Tribological properties and interfacial adsorption behavior of milk protein-based formulations: Roles of whey protein type and thermal processing

Junwei Lian[1], Kun Yang[3*], Jianshe Chen[1,2], and Yang Zhu[1*]

1. Laboratory of Food Oral Processing, School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310018

2. Singapore Institute of Food and Biotechnology Innovation, A*Star, Singapore 138669

3. China College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang, 310018, China

*Corresponding author

Email: zhuyang@mail.zjgsu.edu.cn; yangk@cjlu.edu.cn

Abstract:

Whey protein-associated mouthdrying during oral processing is a driving factor for consumers' dislike of high-protein beverages, however, remains mechanistically underexplored in dairy systems. This study investigated the fundamental mechanisms driving powderiness in whey protein-enriched dairy beverages (8% w/w protein). We conducted a comparative analysis of whey protein concentrate (WPC) versus whey protein isolate (WPI) at various casein-whey ratios (80:20 to 20:80) and evaluated thermal processing effects (63 °C/85 °C for 30 min) from rheological characterization, particle size distribution, tribological properties, and interfacial adsorption kinetics. Results demonstrated that protein composition significantly altered frictional behavior, with WPC-dominated systems (casein-whey protein ratio of 20:80) exhibiting 33.6% higher friction coefficients versus WPI counterparts at 10 mm/s without heating. Thermal treatment at 85 °C induced substantial aggregation in WPI-dominant systems (hydrodynamic diameter increased from 0.150 μm to 0.167 μm) and reduced adsorption capacity by 30.80%, correlating with impaired lubrication. Conversely, WPC maintained structural homogeneity and enhanced interfacial coverage post-heating, preserving lubrication functionality. These findings establish composition-specific thermal processing guidelines for optimizing sensory attributes in high-protein dairy beverages.

Keywords: Whey protein concentrate; Whey protein isolate; QCM-D; Tribology; Lubrication

1. Introduction

High-protein beverages have witnessed a meteoric rise in consumer popularity over the past decade, driven by a confluence of factors including heightened health consciousness, growing interest in fitness and sports nutrition, and the demand for convenient, functional food options (Paterson et al., 2021; Singh et al., 2022). These beverages cater to a diverse consumer base, from athletes seeking muscle repair and growth to individuals aiming for weight management or simply a satiating, nutritious snack (Kew et al., 2021; Leidy et al., 2015; Pasiakos et al., 2013; Veldhorst et al., 2008). Milk proteins, particularly whey and casein, are highly favored ingredients in these formulations due to their excellent nutritional profile, boasting a complete amino acid composition rich in essential and branched-chain amino acids, and high bioavailability (Burd et al., 2009; Singh et al., 2022; Vogel et al., 2021). Whey Protein Concentrate (WPC) and Whey Protein Isolate (WPI) are common whey-derived ingredients, differing primarily in their protein purity and the presence of other milk components like lactose and fat (Fuente et al., 2002; Hernández-Ledesma et al., 2014; Mulet-Cabero et al., 2020).

Despite their nutritional advantages, formulating high-protein beverages with acceptable sensory characteristics, particularly mouthfeel, presents significant challenges. A prevalent sensory defect is "powderiness" or "grittiness," a chalky or sandy sensation in the mouth that severely diminishes consumer acceptance and product appeal (Giles et al., 2024). This undesirable texture is often attributed to the presence of undissolved or aggregated protein particles that are perceived by mechanoreceptors in the oral cavity (Giles et al., 2024; Li et al., 2018). Protein concentration, often reaching 8% (w/w) or higher in high-protein formulations, inherently increases the propensity for such issues due to increased particle interactions and potential for insolubility (Carter et al., 2020; Giles et al., 2024; Graf et al., 2020).

The physicochemical state of proteins in solution, including their size, aggregation state, surface properties, and interactions, is critically influenced by both the intrinsic nature of the protein ingredients and the processing conditions applied, most notably thermal treatment (Lucey, 2017; Zhu et al., 2019). Heat processing, such as pasteurization (e.g., 63 °C for 30 min) or more severe treatments (e.g., 85 °C for 30 min, mimicking aspects of UHT pre-heating or harsher pasteurization), is indispensable for ensuring microbiological safety and extending shelf-life (Arzuaga et al., 2020; Nicolai & Durand, 2013; Zhang et al., 2021). However, these thermal stresses can induce protein denaturation, unfolding, and subsequent aggregation, leading to changes in particle size distribution, viscosity, and interfacial behavior (Corredig et al., 2019; Pan et al., 2022). Whey proteins, particularly β-lactoglobulin, are known for their susceptibility to heat-induced denaturation and aggregation via disulfide bond formation and hydrophobic interactions (Jorgensen et al., 2015; Ren et al., 2024). Caseins, existing predominantly as micelles, are generally more heat-stable but can interact with denatured whey proteins, forming complex co-aggregates (Pan et al., 2022). The extent and nature of these heat-induced changes can vary significantly depending on the specific protein type (e.g., WPC *vs.* WPI, which differ in non-protein components that can affect stability) and the casein-to-whey ratio in the system (Pan et al., 2022; Wijegunawardhana et al., 2025). For instance, WPC, typically produced via ultrafiltration and spray drying, may contain pre-existing aggregates or denatured proteins from its own processing history, which could respond differently to further heating compared to the more purified WPI (Ho et al., 2021; Sun et al., 2016).

The oral perception of whey protein-associated mouthdrying is intimately linked to the tribological properties of the beverage, which describe the friction and lubrication occurring between surfaces in relative motion, in this case, the tongue, palate, and teeth during consumption (Ji et al., 2023; Pradal & Stokes, 2016; Sarkar et al., 2019). Effective lubrication by the fluid film formed in the oral cavity is crucial for a smooth, pleasant mouthfeel. When this lubrication is compromised, often by particles that are too large or that disrupt the lubricating film, sensations like astringency, grittiness, or powderiness can arise (Giles et al., 2024; Li et al., 2018). Therefore, understanding how protein composition and thermal processing affect the lubrication behavior of high-protein solutions is paramount for controlling mouthfeel. Soft tribology, employing compliant surfaces that mimic oral tissues (e.g., PDMS), has emerged as a powerful tool to quantify these lubrication properties under conditions relevant to oral processing (Chen & Stokes, 2012; Wang et al., 2021).

Beyond bulk properties like particle size and viscosity, the interfacial behavior of proteins, including their adsorption onto surfaces and the viscoelastic characteristics of the adsorbed layer, plays a critical role in lubrication (Stokes et al., 2011). QCM-D is a highly sensitive mass-sensing technique capable of real-time, label-free monitoring. This method tracks shifts in resonant frequency and energy dissipation over time. These measurable parameters provide critical insights into the hydration state and characteristic properties of adsorbed protein layers (Glumac et al., 2019; Zembyla et al., 2021). The synergistic application of tribological analysis and QCM-D technology offers profound insights into interfacial protein layer formation (Brown et al., 2025).

While previous studies have investigated individual aspects, such as the effect of heat on whey protein aggregation (Bull et al.,2022; Jorgensen et al., 2015; Ren et al., 2024) or as a control group for the tribological studies of plant protein systems (Brown et al., 2025; Kew et al., 2021), a comprehensive understanding of how the interplay between different whey protein types, casein-whey protein ratios and varying heat treatment collectively impacts the physicochemical, interfacial, and tribological properties for high-protein solutions is still developing. Specifically, the distinct responses of WPC and WPI to thermal processing, given their different purity and pretreatment methods, and how these differences affect lubrication mechanisms, warrant detailed investigation (Brown et al., 2025).

Therefore, this study aimed to systematically investigate the impact of varying casein-to-whey protein ratios (achieved by blending milk protein concentrate powder with either WPC or WPI) and two distinct heat treatments (63 °C and 85 °C for 30 min) on the hydrodynamic diameter, shear viscosity, wettability, interfacial adsorption characteristics (using QCM-D), and soft tribological behavior of 8% (w/w) total protein model solutions. By correlating these multifaceted instrumental measurements, we sought to elucidate the fundamental mechanisms by which protein composition and thermal processing govern lubrication performance. The results of this study will help to promote the elucidation of protein-mediated lubrication mechanisms and the development of innovative formulations of protein-rich foods.

2. Materials and methods

2.1. Materials

The protein materials utilized in this study were obtained from different sources. China Mengniu Dairy Company Limited (Hohhot, China) kindly provided two protein concentrates: Whey Protein Concentrate (WPC) containing 86% protein content and Milk Protein Concentrate Powder (MPC) with a protein concentration of 80.4%. Additionally, Whey Protein Isolate (WPI) with 90% protein purity was commercially acquired from Ueno (USA). All protein samples were employed in their original form without undergoing further purification processes.

2.2. Methods

2.2.1. High protein solutions preparation

Protein solutions were formulated at a constant total protein concentration of 8% (w/w) by blending different proportions of whey and casein proteins. Three distinct casein/whey protein ratios (80:20, 50:50, and 20:80) were achieved through the combination of WPC, WPI and MPC. A detailed description of the samples is present in Table. 1.

Table 1

All the protein powders were dispersed and mixed in ultrapure water and stirred at 40 °C for 4 h. The solution preparation process involved multiple sequential steps. Initial emulsion formation was achieved through high-speed shearing at 12,000 rpm for 2 min, creating a preliminary mixture. This pre-emulsion subsequently underwent high-pressure homogenization using a dynamic microjet system, with two processing cycles at 12,000 bar pressure. Following homogenization, all samples were transferred to a refrigerated environment (4 °C) and maintained for 12 h to facilitate complete hydration. To generate samples containing partially and fully denatured proteins, the solutions underwent thermal treatment after overnight storage. Two distinct heating protocols were applied: exposure to either 63 °C or 85 °C (Pan et al., 2022; Zhang et al., 2021). Each temperature was held constant for a duration of 30 min. All heat-treated samples were then cooled to ambient temperature and stored under refrigeration prior to subsequent analytical procedures.

2.2.2. Contact angle test

Hydrophilicity characterization of high protein solutions was performed through contact angle measurements using a Theta optical tensiometer (Biolin Scientific, Sweden). The sessile drop technique (Mohammadi et al., 2023) was employed to evaluate surface wetting properties on acrylic resin substrates. For measurement preparation, acrylic resin surfaces were horizontally positioned and securely mounted onto glass slides, which were subsequently placed on the instrument stage. A syringe (10 μL, Hamilton, Switzerland) was used to put a fixed volume (4 μL) of liquid sample onto the surface of acrylic resin. When the syringe discharged the liquid and the gun head left the droplet boundary, a camera was used to quickly record the droplet at a frequency of 10 frames/s for 10 seconds. And data acquisition and analysis were conducted using OneAttention software (TGI Technology Co. Ltd.), which obtain the contact angle over time. To minimize the influence of droplet evaporation, all experiments were maintained at a constant temperature of 28 °C. Each test involved triplicate measurements, with each replicate performed at distinct positions to ensure representative sampling.

2.2.3. Particle size

Particle size characterization of the high protein solutions was conducted using a Master sizer 3000 analyzer (Malvern Instruments, Worcester, UK). The instrument parameters were configured with a protein refractive index of 1.47 and an absorption value of 0.001, following established methodology (Kew et al., 2021). Prior to analysis, samples were appropriately diluted using ultrapure water to achieve optimal measurement conditions. For each experimental condition, triplicate measurements were performed to ensure data reliability, with results recorded for both average particle diameter and size distribution profiles.

2.2.4. Shear rheology

Rheological characterization of high protein solutions was performed using a Discovery Hybrid Rheometer-2 (TA Instruments, USA) following established protocols (Van Aken et al., 2007). A cone-plate geometry (40 mm diameter with a 2.017° angle) was employed for viscosity determination at 55 μm operational gap. The experimental temperature was maintained at 15 °C throughout the measurements. Shear rate sweeps were conducted across a range from 0.1 to 1000.0 s^{-1} to evaluate flow properties. To ensure data reproducibility, each measurement was replicated three times.

2.2.5. Soft tribology

Soft friction experiments were carried out using the laboratory's own *in vitro* soft friction apparatus “STAT”. The experimental configuration was implemented according to previously established methodologies (Chen & Stokes, et al., 2012), incorporating operational principles detailed in recent literature (Wang et al., 2021). The apparatus design featured a compliant sensing element comprising three hemispherical PDMS components, simulating the structural characteristics of the hard palate. Additionally, a PDMS-based platform was employed to mimic human tongue. Soft friction experiments were performed at room temperature. Approximately 8 mL of sample was aspirated using a disposable plastic pipette and transferred to the silicone rubber surface in the cuvette. The probe was set to move at a distance of 10 mm and speeds of 0.05, 0.1, 0.3, 1, 3, 6, 10, and 20 mm/s, and the measuring temperature was set to 28 °C. Experimental parameters including force, displacement, velocity, and time were automatically acquired using Exponent 137 software (Stable Microsystems, version 6.1.9.0). Frictional properties were quantified through calculation of the friction coefficient, determined as the ratio between normal force and frictional resistance. To ensure statistical reliability, a minimum of six independent measurements were conducted for every experimental condition for tribological measurement.

2.2.6. Quartz crystal microbalance with dissipation monitoring (QCM-D)

Interfacial adsorption characteristics of high protein solutions were analyzed using QCM-D, following established methodologies (Glumac et al., 2019; Xu et al., 2020).

The QCM-D allowed real-time monitoring of frequency shifts and energy dissipation across multiple overtones, providing comprehensive data regarding adsorption dynamics, deposited mass, and viscoelastic properties of interfacial films (Zembyla et al., 2021). Protein-surface interactions were quantified using QSX301 gold-coated sensors (Biolin Scientific/Q-Sense, Sweden), as previously described (Zou et al., 2018). The gold sensors needed to be clean and free of contamination, and underwent a specific cleaning process before and after the experiment, i.e., soaking in RCA solution (deionized water, concentrated ammonia, and 30% hydrogen peroxide in a 5:1:1 ratio) at 75 °C for 10 min, followed ultrasonic treatment in ultrapure water for 10 min, blowing the surface of the sensors with nitrogen, and drying them in a UV-irradiator for 10 min. The experimental temperature of the quartz crystal microbalance was controlled at 28 ℃, and the injection of protein solution was controlled by a peristaltic pumping system at 100 μL/min flow rate (Kew et al., 2021).

For protein adsorption on the gold sensors, water was passed through in the first stage to maintain a stable baseline, and then the protein solution was passed through and adsorbed on the sensors for 10 min. Finally, water was passed to remove the protein that was not firmly adsorbed, and this phase lasted 5-10 min. All QCM-D data obtained were analyzed using the "Smartfit model" of the Q Sense D find software (Biol in Scientific/Q-Sense, Sweden). Each experimental specimen underwent triplicate analytical determinations to ensure measurement reliability.

2.2.7. Statistical analysis

All experimental findings are reported as mean values accompanied by their corresponding standard deviations, calculated from at least three separate experimental replicates. The distribution characteristics of the obtained data were evaluated through Shapiro-Wilk normality testing. Parametric statistical comparisons were conducted using one-way ANOVA coupled with Tukey's test for datasets satisfying normality requirements. For data sets deviating from normal distribution patterns, appropriate non-parametric analytical techniques were implemented. In examining variable relationships, Pearson correlation coefficients were computed for normally distributed parameters, while Spearman's rank-order correlation analysis was applied to non-parametric data.

3. Results and discussions

3.1. Hydrodynamic diameter

The hydrodynamic diameter (dH) of the prepared samples was tested through dynamic light scattering (DLS), and its particle size distribution under various protein compositions and heat treatments is shown in Fig. 1. It can be seen from the figure that the peak gradually shifts to the right as the increasing of WPC percentage (Fig. 1a), which means the raise of protein particle size in the solution (Table 1). And for different whey proteins, their DLS plots were also different, it was found that if systems contained WPC, it had a much larger particle size than WPI-contained solutions (Fig.1a & c). This is because compared to WPI and casein, WPC has larger particle size (Sun et al., 2016), thus caused the increase of particle size.

Fig. 1.

As shown in Table 2, for WPI-dominant systems, the protein particle size of solutions increased significantly after heating, especially after denaturation (85 °C for 30 min). For example, for WPI-dominated systems (casein-whey protein ratio of 20:80), the hydrodynamic diameter increased from 0.150 ± 0.007 μm to 0.167 ± 0.005 μm. This result was in line with previous studies (Jorgensen et al., 2015; Ren et al., 2024). This phenomenon happened since the polymerisation of milk proteins increase observably during high heating temperature (Wang et al., 2024). However, for WPC, it was found that the heating process decreased the protein particle size (Table. 2), which may be attributed to the fact that the WPC itself has already been aggregated during spray drying and could be depolymerization after heating (Ho et al., 2021; Sun et al., 2016).

Table 2

3.2. Shear viscosity

Fig. 2 shows the shearing rheological property of high protein solutions with various casein to whey protein ratios under different heat treatments. As expected, all samples presented shear thinning under tested shear rate, which is a typical non-Newtonian behaviour (Dickinson, 2001; Fischer et al., 2020; Simmons et al., 2007). Besides, as shown in Fig. 2, the milk protein composition had a more significant effect on its viscosity than heat treatments, especially for samples containing WPC.

Fig. 2.

Since the fluids usually remain briefly in the oral cavity and might be subjected to high intermittent shear stresses when intra-fluid proteins aggregated and merged between the tongue and palatal surfaces, it normally consider the its organoleptic attributes correlate well with the viscosity at 50 s^{-1} shear rate (η_{50}) for food products ranging from Newtonian fluids to oil solutions (Nguyen et al., 2017). Fig. 3 shows the η_{50} of high protein solutions with different milk protein compositions and heat treatments. The observed inverse relationship between viscosity and casein-whey protein ratio (Fig. 3) suggests that reduced viscosity at lower casein proportion likely stems from the comparatively weaker intermolecular forces inherent to whey proteins versus the more robust associative properties of casein micelle (Jorgensen et al., 2015; Solowiej et al., 2014; Warncke et al., 2022). In addition, the viscosity of high protein solutions increased significant after heating, it consistent with former studies (Meletharayil et al., 2015; Warncke et al., 2022). However, for different whey proteins, the influence of heat treatment was different. For sample WPC-20:80, the viscosity raised from 2.6 mPa·s to 2.8 mPa·s, while when WPC replaced by WPI, the viscosity had been increased by a factor of 1.4 (Fig. 3). The observed divergence likely originates from inherent material properties. Unlike WPC, which undergoes spray-drying that stabilizes its components, WPI retains greater levels of heat-labile constituents. Consequently, WPI exhibits heightened susceptibility to thermal denaturation during processing (Warncke et al., 2022; Zhu et al., 2019).

Fig. 3.

3.3. Wettability

The static contact angle was measured by placing the high protein solutions on the surface of the hydrophilic material acrylic resin to compare the hydrophilicity of samples. As can be seen from Fig. 4, the static contact angle for water was ~79.37◦, and the high protein solutions showed significant lower contact angle ($P<0.05$), this indicated that the samples were more hydrophilic. With the decrease of casein-whey protein ratio and heat treatment, the contact angle of high protein solutions raised. All these results indicate that milk protein solutions have a better wetting and lubricating effect than water, which is due to the fact that casein micelles as well as whey proteins have a certain surface energy, which is related to their behaviour and stability at the interface (Effraimopoulou et al., 2024; Michel et al., 1989). In addition, heating and decreasing casein-whey protein ratio had a negative effect on its wetting effect, which suggests that its hydrophobicity can be significantly improved by heat treatment and by lowing casein-whey protein ratio, thus increasing its contact angle (Jafari et al., 2015; Silva & O'Mahony, 2017; White et al., 2013).

Fig. 4.

3.4. QCM-D

3.4.1. Adsorption characteristics

To gain deeper insights into interfacial adsorption characteristics of high protein solutions, QCM-D was employed to quantify both hydrated mass adsorption and viscoelastic properties of protein layers. For QCM-D, the frequency and dissipation displacements over time was recorded to monitor the adsorption of proteins on surfaces (Li et al.,2021). The adsorption and elution curves of high protein solutions under different protein compositions and heat treatments were presented in Fig. 5.

The measurement commenced with establishing a stable baseline through water flow. Upon introduction of high protein solutions (8% w/w), significant shifts in resonance parameters were observed, characterized by a substantial reduction in frequency (F) and corresponding elevation in dissipation (D) values. These pronounced changes in QCM-D signals demonstrated the formation of a viscoelastic interfacial layer, resulting from protein adsorption onto the sensor surface (Feng et al., 2024). When the protein adsorption-desorption rate reached equilibrium, it meant that the proteins on the sensor had formed a stable layer, which was shown in the QCM-D data as the basically constant frequency and dissipation was got. Finally, when the gold chip was eluted by passing water, the adsorption curves of high protein solutions changed significantly, with a rapid raise in frequency and a rapid decrease in dissipation, indicating proteins that were not firmly adsorbed on the chip surface were removed. With the removal of most of the unfastened proteins, the frequency and dissipation gradually levelled off until there was basically no change (Feng et al., 2024; Zou et al., 2018).

Fig. 5.

The hydrated mass of adsorbed protein layers on QCM-D gold sensors was quantitatively determined using the 'Smartfit' analytical model. Quantitative results demonstrating the hydration characteristics of high protein solutions following elution processes are presented in Table 3. As shown in Fig. 5 and Table 3, the frequency signal of the protein on the surface of the gold chip decreases gradually, and the decrease in frequency signals was proportional to the increase in protein adsorption. For example, when bovine serum protein was adsorbed on the gold chip, the decrease of the frequency signal indicated that the adsorption amount was increasing (Stadler et al., 2003). When increasing the amount of whey protein in the system, the influence of heat treatment on high protein solution for WPI and WPC showed different. For WPC, the adsorption amount increased significantly after heating (Table 3), the gradual increase in adsorption may be due to its dissociation upon heating, which leading to the release of hydrophobic groups, thus enhance its adhesion to the gold chip surface (Kim et al., 2007; Ren et al., 2024) . However, the opposite effect was observed with WPI, where adsorption decreased significantly ($P < 0.05$) when got the denaturation temperature. The observed reduction in adsorption capacity could be due to the agglomeration of WPI upon heating, which inhibits surface deposition on the gold sensor and consequently diminishes interfacial binding (Ren et al., 2024; van Lieshout et al., 2020; Zembyla et al., 2021).

Table 3

3.4.2. Differences in properties of hydrated membrane layers

The main parameters measured by the QCM-D technique were the changes in frequency (Δf) and dissipation factor (ΔD), where Δf correlated not only with small mass changes on the sensor, but also with the density, viscosity, etc., and ΔD reflected the change in energy loss in the system on the sensor, whereas the parameter -ΔD/Δf allowed for a more comprehensive understanding about the properties of formed film, with higher -ΔD/Δf correlating with viscous thin films and smaller -ΔD/Δf often corresponding to more rigid films (Ash et al., 2014; Carpenter et al., 2021; Madsen et al., 2016; Xu et al., 2020). The ratio of dissipation and frequency was shown in Fig. 6. The trend of the graph showed the rapidly increasing curve, indicating the passage of the high protein solution leaded the forming of membrane layer, which was loose and viscous at this stage, this might related to the protein arrangement (Xu et al., 2020). As the percentage of whey protein increased, -ΔD/Δf gradually decreased (Fig.6 a & c), suggesting that the protein membrane layer was less viscoelastic (Ash et al., 2014; Kew et al., 2021). When casein-whey protein ratio remained constant, the influence of heating on high protein solutions for WPC and WPI was different (Fig.6 b & d). For WPC, the change in -ΔD/Δf with increasing temperature was not significant, indicating heating had no influence on the protein adsorption layer. While for WPI, -ΔD/Δf increased significantly, especially when reached the denaturation temperature, suggesting a change in its viscoelasticity (Zhou et al., 2023).

Fig. 6.

To check whether any structural rearrangement of proteins bound to the surface occurred during adsorption, ΔD-Δf correlation plots were drawn (Supporting information Fig. S1) with subsequent slope analysis. These plots provide critical information about layer rigidity, where minimal slope values indicate formation of rigid interfacial films, corresponding to limited energy dissipation relative to frequency changes. Conversely, steeper gradients suggest the development of soft, viscoelastic layers (Teo et al., 2016). With the decrease of casein-whey protein ratio, the slope decreased, while all protein solutions exhibit a simple linear relationship, except for the high protein solution at ratio 80:20 for casein to whey protein (Fig. S1). For casein-rich protein solutions, the adsorption process exhibited distinct biphasic behavior in the ΔD/Δf profile. The initial adsorption phase demonstrated minimal slope values, indicating rigid layer formation, followed by a subsequent transition phase characterized by substantial enhancement in the dissipation-to-frequency ratio. The observed slope variation indicates significant structural reorganization within the adsorbed protein layer throughout the deposition process, ultimately generating a more hydrated and compliant interfacial film (Dolatshahi-Pirouz et al., 2008). Casein incorporation modifies adsorption characteristics, initially promoting the development of compact, rigid surface layers. Subsequent molecular rearrangement, involving protein unfolding and water incorporation, facilitates the transition to less densely packed, viscoelastic interfacial structures.

3.5. Soft tribology

Soft friction experiments were performed to measure the friction coefficients of high protein solutions through Soft TA-Tribometer (STAT) to understand the different friction behaviours induced by protein composition and heat treatment. The tribological behavior typically exhibits three distinct lubrication regimes: boundary, mixed, and hydrodynamic phases (Wang et al., 2021), which depended on the lubricant involvement in the surface contact region. Fig. 7 showed the friction behaviours of all samples, and two lubrication states (boundary stage and mixed stage) can be identified. At boundary state showed an almost velocity independent friction coefficient since the two contacting surfaces did not separate from each other in this stage. The shift between lubrication regimes, such as from boundary to mixed conditions, occurs with progressive enhancement of entrainment speed. Within the mixed lubrication phase, viscous interactions create discontinuous pressurized fluid layers that partially disrupt surface contact (Prakash et al., 2013; Sarkar et al., 2019; Stokes et al., 2013), consequently diminishing interfacial friction compared to the boundary lubrication stage.

Fig. 7.

The lubrication effect increased with increasing percentage of WPI (Fig. 7c), which suggested that bonding occurs with the addition of WPI and rolling friction took place, which reduced the coefficient of friction and increased the lubrication effect. The results similar to previous research that increased dimensions of whey protein aggregates can significantly improve tribological performance (Chojnicka et al., 2008). However the friction coefficient raised as the percentage of WPC increased (Fig.7a), suggesting that the raise of protein particle size (Table.1) reduced the lubrication effect of high protein solutions (Warncke et al., 2022). And WPC-dominated systems (casein-whey protein ratio of 20:80) showed 33.6% higher friction coefficients versus WPI counterparts at 10 mm/s before heating. The dynamic light scattering (DLS) analysis revealed that samples with elevated WPC content exhibited greater particle dimensions (Fig. 1). This phenomenon could be attributed to the existence of large protein aggregates, which tend to exhibit particulate rather than polymeric interfacial behavior. In opposite, smaller protein complexes appear to demonstrate enhanced entrapment efficiency, potentially explaining their superior lubrication properties (Liu et al., 2016).

Fig. 7b & d show the friction curves of high protein solutions under different heat treatments. It was found that there were no obvious changes ($P > 0.05$) in the friction curves of heat treatment temperatures for high protein solutions consisting of WPC, meaning thermal treatment appeared to exert negligible influence on the tribological properties of samples. On the other hand, unlike WPC, heating influenced the lubrication of samples containing WPI, and it was found that the friction coefficient raised obviously, especially when denaturation temperature reached (Fig. 7d). WPI undergone denaturation at high temperatures and changed from small particles to large particles (Zhu et al., 2019), which reduced its lubrication effect. Besides, the enhanced concentration of large WPI particles potentially induces aggregation phenomena within the contact region. This aggregation tendency promotes discrete particulate interactions rather than uniform polymeric behavior, potentially causing obstruction in the interfacial zone. Such clogging effects could limit relative motion between surfaces, consequently causing an increase in the coefficient of friction (Sarkar et al., 2017).

3.6. Correlations between various instrumental characteristics

Establishing relationships between tribological behavior and physicochemical attributes offers valuable mechanistic understanding, facilitating the identification and optimization of specific protein fractions while elucidating their structural and functional modifications. Therefore, we evaluated the data from the various instruments used to high protein solutions in this study with the aim of determining the intercorrelation among various physicochemical parameters, including hydrodynamic size, hydrated mass, viscoelasticity, and frictional coefficient (μ) (Fig. 8a). Due to the difference between WPC and WPI, it was clear that the friction characteristics as well as the physical properties were significantly different.

Fig. 8.

The hydration mass measured by QCM-D was positively correlated with the mixing state μ under all conditions ($r = 0.598$). A significant positive correlation ($r = 0.67$) was observed between particle dimensions and their associated hydrated mass. This relationship suggests that macromolecular complexes with greater hydrodynamic size and higher water-binding capacity tend to exhibit particulate characteristics, potentially leading to interfacial obstruction and consequent raising frictional forces (Dai et al., 2016). The negative correlation between hydrodynamic diameter, boundary layer friction, mixed layer friction and -ΔD/Δf suggested that particle size as well as friction magnitude affected changes in protein viscoelasticity (Fig. 8b), i.e., proteins exhibiting greater hydrodynamic dimensions tend to assemble into densely packed, rigid interfacial structures, whereas proteins with a smaller dH form a more viscoelastic layer (Kew et al., 2021; Tellechea et al., 2009). Subsequent analysis demonstrated a significant correlation ($P < 0.01$) between particle dimensions in solution and frictional properties under mixed range, indicating that increased hydrodynamic size correlates with elevated friction coefficients.

The investigation of thermal processing parameters and milk protein composition revealed significant impacts on lubrication characteristics in high protein systems. Alterations in protein formulation influenced colloidal dimensions, rheological properties, and surface adsorption capacity, consequently modifying the solution's tribological behavior. Fig. 9 illustrates distinct frictional patterns among casein, WPI, and WPC, primarily attributed to variations in protein aggregate size. Progressive incorporation of whey protein components resulted in changes of particle dimensions at the interface (Fig. 9a-c), with WPC demonstrating substantially larger aggregates compared to casein and WPI. Interestingly, while WPC-enriched systems exhibited enhanced protein adsorption, this was accompanied by reduced lubrication efficiency. This phenomenon likely stems from persistent interfacial interactions between macromolecular protein complexes and contact surfaces, as depicted in Fig. 9b. Nevertheless, the minimal variation in particle dimensions following WPI replacing corresponded with insignificant alterations in frictional behavior.

Fig. 9.

In addition, casein, WPI and WPC also showed different degrees of changes before and after heat treatment. For the WPC, the viscosity and protein particle size of the high protein system was basically stable before and after heat treatment, but its protein adsorption capacity increased, which led to the retention of protein particles on the contact surfaces, thus slightly improve its lubrication characteristics (Fig. 9e). However, for WPI, the viscosity and protein particle size of the solution increased significantly after heat treatment, and its protein adsorption capacity was reduced, which resulted in the reduction of the lubrication characteristics. For high protein solutions, the formation of a single chain-structured layer might be the reason for good lubrication property, thus when there are too many particles exist, the inter-particles are piled up and extruded, and they are no longer well delaminated but embedded in each other, which in turn limits the rolling ability (Fig. 9f). At the same time, excessive particle extrusion may also cause different degrees of deformation of the substrate and particles, among others, leading the increasing of friction coefficient (Douaire et al., 2014).

4. Conclusion

This study elucidates the fundamental mechanisms governing powderiness formation in high-protein solutions through systematic manipulation of protein composition (WPC *vs.* WPI) and thermal processing. Our findings demonstrate that milk protein ratios directly modulate post-heat treatment physicochemical properties, including protein aggregate size, viscosity, and interfacial adsorption capacity, which collectively determine lubrication behavior. Crucially, compositional selection dictates distinct processing requirements: WPI-enriched solutions showed substantial heat-triggered viscosity/aggregate growth coupled with reduced adsorption capacity. This impairs lubricating properties, necessitating alternative stabilization strategies (e.g., selective emulsifiers or non-thermal pasteurization) to achieve desirable sensory smoothness. While WPC-dominant systems (casein-whey protein ratio of 20:80) exhibited minimal heat-induced changes in viscosity or particle size, but significantly enhanced protein adsorption. This promotes interfacial film retention during oral processing, enabling manufacturers to prioritize thermal efficiency without compromising lubricity. These mechanistic insights provide a predictive framework for optimizing protein beverage processing. By strategically balancing composition and thermal inputs, manufacturers can proactively suppress powderiness defects and engineer products with target mouthfeel characteristics.

Acknowledgements

The research team extends sincere gratitude to all participants involved in this study. Financial support for this work was provided through multiple funding sources:

the National Natural Science Foundation of China (Project Code: 32001833 and 32101931), Chinese Institute of Food Science and Technology Food Science and Technology Fund- Mengniu Nutrition and Health Science and Technology Young Scholar Fund (2023-M02), the Natural Science Foundation of Zhejiang Province (Grant No. LQ21C200005), and Zhejiang Gongshang University Provincial University Basic Research Business Funding Project (Grant No. QRK22007). Thanks Dr. Boualegue Amir for assisting with text editing.

Author Contributions

Junwei Lian: Formal analysis, Investigation, Validation, Writing – original draft; Kun Yang: Funding acquisition, Writing – review & editing; Jianshe Chen: Supervision, Writing – review & editing; Yang Zhu: Conceptualisation, Funding acquisition, Experimental design, Writing – review & editing.

Declaration of competing interest

The researchers confirm the absence of any potential conflicts that might affect this investigation. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work.

References

Arzuaga, M. R., Bosch, A., Añón, M. C., & Abraham, A. G. (2020). Heat induced conformational changes of whey proteins in model infant formulae: Effect of casein and inulin. *International Dairy Journal*, *105*, 104695. http://dx.doi.org/10.1016/j.idairyj.2020.104695

Ash, A., Burnett, G. R., Parker, R., Ridout, M. J., Rigby, N. M., & Wilde, P. J. (2014). Structural characterisation of parotid and whole mouth salivary pellicles adsorbed onto DPI and QCMD hydroxyapatite sensors. *Colloids and Surfaces B: Biointerfaces*, *116*, 603-611. http://dx.doi.org/10.1016/j.colsurfb.2013.10.024

Brown, F., Soltanahmadi, S., Mackie, A. R., He, Q., Pfeifer, J., & Sarkar, A. (2025). Comparing frictional behaviour of plant and dairy proteins: Case study on high protein concentration. *Food Research International*, *209*, 116322. https://doi.org/10.1016/j.foodres.2025.116322

Bull, S. P., Khutoryanskiy, V. V., Parker, J. K., Faka, M., & Methven, L. (2022). Oral retention of thermally denatured whey protein: *In vivo* measurement and structural observations by CD and NMR. *Food Chemistry*, *374*, 131650. https://doi.org/10.1016/j.foodchem.2021.131650

Burd, N. A., Tang, J. E., Moore, D. R., & Phillips, S. M. (2009). Exercise training and protein metabolism: Influences of contraction, protein intake, and sex-based differences. *Journal of Applied Physiology*, *106*(5), 1692-1701. https://doi.org/10.1152/japplphysiol.91351.2008

Carpenter, J., Wang, Y., Gupta, R., Li, Y. L., Haridass, P., Subramani, D. B., Reidel, B., Morton, L., Ridley, C., O'Neal, W. K., Buisine, M. P., Ehre, C., Thornton, D. J., & Kesimer, M. (2021). Assembly and organization of the N-terminal region of mucin MUC5AC: Indications for structural and functional distinction from MUC5B. *Proceedings of the National Academy of Sciences*, *118*(39), e2104490118. https://doi.org/10.1073/pnas.2104490118

Carter, B. G., Foegeding, E. A., & Drake, M. A. (2020). Invited review: Astringency in whey protein beverages. *Journal of Dairy Science*, *103*(7), 5793–5804. https://doi.org/10.3168/jds.2020-18303

Chen, J., & Stokes, J. R. (2012). Rheology and tribology: Two distinctive regimes of food texture sensation. *Trends in Food Science & Technology*, *25(1)*, 4-12. https://doi.org/10.1016/j.tifs.2011.11.006

Chojnicka, A., de Jong, S., de Kruif, C. G., & Visschers, R. W. (2008). Lubrication properties of protein aggregate dispersions in a soft contact. *Journal of Agricultural and Food Chemistry*, *56(4)*, 1274-1282. https://doi.org/10.1021/jf0720988

Corredig, M., Nair, P. K., Li, Y., Eshpari, H., & Zhao, Z. T. (2019). Invited review: Understanding the behavior of caseins in milk concentrates. *Journal of Dairy Science*, *102*(6), 4772–4782. https://doi.org/10.3168/jds.2018-15943

Dai, B., Yang, J., & Zhou, C. (2016). Observed effects of interparticle friction and particle size on shear behavior of granular materials. *International Journal of Geomechanics*, *16*(1), 04015011. https://doi.org/10.1061/(ASCE)GM.1943-5622.0000520

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces B: Biointerfaces*, *20*(3), 197-210. https://doi.org/10.1016/S0927-7765(00)00204-6

Dolatshahi-Pirouz, A., Rechendorff, K., Hovgaard, M. B., Foss, M., Chevallier, J., & Besenbacher, F. (2008). Bovine serum albumin adsorption on nano-rough platinum surfaces studied by QCM-D. *Colloids and Surfaces B: Biointerfaces*, *66*(1), 53–59. https://doi.org/doi:10.1016/j.colsurfb.2008.05.010

Douaire, M., Stephenson, T., & Norton, I. T. (2014). Soft tribology of oil-continuous emulsions. *Journal of Food Engineering*, *139*, 24-30. https://doi.org/10.1016/j.jfoodeng.2014.04.007

Effraimopoulou, E., Kalmár, J., Paul, G., Marchese, L., Ioannou, D., Paraskevopoulou, P., & Gurikov, P. (2024). Whey protein isolate-based aerogels with improved hydration properties for food packaging applications. *ACS Applied Nano Materials*, *7*(1), 618–627. https://doi.org/10.1021/acsanm.3c04784

Feng, G., Wang, G., Li, T, Han, C., Han, K., Guo, J., Wan, Z., & Yang, X. (2024). Phosphatidylcholine surface hydration-dependent adsorption to mucin enhances intestinal mucus barrier function. *Langmuir*, *40*(36), 18977–18987. https://doi.org/10.1021/acs.langmuir.4c01666

Fischer, N., Kieferle, I., Kulozik, U., & Germann, N. (2020). Three-dimensional numerical investigation of the displacement of shear-thinning milk protein concentrates in spacer-filled channel. *Journal of Food Engineering*, *296*, 110459. https://doi.org/10.1016/j.jfoodeng.2020.110459

Fuente, M. A. D. L., Hemar, Y., Tamehana, M., Munro, P. A., & Singh, H. (2002). Process-induced changes in whey proteins during the manufacture of whey protein concentrates. *International Dairy Journal*, *12(4)*, 361-369. https://doi.org/10.1016/S0958-6946(02)00031-6

Giles, H., Bull, S. P., Lignou, S., Gallagher, J., Faka, M., & Methven, L. (2024). A narrative review investigating the potential effect of lubrication as a mitigation strategy for whey protein-associated mouthdrying. *Food Chemistry*, *436*, 137603. https://doi.org/10.1016/j.foodchem.2023.137603

Glumac, M., Ritzoulis, C., & Chen, J. (2019). Surface properties of adsorbed salivary components at a solid hydrophobic surface using a quartz crystal microbalance with dissipation (QCM-D). *Food Hydrocolloids*, *97*, 105195. https://doi.org/10.1016/j.foodhyd.2019.105195

Graf, B., Protte, K., Weiss, J., & Hinrichs, J. (2020). Concentrated whey as protein source for thermally stabilized whey protein-pectin complexes. *Journal of Food Engineering*, *269*, 109760. https://doi.org/10.1016/j.jfoodeng.2019.109760

Hernández-Ledesma, B., García-Nebot, M. J., Fernandez-Tomé, S., Amigo, L., & Recio, I. (2014). Dairy protein hydrolysates: Peptides for health benefits. *International Dairy Journal*, *38*(2), 82-100. https://doi.org/10.1016/j.idairyj.2013.11.004

Ho, T. M., Zhu, J. L., Bansal, N., Boyce, M. C., & Le, T. T. (2021). Effect of pH and heat treatment on physicochemical and functional properties of spray-dried whey protein concentrate powder. *International Dairy Journal*, *119*, 105063. https://doi.org/10.1016/j.idairyj.2021.105063

Jafari, S. M., Khanzadi, M., Mirzaei, H., Dehnad, D., Chegini, F. K., & Maghsoudlou, Y. (2015). Hydrophobicity, thermal and micro-structural properties of whey protein concentrate-pullulan–beeswax films. *International Journal of Biological Macromolecules*, *80*, 506-511. https://doi.org/10.1016/j.ijbiomac.2015.07.017

Ji, L., Otter, D. D., Cornacchia, L., Sala, G., & Scholten, E. (2023). Role of polysaccharides in tribological and sensory properties of model dairy beverages. *Food Hydrocolloids*, *134*, 108065. https://doi.org/10.1016/j.foodhyd.2022.108065

Jorgensen, C. E., Abrahamsen, R. K., Rukke, E. O., Johansen, A. G., Schüller, R. B., & Skeie, S. B. (2015). Improving the structure and rheology of high protein, low-fat yoghurt with undenatured whey proteins. *International Dairy Journal*, *47*, 6-18. https://doi.org/10.1016/j.idairyj.2015.02.002

Kew, B., Holmes, M., Stieger, M., & Sarkar, A. (2021). Oral tribology, adsorption and rheology of alternative food proteins. *Food Hydrocolloids*, *116*, 106636. https://doi.org/10.1016/j.foodhyd.2021.106636

Kim, S. B., Seo, I. S., Khan, M. A., Ki, K. S., Lee, W. S., Lee, H. J., Shin, H. S., & Kim, H. S. (2007). Enzymatic Hydrolysis of Heated Whey: Iron-Binding Ability of Peptides and Antigenic Protein Fractions. *Journal of Dairy Science*, *90*(9), 4033-4042. https://doi.org/10.3168/jds.2007-0228

Leidy, H. J., Clifton, P. M., Astrup, A., Wycherley, T. P., Westerterp-Plantenga, M. S., Luscombe-Marsh, N. D., Woods, S. C., & Mattes, R. D. (2015). The role of protein in weight loss and maintenance. *American Journal of Clinical Nutrition*, *101(6)*, 1320s-1329s. https://doi.org/10.3945/ajcn.114.084038

Li, H., Wu, J., Doost, A. S., Su, J., & Van der Meeren, P. (2021). Electrostatic interaction between whey proteins and low methoxy pectin studied by quartz crystal microbalance with dissipation monitoring. *Food Hydrocolloids*, *113*, 106489. https://doi.org/10.1016/j.foodhyd.2020.106489

Li, Y., Joyner, H. S., Carter, B. G., & Drake, M. A. (2018). Effects of fat content, pasteurization method, homogenization pressure, and storage time on the mechanical and sensory properties of bovine milk. *Journal of Dairy Science*, *101*(4), 1-15. https://doi.org/10.3168/jds.2017-13568

Liu, K., Stieger, M., van der Linden, E., & van de Velde, F. (2016). Effect of microparticulated whey protein on sensory properties of liquid and semi-solid model foods. *Food Hydrocolloids*, *60*, 186-198. http://dx.doi.org/10.1016/j.foodhyd.2016.03.036

Lucey, J. A. (2017). Formation, structural properties, and rheology of acid-coagulated milk gels. *Cheese ： Chemistry, Physics and Microbiology*, 179-197. http://dx.doi.org/10.1016/B978-0-12-417012-4.00007-7

Madsen, J. B., Sotres, J., Pakkanen, K. I., Efler, P., Svensson, B., Abou Hachem, M., Arnebrant, T., & Lee, S. (2016). Structural and mechanical properties of thin films of bovine submaxillary mucin versus porcine gastric mucin on a hydrophobic surface in aqueous solutions. *Langmuir*, *32*(38), 9687-9696. https://doi.org/10.1021/acs.langmuir.6b02057

Meletharayil, G. H., Patel, H. A., & Huppertz, T. (2015). Rheological properties and microstructure of high protein acid gels prepared from reconstituted milk protein concentrate powders of different protein contents. *International Dairy Journal*, *47*, 64–71. https://doi.org/10.1016/j.idairyj.2015.02.005

Michel, B., Marcel, B., & Paul, P. (1989). Estimation of casein micelles' surface energy by means of contact angle measurements. *Journal of Dairy Research*, *56*(2), 223–234. https://doi.org/10.1017/S0022029900026443

Mohammadi, A., Kashi, P. A., Kashiri, M., Bagheri, A., Chen, J. S., Ettelaie, R., Jager, H., & Shahbazi, M. (2023). Self-assembly of plant polyphenols-grafted soy proteins to manufacture a highly stable antioxidative Pickering emulsion gel for direct-ink-write 3D printing. *Food Hydrocolloids*, *142*, 108851. https://doi.org/10.1016/j.foodhyd.2023.108851

Mulet-Cabero, A. I., Torcello-Gómez, A., Saha, S., Mackie, A. R., Wilde, P. J., & Brodkorb, A. (2020). Impact of caseins and whey proteins ratio and lipid content on *in vitro* digestion and *ex vivo* absorption. *Food Chemistry*, *319*, 126514. https://doi.org/10.1016/j.foodchem.2020.126514

Nguyen, P. T. M., Kravchuk, O., Bhandari, B., & Prakash, S. (2017). Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids*, *72*, 90-104. https://doi.org/10.1016/j.foodhyd.2017.05.035

Nicolai, T., & Durand, D. (2013). Controlled food protein aggregation for new functionality. *Current Opinion in Colloid & Interface Science*, *18*(4), 249-256. https://doi.org/10.1016/j.cocis.2013.03.001

Pan, Z., Ye, A. Q., Dave, A., Fraser, K., & Singh, H. (2022). Kinetics of heat-induced interactions among whey proteins and casein micelles in sheep skim milk and aggregation of the casein micelles. *Journal of Dairy Science*, *105(5)*, 3871-3882. https://doi.org/10.3168/jds.2021-21444

Pasiakos, S. M., Cao, J. J., Margolis, L. M., Sauter, E. R., Whigham, L. D., McClung, J. P., Rood, J. C., Carbone, J. W., Combs, G. F., & Young, A. J. (2013). Effects of high-protein diets on fat-free mass and muscle protein synthesis following weight loss: A randomized controlled trial. *The FASEB Journal*, *27*(9), 3837-3847. https://doi.org/10.1096/fj.13-230227

Paterson, D. C., Ramage, K., Moore, S. A., Riazi, N., Tremblay, M. S., & Faulkner, G. (2021). Exploring the impact of COVID-19 on the movement behaviors of children and youth: A scoping review of evidence after the first year. *Journal of Sport and Health Science*, *10*(6), 675-689. https://doi.org/10.1016/j.jshs.2021.07.001

Pradal, C., & Stokes, J. R. (2016). Oral Tribology: Bridging the gap between physical measurements and sensory experience. *Current Opinion in Food Science*, *9*, 34-41. https://doi.org/10.1016/j.cofs.2016.04.008

Prakash, S., Tan, D. D. Y., & Chen, J. (2013). Applications of tribology in studying food oral processing and texture perception. *Food Research International*, *54*(2), 1627-1635. https://doi.org/10.1016/j.foodres.2013.10.010

Ren, Q., Boiani, M., He, T., Wichers, H. J., & Hettinga, K. A. (2024). Heating affects protein digestion of skimmed goat milk proteins with different casein:whey ratios under simulated infant conditions. *Food Hydrocolloids*, *148*, 109452.

https://doi.org/10.1016/j.foodhyd.2023.109452

Sarkar, A., Andablo-Reyes, E., Bryant, M., Dowson, D., & Neville, A. (2019). Lubrication of soft oral surfaces. *Current Opinion in Colloid & Interface Science*, *39*, 61-75. https://doi.org/10.1016/j.cocis.2019.01.008

Sarkar, A., Kanti, F., Gulotta, A., Murray, B. S., & Zhang, S. Y. (2017). Aqueous Lubrication, Structure and Rheological Properties of Whey Protein Microgel Particles. Langmuir, 33(51), 14699-14708.

Silva, J. V. C., & O'Mahony, J. A. (2017). Flowability and wetting behaviour of milk protein ingredients as influenced by powder composition, particle size and microstructure. *International Journal of Dairy Technology*, *70*(2), 277-286. https://doi.org/10.1111/1471-0307.12368

Simmons, M. J. H., Jayaraman, P., & Fryer, P. J. (2007). The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. *Journal of Food Engineering*, *79*(2), 517-528. https://doi.org/10.1016/j.jfoodeng.2006.02.013

Singh, R., Rathod, G., Meletharayil, G. H., Kapoor, R., Sankarlal, V. M., & Amamcharla, J. K. (2022). *Invited review*: Shelf-stable dairy protein beverages- Scientific and technological aspects. *Journal of Dairy Science*, *105(12)*, 9327-9346. https://doi.org/10.3168/jds.2022-22208

Solowiej, B., Cheung, I. W. Y., & Li-Chan, E. C. Y. (2014). Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. *International Dairy Journal*, *37(2)*, 87-94.

https://doi.org/10.1016/j.idairyj.2014.03.003

Stadler, H., Mondon, M., & Ziegler, C. (2003). Protein adsorption on surfaces: Dynamic contact-angle (DCA) and quartz-crystal microbalance (QCM) measurements. *Analytical and Bioanalytical Chemistry*, *375*(1), 53-61. https://doi.org/10.1007/s00216-002-1664-5

Stokes, J. R., Boehm, M. W., & Baier, S. K. (2013). Oral processing, texture and mouthfeel: From rheology to tribology and beyond. *Current Opinion in Colloid & Interface Science*, *18*(4), 349-359. https://doi.org/10.1016/j.cocis.2013.04.010

Stokes, J. R., Macakova, L., Chojnicka-Paszun, A., de Kruif, C. G., & de Jongh, H. H. J. (2011). Lubrication, adsorption, and rheology of aqueous polysaccharide solutions. *Langmuir*, *27*(7), 3474-3484. https://doi.org/10.1021/la104040d

Sun, C., Liu, R., Wu, T., Liang, B., Shi, C., Cong, X., Hou, T., & Zhang, M. (2015). Combined superfine grinding and heat-shearing treatment for the microparticulation of whey proteins. *Food Bioprocess Technology*, *9*, 378-386. https://doi.org/10.1007/s11947-015-1629-2

Tellechea, E., Johannsmann, D., Steinmetz, N. F., Richter, R. P., & Reviakine, I. (2009). Model-independent analysis of QCM data on colloidal particle adsorption. *American Chemical Society*, *25*(9), 5177-5184. https://doi.org/10.1021/la803912p

Teo, A., Dimartino, S., Lee, S. J., Goh, K. K. T., Wen, J. Y., Oey, I., Ko, S., & Kwak, H. S. (2016). Interfacial structures of whey protein isolate (WPI) and lactoferrin on hydrophobic surfaces in a model system monitored by quartz crystal microbalance with dissipation (QCM-D) and their formation on nanoemulsions. *Food*

Hydrocolloids, *56*, 150-160. https://doi.org/10.1016/j.foodhyd.2015.12.002

van Lieshout, G. A. A., Lambers, T. T., Bragt, M. C. E., & Hettinga, K. A. (2020). How processing may affect milk protein digestion and overall physiological outcomes: A systematic review. *Critical Reviews in Food Science and Nutrition*, *60*(14), 2422-2445. https://doi.org/10.1080/10408398.2019.1646703

Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiology & Behavior*, *94(2)*, 300-307. https://doi.org/10.1016/j.physbeh.2008.01.003

Vogel, K. I. I. I., Carter, B. G., Cheng, N., Barbano, D. M., & Drake, M. A. (2021). Ready-to-drink protein beverages: Effects of milk protein concentration and type on flavor. *Journal of Dairy Science*, *104(10)*, 10640-10653. https://doi.org/10.3168/jds.2021-20522

Wang, D., Wang, Y., Bao, A., Xing, M., Ji, M., Li, L., Song, G., Yuan, T., & Gong, J. (2024). Effects of thermal treatment on the formation and properties of whey protein isolate/whey protein hydrolysate-sodium hyaluronate complexes. *Food Research International*, *190*, 114608. https://doi.org/10.1016/j.foodres.2024.114608

Wang, Q., Wang, X., & Chen, J. (2021). A new design of soft texture analyzer tribometer (STAT) for *in vitro* oral lubrication study. *Food Hydrocolloids*, *110*, 106146. https://doi.org/10.1016/j.foodhyd.2020.106146

Warncke, M., Kieferle, I., Nguyen, T. M., & Kulozik, U. (2022). Impact of heat treatment, casein/whey protein ratio and protein concentration on rheological properties of milk protein concentrates used for cheese production. *Journal of Food Engineering*, *312*, 110745. https://doi.org/10.1016/j.jfoodeng.2021.110745

White, S. S., Fox, K. M., Jervis, S. M., & Drake, M. A. (2013). Influence of heating and acidification on the flavor of whey protein isolate. *Journal of Dairy Science*, *96*(3), 1366-1379. https://doi.org/10.3168/jds.2012-5935

Wijegunawardhana, D., Wijesekara, I., Liyanage, R., Truong, T., Silva, M., & Chandrapala, J. (2025). The effect of varying casein to whey protein ratio on physicochemical and structural properties of lactose and maltodextrin modified liquid and concentrated milk-tea blends. *Food Research International*, *213*, 116466. https://doi.org/10.1016/j.foodres.2025.116466

Xu, F., Liamas, E., Bryant, M., Adedeji, A. F., Andablo-Reyes, E., Castronovo, M., Ettelaie, R., Charpentier, T. V. J., & Sarkar, A. (2020). A Self-Assembled Binary Protein Model Explains High-Performance Salivary Lubrication from Macro to Nanoscale. *Advanced Materials Interfaces*, *7*(1), 1901549. https://doi.org/10.1002/admi.201901549

Zembyla, M., Liamas, E., Andablo-Reyes, E., Gu, K. W., Krop, E. M., Kew, B., & Sarkar, A. (2021). Surface adsorption and lubrication properties of plant and dairy proteins: A comparative study. *Food Hydrocolloids*, *111*, 106364. https://doi.org/10.1016/j.foodhyd.2020.106364

Zhang, L., Zhou, R., Zhang, J., & Zhou, P. (2021). Heat-induced denaturation and bioactivity changes of whey proteins. *International Dairy Journal*, *123*, 105175 https://doi.org/10.1016/j.idairyj.2021.105175

Zhou, B., Drusch, S., & Hogan, S. A. (2023). Effects of temperature and pH on interfacial viscoelasticity, bulk rheology and tribological properties of whey protein isolate-stabilized high internal phase emulsions. *Food Hydrocolloids*, *142*, 108756. https://doi.org/10.1016/j.foodhyd.2023.108756

Zhu, Y., Bhandari, B., & Prakash, S. (2019). Tribo-rheology characteristics and microstructure of a protein solution with varying casein to whey protein ratios and addition of hydrocolloids. *Food Hydrocolloids*, *89*, 874-884. https://doi.org/10.1016/j.foodhyd.2018.12.005

Zou, Y., Pan, R., Ruan, Q., Wan, Z., Guo, J., & Yang, X. (2018). Interaction of soybean 7S globulin peptide with cell membrane model via isothermal titration calorimetry, quartz crystal microbalance with dissipation, and langmuir monolayer study. *Journal of Agricultural and Food Chemistry*, *66*(19), 4913-4922. https://doi.org/10.1021/acs.jafc.8b00414