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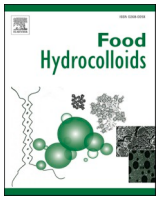

Relating tribology to astringency perception in acidic plant protein-fortified fiber-based smoothies

Ben Kew [a,*], Xinyi Guo [a], Alice Heath [b], Kieran Tuohy [c,d], Anthony Buckley [c], Anwesha Sarkar [a,d,**]

[a] Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK
[b] Innocent Drinks Ltd, Fruit Towers, 342, Ladbroke Grove, London, W10 5BU, UK
[c] Nutritional Sciences and Epidemiology Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK
[d] National Alternative Protein Innovation Centre (NAPIC), UK

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ABSTRACT

With increased need to address environmental sustainability, there has been a pronounced interest on incorporating plant proteins in health-promoting fiber-rich fruit based drinks. Often such matrices are acidic in nature posing challenges for incorporating plant proteins causing undesirable textural issues such as astringency, which is poorly understood in the literature. This study aimed to understand how tribological and rheological characterization can help to explain mouthfeel of plant proteins when incorporated in fiber-based matrices (both model and real smoothies) at pH 3.8. Ten different commercially available isolated plant proteins (5 wt% protein solutions) exhibited significant aggregation being close to their isoelectric point in the fiber-based model smoothie dispersion (0.3 wt% pectin, 0.8 wt% inulin). Particularly, the viscosity of model smoothies spanned across three orders of magnitude, with many, if not, most demonstrating shear-thinning behaviors. Plant proteins exhibited diverse frictional dissipation, with some of the tested commercial fava bean protein, pea protein and chickpea protein concentrates outperforming industry standards, such as soy protein isolate. Model smoothie's effectively mimicked real smoothies in mouthfeel attributes (11 trained panelists), showing plant proteins governing the mouthfeel. Pearson's correlation identified strong relationships between boundary friction, rheology, and sensory attributes, highlighting the predictive value of *in vitro* methods. Notably in legume proteins, %insoluble fraction negatively correlated with all tested undesirable attributes, such as astringency offering a facile screening metric for plant protein performance. Overall, this study validates the use of *in vitro* tools for mouthfeel assessment in complex food matrices, streamlining protein selection for accelerating the development of sustainable plant-based foods.

1. Introduction

A smoothie is typically a blended beverage made by combining various ingredients, such as fruits and vegetables, which inherently come with fibers and water. Smoothies are characterized as smooth, slightly viscous with a high concentration of nutrients, making it a convenient and sensorially pleasing snack or meal addition with a healthy connotation. As modern consumers increasingly prioritize balanced diets that combine nutrition and convenience, whether for general health, athletic performance, or supporting the needs of the elderly, a fiber-rich, protein-fortified smoothie presents an ideal food matrix to meet these demands. (Chermon et al., 2024).

Due to increasing environmental concerns, there is an additional emphasis on using alternative proteins instead of animal based protein. Animal protein production contributes to 15–20 % of total anthropogenic greenhouse gas emissions (GHG) (Poore & Nemecek, 2018) yet little of our total protein contributing to only 37 % in global diet (Makkar, 2018). As a result, the strain on biodiversity from monoculture feed crops, along with excessive water, land, energy, and fertilizer use, leading to issues like eutrophication, is becoming more prevalent (Bryant, 2022) which is exacerbated by the growing population, whose demand for animal protein is expected to double by 2050 (Henchion

* Corresponding author.
** Corresponding author. Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK.
E-mail addresses: B.J.Kew@leeds.ac.uk (B. Kew), A.Sarkar@leeds.ac.uk (A. Sarkar).

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et al., 2017). In stark contrast is the production of plant proteins, whose emissions are half the amount of animal proteins that also contribute to greater amounts of available protein for the global diet (Xu et al., 2021).

There has been prior interest in replacing animal-derived whey protein with plant-based proteins in smoothies, with studies showing comparable benefits in appetite regulation following substitution (Bäuerle & Kühn, 2022). However, the key challenge of the incorporation of alternative proteins is their poor functionality, limited solubility and sensory properties. When used as replacements to dairy proteins, they often impart undesirable mouthfeel characteristics, such as astringency (Sarkar, 2024), necessitating addition of high levels of fats, sugars or other masking additives to improve palatability. In smoothies, which are generally formulated at an acidic pH, plant proteins face an added difficulty where they tend to sediment near their isoelectric point (pI) due to minimal charge repulsion. This promotes protein-protein aggregation, resulting in poor dispersibility, unappealing organoleptic and textural appeal and compromised appearance. Moreover, poor functionality, such as phase separation, coupled with aforementioned sensory challenges, results in significant time and cost spent to develop formulation (Etzbach et al., 2024).

Despite such issues, Bäuerle and Kühn (2022) developed a protein-enriched fruit smoothie using pea protein isolate and conducted hedonic testing with 67 untrained panelists. Although overall perception was negative, nine participants—identified through cluster analysis as athletic, health-conscious, and young—expressed a clear preference for the smoothie containing 20 % pea protein. Dipakkumar Mehta et al. (2017) also utilised soy protein with pectin to develop a high protein, high fiber smoothie yielding an acceptable quality smoothie based on sensory responses. Research into adding new proteins or new varieties of existing plant proteins show promise and may yield unexpected success. Erickson and Slavin (2016) for example, showed that untypical proteins such as puréed red lentil can be used to form smoothies compared to an ice cream base smoothie demonstrating an effective way to improve protein and fiber intake. This growing interest in diversifying protein sources reflects a broader shift away from soy-dominated markets, aiming to address challenges related to sustainability, supply chains, and allergenicity.

Given the wide variety of new, uncharacterized plant proteins available, it would be highly beneficial for the food industry to predict, fast-track, and narrow down promising new proteins for their formulations. Techniques such as tribology, rheology and size have shown to correlate to numerous sensory attributes (Sarkar & Krop, 2019) providing a vital and quick tool set to accelerate new ingredients and products. Firstly, rheology is one of the most utilised methods to characterize food providing structural, flow and textural information (Stokes et al., 2013). For instance, in smoothies Sun-Waterhouse et al. (2014) formulated with high concentrations of apple polyphenols and fiber and used rheology for the purpose of studying the temperature dependence of viscosity in these complex beverage systems. Leal et al. (2021) examined the effects of gelsolin concentration on the physicochemical, rheological, and sensory properties of needle-fruit smoothies. Collectively, these studies illustrate the use of rheology in smoothies providing information on sensory thickness that yield important mouthfeel consequences (Chen & Stokes, 2012). Secondly, particle size within a food plays a crucial role in sensory perception, with several studies correlating smaller particles with smoother, more pleasant textures (Garti & Leser, 2001) whilst larger associated with more grittiness (Shewan et al., 2020). Thirdly, tribology is now seen as a powerful tool to characterize food mouthfeel, as it elucidates surface interactions within the mouth, providing insights into lubrication properties of foods that helps predict attributes such as smoothness, slipperiness, creaminess but also off-mouthfeel, astringency and dryness (Krop et al., 2019; Mehta et al., 2023; Sarkar & Krop, 2019). There is now increasing understanding of how tribology can help to understand frictional dissipation in plant proteins and protein-fiber matrices (Soltanahmadi et al., 2022, 2023) where collectively, these methods offer valuable predictive capabilities into food texture at a multiscale level that can screen and help optimise product formulation.

To summarize, despite the wide range of smoothie products available, there is a significant gap in understanding the mouthfeel of these products mechanistically when plant-based protein are incorporated in such fiber-rich matrices. Moreover, research investigating model smoothies to understand the specific effects of different plant proteins, particularly the mechanisms behind undesirable sensory attributes like astringency under acidic conditions, remains largely unexplored. Hence, the objective of this study was to examine the rheology, tribology and sensory behavior of ten plant protein powders in a model fiber-rich matrix at pH 3.8 as well as real smoothies in hopes of aiding the quick transition of plant proteins into a product whilst making useful correlations across *in vitro* tests and *in vivo* outcomes. Legume proteins such as fava bean protein, pea protein, chickpea protein, lentil protein as well as oil seed protein (rapeseed protein, soybean protein) were a key focus in this study owing to their natural high protein and popular use in food, in addition we explored different variants of the same protein to observe varietal differences. Proteins were compared to whey and soy protein as current industry gold standards for animal and plant protein, respectively. In addition to size, ζ-potential, rheology and tribology, phase separation and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were performed to understand how physicochemical parameters affects mouthfeel perception. The insights from this work aim to validate *in vitro* techniques and their use to fast-track the optimization process of formulation where sustainable alternative proteins are used in a challenging acidic fiber-rich food matrices.

2. Materials and methods

2.1. Materials

Eleven commercial protein concentrates and isolates were sourced from commercial suppliers. The proteins included in this study were two soy protein isolates (SPI1, SPI2) with 90 wt% total protein, three pea protein concentrates (PPC1, PPC2, PPC3) that contained 85 wt%, 83 wt % and 82 wt% protein, respectively), fava bean protein concentrates (FPC1, FPC2) containing 85 wt% and 82 wt% protein, chickpea protein concentrate (CPC) containing 60 wt% protein, red lentil protein concentrate (RLPC) containing 80 wt%, rapeseed protein concentrate (RPC) 50 wt% protein and whey protein isolate (WPI), comprising a minimum of 96 wt% protein. Due to commercial sensitivity we are unable to state supplier. Inulin fiber was obtained from BIOGLAN® (Surrey, UK), while apple, citric acid and sodium citrate, were purchased from Special Ingredients (Chesterfield, UK). All proteins and fibers were food grade.

For sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the reagents employed included Bolt™ 4–12 % Bis-Tris Plus gels, Bolt™ SDS running buffer, Bolt™ LDS sample buffer, and the PageRuler™ Plus pre-stained protein ladder, which were purchased from Thermo Fisher Scientific (Loughborough, UK). All solutions were prepared using analytical grade chemicals unless otherwise stated. The Milli-Q system (Millipore Corp., Bedford, MA, USA) was utilised for the purification of Milli-Q water, which was used to make the buffer.

2.2. Preparation of model smoothie dispersions containing plant proteins and fibers at pH 3.8

10 mM of sodium citrate and 10 mM of citric acid solutions were made by adding the respective powders to Milli-Q water under constant stirring for 2 h. These solutions were combined to form a citrate buffer to obtain a final solution of pH 3.8. 0.3 wt% pectin and 0.8 wt% inulin were added to the citrate buffer and stirred for a minimum of 2 h to ensure the complete dissolution of both the additives to which this buffer is referred to as the model fiber buffer (FB_M). For rheology and tribology, aqueous solutions of 5 wt% total protein (Refer to Table 1 for model smoothie

Table 1
Composition of model and real protein-fortified fiber smoothie formulations.

Protein smoothie	Model/Real (M/R)	Nomenclature	Protein powder (wt %)	Inulin (wt %)	Pectin (wt %)	Citrate Buffer (wt %)	Fruit base (wt %)	Water (wt %)
Soy protein isolate 1	M	$SPI1_M$	5.6	0.8	0.3	93.3	–	–
Soy protein isolate 2	M	$SPI2_M$	5.6	0.8	0.3	93.3	–	–
	R	$SPI2_R$	5.6	0.8	0.3	–	46.7	46.6
Pea protein concentrate 1	M	$PPC1_M$	5.9	0.8	0.3	93.0	–	–
	R	$PPC1_R$	5.9	0.8	0.3	–	46.5	46.5
Pea protein concentrate 2	M	$PPC2_M$	6.0	0.8	0.3	92.9	–	–
Pea protein concentrate 3	M	$PPC3_M$	6.1	0.8	0.3	92.8	–	–
Fava bean protein concentrate 1	M	$FPC1_M$	5.9	0.8	0.3	93.0	–	–
Fava bean protein concentrate 2	M	$FPC2_M$	6.1	0.8	0.3	92.8	–	–
	R	$FPC2_R$	6.1	0.8	0.3	–	46.4	46.4
Chickpea protein concentrate	M	CPC_M	8.3	0.8	0.3	90.6	–	–
Red lentil protein concentrate	M	$RLPC_M$	6.3	0.8	0.3	92.6	–	–
Rapeseed protein concentrate	M	RPC_M	10	0.8	0.3	88.9	–	–
Whey protein isolate	M	WPI_M	5.2	0.8	0.3	93.7	–	–
Control no protein	M	FB_M	–	0.8	0.3	98.9	–	–
	R	FB_R	–	0.8	0.3	–	49.5	49.4

composition) were prepared by dispersing and mixing the protein powders in FB_M for 2 h. The final pH of the model smoothie dispersion containing protein, inulin and pectin was adjusted to pH 3.8 and refrigerated at 5 °C until analysis.

2.3. Preparation of real protein smoothies with fiber at pH 3.8

Real smoothies were formulated with water, pasteurized fruit base (confidential recipe with ascorbic acid, citric acid and natural flavorings), 5 wt% total protein, 0.3 wt% pectin and 0.8 wt% inulin (Refer to Table 1 for real smoothie compositions). Protein and fibers were firstly hydrated with water for 10 min and then blended with fruit using a Thermomix® apparatus (model TM6-1, Vorwerk, Germany) at a speed setting of 2.5. The pH of the smoothies was then adjusted to pH 3.8 using citric acid. Samples were transferred into Polyethylene terephthalate (PET) bottles and refrigerated at 5 °C until analysis.

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Each protein solution was diluted to 1 mg/mL protein. 75 μL of protein sample was mixed with 25 μL of Bolt™ LDS sample buffer and the mixture was heated at 70 °C for 10 min to denature the protein. 10 μL of the mixture was loaded onto the pre-cast polyacrylamide gel. A 5 μL molecular weight marker was loaded into a separate lane. The gel was placed in an Invitrogen™ Mini Gel Tank system (Thermofisher Scientific, Loughrough, UK) filled with running buffer and a constant voltage of 220 V applied for 30 min until the front of the dye migrates to the bottom of the gel. After electrophoresis, the gel was carefully removed and stained overnight in a staining solution containing Coomassie Brilliant Blue R-250. The gel was rinsed with Milli-Q water to remove any remaining staining solution. When staining was complete, the gel was scanned by ChemiDoc™ XRS + System and analysis was performed using Image Lab Software Version 6.0.

2.5. Measurement of %insoluble fraction

Model smoothies containing protein in FB_M were prepared with a total protein concentration of 5 wt% and stirred continuously for at least 2 h at room temperature and adjusted to pH 3.8. The protein dispersions were placed in glass vials for 7 days storage period at 5 °C and then the percentage of the insoluble fraction was calculated by measuring the concentration of sedimented protein compared to the total concentration of the protein in the dispersion.

2.6. Particle size

Each protein was dispersed in citrate buffer without fiber at 0.1 wt% total protein concentration and then filtered through a 0.22 μm syringe filter (PTFE syringe filter, PerkinElmer, USA). The filtered protein solution was transferred to a DTS1070 disposable folded capillary cuvette (PMMA, Brand GmbH, Wertheim, Germany). The cuvette was placed in the Zetasizer Ultra (Malvern Instruments Ltd, Worcestershire, UK), which was pre-equilibrated at 25 °C for 20s. The refractive index (RI) was set to 1.5 with an absorbance of 0.001. The viscosity of water was employed, *i.e.* 1 mPa s where samples were equilibrated for 120 s at 25 °C, which were then analyzed using backscattering technology at a detection angle of 173°. At least three replicates were measured for each protein sample where mean hydrodynamic diameter (d_H) and polydispersity index (PDI) were obtained.

2.7. ζ-potential

The electrophoretic mobilities of the protein dispersions without added fibers were measured in the Malvern Zetasizer Ultra, Malvern instruments Ltd, Worcestershire, UK at 25 °C. Diluted protein dispersions (0.01 wt% total protein) was filtered through a 0.22 μm syringe filter and transferred to a DTS1070 folded capillary electrophoresis cell. Electrophoretic mobility was determined considering the dielectric constant (ε) of the medium, the viscosity of citrate buffer (equivalent to water at 1 mPa s) and Henry's function (F(ka)) using the Smoluchowski approximation, which was taken as 1.5. At least three biological replicates were measured for each protein sample where mean and standard deviation was calculated.

2.8. Apparent viscosity

The flow curves of the model systems containing protein and fibers were recorded at 37 °C using a stress-controlled rheometer (Paar Physica MCR 302, Anton Paar, Austria) equipped with a measuring cone CP50-1 D and an inset plate I-PP50. The model smoothies were prepared by dissolving the protein powder in FB_M in order to achieve a 5 wt% total protein concentration and were stirred continuously for at least 2 h at room temperature before measuring. The prepared samples were carefully loaded onto the rheometer's measuring system, taking care to avoid air bubbles. Silicon oil was pipetted around the sample to prevent evaporative losses. The rheometer was set to perform a shear rate sweep, covering a range from 1 s^{-1} to 1000 s^{-1}. Experiments were repeated at

least three times (n = 3 × 1) to which means and standard deviations were calculated.

2.9. Tribology

Tribological experiments were carried out using a tribology-cell attachment to the rheometer utilizing glass ball (R = 12.7 mm) on three polydimethylsiloxane (PDMS) pins (6 mm height and diameter) inclined at 45° forming a glass ball-PDMS (hard-soft) contact. Samples were added in an enclosed chamber covering PDMS pins with glass ball geometry applying an evenly distributed load of 2.0 N. Upwards sliding speeds of 0.001–1.0 m/s were measured with pins remaining stationary generating three-sliding point contact. Measurements were performed at 37 °C with friction coefficients (μ) of water and citrate buffer with fiber measured as control. Normal force is related to the total normal load acting on the plates as described in equation (1). Furthermore, the torque sensed by the glass ball is related to total frictional force (FF) denoted by equation (2).

$$F_L = \sqrt{2F_N} \tag{1}$$

$$F_F = \frac{\sqrt{2T}}{R} \tag{2}$$

With μ expressed as

$$\mu = \frac{F_F}{F_N} = \frac{T}{F_N R} \tag{3}$$

PDMS pins were cleaned using isopropanol then sonication in detergent for 10 min between samples. Pins were replaced following signs of surface wear. Experiments were biologically repeated at least three times (n = 3 × 1) to which means and standard deviations were calculated from average of all repeats.

2.10. Trained sensory panelists

Sensory analysis was conducted using a trained panel ($n = 11$, 3 males, 8 females) with an average age of 31 at innocent drinks ltd. Attribute definitions were established by three experts during pre-tasting. Panelists underwent training, maintenance and screening to ensure consistent and precise attribute assessment. They were trained on each attribute in several sessions using an unstructured 1–9 scale, refining their use of language, scale, and methodology during the first session. Data collection was carried out over two separate sessions on different days, first with model smoothie samples and then with real smoothie samples. Samples were presented using a balanced design generated using Compusense and presented in closed containers with a randomly selected 3-digit code (Compusense Inc. ON, Canada Version 24.0.60). Participants were not informed of what sample was provided and asked to consume all of a 50 ml sample at 5 °C. Palate cleansers (crackers and water) were consumed after assessment with 5-min breaks between each assessment. Assessed attributes and definitions are provided in Table 2.

Table 2
Sensory attributes used to assess the model and real protein-fortified fiber smoothie formulations and the definitions of the attributes used.

Attribute	Definition
Astringent	A dry, puckering, or tightening mouthfeel present, reminiscent of the sensation experienced after sampling tea or wine
Granular	A particulate gritty mouthfeel characterized by the perception of small detectable particles or grains when consuming food
Creamy	A silky, smooth, rich texture in the oral cavity
Thickness	Perceived viscosity and body of food in the mouth
Powder	A dry, dusty or chalky mouthfeel characterized by fine particles in the mouth

2.11. Statistical analyses

All results are reported as means and standard deviations based on at least three measurements conducted on three independent samples unless stated otherwise. Statistical significance between datasets was assessed using analysis of variance (ANOVA) with a Tukey post hoc test, with a significance level of $p < 0.05$. Pearson's correlation coefficients were calculated to assess relationships between variables, and confidence intervals were estimated using the bootstrap resampling method. All model calculations were performed using Excel 2016 and Origin 2024.

3. Results and discussion

3.1. Composition and physicochemical properties of the proteins

Although commercially available protein powders are generally high in protein content (often >80 % protein), proteins can differ hugely in structure such as protein subunit, degree of aggregation, quaternary structure and isoelectric point brought upon by both natural amino acid sequence (Jumper et al., 2021) but also influenced by processing used to extract and isolate protein (Hall & Moraru, 2021; Lechevalier et al., 2007). Hence, we first characterized the molecular weight (Mw) of each protein, without added fiber, using SDS-PAGE to understand sub-unit proteins and purity of the sample. Soy protein typically consists of four main sub-proteins: 2S albumins, 7S globulin, 11S globulin, and 15S globulin (Medic et al., 2014; Nishinari et al., 2014; Verfaillie et al., 2023). Among these, 7S (β-conglycinin) and 11S (glycinin) serve as the dominant storage proteins (Qi et al., 2011). SPI2 showcases as such in line with previous studies (Zheng et al., 2022). However, in the case of SPI1, only a single faint protein band was observed around 20 kDa containing the basic subunit of 11S globulin, although the darkening of the gel at lower molecular weights may suggest the presence of albumin fractions.

Similarly, differences in PPC types used in this study are evident in the SDS-PAGE gel, highlighting that often commercially available protein powders significantly vary in their composition. Pea protein is typically composed of four major globulin groups: 11S legumin, 7S vicilin, convicilin (72–73 kDa) and water-soluble 2S albumins. Legumin consists of α-subunits (34 kDa) and β-subunits (20 kDa), while the cleavage of the vicilin subunit (~50 kDa) generates can generate various low molecular weight fragments (Fig. 1). PPC1 and PPC3 align with findings from previous studies (Kew et al., 2021), whereas PPC2 displays a single faint band at 20 kDa. Both FPC1 and FPC2 displayed convicilin, legumin, vicilin and β-legumin at approximately 23 kDa, 52 kDa, 58 kDa, and 70 kDa, respectively. Additional faint small Mw bands may indicate albumins might be present and that the two protein variants share a similar structural sub units with well-defined molecular weights (Kimura et al., 2008; Oluwajuyitan & Aluko, 2024).

The electrophoresis results of CPC exhibited four distinct bands with molecular weights of 39 kDa, 45 kDa, 60 kDa, and 71 kDa consisting of globulins, albumins, glutelins and prolamin (Boukid, 2021; Grasso et al., 2022). For RLPC, the SDS-PAGE gel exhibited a major protein group in the region of 50–60 kDa, followed by four faint bands at 23 kDa, 33 kDa, 40 kDa and 48 kDa compromising of albumins, 11S legumin and 7S vicilin like structures in line with reported literature (Lee et al., 2021).

Interestingly, the SDS-PAGE gel did not show any protein bands in the RPC sample, suggesting that the protein concentration might be below the detection limit, interfered with by contaminants due to the sample containing only 50 wt% protein, or possibly composed of very small peptides that could not be captured by the gel. Chmielewska et al. (2021) reported that the primary storage proteins in rapeseed are cruciferin (300 kDa) an 11S globulin, consists of six subunits, each comprising two polypeptides: polypeptide α (~40 kDa) and polypeptide β (~20 kDa) and napin (12–16 kDa). Lastly, WPI exhibited the well-defined three band patterning compromising of bovine serum

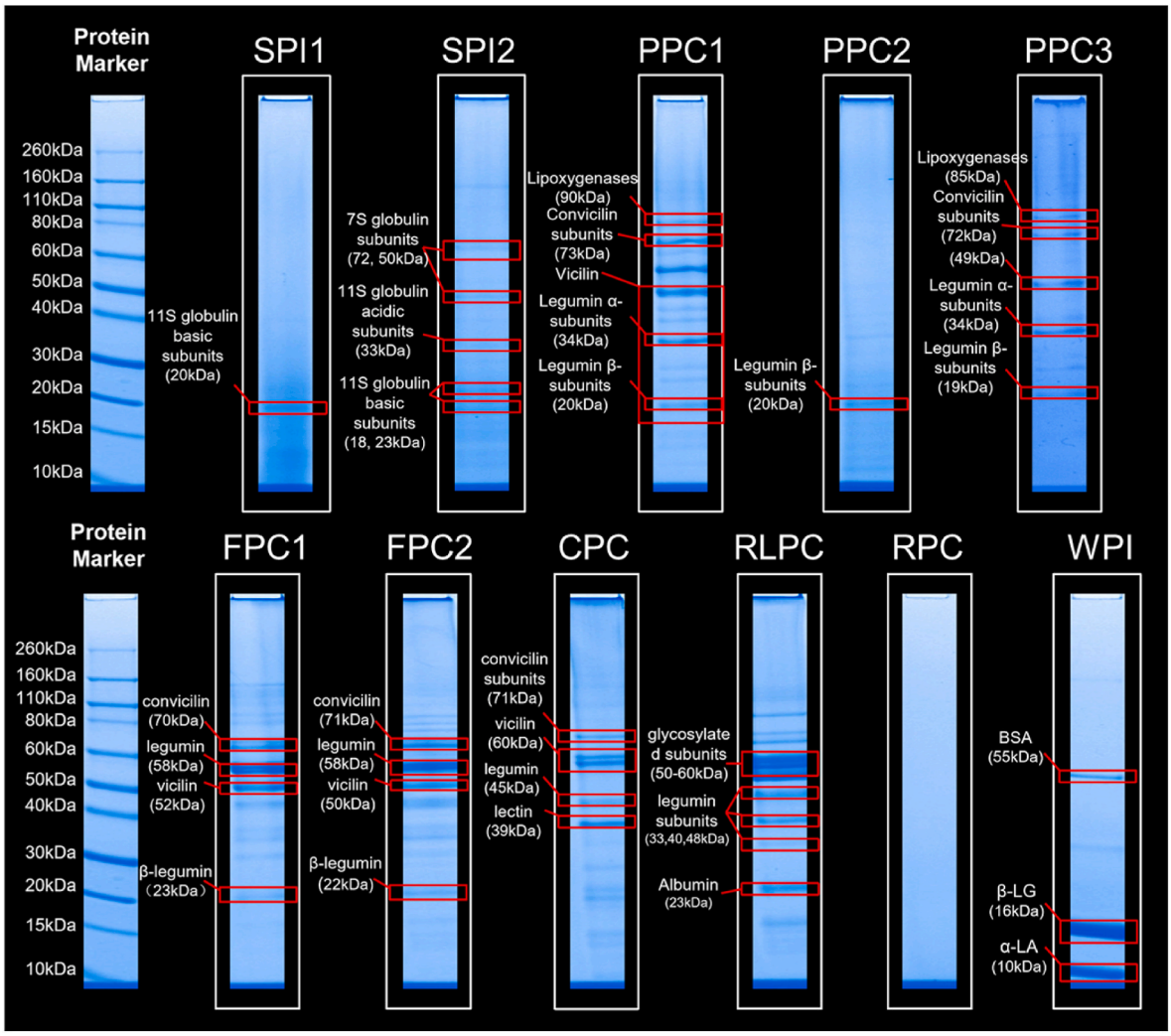


Fig. 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of protein dispersions (without added fibers) at pH 3.8. Protein fraction abbreviations are as follows, beta-lactoglobulin (β-lg), bovine serum albumin (BSA), alpha-lactalbumin (α-la). SPI1-2 are soy protein isolates, PPC1-3 are pea protein concentrates, FPC1-2 are fava bean protein concentrates, CPC is chickpea protein concentrate, RLPC is red lentil protein concentrate, RPC is rapeseed protein concentrate and WPI is whey protein isolate.

albumin, beta-lactoglobulin and alpha-lactalbumin with molecular weights of 10 kDa, 16 kDa, and 55 kDa, reported previously respectively (Kew et al., 2021). Overall despite proteins from similar sources, different subunit compositions may lead to varying material performance and sensory differences, which have been examined in this study.

Next, the physicochemical properties of the commercial protein powders were assessed at pH 3.8. All proteins exhibited a degree of aggregation and polydispersity, with d_H values ranging from 9.7 nm to 328 nm (Table 3, Fig. 2). It is unsurprising that the proteins were highly aggregated at pH 3.8, as the proteins were in vicinity of their isoelectric points (pI) that range from 4.0 to 5.6 for the proteins assessed. Consequently, the low ζ-potential values reported in Table 3 (ranging from

Table 3
Physicochemical properties of protein dispersions (0.1 wt% protein, without added fibers) at pH 3.8 after filtration using 0.22 μm syringe filter represented as average ± standard deviations. %insoluble fraction was performed in combination with fiber (FB_M) at 5 wt% total protein. Different letters in same column represent significant difference ($p < 0.05$). Experiments have been repeated at least three times by which means and standard deviation are stated (n = 3 × 1).

Protein	Isoelectric point (pI)	%Insoluble fraction	Hydrodynamic diameter (d_H) (nm)	Polydispersity Index (PDI)	ζ-potential (mV)
SPI1	4.5 (Puppo & Añón, 1998)	25 ± 4.0^{ef}	17.3 ± 1.8^{g}	1.07 ± 0.080^{b}	2.6 ± 0.1^{a}
SPI2		45 ± 3.0^{c}	9.7 ± 0.1^{g}	0.68 ± 0.018^{c}	2.0 ± 1.3^{a}
PPC1	4.0 (Adal et al., 2017)	20 ± 2.5^{f}	211.3 ± 19.4^{c}	0.21 ± 0.014^{h}	-0.9 ± 1.0^{c}
PPC2		20 ± 3.5^{f}	10.3 ± 9.0^{g}	0.37 ± 0.111^{fg}	0.7 ± 0.5^{b}
PPC3		28 ± 4.3^{e}	54.5 ± 5.1^{f}	1.41 ± 0.059^{a}	0.4 ± 0.1^{b}
FPC1	5.0 (McClements, 2021)	65 ± 4.0^{b}	9.8 ± 1.0^{g}	0.58 ± 0.079^{d}	0.6 ± 0.6^{b}
FPC2		22 ± 1.5^{f}	182.7 ± 2.5^{d}	0.32 ± 0.007^{g}	2.2 ± 0.4^{a}
CPC	4.5 (Ladjal-Ettoumi et al., 2016)	45 ± 3.5^{c}	328.5 ± 17.7^{a}	0.34 ± 0.056^{g}	1.9 ± 0.6^{a}
RLPC	4.5 (Ladjal-Ettoumi et al., 2016)	35 ± 3.3^{d}	300.3 ± 25.4^{b}	0.43 ± 0.005^{e}	2.3 ± 0.5^{a}
RPC	5.6 (Kim et al., 2016)	90 ± 5.5^{a}	111.5 ± 13.0^{e}	0.30 ± 0.029^{g}	-3.2 ± 0.5^{d}
WPI	5.2 (Weinbreck et al., 2003)	18 ± 1.7^{f}	9.3 ± 0.8^{g}	0.44 ± 0.045^{ef}	2.3 ± 0.3^{a}

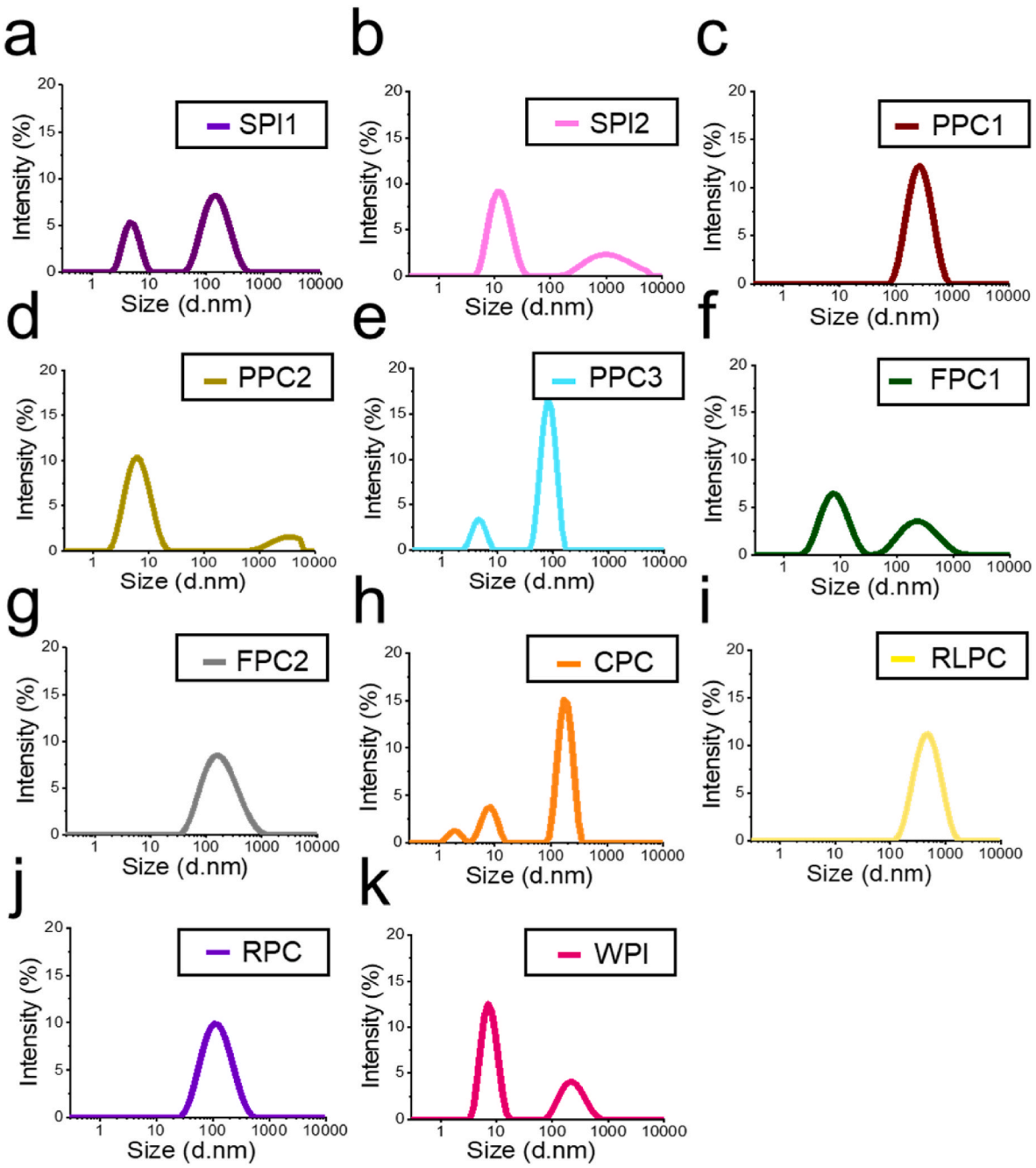


Fig. 2. Mean particle size distribution of protein samples *i.e.* soy protein isolates, a) SPI1, b) SPI2, pea protein concentrates, c) PPC1, d) PPC2, e) PPC3, fava bean protein concentrates, f) FPC1, g) FPC2, chickpea protein concentrates, h) CPC, red lentil protein concentrate, i) RLPC, j), rapeseed protein concentrate, RPC, and whey protein isolate, k) WPI at 0.1 wt% protein at pH 3.8 after filtration using 0.22 μm syringe filter. Experiments have been repeated at least three times (n = 3 × 1).

−3.2 to 2.6 mV) indicate weak electrostatic repulsion, which is likely to promote protein–protein aggregation. This aggregation may persist even after filtration, accounting for particle sizes exceeding the filter's pore size (Fig. 2).

In addition the insoluble fraction was recorded from each sample ranging from 18 to 90 % (Table 3). All proteins showed a degree of protein sediment and therefiore %sinoluble fraction due to the high level of protein-protein aggregation. Interestingly RPC showed highest levels of %insolubility, which may be attributed to its lower total protein concentration and the presence of non-proteinaceous impurities. Although from the same botanical source, sedimentation and physico-chemical differences were apparent for variety of SPI, PPC and FBC powders. Particularly, for SPI1 and SPI2, the latter exhibited much larger aggregates and notable differences in polydispersity index (PDI), such differences are expected from differing protein compositions from SDS-PAGE (Fig. 1). Recently, Schmid et al. (2024) also found such variation in physiochemical attributes when investigating six different soy protein powders highlighting the complexities of commercial protein powder, which must be considered when comparing properties in the literature.

Among the pea protein concentrates, PPC1 had the lowest PDI with a single peak and d_H of 211 nm (Fig. 2), this was in contrast to PPC2 and PPC3 containing multiple larger aggregates and significantly higher PDI (Table 3). Additionally PPC2 and PPC3 contained smaller sized particles with PPC2 containing aggregates beyond 1000 nm (Fig. 2). Similarly, FBP1 and FBP2 showed considerable variation in size distribution,

reflecting significant differences in d_H, PDI, and ζ-potential (Table 3, Fig. 2). Among the other proteins, CPC exhibited three distinct size peaks, including the largest d_H while RLPC and RPC each formed single peaks, and WPI exhibited two peaks (Fig. 2).

Overall, all protein solutions at pH 3.8 exhibited high degree of aggregation, low ζ-potential, and noticeable sedimentation with noticeable %insoluble fraction. Additionally despite their similarities, proteins of the same type varied not only in the fractions of their protein subunits but also in their physicochemical properties and aggregation behavior. In a typical smoothie formulation, these proteins would likely exhibit poor functionality due to limited solubility and sedimentation. However, the combination of proteins with fiber, an area that has not been extensively explored across such a diverse range of proteins (Sá et al., 2022), may either improve or deteriorate functionality, which needs to be evaluated. Additionally, comparisons of such a wide range and variety of proteins are relatively underexplored, and their physicochemical properties in relation to sensory perception continue to be a subject of ongoing debate. This warrants further assessment of their rheological, tribological, and sensory properties, these are characterized in the following sections.

3.2. Rheological properties of model protein-fortified fiber-based smoothies

Rheology plays a crucial role in smoothies, influencing key organoleptic qualities such as appearance, taste, and mouthfeel (Chen & Stokes, 2012). While the characteristic viscosity of smoothies enhances their texture and enjoyment, it must be carefully balanced to ensure flow during consumption. Proteins effectively increase viscosity through their water-binding capacity when solublised, which in addition to enhancing the nutritional value, make them excellent additions to smoothies. Fig. 3 presents the viscosity as a function of shear rate for the eleven model smoothie samples containing 5 wt% total protein combined with fiber at pH 3.8 with sample containing whey protein as an animal protein comparison. Firstly the addition of fiber alone (FB_M) to the buffer increases viscosity by one order of magnitude to 10 mPa s, displaying Newtonian behavior (Fig. 3). In this study, we used a mixture of inulin (0.3 wt%) and pectin (0.8 wt%). While inulin's use in juices and smoothies has been rarely explored, it has been reported in dairy drinks (Meyer, Bayarri, et al., 2011). These studies demonstrate a similar viscosity increasing effect similar to our findings, but typically exhibiting shear-thinning behavior, however this is observed because higher concentrations are usually studied unlike the current work. Pectin on the other hand has been widely studied in its rheology (Chan et al., 2017; Dickinson, 2003) and is commonly examined in fruit analogues due to its natural abundance in the middle lamella of plant cell walls. It is reported that at concentrations below 3 %, pectin typically exhibits Newtonian behavior, which aligns with our findings (Dickinson, 2003).

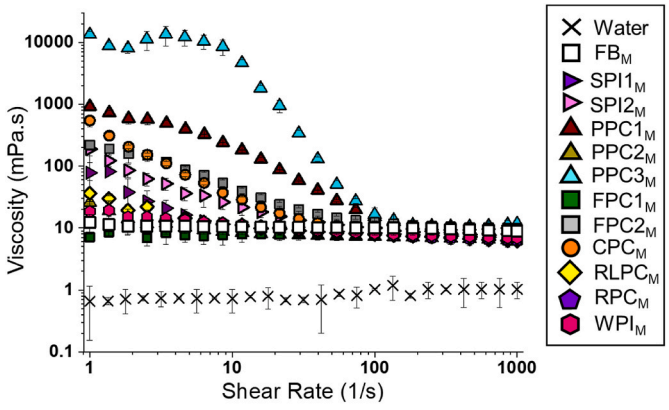


Fig. 3. Mean apparent viscosity as a function of shear rates of model smoothie samples containing protein and fibers (5 wt% total protein with 0.3 wt% pectin and 0.8 wt% inulin) at pH 3.8. Error bars represent standard deviations. Experiments have been repeated at least three times (n = 3 × 1). FB_M is the buffer containing the fiber without any added proteins. Nomenclature of added protein to model smoothies are: SPI_M1-2, soy protein isolates, PPC_M1-3, pea protein concentrates, FPC_M1-2, fava bean protein concentrates, CPC_M, chickpea protein concentrate, $RLPC_M$, red lentil protein concentrate, RPC_M, rapeseed protein concentrate and WPI_M, whey protein isolate (For compositions refer to Table 1).

Next, when we incorporate proteins into the FB_M, we observe stark differences dependent on protein type. For most, the model smoothies *i.e.* the protein-fiber dispersions, exhibited shear-thinning behavior, characterized by a decrease in viscosity as the shear rate increased from 1 to 1000 s^{-1} (Fig. 3) with the largest variations in viscosity observed at low shear rates (1–10 s^{-1}), where differences spanned several orders of magnitude. These significant differences at low shear, combined with shear-thinning behavior, suggest that addition of protein at pH 3.8 leads to aggregation which might be due to protein-protein or protein-fiber aggregation. Corroborating the data with respective d_H, low ζ-potential values and PDI values (Table 3, Fig. 2), the protein-protein aggregation seems to have a clear effect on this shear thinning behavior. As shear increases, these aggregates progressively break down, a desirable characteristic that improves flow and often known to enhance mouthfeel (Greis et al., 2022).

Uniquely however is $FPC1_M$ which was least viscous and that was not significantly different to FB_M ($p > 0.05$). This was due to the large insoluble fraction that this protein likely sedimented out without associating with the FB_M continuous phase (Table 3). $SPI1_M$, $SPI2_M$, $PPC1_M$, $FPC2_M$ and CPC_M exhibited similar rheological profiles, starting with a higher initial viscosity of 100–1000 mPa s that decreased with shear plateauing around 70 s^{-1}. In contrast, $PPC3_M$ exhibited a distinct viscosity profile, with the highest initial viscosity exceeding 10,000 mPa s, followed by a slight increase in viscosity as the shear rates increased from 2 to 3 s^{-1}. $PPC3_M$ was characterized as the lowest surface charge measured by ζ-potential and the highest PDI (Table 3), minimal repulsion between particles likely suggest protein-protein interactions, which may explain the initially elevated viscosity observed before it subsequently decreased as a function of shear. Finally, RPC_M and WPI_M exhibited minimal reactivity, with viscosity not undergoing any significant alterations when subjected to varying shear rates.

Overall, we showcase the diverse rheological impact of plant proteins when incorporated into low pH solutions with fiber achieving different viscosities with shear thinning behavior. Despite similarities in psychochemical characteristics (*i.e.* aggregated particles, high PDI and low ζ-potential) how the protein interacts with food matrix, in this case fiber, may yield unexpected rheological outcomes. In this case $PPC3_M$ with its elevated initial viscosity indicates that PPC3 may be more appropriate for the production of high-viscosity drinks whilst $FPC1_M$, WPI_M and RPC_M demonstrated minimal viscosity alteration with shear rate that may make the corresponding proteins, a suitable addition for smoothies that do not require rheological modification. However, their stability and sedimentation must also be taken into account as a factor that may influence mouthfeel.

3.3. Tribological properties of model protein-fortified fiber-based smoothies

In addition to rheology, the surface properties of plant proteins may play a crucial role in formulating products with optimized mouthfeel, where the boundary friction is key in understanding sensory attributes such as slipperiness, astringency, and graininess (Fan et al., 2021; Krop et al., 2019; Nguyen et al., 2017; Sarkar & Krop, 2019; Zembyla et al., 2021). In line with previous literature, water showcases a high friction coefficient in Fig. 3 at the boundary regime ($U = 0.001–0.01$) as a result of little lubrication between glass ball and PDMS pin contact (Kew et al., 2021). With FB_M alone, a significant reduction in friction is observed at boundary regime, highlighting its potential to enhance lubricity purely via viscosity effects as observed previously in polysaccharide systems (Li

et al., 2022; Stokes et al., 2011; Torres et al., 2019). For inulin, previous studies have shown effective lubrication in dairy systems across a range of concentrations, from as low as 1 wt% to as high as 7–9 wt% (Meyer, Bayarri, et al., 2011; Ng et al., 2018), this also holds true in pH 3.8 model smoothie environment. Pectin's tribological behavior in both model and food systems at low pH has been more extensively studied, with concentrations as low as 0.2 % shown to reduce friction, primarily due to the formation of a viscous lubricating film (Ng et al., 2018; Stokes et al., 2011). Together these fibers show similar scales of lubricity improvements, however it is scarcely reported the impact of such fibers and proteins together, especially in respect to a model smoothies.

At a glance when adding protein, similar to the large variation in rheology, we observe a vast range of frictional dissipation between types of protein (Fig. 4a). In previous studies, the high shear rate viscosity of the solution has been included to complement the frictional understanding of proteins (Andablo-Reyes et al., 2019; Kew et al., 2021) as elastohydrodynamic lubrication theory can explain the viscous component of the frictional behavior (De Vicente et al., 2005). Given the variation in rheology across solutions (Fig. 3), the viscosity and material properties of the glass ball–PDMS contact at high shear rate viscosity of 1000 s^{-1} were used to scale the tribology data (Fig. 4b), enabling direct friction comparisons between proteins without viscous contribution. The poorest lubricating proteins at boundary regime ($\eta_{1000}U = 1 \times 10^{-9}$ - 1×10^{-8}) were $RLPC_M$ and RPC_M which were significantly higher in friction than just FB_M alone ($p < 0.05$) (Fig. 4b). In contrast, the most lubricating *at* $\eta_{1000}U = 1 \times 10^{-9}$ followed in order of $PPC2_M > FPC2_M > PPC1_M > SPI1_M > FPC1_M > CPC_M > WPI_M > PPC3_M$. Interestingly, we observe large differences between protein of the same origin with $PPC2_M$ and $PPC1_M$ ($\mu = 0.14$–0.20) significantly lower in μ compared to $PPC3_M$ ($\mu = 0.30$), $p < 0.05$). However, this did not hold true for fava bean protein concentrates ($FPC1_M$ and $FPC2_M$) or soy protein isolates ($SPI1_M$ and $SP2_M$), where minor compositional differences did not show differences in frictional dissipation particularly in boundary regime ($p > 0.05$). $PPC3_M$ displayed exceptionally high viscosity (Fig. 3) and were highly polydisperse (Table 3) in comparison to all other proteins tested and the higher friction may be attributed to the high degree aggregation causing jamming in the contact (Fig. 4b) (Kew et al., 2021)

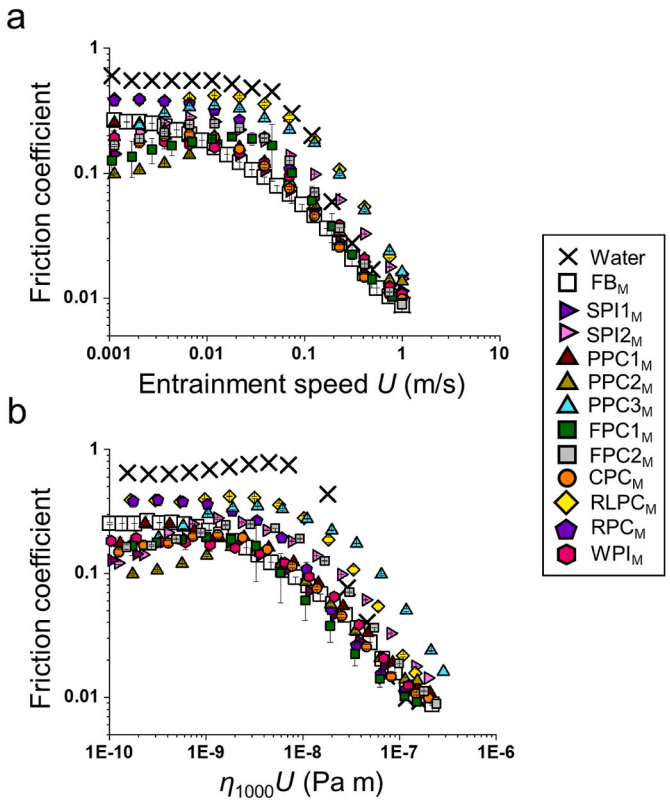


Fig. 4. Mean friction coefficient as a function of entrainment speed (a) and as function of product of entrainment speed and high shear rate viscosity (b), respectively of model smoothie samples containing protein and fibers (5 wt% protein with 0.3 wt% pectin and 0.8 wt% inulin) at pH 3.8. Error bars represent standard deviations. Experiments have been repeated at least three times (n = 3 × 1). FB_M is the buffer containing the fiber without any added proteins. Nomenclature of added protein to model smoothies are: SPI_M1-2, soy protein isolates, PPC_M1-3, pea protein concentrates, FPC_M1-2, fava bean protein concentrates, CPC_M, chickpea protein concentrate, $RLPC_M$, red lentil protein concentrate, RPC_M, rapeseed protein concentrate and WPI_M, whey protein isolate (For compositions refer to Table 1).

Overall, $PPC2_M$, $FPC2_M$, $PPC1_M$, $FP1_M$, $SP1_M$ and CPC_M can be grouped into the model protein solutions with similar and more importantly, best lubrication performance ranging from $\mu = 0.14$–0.2 ($\eta_{1000}U = 1 \times 10^{-9}$, Fig. 4b), despite such large variation in viscosity ranging from 8 to 1000 mPa s (Fig. 2). In other words, the aforementioned plant proteins can effectively lubricate in fiber-based acidic food matrix with a range of viscosity modifying properties. Generally, WPI and SPI are considered as the gold standards in product formulation to which we showcase a number of alternative proteins with either similar or better mouthfeel performance (Fig. 4b). Overall, the interplay between proteins and fiber has led to both increasing and decreasing lubricity, resulting in surprising and significant differences between proteins, despite their similarity in aggregation and vastly different viscosities. A deeper investigation is warranted to understand the biopolymer interactions occurring, as this knowledge would be crucial for optimizing plant protein and matrix interactions to enhance food mouthfeel. Next we assess the *in vivo* performance *i.e.* examine the sensory performance of selected plant proteins with fiber in model as well as real smoothies.

3.4. Sensory performance of model and real protein smoothies

Proteins were narrowed down based on their functionality, prior *in vitro* performance, as well as their current and future market appeal. The chosen proteins were pea protein (PPC1) and fava protein (FBC2), which were compared to soy protein (SPI2), the most widely used and characterized plant proteins in food. In addition to model smoothie assessment, used throughout this work, we conducted sensory on real smoothie recipes fortified with afore-mentioned plant proteins (For compositions refer to Table 1). This was conducted to evaluate the effectiveness and suitability of model systems in analyzing protein performance as well as comparing *in vivo* to *in vitro* study. Finally, as controls, we evaluated the model fiber solution (FB_M) and a real smoothie (FB_R) without any fortification with plant proteins to determine the overall impact of plant proteins within the system.

Firstly, the impact of SPI2 was assessed in the smoothies ($SPI2_M$ and SPI_R). We observe no significant difference to astringency, granularity or powder when compared to FB_M and FB_R (Fig. 5a, b and 5e, $p > 0.05$). These three attributes coincide closely to mouthfeel and surface interactions in the mouth where such low values suggest soy as an effective protein addition when combined with fiber in these food systems. The addition of SPI2 also increases creaminess (Fig. 5c). This trend is observed across all proteins, as a result of proteins' water-holding capacity to enhance viscosity and often viscosity-driven lubrication, which coincides with our findings in Figs. 3 and 4. When assessing thickness, a primarily rheology-driven attribute, $SPI2_M$ observed an expected increase with the protein addition to FB_M solution. However, in real smoothies, i.e. FB_R compared to $SPI2_R$, this effect was not significant, and thickness values even decreased, suggesting a non-synergistic relationship in which other ingredients interacting with the protein, counteracting the anticipated increase in thickness (Fig. 5d). A similar decrease in smoothie thickness with protein was observed for both $FPC2_R$ and $PPC1_R$. This may be attributed to phase separation or aggregation of plant proteins, potentially linked to competition for water

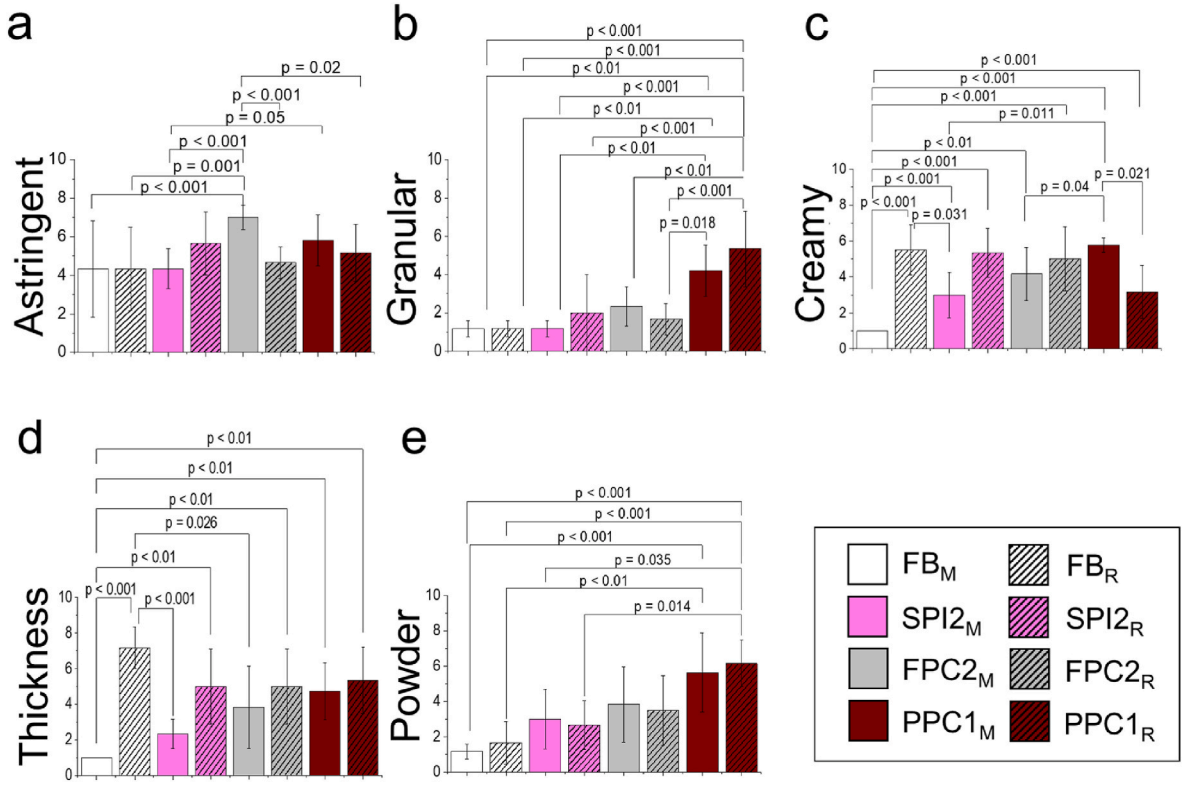
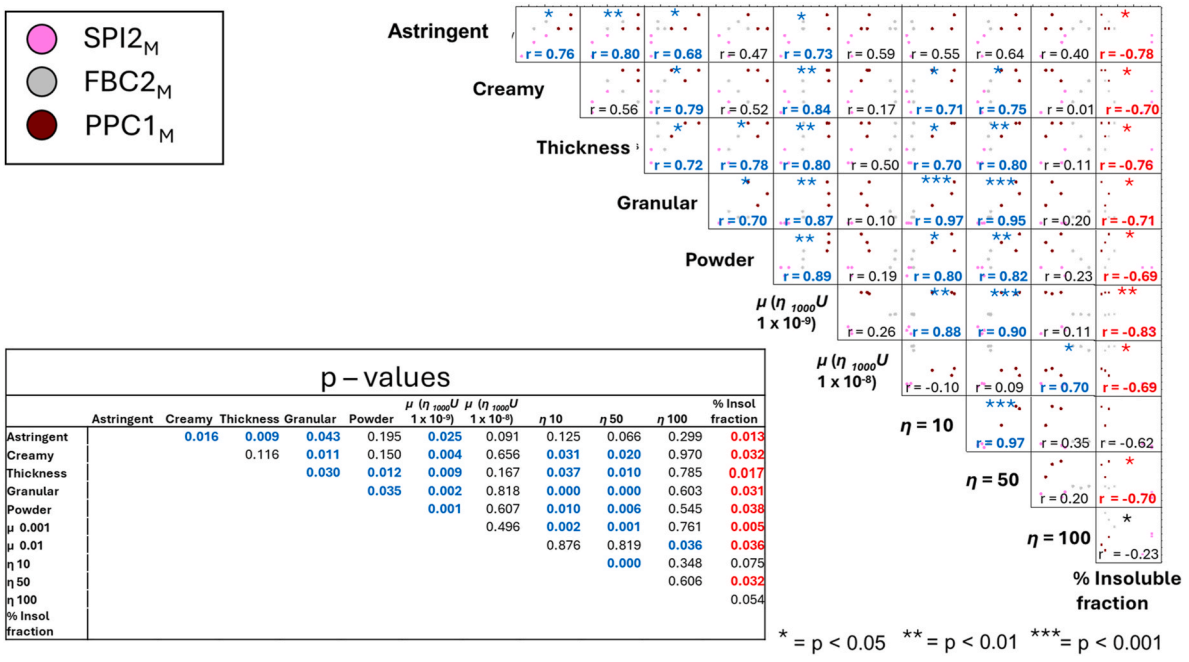


Fig. 5. Mean sensory attributes of model and real plant protein smoothies (5 wt% total protein with 0.3 wt% pectin and 0.8 wt% inulin) at pH 3.8, compared against no-protein model fiber and real smoothie. Error bars represent standard deviations; experiments were conducted with trained panelists (n = 11). FB_M and FB_R are the buffers containing the fiber without any added proteins in model and real smoothies, respectively. Nomenclature of model and real smoothies (S) are: $SPI2_M$ and $SPI2_R$, soy protein isolate added, $FPC2_M$ and $FPC2_R$, fava bean protein concentrate added, $PPC1_M$ and $PPC1_R$, pea protein concentrate added (For compositions refer to Table 1).



p – values

	Astringent	Creamy	Thickness	Granular	Powder	μ (η 1000U 1 x 10-9)	μ (η 1000U 1 x 10-8)	η 10	η 50	η 100	% Insol fraction
Astringent		0.016	0.009	0.043	0.195	0.025	0.091	0.125	0.066	0.299	0.013
Creamy			0.116	0.011	0.150	0.004	0.656	0.031	0.020	0.970	0.032
Thickness				0.030	0.012	0.009	0.167	0.037	0.010	0.785	0.017
Granular					0.035	0.002	0.818	0.000	0.000	0.603	0.031
Powder						0.001	0.607	0.010	0.006	0.545	0.038
μ 0.001							0.496	0.002	0.001	0.761	0.005
μ 0.01								0.876	0.819	0.036	0.036
η 10									0.000	0.348	0.075
η 50										0.606	0.032
η 100											0.054
% Insol fraction											

Fig. 6. Pearson correlation (*r*) of *in vitro* and *in vivo* data for model smoothies containing 5 wt% total plant protein ($SP2_M$, soy protein isolate; FBC_M, fava bean protein concentrate, $PPC1_M$, pea protein concentrate). Samples underwent a bootstrap resampling method to standardize repeats. Spearman's rank was used to obtain *p* values as an inset table translating into *, ** and *** indicating *0.05-0.001* order of significance where a negative correlation is indicated in red and positive correlation in blue.

by the sugars in the fruit base, leading to lower perceived homogeneity (Çakır & Foegeding, 2011). Next incorporation of favabean was assessed *i.e.* $FPC2_M$ and $FPC2_R$. Similar to $SPI2_M$ and $SPI2_R$ smoothies, FPC2 incorporation exhibited low levels of powder and granularity, along with comparable creaminess and thickness. However, $FPC2_M$ exhibited a significantly higher astringency, reaching the highest score of 7, which initially suggests that fava bean protein may have limited usability (Fig. 5a). But notably, when incorporated into a real smoothie ($FPC2_R$), astringency decreased back to baseline FB_R (Fig. 5a), highlighting the ability of other ingredients present in real smoothie which may often reduce its intensity or mask astrignency, an important consideration when testing model solutions. Lastly, PPC1 is evaluated in both model and real smoothies. Similarities in astringency are observed with other proteins; however, in terms of granular and powder, $PPC1_M$ and $PPC1_R$ shows notable increase, achieving the highest scores among the proteins in both formats (Fig. 5b and e). As a result, PPC1 can be presumed be the least favorable protein from a sensory perspective.

3.5. Relating in vitro assessments to in vivo mouthfeel evaluation

Pearson's correlation was conducted to examine the relationship between the *in vitro* characteristics of proteins and their influence on *in vivo sensory* response including data from SPI2, FPC2 and PPC1 utilised proteins. We aimed to identify relationships between viscosity, μ at different regimes scaled to viscosity, %insoluble fraction and the sensory attributes *i.e.* astringency, creamy, thickness, granular and powder.

Astringency stands out as a particularly challenging sensory barrier in the replacement of animal proteins with plant proteins (Sarkar, 2024). In Fig. 6 Astringency demonstrated several significant correlations ($p = 0.01$-0.05) in both *in vitro* and *in vivo* attributes, highlighting its interconnected relationship. Astrignency presented positive correlations with creamy, thickness, granular, boundary friction coefficient ($\eta_{1000}U = 1 \times 10^{-9}$) (r = 0.68–0.80) while negatively correlating to % insoluble fraction ($r = -0.78$) ($p = 0.05$-0.01). Interestingly, creaminess, a sensory attribute influenced by both surface and macroscale interactions in the mouth is perceived more with increasing astringency. Thickness is likely to play the dominant role in the perception of creaminess in plant proteins (Kokini et al., 1977) where the latter attributes both show strong correlation ($r = 0.79$) ($p < 0.05$). We also show astringency was positively correlated with the protein's boundary friction (Fig. 4b), aligning with emerging evidence that increasingly supports this *in vivo* and *in vitro* relationship (Corvera-Paredes et al., 2022; Gamaniel et al., 2024; Rudge et al., 2021; Sarkar & Krop, 2019). Granularity was additionally correlated to astringency perception, this is likely to occur due to salivary-protein interactions leading to aggregation driving such an effect (Vlădescu et al., 2023). Remarkably, % insoluble fraction was seen to be a key indicator of astringency in these three plant proteins with lower levels of %insoluble fraction indicating higher astringency. Recently, it was found when assessing protein subunits, the soluble albumin protein fraction was rated as the most astringent fraction (Lesme et al., 2024) largely associated with protein-protein aggregation and may explain such a relationship in our study. Overall, the extent of %insoluble fraction at acidic pH serves as an underestimated and facile metric of all sensory attributes ($r = -0.69$ - -0.78), friction coefficient ($r = -0.69$ - -0.83) and viscosity (η_{50}) ($r = -70$) response ($p = 0.01$-0.05) (Fig. 6). This relatively accessible and quick method could therefore be useful to evaluate and predict plant protein performance in foods under acidic conditions. This however is likely dependent on protein type and subunit fraction. Additionally, the association with fiber is not fully understood and warrants further research. We next evaluate creamy and thickness attributes (Fig. 5c and d) where an expected positive correlation is observed when compared to η_{10} and η_{50} (r = 0.70–0.80) ($p < 0.05$). However, unexpectedly the attributes were positively correlated to $\eta_{1000}U = 1\ x10^{-9}$. Often an inverse relationship is expected with creamy, but in the case of plant proteins, complex temporal interactions with saliva and oral surfaces may influence sensory perception, resulting in a more intricate relationship. We recommend that future research validate these findings by measuring the frictional behavior of saliva–protein mixtures which needs further investigation.

Granularity displays some of the strongest correlations to *in vitro* data in this study (Fig. 6) ($r = 0.87$-0.97) relating significantly to $\eta_{1000}U = 1 \times 10^{-9}$, η_{10} and η_{50} ($p < 0.001$-0.01) mentioned previously as a result of the formation of large viscous aggregates that also increase friction via jamming of the contact preventing surface motion (Kew et al., 2021). Similarly, the perception of powder aligns with granularity, suggesting they were likely interpreted as related sensory attributes. Finally, a holistic overview is provided in evaluating the relevance of friction ($\eta_{1000}U = 1 \times 10^{-9}$) and viscosity ($\eta_{10}$ and η_{50}) measurements in relation to sensory perception. $\eta_{1000}U = 1 \times 10^{-9}$ is shown to characterize sensory perception excellently, producing significant correlations with all sensory attributes assessed ($r = 0.73$-0.89, $p = 0.05$-0.01). We shows that lower $\eta_{1000}U = 1 \times 10^{-9}$ to be of lower astringency, creamy, thick, granular and powder which may indicate issues where plant proteins are used and able to thicken acidic beverages. Viscosity emerged as a key predictor for several sensory attributes; however, notably, it showed no correlation with astringency. This supports the notion that plant protein-induced astringency is primarily a surface-driven phenomenon, as previously hypothesized (Sarkar, 2024). This distinction helps explain why astringency often arises unexpectedly or overlooked in product development, particularly when plant proteins are used to replicate the creaminess of dairy through thickening alone.

Overall, this data supports the utility of *in vitro* methodology for predicting sensory attributes. However, the study is limited by its focus on acidic pH, a small sample size, and limited repetitions. While trends are clearly demonstrated, future research should assess a broader range of non-legume plant proteins across different model environments.

4. Conclusions

In total, ten alternative proteins spanning across pea, fava, chickpea, red lentil and rapeseed were assessed at pH 3.8 with fiber in smoothies utilizing both model and real food matrices in comparison to whey protein and soy protein. At acidic pH and singly, proteins faced numerous physicochemical challenges including high degree of aggregation, low ζ-potential and significant sedimentation. Plant proteins in combination with pectin and inulin fiber showcased a vast range of shear thinning viscosity profiles spanning across three orders of magnitude dependent on the protein type, in line with the aggregation. As expected, plant proteins also demonstrated diverse frictional dissipation. Significant differences were found in variations of the same protein where, the most lubricating proteins were variants of fava bean protein, pea protein and chick pea protein that outperformed soy protein isolate and whey protein isolate, that are the typical industry used standards. In tactile evaluation, model smoothies effectively replicated the behavior of real smoothies when formulated with plant proteins, highlighting the significant influence of plant proteins on mouthfeel in these products. Regardless of plant protein type astringency, granularity, powder increased when protein was added. Finally a number of strong and significant correlations were found in our study, notably with boundary friction and rheology parameters where we showcase the effectiveness in its ability to predict these mouthfeel attributes. Remarkably, we demonstrate that measuring %insoluble fraction offers a quick, facile metric which significantly correlated to all tactile sensory attributes as well as viscosity and boundary friction coefficient in the Pearson's matrix. Overall we showcase that measuring tribology, rheology and simply assessing %insoluble fraction can be powerful *in vitro* techniques that can directly help predict textural sensory attributes. In addition, our findings validate the use of model smoothies to effectively narrow down a vast range of plant proteins, which can be helpful in fast tracking product development to transition towards more sustainable protein foods with increased food protein security.

CRediT authorship contribution statement

Ben Kew: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xinyi Guo:** Writing – original draft, Formal analysis, Data curation. **Alice Heath:** Project administration, Formal analysis. **Kieran Tuohy:** Writing – review & editing. **Anthony Buckley:** Writing – review & editing. **Anwesha Sarkar:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

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Data availability

Data will be made available on request.

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