work is needed to see if these alternative mechanisms apply to other proteins as well. In addition, further incorporation of *in vivo* oral processing studies, bolus analysis and sensory analysis to see if they contribute to sensory mouthfeel in the same way as electrostatic interactions is warranted.

Protein concentration appears to influence the development of saliva-protein interactions. However, the effect of concentration cannot be fully elucidated due to limited variability in protein type and concentration range. The present review is the first to identify a clear gap in research on protein-saliva interaction at higher protein concentration relating to mouthfeel perception relevant for claiming “high protein” source. Moreover, the present review identified concerns over methodology used in studies. Most studies analysed used a 1:1w/w saliva:protein ratio, however, previous research has suggested a 1:4w/w ratio is more physiologically relevant and using this different ratio yielded different results in zeta-potential and viscosity compared to that of 1:1w/w. For similar reasons, methodological development to accurately simulate saliva’s continuous secretion with swallowing and oral residual analysis are warranted. Another methodological

concern was the poor study similarity making comparison difficult because of inconsistency or missing information such as saliva type, protein processing method, temperature and lack of limitations in general. It would be recommended that future studies take more consideration into transparent reporting to improve quality and minimize bias. In addition, it is recommended that future research employ a variety of newer techniques such as tribology, QCM-D and work towards standardizing approaches to improve comparability between studies. Finally, there is a key gap in the literature for analysing plant protein-saliva interaction to predict mouthfeel perception, as plant proteins are becoming increasingly popular.

Table 1. Study characteristics of articles where bulk protein solutions interact with model or real human saliva.

Reference (Author, Year)	Protein			Saliva			Experimental set up		Main findings	Quality score
	Protein type (*pI*)	pH	Protein concentration	Model or Real human saliva	Type of saliva (or mucins)	Number of saliva donors, gender, age	Methods	Saliva: protein solution ratio (w/w)		
Çelebioğlu, et al. (2016)	*β*-lg (*5.2)*	3.0-7.4	1mg/mL	MS	BSM or PGM	NA	BAA, Tribology, ZP	1:1	pH-dependent lubricating effects are dominated by competitive absorption of the two proteins and *β*-lg+BSM does not form strong aggregates.	96%
Çelebioğlu, et al. (2015)	*β*-lg (*5.2*)	3.0-7.4	1 or 10mg/mL	MS	BSM	NA	CD, DLS, NMR, ZP	1:1	NMR indicated polar interactions at pH3.0 and 5.0 with no interaction visible at pH7.4.	96%
Celebioglu, et al. (2017)	*β*-lg (*5.2*)	3.0-7.4	1 or 2mg/mL	MS	BSM	NA	Rheology	1:1	At pH closest to *β*-lg's pI, electrostatic repulsions between *β*-lg was reduced thus aiding the formation of a stable absorbed layer which had a high elastic modulus.	63%
Vardhanabhuti, et al. (2011)	*β*-lg (*5.2*)	3.5 and 7.0	0.5-10%w/w	HS	SWHS	n=1, F, ND	Sensory analysis (n=ND, ND), Tribology	ND	Friction coefficient of SWHS increased more substantially when *β*-lg was added at pH5.2 compared to pH3.5. The increase in friction coefficient was unaffected by when *β*-lg was added concentration (0.5-4 %w/w). Sensory analysis showed an increase in astringency ratings with increasing protein concentration (up to 4 %w/w), after this point, ratings plateaued, astringency did not correlate with tribology.	74%
Withers, et al. (2013)	*β*-lg (*5.2*) or casein (*4.6*)	7.4	8.7%w/w (rheology); 2% w/v (force	MS	AS	NA	Adhesion, Fluorescence microscopy, Rheology, Thiol content analysis, Retention, ZP	0.088:1, 1:1 (bioadhesion)	Casein bound more efficiently than *β*-lg to the epithelial lining or porcine oral mucosa. The force of bioadhesion measurements was greater for *β*-lg compared to casein suggesting *β*-lg but not casein interacts with mucin-rich AS.	83%

			and bioadhesion)							
Vardhanabhuti, et al. (2010)	*β*-lg (*5.2*) or lactoferrin (*8.7*)	*β*-lg 3.4. or 6.0; lactoferrin 3.5 to 7.0	4%w/w (2%w/w for SDS-PAGE)	HS	ND	n=2, ND, ND	SDS-PAGE, Sensory analysis (*β*-lg; trained panel, n=10; lactoferrin, trained panel, n=12)	1:1	At pH7.0, *β*-lg had low astringency which increased with decreasing pH. Whereas lactoferrin was astringent at all pH values with no pH effect on the ratings.	91%
Ye, et al. (2011)	*β*-lg (*5.2*), lactoferrin (*8.7*)or WPI (*4.5*)	2.0-7.0	WPI 5%w/w, β-lg 2%w/w or lactoferrin 2%w/w	HS	SWHS	n=5, ND, ND	DLS, SDS-PAGE, Sensory analysis (trained panel, n=12), Turbidity, ZP	1:1	*β*-lg+SWHS had increased particle size and turbidity at pH of 3.4. Turbidity decreased with further pH reductions until pH2.5. Larger particle size was found between pH3.0-4.0. Sensory analysis showed intense astringency between pH3.0-4.0, which hypothesized *β*-lg-saliva interaction to be electrostatically-driven. Lactoferrin+SWHS mixtures were smaller in particle size range and turbidity than *β*-lg+SWHS mixtures between pH8.3 and 3.0. Sensory analysis showed little/no astringency at neutral pH, ratings were greatest at pH3.4.	*96%*
Ahmad, et al. (2020)	Gelatine (*4.85*)	3.0 or 7.0	0-8mM	MS	PGM	NA	Fluorescence spectroscopy, Rheology, ZP	Various ratios (8:2, 6:4, 5:5, 4:6, and 2:8)	At pH7.0, there was aggregation and phase separation between PGM and gelatine. At pH3.0, there was an indication of electrostatic interaction with binding regimes exhibited between PGM and gelatine at both pH3.0 and 7.0.	96%
Sano, et al. (2005)	Gelatine (*4.85*) or WPI (*4.5*)	3.5 or 7.0	*in vitro* experiments 50mg/mL, *n vivo* 0.13-5mg/mL	HS	SWHS	n=7, M, 28-37 years	BAA, Sensory analysis	ND	WPI was rated more astringent than gelatine, latter was not rated astringent at pH3.5. For WPI, astringency increased with protein concentration.	85%
Biegler, et al. (2016)	Lysozyme (*10.7*)	7.0	0.25 mM	HS or MS	ND or BSM	n=12, ND, 25-35 years	Rheology, SEC, Tribology	1:1	The addition of lysozyme to saliva (HS or MS) increased friction coefficient. Lysozyme had a low tendency to precipitate BSM (but still induced a loss of lubrication).	73%
Ritzoulis, et al. (2012)	Sodium caseinate (*4.2*)	1.0-7.0	0.00-0.05 wt%	MS	PGM	NA	LD, Microscopy, ZP	1:1	Sodium caseinate and PGM interacted around the pI of sodium caseinate (pH3), whereas they repelled at pH 7.0. Interactions also occurred at pH 1.0 but aggregation was much smaller than at pH 3.0.	88%

Ahmad, et al., (2020)	WPI (*4.5*)	3.0-7.0	1%w/w	MS	PGM	NA	Fluorescence spectroscopy, Rheology, ZP	Various ratios (8:2, 6:4, 5:5, 4:6 and 2:8)	At pH3.0 and 7.0, there was phase separation between PGM and WPI. At pH3.0, interactions where electrostatically driven. Conversely, at pH7.0, interactions were entropically and enthalpically driven.	100%
Andrewes, et al. (2011)	WPI (*4.5*)	3.0-4.0	1-10%w/w	MS or HS	PGM or SWHS	n=5, ND, ND	pH, Turbidity	Dynamic model with saliva added; 2 different conditions.	Increasing protein concentration delayed time to reach maximum turbidity. At higher protein concentrations, there was not enough saliva to increase the pH to the point of aggregation. Increasing pH reduced turbidity duration and time to reach maximum turbidity, as less saliva was needed to raise the pH to the pI of WPI.	87%
Beecher, et al. (2008)	WPI (*4.5*)	3.4-7.0*	6%w/w	HS	ND	n=3, ND, ND	Sensory analysis (trained, n=8), Turbidity	1:1	WPI interacted with HS proteins leading to increased turbidity. At neutral pH, the change in turbidity was small compared to pH3.4. The increase in turbidity was correlated with levels of astringency (sensory analysis).	74%
Hsein, et al. (2015)	WPI (*4.5*)	3.5 or 6.8	0.25-10.8%w/w	MS	PGM	NA	Isothermal calorimetry. Rheology, Turbidity	Various ratios (1:4, 1:2, 1:1, 2:1, and 4:1)	From pH1.2 to 4.5, there was an increase in turbidity of all WPI+BSM, which did not occur at pH6.8, where no interaction was found. Bio-adhesion forces were greatest for pH6.8 for high concentration denatured WPI. Use of chemical blockers demonstrated hydrogen bonding and disulphide bridges were the primary interaction mechanisms with PGM.	96%
Kelly, et al. (2010)	WPI (*4.5*)	2.6-4.2	0.25-13%w/w	HS	SWHS	n=3, F, ND	pH, Salivary flow rate (n=10), Sensory analysis (trained panel, n=10), Turbidity	1:1	Astringency increased with increasing protein concentration from 0.05-4%w/w and then a plateau occurred (4-13 %w/w). Time to maximum astringency was indifferent between protein concentrations ($p>0.5$). Increasing protein concentration also increased turbidity. Maximum turbidity occurred at pH4.6-5.2 which is near the pI of WPI.	93%
Lee and Vickers (2008)	WPI (*4.5*)	3.4	1 or 6%w/v	HS	SWHS	n=20, 5 M, 15F, ND	Sensory analysis (trained panel, n=20)	ND	WPI was rated less astringent than acid-only solutions matched for total acidity. WPI was rated more astringent than controls matched for pH. Sourness ratings were reduced in WPI solutions. Collectively, this is indicative of astringency ratings resulting from high acidity not WPI in acidic solutions.	74%

Note. 7.0*: 'mentioned in the literature as neutral pH', AS: Artificial saliva (commercial formulation), BAA: bicinchoninic acid assay, β-lg: beta-lactoglobulin, BSM: bovine submaxillary mucin, CD: circular dichroism, DLS: dynamic light scattering, F: female, HS: human saliva, LD: laser diffraction, M: male, MS: model saliva, NA: Not applicable, ND: not disclosed, NMR: nuclear magnetic resonance, pI: Isoelectric point, PGM: porcine gastric mucin, SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis, SEC: size exclusion chromatography, SWHS: stimulated whole human saliva, WPI: whey protein isolate, ZP: zeta potential.

Table 2. Study characteristics of articles where proteins in emulsions and food matrices interact with model or real human saliva.

Reference (Author, Year)	Protein			Saliva			Experimental set up		Main findings	Quality score
	Protein type	pH	Protein concentration	Model or Real human saliva	Type of saliva (or mucins)	Number of saliva donors, gender, age	Methods	Saliva: protein solution ratio		
FOOD EMULSIONS										
Sarkar, et al. (2009)	β-lg or Lactoferrin	6.8	Soy oil O/W (20 %w/w) emulsion stabilized by 1.0%w/w protein	MS	PGM	NA	CSLM, LS, Rheology, ZP	1:1, different concentrations of mucin used (0.0-3.0%w/w mucin)	There was reversible droplet aggregation between β-lg-stabilized emulsions and MS containing PGM, which was not observed in MS without mucin. When lactoferrin was combined with MS (no mucin), pseudoplastic flow behaviour was found due to bridging flocculation, when mucin was added viscosity increased. ZP results showed a decrease from +27mV to -27mV when mucin concentration was increased from 0.1 to 1.5wt%.	100%
Silletti, et al. (2007, 2007, 2008, 2010)	β-lg or Lysozyme	6.7 and 3.0	Sunflower oil O/W emulsions (40 %w/w) stabilized by 1%w/w protein	HS	SWHS or UWHS	n=11, 5F, 6M, age 20-45	CLSM, LS, Rheology, ZP, Microscopy, ATR-FTIR, SDS-PAGE, Western blotting	1:1	When emulsions were mixed with saliva, pronounced flocculation was found for β-lg at pH6.7 and at pH3.0, as well as with lysozyme. Flocculation was reversible for β-lg at pH3.0 and lysozyme 6.7 suggesting flocculation is weak. β-lg stabilized emulsions at pH3.0 had irreversible flocculation, as this pH was close to the pI of β-lg. ZP suggest flocculation is electrostatically-driven.	87%, 94%, 67%, 63%

Vingerhoeds, et al. (2005)	β-lg, casein, sodium caseinate or WPI	6.7	Sunflower oil O/W emulsions (40%w/w) stabilized by 1%w/w protein	HS or MS	UWHS, PGM	n=6, 2M, 4F, age 28-43	Demixing (Turbiscan), LS, Microscopy, and Rheology	UWHS1:1	Parotid saliva (no mucin) only caused reversible aggregation with WPI-and β-lg stabilized emulsions. PGM induced flocculation of emulsions, and human saliva also resulted in aggregation of emulsions. All proteins when mixed with PGM concentration greater than 0.4±0.1wt% had phase separation suggesting interactions were depletion flocculation.	79%
Koukoura, et al. (2019)	Sodium caseinate	ND	Medium chain triglyceride O/W emulsions (30%w/w) stabilized by 2% w/w protein	MS	PGM	NA	CLSM, LS, ZP,	1:1	Sodium caseinate-stabilized emulsions size were not affected by addition of saliva. Caseinate-stabilized emulsions initially had a highly negative zeta-potential which was reduced when mixed with saliva. As sodium caseinate-stabilized emulsions had a strong negative zeta-potential, and saliva had a negative (but not as strong) zeta potential; this reduction was expected.	96%
Ritzoulis, et al. (2012)	Sodium caseinate	1.0-7.0	n-Hexadecane O/W emulsions (30%w/w) stabilized by 1-1.25%w/w protein	MS	PGM	NA	LS, Microscopy, ZP	1:1	Caseinate-stabilized emulsions flocculated in the presence of model saliva. Flocculation was bridging and electrostatic at lower pH (pH3.0) whereas at greater *i.e.* pH5.0 and above, depletion flocculation occurred. Average particle size increased with increasing pH, however at pH7.0, flocs separated due to electrostatic repulsion. At pH3.0 and 5.0, flocculation was irreversible.	88%
Fuhrmann, et al. (2019)	Gelatine or WPI	5.0 or 7.0	Sunflower oil O/W emulsions (20 %w/w) stabilized by 0.0-0.6%w/w gelatine or 0.0-0.19%w/w WPI	HS	UWHS	n=10 (ND, ND)	Rheology, LS, Tribology, Sensory analysis (n=83, 62F, 21M, mean age 23.5±3.8), ZP	1:1	For gelatine-stabilized emulsions, viscosity and droplet size increased from 1-2mm to >25mm likely because of flocculation. When WPI-stabilised emulsions was combined with saliva, no change in droplet size was observed. Sensory analysis showed correlations between thickness and consistency (viscosity) and friction properties correlated with creaminess when saliva was not present.	73%
Vingerhoeds, et al. (2009)	Lysozyme or WPI	6.7	Sunflower oil O/W emulsions (40 %w/w) stabilized by 1%w/w WPI;	HS	SWHS	Oral processed (OP) emulsions spitting out oral processed	ATR-FTIR, LS, Western Blotting, SDS PAGE, OP emulsions spitting out oral	NA	Lysozyme-stabilised emulsions had larger flocs than WPI-stabilised emulsions after oral processing. Before oral processing, both lysozyme and WPI non thickened emulsions had similar viscosity. Tongue oil retention was found to be greater for lysozyme-stabilized over WPI stabilized emulsions.. Sensory	79%

			Sunflower oil O/W emulsions (20%w/w) stabilized by 1%w/w Lyzozyme			samples; Sensory analysis (n=9, 9F, age 30-60)	processed samples, Trained Sensory Analysis, Rheology		analysis showed WPI were associated with greater thickness, creaminess, fattiness and slipperiness attributes compared to lysozyme, whereas lysozyme was associated with dryness, roughness and astringency.	
Dresselhuis, et al. (2008)	WPI	ND	Sunflower oil O/W emulsions (40 %w/w) stabilized by 0.3 or 1%w/w protein	HS	UWHS	n=30, ND, ND	LS, Microscopy, Retention (pig tongue-CLSM), Rheology, Swallow and spit out	Raman spectroscopy and CSLM= 1:1	Emulsions stabilized by 0.3%w/w WPI emulsions were less stable, with more fat remaining, which was harder to remove on the tongue surface than the ones stabilized by 1.0%w/w WPI. In addition, spectroscopy showed more emulsion droplets visible for higher protein concentrations (1.0vs 0.3%w/w).	84%
Hu, et al. (2019)	WPI	pH 7.0*	Sunflower oil O/W emulsions (20 %w/w) or Orange oil O/W emulsions (0.0033 %w/w) stabilized by 1.0%w/w protein	MS+HS	PGM or SWHS	n=10, 6F, 4M, age range 20-25	Optical microscopy, OP (*in vitro*), LS, Rheology, Turbidity	4:5	WPI emulsion mixed with model saliva in conditions with and without α-amylase. Flow analysis showed WPI+model saliva mixtures were shear thinning (non-Newtonian). There was no viscosity differences with and without α-amylase.	90%
FOOD MATRICES										
Campbell, et al. (2017)	WPI	6.1 to 6.9	11 %w/w protein solutions formed into model foods by manipulating pH, ionic strength and heating time	HS	WSHS or UWHS	n=4, ND, age range 23-31	Rheology, Tribology	ND	Different processing affected rheology and textures of the model food. Friction values were reduced for saliva alone compared to model foods. Viscosity of saliva-model foods was increased compared to model food alone with reduced friction. Variation in tribology methods (elliptical versus linear motion) had different results.	63%

Childs and Drake (2010)	WPI	2.6 to 3.4	Acidified whey beverage (0-6.0 %w/w) with added citric acid (0.43-1.8%w/w)	HS	SWHS	Sensory, n=49 (30F, 19M, age ≥25); ODT n=25 (ND, ND), Taste threshold, n=25 (ND, ND) Acceptance tests, n=120 (ND, ND)	Sensory analysis (FG, ODT, taste threshold, consumer acceptance tests)	NA	FG 95% chose WP sports drink as their least favourite, and 40/49 suggest it was very astringent. Terrible aftertaste was also recorded. Consumer acceptance was reduced as protein concentration increased without nose clips.	63%
Morell, et al. (2017)	WPI	4.5-4.6	Milk powder or WPC yogurts; 10%w/w skimmed milk powder versus 4.3%w/w WPC	MS or HS	PGM or SWHS	n=1, ND, ND	Sensory analysis (n=13), Tribology	1:4	The addition of saliva led to a reduction in yogurt friction values, with the reduction greater in yogurts without added starch. At low sliding speeds, WPC and milk powder had similar friction coefficient values (boundary lubrication). WPC-based yoghurts were described as rough, gritty, grainy and astringent whereas milk powder yoghurts were described as smooth and creamy.	95%
Morell, et al. (2015)	WPI	4.5-4.6	1.9%w/w of WPI or 10%w/w skimmed milk formulated into milk yogurts	MS or HS	PGM + SWHS	Sensory n=2, 16F, 5M, age range 21-45 years; liking and satiating n=121, ND, ND	LTSEM, Microscopy, Rheology, Sensory analysis (FP and liking and satiating tests),	1:4	After *in vitro* OP, channel like formations of saliva was observed in all samples, with aggregation of protein network forming dense and opaque areas. Saliva reduced viscosity values in all samples. Milk protein samples were rated as creamy, thick and dense, whereas whey protein samples were rated as lumpy, grainy and gritty. WPI-rich yoghurts was the least popular, followed by milk protein and the control which had half the protein content.	96%
Wang, et al. (2016)[50]	WPI	2.2-3.9	21.g//L protein with 8%wt/wt sucrose, phosphoric acid 40% or potassium carbonate 20%	HS	SWHS	n=10, ND, ND	Chemometric Analysis, (ATR-FTIR spectrometer), Quantitative sensory analysis,	ND	Comparing WPC, WPI and WPH, Whey protein beverages had increased astringency when pH was reduced from 3.9 to 2.2. WPH had highest variability in astringency, and WPI had lowest variability of scores. pH affected WPC and WPH with lower pH (more acidic) correlated to greater astringency- whereas pH only affected 1/3 WPI samples.	49%

Note. 7.0*: 'mentioned in the literature as neutral pH', ATR-FTIR Spectroscopy: Attenuated total reflection–Fourier transform infrared Spectroscopy, β-lg= beta-lactoglobulin, CLSM: Confocal laser scanning microscopy, F: female, FG: Focus group, FP: Flash profiling, HS: human saliva, LS= light scattering, LTSEM: Low-temperature scanning electron microscopy; M: male, MS: model saliva, NA: Not applicable, ND: Not disclosed, ODT: Orthonasal detection threshold, OP: Oral processing, O/W: Oil in water, pI: Isoelectric point, PGM: porcine gastric mucin, PNA: Protein non-associated; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis, SWHS: stimulated whole human saliva, UWHS: Unstimulated whole human saliva, WPH: whey protein hydrolysate, WPI: whey protein isolate, WPC: whey protein concentrate, ZP: zeta potential.

Chapter 3. Comparing frictional behaviour of plant and dairy proteins: role of high protein concentration and interactions with mucins

Abstract

The aim of this study is to understand the frictional behaviour of non-purified plant proteins compared to dairy proteins. Particularly, the effect of higher concentrations (10-20wt% protein) and interaction with model saliva were examined using soft tribology, rheology, and adsorption measurements. Aqueous dispersions of plant proteins (pea protein concentrate (PPc) and soy protein isolate (SPI) are compared with dairy proteins (whey protein isolate (WPI) and sodium caseinate (NaCas) in presence and absence of model saliva (MS) at pH6.8. Owing to protein-protein aggregation and lower solubilities, plant proteins show a prominent shear thinning with one-to-three orders of magnitude decay in viscosities with increasing shear rates, even when diluted with saliva, unlike dairy proteins showing less shear dependence. Addition of proteins reduces the boundary friction coefficients (μ) with NaCas showing the lowest boundary μ ($p<0.05$). In general, the dairy proteins show better lubricity whilst the plant proteins demonstrated surface de-wetting characteristics increasing the mixed μ with twice as higher calculated film thickness (h_{min}) required for onset of elastohydrodynamic regime as compared with that of dairy proteins, irrespective of concentration or saliva addition. Such low μ values in dairy proteins might be attributed to increased adsorption (~1.5-5× more) of the elastic films as compared to that by plant proteins. Increasing protein concentration to 20wt% further improves lubrication performance of dairy unlike plant proteins. Findings suggest that product development towards more sustainable formulations, requires

innovative strategies to reduce high friction and aggregation when using larger proportions of plant proteins.

3.1. Introduction

Food is essential for human existence; however global resources are struggling to meet current food demands (Miraglia et al., 2009). Methods for alleviating this pressure include the development of more sustainable foods (UN, 2022, Granato et al., 2022, Abbasi and Abbasi, 2016). An area of recent development and interest is to design foods with sustainable plant-based ingredients (Brown et al., 2021). Targeting proteins is crucial because they are essential macronutrients associated with several health benefits including increased satiation and lower calorie density than carbohydrates and fats (Górska-Warsewicz et al., 2018, Weigle et al., 2005). It is now well-acknowledged that animal proteins including dairy proteins are associated with high environmental and climate impact due to their elevated greenhouse gas emissions, large demands for water and increased land required for livestock rearing (Aschemann-Witzel et al., 2021).

Plant proteins are environmentally attractive as protein source as they emit nearly half of the greenhouse gases compared to animal proteins (Xu et al., 2021). However, their use in food products has been associated with unpleasant sensory mouthfeel and flavour characteristics including dryness, astringency, bitterness, and beany flavours *etc.* (Canon et al., 2021, Onwezen et al., 2021, Tanger et al., 2021, Xia et al., 2022, Wang et al., 2009). For example, when animal milk is proportionally substituted with pea milk, a higher proportion of pea milk is correlated with reduced acceptability (Omrani Khiabanian et al., 2020). For this reason, understanding the fundamental mechanisms behind the mouthfeel of plant proteins is of key interests for the development of more sustainable protein-based foods and beverages.

Mouthfeel is a multifaceted tactile sensation with no single method developed yet capable of capturing the full sensation (Stokes et al., 2013, Sarkar and Krop, 2019, Vlădescu et al., 2023). As such, a range of methods have been used to try understand mouthfeel, ranging from human sensory panels to instrumental measurements of objective material properties (Sarkar and Krop, 2019). Throughout all these methods, the integration of saliva within the objective measurement techniques is gaining importance to understand mouthfeel (Brown et al., 2021). The way food or beverages interact with saliva determines the subsequent tactile sensations experienced in the mouth (Stokes et al., 2013, Agorastos et al., 2023). Previous research on protein-saliva mouthfeel has focussed on understanding frictional dissipation and physicochemical properties of protein, saliva and protein-saliva mixtures. (Brown et al., 2021, Çelebioğlu et al., 2019). In particular, rheology and tribology have been correlated with human sensory measurements, with correlations being stronger for model food/saliva mixtures than model foods with no saliva added (Upadhyay and Chen, 2019b).

Regarding understanding protein-based mouthfeel, the majority of research has been conducted on dairy proteins and more specifically, whey protein isolate (WPI) (Brown et al., 2021). Human sensory studies show that the replacement of skimmed milk powder with WPI reduced consumer acceptability with 'dry' aftertastes noted (Childs and Drake, 2010). Research trying to understand these 'dry' tastes suggest that WPI interacts with saliva via depletion interactions (Çelebioğlu et al., 2019). The interaction consequentially leads to de-lubrication which can be seen through higher friction in tribological analysis (Vlădescu et al., 2023). Regarding other types of protein, there is a lack of clarity due to the scarcity or inconsistency of research. For example, one study has reported intense astringency for the dairy protein lactoferrin (Vardhanabhuti et al., 2010), whereas another has reported little astringency (Ye et al., 2011).

Despite their increasing significance, research on plant proteins has gained attraction only recently. A systematic review published in 2020 with a search date between 2000-10.07.2020 found no studies on plant protein-saliva interaction (Brown et al., 2021). Since then, investigations using both tribology and adsorption using quartz crystal microbalance with dissipation monitoring (QCM-D) have surfaced. When pea and whey proteins are compared using tribology and QCM-D on model saliva-coated surfaces, WPI is a better lubricant (Zembyla et al., 2021). In a recent study using both sensory and instrumental methods, sensory analysis shows two separate pea proteins are astringent. Interestingly, specifically tribology results combined with *in situ* imaging of the tribofilms have different frictional behaviours between the two pea proteins (Vlădescu et al., 2023). Combining QCM-D and tribology is thus offering a more comprehensive mechanistic insights behind sensory perception. Nevertheless, more work is needed to quantify plant protein + saliva interactions and compare such behaviour instrumentally between dairy and plant proteins to derive mechanistic understanding before such knowledge can be used for product development.

As limited solubility is often advocated as a clear driver behind unpleasant mouthfeel in plant proteins, studies have frequently used purer, soluble fractions of plant proteins *via* centrifugation and filtration (Zembyla et al., 2021, Kew et al., 2021), thus overlooking comparison of animal and plant proteins without purification. Consideration of the environmental impact of processing steps regarding proteins should be acknowledged. Every additional purification step to try to improve properties will increase resources and therefore environmental cost of production as shown recently shown via the life cycle assessment (Lie-Piang et al., 2021). Hence, it is important to understand the properties of proteins without purification.

When examining protein mouthfeel, concentration is an important factor for consideration which is often underestimated. Human sensory studies showed that by increasing concentration up to 6wt% in WPI protein beverages, the

consumer acceptance decreased (Childs and Drake, 2010). Studies using instrumental methods found no change in lubrication behaviour when WPI concentration was raised between 0.5 to 4wt% (Vardhanabhuti et al., 2010). However, when concentration was further increased to 10wt%, a significant reduction in friction was found (Vardhanabhuti et al., 2010). To claim high protein on food labels in Europe protein must account for at least 20wt% of the total energy value (European Commission, No Date). However, the majority of research on protein-saliva interactions has been conducted at low concentrations (<5wt%) (Brown et al., 2021). Therefore, to fully comprehend the impact of concentration on mouthfeel in 'high protein' foods, further evidence comparing plant proteins with dairy proteins at higher concentrations is necessary.

Hence, the aim of this study was to compare the *in vitro* mouthfeel properties of high concentrations of plant and dairy proteins in presence or absence of model saliva. The proteins are examined individually and in conjunction with model saliva to understand the role of saliva in their material performance. A comprehensive group of objective measurements including rheology, tribology, scanning electron microscopy (SEM) and QCM-D and calculation of film thickness are used to determine the mechanism behind frictional dissipation of plant proteins (pea and soy) and compared with those of dairy proteins (sodium caseinate and whey protein isolate). Additionally, two high protein concentrations (10wt% and 20wt%) are compared to simulate high-protein fortified foods.

3.2. Materials and Methods

3.2.1. Materials

Whey protein isolate (WPI) was purchased from Bulk Powders.com (Colchester, UK) and sodium caseinate (NaCas) was purchased from Arcos Organics (Netherlands). Pea protein concentrate (PPc) and soy protein isolate (SPI) were kindly gifted by ADM (United States). The proteins were used without any further purification. 4-(2-hydrozyethyl)-1-piperazineethanesulfanoic acid (HEPES) buffer was purchased from PanReac AppliChem (Germany) and both bovine submaxillary mucin (BSM) and porcine gastric mucin Type II (PGM) were purchased from Sigma Aldrich (Dorset, UK). The mucins were used to replicate the lubrication behaviour of human saliva (Sarkar et al., 2019b). Polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Midland, MI, USA) base fluid and cross-linker (10:1w.w) was used in the QCM-D experiments to create PDMS-coated sensors to replicate the surfaces used in the tribological tests. The PDMS coating of silica coated crystals (QSX-303, Q-Sense) was performed in line with previous studies (Xu et al., 2020b). Ammonia solution (25%) and hydrogen peroxide solution (30%) were purchased from Fisher Chemicals (UK) and Sigma-Aldrich (Dorset, UK). MilliQ water purified by a Milli-Q apparatus (Millipore, Bedford, UK), with an electrical resistivity not less than 18.2MΩ cm was used to make HEPES buffer in this study before any protein dissolution.

3.2.2. Methods

3.2.2.1. Preparation of aqueous dispersions of plant and dairy proteins

Protein solutions were prepared by dissolving WPI, SPI, NaCas and PPc powders of various size, shape and polydispersity (see SEM images in **Figure 1.**) in 10 mM HEPES buffer and adjusted to human salivary pH (6.8) using 0.1 M NaOH. To ensure dissolution, the solutions were allowed to hydrate for a minimum of 2 h at room temperature (21°C). The protein concentration (10wt% and 20wt%) was based on manufacturer information of the protein concentration provided and calculated accordingly. Manufacturer information stated WPI, PPc, SPI and NaCas contained 97%, 88.6%, 87.9% and 92% protein, respectively. Concentration was kept constant as it is a common parameter for product design of high-protein foods and when animal proteins are replaced by plant proteins (Zembyla et al., 2021).

3.2.2.2. Preparation of model saliva

Model saliva was prepared following the protocol of Sarkar et al, 2009 (Sarkar et al., 2009). This contained 1.594 g/L NaCl, 0.328 g/L NH_4NO_3, 0.636 g/L KH_2PO_4, 0.202 g/L KCl, 0.308 g/L $K_3C_6H_5O_7H_2O$, 0.021 g/L $C_5H_3N_4O_3Na$, 0.198 g/L H_2NCONH_2, 0.146 g/L $C_3H_5O_3Na$, 3 g/L PGM or (BSM), made up to 1 lL with MilliQ water. This was stirred for 4h to allow for the salts to dissolve and protein dispersion-saliva mixtures were created at 4:1w/w ratio (Krop et al, 2019) simulating real oral processing conditions (de Lavergne et al., 2015). For rheology and tribology of protein+saliva mixtures, PGM was mainly used as the source of mucins to simulate oral processing where lubricating performance of saliva is often poor in stimulated (fed) conditions. Nevertheless, the lubricating

performance of model saliva containing PGM and BSM was similar across the lubrication regimes ($p<0.05$) (see **Supplementary Figure B.1.**).

Bovine submaxillary mucin (BSM) was used for QCM-D experiments to mimic the salivary pellicle (Xu et al., 2020b). Prior to its use, BSM was purified through dialysis and freeze drying as previously recommended (Xu et al., 2020b). Dialysis involved a 30mg/mL stock solution of commercially purchased BSM in 100kDa molecular weight cut-off membranes (Spectrum Laboratories, USA). The filled membranes were placed in a 1L beaker of Milli-Q water under constant magnetic stirring condition for 10 days. The Milli-Q water was replaced twice on day 1 (morning and afternoon) and hereafter once daily. After the dialysis, the purified BSM was extracted with a Pasteur pipette and freeze-dried before being stored at -20°C.

3.2.2.3. Zeta-potential

Protein solutions of 0.1wt% were placed into a folded capillary electrophoresis cell (DTS1070) and placed into the Zetasizer (Zetasizer Nano ZS instrument, Malvern Instruments Ltd., Worcester, UK). Within the cell, particles gravitate towards oppositely charged electrodes at certain velocities. This velocity can then be converted to zeta-potential (ζ) by using Henry's equation, $u_e = \frac{2\varepsilon_{rs}\,\varepsilon 0}{3\eta}\,\zeta f_1(\kappa\alpha)$. Where f_1 is the Henry function, κ is the inverse of the Debye screening length, α is the particle radius, and η is the viscosity of the solvent. The value of f (κα) is determined by the medium, the electrolyte concentration, and the size of the proteins. In aqueous protein dispersions, where κα ≫1,f(κα) was 1 according to Smoluchowski approximation.

3.2.2.4. Particle sizing

Protein solutions were diluted (1:1000w/w with buffer and filtered using a 0.22μm syringe filter (PTFE Syringe filters, PerkinElmer,USA) and placed in disposable cuvettes (PMMA, Brand Gmbh, Wertheim Germany). Mean hydrodynamic diameter (d_H) were measured using a Zetasizer Nano ZS Instrument (Malvern Instruments Ltd, Worcestershire, UK) via dynamic light scattering (DLS). The refractive index (RI) of proteins and buffer was set at 1.52 and 1.33, respectively, with an absorption value of 0.001. The samples were equilibrated for 120 s at 25°C and analysed using back-scattering technology at a detection angle of 173°.

3.2.2.5. Solubility

The concentration of soluble protein was determined using the DC protein assay kit (Bio-rad Laboratories, Hercules, Ca) using the Lowry method (Lowry et al., 1951). This incorporated a UV-Vis Spectrophotometer with an absorption wavelength of 750nm and Bovine serum albumin was used as a standard.

3.2.2.6. Scanning electron microscopy (SEM)

The microstructure of proteins were analysed using scanning electron microscopy (SEM). The sample was frozen in slush nitrogen using a Quorum Technologies PP3010 (Quorum Technologies Ltd., East Sussex, UK), cryo-system, then transferred to the precooled preparation chamber under vacuum and sputter coated with platinum. The sample was imaged using a Tescan

AmberX (Tescan, Brno, Czech Republic) dual beam SEM, under high vacuum while being kept at -140°C.

3.2.2.7. Shear rheology

Apparent viscosity of protein solutions and their mixtures with the model saliva were measured using a stress-controlled rheometer (Anton Paar MCR-302, Ostfildern, Germany). A 50mm-diameter circular cone−plate geometry was used, with an angle of 2°. Viscosity measurements were conducted in the range of 0.01−2,000s^{-1} at a constant temperature (37°C) to mimic oral conditions and flow curves were obtained. The gap was set to 1 mm and 350cSt silicone oil was used as a solvent trap in addition to a Peltier hood. The solutions comprised of model saliva and protein solutions (4:1 w/w ratio) was mixed for 5 minutes prior to viscosity measurements.

3.2.2.8. Oral tribology

The Mini Traction Machine (MTM2) from PCS instruments (UK) was used to measure rolling/ sliding friction coefficient- (μ) results using PDMS surfaces in presence of protein dispersions with/ without model saliva. The set-up used a PDMS ball (19mm diameter) on a PDMS disc (46mm diameter). The temperature was controlled at 37°C and a normal force of 2.0 N (~200kPa Hertzian contact pressure) was applied (Sarkar et al., 2019a). The μ values were measured as a function of entrainment speed (mm s^{-1}). This can be attained by $u = \frac{(U_B + U_D)}{2}$. U_B denotes the linear speed of the ball, and U_D denotes the linear speed of the disc at contact points in the direction of the fluid flow. The sliding to rolling ratio (SRR)

equates the proportions of either rolling or sliding. This is given by $SRR = \frac{[U_B - U_D]}{(U_B + U_D)}$. This was fixed at 50% with the entrainment speed from 0.1 to 1000mm s $^{-1}$. The results are presented in the form of as a function of entrainment speed (u) or the product of the limiting high-shear viscosity obtained at 2000s^{-1} (*i.e.*η_{2000}) and the u (i.e.η_{2000}×U). The cleaning protocol employed used between each experiment included sonication steps in sodium dodecyl sulphate (2wt% in DI water), IPA and DI water for 10min at each step and also ethanol after samples containing model saliva.

3.2.2.9. QCM-D measurements

Silica-coated sensors (QSX-303, Q-Sense) were coated with PDMS to allow for better comparison to tribological data which also used PDMS contact surfaces (Xu et al., 2020b). To coat surfaces the following protocol was used based on (Kew et al., 2021, Zembyla et al., 2021). 10wt% PDMS in toluene solution was prepared and stirred for 24h before being further diluted to 0.5wt% and again stirred 24h at room temperature. For removal of organic material and insoluble particles on crystals a RCA solution was prepared by using 5 parts deionized water, 1 part ammonia and 1 part aqueous hydrogen peroxide (30%). Silica sensors were then immersed in the cleaning solution on a heated stirring plate set to 80°C for 15 min. The crystals were then placed in ultrapure water and sonicated for 10 minutes three times before drying with nitrogen gas. To coat, the crystals were placed in a spin coater. 100mL of the 0.5wt% PDMS solution was pipetted onto the crystals and spin coated at 5,000rpm for 60s. Crystals were dried for 10min each again on an 80°C heated plate. Finally, crystals were transferred into a vacuum oven set to 80°C overnight.

After running the QCM-D experiments, crystals were then immersed in sulphuric acid for 30min and recoated following the above protocol. Prior to use on the day of using, PDMS sensors were cleaned by immersing for 30 s in toluene, then 30s in isopropanol and 2min in ultrapure water. The crystals were then dried with nitrogen gas, before being left for a minimum of 2h. This allowed time for all the solvent molecules to evaporate.

QCM-D was used to measure real time absorption behaviour of proteins. Both changes in frequency and dissipation are measured concurrently during adsorption onto the surfaces. Thus, it can provide data on absorption kinetics, mass, viscoelasticity, and adsorbed film thickness. Firstly, protein solutions alone (without any BSM) of 0.1mg/mL solutions were used to understand their adsorption behaviour. A peristaltic pump set to a flow rate of 100 mL/ min at 25°C pumped solutions into chambers containing the PDMS-coated sensors. HEPES buffer was first pumped over the surfaces for a minimum of 30 min or until when a stable baseline was observed. Following this, protein solutions were pumped into the system for 1h minimum or until when a stable baseline had been observed. Then the outer pump lines were rinsed with ultrapure water before the buffer was again injected into the chambers for a further 30 min.

For experiments using model saliva containing BSM, first mucin alone was measured. This was done by first injecting HEPES buffer followed by injecting 0.1mg/mL solution of BSM for a minimum of 1h or until a stable baseline was observed. Then the protein solutions were injected to coat the BSM-coated sensors the following the afore-mentioned protocol. HEPES buffer was injected into the system for 30 minutes or until a stable baseline had been observed. Next BSM was injected for a minimum of 1h or until a stable baseline had been observed. HEPES was injected for 30min to rinse away any unabsorbed mucin or proteins after each protein/ mucin injections. To analyse the data, Dfind software (Q-Sense, Sweden) and the 'Smartfit Model' was used and in most

cases fifth overtones of frequency and dissipation were used to fit the data to get the film thickness and the viscoelastic parameters.

3.2.2.10. Statistical analyses

Each sample was prepared in triplicate and measured at least three times with means and standard deviations reported unless otherwise specified. One way ANOVA was used to study the effect of protein source on the rheological properties and tribological properties. The significance of the differences among mean values of the samples were determined by Bonferroni test (with $p<0.05$) using SPSS software (IBM, SPSS statistics).

3.3. Results and Discussion

3.3.1. Physicochemical characteristics of protein dispersion

Firstly, we compare the physicochemical properties of the plant protein with the dairy protein dispersions. As one can appreciate from the morphology of the dry powders in **Figure 12.**, both the dairy proteins (NaCas, WPI) show spherical particles compared to the plant proteins (PPc, SPI), in particular PPc is irregular-shaped with sharp edges. The SEM images were similar to those obtained by

(Vlădescu et al., 2023). NaCas shows large, aggregated particles whereas WPI has much smaller particles. The measured solubility, hydrodynamic diameter (d_H) and ζ-potential of aqueous dispersions of all four protein dispersions are shown in (**Figure 12.**).

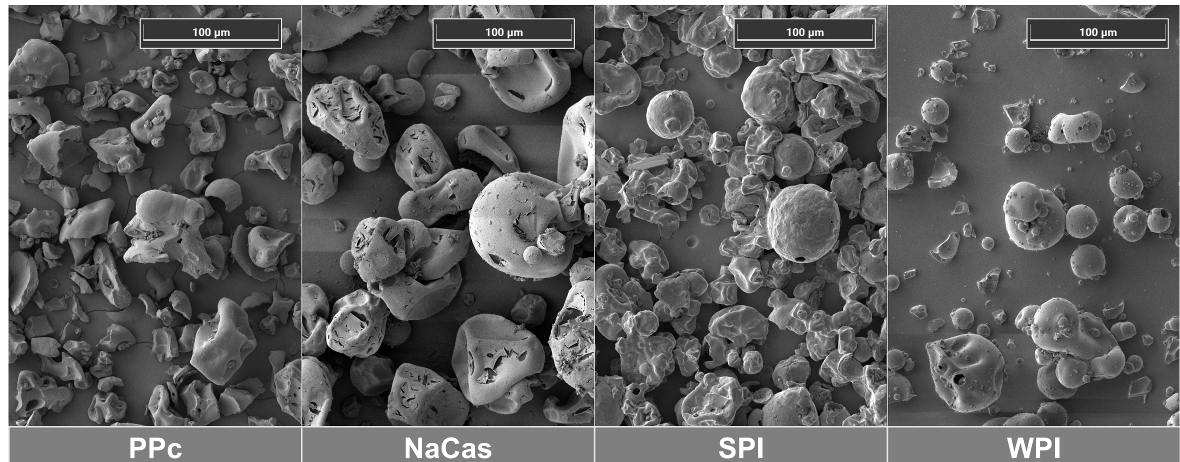


Figure 12. SEM images of the protein powders. Scale bar represents 100 µm.

As one might expect, both the tested plant proteins have considerably lower solubility than the dairy proteins ($p<0.05$) (Figure 13.a.). The lower solubility shown for plant proteins is in close agreement with previous literature (Kew et al., 2021, Kim et al., 2020) owing to the aggregation of these storage proteins. Protein solution aggregates differed in size, with WPI having the largest (366.0nm) and PPc having the smallest (81.1nm) d_H (Figure 13.b.). However, the particle size of protein samples should be taken with caution as the polydispersity indices are high (>0.25).

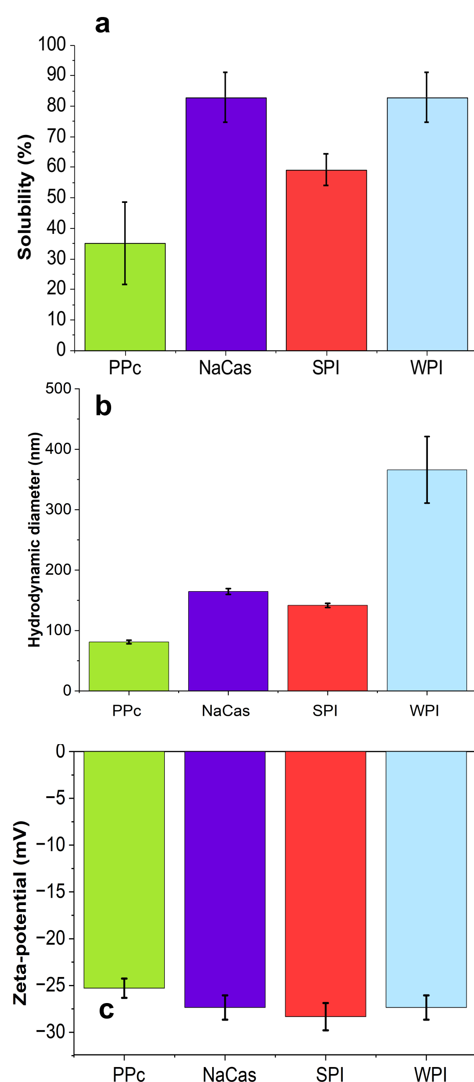


Figure 13. Mean solubility (a), hydrodynamic diameter (b) and zeta-potential (c) for the tested proteins. PPc refers to pea protein concentrate, NaCas sodium caseinate, SPI soy protein isolate and WPI whey protein isolate. Error bars represent standard deviations for triplicate measurements (n=3×3).

Further, DLS is biased to larger aggregates as this will obscure the light refracted from smaller particles (Maguire et al., 2018). All proteins used have isoelectric point (pI) of around 4.5 (Strange et al., 1993, Ma et al., 2009, Sumner et al., 1981, Wang et al., 2009, Pelegrine and Gasparetto, 2005) and thus the ζ-potential of all the proteins tested in this study at pH6.8 was negative (**Figure 13.c.**) as

expected and in agreement with previous reports (Khalesi et al., 2016, Freitas et al., 2017, Ladjal-Ettoumi et al., 2016).

3.3.2. Apparent viscosity

Unlike the dairy proteins (WPI and NaCas), the plant proteins show a much greater shear thinning tendency as shown in (**Figure 14.a.).** In particular, SPI shows three orders of magnitude decay in apparent viscosity whilst PPc shows one order reduction as a function of shear rates tested. Such shear thinning behaviour is not a surprise for SPI (O′ Flynn et al., 2021) and is also in line with the low solubility (**Figure 14.a.**). Shear thinning behaviour has previously been shown for plant proteins due to aggregation breaking in the direction of flow which has been shown previously for both soy protein (O′ Flynn et al., 2021) and pea protein (Zembyla et al., 2021).

Nevertheless, comparing the apparent viscosities at orally relevant shear rates ($50 s^{-1}$) (Wood, 1968, Ross et al., 2019), with the exception of SPI which had relatively large deviations between measurements, apparent viscosities were similar among the protein types ($p>0.05$) (see **Supplementary Table B.1.** for statistics). The addition of saliva to the protein solutions lowered the viscosity for PPc by 45.33% and by 50.74% for NaCa’s ($p>0.05$) (**Figure 14.b.**). For WPI and SPI, the viscosities remains similar despite mixing with saliva. As model saliva has a lower viscosity than all the proteins tested, a dilution effect may account for the lowered viscosity in PPc and NaCas solutions mixed with model saliva. The lack of such dilution effect for WPI and SPI may be compensated by interactions between SPI, WPI and mucins. Previous studies have reported that interactions between WPI and mucin increased formation of aggregates, which impacted flow behaviour (Ahmad et al., 2020a). In summary, the rheological analyses suggests that although the plant proteins show shear thinning behaviour, the apparent

viscosities were similar at orally relevant shear rates with/ without added model saliva.

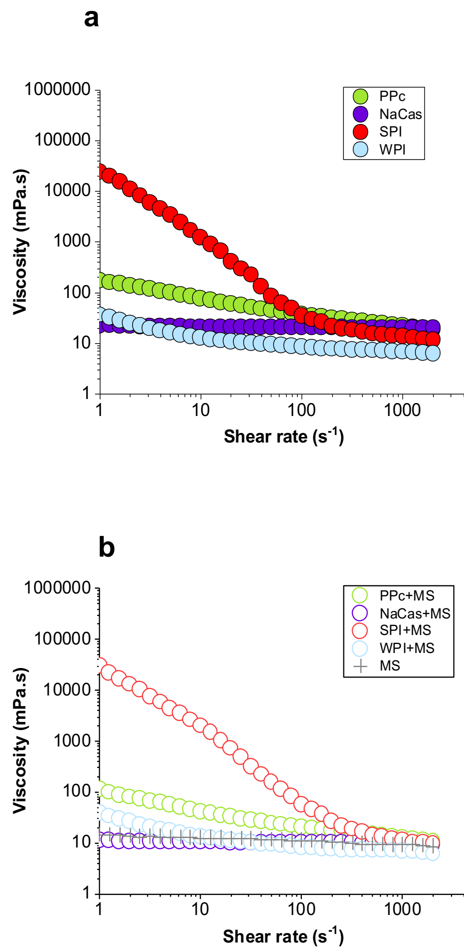


Figure 14. Mean apparent viscosities in presence of protein (a) and protein + model saliva (MS) mixtures (b) as a function of shear rate. Data are shown for three independent readings on triplicate measurements. PPc refers to pea protein concentrate, NaCas sodium caseinate, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be followed in Supplementary Table B.1. (n=3x3).

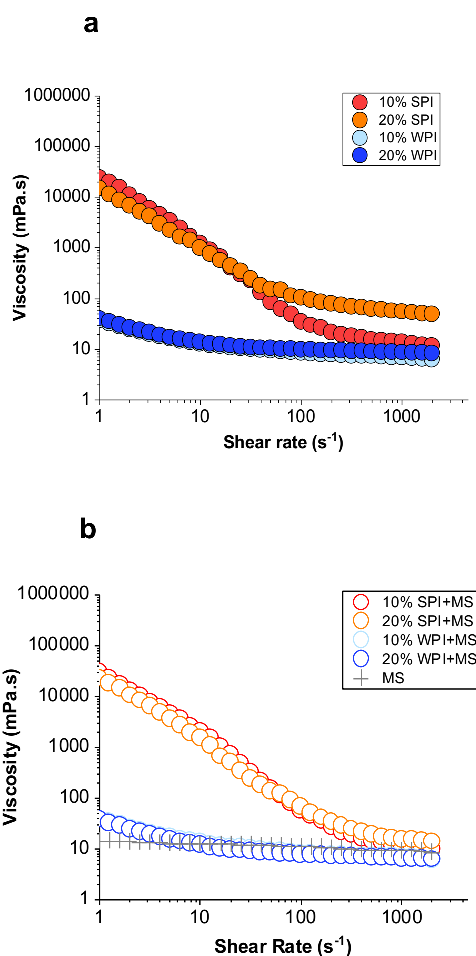


Figure 15. Mean apparent viscosities in presence of higher concentrations of protein (10-20 wt%) (a) and protein (10-20 wt%) + model saliva (MS) mixtures (b) as a function of shear rate. Data are shown for three independent readings on triplicate measurements. SPI refers to soy protein isolate, WPI refers to whey protein isolate and MS model saliva. Statistics can be followed in Supplementary Table B.1. (n=3x3).

Concentration is increased to 20wt% for SPI and WPI. Concentration is not increased for PPc or NaCa's due to their low solubility so they are unable to be mixed at these high concentrations. Increasing the concentration to 20wt% shows a significant influence on second-limiting plateau shear viscosities of SPI ($p<0.05$) (Figure 15.a.), which might affect the tribological performance, this is

discussed later. However, there is a small and insignificant increase of apparent viscosity for both WPI and SPI between 10wt% and 20wt% at orally relevant shear rates ($50s^{-1}$) in presence of saliva (**Figure 15.b.**) (see statistics in **Supplementary Table B.1.**).

3.3.3. Soft tribology

Figure 16. displays the friction curves obtained for 10wt% protein solutions and protein + model saliva mixtures. With increasing entrainment speeds, all samples reach mixed lubrication regime with a reduction in friction. All the protein samples tested with or without model saliva (**Figure 16.a.1.** and **Figure 16.b.1.**) show the boundary and the mixed lubrication regimes with most showing the onset of the elastohydrodynamic regime (EHL). The addition of proteins to the buffer shifts the onset of the mixed and the EHL regimes to lower *u* values suggesting the viscous-driven lubricity (Sarkar et al., 2021, Soltanahmadi et al., 2023, Shewan et al., 2020). At the boundary lubrication regime (*u*=5 mm/s), all protein solutions have lower friction than buffer with SPI showing the highest μ (~0.518), which is not statistically different to WPI ($p>0.05$) (**Supplementary Table B.2.**), whilst NaCas (μ~0.111) shows the lowest friction values (μ~0.111).

The similarity of PPc with WPI at 10 wt% ($p>0.05$) is in sharp contrast to previous literature looking at soluble fractions. Protein concentration may vary as sample preparation included centrifugation of solutions with insoluble proteins in the pellet removed. Notwithstanding this difference, the study reported WPI to have much lower boundary friction (0.05) compared to PPc (0.21) at 10wt%. This was attributed to pea protein increases μ between hydrophobic contact surfaces across entrainment speeds versus whey protein (Kew et al., 2021). However, the study reported similar boundary lubrication values (*μ)* were shown at low concentrations (1wt%) of (0.33 and 0.39), corresponding to results observed in this study. In addition, the tribometer used in this study only looked at sliding

friction. A separate study also using 10wt% concentrations of pea protein and WPI (again soluble fraction) reported WPI to have a boundary friction of 0.4. Whereas pea protein isolate (PPI), friction started at 0.62, which authors posited was the mixed regime. The high PPI friction was hypothesized to be due to PPI-aggregates causing particle-like behaviour. Interestingly, when PPI concentration was lowered to 0.1wt% friction was similar to 10wt% (Zembyla et al., 2021), due to PPI behaving like a polymer which has been shown to enhance lubrication (Zembyla et al., 2021, de Vicente et al., 2005). Whereas for WPI, at 0.1wt% friction (*μ)* was two-folds higher at 1.05, with authors suggesting WPI requires larger concentrations to saturate lubricated surfaces compared to PPc (Zembyla et al., 2021).

Looking at protein + model saliva mixtures (**Figure 16.b.1.**), we can see similar lubrication behaviour to the protein alone except for NaCas. NaCas had the lowest overall *μ* across entrainment speeds ($p<0.05$) (**Supplementary Table B.2.**) with smaller differences between the other proteins. Although lower than buffer, model saliva has the highest overall *μ* values across boundary and mixed regimes. It was hypothesized that mixing proteins with saliva would result in increased friction. The frictional curves for SPI and WPI remained similar with and without model saliva, which is in line with the behaviour observed in the viscosity curves at the high-shear rate limiting plateau. In general, the poor lubrication of model saliva has been previously attributed to a poor adsorption of PGM onto PDMS surfaces (Çelebioğlu et al., 2016). Nevertheless, lubricity of real stimulated saliva is often lower as compared to unstimulated saliva (Sarkar et al., 2019b) and hence the poor lubrication of PGM-containing model saliva in this study can be considered to be a reasonable representation of real oral processing in fed condition. In any case, the frictional profiles of BSM and PGM-containing model saliva are also comparedand there are no significant difference in the frictional curves (**Supplementary Figure B.1.**) highlighting that changing the mucin-type would probably not have affected the MTM tribometer-generated friction curves. In addition, the large error bars displayed for all protein solutions

have been previously attributed to insoluble particles which create 'noise' through their spontaneous tendencies (Vlădescu et al., 2023).

Recently, a novel dynamic tribology approach has been used to compare two pea proteins, two whey proteins and one casein interacting with human saliva (Vlădescu et al., 2023). Interestingly the dynamic set up exhibited different results for two different types of pea protein with greater binding affinity of one PPc over another. When comparing to results of this previous study, the good lubrication observed in the current study may be a result of the higher protein concentration (Vlădescu et al., 2023). However, the dilution effect observed in the present study harmonizes with the unchanged friction of human saliva (Vlădescu et al., 2023). Disparities in saliva mixing ratio should also be mentioned, the present investigation uses a 4:1w/w protein: saliva ratio whereas (Vlădescu et al., 2023) used 1.5mL human saliva followed by 30mL protein solution so friction results of the mentioned study will be more influenced by the protein solution than the saliva.

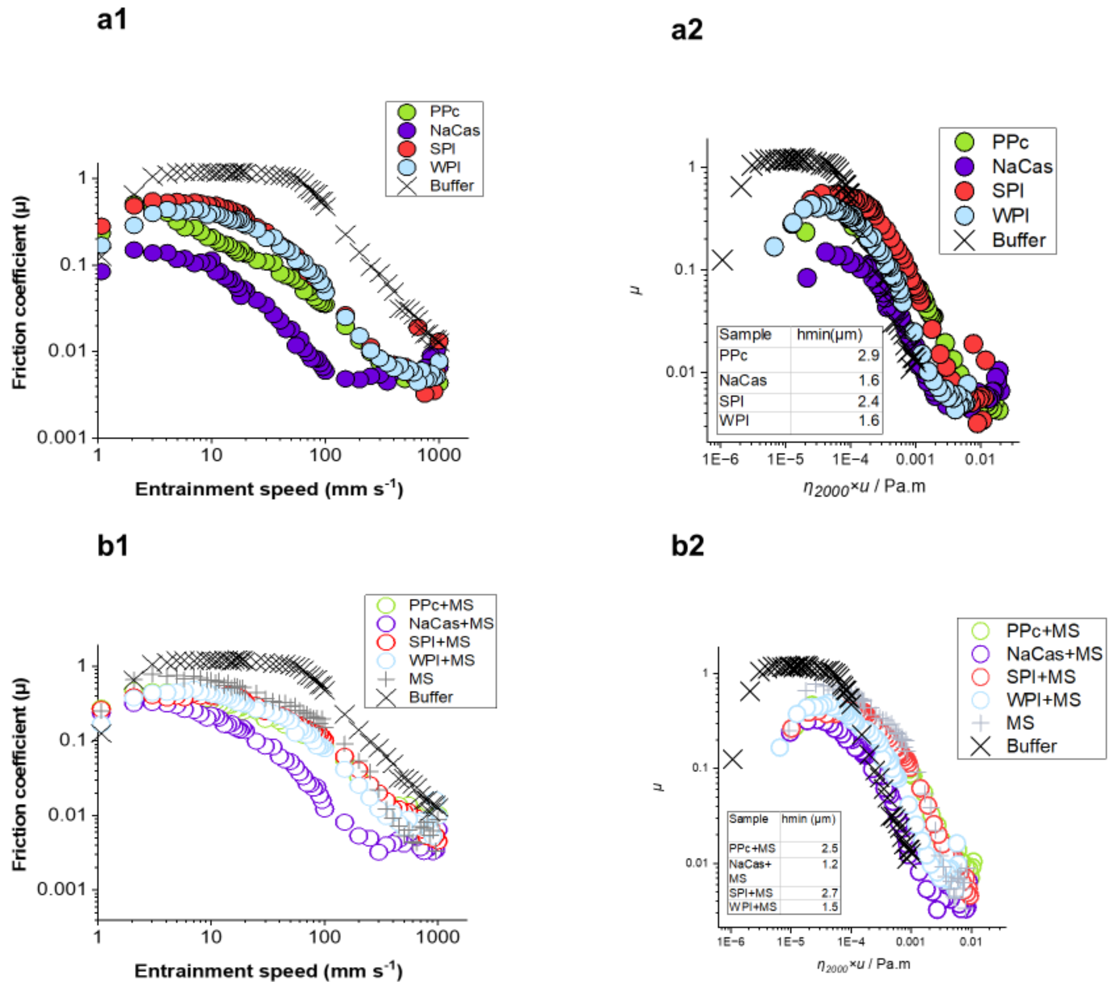


Figure 16. Mean friction coefficients in presence of protein (a) and protein + model saliva (MS) mixtures (b) as a function of entrainment speed (1) and as a function of entrainment speed × high shear rate viscosity (2). Data is shown for three independent readings on triplicate measurements. PPc refers to pea protein concentrate, NaCas sodium caseinate, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be found in Supplementary Table B.2. (n=3x3).

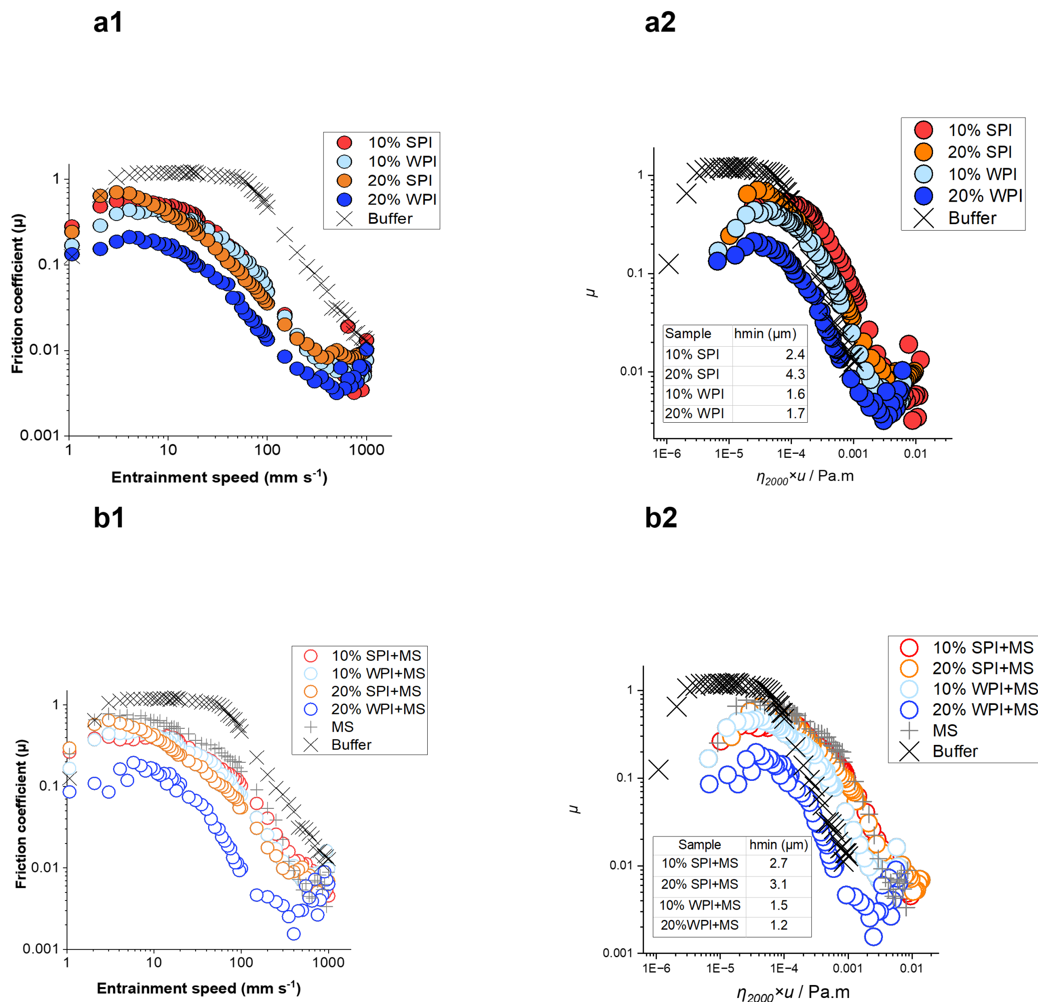


Figure 17. Mean friction coefficients in presence of higher concentrations of protein (10-20 wt%) (a) and protein (10-20 wt%) + model saliva (MS) mixtures (b) as a function of entrainment speed (1) and as a function of entrainment speed × high shear rate viscosity. Data is shown for three independent readings on triplicate measurements. SPI refers to soy protein isolate, WPI refers to whey protein isolate and MS model saliva. Statistics can be followed in Supplementary Table B.2. (n=3x3).

To further understand the frictional behaviour mechanisms of the proteins and pinpoint the importance of viscosity in such frictional data, the μ curves are scaled

to second plateau-shear viscosity *i.e.* as a function of reduced speed parameter *i.e.*, η_{2000}x*u* as reported previously (Soltanahmadi et al., 2022, de Vicente et al., 2005). When scaled to viscosity, NaCas still shows improved boundary lubricity compared to other protein types (**Figure 16.a.2.**), highlighting the importance of surface forces rather than fluid film lubrication. Interestingly, the PPc and SPI appear to overlap and show an onset of EHL at higher *u* and higher μ at the mixed regime compared to the master curve and WPI/ NaCaS curves. This suggests faster squeeze out or de-wetting of the lubricating film formed by plant proteins *i.e.,* PPc and SPI from the contact interfaces which is in agreement with the de-lubricating behaviour of plant proteins observed previously (Vlădescu et al., 2023). Although such differences at the boundary and mixed regimes diminished in presence of model saliva, but the trend was still the same (**Figure 16.b.2.**). This suggests that indeed all proteins studied here reduced the boundary friction but differed in their viscous lubrication capacity which influences the mixed lubrication regime.

Similarly, the *μ* curves were fitted as a function of film thickness (h_{min}) which also takes viscosity into account (**Supplementary Figure B.2.).** The h_{min} was almost 2x higher for plant proteins than those of dairy proteins irrespective of addition of model saliva (**Figure 16.a. and Figure 16.b.)** which means onset of EHL requires double the film thickness as compared to those for dairy proteins. This raises the questions that do plant proteins a) have thinner absorbed film on surface or b) are poorer in surface wetting and/or retaining properties, this is further discussed in the QCM-D results.

As anticipated, increasing concentration to 20 wt% (**Figure 17.a.1.)** reduces friction for WPI at lower boundary friction (speeds between 5-10mms^{-1}) by 55.93% and an order of magnitude lower in the mixed regime (speeds (100-150mms^{-1}) as compared to 10wt%($p<0.05$) (**Supplementary Table B.2.**). Such behaviour was restored in the presence of model saliva (**Figure 17.b.1.**). These findings are in line with previous literature, which suggests that increasing protein

concentration in dairy proteins leads to greater lubrication attributed to formation of surface films capable of separating the PDMS contact surfaces effectively (Liu et al., 2016, Kew et al., 2021). However, such behaviour is not observed for plant protein *i.e.* SPI, where increasing protein concentration did not give any benefit to lubrication performance in presence or absence of saliva. As mentioned previously, both NaCas and PPc were unable to form a solution higher 20wt% concentrations therefore were omitted from investigation.

When μ is plotted as a function of reduced speed parameter, η_{2000}xu, differences still remain in boundary friction for WPI ($p<0.05$) in the absence or presence of saliva (**Figure 17.a.2.** and **Figure 17.b.2.**). Interestingly, WPI at 20 wt% shows lower mixed μ values than the master curve (*i.e.* buffer) with lower h_{min} required for onset of EHL (**Supplementary Figure B.3.**), indicating improved wetting of the surfaces by WPI molecules and a reduced rate of squeeze out (Sarkar et al., 2021, Soltanahmadi et al., 2023, Shewan et al., 2020). However, for SPI, the μ curves collapsed irrespective of concentration or interaction with PGM. Overall, this highlights the importance of surface adsorption phenomena driving frictional differences between plant and dairy proteins, which is discussed in the following section.

3.3.4. Surface adsorption

Figure 7. shows the frequency shifts and consequently adsorbance of PPc, NaCas, SPI and WPI on PDMS-coated surfaces before and after the addition of BSM-containing model saliva as a base layer to mimic salivary pellicle in the latter (see the corresponding dissipation curves in (**Supplementary Figure B.5.**). For protein alone, after the injection of buffer, a stable baseline is measured with no sharp change in frequency. After all protein solutions are subsequently introduced, a sharp change in frequency is detected with the exception of SPI (**Figure 18.a.**). Although QCM-D is relatively new tool used for understanding

adsorption behaviour of food proteins, WPI is well studied. Previous reports showed a similar frequency shift (-20 Hz) with little change after rinsing with buffer (Zembyla et al., 2021). The change in frequency differed according to protein type (**Figure 18.a.**), with dairy proteins (WPI and NaCas) eliciting the greatest frequency change and therefore greatest adsorption onto the PDMS surfaces. On the other hand, the low frequency shift and dissipation for plant proteins (PPc and SPI) highlights poor adsorption of these proteins. It appears SPI is poorly adsorbed and rapidly desorbed (**Figure 18.a.**), which corroborates with the high friction observed across the entrainment speeds (**Figure 16. and Figure 17.**). Dairy proteins on the other hand show lower friction at the mixed regime for a given h_{min} which corroborates with the QCM-D data (**Figure 18.a.**) indicating that dairy proteins remain at the contact for longer due to the formation of a thicker adsorbed film, which subsequently reduces the overall surface roughness and prevents direct contact of the PDMS ball against the PDMS disc and also enables squeezing out (if any) at a lower rate than plant counterparts. Looking at the dissipation data it reveals that WPI forms the most viscous film whereas SPI forms a rather "dehydrated" film with minimal dissipation (**Supplementary Figure B.5.**).

It is important to compare the present results with a previous study that utilized soluble fractions of whey and pea protein isolate (Zembyla et al., 2021) using similar characterization techniques. When comparing WPI results are as expected, showing purified WPI (WPI_Sol) to have lower friction in low entrainment speeds than the WPI in the present study (Zembyla et al, 2021). Of more importance here is to compare PPc in the current study with soluble fractions of pea protein (PPI_Sol), as the former contains aggregates and shows limited solubility of ~30% (**Figure 13.a.**). As one might expect, unpurified PPc had an order of magnitude higher viscosity versus PPI_Sol (**Supplementary Figure B.4.a.**), irrespective of the shear rates tested, owing to the afore-mentioned pea protein aggregates present in PPc. In line with the flow behaviour, the hydrated mass was nearly two-times higher in PPc as compared to that of PPI_Sol (*$p<0.05$*) (**Supplementary Figure B.4.b.**) suggesting that PPc was able to form

a thicker adsorbed film on a hydrophobic surface. This in fact corroborates with viscosity-mediated friction reduction in PPc, which is slightly lower as compared to PPI_Sol in the mixed regime (**Supplementary Figure B.4.c.**). Nevertheless, a marked reduction in friction in PPc versus PPI_Sol is not observed (**Supplementary Figure B.4.c.).** One might hypothesize this to be associated with the surface roughness of the tribofilm formed by the aggregated PPc, which despite the higher hydrated mass and consequently film thickness (**Supplementary Figure B.4.b.**) did not reduce the friction significantly. To sum it up, the difference between PPc and PPc_Sol is largely associated with the viscous behaviour and roughness, latter needs further investigation in the future using sophisticated techniques such as atomic force microscopy to observe the film roughness.

BSM not PGM was chosen for QCM-D due to the inadequate adsorption observed using the same non-purified PGM had poor adsorption. Commercial PGM can contain impurities such as salts, other proteins, which may have contributed to the lack of adsorption shown (Lee et al., 2005). Prior investigation using both purified BSM and PGM demonstrated spontaneous adsorption to PDMs. Specifically, PGM adsorption was characterized by a tail-like structure, with a single end anchored to the surface. Whereas, BSM exhibited a loop conformation resulting in a more elastic film (Madsen et al., 2016). Considering BSM’s enhanced lubrication properties stemming from the higher elastic film formation, and increased reliability, it is recommended as the preferred choice for adsorption experiments (Sarkar et al., 2019b). BSM alone without salivary salts is chosen as BSM with salivary salts (detailed as model saliva) had poor adsorption (**Supplementary Figure B.6.).**

On adding BSM (**Figure 18.b.**), the initial frequency shift was lower and correspondingly the adsorbed mass was approximately 2mg/m^2 which was lower than previously reported using 10x the concentration (1mg/mL) of BSM (Zembyla et al., 2021) used for this study. Interestingly, the addition of this BSM pre-coating

supported SPI to adsorb more strongly. However, for all other proteins, presence of BSM depletes the proteins from the surface owing to mutually opposite charges of the proteins and the BSM at pH6.8 (see **Figure 13.c.**). This suggests that there might be some hydrophobic interactions between SPI and BSM, even electrostatic interaction between some positive patches of SPI and BSM cannot be ignored despite the overall net charge for both BSM and SPI being negative. Of more importance, such film adsorption by dairy proteins (WPI and NaCas), though reduced in magnitude (**Figure 18.b.**) in presence of BSM was still higher than the plant counterparts. Although QCM-D is a quiescent technique unlike dynamic tribology where films are continuously replenished between contacts during tribo-shear, our QCM-D data shows a clear mechanism of higher adsorption abilities of dairy proteins as compared to plant proteins (**Figure 18.b.**), corroborating the friction reduction in presence of the former (**Figure 16.b.**). Although it is apparent from the adsorption behaviour, it is imperative to understand the quantitative differences between plant proteins and dairy proteins in terms of viscoelasticity and film thickness.

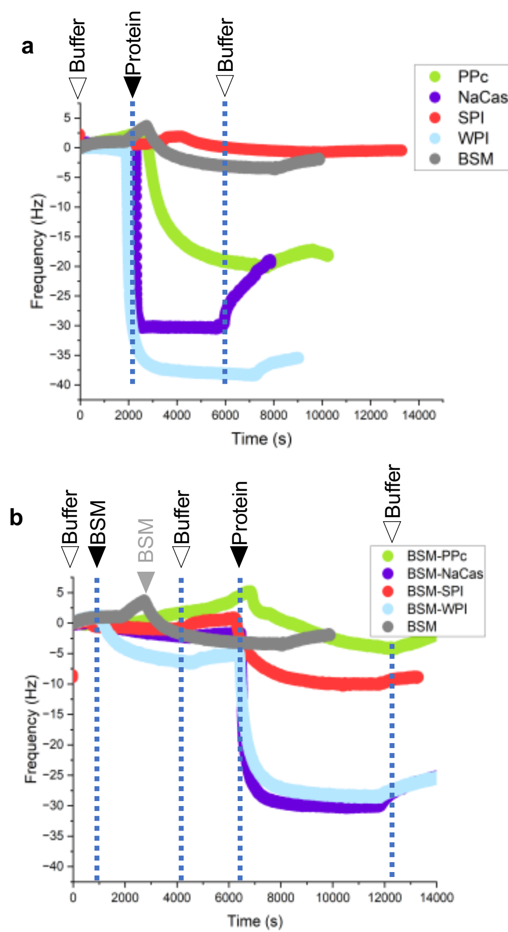


Figure 18. Frequency shift (5th overtone) as a function of time of a) 0.1mg/mL protein on PDMS-coated surfaces (a) and 0.1mg/mL BSM-coated PDMS surfaces (b) followed by addition of 0.1mg/mL protein on PDMS-surfaces. PPc refers to pea protein concentrate, NaCas sodium caseinate, SPI soy protein isolate, WPI whey protein isolate and BSM bovine submaxillary mucin. Measurements were repeated in triplicate (n=1x3). Dissipation shift is shown in Supplementary Figure B.

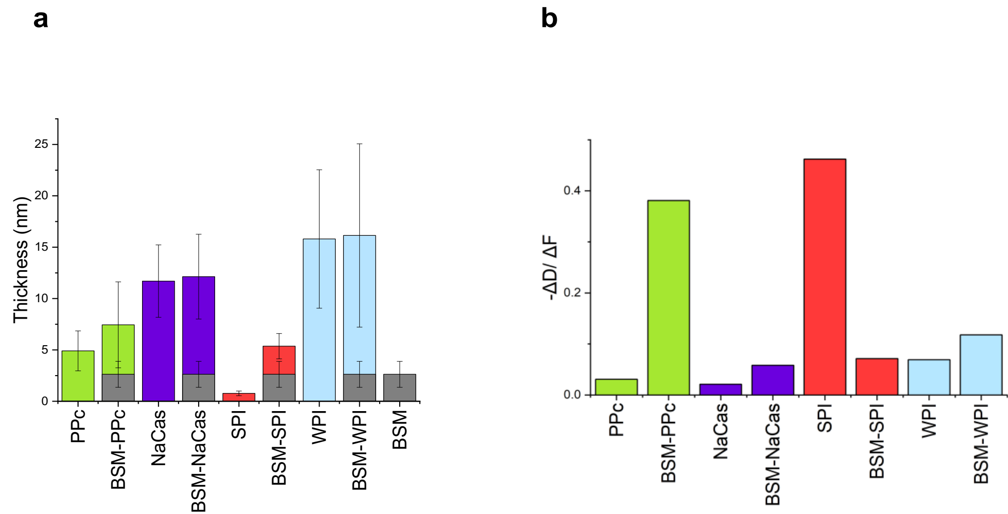


Figure 19. Fitted film thickness (nm) (a) and viscolelasticity i.e. dissipation/ frequency shift (–ΔD/ ΔF) (b) for all the protein samples. PPc refers to pea protein concentrate, NaCas sodium caseinate, SPI soy protein isolate, WPI whey protein isolate and BSM bovine submaxillary mucin. The average BSM alone value has been overlayed on BSM-Protein measurements to indicate the contribution of BSM to values obtained. Measurements were taken in triplicate (n=3), and error bars represent ± standard deviation.

Figure 19. shows the quantitative estimation of film thickness and viscoelasticity (-ΔD/Δf) by fitting the frequency and dissipation data with Voigt's viscoelastic model (see method section). As can be inferred from the aforementioned discussion, higher film thickness ($p<0.05$) is obtained from both NaCas and WPI compared to PPc and SPI with or without the BSM conditioning layer (Figure 19.a.). A higher –ΔD/ ΔF implies a more viscoelastic film (Xu et al., 2020b). It appears that both WPI and NaCas form elastic thick films (Figure 19.b.) that remained in the contact zone unlike the plant protein films, which adsorbed to a small extent. More importantly, both SPI and PPc formed thin viscous films which is depleted from the contact region easily while shearing results in such high friction as observed in **Figure 16.** and **Figure 17.** and consequently high h_{min} to achieve EHL.

3.4. Limitations

Limitations include the test conditions; proteins were analysed at neutral pH however when proteins are embedded within a food matrix pH can be lower. In addition, food was cooked, the behaviour of the proteins may again change. Therefore, behaviour observed in this study may only apply to a narrow range of conditions with further work required in a wider range of contexts. Looking at how protein is actually consumed as present results may only reflect a narrow range of 'protein food products' therefore looking at more complex systems may find different results.

3.5. Conclusions

To summarize, unpurified plant proteins, at neutral pH, tested in this study had low solubility, increased aggregation and showed greater shear thinning behaviour than dairy proteins. The dairy proteins, in particular sodium caseinate had excellent lubrication ability with a low boundary friction. Scaling tribology data to viscosity highlighted higher friction in plant proteins, which does not diminish with increased protein concentration with or without saliva unlike dairy proteins. Adsorption analysis reveals that such low friction in dairy proteins might be attributed to formation of an elastic boundary film with high film thickness that was capable of bearing the load in the hydrophobic contact surfaces unlike the plant proteins, latter had limited adsorption to hydrophobic contact surfaces. Although the addition of saliva does not appear to change the lubrication ability between protein types, adsorption analysis show that precoating with saliva aided adsorption particularly for soy protein. To conclude, a combination of tribology with adsorption techniques and rheology offers a powerful approach to identify differences between plant and animal proteins. Overall, our results suggest that SPI in the tested conditions, has very poor lubrication performance with PPc having slightly superior lubricity among the two plant proteins tested. More

importantly, careful formulation engineering is needed if larger concentrations of dairy proteins are to be replaced with plant proteins to reduce high friction and insolubility which can impair mouthfeel. Ongoing work is focusing on understanding the behaviour of these proteins when added in emulsion stabilizing liquid-liquid interfaces as well as sensory properties of these proteins when added in food matrix.

Chapter 4. Comparing emulsion characteristics and lubrication behaviour of plant vs dairy protein emulsions

Abstract

The aim of this chapter is to understand the lubrication and material properties associated with different proteins when added as emulsifiers to oil-in-water emulsions. Oil-in-water emulsions containing 20wt% sunflower oil and 5wt% protein were prepared using high pressure homogenization. Two dairy proteins (skimmed milk powder, SMP, and whey protein isolate, WPI) are chosen in conjunction with two alterative proteins (pea protein concentrate, PPc, and soy protein isolate, SPI). Emulsion characteristics and material behaviour are compared to 5wt% protein dispersions as a reference. The results indicate plant protein-stabilized emulsions exhibited poor kinetic stability, which is likely due to their limited solubility, cause proteins to aggregate instead of forming a thick adsorbed layer at the interface. SMP also shows poor stability, whilst WPI remained stable even after 1 week of storage. All the kinetically unstable emulsions (PPc, SMP and SPI) display shear thinning behaviour. At orally relevant shear rates, PPc has significantly higher viscosity than all other emulsions, potentially due to a high degree of insoluble protein aggregation and droplet flocculation. Conversely, WPI exhibits notably lower viscosity irrespective of shear rates. Lubrication performance varies between the protein types. As compared to proteins in solution, the lubrication behaviour in emulsions is dominated by the oil phase in all emulsions. WPI, SMP and SPI-stabilized droplets creating a lubricating coalesced oil film in the contact region, accelerating the onset of mixed lubrication regime at very low speeds (<5mm/ s). In contrary, PPc shows a boundary regime and delayed onset into the mixed regime. It is hypothesized PPc aggregates in the continuous phase is somehow jamming the contact and interfering with coalesced oil film (if any) to form a continuous

lubricating film. Overall, this suggests that plant protein in the continuous phase may affect lubrication performance despite presence of oil in model emulsion system.

4.1. Introduction

Within food science, research on emulsions is synonymous with protein. Protein has gathered significant attention due to high desirability and natural emulsifying ability (Hinderink et al., 2021). Emulsions are described as unstable colloidal systems composed of at least two immiscible liquids. One liquid is dispersed as small droplets within the other liquid, forming the continuous phase through external shear energy (Anvari and Joyner, 2017). Emulsions are present in a wide range of every day applications including food products (Silletti et al., 2007b). Most commonly for food, depending on the dispersant phase emulsions are either oil in water (O/W) or water in oil (W/O) mixtures. Common examples are milk, creams, mayonnaise, soup (O/W) and margarine (W/O) (Lam and Nickerson, 2013). More intricate systems may also be produce including multiple emulsions e.g., oil in water in oil (Ma and Chatterton, 2021). Emulsions are inherently unstable meaning they will destabilize and reorder into a lower energy state (Anvari and Joyner, 2017, Ma and Chatterton, 2021). Therefore, it is imperative to create emulsion structures that not only satisfy sensory perception but also exhibit sufficient stability for commercial feasibility (Anvari and Joyner, 2017) and optimization of shelf-life (Galani et al., 2023).

Proteins can play pivotal roles in structure stabilization and formation of emulsions at colloidal scale. Due to proteins amphiphilic nature, meaning they contain both a hydrophobic and a hydrophilic component, proteins are natural emulsifiers (Hinderink et al., 2021). Commonly used proteins include WPI, casein, ovalbumin and soy (Lam and Nickerson, 2013). A protein’s physicochemical properties will affect their ability to stabilize an emulsion and therefore are important to take note of. These include surface hydrophobicity, which influences

proteins adsorption onto oil, with greater hydrophobicity allowing for better stabilization (Lam and Nickerson, 2013). Similarly, higher protein solubility increases the capacity of protein adsorb at the interface which enhances stability of the interfacial layer (Hailing and Walstra, 1981, Millqvist-Fureby et al., 2001). As proteins are amphoteric, containing both positive and negative charges, pH is another important consideration (Amine et al., 2014). When pH is around the isoelectric point of a protein, meaning overall net charge is near zero, proteins have compact globular structures and aggregation is more likely to occur, leading to instability (Amine et al., 2014, Lam and Nickerson, 2013). Alternatively, at pH's above this, proteins partially unfold and acquire negative charges (Amine et al., 2014). The repulsion between oil droplets will be greater so emulsion stability is naturally improved (Lam and Nickerson, 2013). It should be noted that the two phases within an emulsion (the dispersed and the continuous) will naturally move towards a low energy state which is often phase separation. With time, any protein being used as an emulsifier will also change as non-covalent bonds may be formed, reducing emulsion stability (Lam and Nickerson, 2013).

Dairy proteins have been a traditionally popular choice of protein emulsifier. This popularity is due to industrial versatility and accepted taste and therefore dairy protein-emulsions exist in a wide range of common foods such as soups, sauces, confectionary and baking (Brown et al., 2021, Kim et al., 2020, Oliveira et al., 2022). As mentioned in previous chapters, consumers and food manufactures are under increasing pressure to turn to legume proteins with lower carbon cost (McClements, 2020). As a proteins structure denotes its ability to stabilize emulsions, it is important to the structural differences between plant and dairy proteins. Plant proteins are typically storage proteins, held together with disulphide bonds and can be less flexible than dairy proteins (Day et al., 2022). When a protein is at the interface between oil and water, it often needs to partially unravel to expose the buried hydrophobic amino acids to the surface. Therefore, plant proteins inflexibility may hinder their emulsification ability. Plant proteins additionally tend to have lower solubility compared to dairy proteins which may also affect their ability to adsorb on the interface (Nishinari et al., 2014).

Gaining a comprehensive understanding of the relationship between different types of proteins and emulsions in relation to mouthfeel is important for the development of novel foods. Previous studies have utilized mechanistic techniques such as rheology and tribology (Fuhrmann et al., 2019). These properties are influenced by the volume fraction of oil and size of oil droplets. It has previously been demonstrated that increasing the oil volume fraction leads to increased viscosity, related to desirable fat-related sensory attributes (Fuhrmann et al., 2019). Although research on legume proteins and emulsions is growing, there remains a lack of comparative studies among protein focussing on sensory perception. This work aims to address this research gap by investigating two dairy and milk proteins, whey and skimmed milk powder, in comparison to two legume proteins, pea and soy.

4.2. Methods

4.2.1. Materials

The o/w emulsions containing 20wt% sunflower oil were prepared using the following procedure. The emulsions were stabilized by either 5wt% SMP, WPI, PPc or SPI. Sunflower oil (SF), purchased from a local supermarket, the Co-operative, and was used at 20wt%. Pea protein concentrate (PPc) and soy protein isolate (SPI) were kindly provided by Archer Daniels Midland Company (ADM, Decatur, Illinois), the skimmed milk powder (SMP) was kindly obtained from Lactalis (Lactalis Industrie, Bourgbarre, France) and the whey protein isolate (WPI) kindly from Sachsen Milch (Sachsen Milch, Saxony, Germany). The PPc was composed of 82.9%w.w, SPI 88.10% protein, WPI 92.84% protein and SMP 32.4% protein information was provided by the manufacturers. Sodium azide was added at 0.03%w/w to prevent microbial growth.

4.2.1.1. Bulk solution preparation

Aqueous protein solutions were used to compare against emulsions. These were prepared by dissolving WPI, SPI, and PPc powders in Milli-Q and hydrated for 2h at room temperature (21°C) with stirring at 500rpm. The protein concentration of 5wt% was based on the manufacture information provided on protein content. Protein information detailed PPc was composed of 82.9%w.w, SPI88.10% protein and WPI92.84% protein.

4.2.1.2. Emulsion preparation

To prepare the emulsions, the protein powder was dissolved in Milli-Q water (purified by Milli-Q apparatus, Millipore Crop., Bedord, MA, USA), which served as the continuous phase. This was obtained through continual stirring at room temperature for 2 hours. The pH of the solution was adjusted to 6.7 when necessary. Next, these protein suspensions were pre-mixed with oil using a rotor-stator type mixer (Silverson Shear Mixer, L5M-A, UK) at 5,000rpm for 3 minuets. This pre-emulsion mixture was subsequently homogenized using a two valve Panda homogenizer (Panda Plus 2000l, Niro Saovi Homogeneizador Parma, Italy) for 3 passes at a pressure of 300/50 bar. Emulsions were analysed through size, rheology, tribology on the same day. The final concentration was 5wt% protein, 75wt% water and 20wt% oil.

4.2.1.3. Preparation of model saliva

Bulk solutions were mixed with model saliva containing bovine-submaxillary mucin. Emulsions were mixed with model saliva containing porcine-gastric mucin. Both PGM model saliva and BSM model saliva were prepared using the same protocol detailed in **2.2.2. Preparation of model saliva**. As detailed in **Chapter**

3, BSM and PGM model saliva showed comparable lubrication results. Emulsion/saliva mixtures and solution/saliva mixtures were prepared by mixing the required amount of emulsion/solution with model saliva 4.1.

4. 2.1.4. Zeta-potential

The Zeta-potential of protein solutions were measured using the same protocol detailed in **Chapter 3 (2.2.3. Zeta-potential).** Briefly, 0.1wt% solutions were placed into a folded capillary electrophoresis cell (DTS1070) and placed into the Zetasizer (Zetasizer Nano ZS instrument, Malvern Instruments Ltd., Worcester, UK).

4.2.1.5. Solubility

Solubility was calculated as the percentage of soluble protein content compared to the total protein content. All 1wt% protein stock solutions were analysed before and after centrifugation using the Bradford dye-binding method. The solutions were centrifuged (Hettich Zentrifugen, Rotina 380R Germany) at 4,000rpm for 10 minutes and the concentration of the supernatant was measured. 30mL of sample was combined with 1500mL of Coomassie brilliant blue G-250 reagent, stirred well and left for 10 minutes. The absorbance was measured at a wavelength of 595nm using a spectrophotometer (Jenway 6715 UV/Vis, USA). Bovine serum albumin (BSA) was used to make the standard curve at 0mg/mL, 0.2mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1mg/mL.

4.2.1.6. Droplet size distribution

The droplet size distribution was analysed using light scattering techniques (Mastersizer, 2000, Malvern, Worcestershire, UK). To determine the size

distribution, surface-averaged diameter ($d_{3,2}$) and volume-averaged diameter ($d_{4,3}$), the samples were diluted in demineralised water and analysed by laser diffraction. To calculate the particle size distribution in accordance with the Mie theory a refractive index of 1.469 (sunflower oil) was used.

4.2.1.7. Stability

For stability, sodium azide was added to prevent microbial growth and emulsions were either stored at room temperature (21°C) or at 4°C in a refrigerator. Photographs of the emulsions were taken using a mobile phone camera to evaluate phase behaviour and assess storage stability.

4.2.1.8. Rheology

Apparent viscosity of emulsions and their mixtures were measured using the same protocol detailed in **2.2.7 Shear rheology**. To summarize a stress-controlled rheometer (Anton Paar), with a 50mm-diameter circular cone-plate geometry was used to conduct viscosity measurements in the shear-rate range from 0.01-2000s^{-1}. The physical-chemical characterisation of the emulsions was performed again on the same day to eliminate time variability.

4.2.1.9. Tribology

The tribological performance of emulsions and their mixtures with model saliva was measured in accordance with **2.2.8 Oral tribology**. Briefly, hydrophobic PDMS ball and discs were with a rolling/ sliding friction coefficient (μ) with a normal force of 2.0N using a Mini Traction Machine (MTM2). The physical-

chemical characterisation of the emulsions was performed on the same day to eliminate time variability.

4.2.1.10. Statistical Analysis

Each sample was prepared in duplicate and measured at least three times with means and standard deviations reported unless otherwise specified. One way ANOVA was used to study the effect of protein source on the rheological properties and tribological properties. The significance of the differences among mean values of the samples were determined by Bonferonni test (with $p<0.05$) using SPSS software (IBM, SPSS statistics).

4.3. Results and Discussion

4.3.1. Protein Characteristics

As different sources of proteins were used compared to **Chapter 3** (3.1. Physicochemical characteristics of protein dispersion), physical characteristics are reanalysed as they have been shown to vary between different manufacturers (Carter and Drake, 2021). As expected, and in accordance with **Chapter 3**, plant proteins had lower solubility. PPc had the lowest at 30.3%, then SPI 33.4% whereas SMP had 63.7% with WPI 93.0%, (**Figure 20.a.)**. In accordance with **Chapter 3**, all proteins again have a stable negative charge in near neutral pH's (**Figure 20.b.**). The negative charge should improve the emulsifying ability of proteins as it will enable electrostatic repulsion and proteins to partially unfold and adsorb at the oil/water interface (Amine et al., 2014).

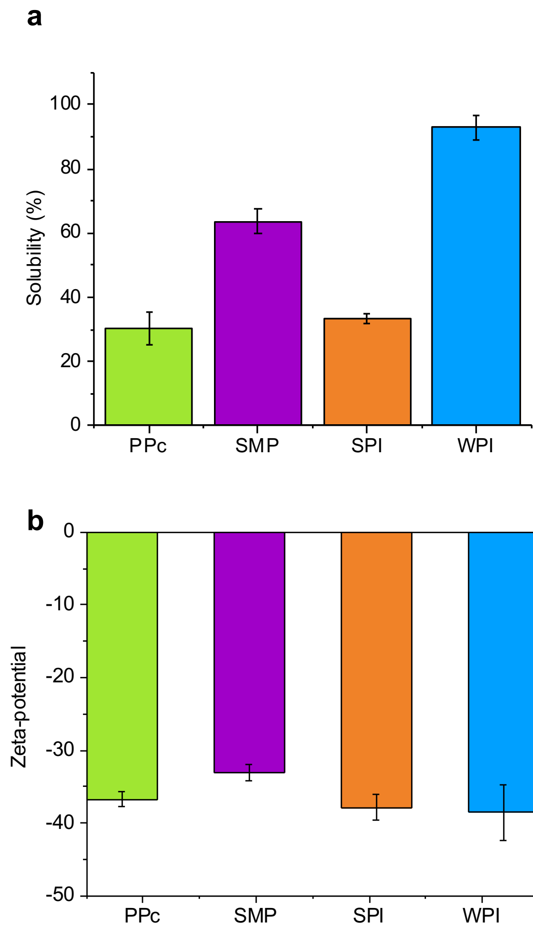


Figure 20. Mean solubility (a), and zeta-potential (b) for the 0.001wt% aqueous protein solutions. PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate and WPI whey protein isolate. Error bars represent ± standard deviations for triplicate measurements (n=3×3).

4.3.2. Emulsion Characteristics

Next, the characteristics of the emulsions are discussed. **Figure 20.** shows the particle size distribution of the emulsions. Throughout, the average surface area weighted diameter ranges from 1µm to 1000µm. As shown in **Figure 21.**, the average diameter for each protein type appears not to change much between the

measurements taken (0h, 48h and 1 week). WPI has a unimodal distribution and the smallest average surface diameter out of all emulsions. This small droplet size and narrow distribution is indicative of a kinetically stable emulsion (Burger and Zhang, 2019). In comparison legume emulsions, PPc and SPI and SMP shows a bimodal distributions, indicating flocculation or coalescence.

These results are in accordance with previous investigation of 5wt% protein o/w emulsions with a lower lipid concentration phase of 10wt% (lipid, medium chain triglycerides) at pH7. The investigation reported the WPI emulsion to have an average size of 170.4µm, which was smaller than SPI and PPc (Amine et al., 2014), as observed in this study. Regarding SMP, the bimodal distribution and wider particle range observed is also previously reported. This trend suggests there are distinct populations with different sizes which could be due to either flocculation or coalescence (Euston and Hirst, 1999). The bimodal distributions for legume emulsions is again previously reported in several studies using soy-protein and pea protein in o/w emulsions (Ningtyas et al., 2021, Kutzli et al., 2021, Chen et al., 2019, Amine et al., 2014, Shao and Tang, 2014). The large size of SPI is reported in similar investigation using 5wt% protein and 10wt% lipid o/w emulsions at pH7. The investigation reports SPI to be nearly 4x the size of WPI at 675.3µm vs 170.4µm, and larger than pea at 251.9µm(Amine et al., 2014).

The wide range of particle sizes for legume emulsions is linked to the low solubility of these plant proteins. Low solubility increases protein aggregation, which can lead to incomplete coverage at the interface (Nishinari et al., 2014, Ningtyas et al., 2021). Additionally, the variance in particle sizes can be influenced by electrostatic forces causing repulsion between protein particles, as well as inadequate flexibility of proteins to effectively stabilize emulsions (Ningtyas et al., 2021, Nishinari et al., 2014). In contrast, high solubility is advantageous as it allows a protein to migrate quickly to the oil/water interface as well as giving the protein greater flexibility to rearrange the interfacial film (Burger and Zhang, 2019).

Most emulsions do not maintain their stability despite the relatively minor size fluctuations as observed in the stability images shown in **Figure 22**. In detail, following a 48-hour period, similarities in appearance between WPI and PPc are noticeable when stored both at room temperature and in the refrigerator. Notably, there are distinct phase separation observed for SMP, with SPI similarly beginning to separate. After 72h, samples, specifically PPc, showed a pronounced boundary which are indicative of flocculation processes. Meanwhile, WPI had the best stability, which only began to separate at 2 weeks (room temperature) through what appears is creaming.

The poor stability of SMP may be due to its components as well as its low solubility (**Figure 20.a.**). SMP exists as a blend of milk proteins, mainly whey protein and non-aggregated casein. For SMP stabilized emulsions it is suggested whey protein competes against aggregated caseins which can affect stability. The caseins often dominate adsorption to the surface due to their greater flexibility and surface activity (Euston and Hirst, 1999). In addition, the amount of SMP added was matched to PPc, it contained 1.96wt% protein, which was lower than the other emulsions used. It has previously been stated that when there is insufficient protein compared to oil bridging flocculation can occur (Ma and Chatterton, 2021).

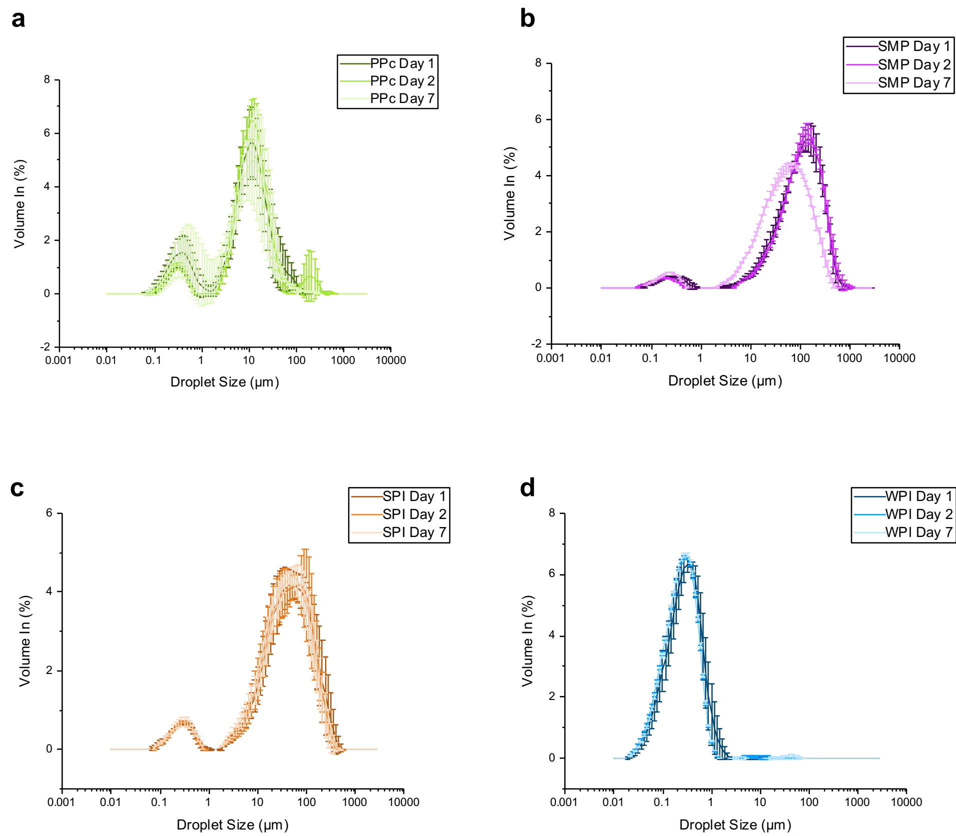


Figure 21. Mean particle size distribution of 5wt% protein oil (20wt%) in water emulsions after 0h, 48h and 1 week. A) represents PPc, pea protein concentrate, stabilized emulsions, b) SMP, skimmed milk powder, c) SPI, soy protein isolate and d) WPI, whey protein isolate. Error bars represent ± standard deviations of two repeated triplicate measurements (n=2x3).

Figure 22. Photographs showing 0h, 48h, 72h, 1 week and 2-week storage of protein emulsions. Left column shows storage 21°C, right column shows storage at 4°C.

4.3.3. Rheology

Figure 23.a.1. shows the apparent viscosity (η) of bulk-protein solutions and **Figure 23.b.1.** emulsions with and without model saliva (**Figure 23.a.2.** and **Figure 23.b.2.**). Looking at 5wt% protein solutions first, the viscosities are consistent with those data acquired in **Chapter 3** at the higher protein concentrations of 10wt and 20wt% (**3.2. Apparent viscosity**). Whereby dairy proteins, WPI and SMP, show low shear dependence and plant-proteins, SPI and PPC, had high shear dependence. In addition, at orally relevant shear-rates (50s^{-1}). SPI again has a significantly higher viscosity than all other proteins (p<0.05) (**Supplementary Table C.1.)**. When bulk solutions are mixed with model saliva

(BSM) there is no change in pattern of viscosity between protein types, with SPI again having higher viscosity (see **Supplementary Table C.2.**).

Next emulsions are compared with sunflower oil as a reference using data obtained from a similar investigation (Torres et al., 2018). The data reported shows sunflower oil to have limited shear-dependence. WPI also exhibited limited shear dependence, whereas SMP, SPI and PPc all display shear thinning behaviour (**Figure 23.b.1.**). Similar flow characteristics have been reported for both soy and pea o/w emulsions containing 2-4% protein (Ningtyas et al., 2021). At orally relevant shear rates ($50s^{-1}$), between protein types, WPI has significantly ($p<0.05$) lower viscosity than all other emulsions, specifically 92.62% lower than PPc (**Supplementary Table C.2.**). Contrastingly, the PPc emulsion has significantly ($p<0.05$) higher viscosity than all other emulsions. After mixing with model saliva, all emulsions have significantly lower ($p<0.05$) viscosity as expected (**Supplementary Table C.2.**). In the case of the WPI emulsion, the addition of saliva does not seem to affect its flow behaviour. Whereas, mixing

legume emulsions with model saliva reduces the decay rate in shear thinning behaviour (e.g. reducing the order of magnitude).

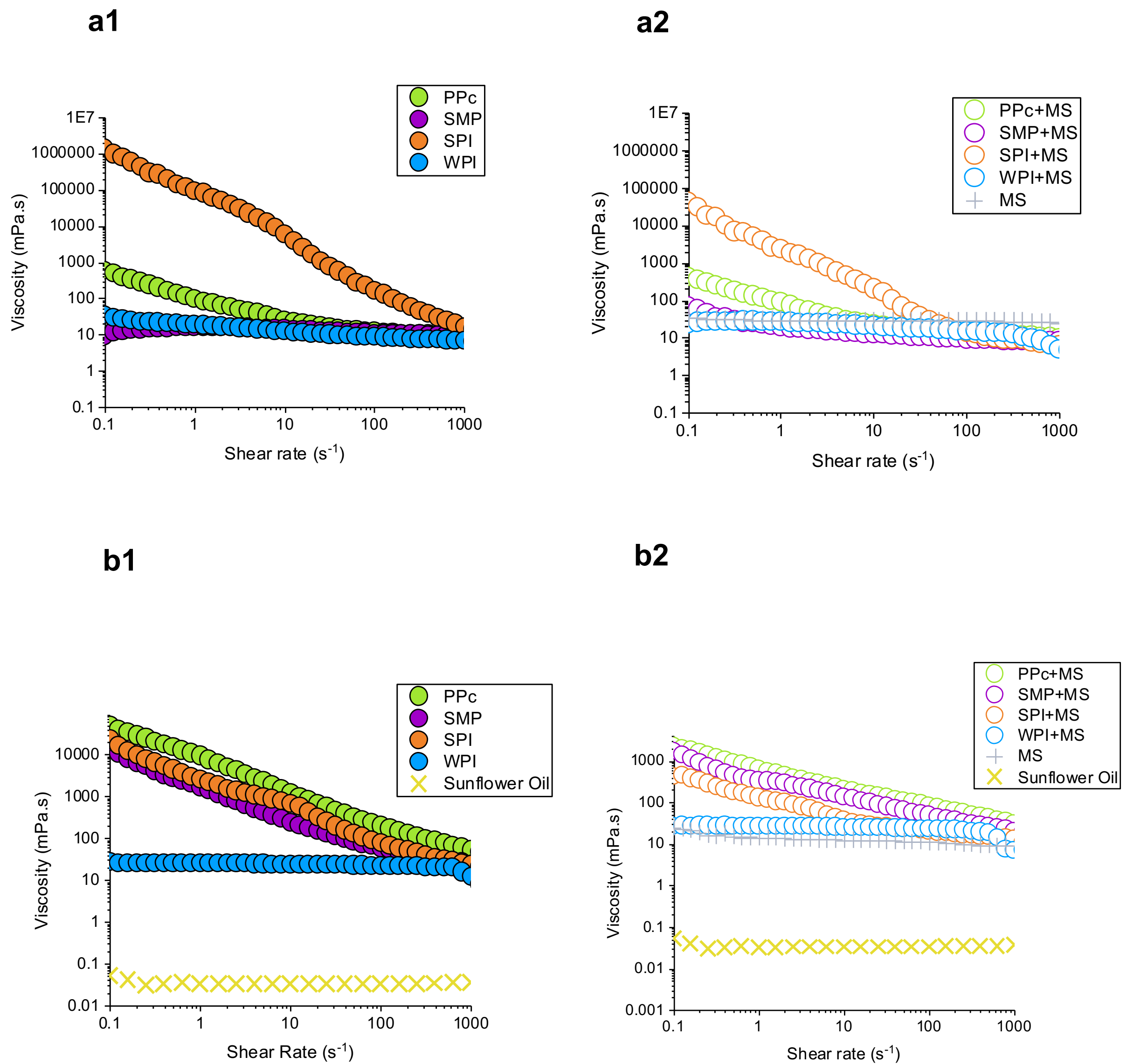


Figure 23. Mean apparent viscosities of aqueous 5wt% protein solutions (a) in the absence (a1) or presence (a2) of model saliva (MS). Mean apparent viscosities of 5wt% protein-enriched emulsions (b) in the absence (b1) or presence (b.2.) of model saliva (MS). PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be found in Supplementary Table C.1. and Supplementary Table C.2. (n=2×3).

4.3.4. Tribology

Figure 24. displays the friction curves obtained for protein solutions and protein + model saliva mixtures. With increasing entrainment speed, all samples reached the mixed lubrication regime which is characterized by a reduction in friction. WPI, SPI and PPc solutions are all more lubricating than the buffer as expected and in accordance with **Chapter 3** results (for details see **3.3 Soft tribology**). Interestingly, however the 5wt% SMP solution, which contains less protein (1.96wt%), is unable to form a lubricating film, which results in a significantly higher friction than other proteins ($p<0.05$). This is surprising, as even at low concentrations such as 0.1wt% both plant and dairy proteins have been shown to have lubricating effect compared to buffer/water (Zembyla et al., 2021). Therefore, the lack of lubricating film for SMP is likely due to SMP protein characteristics and not lower protein concentration. In addition, SPI appeared to have an absence of a boundary regime. This has previously been shown for pea protein at 5wt% and was indicative of effective viscosity separating surfaces (Zembyla et al., 2021). The effect of model saliva however was consistent with **Chapter 3** and did not significantly change friction readings ($p>0.05$).

Aside from SMP, all proteins chosen are also studied in higher concentration (10wt%) in **Chapter 3.** Overall, both chapters showed no significant differences between protein type in boundary friction ($p>0.05$). It is worth noting the lubrication trend was different between chapters. The most lubricating protein to least in **Chapter 3** was PPc> WPI >SPI (**Supplementary Table B.2.**). Whereas, in the present chapter it was SPI>PPc>WPI (**Supplementary Table C.3.**). No difference between type was significant, and source of protein varied between chapters. Previous research has shown different sources of protein for the same type can have different lubricating effects (Vlădescu et al., 2023), therefore may of contributed to the different order in lubrication between chapters. The effect of

saliva also did not significantly change friction readings in the present results and **Chapter 3** (see **Supplementary Table B.2.**).

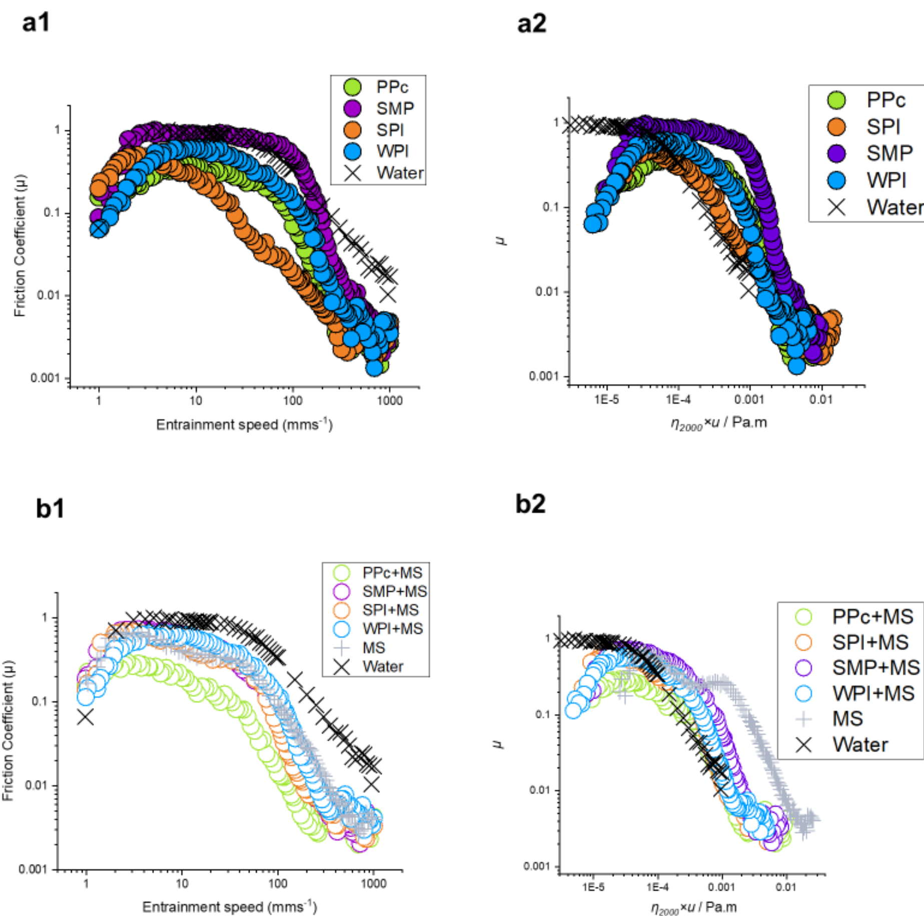


Figure 24. Mean friction coefficients of aqueous 5wt% protein solutions (a) in the absence (a.1.) or presence (a.2.) of model saliva (MS). Mean apparent viscosities of 5wt% protein-enriched emulsions + model saliva (BSM) mixtures (b) as a function of entrainment speed (b.1.) and as a function of entrainment speed × high shear rate viscosity (b.2.). Data is shown for three independent readings on duplicate measurements. PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be found in Supplementary Table C.3. (n=2×3).

To gain a deeper understanding of the mechanisms governing frictional behaviour of protein solutions and identify the importance of viscosity, the μ curves are scaled to second plateau-shear viscosity *i.e.* as a function of reduced speed parameter *i.e.*,$\boldsymbol{\eta}_{\mathbf{2000}}\mathrm{x}u$ as reported previously (Soltanahmadi et al., 2022, de Vicente et al., 2005), and in **Chapter 3** (see **Figure 16.2.**). When scaled to viscosity, the lubricating properties of plant proteins, SPI and PPc, are reduced (**Figure 24.a.2.**). This highlights the likelihood of fluid film lubrication mechanisms playing a more significant role than surface forces in boundary lubrication. Similarly, SMP still has poor boundary lubrication which also suggests fluid-film lubrication may be responsible for boundary lubrication. Overall, protein solutions tend to converge into a unified master curve for the mixed and EHL (Elastohydrodynamic Lubrication) regimes. This phenomenon suggests the dispersion is entrained within the contact zone. Similar observations are reported by (de Vicente et al., 2005).

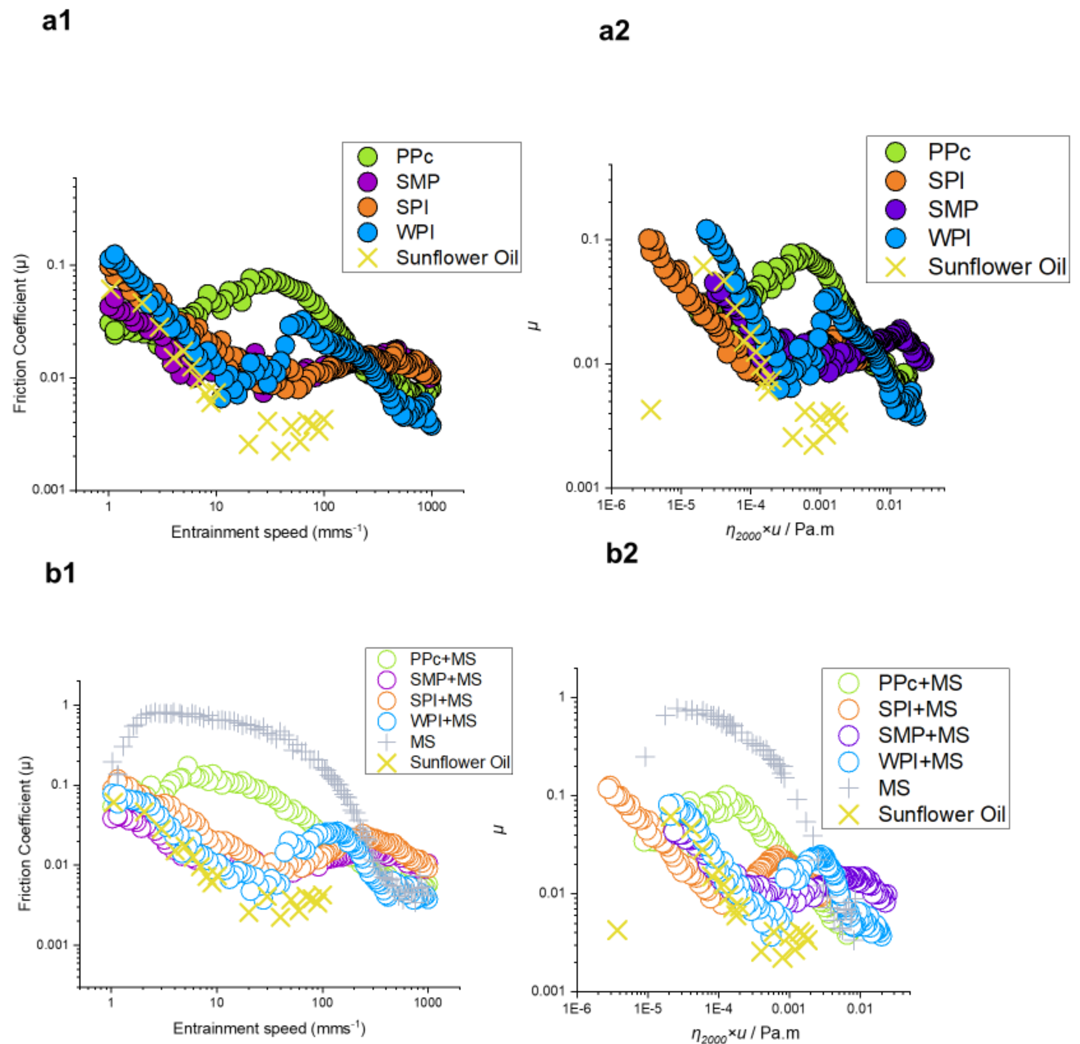


Figure 25. Mean friction coefficients in presence of 5wt% protein emulsions (a) as a function of entrainment speed (1.a.) and as a function of entrainment speed × high shear rate viscosity (2.a.). 5wt% protein emulsions + model saliva (PGM) mixtures (b) as a function of entrainment speed (1.b.) and as a function of entrainment speed × high shear rate viscosity (2.b.). Data is shown for three independent readings on duplicate measurements. PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be found in Supplementary Table S.2. (n=2×3).

The lubrication behaviour of protein-emulsions (Figure **25.a.1.**) shows no boundary lubrication for WPI, SMP and SPI emulsions over the speed range analysed. This behaviour has been attributed to characteristics of the emulsion systems used. As load increases, the thickness of the fluid layer between interacting surfaces diminishes. Consequently, fewer droplets penetrate this region and engage in interactions with each other (Douaire et al., 2014). Collectively, the friction for WPI, SMP, SPI exhibit characteristics of working in the mixed lubrication regime with the oil-phase dominating friction, which has been seen previously in different oil-in-water emulsion systems (Torres et al., 2018, Upadhyay and Chen, 2019a, Wang et al., 2021b).

Oil/fat characteristics such as amount and viscosity, are well established to be the main driver of friction in o/w emulsions stabilised by protein (Fuhrmann et al., 2020, Olivares et al., 2019). Fat droplets entrained between the two surfaces provide a lubricating layer as well as conceal surface irregularities, therefore proteins which facilitate fat coalescence will have lower friction (Fuhrmann et al., 2020). For example, WPI (7.5mg/mL) was used to stabilise o/w emulsions but oil droplet size was controlled through clustering (through cross-linking with proanthocyanidins), increasing oil droplet clusters reduced friction, which was correlated with increased sensory creaminess, coating and thickness (Fuhrmann et al., 2020). Similarly, fat coalescence which adhered to tribopair surfaces are attributed to reduce friction in boundary/mixed regime (Olivares et al., 2019).

In contrast to SPI, SMP and WPI, the PPc emulsion has a boundary regime with friction reducing at higher speeds as emulsions entered the contact (**Figure 25.a.**). At entrainment speeds was of $52.33s^{-1}$, PPc has significantly ($p>0.05$) higher friction than all other emulsions (see **Supplementary Table C.5.**). The presence of a boundary regime is thought to be driven by PPc aggregates. The observation of a boundary regime for SPI and pea protein isolate (PPI) is attributed to protein aggregates in a previous study of cream emulsions. The study also reported fava-bean emulsions in contrast did not have distinct

boundary regions at sliding speeds between 0.1-3mm/s (Ningtyas et al., 2021). The authors further hypothesized the higher friction was due to low solubility of plant proteins, which lead to incomplete surface coverage. This in turn hindered the formation of a robust film (Ningtyas et al., 2021). As PPc in this study had low solubility (**Figure 20.a.**), it is likely a similar phenomenon is occurring in the current study.

Looking at high entrainment speeds, **Figure 25.a.1**, friction begins to increase again for dairy protein emulsions. Friction in higher entrainment speeds is no longer dependent on surface forces/ asperities, therefore emulsion structure and viscosity will play a role. As speed increases, it has been suggested the adherent oil layer in an emulsion is broken and emulsified. This leads to an increase in friction because of the bulk fluid's high pressure in the contact zone and the higher shear required to break the adherent film (Upadhyay and Chen, 2019a). Despite the increase in μ for dairy proteins, and the differences in viscosity between emulsions, there are no significant differences in friction at entrainment speeds of 151.28mms^{-1}. Similarly, at the highest entrainment speeds 950-1000mms^{-1} there are no significant differences in friction between any protein type and emulsion (see **Supplementary Table C.5.)**.

When emulsions are combined with model saliva there are no statistically significant differences for any protein type (p>0.05). Furthermore, between protein types, emulsions had the same lubrication pattern. Which showed PPc+MS to still have higher boundary friction and delayed onset into the mixed regime, suggesting insoluble pea protein aggregates may still impair lubricity. Although not shown in present results, the addition of (human) saliva has been previously shown to influence o/w emulsion boundary and mixed regime friction. It has been proposed that by adhering to PDMs surfaces, saliva can change the surface properties towards a less hydrophobic state. Therefore, friction may increase as oil droplets wetting abilities will be reduced (Fuhrmann et al., 2019, Dresselhuis et al., 2007).

To gain a deeper insight into the underlying mechanisms governing frictional behaviour of emulsions, the μ curves are scaled to second plateau-shear viscosity *i.e.* as a function of reduced speed parameter *i.e.*, $\boldsymbol{\eta_{2000}}$xu as reported previously (Soltanahmadi et al., 2022, de Vicente et al., 2005). As all emulsions bar WPI are non-Newtonian, for PPc an estimated value of effective viscosity was obtained according to (de Vicente et al., 2005). For SMP and SPI, the fitting was poor therefore viscosity at a shear rate of $1000s^{-1}$ was taken (see **Supplementary Table C.6.**). When emulsions are scaled, after speeds of 0.001, they do not converge into a master curve as was the case for the protein solutions discussed earlier (**Figure 24.a.2.**). This may suggest in higher speed ranges, the bulk emulsion system is not fully present at the contact interface. Whereas below speeds of 0.001, SMP and WPI emulsions collapse into a master curve similar to sunflower- oil. However, the SPI emulsion have an earlier reduction in friction, which may be due to slower ‘squeezing-out’ from the contact interfaces. The PPc emulsion still has higher friction at the contact interfaces at speeds of around 0.001, indicating PPc is dominated by a different lubrication mechanism compared to SPI, SMP and WPI emulsions. Previous research also failed to establish a collapsed curve in in skimmed milk and whey protein emulsions (Olivares et al., 2019).

Further analysis fitting to the $\boldsymbol{\eta_{2000}}$xu using the viscosity of solutions to the tribology of emulsions also did not show a master curve collapse (**Supplementary Figure C.1.**), with PPc again having higher friction at speeds of around 0.001. Overall, this may suggest protein in the dispersed phase alone is not responsible for the lubrication differences observed in the emulsions. Therefore, other factors such as protein aggregation may be responsible for the high friction obtained for the PPc emulsion. Comparing fitted emulsions to fitted solutions in **Chapter 3** (see **Figure 16.**) and in this present chapter, the consistent higher boundary friction may be attributed to de-wetting properties of PPc aggregates.

4.4. Conclusions

In summary, among the various protein types examined, whey protein isolate exhibited superior stability which is beneficial for food manufacturers. The poor stability of plant proteins may be attributed to the impact of insoluble proteins, which are used in their native state without additional processing. In terms of material behaviour, proteins display distinct flow characteristics that are more similar when combined with model saliva. Lubrication behaviour of emulsions varied by protein type. WPI, SPI, SMP all have favourable lubrication, demonstrating an absence of the boundary regime with the oil phase dominating friction. Contrastingly PPc displays high boundary friction hypothesized due to be from PPC protein aggregates in the continuous phase increasing friction.

Chapter 5. Comparing sensory and lubrication behaviour of plant vs dairy protein in a model food system

Abstract

Alternative proteins are becoming increasingly popular with consumers and food manufacturers due to environmental concerns. However, they often have poor consumer acceptance due to unpleasant mouthfeel. Material properties provide insight into mouthfeel, but it is important to combine these with sensory understanding. The following study aims to compare different types of protein in a model food system using a combination of material property and sensory analysis. Aqueous protein dispersions and a syrup-based food matrix are prepared with pea protein concentrate, soy protein isolate or whey protein isolate. Protein concentration is kept consistent at 5wt% with skimmed milk powder was used as the control. WPI at 10wt% is additionally added to look at the effect of higher concentrations. Aqueous dispersions and food matrices are then measured in the presence or absence of model saliva (MS) using rheology and tribology with a polydimethylsiloxane (PDMS) surface. A trained sensory panel (n=8) is used for qualitative descriptive analysis (QDA) of the food matrices, which established different texture and mouthfeel properties by protein type of the fillings. Legume fillings, pea and soy, rated as powdery, whey the smoothest and skimmed milk powder was rated as gritty. One of the key findings was no clear relationship between instrumental material properties (viscosity and tribology) of the food matrices and sensory attributes. Nevertheless, when examining material properties of bulk solutions, a connection emerged between these properties and sensory results. Skimmed milk powder in a bulk solution has significantly higher boundary friction and a delayed onset of the mixed regime that may relate to the gritty sensations. As legume proteins did not show differences in bulk-solution lubrication compared to whey, powdery sensations may be linked to protein characteristics such as poor solubility. In summary, this chapter underscores the

difficulties in using instrumental analysis for more intricate real food systems, where multiple ingredients can impact lubrication. Moreover, the aggregation of proteins in the continuous phase, even within mouthfeel, could potentially a crucial role in mouthfeel of food matrices.

5.1. Introduction

Now, more than ever, consumers are thinking about the environmental cost of food production. The growing awareness of environmental and food security concerns are putting pressure on food manufacturers to turn to low carbon sources and novel formulations (McClements, 2020). However, for these lower-carbon foods to gain widespread uptake and acceptance, they must be desirable, affordable, and convenient (Ismail et al., 2020). Therefore, it is fundamental food manufacturers to understand how different ingredients affect consumer's sensory perception. Legume proteins are a relatively new ingredient associated with lower carbon emissions. These have become popular, with a variety of products incorporating them such as bakery, cereal products, confectionary, dairy products, and deserts (Kumar et al., 2021, Ismail et al., 2020). However, using legume proteins has been associated with reduced acceptability. Legume proteins have been reported to elicit unpleasant flavours (e.g. bitter, earthy) and unpleasant mouthfeel including gritty and pasty (Saint-Eve et al., 2019, Roland et al., 2017). For example, when legume proteins are compared against traditional dairy proteins in yoghurts, texture differences are reported which related to sensory panels liking (Greis et al., 2020). Thus, it is important to understand mouthfeel as it is likely to affect legume proteins uptake (Saint-Eve et al., 2019).

The findings discussed in the previous chapters highlight lower adsorption and high insolubility of the plant proteins soy protein isolate (SPI) and pea protein concentrate (PPc), in comparison to dairy proteins in bulk-solutions. In addition, **Chapter 4** found plant proteins when added to the continuous phase, to have different lubrication behaviour in more complex oil-in-water emulsions. PPc emulsions have particularly poor lubrication, hypothesized to be due to pea disrupting the formation of a lubricating film with the oil droplets. Increasing whey protein isolate (WPI) concentration has additionally been well established to

increase boundary lubrication in bulk solutions (**Chapter 3.** see **3.3 Soft tribology**) (Kew et al., 2021, Zembyla et al., 2021).

Validating these findings, using more realistic systems, which people are more likely to consume is important for several reasons. Firstly, the behaviour of proteins in the aqueous dispersions and emulsions may not accurately represent their behaviour in more complex food matrices. Food systems often contain various components including fats, carbohydrates and fibres that can interact with proteins and alter their subsequent sensory properties (Yang et al., 2020). Through analysis of the behaviour of proteins in more complex systems, like confectionary fillings, we can gain insights into how these interactions influence the behaviour of proteins and their overall impact on mouthfeel in food products. By looking at protein in both simplistic systems (e.g. bulk protein dispersions) as well as through more realistic food systems, we aim to account for the complexity of food matrices and see if protein alone, or its interaction with other components is driving mouthfeel. Secondly, mouthfeel plays a critical role in determining overall consumer acceptability. To fully elucidate how mouthfeel arises, it is necessary to integrate material understanding, including tribology and rheology, with sensory panels (Sarkar and Krop, 2019). This can help identify key factors which contribute to mouthfeel attributes of food formulated with protein.

The aim of the following study is to evaluate the impact of replacing skim milk powder (SMP) with different proteins (whey protein isolate, pea protein concentrate and soy protein isolate) on structure formation, textural properties, and sensory properties. A syrup-based filling enriched with protein is prepared and compared to a model syrup filling recipe (SMP) as a reference. In addition, a food matrix is compared to a simpler system, aqueous protein dispersions to see if protein is driving different sensory perceptions. To the best of our knowledge, no previous study has investigated textural/ sensory perceptions in protein-enriched fillings.

5.2. Methods

5.2.1. Materials

The same proteins used in **Chapter 3** are used here. Notably, pea protein concentrate (PPc) and soy protein isolate (SPI) are kindly provided by Archer Daniels Midland Company (ADM, Decatur, Illinois), the skimmed milk powder (SMP) was kindly obtained from Lactalis (Lactalis Industrie, Bourgbarre, France) and the whey protein isolate (WPI) was kindly provided from Sachsen Milch (Sachsen Milch, Saxony, Germany). The PPc was composed of 82.9%w.w, SPI88.10% protein, WPI92.84% protein and SMP32.4% with this based on information provided by the manufacturers.

5.2.2. Preparation of protein dispersions

Preparation of aqueous dispersions of plant and dairy proteins are prepared by dissolving WPI, SPI, and PPc powders in Milli-Q and hydrated for 2h at room temperature (21°C) with stirring at 500rpm. The protein concentration of 5wt% was based on the manufacture information provided on protein content. A 10wt% solution was also made for WPI. A control SMP solution was made matching the same dry powder weight to that of PPc, which had the lowest protein content. As SMP contained 32.4%, the SMP solution was 1.96wt% protein. The pH of solutions was measured using a pH stat (Jenwway 3520, England) and were the following: WPI6.5, SMP, 6.67, SPI6.35 and PPc7.3.

5.2.3. Preparation of food filling samples

Five food-based filling samples were made using compositions outlined in **Table 3**. The fillings are a food matrix consisting of fat and syrup. The syrups are a blend of invert sugar syrup which is a mixture of glucose and fructose and C*Sweet which is a glucose syrup. (Invert1, British Sugar, London, United Kingdom and C*Sweet-, Cargill, Minneapolis, United States). The ratio between syrups was approximately 8:5 Invert 1: C*sweet. All fillings contained solid vegetable fat (Deliair, NH50, AAK, Sweden, 20 wt%), emulsifier (1.2wt%), water (5.5-7wt%), a blend of two types of syrup (Syrup 1≈40wwt%, syrup 2≈27wt%). Proteins including plant and dairy proteins are tested with skimmed milk powder (SMP) acted as the control. In the test filling samples, protein concentration was kept consistent at 5 wt% with one exception. The one exception was the 10wt% whey protein condition. The concentration was calculated using the protein content provided by the manufacturer. SMP had a much lower protein concentration (32.4%), therefore this was just kept at 6wt% which equates to 1.921g of protein for the 5.93g added. SMP was kept at 6wt% to keep total solid consistent, as SMP is the ingredient which would be typically substituted for a protein concentrate in the manufacturing of protein-added filling products.

Whey permeate was added to equilibrate the solid content amongst all samples. Water was adjusted per protein filling to maintain a similar consistency/ texture between samples. The skimmed milk powder added to the control was matched to the protein with the lowest protein percentage, in this case pea. The total solid content for the control was adjusted to the amount which would be required to make a 10wt% pea protein-based filling. 500g of sample was prepared on one occasion and kept in plastic storage containers. The same 500g sample was used for sensory and instrumental analysis. The samples are kept at room temperature and analysed within 3 months of creation as the water activity for samples was determined to be bellow 0.7 using an AquaLab 4TEV (Decagon Devices, Labcell, Basingstoke, UK).

Table 3. Composition of the filling samples containing protein with SMP acting as the control protein-based ingredients.

Protein Type	Protein powder Added	Whey Permeate Added	Water Added (wt%)	Syrup blend (wt%)	Solid Fat (wt%)	Emulsifier
PPc	6.03	4.71	5.5	62.56	20	1.2
SMP	6.03	6.03	6	60.74	20	1.2
SPI	5.68	5.06	7	61	20	1.2
WPI 5%	5.37	5.37	5.5	62.56	20	1.2
WPI 10%	10.74	0	7	61.06	20	1.2

The process of creating the fillings involved heat treatment of the syrups and water to 50°C and shearing using a Thermomix (Thermomix TM31, Vorwerk, Germany) at speed 4 (1100RPM) for 2 minutes. The protein powders (or the SMP in case of the control) and the whey permeate powder are then added gradually over a minute at 500RPM (Speed 3). The shearing speed was then increased back to 1100RPM (Speed 4) temperature was set to 70°C, and the sample mixed for 5 minutes. The solid fat was melted using a microwave (KS25MSS11, Kenwood, UK, 900W,273°C). The fat was mixed with the emulsifier Lactem (Lactem 0410, supplied via Palsgaard,Juelsminde, Denmark) at 40°C. The combined fat and emulsifier mixture was added to the Thermomix on a 500 RPM (Speed 3) over the course of a minute. Finally, the Thermomix was set to 80°C on a high-speed setting 8 (5800 RPM) and mixed for 5 minutes. The fillings were left to cool overnight at 20°C.

5.2.4. Preparation of model saliva.

Model saliva was mixed 1:4 with aqueous protein solutions and separately 1:4 with fillings. Unlike **Chapter 3** and **Chapter 4,** the model saliva contained BSM (Bovine Submaxillary Mucin). BSM was chosen over PGM as it has been established to have more similar lubricating properties to human saliva (Sarkar et al., 2019b). Model saliva was prepared following the same protocol described in **Chapter 3 (see 2.2.2 Preparation of model saliva).** BSM purification was through dialysis and freeze drying as described in **Chapter 3.** To summarize, model saliva contained 3g/L BSM and 1.594 g/L NaCl, 0.328 g/L NH_4NO_3, 0.636 g/L KH_2PO_4, 0.202 g/L KCl, 0.308 g/L $K_3C_6H_5O_7H_2O$, 0.021 g/L $C_5H_3N_4O_3Na$, 0.198 g/L H_2NCONH_2, 0.146 g/L $C_3H_5O_3Na$, 3g/L PGM or (BSM), made up to 1lL with Milli-Q water. This was stirred for 4 h to allow the salts to dissolve and protein dispersion-saliva mixtures were created at 4:1w/w ratio (Krop et al, 2019) simulating real oral processing conditions (De Lavergne et al., 2015). Sodium azide was added at 0.03% w/w to prevent microbial growth.

5.2.5. Confocal Laser Scanning Microscopy (CLSM)

Microstructure and morphology of protein-enriched filling matrices are characterized using a Zeiss LSM 880inverted microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany). After 1 month of storage at 21°C, fillings were dyed with 10µL/g Nile Red (0.02 mg/mL in dimethyl sulfoxide, final concentration) and 10µL/g Fast Green (1mg/mL in Milli-Q water). The stained matrices were placed into a concave confocal microscope slide and covered with a glass coverslip and observed in the microscope after 10 minutes. The images were acquired using an oil immersion 63x lens and the pinhole was kept at 1 Airy unit to filter out most of the light scattering. A wavelength of 488nm was used to excite the Nile Red used to stain fat droplets and 555 nm for the Fast Green used to stain the protein molecules.

5.2.6. Zeta-potential

The Zeta-potential of protein solutions were measured using the same protocol detailed in **Chapter 3** (see **2.2.3. Zeta-potential**). Briefly, 0.1wt% solutions were placed into a folded capillary electrophoresis cell (DTS1070) and placed into the Zetasizer (Zetasizer Nano ZS instrument, Malvern Instruments Ltd., Worcester, UK).

5.2.7. Solubility

Solubility was calculated as the percentage of soluble protein content compared to the total protein content. All 1wt% protein stock solutions were analysed before and after centrifugation using the Bradford dye-binding method. The solutions were centrifuged (Hettich Zentrifugen, Rotina 380R Germany) at 4,000rpm for 10 minutes and the concentration of the supernatant was measured. 30mL of sample was combined with 1500mL of Coomassie brilliant blue G-250 reagent, stirred well and left for 10minutes. The absorbance was measured at a wavelength of 595nm using a spectrophotometer (Jenway 6715 UV/Vis, USA). Bovine serum albumin (BSA) was used to make the standard curve at 0mg/mL, 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL and 1mg/mL.

5.2.8. Tribology

The rolling/ sliding friction of aqueous protein solutions, protein fillings and mixtures with model saliva (BSM) were measured following a similar protocol to that described in **Chapter 3** and **Chapter 4** (see **2.1.9. Tribology**). The protocol was the same except entrainment speed was measured from 0.1 to 2000mms^{-1}. Briefly, µ values were measured as a function of entrainment speed (U*)* at a normal force of 2.0N at 37°C.

5.2.9. Rheology

The apparent viscosity of aqueous protein solutions, protein fillings and mixtures with model saliva (BSM) were measured following the same protocol as described in **Chapter 3** and **Chapter 4.** To summarize a cone-plate geometry (angle 2°) measured viscosity at shear rates of 0.01-2,000s^{-1} at 37°C.

5.2.10. Descriptive sensory analysis

An internally trained panel (n=8, 28-65 years old) recruited by Mondelez was used for sensory analysis. The panel were familiarized with the filling samples followed by generation of attributes and introduction to rating scale. The method of evaluation was qualitative descriptive analysis (QDA™). Only filling samples were assessed over four replicates for their texture and aftertaste over a period of 2 days between the 3rd-6th of October 2022. The aim of the testing was to understand the differences in intensity ratings between the filling samples versus the control filling (containing SMP).

Panellists had 8+ years of prior QDA experience in filling and other confectionery products. The vocabulary was generated by the panel during the round table discussion (see descriptors in **Table 4.**). The order of the sample presentation was balanced across assessors. Each sample was evaluated on 4 separate occasions by each participant over two days. The results were collected via the Compusense data capture system. Fillings (30g) were dispensed into sample cups with 3-digit codes and evaluated at room temperature. Sensory analyses were conducted individually in odour free room with panellists asked to evaluate the basic texture and mouthfeel of samples. Between samples, panellists were required to rinse with water.

Table 4. Descriptors and their definitions as devised by trained sensory panel.

	Attribute	Definition
Mouthfeel	Drying	Measure of how much the product leaves the mouth feeling dry, like you need a drink of water.
Mouthfeel	Melt Rate	Measure of how slowly or quickly the sample melts in the mouth, the speed until which you swallow the product.
Mouthfeel	Mouthcoating	Degree to which the sample coats the inner surface of the mouth.
Texture	Adhesive	Measure of how adhesive/ sticky the sample feels; this can be measured by how much the sample sticks to your teeth.
Texture	Grainy/Gritty	Measure of the amount of gritty/grainy particles from slightly which is few particles to very which is mainly particles.
Texture	Powder	Measure of powder perceived in the mouth similar to biting icing sugar as the sample is melting in the mouth.
Texture	Smoothness	Measure of how smooth the product feels as it is melting in the mouth in a liquid phase.
Texture	Viscosity	Measure of how thick the sample feels in the mouth, from thin and fluid to thicker.

5.2.11. Statistical Analysis

Regarding instrumental tests, the food matrix was prepared once and measured at least three times in duplicate with means and standard deviations reported. The accompanying solutions were prepared in duplicate and measured at least three times in duplicate again with means and standard deviations reported. One way ANOVA was used to analyse the effect of protein source and concentration on the rheological and tribological properties. The significance of the differences among the mean values of the samples were determined by Bonferroni test, using SPSS software (IBM, SPSS statistics) with $p<0.05$ used for significance. The sensory results were collected via the Compusense data capture system and were analysed with QDA^{TM} software (RedJade) using Analysis of variance (ANOVA). The Duncan minimum significant difference was calculated to determine those samples which were significantly different ($p<0.05$). Correlations between measured rheology and tribology results with sensory attributes were obtained using the CORREL function in Excel (Microsoft Office 2023).

5.3. Results and Discussion

5.3.1. Characteristics

The structure and visual composition of the model foods are displayed in **Figure 26**. The fluorescence was obtained with Fast green and Nile red. The dispersed lipid droplets is most observable for the soy protein matrix. Protein particles appear to differ in size and are non-uniformly distributed throughout food matrices. The source of proteins was the same as used in **Chapter 4** but differed from **Chapter 3.** As detailed in Chapter 4 (see **Protein Characteristics** and **Figure 20.**), plant proteins again have lower solubility than dairy proteins with SPI having 33.35% and PPc 30.26%. The new source of WPI had higher solubility at 93.00%, and SMP had 63.68%. All proteins have a stable negative charge, in line with **Chapter 3 (see Figure 13.a.)**, despite variation in pH (6.35-7.3) between solutions.

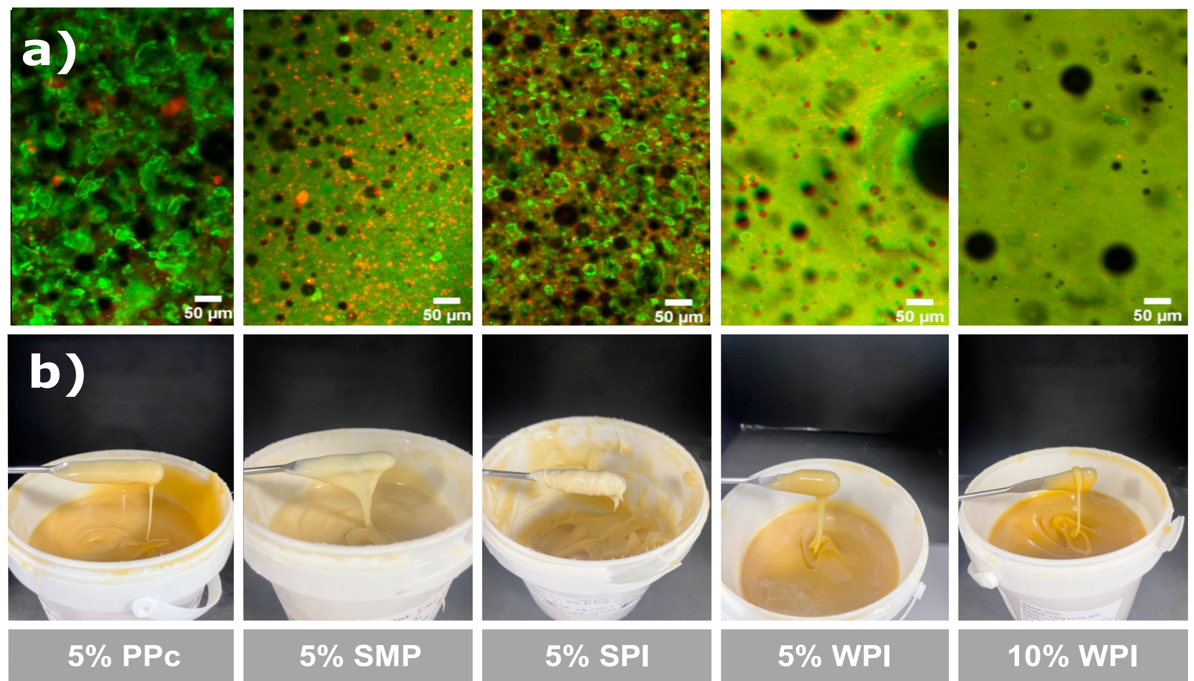


Figure 26. Confocal images of food filling samples, showing multiscale the structure (a). Scale bar represents 50µm, lipids appear in red and proteins in green(a) visual images taken with a standard phone camera (b).

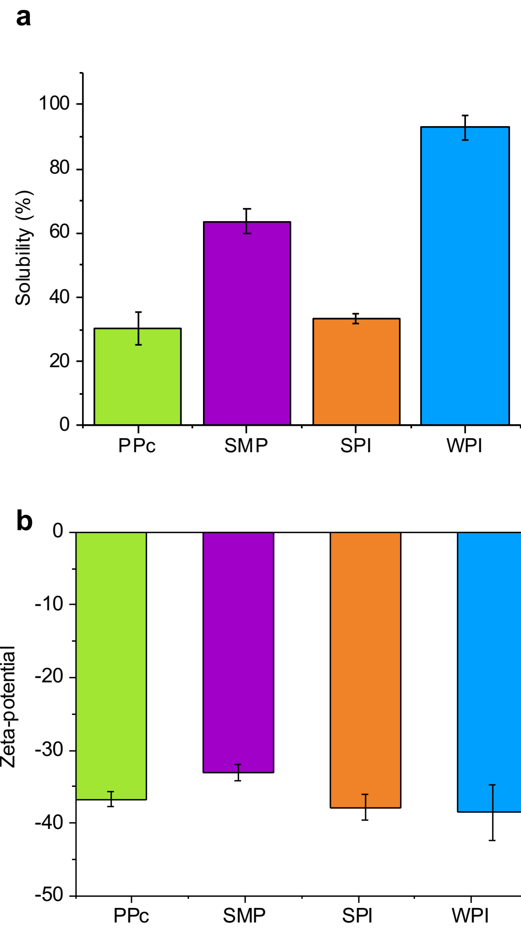


Figure 27. Mean solubility (a), and zeta-potential (b) for the tested proteins in aqueous solutions (0.001wt%) for triplicate measurements. PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate and WPI whey protein isolate. Error bars represent ± standard deviations for triplicate measurements (n=3×3).

5.3.2. Apparent viscosity

As discussed in **Chapter 4** (**3.3. Rheology**), the viscosity of the 5wt% bulk-protein solutions is consistent with those acquired in **Chapter 3** (**3.2. Apparent viscosity**) at 10wt and 20wt%. Whereby dairy proteins, WPI and SMP, show low shear dependence and plant-proteins, SPI and PPC, having high shear dependence (**Figure 28.a.1.**). At orally relevant shear-rates ($50s^{-1}$) SPI again has significantly higher viscosity than all other proteins (p<0.05) (see **Supplementary Table D.1.)**. When bulk solutions are mixed with model saliva (BSM) there is no change in the pattern of viscosity between protein types, with SPI again having higher viscosity (see **Supplementary Table D.1.**).

When looking at model food systems, all protein food matrices appeared to be shear thinning (**Figure 28.b.1.**). Consistent with solution and emulsion results, WPI has the least shear dependence. Invert1 syrup in the absence of protein/ fat has been shown to have Newtonian behaviour (Lau and Dickinson, 2004), whereas glucose syrup has been shown to be shear-thinning (Fakayode et al., 2019). At orally relevant shear rates ($50s^{-1}$) the 5wt% protein food matrices have significantly different viscosity except for the legume fillings SPI and PPc (see **Supplementary Table D.2.**). WPI had the highest viscosity then SMP, then PPc with SPI having the lowest. Water was added in different proportions (5-7wt%) to protein-matrices to try and match viscosity, therefore it is difficult to ascribe any differences among samples to protein type. However, as water alone is Newtonian and has a much lower viscosity (≈1mPas), it would be expected that matrices with more water would have lower viscosity. As the WPI matrices have the joint-least amount of water added (5.5wt%), the high viscosity is likely related to the low water proportion. Comparing across systems, WPI has consistently shown low viscosity in both emulsions and solutions (see **Chapter 3 Figure 14.** and **Chapter 4 Figure 23.**). Interestingly, SPI has the joint-least amount of water added (5.5wt%) but has the lowest viscosity whereas it would have been expected to have the highest (**Supplementary Table D.2.**). The low viscosity is

interesting as SPI in solutions and emulsions has consistently shown high viscosity (**Chapter 4** see **Figure 23.**). Overall, this highlights viscosity in simpler systems cannot always predict more complex systems.

As expected, when matrices are mixed with model saliva, the viscosity reduced for all matrices (see **Figure 28.b.2.**). At orally relevant shear rates ($50s^{-1}$), the order of viscosity changed between samples changed (see **Supplementary Table D.2.**). Most notably, alone WPI had the highest viscosity, but when mixed with model saliva it had the lowest. As discussed earlier, the high viscosity is thought to be related to the low amount of water used in WPI matrices preparation.

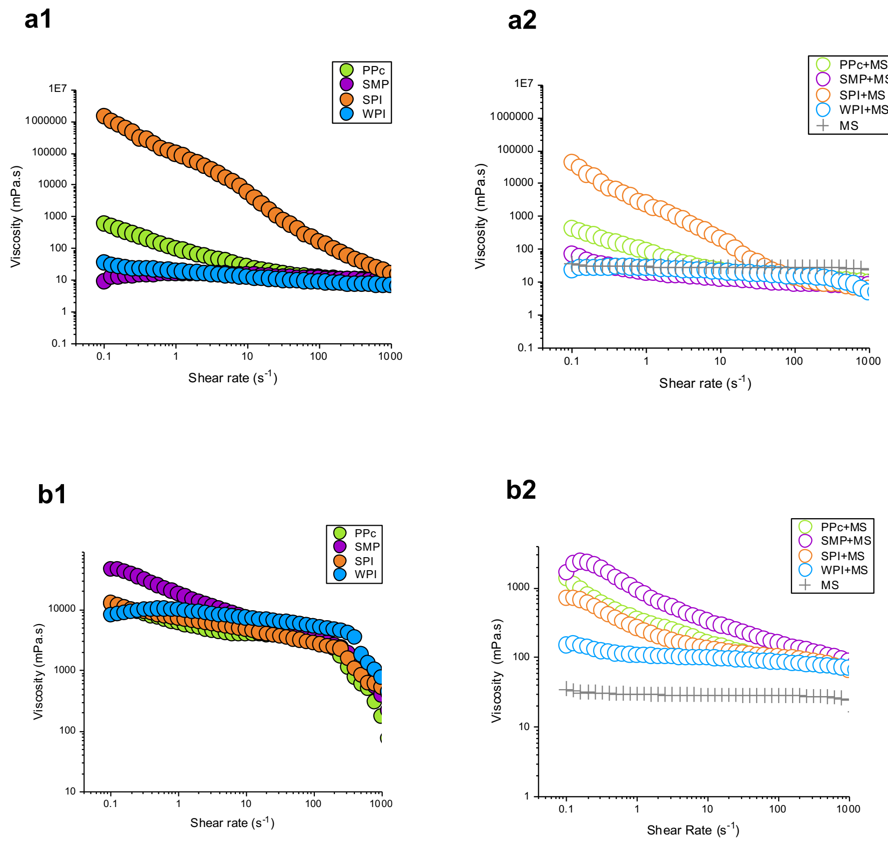


Figure 28. Mean apparent viscosities of 5wt% aqueous protein solutions (a) in the absence (a.1.) or presence (a.2.) of model saliva (MS). Mean apparent viscosities of 5wt% protein-enriched filling (b) in the absence (1b) or presence (2.b.) of model saliva (MS). PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be found in Supplementary Table D.1. and Supplementary Table D.2. (n=2×3).

Increasing concentration for WPI solutions with and without model saliva, has no significant effect on viscosity with both solutions shear thinning (**Figure 29.**). For food matrices, 10wt% WPI had 74.50% greater viscosity at orally relevant shear rates, which was found to be statistically significant (p<0.05) (see **Supplementary Table D.2.**). This occurred despite modification of the water content, the 10wt% WPI food matrix had 7wt% water, whilst the 5wt% WPI matrix

contained 5wt% to try to achieve uniform viscosity between the two (see **Table 3.**). Again, when mixed with model saliva viscosity reduced, and 10wt% WPI still had higher viscosity (see **Supplementary Table D.2.**).

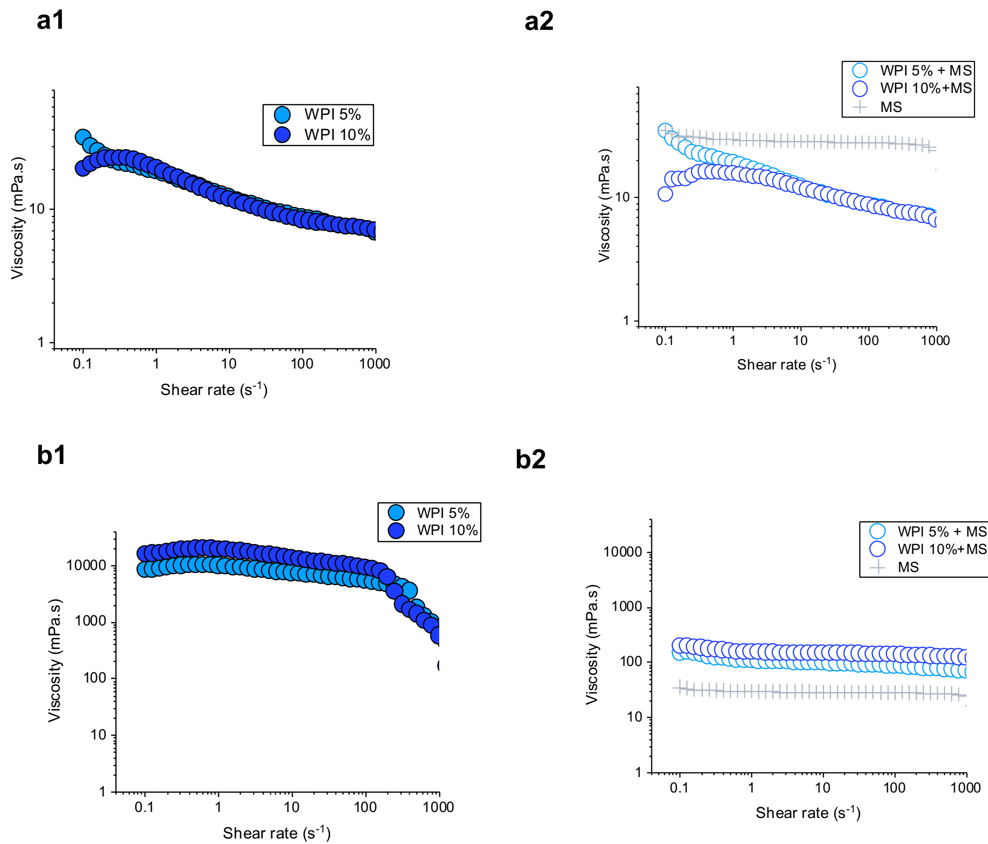


Figure 29. Mean apparent viscosities in presence of higher concentrations of protein (5-10wt%) in aqueous solutions (a) in the absence (a.1.) or presence (a.2.) of model saliva (MS). Mean apparent viscosities of protein-rich filling samples (5-10wt%) in the absence (b.1.) or presence (b.2.) of model saliva (MS). WPI refers to whey protein isolate and MS model saliva. Error bars represent ±standard deviations for triplicate measurements (n=2×3).

5.3.3. Tribology

Figure 30.a. displays the friction curves obtained for protein solutions and protein+model saliva mixtures. As discussed in **Chapter 4** and in accordance with **Chapter 3** (see **Figure 16.**), all samples reach the mixed lubrication regime with a reduction in friction. PPc, SPI and WPI solutions are more lubricating than the buffer. The 5wt% SMP, which contained less protein 1.96wt%, was unable to form a lubricating film resulting and has a significantly higher friction than other proteins (p<0.05) (see **Supplementary Table D.3.).** The effect of model saliva however is consistent with **Chapter 3** and did not significantly change friction readings (p>0.05) (see **Supplementary Table D.4.**).

Looking at the friction curves for the food matrices (**Figure 30.b.1.**) we can see a different lubrication pattern. Unlike protein solutions and emulsions (see **Figure 24.**), there is no obvious difference between protein type, therefore friction appears to be dominated by the syrup and fat phases. All food matrices have an absence of the boundary lubrication regime which shows that even at low speed, the food matrices are entrained and providing lubrication between the two surfaces. In addition to no boundary regime, the food matrices transitioned much faster into the hydrodynamic regime with friction increasing at speeds around 100mms^{-1} (**Supplementary Table D.5.).** The increase may be attributed to shear of the tribometer disrupting and breaking up the lubricating layer, which has been shown in milks of varying fat content (Chojnicka et al., 2008, Chojnicka-Paszun et al., 2012).

Since tribological studies examining the material behaviour of model-food systems are relatively uncommon in the broader context and given the intricate and unique nature of the model foods employed, there are limitations in terms of making direct comparisons to existing literature. None the less, looking at tribology of model-foods with high sugar content we can see similar lubrication

behaviour to the food matrices used in the present study which contain a high syrup proportion (≈60wt%, see **Table 3.**). Agar filled gels with a glucose concentration of 30%w/w also show no boundary regime and an early transition at approximately 50mm/s to the hydrodynamic regime (Fernández Farrés and Norton, 2015). Similarly, no boundary lubrication is observed for protein semi-solid gels whereas the same protein in solutions had boundary lubrication (Liu et al., 2016). Hence, it is unsurprising that varying protein type does not have an obvious role in tribology of the model foods used in this study as the syrup and fat phase will be dominating lubrication. When fillings are mixed with model saliva, all except 5wt% WPI has a significantly lower friction (p>0.05) in the hydrodynamic regime (**Supplementary Table D.6.**). This is as expected as viscosity was shown to be significantly lower for model-foods mixed with saliva (**Supplementary Table D.2.**).

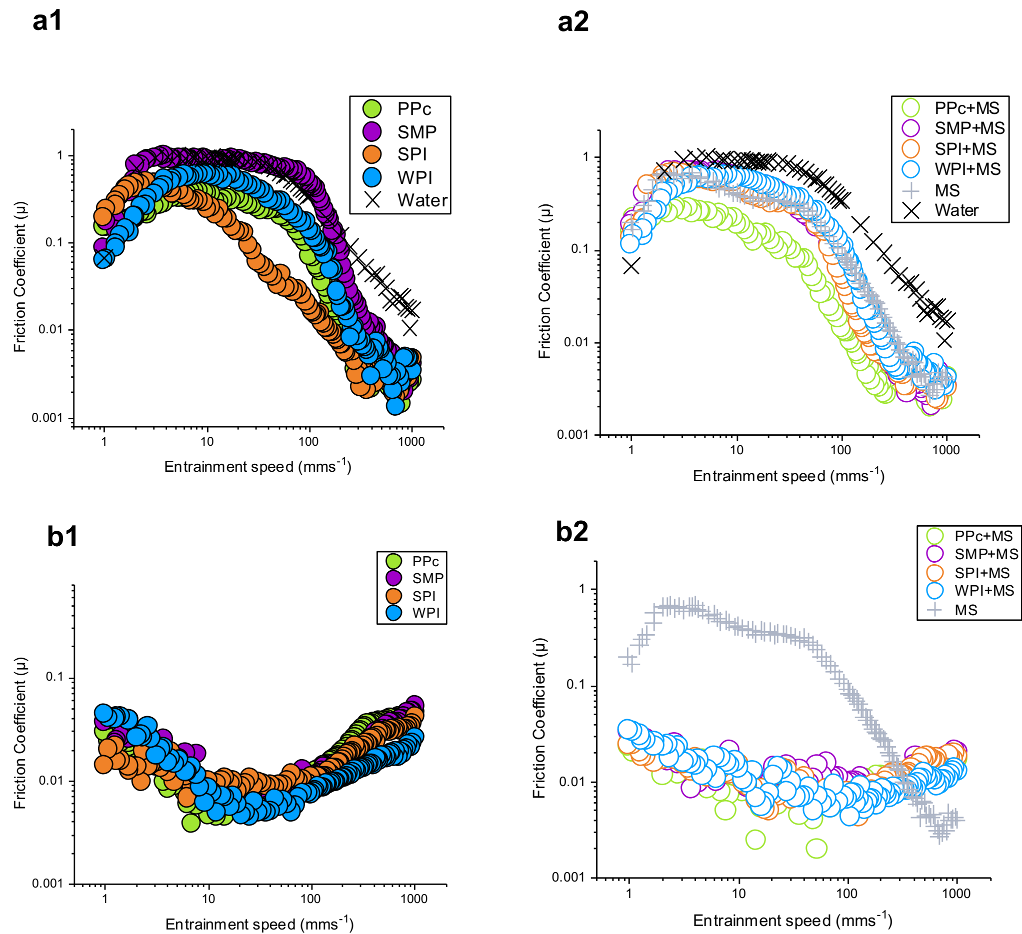


Figure 30. Mean friction coefficients of 5wt% aqueous protein solutions (a) in the absence (a.1.) and in the presence of model saliva (MS) (a2). 5wt% protein-rich filling samples (b) in the absence (b.1.) and in the presence of model saliva (MS) (b.2.). PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate, WPI whey protein isolate and MS model saliva. Statistics can be followed in Supplementary Table D.3., Supplementary Table D.4., Supplementary Table D.5. and Supplementary Table D.6. (n=2×3).

Next looking at the influence of protein concentration for WPI (**Figure 31.a.1.**). In aqueous solutions, in the boundary regime and mixed regime increasing concentration led to significantly lower friction (p>0.05) (see **Supplementary Table D.3.**). In detail the 10wt% WPI solution is 80.52% lower in the boundary regime and 94.16% lower in the mixed regime. There are no significant

differences in the hydrodynamic regime. The improved lubrication of WPI with increasing concentration is consistent with results obtained in **Chapter 3** (see **Figure 17.**) between 10wt% and 20wt% and previous studies (Vardhanabhuti et al., 2011). For example, when WPI solutions are varied between 3 to 9% a reduction in boundary lubrication was observed. Improved lubrication is hypothesized to result from layer protein deposition creating thicker adhered layers and surface 'voids' being filled with additional protein aggregates reducing roughness (Chojnicka et al., 2008).

For food matrices (**Figure 31.b.1.**) increasing concentration from 5wt% to 10wt% leads to lower friction at low speeds (0-10mms^{-1}). Although, unlike aqueous solutions, differences are not significant between the samples (see **Supplementary Table D.5.**). It should be noted that the 10wt% model food does not include whey-permeate, whereas the 5wt% model foods incorporates whey permeate to achieve the same solid content as the 10wt% variant (see **Table 3.**). Therefore, the lower low-speed friction may indicate whey-protein is providing greater lubrication than whey permeate. This outcome is as expected because whey permeate is a biproduct of whey processing, containing significantly less protein (≈1%) and is primarily composed of lactose (≈90%) and minerals (≈9%) (Božanić et al., 2014) which are not known to be lubricants. Therefore, the difference in friction at low-speeds which are less dependent on bulk-properties (Stokes et al., 2013), is unsurprising.

Focusing on effect of model saliva on protein-solutions (**Figure 31.a.2.**), it is apparent that the behaviour was almost overlapping the system containing no saliva (**Figure 31.a.1.**). This was expected given the similar lubrication behaviour of solutions to that of model saliva. Whereas when the food matrices are mixed with model saliva, the filling boli (**Figure 31.b.2.**) did not behave like the systems without saliva addition (**Figure 31.b.2.**). In fact, the lubrication behaviour in fillings is largely driven by no-boundary-immediate mixed lubrication regime was transformed into a three-regime-type system in the boli samples (**Figure 31.b.2.**).

Particularly, at low-speeds (up to 10mms^{-1}), friction coefficients are higher for 10wt% WPI containing filling boli versus the 5wt% one (see **Supplementary Table D.5.** and **Supplementary Table D.6.**). Although the effect of protein concentration are not apparent in oil-driven lubrication in the filling samples, high content of protein (10wt% vs 5wt%), became very important in boli driving protein protein aggregation, preventing entrainment, and consequently leading to high frictional dissipation particularly in boundary regime in the presence of model saliva.

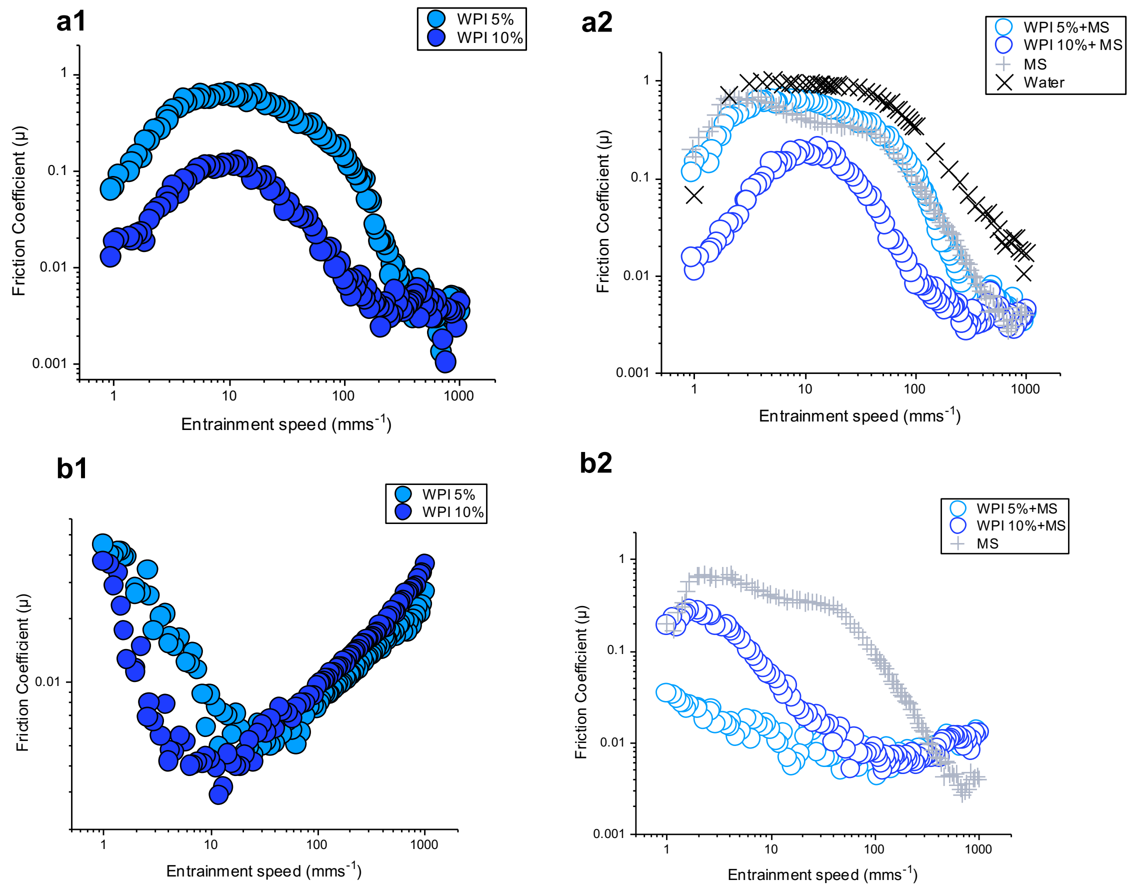


Figure 31. Mean friction coefficients in presence of higher concentrations of protein (5-10wt%) in aqueous solutions (a) in the absence (a.1.) and in the presence of model saliva (a.2.). Protein-rich food matrixes (5-10wt%) (b) in the absence (b.1.) and in the presence of model saliva (b.2.). WPI refers to whey protein isolate and MS model saliva. Statistics can be found in Supplementary Table D.3., Supplementary Table D.4., Supplementary Table D.5. and Supplementary Table D.6. (n=2×3).

5.3.4. Sensory Analysis

Figure 32. shows the results of the sensory analysis on the attributes generated **Table 4**. Five texture and three mouthfeel attributes are selected. Looking first at texture attributes, there are no significant differences between protein type and sensory 'viscosity' (**Figure 32.a.**). This is unexpected, as discussed earlier, all protein fillings have significantly different viscosity except for SPI and PPc (see **Supplementary Table D.2.**). However, what does relate to the instrumental rheology measurements is sensory 'smoothness'. 5wt% WPI is significantly smoother than the legume fillings PPc and SPI, corresponding to the lower 5wt% whey viscosity measurements of model foods.

Another important texture finding is that legume fillings are more powdery than dairy fillings which may be attributed to the presence of insoluble particles (**Figure 32.**, **Figure 27.a.** and **Figure 26.**). Poor plant protein solubility has previously been proposed to impair mouthfeel (Grossmann and McClements, 2023), however there is a lack of studies directly comparing solubility to mouthfeel in protein. Solubility was shown to be a key determinant of texture and mouthfeel sensation in oat-bran fibres. Soluble fibres were compared to insoluble fibres with the insoluble eliciting increased 'chalkiness' and 'particle perception' dominating overall mouthfeel and textural sensations (Chakraborty et al., 2019).

The skimmed milk powder model food is rated significantly more gritty and less smooth than all other fillings (**Figure 32.a.**). This finding appears to relate to tribology results for aqueous solutions and not tribology of food-matrices (see **Figure 30.**). As discussed earlier, the 5wt% SMP solution has poor lubrication compared to the other protein-solutions. Gritty/grainy textures for whey-protein yoghurts are previously hypothesized to come from particle characteristics (Morell et al., 2017). The presence of particles has been previously shown to increase roughness sensations as well as reduce texture attributes including

‘smoothness, creamy, fatty and slippery’ (Engelen et al., 2005a). Particle characteristics have also been shown to influence texture, with harder particles and more irregular particles perceived as larger by a trained panel (Engelen et al., 2005b). Therefore, the high grittiness as well as low smoothness despite high instrumental viscosity, of SMP is likely a reflection of SMP particles in the food matrices. Finally, there are no significant differences for ‘adhesive’ between pea and soy fillings. Regarding the influence of concentration, comparing 5wt% whey versus 10wt% did not yield any significant sensory differences in all texture attributes (**Figure 32.a.**).

Regarding mouthfeel attributes (**Figure 32.b.**) there are no significant different between protein type for melt-rate and mouthcoating. For drying, whey was significantly less drying than all other protein types. There are no significant sensory mouthfeel differences between legume fillings and skimmed milk protein or between soy and pea themselves. Finally, despite differences in material properties, there are also no significant sensory differences in mouthfeel between different concentrations of WPI filling.

In general, there appeared to be no clear association between the instrumental rheology and tribology results of the food matrices (see **Supplementary Figure D.1.** and **Supplementary Figure D.2.**). Further correlations between sensory and instrumental methods appeared to be inconsistent between model food measured alone and model food+ saliva boli, further highlighting the lack of clear relationship. The lack of clear relationship is consistent with previous findings, with previous studies using tribology and sensory analysis producing equivocal findings. First looking at protein solutions, a recent investigation using both sensory evaluations (visual analogue scale) and a dynamic tribology set-up showed astringency via sensory analysis in two different sources of pea proteins. However, the tribology results show different friction behaviours between the two pea proteins (Vlădescu et al., 2023). In model-food systems a link between tribology and sensory was established in chocolate milk. The study varied casein

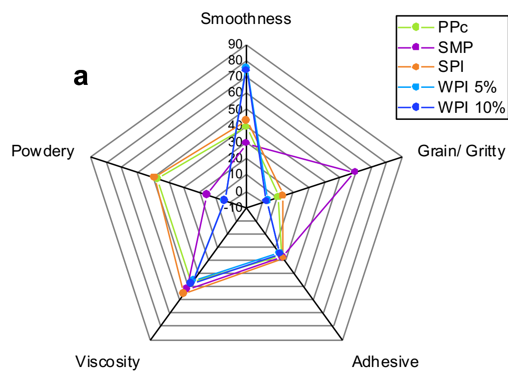
to whey protein isolate ratio found replacement of casein by WPI reduced friction in speed ranges of 0.5-10mms^{-1}. Sensory analysis (TDS) found these samples with higher WPI to have higher perceived creaminess and lower astringency, linking to tribology results (Zhu et al., 2020). However, a separate study comparing whey protein concentrate yoghurts compared to skimmed milk-powder yoghurts did not establish a clear relationship. Despite skimmed milk powder yoghurts having higher friction at speeds between 1-3mms^{-1}, they are perceived as 'creamier' and 'smoother' than whey protein yoghurts (Morell et al., 2017). Instead, sensory mouthfeel is proposed to be related to particle characteristics (Morell et al., 2017). Collectively, these previous study results in conjunction with present findings may suggest texture/mouthfeel is driven by friction differences (which can be observed in tribology experiments) but it may also be driven different factors which do not result in different lubrication. Therefore, it is important to consider protein characteristics such as solubility, and adsorption when trying to understand different texture/mouthfeel perceptions.

Table 5. Mean and standard deviations of sensory texture attributes. Data reported for 4 repeats by 8 panellists (n=4x8). Statistics can be found in Supplementary Table D.7.1.

	Smoothness		Grainy/ Gritty		Adhesive		Viscosity		Powdery	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Pea Protein Concentrate	39.21	19.75	10.61	12.72	26.08	16.89	46.9	16.93	46.91	16.49
Skimmed Milk Powder	29.26	18.23	59.78	9.90	27.49	17.03	51.71	10.68	15.36	16.72
Soy Protein Isolate	43.35	22.9	13.58	15.46	28.33	17.87	55.25	17.74	48.91	13.61
Whey Protein Isolate (5wt%)	75.54	11.74	3.60	7.47	24.67	15.43	45.18	5.45	3.93	5.16
Whey Protein Isolate (10wt%0	73.95	14.75	2.73	4.17	24.93	18.66	47.45	6.87	3.84	3.98

Table 6. Mean standard deviations of sensory mouthfeel attributes. Data reported for 4 repeats by 8 panellists (n=4x8). Statistics can be found in Supplementary Table D.7.1.

	Mouthcoating		Melt Rate		Drying	
	Average	SD	Average	SD	Average	SD
Pea Protein Concentrate	48.24	10.94	54.97	15.52	48.08	17.33
Skimmed Milk Powder	53	7.7	55.08	11.77	47.84	12.75
Soy Protein Isolate	50.92	11.24	53.37	11.11	46.93	16.73
Whey Protein Isolate (5wt%)	54.6	13.23	58.89	14.8	35.55	19.11
Whey Protein Isolate (10wt%)	53.12	11.92	59.72	15.23	36.56	18.63



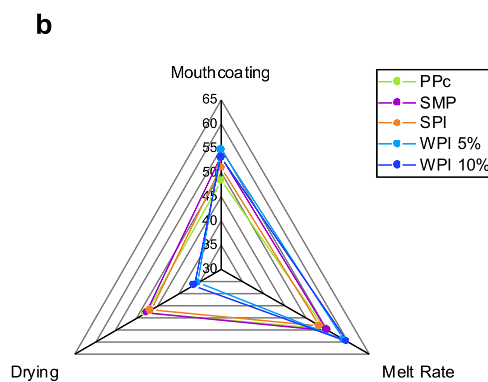


Figure 32. Radar plot of mean sensory panel scores (a) texture attributes (b) mouthfeel attributes and n= 8x4 (8 participants with four replicates). PPc refers to pea protein concentrate, SMP skimmed milk powder, SPI soy protein isolate, WPI whey protein isolate protein model foods (5wt%-10wt%).

5.4. Conclusion

The aim of this study was to analyse the effect of protein type and concentration in real food systems. The sensory results of the food systems are compared to material behaviours to verify instrumental methods for mouthfeel understanding.

Between protein types, skimmed milk powder is rated as 'gritty' which is hypothesized to be related to poor boundary lubrication (observed in tribology) and the presence of particles. The plant-protein foods are rated as powdery, which do not clearly link to material behaviour (rheology/tribology) but may be resultant from poor solubility. Whey protein was rated as most favourably, which is likely a combination of good solubility, good lubricity, and stable viscosity. Interestingly, although increasing whey concentration improved material behaviour it was not large enough to be also shown in sensory ratings. Overall, the different sensory attributes appeared to relate to different instrumental methods, highlighting the importance of adopting an integrative approach instead of relying on a single parameter. Secondly, understanding sensory characteristics of the more complex (real) food systems for some attributes was easier understood in simpler systems. This highlights the challenges systems with several ingredients which can affect lubrication differently and often the protein aggregating in the continuous phase might play an important role even in the mouthfeel of the food matrix.

Chapter 6. Summary and Discussion

The main objective of this research was to gain insights into the mouthfeel and perception associated with various types of plant and dairy proteins either as solutions or in food matrices by analysing their flow and lubrication characteristics. Given that plant proteins evoke distinct mouthfeel sensations compared to dairy proteins (Vlădescu et al., 2023, Mehta et al., 2023, Canon et al., 2021, Tanger et al., 2021, Omrani Khiabanian et al., 2020), comprehending the sensory impact of these ingredients holds significance for food development. In contrast to resource-intensive sensory evaluations, researchers have sought to employ instrumental techniques to provide mechanistic information behind sensory experiences. This research stems from unresolved question: how protein affects mouthfeel? This discussion chapter first summarizes contributions from each thesis chapter, then overall findings before providing recommendations for future research.

6.1. Chapter summaries and novelty of thesis

To first assess the current evidence, a systematic review was conducted (**Chapter 2: Protein-saliva interactions: a systematic review**[a]). The following knowledge gaps were identified and targeted to ensure novelty of the thesis; plant proteins, high protein concentrations, plant protein + model saliva interactions in realistic protein: saliva ratios and comparing protein through different systems (solutions/emulsions/ food matrices).

The first series of experiments used aqueous suspensions to compared higher concentrations of protein (10.0wt% and 20.0wt%). Four proteins were selected including whey protein, soy protein, pea protein, and sodium caseinate. Key

results showed plant proteins had a more a prominent shear thinning behaviour as well as higher friction coefficients (**Figure 14.**). Increasing concentration improved lubricity for whey proteins but not for soy proteins.

It was next imperative to understand whether such high frictional behaviour of plant proteins was replicated in more complex systems, such as emulsions, as these are more indicative of real-food systems. Protein(s) chosen also included skimmed-milk-powder, which had a lower protein content (≈33%) but is common in foods and typically substituted for fortified proteins. Key findings showed distinct emulsion characteristics by protein type. All emulsions except the ones stabilized by whey protein isolate had shear-thinning behaviour (see **Figure 23.**). For lubrication, PPc had high friction in boundary regimes (entrainment speed 9.97mms^{-1}, 0.0498µ), whereas SPI and WPI both had the lowest (0.0176µ for SPI and 0.0134µ WPI) (see **Supplementary Table C.5.**). Overall, this indicated that although in the presence of oil, the tribology is largely driven by oil film lubrication, the protein aggregation particularly in pea still had a distinct effect on increasing frictional dissipation.

Finally, the effects of the proteins in real food systems were investigated using both instrumental and sensory analysis (**Chapter 5**). Sensory findings showed model foods varied in texture and mouthfeel by protein type, with both legume and skimmed milk powder rated least favourably (**Figure 32.**). Another key finding was the lack of correlation between instrumental material properties (viscosity and tribology) of the food matrices and sensory attributes. However, material properties of bulk protein solutions linked to sensory findings, as skimmed milk powder in a bulk solution had significantly higher boundary friction and a delayed onset of the mixed regime which may relate to the gritty sensations (**Supplementary Table D.3.**). As legume proteins did not have differences in bulk-solution lubrication compared to whey, powdery sensations may be linked to protein characteristics such as poor solubility.

6.2. Collective summary

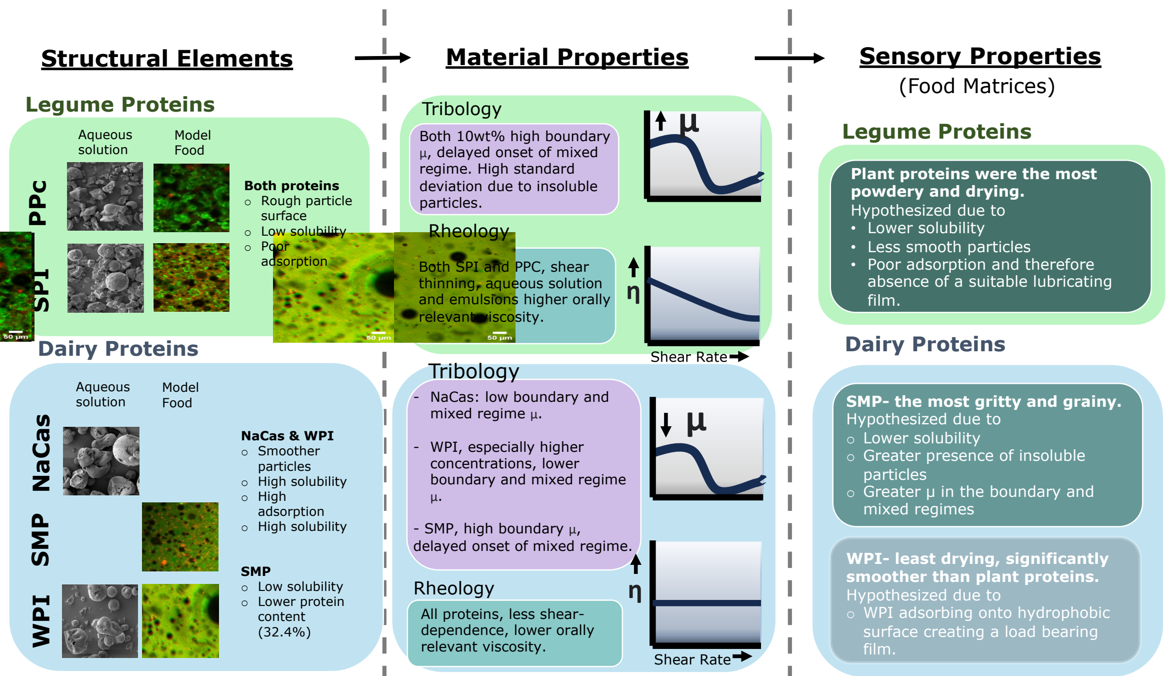


Figure 33. Summary of results by protein type across different systems throughout chapters.

The differences between protein types are summarized in **Figure 33.**, showing plant proteins tended to have higher boundary friction, greater shear thinning behaviour and unpleasant sensory mouthfeel. As mentioned in previous chapters, the greater friction and shear thinning behaviour is hypothesized to arise from aggregation of plant proteins. Aggregates have previously been proposed to jam the contact zone, resulting in higher friction (Kew et al, 2021). In contrast, the dairy protein WPI was hypothesized to have lower aggregation, creating a smoother film leading to lower boundary friction. These hypothesized mechanisms are shown in **Figure 34**. This jamming of aggregates is also shown for the pea protein emulsion, with pea protein aggregates disrupting the oil film lubrication leading to high friction. Looking at food matrixes, there are no observable differences between protein type and lubrication.

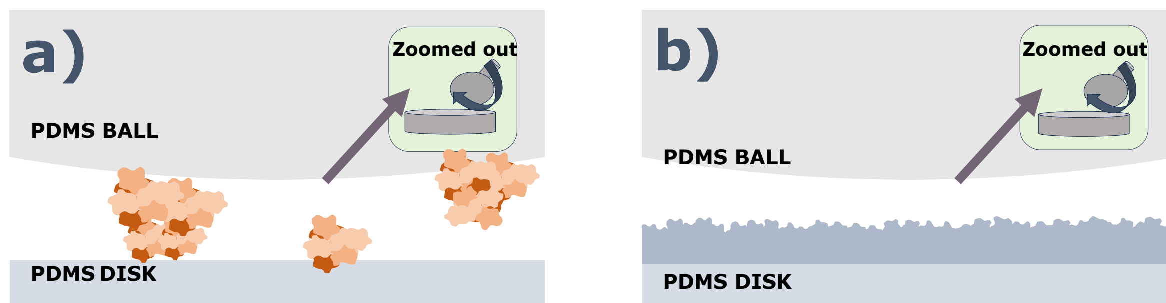


Figure 34. Hypothesized mechanisms behind lubrication differences of a) plant proteins and b) dairy proteins

When looking at concentration effects, when concentration was increased boundary friction was reduced for WPI in both Chapter 3 (10-20wt%) and Chapter 4 (5-10wt%) aqueous suspensions. For the legume protein SPI, no such change was observed. However, sensory analysis did not establish a difference in mouthfeel or texture when concentration was increased. Therefore, further analysis is required to fully elucidate the effect of protein concentration.

Finally, looking across the different systems utilized, aqueous protein solutions had the lowest viscosity and highest friction. Adding sunflower oil for emulsions and polysaccharides and fat for model foods lead to a reduction in friction. The overall combined effect of medium (dispersion vs emulsion vs model food) and protein type on tribology/rheology was tested using univariate analysis. The marginal means showed differences between media, whereas means were relatively consistent between different proteins for each medium in both rheology and tribology results (see **Supplementary Figure E.1.** and **Supplementary Figure E.2.**). Testing for between subjects' effects confirmed these visual interpretations with lower p values for the medium than the protein type (see **Supplementary Table E.1., Supplementary Table E.2., Supplementary Table E.3., Supplementary Table E.4.**, **Supplementary Table E.5.** and **Supplementary Table E.6.** for full details). Therefore, while all were significant, the much greater significance for the medium implies that as expected, the form

protein was measured in affected tribology and rheology results more substantially compared to protein type. Out of protein types, PPc had particularly poor lubrication in both protein dispersions and emulsions (**Figure 16., Figure 18., Figure 24. and Figure 25.**). This was also shown through unpleasant sensory ratings (**Figure 32.**). Therefore, food manufacturers may find the use of PPc particularly challenging compared to other protein types.

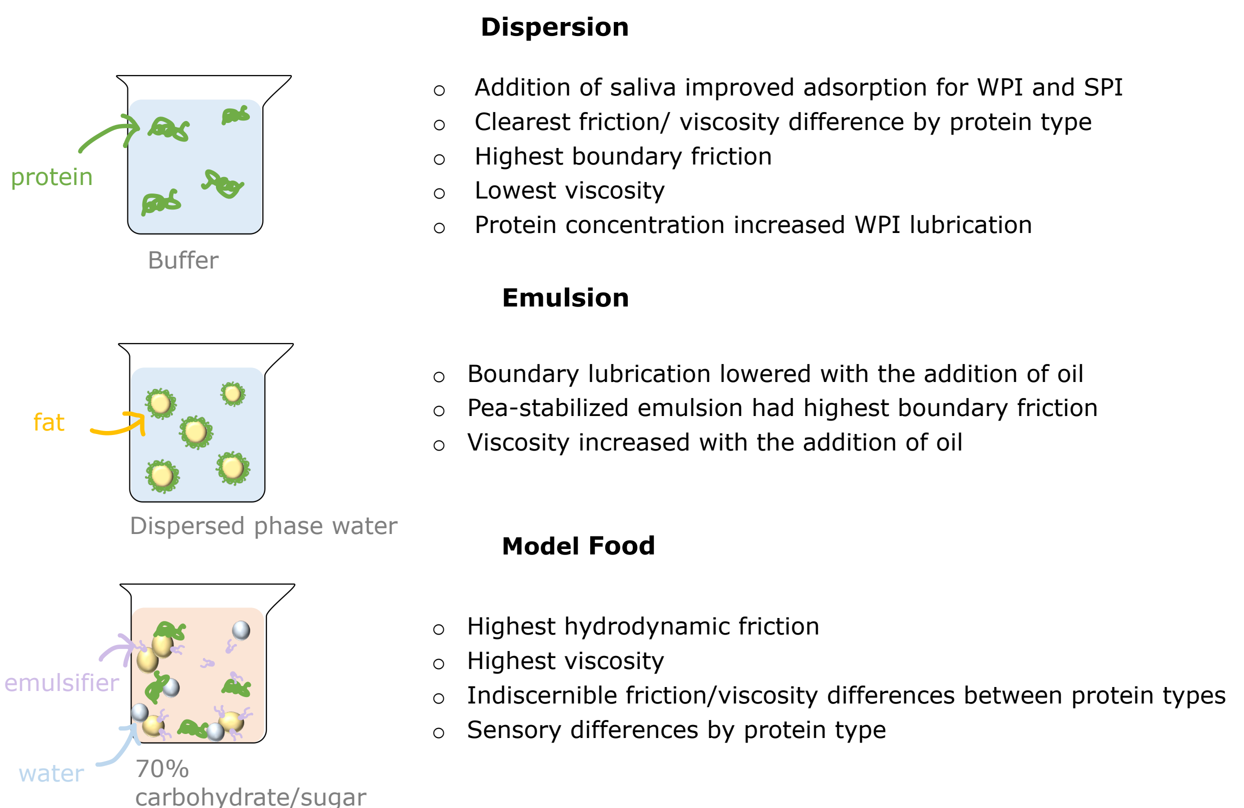


Figure 35. Schematic showing differences collectively between systems used.

6.3. Limitations

Although model saliva was utilized solely throughout this thesis, it is important to acknowledge that model saliva differs from human saliva significantly in lubrication and adsorption performance (Sarkar et al., 2019b). Human saliva, being composed of several proteins which also contribute to lubrication, although not in a smaller proportion compared to mucin (Sarkar et al., 2019b). The decision to avoid human saliva was driven by its variability, as discussed in the introduction, along with inherent challenges in handing and processing. Human saliva degrades rapidly and necessitates both fast and careful processing. In particular lubrication properties of saliva change upon freezing, centrifugation and dilution (Takehara et al., 2013, Schipper et al., 2007). Furthermore, the acquisition of a sufficiently large human saliva sample to use in the MTM-tribometer posed challenges. The smallest feasible sample was 10mL, implying that acquiring 6 repetitions for saliva alone would require 60mL. Given that participants typically provide 2mL (Wang et al., 2021c, Sarkar et al., 2019b), this approach would have imposed considerable resource constraints and dilution when conducting experiments involving both saliva and multiple samples.

In the course of this thesis, a saliva-to-sample ratio of 1:4 was used to align with practices observed in previous literature (Devezeaux de Lavergne et al., 2015, Stribiţcaia et al., 2020). However, recent developments suggests that this ratio may be an overestimation of saliva, as more recent studies have used a lower ratio of 1:12 (Laguna et al 2021b, Turcanu et al, 2018). While the primary focus of this thesis was on astringency/after feel, therefore additional saliva secretion is likely to occur to 'wash off' the protein. At present, there is no consensus in the literature regarding how to select an appropriate saliva-sample ratio, leading to suggestions to develop a clear criterion. In addition, the amount of saliva is known to depend on a range of factors including volume ingested per sip/bite, mouth residue time and the characteristics of the food. It is recommended that studies

should incorporate preliminary tests to calculate ratios for the desired food of investigation (Laguna et al, 2021a).

This thesis used a tribometer to try capture dry astringent high friction sensations, however as shown through the lack of linking to sensory results, different methods may be more appropriate for detecting these complex sensations. As mentioned in the introduction a variety of innovative tribometer set ups have been established. For example, a tongue substrate was created by replicating the topography of the tongue using fungiform and filiform from papillae images. The tongue substrate was shown to have reproducible sliding-lubrication results in a variety of liquids including water and diluted syrup solutions (Wang et al., 2021a). Previous research has also used a biomimetic tongue (created from moulds of a human tongue) apparatus in conjunction with the MTM to find significant differences due to the absence of rolling-friction in biomimetic tongue set-ups. Authors subsequently recommend further development of these methods to include rolling friction (Soltanahmadi et al., 2022).

Similarly, taking a more temporal approach to studying lubrication in different stages could provide insights into its build-up over time. Innovative setups involving pre adsorption of saliva have already been initiated to explore this aspect (Vlădescu et al., 2023, Selway and Stokes, 2013). It was shown that when human saliva is pre-adsorbed, different foods affected the salivary film contrastingly (Selway and Stokes, 2013). The study used low fat vs high fat food products (yoghurts/custards/creams), which exhibited similar rheological attributes but different tribological properties. Specifically, when saliva was exposed to yoghurt higher in fat, friction remained constant. In contrast, lower-fat samples exhibited a decrease in lubrication, attributed to the interaction between the food sample and the deconstruction of the adsorbed salivary film. Furthermore, lower fat yoghurt had an accelerated rise in friction between saliva-coated surfaces compared to two custard samples (Selway and Stokes, 2013). Another study which looked at protein-mouthfeel in a similar set up again found

differences by protein type. Dry friction was first measured, before human saliva added and allowed to adsorb for 10 minutes. Following this a protein solution was added and sliding friction was recorded (Vlădescu et al., 2023). Proteins were found to reduce friction at different rates over time (Vlădescu et al., 2023). Considering these results, authors recommended further exploration incorporating dynamic sensory analysis to understand the transient lubrication mechanisms which occur during oral processing (Selway and Stokes, 2013).

Sensory methods which can measure attributes temporally include progressive profiling or temporal dominance methods that allow the assessment of attribute intensity at various time intervals during oral processing. These methods were used in protein-yoghurts to confirm the dynamic mouthfeel perception. Interestingly, attributes dominant earlier on had a stronger impact on mouthfeel liking than later dominants (Greis et al., 2020), although there is further scope to look at different food systems as well as lubrication sensations in isolation. In addition, as saliva flow depends on food/beverage stimuli, it was suggested accounting for the flow could improve mouthfeel understanding (Davies et al., 2009). Building on the dynamic techniques discussed earlier, future investigations may seek to examine the continuous impact of added saliva to further enhance the realism methodologies.

6.4. Future directions and recommendations

6.4.1. Protein types

A range of proteins were used in this thesis. However, given the rapidly evolving nature of the food industry, there is scope to transition towards proteins with lower carbon footprints. Embracing innovative, lesser-known protein sources such *in*

vitro cell cultured meat, insect, algae and microbes becomes crucial for the future of food design (Collett et al., 2021). The ultimate goal of optimizing food production to effectively address the ongoing challenges posed by climate change.

The present study used commercial proteins. Proteins through different suppliers, especially within pea protein, were shown to vary in this thesis as also shown in previous literature. Previous literature established two separately sourced pea proteins had different lubrication and friction behaviour despite similar sensory scores (Vlădescu et al., 2023). More specifically, past investigation established differences in starch and protein content and composition among pea varieties. The quantification of protein content across 59 pea lines was measured using Dumas combustion and SDS-PAGE, revealing protein content varied from 13.7% to 30.7%. Th variations were found to be influenced by the type of pea, with wild relatives having higher average protein content (28.5%) than yellow peas (21.8%). Protein composition also varied, with globulin protein content varying between 49.2% to 81.8% (Tzitzikas et al., 2006). These differences hold significance considering recent advances which have demonstrated interactions of specific pea-proteins with saliva. The study looked at raw yellow pea when mixed with whole human unstimulated saliva. Analysis was conducted using Shotgun Liquid Chromatography-Mass Spectrometry (LC-MS) and Native-Polyacrylamide Gel Electrophoresis (PAGE). The specific globulins namely legumins, Vicalins, Albumins were established to aggregate with salivary proteins (Assad-Bustillos et al., 2023).

Similarly, there have been attempts to link specific phytochemical components of pea protein to sensory attributes. The study combined sensory analysis (check-all-that-apply) with phytochemical profile characterization via ultra-high-performance liquid chromatography-diode array detector-tandem mass spectrometry (UPHLC). Findings identified a total of 29 compounds including phenolic acids, flavonoids and saponins were correlated with either perceived

bitterness or astringency (or both). It was suggested the mixture of compounds can contribute to astringency and it can be difficult to isolate specific compounds as it may be due to a mixture. (Cosson et al., 2022). Collectively, there is scope for future research to combine methodologies to diverse pea types to see if different binding to saliva occurs between types of peas.

6.4.2. Protein-human saliva interactions

Recent developments in non-protein astringency have however begun to isolate specific components related to astringency. Developments used a cell-based model with human saliva, mucosa pellicle and oral cell lines to unravel astringency perceptions (Soares et al., 2019). This set up determined the interactions of procyanidins with distinct oral components (oral cells, human salivary proteins, and mucosa) in relation to astringency (Soares et al., 2019). A second study used the same protocol and subsequently proposed two distinct drivers of astringency in phenolic compounds (Guerreiro et al., 2022). Results show differences in binding between gallotannins and flavonols. As gallotannins have previously been described as harsh, the shown interactions between salivary proteins were suggested as responsible for more harsh astringent sensations. As flavonols have been described as velvety and silk, authors posited interactions (Huang and Xu, 2021) of the tongue cell, may therefore, be more related to velvet and silky sensations (Guerreiro et al., 2022). Expanding this methodology into the realm of protein may provide valuable insights into the origins of protein-saliva interactions. Moreover, it is important to note the mentioned studies did not incorporate sensory analysis as a validation of astringency. Therefore, an approach combining cell-based model with advanced sensory investigation could offer an elevated understanding.

6.4.3. Strategies of reducing friction

Finally, this thesis concluded the high degree of insolubility of plant proteins was likely a cause of the high friction and poor palatability observed. Strategies to improve protein properties include the development of different processing methods which have been shown to affect sensory mouthfeel in dairy systems (Li et al., 2018). For example, ultra-pasteurization compared to high-temperature short-time pasteurization was found to have higher sensory astringency and mixed regime friction (Li et al., 2018). Processing methods which improve protein functionality include glycation. This technique involves covalent bonds forming between proteins and carbohydrates in the initial stages of the Maillard reaction (Kutzli et al., 2021) and has enhanced protein characteristics within the food domain (Soltanahmadi et al., 2022). The method increased protein resilience when exposed to environmental stresses (such as pH and ionic strength) and as well as increasing protein solubility and stability (Soltanahmadi et al., 2022). From an environmental perspective the approach is advantageous as it does not use hazardous chemicals which have been used in the past and often produce harmful by-products (Soltanahmadi et al., 2022). For soy protein, which was suggested as the most extensively studied, glycation has proved a successful method in mediating poor solubility and bitter off tastes (Kutzli et al., 2021). Although, the structure-functional relationship using this processing technique has not yet fully understood for many plant proteins with further research required.

Another promising processing method is micro-gelation which have been used to increase protein concentration as well as improve lubrication properties of proteins (Sarkar et al., 2017). Microgels involve the processing steps of hydrating proteins, thermally denaturing protein to form a gel shearing and homogenization. This causes hydrophobic interactions to occur between protein and water molecules and cross linking proteins, which are converted to gel like particles

(Sarkar et al., 2017, Kew et al., 2023). Both plant and dairy protein microgels have been shown to have effective boundary lubrication (Kew et al., 2021, Sarkar et al., 2017), and therefore gained substantial attention as substitutes for fats. Further research optimizing this micro-gelation technology in a varied of model foods with consumer testing is an interesting area of future development.

6.5. Implications of current findings

In conclusion this thesis has contributed significantly to a heightened understanding of the different material behaviour of food proteins which influence sensory perception. The knowledge gained in this thesis showed plant proteins to have poor adsorption and lubrication properties, particularly pea protein concentrate. This was shown to impair their mouthfeel when implemented in a model food system. Careful formulation may thus be required to mitigate differences compared to dairy proteins. In addition, increasing protein concentration is shown to have beneficial lubricating properties. Understanding lubrication mechanisms in more complex systems remains challenging.

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Appendix A: Supplementary Information for Chapter 2

Supplementary Table A. 1. Risk of Bias Criteria

Question Number used Table A.2	Criteria
	1. Reporting Quality
1.A	Disclosed protein origin and type used
1.B	Disclosed (Model) Saliva origin and type used
1.C	Disclosed protein processing method
	Only Applicable if using human saliva:
1.D	Reported how many saliva donors
1.E	Reported age range and sex
1.F	Reported health status of donors (and how they were deemed healthy)
1.G	Reported how saliva was stimulated
1.H	Reported how saliva was stimulated
	2. Performance Bias
2.A	Reported temperature
2.B	Reported ratio of mixture between saliva and protein
2.C	Reported the makes of apparatus used for methodology
	3. Selection Bias
3.A	Kept the food system/ delivery of the protein the same in all conditions except for 1 variable
3.B	Blinded sensory panel to the true objectives of the study
	4. Detection Bias

4.A	Used the same methods for the control and exposure
4.B	Reported the number of replicates

Supplementary Table A.1. Assessment of risk per included study

Reporting quality				Only applicable if using human saliva					Performance Bias			Selection bias		Detection Bias		Totals				
Reference	1.A	1.B	1.C	1.D	1.E	1.F	1.G	1.H	2.A	2.B	2.C	3.A	3.B	4.A	4.B	RQ	PB	SB	DB	Total
Ahmad, et al. (2020)	2	2	NA	NA	NA	NA	NA	NA	2	2	2	2	NA	2	2	100%	100%	100%	100%	100%
Ahmad, et al. (2020)	2	2	NA	NA	NA	NA	NA	NA	1	2	2	2	NA	2	2	100%	83%	100%	100%	96%
Andrewes, et al. (2011)	2	2	2	2	0	1	2	2	0	2	2	2	NA	2	2	81%	67%	100%	100%	87%
Beecher, et al. (2008)	2	NA	2	2	2	1	0	0	1	2	2	2	2	2	0	64%	83%	100%	50%	74%
Biegler, et al. (2016)	2	2	NA	2	1	1	0	0	1	2	2	2	0	2	2	57%	83%	50%	100%	73%
Campbell, et al (2017)	2	NA	2	2	1	2	0	2	1	NA	2	0	NA	2	2	79%	75%	0%	100%	63%

Çelebioğlu, et al. (2015)	2	2	2	NA	NA	NA	NA	NA	1	2	2	2	NA	2	2	100%	83%	100%	100%	96%
Çelebioğlu, et al. (2016)	2	2	NA	NA	NA	NA	NA	NA	1	2	2	2	NA	2	2	100%	83%	100%	100%	96%
Çelebioğlu, et al. (2017)	2	2	2	NA	NA	NA	NA	NA	2	2	2	2	NA	2	0	100%	100%	100%	50%	88%
Childs, et al. (2010)	0	NA	2	2	1	0	NA	NA	1	NA	2	0	2	2	2	50%	50%	50%	100%	63%
Dresselhuis, et al. (2008)	2	NA	NA	2	0	1	2	NA	0	2	2	2	2	2	2	70%	67%	100%	100%	84%
Fuhrmann, et al. (2019)	2	NA	2	2	0	0	0	0	2	2	2	0	2	2	2	43%	100%	50%	100%	73%
Hsein, et al. (2015)	2	2	2	NA	NA	NA	NA	NA	1	2	2	2	NA	2	2	100%	83%	100%	100%	96%
Hu, et al. (2019)	2	0	NA	2	2	1	NA	2	1	2	2	0	NA	2	2	75%	100%	0%	100%	90%
Kelly, et al. (2010)	2	NA	2	2	2	1	2	2	1	2	2	2	2	2	2	93%	83%	100%	100%	94%
Koukoura, et al. (2019)	2	2	NA	NA	NA	NA	NA	NA	1	2	2	2	NA	2	2	100%	83%	100%	100%	96%
Lee, et al. (2008)	2	NA	2	2	1	0	NA	NA	1	NA	2	2	2	2	0	70%	75%	100%	50%	74%
Morell, et al. (2015)	2	2	2	2	2	0	NA	NA	2	2	2	2	2	2	2	83%	100%	100%	100%	96%

Morell, et al. (2017)	2	2	2	2	0	1	2	2	2	2	2	2	2	2	2	81%	100%	100%	100%	95%
Ritzoulis, et al. (2012)	2	2	2	NA	NA	NA	NA	NA	1	2	2	2	NA	2	2	100%	50%	100%	100%	88%
Sano, et al. (2005)	2	NA	2	2	2	1	NA	NA	0	NA	2	2	2	2	2	90%	50%	100%	100%	85%
Sarkar, et al. (2009)	2	2	2	NA	NA	NA	NA	NA	2	2	2	2	NA	2	2	100%	100%	100%	100%	100%
Silletti, et al. (2010)	2	NA	2	0	0	0	2	1	1	0	2	2	NA	2	0	50%	50%	100%	50%	63%
Silletti, E., et al. (2007)	2	NA	2	1	1	1	1	1	1	2	2	2	NA	2	2	64%	83%	100%	100%	87%
Silletti, E., et al. (2007)	2	NA	2	2	2	1	2	2	1	2	2	2	NA	2	2	93%	83%	100%	100%	94%
Silletti, et al. (2008)	2	NA	2	NA	0	2	2	2	1	2	2	0	NA	2	2	86%	83%	0%	100%	67%
Vardhanabhuti, et al. (2010)	2	NA	2	2	2	0	NA	NA	1	2	2	2	2	2	2	80%	83%	100%	100%	91%
Vardhanabhuti, et al. (2011)	2	NA	2	2	0	0	2	2	1	0	2	2	1	2	2	71%	50%	75%	100%	74%
Vingerhoeds, et al. (2009)	2	NA	2	0	0	0	NA	NA	1	NA	2	2	2	2	2	40%	75%	100%	100%	79%
Vingerhoeds, et al. (2005)	2	2	2	2	2	1	0	2	1	2	2	2	NA	2	0	81%	83%	100%	50%	79%

Wang, et al. (2016)	0	NA	0	2	0	0	NA	NA	0	NA	2	0	1	2	2	20%	50%	25%	100%	49%
Withers, et al. (2013)	2	2	2	NA	NA	NA	NA	NA	1	2	2	2	NA	2	0	100%	83%	100%	50%	83%
Ye, et al. (2011)	2	NA	2	2	0	2	2	2	2	2	2	2	2	2	2	86%	100%	100%	100%	96%

Yes= 2, No= 0, Ambiguous/ indirect =1, NA did not count towards scores, DB: Detection bias, PB: Performance Bias, RQ: Reporting quality ,SB: Selection Bias,

Appendix B: Supplementary Information for Chapter 3

Supplementary Table B. 1. Means and standard deviations of apparent viscosity values of samples in presence or absence of model saliva reported at orally relevant shear rate of 50 s^{-1}. Data is reported for three repeats for triplicate measurements (n=3x3). Different lower case subscript letters in the same column indicate a statistically significant difference (*p<0.05*), obtained from ANOVA with multiple comparisons (Bonferroni).

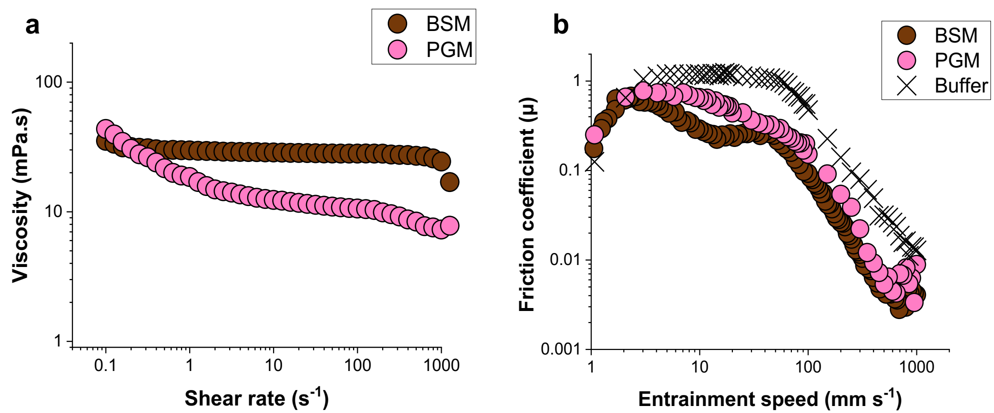
Samples	Apparent Viscosity (mPa.s)	Standard Deviation (mPa.s)
10wt% WPI	9.19^{a}	2.04
10wt% Soy	85.33^{b}	84.56
10wt% Pea	44.94^{b}	5.97
10wt% NaCas	20.85^{a}	3.23
20wt% WPI	$20.42.^{a}$	1.65
20wt% SPI	153.42^{c}	99.85
Model Saliva (MS)	10.81^{a}	1.12
10 wt% WPI +MS	9.32^{a}	1.24
10 wt% SPI +MS	154.99^{c}	128.37
10wt% PPc + MS	24.57^{a}	4.67
10wt% NaCas + MS	10.27^{a}	1.57
20wt% WPI + MS	8.40^{a}	1.24
20wt% SPI + MS	133.47^{bc}	143.90

Supplementary Table B. 2. Means and standard deviations of friction coefficients reported for protein samples at various lubrication regimes. Data are reported for three repeats for triplicate measurements (n=3x3). Different lower case subscript letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

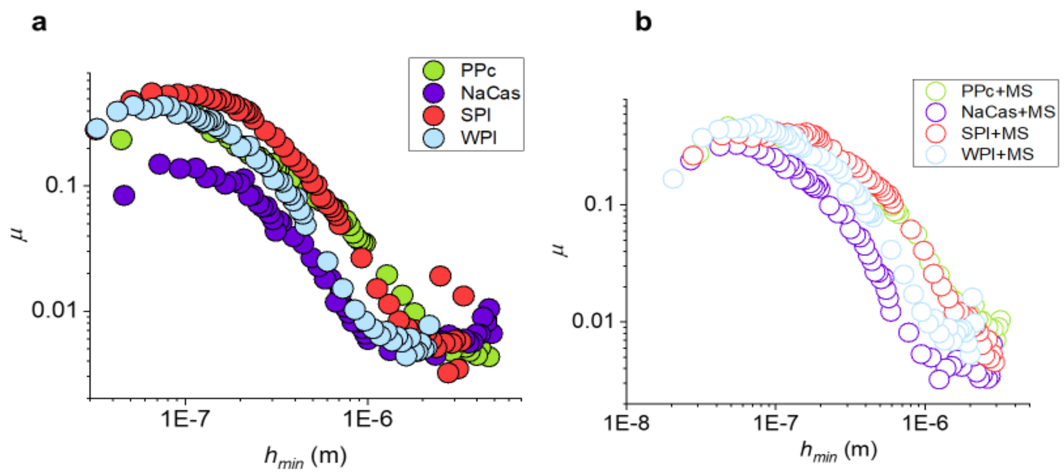
	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(5-10 mms^{-1})		(100-150 mms^{-1})		(700-900 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
10wt% PPc	0.253 ^{a}	0.065	0.027 ^{ab}	0.012	0.005 ^{a}	0.004
10wt% NaCas	0.111 ^{a}	0.116	0.005 ^{a}	0.002	0.007^{ab}	0.004
10wt% SPI	0.518 ^{b}	0.124	0.038 ^{b}	0.015	0.005 ^{a}	0.003
10wt% WPI	0.413 ^{ab}	0.148	0.037 ^{b}	0.024	0.005 ^{a}	0.002
20wt% SPI	0.501 ^{b}	0.074	0.028 ^{ab}	0.014	0.009 ^{b}	0.003
20wt% WPI	0.182 ^{a}	0.092	0.011 ^{a}	0.006	0.005 ^{a}	0.003

Supplementary Table B. 3. Means and standard deviations of friction coefficients reported for protein samples + model saliva mixtures (4:1 w/w) at various lubrication regimes. Data are reported for three repeats for triplicate measurements (n=3x3). Different lower-case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

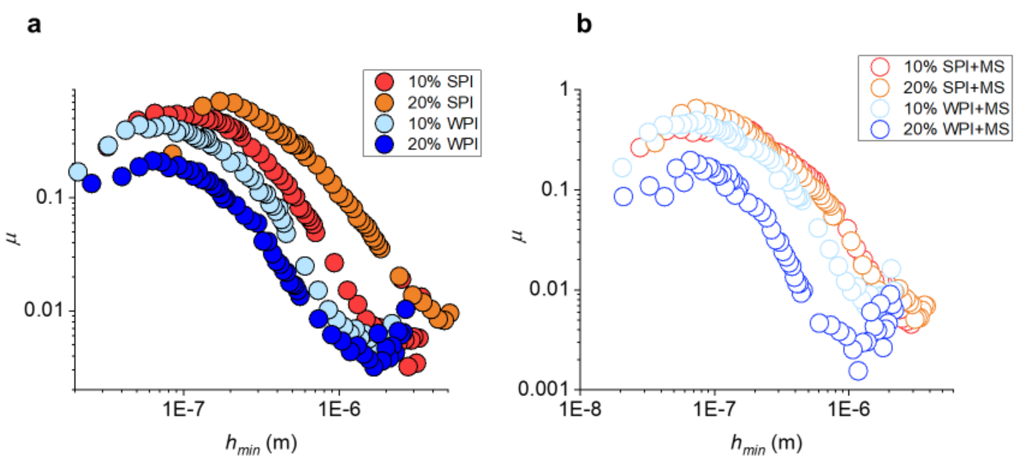
	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(5-10 mms^{-1})		(100-150 mms^{-1})		(700-900 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
10% PPc + MS	0.389	0.079	0.0696	0.029	0.007	0.005
10% NaCas+MS	0.245	0.064	0.010	0.005	0.004	0.003
10% SPI+MS	0.399	0.064	0.082	0.059	0.006	0.007
10% WPI+MS	0.454	0.164	0.059	0.047	0.007	0.005
20% SPI+MS	0.485	0.095	0.043	0.033	0.005	0.002
20% WPI+ MS	0.229	0.099	0.019	0.008	0.006	0.004



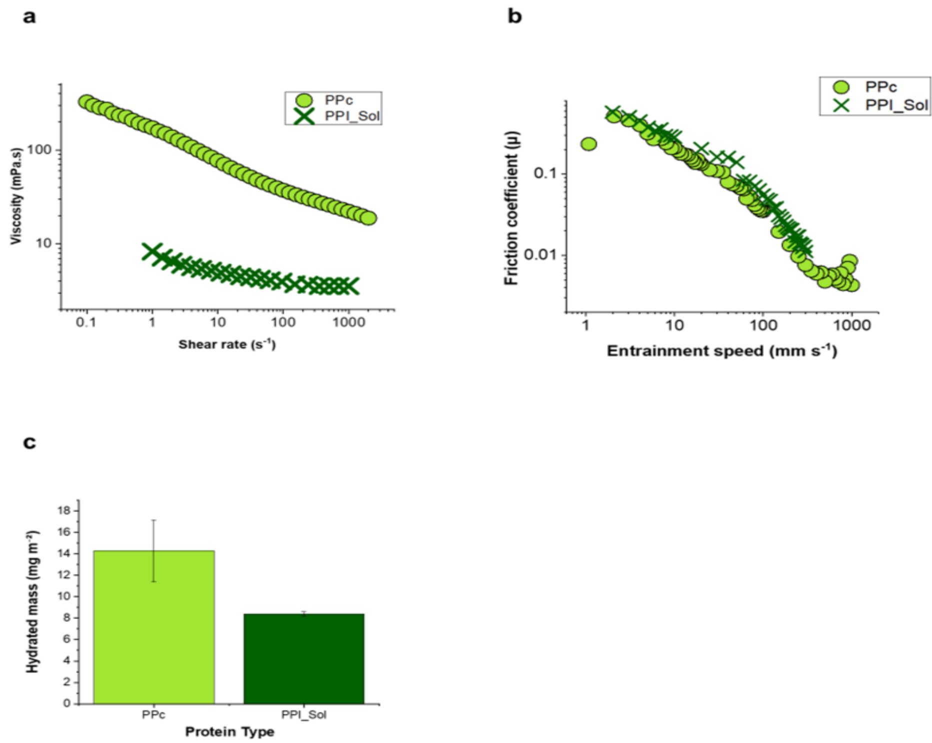
Supplementary Figure B. 1. Mean apparent viscosities of model saliva containing either PGM or BSM as a function of shear rate (a) and mean friction coefficients in presence of model saliva containing either PGM or BSM as a function of entrainment speeds (b). Data is reported for average of three repeats for at least duplicate samples (n=2x3).



Supplementary Figure B. 2. Mean friction coefficients plotted as a function of film thickness (h_{min}) on proteins in absence (a) and presence (b) of model saliva. Data shown for three independent readings on triplicate measurements (n=3x3).

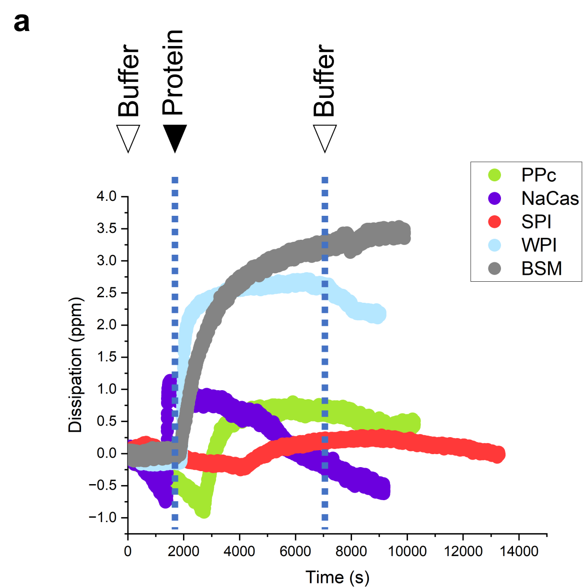


Supplementary Figure B. 3. Mean friction coefficients plotted as a function of film thickness (hmin) on high concentration of proteins in absence (a) and presence (b) of model saliva. Data are shown for three independent readings on triplicate measurements (n=3x3).

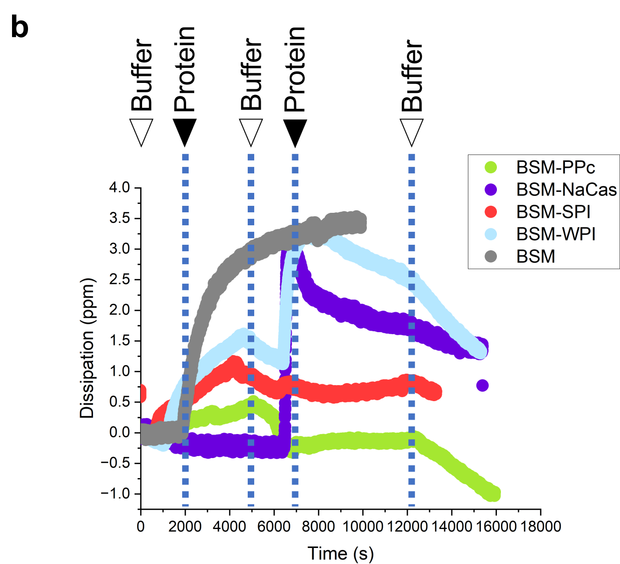


Supplementary Figure B. 4. Mean viscosity (a) friction (b) and hydrated mass (c). Comparing own data (PPc) to data reported by Zembyla et al., 2021 using soluble fraction (PPc_Sol). Error bars represent ± standard deviations.

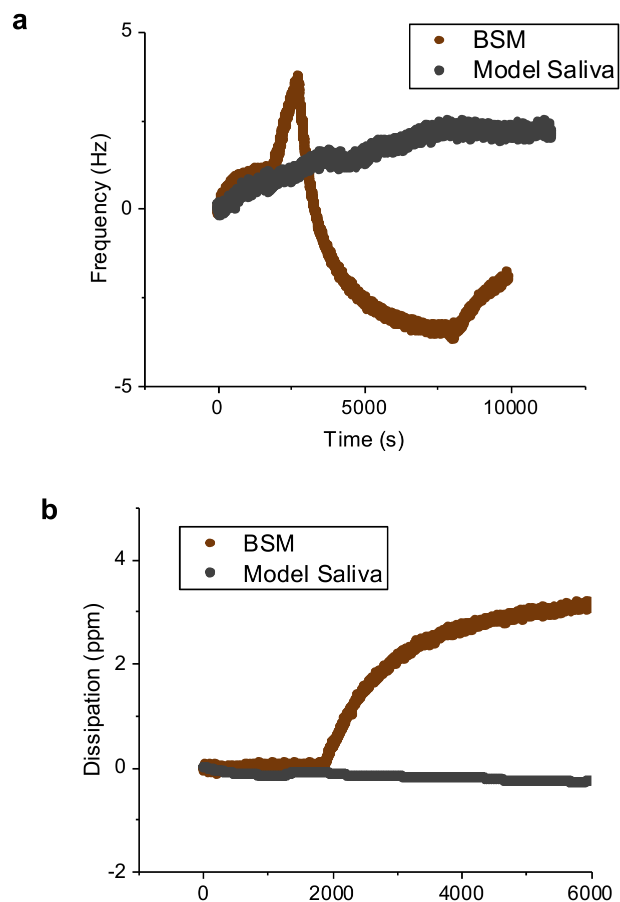
a



b



Supplementary Figure B. 5. Dissipation shift (5th overtone) as a function of time of a) 0.1mg/mL protein on PDMS-coated surfaces and b) 0.1mg/mL BSM followed by addition of 0.1mg/mL protein on PDMS surfaces. Measurements were repeated in triplicates (n=1x3).



Supplementary Figure B. 6. Frequency shift (5th overtone) as a function of time of 0.1mg/mL BSM or model saliva a) and dissipation shift (5th overtone) on PDMS surfaces. Measurements were repeated in duplicate (n=2).

Appendix C: Supplementary Information for Chapter 4

Supplementary Table C. 1. Means and standard deviations of apparent viscosity values of aqueous solutions in presence or absence of model saliva (BSM) reported at orally relevant shear rate of $50s^{-1}$. Data are reported for two repeats for triplicate measurements (n=3x2). Different lower case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

Samples	Apparent Viscosity (mPa.s)	Standard Deviation (mPa.s)
5wt% PPc	13.94_{SPI}	2.81
5wt% SPI	$424.91_{\text{PPC, SMP, 5wt\% WPI, 10wt\% WPI, SPI+MS, PPc+MS, SMP+MS, 5wt\% WPI+MS, 10wt\% WPI+MS, MS}}$	416.12
5wt% SMP	11.84_{SPI}	3.16
5wt% WPI	9.16_{SPI}	3.49
10wt% WPI	9.75_{SPI}	2.46
Model Saliva (MS)	27.15_{SPI}	7.08
5wt% PPc + MS	14.20_{SPI}	6.35
5wt% SPI + MS	22.63_{SPI}	17.32
5wt% SMP + MS	10.13_{SPI}	2.42
5wt% WPI + MS	16.89_{SPI}	9.76
10wt% WPI +MS	9.59_{SPI}	2.78

Supplementary Table C. 2. Means and standard deviations of apparent viscosity values of emulsions in presence or absence of model saliva (PGM) reported at orally relevant shear rate of $50s^{-1}$. Data are reported for two repeats for triplicate measurements (n=3x2). Different lower case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

Samples	Apparent Viscosity (mPa.s)	Standard Deviation (mPa.s)
PPc	$326.11_{\text{SPI, SMP, WPI, MS, PPc+MS, SPI+MS, SMP+MS, WPI+MS}}$	13.41
SPI	$149.06_{\text{PPc, WPI, MS, PPc+MS, SPI+MS, SMP+MS, WPI+MS}}$	25.88
SMP	$117.56_{\text{PPc, WPI, MS, SPI+MS, SMP+MS, WPI+MS}}$	19.66

WPI	24.02PPc, SPI, SMP, PPc+MS, SMP+MS	8.00
Model Saliva (MS)	11.68PPc, SPI, SMP, PPc+MS, SMP+MS	0.78
PPc + MS	111.53PPc, SPI, WPI, MS, SPI+MS, SMP+MS, WPI+MS	35.31
SPI + MS	22.83PPc, SPI, SMP, PPc+MS, SMP+MS	3.58
SMP + MS	65.97PPc, SPI, SMP, WPI, MS, PPc+MS, SPI+MS, WPI+MS	19.38
WPI + MS	23.74PPc, SPI, SMP, PPC+MS, SMP+MS	8.24

Supplementary Table C. 3.Means and standard deviations of friction coefficients reported for aqueous solutions at various lubrication regimes. Data are reported for two repeats for triplicate measurements (n=2x3). Different lower-case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(10 mms^{-1})		(150 mms^{-1})		(1000 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
5wt% PPc	0.3677SMP	0.1327	0.0254SMP	0.0130	0.0027	0.0018
5wt% SMP	0.8609SPI, PPc,5wt% WPI, 10wt% WPI, SPI+MS, PPc+MS, 10wt% WPI+MS	0.2076	0.2003MS, SPI, PPc, 5wt% WPI, 10wt% WPI, SPI+MS, PPc+MS, SMP+MS, 5wt% WPI+MS, 10wt% WPI+MS	0.1030	0.0042	0.0007
5wt% SPI	0.3446SMP, 5wt% WPI+MS	0.1941	0.0178SMP	0.0175	0.0047	0.0072
5wt% WPI	0.5841SMP, 10wt% WPI, PPc+MS, 10wt% WPI+MS	0.1361	0.0805SMP, 10wt% WPI, SPI+MS, PPc+MS, 10wt% WPI+MS	0.0894	0.0035	0.0013

Supplementary Table C. 4.Means and standard deviations of friction coefficients reported for aqueous solutions + model saliva (BSM) mixtures (4:1 w/w) at various lubrication regimes. Data are reported for two repeats for triplicate measurements (n=2x3). Different lower case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(10 mms^{-1})		(150 mms^{-1})		(1000 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
MS	0.3922$_{\text{SMP, 10wt\% WPI}}$	0.3646	0.0435$_{\text{SMP}}$	0.0288	0.0040	0.0025
5wt% PPc + MS	0.1761	0.0513	0.0067$_{\text{SMP, 5wt\% WPI}}$	0.0026	0.0027	0.0018
5wt% SMP + MS	0.6016$_{\text{10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.0843	0.0327$_{\text{SMP}}$	0.0119	0.0034	0.0008
5wt% SPI + MS	0.5111$_{\text{SMP, 10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.1467	0.0159$_{\text{SMP, 5wt\% WPI}}$	0.0048	0.0032	0.0020
5wt% WPI + MS	0.6199$_{\text{5wt\% SPI, 10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.1034	0.0370$_{\text{SMP}}$	0.0206	0.0042	0.0025

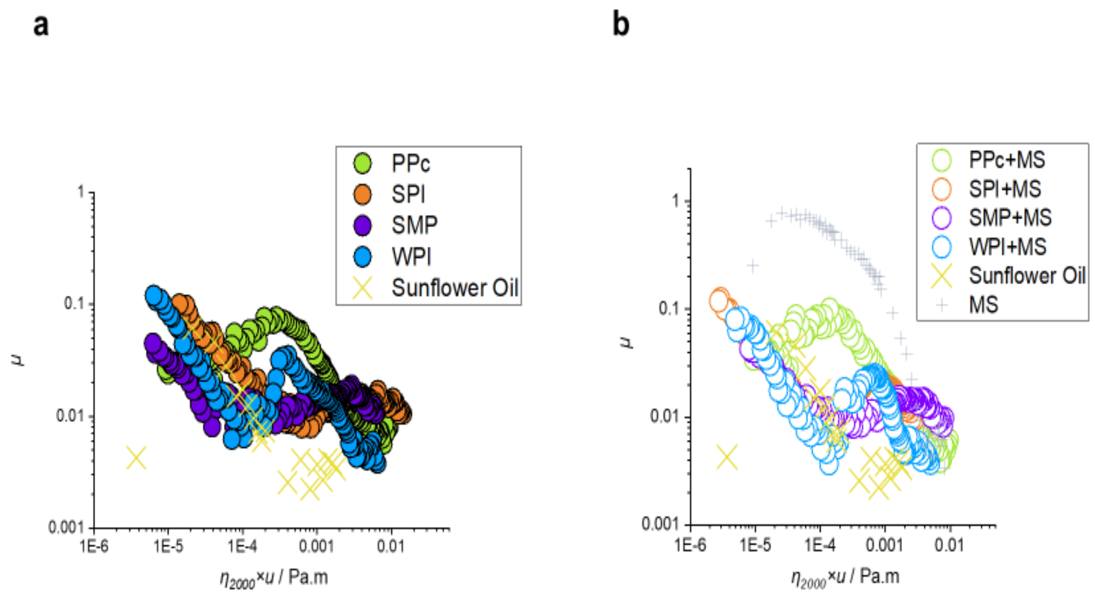
Supplementary Table C. 5. Means and standard deviations of friction coefficients reported for emulsions at various lubrication regimes in presence or absence of model saliva (PGM). Data are reported for two repeats for triplicate measurements (n=2x3). Different lower-case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime						Hydrodynamic lubrication regime	
	(1.14 mms^{-1})		(9.97 mms^{-1})		(52.33 mms^{-1})		(151.281 mms^{-1})		(954.89mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PPc	0.0272 $_{SPI, WPI, MS, SPI+MS}$	0.0047	0.0498 $_{MS}$	0.0176	0.0646 $_{SPI, SMP, MS, SPI+MS, SMP+MS, WPI+MS}$	0.0081	0.0227 $_{MS}$	0.0058	0.00778	0.00152
SPI	0.0980 $_{PPC, MS}$	0.0435	0.0176 $_{Ms, PPc+MS}$	0.0086	0.0113 $_{PPc, MS}$	0.0049	0.0129 $_{MS}$	0.0011	0.01113	0.00292
SMP	0.04334 $_{MS}$	0.0117	0.0152 $_{MS, PPc+MS}$	0.0073	0.0118 $_{PPc, MS}$	0.0054	0.0130 $_{MS}$	0.0015	0.00988	0.00249
WPI	0.1030 $_{PPC, MS}$	0.0221	0.0134 $_{MS, PPc+MS}$	0.0111	0.0351 $_{MS}$	0.0130	0.0150 $_{MS}$	0.0078	0.00392	0.000930412
MS	0.2033 $_{PPC, SPI, SMP, WPI, PPC+MS, SPI+MS, SMP+MS, SMP+MS, WPI+MS}$	0.0701	0.7047 $_{PPc, SPI, SMP, WPI, PPc+MS, SPI+MS, SMP+MS,}$	0.0529	0.2770 $_{PPc, SPI, SMP, WPI, PPc+MS, SPI+MS, SMP+MS,}$	0.0500	0.0896 $_{PPc, SPI, SMP, WPI, PPc+MS, SPI+MS, SMP+MS,}$	0.0206	0.00538	0.00202

			WPI+MS		WPI+MS		WPI+MS			
PPC+MS	0.0516$_{MS}$	0.0297	0.0996$_{SPI, SMP, WPI, MS, SPI+MS, SMP+MS, WPI+MS}$	0.0661	0.472$_{MS}$	0.0150	0.0168$_{MS}$	0.0071	0.00527	0.00154
SPI+MS	0.1006$_{PPC, MS}$	0.0297	0.0209$_{MS, PPc+MS}$	0.0031	0.010$_{PPc, MS}$	0.0030	0.0182$_{MS}$	0.0057	0.01052	0.00258
SMP+MS	0.0421$_{MS}$	0.0125	0.0133$_{MS, PPc+MS}$	0.0080	0.0090$_{PPc, MS}$	0.0032	0.0016$_{MS}$	0.0007	0.00893	0.00142
WPI+MS	0.0818$_{MS}$	0.0192	0.0143$_{MS, PPc+MS}$	0.0074	0.0167$_{PPc, MS}$	0.0276	0.0059$_{MS}$	0.0024	0.00423	0.00142

Supplementary Table C. 6. Emulsion viscosity values used for entrainment speed × high shear rate viscosity (a2 and b2)

Samples	Apparent Viscosity (mPa.s) at 1000s^{-1}
PPc	18.00*obtained from fitting
SPI	24.99
SMP	30.49
WPI	23.51
Model Saliva (MS)	8.53*obtained from fitting
Sunflower Oil	0.04 *data used from (Torres et al., 2018)
PPc + MS	9.00 *obtained from fitting
SPI + MS	12.14
SMP + MS	23.71
WPI + MS	19.95



Supplementary Figure C. 1. Mean friction coefficients in presence of protein emulsions (a) and protein emulsions + model saliva (PGM) mixtures (b) as a function of entrainment speed × high shear rate bulk-protein solution viscosity. Data are shown for three independent readings on duplicate measurements (n=3x2).

Appendix D: Supplementary Information for Chapter 5

Supplementary Table D. 1. Means and standard deviations of apparent viscosity values of aqueous solutions in presence or absence of model saliva reported at orally relevant shear rate of $50s^{-1}$. Data are reported for two repeats for triplicate measurements (n=3x2). Different lower case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

Samples	Apparent Viscosity (mPa.s)	Standard Deviation (mPa.s)
5wt% PPc	13.94_{SPI}	2.81
5wt% SPI	$424.91_{PPC,\ SMP,\ 5wt\%\ WPI,\ 10wt\%\ WPI,\ SPI+MS,\ PPc+MS,\ SMP+MS,\ 5wt\%\ WPI+MS,\ 10wt\%\ WPI+MS,\ MS}$	416.12
5wt% SMP	11.84_{SPI}	3.16
5wt% WPI	9.16_{SPI}	3.49
10wt% WPI	9.75_{SPI}	2.46
Model Saliva (MS)	27.15_{SPI}	7.08
5wt% PPc + MS	14.20_{SPI}	6.35
5wt% SPI + MS	22.63_{SPI}	17.32
5wt% SMP + MS	10.13_{SPI}	2.42
5wt% WPI + MS	16.89_{SPI}	9.76
10wt% WPI +MS	9.59_{SPI}	2.78

Supplementary Table D. 2. Means and standard deviations of apparent viscosity values of food matrices in presence or absence of model saliva reported at orally relevant shear rate of $50s^{-1}$. Data are reported for two repeats for triplicate measurements (n=3x2). Different lower case letters in the same column indicate a statistically significant difference ($p<0.05$) obtained from ANOVA with multiple comparisons (Bonferroni).

Samples	Apparent Viscosity (mPa.s)	Standard Deviation (mPa.s)
5wt% PPc	$3606.69_{\text{SMP, 5wt\% WPI, 10wt\% WPI, SPI+MS, PPc+MS, SMP+MS, 5wt\% WPI+MS, 10wt\%+MS, MS}}$	258.00
5wt% SMP	$4276.87_{\text{SPI,PPc,5wt\% WPI, 10wt\%WPI, SPI+MS,PPc+MS, SMP+MS, 5wt\% WPI+MS, 10wt\%WPI+MS, MS}}$	314.92
5wt% SPI	$3283.90_{\text{5wt\% WPI, 10wt\% WPI, SMP, MS, SPI+MS, PPc+MS, 5wt\%WPI+MS. 10wt\% WPI+MS, SMP+MS, MS}}$	156.45
5wt% WPI	$5884.81_{\text{PPC, SPI, SMP, 10wt\% WPI, PPC+MS, SPI+MS, SMP+MS, 5wt\% WPI+MS, 10wt\% WPI+MS, MS}}$	138.22
10wt% WPI	$10444.58_{\text{PPC, SPI, SMP, 5wt\% WPI, PPC+MS, SPI+MS, SMP+MS, 5wt\% WPI+MS, MS, 10wt\% WPI+MS}}$	382.56
Model Saliva (MS)	$27.15_{\text{PPc, SMP, SPI, 5wt\%WPI, 10wt\% WPI}}$	7.08
5wt% PPc + MS	$117.22_{\text{PPc,SPI,SMP,5wt\%WPI,10wt\%WPI}}$	26.05
5wt% SPI + MS	$97.25_{\text{PPc, SPI, SMP, 5wt\% WPI, 10wt\% WPI}}$	16.04
5wt% SMP + MS	$168.07_{\text{PPc,SPI, SMP, 5wt\%WPI, 10wt\% WPI}}$	37.59
5wt% WPI + MS	$90.10_{\text{PPc,SPI, SMP, 5wt\%WPI, 10wt\% WPI}}$	25.20
10wt% WPI +MS	$128.77_{\text{PPc,SPI, SMP, 5wt\%WPI, 10wt\% WPI}}$	36.75

Supplementary Table D. 3.Means and standard deviations of friction coefficients reported for aqueous solutions at various lubrication regimes. Data are reported for two repeats for triplicate measurements (n=2x3). Different lower-case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(10 mms^{-1})		(150 mms^{-1})		(1000 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
5wt% PPc	0.3677$_{\text{SMP}}$	0.1327	0.0254$_{\text{SMP}}$	0.0130	0.0027	0.0018
5wt% SMP	0.8609$_{\text{SPI, PPc,5wt\% WPI, 10wt\% WPI, SPI+MS, PPc+MS, 10wt\% WPI+MS}}$	0.2076	0.2003$_{\text{MS, SPI, PPc, 5wt\% WPI, 10wt\% WPI, SPI+MS, PPc+MS, SMP+MS, 5wt\% WPI+MS, 10wt\% WPI+MS}}$	0.1030	0.0042	0.0007
5wt% SPI	0.3446$_{\text{SMP, 5wt\% WPI+MS}}$	0.1941	0.0178$_{\text{SMP}}$	0.0175	0.0047	0.0072
5wt% WPI	0.5841$_{\text{SMP, 10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.1361	0.0805$_{\text{SMP, 10wt\% WPI, SPI+MS, PPc+MS, 10wt\% WPI+MS}}$	0.0894	0.0035	0.0013
10wt% WPI	0.1138$_{\text{MS, SMP, 5wt\% WPI, 5wt\% SPI+MS}}$	0.0640	0.0047$_{\text{SMP, 5wt\% WPI}}$	0.0012	0.0043	0.0030

Supplementary Table D. 4. Means and standard deviations of friction coefficients reported for aqueous solutions + model saliva mixtures (4:1 w/w) at various lubrication regimes. Data are reported for two repeats for triplicate measurements (n=2x3). Different lower case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(10 mms^{-1})		(150 mms^{-1})		(1000 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
MS	0.3922$_{\text{SMP, 10wt\% WPI}}$	0.3646	0.0435$_{\text{SMP}}$	0.0288	0.0040	0.0025
5wt% PPc + MS	0.1761	0.0513	0.0067$_{\text{SMP, 5wt\% WPI}}$	0.0026	0.0027	0.0018
5wt% SMP + MS	0.6016$_{\text{10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.0843	0.0327$_{\text{SMP}}$	0.0119	0.0034	0.0008
5wt% SPI + MS	0.5111$_{\text{SMP, 10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.1467	0.0159$_{\text{SMP, 5wt\% WPI}}$	0.0048	0.0032	0.0020
5wt% WPI + MS	0.6199$_{\text{5wt\% SPI, 10wt\% WPI, PPc+MS, 10wt\% WPI+MS}}$	0.1034	0.0370$_{\text{SMP}}$	0.0206	0.0042	0.0025
10wt% WPI + MS	0.1760$_{\text{SMP+MS, 5wt\% WPI_MS, 5wt\% SPI+MS}}$	0.1332	0.0076$_{\text{SMP, 5wt\% WPI}}$	0.0043	0.0045	0.0014

Supplementary Table D. 5.Means and standard deviations of friction coefficients reported for food matrices at various lubrication regimes. Data are reported for two repeats for triplicate measurements (n=2x3). Different lower-case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(1 mms^{-1})		(10 mms^{-1})		(1000 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
5wt% PPc	$0.0241_{10wt\% WPI+MS}$	0.109	0.0059_{MS}	0.0023	$0.0461_{5wt\% WPI, SPI+MS, PPc+MS, SMP+MS, 5wt\% WPI+MS, 10wt\% WPI+MS, MS}$	0.0060
5wt% SMP	$0.0429_{10wt\% WPI+MS}$	0.0179	0.0065_{MS}	0.0026	$0.0503_{5wt\% WPI, SPI+MS, PPc+MS,SMP+MS, 5wt\% WPI+MS, 10wt\% WPI+MS, MS}$	0.0273
5wt% SPI	$0.0120_{10wt\% WPI+MS, MS}$	0.0076	0.0028_{MS}	0.0030	$0.0412_{SPI+MS, PPc+MS, 5wt\% WPI+MS, 10wt\%+MS, MS}$	0.0184
5wt% WPI	$0.0373_{10wt\% WPI+MS}$	0.0261	0.0075_{MS}	0.0094	$0.0257_{PPc, SMP, MS}$	0.0063
10wt% WPI	$0.0362_{10wt\% WPI+MS}$	0.0178	0.0039_{MS}	0.0021	$0.0363_{5wt\% WPI+MS, 10wt\% WPI+MS, MS}$	0.1698

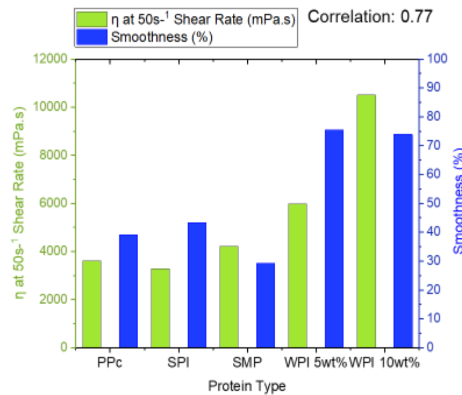
Supplementary Table D. 6. Means and standard deviations of friction coefficients reported for food matrices + model saliva mixtures (4:1 w/w) at various lubrication regimes. Data are reported for two repeats for triplicate measurements (n=2x3). Different lower case letters in the same column indicate a statistically significant difference (*p<0.05*) obtained from ANOVA with multiple comparisons (Bonferroni).

	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(1 mms^{-1})		(10 mms^{-1})		(1000 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
MS *shouldn't be different from other	0.1667$_{\text{SPI}}$	0.2022	0.3922$_{\text{PPc, SMP, SPI, 5wt\% WPI, 10wt\% WPI, PPc+MS, SMP+MS, SPI+MS, 5wt\% WPI+MS, 10wt\% WPI+MS}}$	0.3646	0.0040$_{\text{SPI, PPc, SMP, 5wt\% WPI, 10wt\% WPI}}$	0.0025
5wt% PPc+MS	0.0249$_{\text{10wt\% WPI+MS}}$	0.0210	0.0096$_{\text{MS}}$	0.0065	0.0050$_{\text{SPI, PPc, SMP}}$	0.0035
5wt% SMP+MS	0.030$_{\text{10wt\% WPI+MS}}$	0.0259	0.0124$_{\text{MS}}$	0.0074	0.0034$_{\text{PPc, SMP}}$	0.0008
5wt% SPI+MS	0.0248$_{\text{10wt\% WPI+MS,}}$	0.0328	0.0098$_{\text{MS}}$	0.0057	0.0032$_{\text{SPI, PPc, SMP}}$	0.0020
5wt% WPI+MS	0.0331$_{\text{10wt\% WPI+MS}}$	0.0138	0.0105$_{\text{MS}}$	0.0064	0.0042$_{\text{SPI, PPc, SMP, 10wt\% WPI}}$	0.0025
10wt% WPI+MS	0.2398$_{\text{SPI, PPc, SMP, 5wt\% WPI, 10wt\% WPI, SPI+MS< PPc+MS, SMP+MS, 5wt\% WPI+MS}}$	0.1639	0.0552$_{\text{MS}}$	0.0684	0.0045$_{\text{SPI, PPc, SMP, 10wt\% WPI}}$	0.0014

Supplementary Table D. 7. Means Matrix statistical results for sensory attributes. Data are reported for 4 repeats with eight panellists (n=4x8). Statistical comparisons were made using Duncan's test, sig represents significance and non-sig represents not significant.

Means Matrix	Smoothness	Grainy/Gritty	Mouthcoating	Adhesive	Viscosity	Melt Rate	Drying	Powdery
Skimmed Milk Powder	29.26	59.78	53.00	27.49	51.71	55.08	47.84	15.36
5wt% WPI	75.54	3.60	54.60	24.67	45.18	58.89	35.55	3.93
10wt% WPI	73.95	2.73	53.12	24.93	47.45	59.72	36.56	3.84
5wt% PPc	39.21	10.61	48.24	26.08	46.90	54.97	48.08	46.91
5wt% SPI	43.35	13.58	50.92	28.33	55.25	53.37	46.93	48.91
Minimum	29.26	2.73	48.24	24.67	45.18	53.37	35.55	3.84
Maximum	75.54	59.78	54.60	28.33	55.25	59.72	48.08	48.91
Significance	Sig	Sig	Not Sig	Not Sig	Not Sig	Not Sig	Sig	Sig

a1



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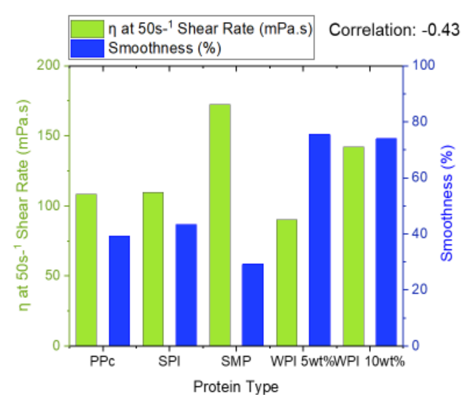

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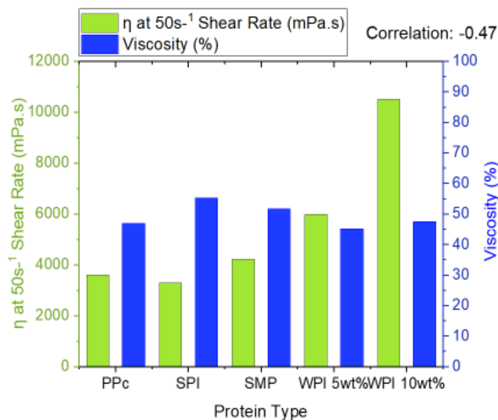

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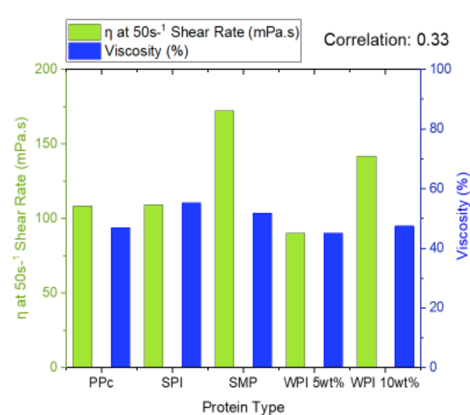
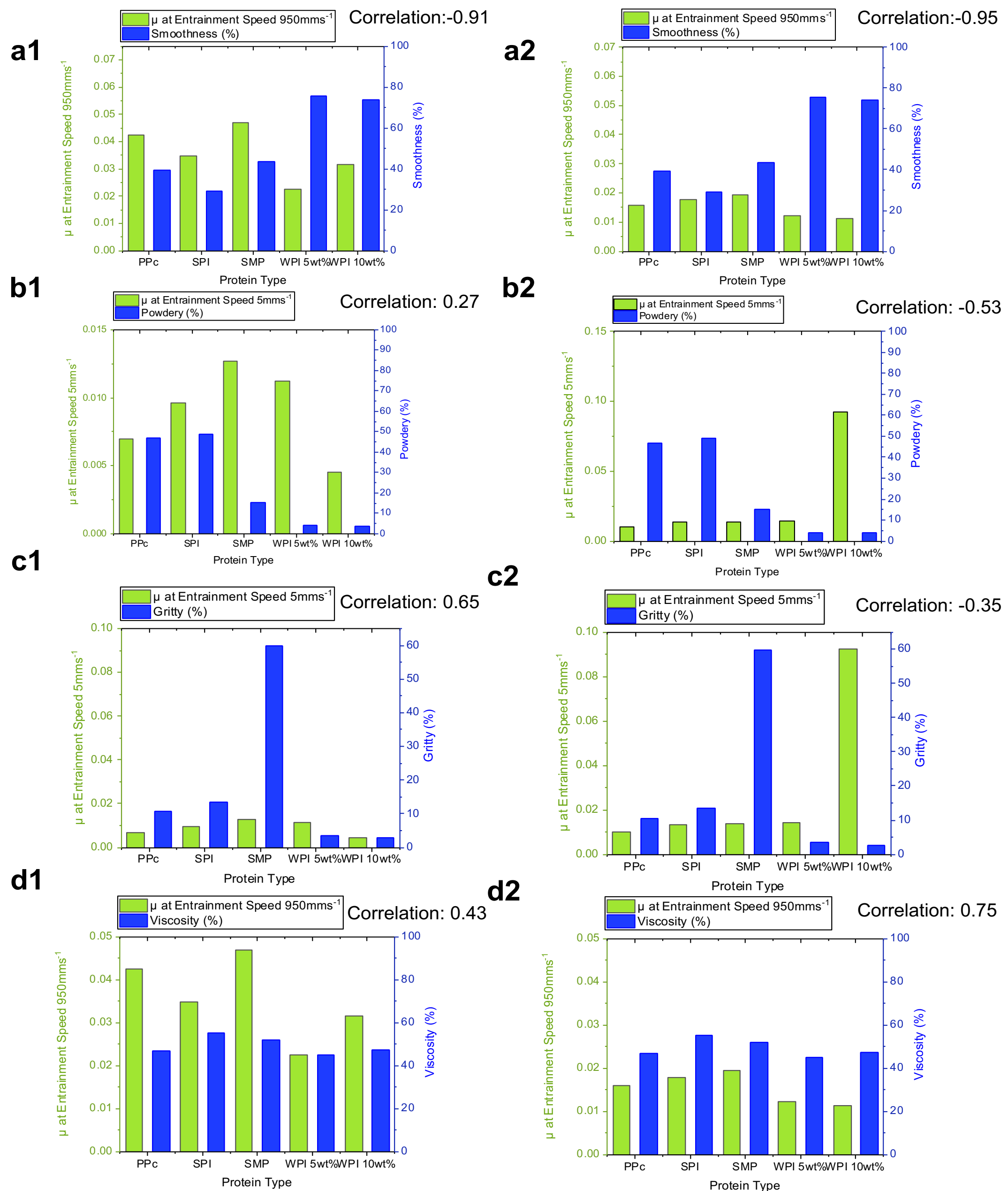


Supplementary Figure D. 1. Dual-Axis Bar charts comparing viscosity a shear rate of $50s^{-1}$ and different sensory ratings by protein type. Comparisons were made between viscosity and sensory smoothness (a) and instrumentally measured viscosity and sensory measured viscosity (b). Instrumental viscosity measurements are taken from model food alone (1) and from model food-saliva boli (2). Both sensory and instrumental viscosity measurements were taken for the same sample, with sensory repeated for 8 panellists on three occasions (n=1x3x8) and instrumental analysis was measured three times on duplicate days (n=2x3). Correlation coefficients were generated using Excel's CORREL function.



Supplementary Figure D. 2. Dual-Axis Bar charts comparing friction (μ) taken from early speeds/boundary lubrication 5mms^{-1}or high speeds/hydrodynamic lubrication (950mms^{-1}) 50s^{-1}and different sensory ratings by protein type. Comparisons were made between high speed friction and sensory smoothness (a) and low speed friction and sensory powderiness (b), low speed friction and sensory grittiness (c) and high speed friction and sensory viscosity (d). Instrumental friction measurements are taken from model food alone (1) and from model food-saliva boli (2). Both sensory and instrumental viscosity measurements were taken for the

same sample, with sensory repeated for 8 panellists on three occasions (n=1x3x8) and instrumental analysis was measured three times on duplicate days (n=2x3). Correlation coefficients were generated using Excel's CORREL function.

Appendix E: Supplementary Information for Chapter 6

Supplementary Table E. 1. Tests of between-subject effects on viscosity at shear rate 50s^{-1}

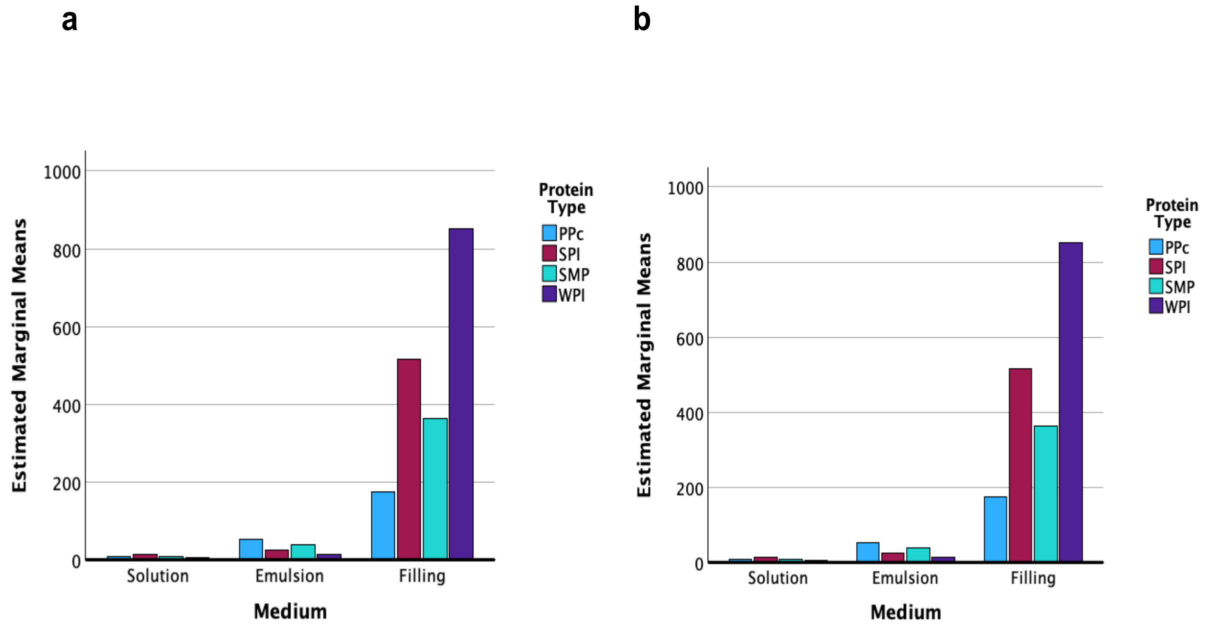
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	359621156.339[a]	11	32692832.394	1094.792	<.001
Intercept	181146904.560	1	181146904.560	6066.105	<.001
Medium	344458372.062	2	172229186.031	5767.476	<.001
Protein Type	6043687.747	3	2014562.582	67.462	<.001
Medium * Protein Type	20639549.348	6	3439924.891	115.194	<.001
Error	2179936.429	73	29862.143		
Total	637373555.239	85			
Corrected Total	361801092.767	84			

a. R Squared = .994 (Adjusted R Squared = .993)

Supplementary Table E. 2. Tests of between-subject effects on viscosity at shear rate 1000s^{-1}

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5423340.612[a]	11	493030.965	27.467	<.001
Intercept	2405151.625	1	2405151.625	133.992	<.001
Medium	4151486.457	2	2075743.229	115.640	<.001
Protein Type	470615.367	3	156871.789	8.739	<.001
Medium * Protein Type	1184782.009	6	197463.668	11.001	<.001
Error	1310350.361	73	17950.005		
Total	10072250.884	85			
Corrected Total	6733690.974	84			

a. R Squared = .805 (Adjusted R Squared = .776)



Supplementary Figure E. 1. Estimated marginal means of viscosity at shear rate of $50s^{-1}$ (a) and $1000s^{-1}$ (b). Data are shown for three independent readings on duplicate measurements (n=3x2).

Supplementary Table E. 3. Tests of between-subjects effects on friction for entrainment speed $1mms^{-1}$. p<0.05 used as statistically significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.329[a]	11	.030	16.618	<.001
Intercept	.564	1	.564	313.014	<.001
Medium	.224	2	.112	62.241	<.001
ProteinType	.020	3	.007	3.717	.015
Medium * Protein Type	.088	6	.015	8.121	<.001
Error	.131	73	.002		
Total	.953	85			
Corrected Total	.461	84			

Supplementary Table E. 4. Tests of between-subjects effects on friction for entrainment speed 5mms^{-1}. p<0.05 used as statistically significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.131[a]	11	.557	75.338	<.001
Intercept	3.125	1	3.125	422.373	<.001
Medium	5.033	2	2.517	340.179	<.001
Protein Type	.398	3	.133	17.953	<.001
Medium * Protein Type	.853	6	.142	19.219	<.001
Error	.540	73	.007		
Total	9.168	85			
Corrected Total	6.671	84			

a. R Squared = .919 (Adjusted R Squared = .907)

Supplementary Table E. 5. Tests of between-subjects effects on friction for entrainment speed 10mms^{-1}. p<0.05 used as statistically significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.832[a]	11	.530	63.871	<.001
Intercept	2.924	1	2.924	352.284	<.001
Medium	4.839	2	2.419	291.481	<.001
Protein Type	.355	3	.118	14.263	<.001
Medium * Protein Type	.764	6	.127	15.332	<.001
Error	.606	73	.008		
Total	8.769	85			
Corrected Total	6.438	84			

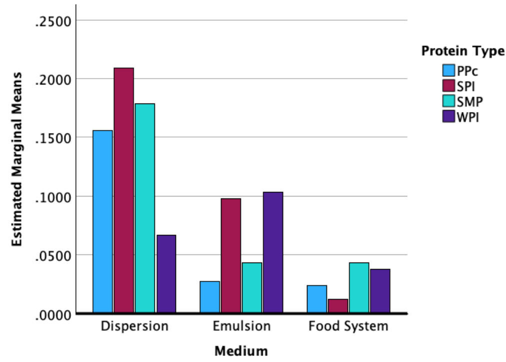
a. R Squared = .906 (Adjusted R Squared = .892)

Supplementary Table E. 6. Tests of between-subjects effects on friction for entrainment speed 950mms^{-1}. p<0.05 used as statistically significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.024[a]	11	.002	21.782	<.001
Intercept	.023	1	.023	226.490	<.001
Medium	.022	2	.011	109.321	<.001
Protein Type	.001	3	.000	3.442	.021
Medium * Protein Type	.002	6	.000	3.366	.006
Error	.007	73	.000		
Total	.062	85			
Corrected Total	.031	84			

a. R Squared = .766 (Adjusted R Squared = .731)

a



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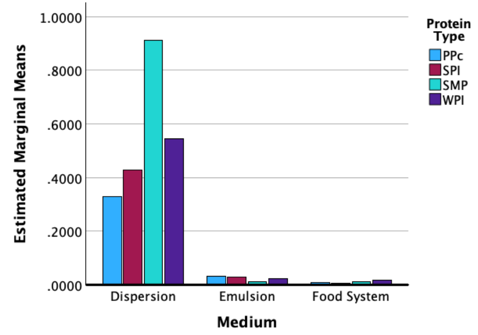

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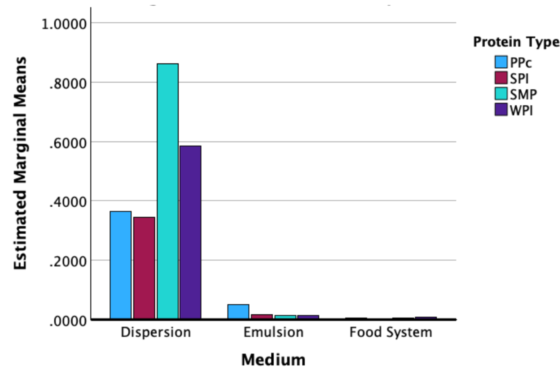

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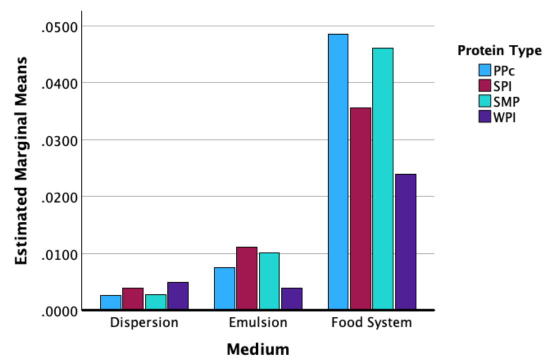

Supplementary Figure E. 2. Estimated marginal means of friction at entrainment speeds of 1mms^{-1} (a), 5 mms^{-1} (b), 10mms^{-1} (c) and 950mms^{-1} (d). Data are shown for three independent readings on duplicate measurements (n=3x2).