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Comparing frictional behaviour of plant and dairy proteins: Case study on high protein concentration

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ABSTRACT

The aim of this study was to understand the frictional behaviour of non-purified plant proteins (*i.e.* pea protein concentrate (PPc) and soy protein isolate (SPI)) compared to dairy proteins (whey protein isolate (WPI) and sodium caseinate (NaCas)). The comparison was conducted for aqueous dispersions of the proteins at 10 and 20 wt% at pH 6.8. Owing to protein-protein aggregation and lower solubilities, plant proteins showed prominent shear thinning behaviour, unlike dairy proteins, which showed less shear dependence. Addition of proteins reduced the boundary friction coefficients (μ) with NaCas showing the lowest boundary μ ($p < 0.05$). In general, the dairy proteins showed larger hydrodynamic size and better lubricity whilst the plant proteins increased the mixed μ with twice as high a calculated fluid film thickness (h_{min}) required for onset of the elastohydrodynamic regime as compared with that of dairy proteins. Such low μ values in dairy proteins might be attributed to increased adsorption (~1.5–5× more) of the elastic films as compared to plant proteins. Findings suggest that product development towards more sustainable formulations, requires innovative strategies to reduce poor lubrication and aggregation when using higher concentrtaions of plant proteins.

1. Introduction

Food is essential for human existence; however global resources are struggling to meet the growing food demands (Miraglia et al., 2009). Methods for alleviating this pressure include the development of more sustainable foods (Abbasi & Abbasi, 2016; Granato et al., 2022; UN, 2022). An area of recent development and interest is to design foods with sustainable plant based ingredients (Brown, Mackie, He, Branch, & Sarkar, 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, Gantriis, Fraga, & Perez-Cueto, 2021).

Plant-sourced proteins are environmentally attractive as they are associated with emission of 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 mouthfeel including dryness, astringency and flavour characteristics such as bitterness, and beany flavours (Canon et al., 2021; Onwezen, Bouwman, Reinders, & Dagevos, 2021; Tanger et al., 2021; Wang, Sun, Yang, & Wang, 2009; Xia et al., 2022). For example, when animal milk was proportionally substituted with pea milk, a higher proportion of pea milk was correlated with reduced acceptability (Omrani Khiabanian, Motamedzadegan, Naghizadeh Raisi, & Alimi, 2020). For this reason, understanding the fundamental mechanisms behind the undesirable mouthfeel of plant proteins is of paramount interest for the development of more sustainable protein-based foods and beverages.

Mouthfeel is a multifaceted tactile sensation. No single method has yet been developed that captures this full sensation (Sarkar & Krop, 2019; Stokes, Boehm, & Baier, 2013; Vlădescu et al., 2023). As such, a range of methods have been used to quantify and understand mouthfeel, ranging from human sensory panels to instrumental measurements of objective material properties (Sarkar & Krop, 2019). Previous research on mouthfeel of protein has focussed on understanding frictional

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dissipation and physicochemical properties of proteins (Brown et al., 2021; Çelebioğlu, Lee, & Chronakis, 2020). In particular, rheology and tribology have been correlated with human sensory measurements (Upadhyay & Chen, 2019).

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 have shown that the replacement of skimmed milk powder with WPI reduced consumer acceptability with 'dry' aftertastes noted (Childs & Drake, 2010). There is a lack of clarity regarding other types of proteins due to the scarcity of and/or inconsistencies between studies. For example, one study has reported intense astringency for the dairy protein lactoferrin (Vardhanabhuti, Kelly, Luck, Drake, & Foegeding, 2010), whereas another has reported little astringency (Ye, Streicher, & Singh, 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 and 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. In a recent study using both sensory and instrumental methods, sensory analysis found two separate pea protein concentrates were astringent (Vlădescu et al., 2023). Interestingly, tribology results combined with *in situ* imaging of the tribofilms showed different frictional behaviours between the two pea proteins. Combining QCM-D and tribology is thus offering a more comprehensive mechanistic insight into sensory perception. Nevertheless, more research is needed to quantify mouthfeel of plant proteins and compare 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 acknowledged as a clear driver behind unpleasant mouthfeel in plant proteins, studies have frequently used either purer, soluble fractions of plant proteins obtained by centrifugation and filtration or used processing such as microgelation (Kew et al., 2023; Kew, Holmes, Stieger, & Sarkar, 2021; Zembyla et al., 2021), thus overlooking comparison of animal and plant proteins without purification. Consideration of the environmental impact of processing steps used in producing plant proteins should be acknowledged. Every additional purification step to try to improve properties will increase the resources required and therefore environmental cost of production as shown recently *via* the life cycle assessment (Lie-Piang, Braconi, Boom, & van der Padt, 2021). Hence, it is important to understand the properties of proteins without purification, aligning with the aim of this study.

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 6 wt% in WPI protein beverages, the consumer acceptance decreased (Childs & Drake, 2010). Studies using instrumental methods found no change in lubrication behaviour when WPI concentration was raised between 0.5 and 4 wt% (Vardhanabhuti et al., 2010). However, when concentration was further increased to 10 wt%, 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 20 wt% of the total energy value (European Commission, 2006). However, the majority of research on protein has been conducted at low concentrations (< 5 wt%) (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 plant and dairy protein solutions at high concentrations. A comprehensive group of objective measurements including rheology, tribology, scanning electron microscopy (SEM) and QCM-D and calculation of fluid film thickness were 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 (10 wt% and 20 wt%) were compared to simulate high-protein fortified foods.

2. Materials and methods

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). Polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Midland, MI, USA) base fluid and cross-linker (10: 1 *w*/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., 2020). Ammonia solution (25 %) and hydrogen peroxide solution (30 %) were purchased from Fisher Chemicals (UK) and Sigma-Aldrich (Dorset, UK). Milli-Q water purified by a Milli-Q apparatus (Millipore, Bedford, UK), with an electrical resistivity not less than 18.2 MΩ cm was used to make HEPES buffer in this study before any protein dissolution.

2.2. Methods

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 Fig. 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 (10 wt% and 20 wt%) 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).

2.2.2. Zeta-potential

Protein solutions of 0.1 wt% 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, $u_e = \frac{2\varepsilon_R\varepsilon 0}{3\eta}\zeta f_1(\kappa\alpha)$. 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 $\kappa\alpha \gg 1$, f (κα) was 1 according to Smoluchowski approximation.

2.2.3. Particle sizing

Protein solutions were diluted [1:1000 *w*/w] with buffer and filtered using a 0.22 μm 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 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°.

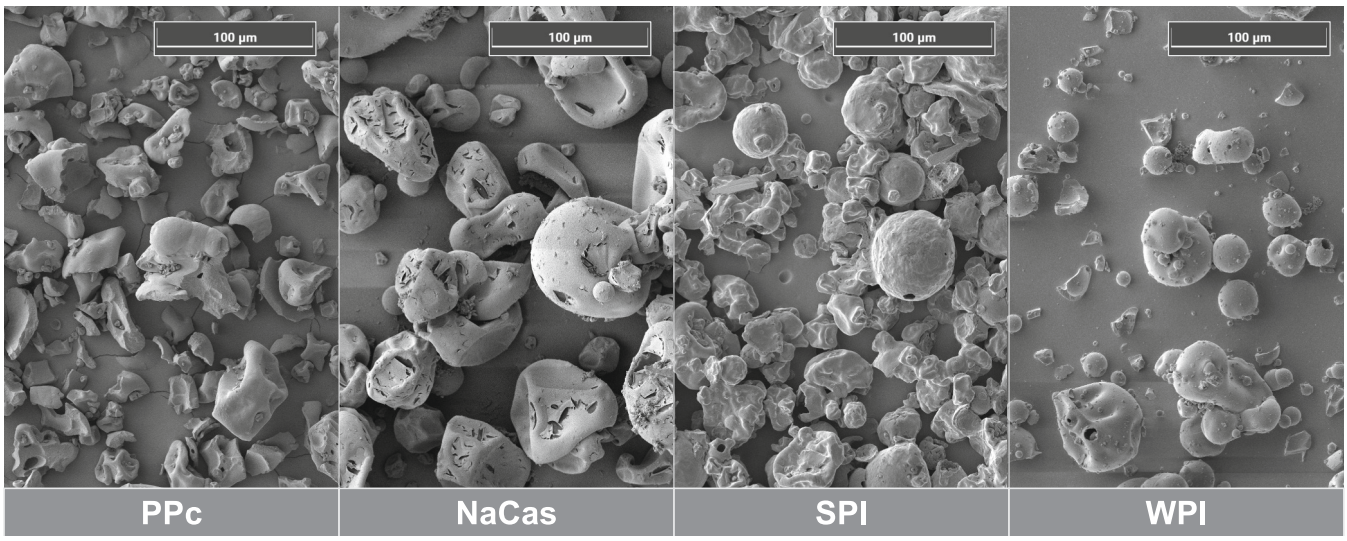

Fig. 1. SEM images of the protein powders. Scale bar represents 100 µm.

2.2.4. Solubility

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

2.2.5. Scanning electron microscopy (SEM)

The microstructure of proteins was 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 vacuum while being kept at −140 °C.

2.2.6. Rheology

Apparent viscosity of protein solutions was measured using a stress-controlled rheometer (Anton Paar MCR-302, Ostfildern, Germany). A 50 mm-diameter circular cone−plate geometry was used, with an angle of 2°. Viscosity measurements were conducted in the range of 0.01–2000 s^{-1} shear rates at a constant temperature (37 °C) to mimic oral conditions and flow curves were obtained. The gap was set to 1 mm and 350 cSt silicone oil was used as a solvent trap in addition to a Peltier hood.

2.2.7. Oral tribology

A Mini Traction Machine (MTM2) from PCS Instruments was used to measure rolling-sliding friction coefficient (μ) results using PDMS surfaces in presence of protein dispersions. The set-up used a PDMS ball (19 mm diameter) on a PDMS disc (46 mm diameter) configuration. The temperature was controlled at 37 °C and a normal force of 2.0 N (~200 kPa Hertzian contact pressure) was applied (Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019). 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 the contact point in the direction of the fluid flow. The sliding to rolling ratio (SRR) indicates the proportions of either rolling or sliding. This is given by $SRR = \frac{(U_B-U_D)}{(U_B+U_D)}$. The SRR was fixed at 0.5 and the entrainment speed ranged between 0.1 and 1000 mm s^{-1}. The tribology results are presented as μ against entrainment speed (u), the product of the limiting high-shear viscosity obtained at 2000 s^{-1} (*i.e.* η_{2000}) and the u (*i.e.* $\eta_{2000} \times u$) and the theoretical minimum fluid film thickness (h_{min}). h_{min} was estimated using the Hamrock & Dowson eq. (1) (Myant, Fowell, Spikes, & Stokes, 2010; Sarkar, Soltanahmadi, Chen, & Stokes, 2021):

$$h_{min} = 2.8U^{0.68}W^{-0.20}$$

where, U is the dimensionless speed parameter ($\frac{u\eta}{E'R'}$), W is the dimensionless load parameter ($\frac{F}{E'R'^2}$). Terms u, η and F are the lubricant entrainment speed, viscosity of the lubricant and the normal applied load, respectively. R' and E' are the combined radius of curvature and the equivalent modulus of elasticity of PDMS ball and disc, respectively. The cleaning protocol used between each experiment included sonication steps in sodium dodecyl sulphate (2 wt% in deionised (DI) water), isopropanol and DI water for 10 min at each step and also ethanol after samples containing model saliva.

2.2.8. QCM-D measurements

Silica 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., 2020). To coat surfaces the following protocol was used based on (Kew et al., 2021; Zembyla et al., 2021). 10 wt% PDMS in toluene solution was prepared and stirred for 24 h before being further diluted to 0.5 wt% and again stirred 24 h at room temperature. For removal of organic material and insoluble particles on crystals a RCA solution was prepared by using 5 parts DI 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 DI water and sonicated for 10 min three times before drying with nitrogen gas. To coat, the crystals were placed in a spin coater. 100 mL of the 0.5 wt% PDMS solution was pipetted onto the crystals and spin coated at 5000 rpm for 60 s. Crystals were dried for 10 min each again on a heated plate at 80 °C. 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 30 min and recoated following the above protocol. Immediately before measurements, PDMS sensors were cleaned by immersing for 30 s in toluene, then 30 s in isopropanol and 2 min in ultrapure water. The crystals were then dried with nitrogen gas, before being left for a minimum of 2 h. This allowed time for all the solvent molecules to evaporate.

QCM-D was used to measure real time adsorption behaviour of proteins. Both changes in frequency and dissipation are measured concurrently during adsorption onto the surfaces. Thus, it can provide data on adsorption kinetics, mass, viscoelasticity, and adsorbed film

thickness. QCM-D measurements were conducted for protein solutions at 0.1 mg/mL protein. 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 at least 1 h or until a stable baseline had been observed. The outer pump lines were then rinsed with ultrapure water before the buffer was again injected into the chambers for a further 30 min to rinse away any unadsorbed proteins. To analyse data, Dfind software (Q-Sense, Sweden) and the Voigt viscoelastic model were used and in most cases fifth overtones of frequency and dissipation were used to fit data to get the film thickness and the viscoelastic parameters (Dunér, Thormann, & Dėdinaitė, 2013; Rodahl et al., 1997; Voinova, Rodahl, Jonson, & Kasemo, 1999).

2.2.9. 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. Results & discussion

3.1. Physicochemical characteristics of protein dispersion

Firstly, we compared the physicochemical properties of the plant protein with the dairy protein dispersions. As one can appreciate from the morphology of the dry powders in Fig. 1, both the dairy proteins (NaCas, WPI) showed spherical particles compared to the plant proteins (PPc, SPI), in particular PPc paticles were irregular-shaped with sharp edges. The SEM images were similar to those obtained previously (Vlădescu et al., 2023). NaCas showed large aggregated particles whereas WPI had much smaller particles. The measured solubility, hydrodynamic diameter (d_H) and ζ-potential of aqueous dispersions of all protein dispersions are shown in Fig. 2.

As one might expect, both the tested plant proteins had considerably lower solubility than the dairy proteins ($p < 0.05$) (Fig. 2a). The lower solubility shown for plant proteins is in close agreement with previous literature (Kew et al., 2021; Kim, Wang, & Selomulya, 2020) owing to the aggregation of these storage proteins. Protein solution aggregates differed in size, with WPI having the largest (366.0 nm) and PPc having the smallest (81.1 nm) d_H (Fig. 2b). However, the particle size of protein samples should be taken with caution as the polydispersity indices were high (> 0.25). Further, DLS is biased to larger aggregates as this will obscure the light refracted from smaller particles (Maguire, Rösslein, Wick, & Prina-Mello, 2018). All proteins used have isoelectric point of around 4.5 (Ma et al., 2009; Pelegrine & Gasparetto, 2005; Strange, Holsinger, & Kleyn, 1993; Sumner, Nielsen, & Youngs, 1981; Wang et al., 2009) and thus the ζ-potential of all the proteins tested in this study at pH 6.8 was negative (Fig. 2c) as expected and in agreement with previous reports (Freitas, Albano, & Telis, 2017; Khalesi, Emadzadeh, Kadkhodaee, & Fang, 2016; Ladjal-Ettoumi, Boudries, Chibane, & Romero, 2016).

3.2. Apparent viscosity

The flow behaviour of dispersions of dairy and plant proteins is shown in Fig. 3. SPI showed the most pronounced shear thinning behaviour and NaCas the least (Fig. 3a). In particular, SPI showed three orders of magnitude decay in apparent viscosity over the range of shear rates tested, whilst PPc and WPI showed approximately one order reduction in apparent viscosity. Shear thinning behaviour has

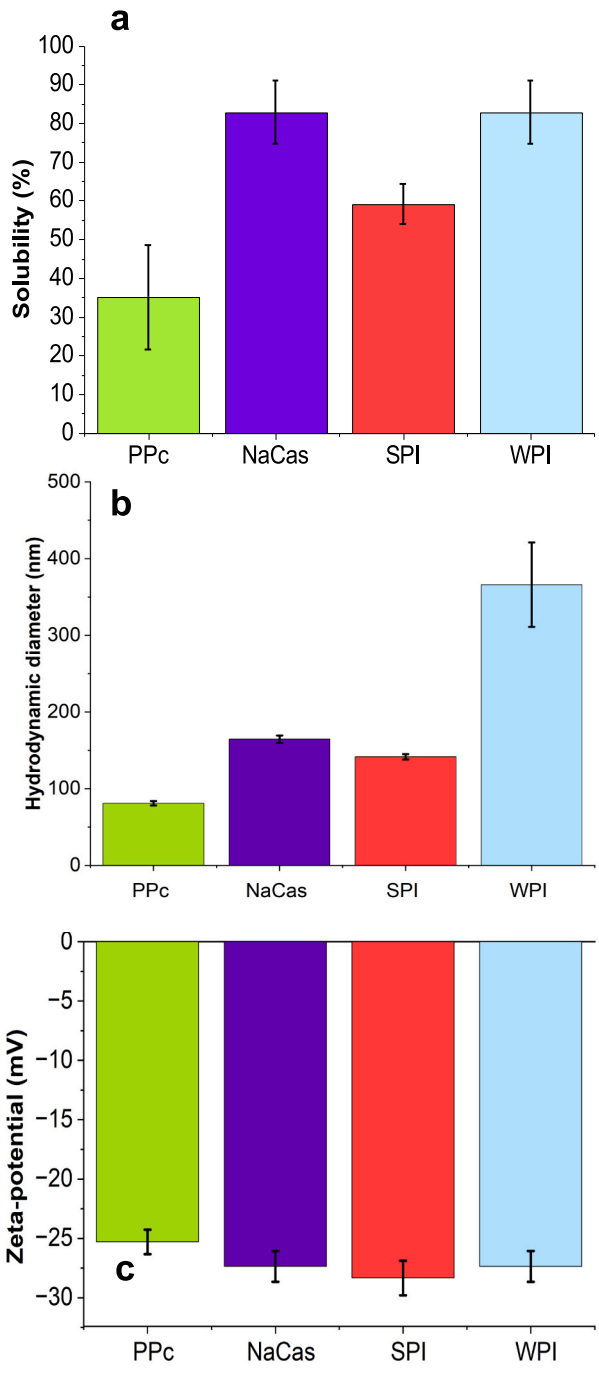

Fig. 2. Mean solubility (a), hydrodynamic diameter (b) and zeta-potential (c) for the tested proteins. Error bars represent ± standard deviations for triplicate measurements ($n = 3 \times 3$).

previously been shown for both soy protein (OFlynn, Hogan, Daly, OMahony, & McCarthy, 2021) and pea protein (Zembyla et al., 2021), which was attributed to plant protein aggregates breaking in the direction of flow. The substantially greater shear thinning behaviour of SPI compared to that of PPc has been reported previously and can be attributed to a higher degree of globular proteins in SPI (> 90 % in SPI compared to 55 % in PPc) that tend to cause protein-protein aggregation (Badjona, Cherono, Bradshaw, & Dubey, 2025; Tiong et al., 2024). Such structural differences make SPI more prone to shear-induced disruption of intra-molecular entanglements and particle-particle aggregates, which bring about extensive shear thinning behaviour. Therefore, the

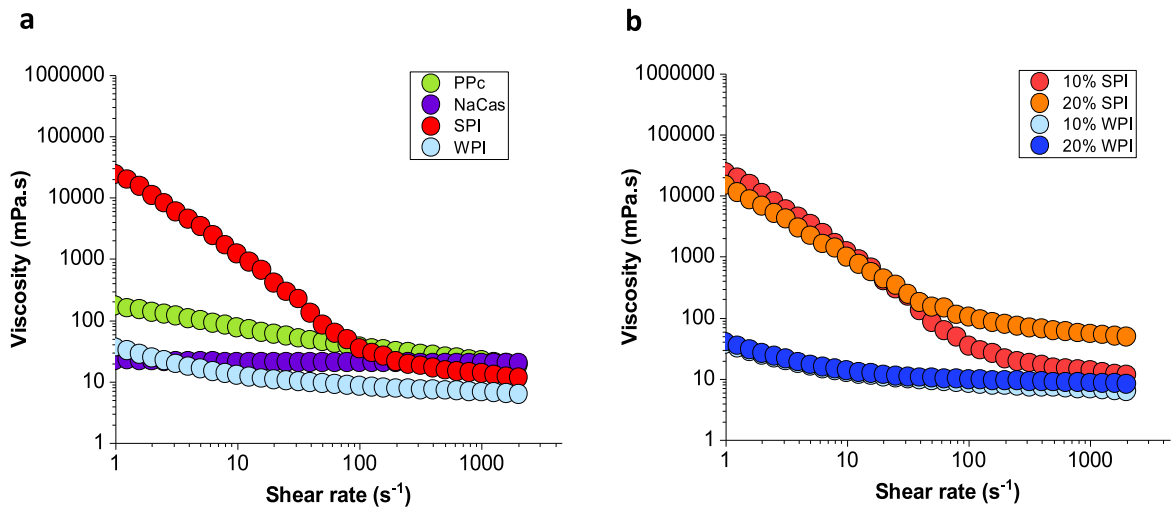

Fig. 3. Mean apparent viscosities of protein dispersions as a function of shear rate. Showing a) all four protein dispersions at 10 wt% and b) soy protein isolate (SPI) and whey protein isolate (WPI) at 10 and 20 wt%. Data are shown for three independent readings on triplicate measurements. Statistics can be followed in Table 1 ($n = 3 \times 3$).

shear thinning behaviour observed for SPI (OFlynn et al., 2021) was not a surprise and is in line with the low solubility (Fig. 3a). Nevertheless, the apparent viscosities at orally relevant shear rates ($50s^{-1}$, η_{50}) (Ross, Tyler, Borgognone, & Eriksen, 2019; Wood, 1968) were similar among the protein types ($p > 0.05$), with the exception of SPI, which showed relatively large deviations between measurements (see Table 1).

It was interesting to check how increase in concentration may influence the flow behaviour of protein dispersions. Although the concentration effect (10–20 wt%) was tested for SPI and WPI, such effects were not investigated for PPc and NaCas due to their incomplete dispersibility at such high concentrations. Increasing the concentration to 20 wt% showed a significant influence on η_{50} and/or η_{2000} of SPI ($p < 0.05$) but no effect was seen on WPI ($p > 0.05$), respectively (Fig. 3b). The increase in η_{2000} of SPI might affect the tribological performance, this is discussed later.

3.3. Oral tribology

Fig. 4 displays the friction curves obtained for 10 wt% protein solutions. All the protein samples tested showed the boundary and the mixed lubrication regimes with most showing the onset of the elasto-hydrodynamic regime (EHL). Representative speed ranges were selected enabling the comparison of the friction curves at the boundary, mixed and hydrodynamic lubrication regimes (Table 2). The addition of proteins to the buffer shifted the onset of the mixed and the EHL regimes to lower u values suggesting the viscous-driven lubricity (Sarkar et al., 2021; Shewan, Pradal, & Stokes, 2020; Soltanahmadi, Bryant, & Sarkar, 2023) (Fig. 4a). At the boundary lubrication regime ($u = 5$ mm s^{-1}), all protein solutions showed lower μ than buffer with SPI showing the highest μ (~0.518). This was not statistically different to WPI ($p > 0.05$) (Table 2), whilst NaCas showed the lowest values ($\mu \sim 0.111$). The similarity of PPc with WPI at 10 wt% ($p > 0.05$) in the boundary regime is in sharp contrast to previous literature looking only at soluble fractions (Kew et al., 2021; Zembyla et al., 2021). Protein concentration may vary as sample preparation included centrifugation of solutions with insoluble protein aggregates in the previous studies. Notwithstanding this difference, the study reported WPI to have much lower boundary friction (0.05) compared to PPc (0.21) at 10 wt%. This was attributed to pea protein increasing μ between hydrophobic contact surfaces across entrainment speeds *versus* whey protein (Kew et al., 2021). In addition, the tribometer used in the previous study only looked at sliding friction as opposed to the current work using a combination of sliding and roling friction. The comparison of PPc with soluble fractions is further discussed later on.

Table 1
Means and standard deviations of apparent viscosity values of samples at orally relevant shear rate of 50 s^{-1}. Data is reported for three repeats for triplicate measurements (n = 3 × 3). Different lower case subscript letters in the same column indicate a statistically significant difference ($p < 0.05$).

Samples	Apparent Viscosity (mPa.s)
10 wt% WPI	9.2 ± 2.0 [a]
10 wt% SPI	85.3 ± 84.6 [b]
10 wt% PPc	44.9 ± 6.0 [b]
10 wt% NaCas	20.8 ± 3.2 [a]
20 wt% WPI	20.4 ± 1.7 [a]
20 wt% SPI	153.4 ± 99.9 [c]

To further understand the frictional behaviour mechanisms of the proteins and pinpoint the importance of viscosity in such frictional data, the μ curves were scaled to second plateau-shear viscosity *i.e.*, $\eta_{2000} \times u$ as reported previously (de Vicente, Stokes, & Spikes, 2005; Soltanahmadi, Murray, & Sarkar, 2022). When scaled to viscosity, NaCas still surpassed the other protein types in boundary lubricity (Fig. 4b), highlighting the importance of surface-solution interactions rather than fluid film lubrication. Interestingly, the PPc and SPI appeared to overlap and showed an onset of EHL at higher u and higher μ at the mixed regime compared to the buffer and WPI/ NaCaS curves. This suggests faster squeeze out of the lubricating film formed by plant proteins *i.e.* PPc and SPI from the contact interfaces or aggregation-induced starvation of contact interfaces (*i.e.* exclusion of fluid entrainment into the contact), which is in agreement with previous findings showing de-lubricating behaviour of plant proteins observed previously (Vlădescu et al., 2023). Collectively, 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 (Fig. 4c). The h_{min} was almost 2× higher for plant proteins than those of dairy proteins (Fig. 4c) which means onset of EHL requires double the film thickness as compared to those for dairy proteins. This raises the questions of whether plant proteins a) have thinner absorbed film on surface (poorer in surface adsorption) or b) promote starvation conditions requiring thicker EHL films to separate contacting surfaces. This is further discussed in the QCM results. Furthermore, occurrence of EHL for PPc and SPI at higher u values, can be associated with their inferior solubility.

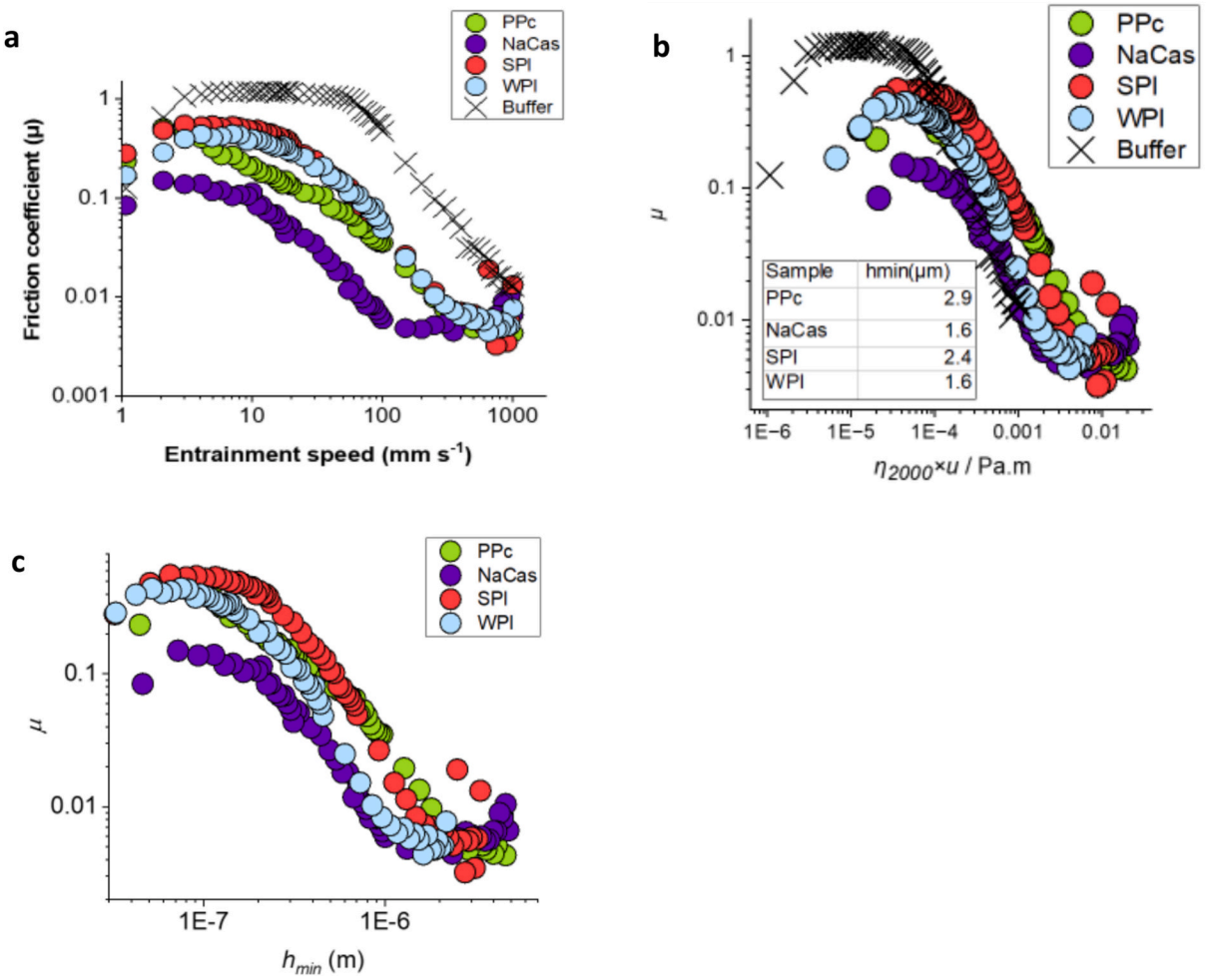

Fig. 4. Mean friction coefficients of protein dispersions at 10 wt% as a function of a) entrainment speed, b) entrainment speed × high shear rate viscosity and c) minimum fluid film thickness. Data are shown for three independent readings on triplicate measurements. Statistics can be followed in Table 2 ($n = 3 \times 3$).

Table 2
Means and standard deviations (SD) of friction coefficients reported for protein samples at various lubrication regimes. Data is reported for three repeats for triplicate measurements ($n = 3 \times 3$). Different lower case subscript letters in the same column indicate a statistically significant difference ($p < 0.05$).

Samples	Boundary lubrication regime		Mixed lubrication regime		Hydrodynamic lubrication regime	
	(5–10 mms^{-1})		(100–120 mms^{-1})		(700–900 mms^{-1})	
	Mean	SD	Mean	SD	Mean	SD
10 wt% PPc	0.253 ^{a}	0.065	0.027 ^{ab}	0.012	0.005 ^{a}	0.004
10 wt% NaCas	0.111 ^{a}	0.116	0.005 ^{a}	0.002	0.007^{ab}	0.004
10 wt% SPI	0.518 ^{b}	0.124	0.038 ^{b}	0.015	0.005 ^{a}	0.003
10 wt% WPI	0.413 ^{ab}	0.148	0.037 ^{b}	0.024	0.005 ^{a}	0.002
20 wt% SPI	0.501 ^{b}	0.074	0.028 ^{ab}	0.014	0.009 ^{b}	0.003
20 wt% WPI	0.182 ^{a}	0.092	0.011 ^{a}	0.006	0.005 ^{a}	0.003

*Note the speed ranges and corresponding regimes are chosen based on the friction curve of the buffer.

The aggregates resulting from insoluble proteins can be confined at the contact inlet and restrict the flow of aqueous solution into the contact interface hindering lubrication and hence requiring higher hydrodynamic pressure (*i.e.* higher u values) to reach a certain lubrication regime.

As anticipated, increasing concentration to 20 wt% (Fig. 5a) led to reduced μ for WPI in the boundary regime (speeds between 5 and 10 mm s^{-1}) by 55.9 % and in the mixed regime (speeds (100–120 mm s^{-1}) by an order of magnitude as compared to 10 wt% ($p < 0.05$) (Table 2). 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 (Kew et al., 2021; Liu, Tian, Stieger, van der Linden, & van de Velde, 2016). However, such behaviour was not observed for SPI, where increasing protein concentration did not give any benefit to the lubrication performance.

When μ was plotted as a function of reduced speed parameter, $\eta_{2000} \times u$, differences still remained in the boundary μ for WPI ($p < 0.05$) (Figs. 5b). WPI at 20 wt% showed lower mixed μ values than the master curve (*i.e.* buffer) with lower h_{min} required for onset of the EHL (Fig. 5c), indicating improved wetting of the surfaces by WPI molecules and a reduced rate of squeeze out (Sarkar et al., 2021; Shewan et al., 2020; Soltanahmadi et al., 2023). However, for SPI, the μ curves collapsed irrespective of concentration. Overall, this again highlights the importance of surface adsorption phenomena driving frictional differences between plant and dairy proteins, which is discussed in the following section.

It is important to compare the present results with a previous study that utilized soluble fractions of pea protein (PPc_Sol) (Zembyla et al., 2021) using similar characterization techniques. Unlike PPc in the current study which contains aggregates, PPc_Sol was composed of only the soluble fractions of pea protein. As one might expect, unpurified PPc had an order of magnitude higher viscosity *versus* PPI_Sol (Fig. 6a), irrespective of the shear rates tested, owing to the afore-mentioned pea protein aggregates present in PPc. This in fact, corroborated with viscosity-mediated friction reduction in PPc, which was slightly lower as

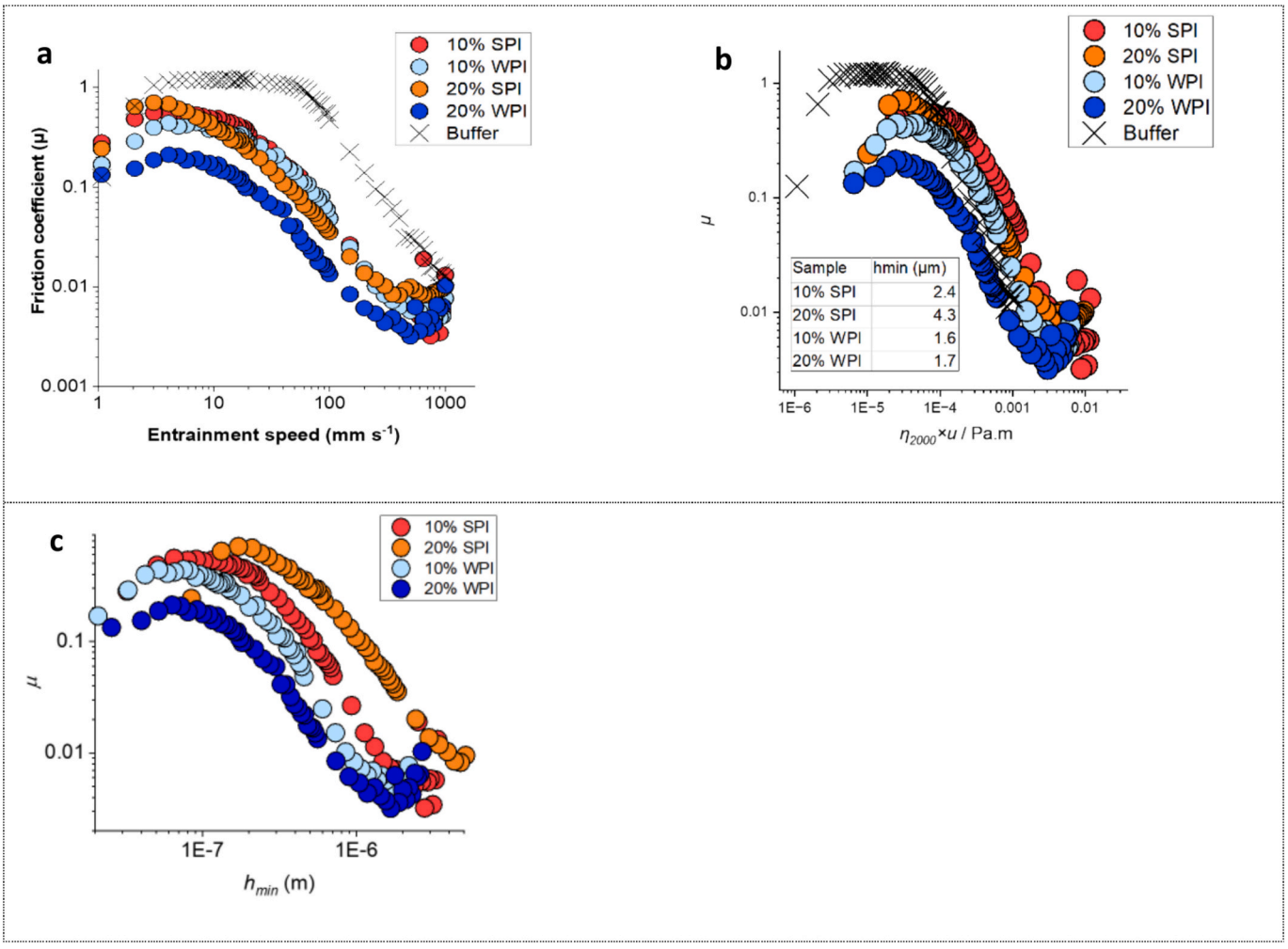

Fig. 5. Mean friction coefficients in presence of higher concentrations of protein (10–20 wt%) as a function of a) entrainment speed, b) entrainment speed × high shear rate viscosity, and c) minimum fluid film thickness. Data are shown for three independent readings on triplicate measurements. Statistics can be followed in Table 2 (n = 3 × 3).

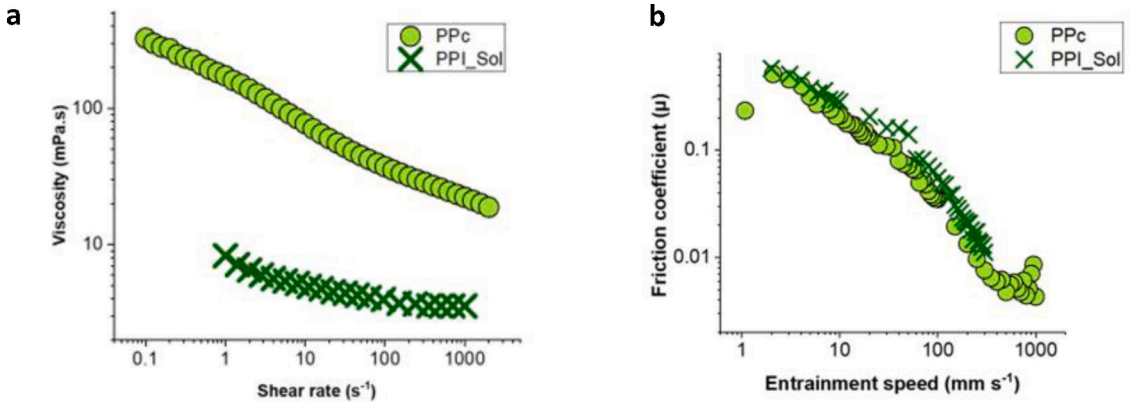

Fig. 6. Mean apparent viscosities (a) and friction coefficients (b). Comparing own data (PPc) to data reported by Zembyla et al., 2021 using soluble fraction (PPc_Sol). Error bars represent standard deviations.

compared to PPc_Sol in the mixed regime (Fig. 6b). Nevertheless, a marked reduction in friction in PPc *versus* PPc_Sol was not observed (Fig. 6b). One might hypothesize this to be associated with the surface roughness of the tribofilm formed by the aggregated PPc. To summarise, the difference between PPc and PPc_Sol is largely associated with the viscous behaviour and roughness. The latter needs further investigation in the future using sophisticated techniques such as atomic force microscopy to observe the roughness of the tribofilm.

3.4. Surface adsorption

Fig. 7a shows the frequency shift and reflects the adsorption of PPc,

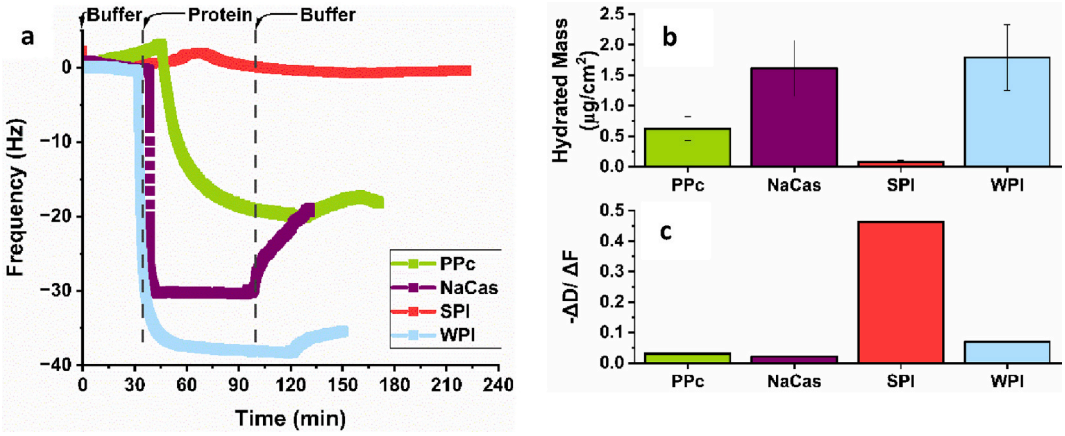

Fig. 7. a) Frequency shift (5th overtone) as a function of time of 0.1 mg/mL protein on PDMS-coated surfaces, b) Fitted hydrated mass and c) viscoelasticity (*i.e.* dissipation/ frequency shift: –ΔD/ ΔF) for all the protein samples. Measurements were repeated in triplicate ($n = 1 \times 3$) and error bars represent standard deviation.

NaCas, SPI and WPI on PDMS-coated surfaces. After the injection of buffer, a stable baseline was measured with no sharp change in frequency. After protein solutions were introduced, a sharp drop in frequency was detected with the exception of SPI (Fig. 7a). Although QCM-D is a relatively new tool used for understanding adsorption behaviour of food proteins, WPI has been 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 (Fig. 7a), with dairy proteins (WPI and NaCas) eliciting the greatest frequency change and therefore greatest adsorption onto the PDMS surfaces. On the other hand, the small frequency shift and dissipation for plant proteins (PPc and SPI) highlights poor adsorption of these proteins. It appears SPI was poorly adsorbed and rapidly desorbed (Fig. 7a), which corroborates with the high friction observed across the entrainment speeds (Figs. 4 and 5). Dairy proteins on the other hand showed lower friction at the mixed regime for a given h_{min} (Fig. 4c), which in agreement with the QCM-D data (Fig. 7a). Overall, this indicates that dairy proteins remained at the contact for longer due to the formation of a thicker adsorbed film, which subsequently reduced the direct contact of the PDMS ball against the PDMS disc and also enabled squeezing out (if any) at a lower rate than plant counterparts.

Fig. 7b and c show the quantitative estimation of hydrated mass and viscoelasticity (−ΔD/Δf), respectively. As stated above, higher hydrated mass ($p < 0.05$) was obtained from both dairy proteins, NaCas and WPI as compared to the plant proteins, PPc and SPI (Fig. 7b). A higher –ΔD/ΔF implies an adsorbed film with a higher contribution of viscous component (Xu et al., 2020). It appears that both WPI and NaCas formed elastic thick films (Fig. 7c) that remained in the contact zone unlike the plant protein films. These adsorbed to a lesser extent, and with a slightly higher elasticity for PPc than SPI. More importantly, both SPI and PPc formed thin adsorbed films which were depleted from the contact region easily during shearing and resulted in such high μ observed in Figs. 4 and 5. The poor adsorption characteristics of SPI and PPc corroborate with the high h_{min} required to achieve the EHL regime (Figs. 4c and 5c).

4. Limitations

Limitations to this study include the test conditions; proteins were analysed at neutral pH however when proteins are embedded within a food matrix, pH can be lower, particularly for fermented foods. Further, the presence of salivary film or mixing with saliva can impose strong influence on the lubrication and flow behaviour of proteins. Moreover, processing techniques, including each step in protein extraction and subsequent purification, may influence the tribological behaviour of plant proteins. Understanding the effects of extraction and purifictaion of plant proteins on tribological properties require further attention in the literature and was not investigated in this study due to the commercial nature of the protein ingredients tested. In addition, when food with added protein is processed, the structure and consequently the tribological behaviour of the plant proteins may further alter due to heat-induced denaturation and such changes in protein structure might affect their interactions with mucins as observed in soluble fractions of pea protein and whey protein (Zembyla et al., 2021) previously. Also, food is a composite matrix consisting of other macro and micronutrients which may influence the tribological properties of plant proteins during oral processing. Therefore, tribological and adsorption properties in this study set the scene for understanding the behaviour of high concentrations of plant proteins in comparison to dairy proteins, with further work required in a wider range of food environmental conditions. Future studies should be carried out with real stimulated and unstimulated saliva (*ex vivo*) to give insights of true oral processing behaviour of higher concentrations of plant proteins.

5. Conclusions

The 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 did not diminish with increased protein concentration. Adsorption analysis revealed 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. The latter had limited adsorption to hydrophobic contact surfaces. 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 higher 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 real food matrices.

CRediT authorship contribution statement

Frances Brown: Writing – original draft, Validation, Methodology,

Investigation, Formal analysis, Data curation. **Siavash Soltanahmadi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation. **Alan R. Mackie:** Writing – review & editing, Supervision, Funding acquisition. **Qi He:** Writing – review & editing. **Jochen Pfeifer:** Writing – review & editing. **Anwesha Sarkar:** Writing – review & editing, Visualization, Project administration, Methodology, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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