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Protein concentration and hydrocolloid effect on the rheological and tribological of resulting protein solution

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Abstract

In recent years, the consumption of high protein beverages has increased due to the consciousness among consumers about their body weight. This study investigated the rheological, tribological and visual properties of pure proteins solutions with variable protein concentrations and with/without hydrocolloids (gelatin, κ-carrageenan, low methoxy pectin and curdlan). Although whey protein addition did not have any obvious influence on the appearances of protein solutions, it increased the stability against agglomeration and improved viscosity and lubrication property (measured as a friction coefficient) to some extent. The protein solutions became less stable with addition of the hydrocolloids under investigation, however the flow and lubrication behaviour of the protein solutions improved as the amount of hydrocolloids increased. The protein solution containing 0.25g/100g of curdlan showed the best lubrication property at both 15 and 37 °C.

Keywords: Hydrocolloids; Protein; Tribology; Rheology

1. Introduction

The macronutrient protein can give a stronger feeling of satiety and increased energy expenditure following consumption in comparison to carbohydrates and fats (Anderson & Moore, 2004; Halton & Hu, 2004; Paddon-Jones et al., 2008). Therefore, for body-weight control and treatment of obesity, high protein foods are considered potential candidates. High-protein products are increasingly being developed in several dairy and non-dairy areas, such as cheese, yoghurt, ice creams, fruits, beverages, and specialized health products because of their unique health benefiting properties (Uluko, Liu, Lv, & Zhang, 2015).

Milk that contains approximately 3.0g/100g protein is ideally thought as high protein food. Dairy proteins such as caseins and whey proteins are common in high protein liquid formulations with; and the ratio of casein to whey protein in milk is 4:1 (O'Mahony & Fox, 2014). Although like most proteins, whey proteins are not stable at high protein concentrations with increased viscosity and gelation after heat treatment resulting in undrinkable products (Sağlam, Venema, Vries, & van Der

Linden, 2014). However, whey is still widely used as an important ingredient in many foods due its functionalities such as gelation, emulsification, thickening, foaming and fat & flavour binding capacity (Foegeding, 2015; Onwulata, Huth, & Huth, 2008). Hydrocolloids are normally used to help maintain a desired product property when developing high protein foods since unwanted interactions always occurs between the ingredients (Nguyen, Kravchuk, Bhandari, & Prakash, 2017; Sağlam et al., 2014). In this study, LM pectin, gelatin and κ-carrageenan that are commonly used in the dairy industry and curdlan that is gradually being introduced in dairy products as it can enhance the mouthfeel properties were used (Nakao et al., 1991; Phillips & Williams, 2009; Salvador & Fiszman, 1998). The concentrations of hydrocolloids recommended to be used in dairy products is <0.3g/100g (Laaman, 2011; Phillips & Williams, 2009), thus we chose 0.01, 0.05 and 0.25 g/100g in this study.

Rheological (bulk) and tribological (thin-film) properties are essential characteristics that provide cues of the viscosity (thickness) and mouthfeel attributes (smoothness and creaminess) of the high protein solutions with and without hydrocolloids (Baier et

al., 2009; Kokini, 1987; Malone, Appelqvist, & Norton, 2003; O'Mahony & Fox, 2014). Particularly there is limited information on the oral sensations (described by frictional forces) imparted by pure proteins and protein-hydrocolloid complexes that are essential to understand for product formulations.

The aim of the current study is to investigate the rheological, tribological and visual properties of pure protein solutions containing casein and whey proteins at different proportions with and without four different hydrocolloids, including gelatin, κ-carrageenan, low methoxy pectin and in particular curdlan to explore the possibility of their use in dairy high protein beverage. Besides, the effect of whey protein addition on the protein solution was also investigated.

2. Materials and methods

2.1. Materials

Three hydrocolloids (gelatin, carrageenan and pectin) used in this study were obtained from Melbourne Food Depot (Melbourne, Australia). The gelatin was a light colored

edible bovine skin (type B) powder with bloom 220. The Kappa type carrageenan and low methoxyl (LM) pectin were also commonly used in the food industry. The curdlan (Molecular weight is 74000) was bought from Shaanxi Orient Industrial Co., Ltd (Shaanxi province, China). The milk protein ingredients, whey protein isolate (WPI, protein 94.4g/100g, moisture 4.5g/100g, fat 0.3g/100g, lactose 0.1g/100g and ash 1.9g/100g) was obtained from Fonterra Co-operative Group Ltd (Auckland, New Zealand) and micellar casein (protein 82.0g/100g, moisture 7.3g/100g, fat 1.3g/100g, lactose 4.6g/100g and ash 4.8g/100g) was purchased from Bulk Nutrients (Sydney, Australia). All the reagents were of food grade and used without further purification.

2.2. Protein solution preparation

Protein solutions were formulated by adding micellar casein and WPI to ultra-pure water. The weight of micellar casein was 2.4g/100g in all samples while varying amount of WPI was added to achieve the final protein concentration of 3.0, 4.0, 6.0, 8.0 and 10.0g/100g, respectively. The following preparation procedure was used for all protein solutions. To ensure that no clumping occurred, the protein powder was

added slowly to the water in a beaker placed on a Fisher Thermix 310T stirring plate (American Instruments Exchange, Inc., Haverhill, MA) and kept for 2 hours at 25 °C under moderate agitation at 400 rpm. A second dispersion and hydration step consisted of using an UltraTurrax Model T25 fitted with an S25N-18G dispersion tool (IKA Works Inc., Wilmington, NC) for 5 min at 10,000 rpm and then sonicated in an ultrasonic bath for 20 min at 300 W. The solutions were kept in 4 °C overnight for further hydration. The next day the stock protein solution was mixed with the selected hydrocolloids before heating (95 °C for 10 min), and the final concentration of hydrocolloids in the system was 0.01, 0.05 and 0.25g/100g. A sample with no hydrocolloids was treated as control. After heating the samples were cooled with running water immediately and stored at 4 °C for 48 hours for instrumental analysis and appearance evaluation.

2.3. Zeta-potential & particle size measurement

The zeta-potential and particle size of protein solution was measured by dynamic light scattering using the Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom).

For the measurement, samples were diluted 500 times with deionised water before measurement. The zeta-potential and particle size was reported as the average and standard deviation of measurements made on three freshly prepared samples.

2.4. Rheological measurement

Viscosities of protein samples were measured under steady state shear conditions by stress-controlled rheometer (Discovery Hybrid Rheometer, TA Instrument, USA) using 40 mm aluminium parallel plates at 1300 μm gap, with shear rate ranging from 0.1 to 100 s^{-1}. At the beginning of each test, the samples were equilibrated again for 120 s at 15 or 37 °C between the plates at the measurement gap and subjected to a pre-shear for 60 s at a shear rate of 0.1 s^{-1}. All tests were performed at 15 and 37 °C.

2.5. Tribological measurement

Lubrication properties of protein samples were measured on a Discovery Hybrid Rheometer, using ring on plate tribo-rheometry (TA Instrument, USA) on a rough plastic surface of 3M Transpore Surgical Tape 1527-2 (3M Health Care, USA). A

3-ball spherical geometry was used to measure tribology in this work.

The tribology measurement was performed at 15 and 37 °C. Since the in-mouth force was reported to be between 0.01 and 10 N (Miller & Watkin, 1996), we used a constant normal force of 2 N to represent the moderate normal force applied on samples during oral processing. The samples were pre-sheared at the speed of 0.01 rad/s for 1 min, and then equilibrated for another 1 min before each measurement. The results were recorded for rotational speeds from 0.01 to 100 mm/s with 10 points per decade.

2.6. Appearance evaluation

After 48 hours of storage at 4 °C the protein solutions were equilibrated at room temperature (22–25 °C) for 1 h followed by evaluation of appearance.

2.7. Statistical analysis

All the experiments were performed in triplicates and the results are expressed as the

mean of independent experiments ± standard deviation. Experimental data were subjected to one-way ANOVA (pairwise comparison of means with Tukey HSD post-hoc test) in order to find differences in samples. Data was analyzed using SPSS software 22.0 (SPSS, Chicago, IL). A *p*-value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. Zeta-potential, particle size and appearance of protein solution

Tables 1 and 2 show the zeta potential and particle size of different protein solution with and without hydrocolloids. With the exception of 10.0g/100g protein, the addition of WPI increased the absolute zeta-potential (Table 1) and decreased the particle size (Table 2) of the protein solution, suggesting improved stability of the solution. Casein and WPI interact during heating and WPI will absorb on the surface of casein to form a casein-WPI complex (Donato & Guyomarc'H, 2009), this may prevent congregation and help the protein to be well dispersed in the solution. Additionally, disulfide bridges formed between milk proteins due to heating (Pakseresht, Mazaheri Tehrani,

156 & Razavi, 2017), binds the proteins together hindering protein precipitation, thereby
157 improving the stability.
158
159 **Table 1**
160
161 The particle size of protein solution increased with the addition of hydrocolloids,
162 while the zeta potential value was the same with 0.01 and 0.05g/100g hydrocolloids
163 (Table 1 and 2), suggesting hydrocolloid addition decreased the protein stability in the
164 system. Moreover, for protein solution (<4.0g/100g) with 0.25g/100g gelatin, the zeta
165 potential value changed from -22.37±1.10 mV to -15.70±0.87 mV (Table 1), the
166 absolute zeta potential value decreasing significantly. It has been reported that gelatin
167 has some interaction with milk protein (Pang, Deeth, Sopade, Sharma, & Bansal,
168 2014), that causes aggregation of protein in the system thereby making the protein
169 less stable. The non-ionic hydrocolloid, curdlan, may have segregative interaction
170 with milk protein since it absorbs water and expands at the beginning of heating
171 (Funami & Nishinari, 2007), which is not ideal for the stability of protein solution.

While in this study, although the particle size increased, the zeta potential of protein solutions with curdlan was almost the same compared with control, even with 0.25g of curdlan (Table 1). Curdlan has thermal irreversible interactions between itself during heating at high temperature (80 °C) (Nakao et al., 1991) that may help with the stability of protein. Therefore, for the application in high protein dairy drinks curdlan may have an advantage over gelatin because it has less influence on the stability of milk protein system, even at high concentration. In addition, when the protein solution reached 10.0g/100g protein, it showed large absolute zeta potential value even the particle size in the solution was also big, especially after hydrocolloids were added. Since the WPI has heat-gelling property, at high concentration (10.0g/100g) the protein solution becomes semi-solid and very viscous after heating, hindering the conglomeration and moving of particles in the system, that may be responsible for the high absolute zeta potential value although the particle size were large.

Table 2

188 The visual appearance of the protein solutions with/without the hydrocolloids (gelatin,
189 κ-carrageenan, LM pectin and curdlan) after 48 hours storage are shown in Fig. 1. As
190 can be seen, higher concentration of WPI (8.0g/100g and 10.0g/100g w/w) made the
191 protein solution more transparent. It is well known that the white color of milk is
192 largely due to the scattering of light by the casein micelles and pure whey protein is
193 transparent (O'Mahony & Fox, 2014), thus adding more WPI reduced the whiteness
194 to some extent. The addition of gelatin/curdlan did not change the visual aspect at all
195 concentrations of proteins, suggesting a possibility of curdlan being used in place of
196 gelatin in high protein drinks. However, with the addition of κ-carrageenan and LM
197 pectin all the protein solutions formed gel when the concentration reached 0.05 and
198 0.25g/100g, respectively (Fig. 1). This suggests that κ-carrageenan and LM pectin
199 easily interact with milk protein (κ-casein) and form gel, as previously observed by
200 Pang, Deeth and Bansal (2015). Overall, the gelation ability of the four hydrocolloids
201 was in the order gelatin/curdlan < LM pectin < κ-carrageenan, and for the utilization
202 of hydrocolloids in high-protein dairy drinks, the usage amount of κ-carrageenan and
203 LM pectin should be below 0.05 and 0.25g/100g, while it could be up to 0.25g/100g

for gelatin and curdlan.

Fig.1.

3.2. Viscosity of protein solution

In order to discuss the effect of the addition of WPI and four different hydrocolloids on the viscosity of protein solutions, the viscosity values at the shear rate 50 s^{-1} (η_{50}) was chosen since the viscosity at shear rate of 50 s^{-1} has been suggested to relate to perceived thickness, stickiness and sliminess for a wide range of food products from Newtonian fluid to thick emulsion (Shama & Sherman, 1973). Besides, in order to show the property during consumption, the measurements were done at 15 and 37 °C, which are the two temperatures at the start and end of consumption. The η_{50} for all the protein solution are showed in Table 3 and 4.

Table 3

220 The viscosity of 3.0g/100g protein solution was 4.84±0.02 mPa·s under 15 °C, and
221 increased to 13.91±0.10 mPa·s when the concentration reached 8.0g/100g, and got
222 even significantly higher at 10.0g/100g (212.50±6.12 mPa·s), suggesting viscosity of
223 protein solution is concentration dependent. With WPI added, more protein denatured
224 during heating and they tended to interact with each other that network more closely
225 (Zhao, Wang, Tian, & Mao, 2016), which increased the viscosity. In addition, the
226 addition of hydrocolloids increased the viscosity of protein solution. For example, as
227 for 8.0g/100g protein solution, the viscosity increased from 13.91±0.10 mPa·s to
228 37.58±0.60 mPa·s, 26.69±0.98 mPa·s, 18.46±0.59 mPa·s and 27.82±0.47 mPa·s with
229 addition of 0.25g/100g gelatin, 0.01g/100g κ-carrageenan, 0.05g/100g LM pectin, and
230 0.25g/100g curdlan (Table 3). It was reported that gelatin, κ-carrageenan and LM
231 pectin had associative interaction with milk proteins (Pang et al., 2015), which
232 increased the viscosity. For curdlan, the swelling of the molecules occurs as heating
233 begins followed by interaction with each other to form network (Funami, Funami,
234 Yada, & Nakao, 2000), increasing the viscosity of the protein solution. In addition,
235 depletion flocculation of the casein micelles occurs at pH 6.7 when the exclusion of

236 the polymer hydrocolloids chains from the space between colloidal particles (casein
237 micelles), inducing an effective attractive interaction between the colloidal particles
238 (Maroziene & de Kruif, 2000), raising the viscosity. Besides, all the four
239 hydrocolloids have cold-gelling property that is the viscosity increases when stored at
240 4 °C. When the total protein concentration reached 10.0g/100g (w/w), the solution
241 transforms to a semi-solid state after heating at 95 °C for 10min due to the denaturing
242 and gelling property of WPI, thus the viscosity at this concentration is quite high.
243
244 **Table 4**
245
246 The viscosity of the protein solutions at 37 °C was lower compared to that at 15 °C
247 (Table 3 and 4). This was markedly obvious for gelatin added protein solution. For
248 instance, the viscosity of 8.0g/100g protein solution with 0.25g/100g gelatin added
249 decreased from 37.58±0.60 mPa·s to 9.18±0.27 mPa·s when the temperature
250 increased from 15 to 37 °C. As the temperature increases, the weak bonds (hydrogen
251 bonds) that is sensitive to temperature break, which may cause the decrease in

252 samples' viscosity. However, it should be noted that the viscosity of protein solution
253 with κ-carrageenan and curdlan was still nearly twice compared to pure protein
254 solution (Table 4). Previous studies showed that κ-carrageenan presents strong
255 interaction with κ-casein (Pang et al., 2015) and curdlan has a thermo-irreversible
256 interaction with itself at high temperature (Funami et al., 2000; Funami & Nishinari,
257 2007), thus although the viscosity decreased due to temperature it is still significantly
258 higher than control (Table 4). Through viscosity results, we can say that
259 κ-carrageenan and curdlan could be an ideal choice for high-protein drinks since they
260 could even raise the viscosity of solutions under oral temperature conditions (37 °C).
261

262 **3.3. Tribological properties of protein solution**

263 Lubrication properties of the protein solution with/without hydrocolloids, measured
264 using the tribo-rheometer at 2N set at 15 or 37 °C is presented in Fig. 2-3 and
265 Supplementary Fig.1.
266

267 **(a) Effect of the addition of WPI and hydrocolloids**

From Fig. 2, it can be seen that all protein solution showed a ‘‘stick and slide’’ pattern (traditional Stribeck curve) that is the friction coefficient was constant at low sliding speed (0.01~0.5 mm/s) and decreased with increasing sliding speed (Prakash, Tan, & Chen, 2013). The reason for this pattern is at low speeds, the protein solution acts as a thin lubricating film and the friction depends on the asperity interaction between the two surfaces while at higher speeds more fluid is drawn into the contact zone to partly separate the two surfaces in the mixed regime to decrease the friction coefficient. At low sliding speeds (0.01~0.5 mm/s), the friction coefficient of protein solutions is almost the same for all concentration. And during the medium speed range (0.5~10 mm/s), the coefficient of friction reduced with the addition of WPI (<10.0g/100g), meaning the additional WPI in the protein solution contributes towards better lubrication property. As mentioned in section 3.2, the WPI denature at high temperature and easily form intramolecular and intermolecular interactions (O’Mahony & Fox, 2014), which strengthens the absorbability of the protein solution promoting the formation of thin layer separating the surfaces as the sliding speed increases. Combined with the results in section 3.2, we can infer that higher viscosity

284 may lead to better lubrication property. Similar results were obtained previously by
285 Nguyen et al. (2017), who found the dairy samples with higher viscosity showed
286 better lubrication property (lower friction coefficient). However, when the
287 concentration of protein solution reached 10.0g/100g, the solution became semi-solid
288 and hindered the movement between the two surfaces, thus increasing the friction
289 coefficient.
290
291 **Fig.2.**
292
293 Besides, at high sliding speeds (> 10mm/s), the friction coefficient was almost the
294 same for protein solutions ≤ 6.0g/100g (w/w) protein and increased with further
295 increase in the protein concentration (Fig. 2). Based on the Stribeck curve, at the end
296 of mixed regime the friction coefficient decreases slowly that gradually increases
297 when the sliding speed reaches the hydrodynamic regime (Nguyen, Bhandari, &
298 Prakash, 2016). Since the surface adsorption force may increase with WPI addition,
299 the protein solutions were faster to form thick lubricating film, thus entering the

hydrodynamic regime earlier. This may explain the increasing friction coefficient as the protein concentration increased.

For the protein solution with added hydrocolloids (gelatin, κ-carrageenan, LM pectin and curdlan), the friction coefficients were lower than the pure protein solution at 37 °C (Supplementary Fig. 1). Considering the friction coefficient of 3.0g/100g protein (casein/WPI=2.4/0.6) solution at 37ºC as an example, we can discuss the friction coefficient at sliding speed of 0.1, 1 and 10 mm/s that are presented in Table 5. At the beginning (0.1 mm/s), all the samples have similar coefficient of friction, however at sliding speed 1 and 10 mm/s, the protein solution with hydrocolloids have lower friction coefficient compared to control sample. This means the protein solution containing hydrocolloids have better lubrication property. This is in agreement with the results that adding hydrocolloids (κ-carrageenan and gelatin) improves the lubrication properties of yoghurt (Nguyen et al., 2016). The interaction between dairy protein and hydrocolloids possibly improves the surface adhesion of the resulting solution, allowing formation of a thin film between the two surfaces with ease and

316 lowering the friction coefficient.

317

318 **Table 5**

319

320 In addition, the addition of curdlan has the best influence on the tribological property
321 of protein solution (Supplementary Fig.1). Adding 0.25g/100g curdlan to the protein
322 solution improved the lubrication property, showing the lowest friction coefficient.
323 Although curdlan is partly soluble or insoluble in neutral condition, it swells and
324 forms thermo-irreversible and thermo-reversible bonds during heating and cooling
325 (Funami et al., 2000), which may be the reason for the improved lubrication property.
326 Thus, curdlan may be the ideal choice for developing smooth and creamy foods.
327 However, the protein solutions with added gelatin did not show any significant
328 difference in friction curves compared with pure protein solution that was contrary to
329 our previous hypothesis. To investigate whether temperature affects the tribological
330 property, the protein solution with added gelatin/curdlan was also studied at 15 °C
331 that is discussed below.

(b) Effect of temperature

Fig.3.

Fig. 3 shows the friction curves of 3.0g/100g protein solution (casein/WPI=2.4/0.6) with the addition of gelatin and curdlan at 15 and 37 °C. The friction curves showed a classical ‘‘stick and slide’’ pattern (traditional Stribeck curve) at both 15 and 37 °C. For friction curves at 15 °C, the friction coefficient decreased as the gelatin concentration increased in the protein solution, especially at low sliding speed range (0.01~0.5 mm/s) and the initial part of medium speed (0.5~6 mm/s). Although the friction coefficient was lower than the pure protein solution at the later part of medium speed (6~10 mm/s), it was almost the same for gelatin added protein solution. In addition, when the speed reached high (> 10 mm/s), the friction curves overlapped for all solutions (Fig. 3A). Gelatin has a good hydrophilic property and can form hydrogen bonds with milk protein or itself (Fiszman & Salvador, 1999), thus adding gelatin improved the surface adhesion of protein solution, forming a thick lubrication

348 film between the two surfaces. Therefore, the friction coefficient decreased. And for
349 protein solution with curdlan, the coefficient of friction was lower than the control
350 during the sliding speed measured in this study (Fig. 3C), especially for 0.25g/100g
351 curdlan added. It means the curdlan addition had similar influence on the lubrication
352 property of protein solution as gelatin addition at 15 °C.
353
354 However, Fig. 3B shows that there was almost no difference between pure 3.0g/100g
355 protein solution and gelatin added protein solution at 37 °C. This may be due to the
356 weak hydrogen bonds formed between gelatin and protein that easily break. As
357 temperature increases from 15 to 37 °C, the interactions in the protein solution
358 decreases, thus the friction curves show less differences between pure protein solution
359 and protein solution with gelatin. While the protein solutions with curdlan added still
360 showed better lubrication property compared to pure protein solution (Fig. 3D). Thus,
361 curdlan addition had better influence on the lubrication property of protein solution
362 than gelatin added at 37 °C. Besides, during the sliding speed from 0.01~6 mm/s, the
363 friction coefficient of 3.0g/100g protein solution at 37 °C was 0.8~1.0, which was

higher than the friction coefficient (0.6~0.8) at 15 °C (Fig. 3).

4. Conclusion

According to the results of this study, adding WPI to raise the protein concentration did not have obvious effect on the visual appearance of protein solution, but increased the protein stability against agglomeration and the viscosity of protein solution to some extent. Besides, within a certain range of total protein (< 8.0g/100g), the addition of WPI led to better lubrication property.

The addition of hydrocolloids had no influence on the visual aspect of protein solution, while the protein solution formed gel after adding 0.05g/100g κ-carrageenan and 0.25g/100g LM pectin for all protein concentrations investigated in this research. Although adding hydrocolloids decreased the stability of protein solution, it increased the viscosity of protein solution, and the viscosity of κ-carrageenan and curdlan added protein solution was still nearly twice more than control under 37 °C. The effect of gelatin on the tribological property of protein solution showed obviously under 15 °C,

while almost had no influence when the temperature increased to 37 °C. Interestingly, curdlan addition improved the lubrication property both at 15 and 37 °C. Therefore, curdlan might be an ideal choice for high-protein dairy drinks since it could increase the viscosity and lubrication property of protein solution without effecting the visual of solution. In the future, we will investigate the application of curdlan in real dairy products. Since the addition of WPI alters the casein to WPI ratio, the influence of fractions of casein to WPI at fixed protein concentration will also be studied in the future.

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475

Fig. 1. The appearance of protein solution with/without different concentration (0.01, 0.05 and 0.25g/100g) of gelatin, κ-carrageenan, LM pectin and curdlan. (A) 3.0g/100g Protein solution (casein/WPI=2.4/0.6); (B) 4.0g/100g Protein solution (casein/WPI=2.4/1.6); (C) 6.0g/100g Protein solution (casein/WPI=2.4/3.6); (D) 8.0g/100g Protein solution (casein/WPI=2.4/5.6); (E) 10.0g/100g Protein solution (casein/WPI=2.4/7.6). * Means gel formed and the other samples without mark kept solution after 4 °C storage 48 hours.

Fig. 2. The tribology curve of different pure protein solution at 37 °C. ×3.0g/100g Protein solution (casein/WPI=2.4/0.6); □4.0g/100g Protein solution (casein/WPI=2.4/1.6); ◇6.0g/100g Protein solution (casein/WPI=2.4/3.6); ○8.0g/100g Protein solution (casein/WPI=2.4/5.6); △10.0g/100g Protein solution (casein/WPI=2.4/7.6)

Fig. 3. The tribology curves of 3.0g/100g protein solution (casein/WPI=2.4/0.6) with/without added gelatin or curdlan (0.01, 0.05 and 0.25g/100g) under (A and C) 15 °C and (B and D) 37 °C. ×Control; ◇0.01g/100g Gelatin; ◆0.05g/100g Gelatin; ★0.25g/100g Gelatin; ○0.01g/100g Curdlan; ● 0.05g/100g Curdlan; ◐0.25g/100g Curdlan

Supplementary Fig. 1. The tribology curve of different protein solution with/without gelatin, κ-carrageenan, LM pectin and curdlan (0.01, 0.05 and 0.25g/100g) at 37 °C. (A) 3.0g/100g Protein solution (casein/WPI=2.4/0.6); (B) 4.0g/100g Protein solution (casein/WPI=2.4/1.6); (C) 6.0g/100g Protein solution (casein/WPI=2.4/3.6); (D) 8.0g/100g Protein solution (casein/WPI=2.4/5.6); (E) 10.0g/100g Protein solution (casein/WPI=2.4/7.6). ×Control; ◇0.01g/100g Gelatin; ◆0.05g/100g Gelatin; ★0.25g/100g Gelatin; △0.01g/100g Carrageenan; □0.01g/100g Pectin; ■0.05g/100g Pectin; ○ 0.01g/100g Curdlan; ●0.05g/100g Curdlan; ◐ 0.25g/100g Curdlan

Table 1 The zeta potential of different protein solution with/without various concentrations (0.01, 0.05 and 0.25g/100g) of gelatin, κ-carrageenan, LM pectin and curdlan.

Samples (g/100g)	3.0g/100g protein (casein/WPI=2.4/0.6)	4.0g/100g protein (casein/WPI=2.4/1.6)	6.0g/100g protein (casein/WPI=2.4/3.6)	8.0g/100g protein (casein/WPI=2.4/5.6)	10.0g/100g protein (casein/WPI=2.4/7.6)
Control	-22.37 (1.10) ^{Abc}	-22.87 (0.97) ^{ABc}	-23.53 (0.73) ^{ABb}	-24.30 (0.65) ^{Bc}	-25.80 (0.31) ^{Cab}
0.01 Gelatin	-20.37 (0.93) ^{Ab}	-21.00 (0.61) ^{Ab}	-21.57 (0.49) ^{Aa}	-23.33 (0.36) ^{Bc}	-26.83 (0.48) ^{Cb}
0.05 Gelatin	-20.33 (0.74) ^{Ab}	-20.83 (0.35) ^{Ab}	-21.47 (0.90) ^{Aa}	-23.47 (0.36) ^{Bc}	-24.53 (0.41) ^{Ca}
0.25 Gelatin	-15.70 (0.87) ^{Aa}	-17.63 (0.71) ^{Ba}	-21.10 (0.85) ^{Ca}	-23.50 (0.74) ^{Dbc}	-24.83 (0.76) ^{Da}
0.01 Carrageenan	-21.13 (0.49) ^{Abc}	-21.57 (0.30) ^{ABc}	-22.00 (0.17) ^{Ba}	-22.87 (0.18) ^{Cbc}	-27.57 (0.18) ^{Dc}
0.05 Carrageenan	-	-	-	-	-
0.25 Carrageenan	-	-	-	-	-
0.01 Pectin	-20.73 (0.35) ^{Ab}	-21.27 (0.59) ^{ABbc}	-21.90 (0.68) ^{Ba}	-24.57 (0.41) ^{Cd}	-24.93 (0.66) ^{Ca}
0.05 Pectin	-21.73 (0.36) ^{Ac}	-22.33 (0.65) ^{Ac}	-22.33 (0.66) ^{Aab}	-23.97 (0.33) ^{Bc}	-25.30 (0.43) ^{Ca}
0.25 Pectin	-	-	-	-	-
0.01 Curdlan	-20.93 (0.66) ^{Abc}	-21.10 (0.57) ^{Ab}	-21.33 (0.58) ^{Aab}	-21.80 (0.40) ^{Aa}	-26.37 (0.71) ^{Bb}
0.05 Curdlan	-20.13 (0.41) ^{Ab}	-20.83 (0.93) ^{ABbc}	-21.87 (0.13) ^{Ba}	-22.60 (0.30) ^{Cb}	-25.77 (0.97) ^{Da}
0.25 Curdlan	-20.17 (0.48) ^{Ab}	-20.23 (0.59) ^{Ab}	-20.80 (0.40) ^{Aa}	-22.17 (0.30) ^{Bab}	-26.17 (0.20) ^{Cb}

Mean value (n=9) in mV and standard deviation shown in parenthesis. Different superscript capital letters in the same line indicate significant differences between different protein concentrations ($P < 0.05$).

Different superscript lower-case letters in the same column indicate significant differences between additions of hydrocolloids ($P < 0.05$).

Table 2 The particle size of different protein solution with/without various concentrations (0.01, 0.05 and 0.25g/100g) of gelatin, κ-carrageenan, LM pectin and curdlan.

Samples (g/100g)	3.0g/100g protein (casein/WPI=2.4/0.6)	4.0g/100g protein (casein/WPI=2.4/1.6)	6.0g/100g protein (casein/WPI=2.4/3.6)	8.0g/100g protein (casein/WPI=2.4/5.6)	10.0g/100g protein (casein/WPI=2.4/7.6)
Control	184.1 (2.46) ^{Afg}	175.9 (1.91) ^{Bf}	150.6 (0.33) ^{Dg}	134.3 (1.31) ^{Ef}	168.9 (0.99) ^{Cf}
0.01 Gelatin	197.4 (1.69) ^{Ad}	179.1 (2.47) ^{Bef}	171.3 (2.17) ^{Cd}	141.0 (1.87) ^{De}	183.0 (1.60) ^{Be}
0.05 Gelatin	201.8 (0.23) ^{Ac}	186.6 (0.58) ^{Bc}	175.3 (2.75) ^{Dcd}	152.5 (0.56) ^{Ed}	182.4 (1.05) ^{Ce}
0.25 Gelatin	260.8 (5.34) ^{Aa}	225.7 (0.60) ^{Ca}	178.3 (1.52) ^{Dc}	164.4 (1.39) ^{Eb}	246.1 (0.69) ^{Bb}
0.01 Carrageenan	208.9 (0.79) ^{Bb}	199.0 (1.85) ^{Cb}	194.8 (0.95) ^{Da}	184.1 (6.99) ^{Ea}	325.1 (6.78) ^{Aa}
0.05 Carrageenan	-	-	-	-	-
0.25 Carrageenan	-	-	-	-	-
0.01 Pectin	186.9 (0.66) ^{Bf}	184.4 (0.71) ^{Cd}	166.6 (1.88) ^{De}	165.2 (1.85) ^{Db}	201.2 (2.46) ^{Ad}
0.05 Pectin	186.0 (1.44) ^{Bfg}	184.8 (1.08) ^{Bd}	163.4 (2.47) ^{Cf}	158.4 (0.61) ^{Dc}	202.0 (5.25) ^{Ad}
0.25 Pectin	-	-	-	-	-
0.01 Curdlan	181.5 (2.25) ^{Bg}	181.6 (0.53) ^{Be}	178.8 (0.39) ^{Cc}	178.6 (2.13) ^{BCa}	206.5 (3.75) ^{Acd}
0.05 Curdlan	184.0 (2.12) ^{Bg}	182.9 (1.51) ^{Bde}	180.0 (2.50) ^{BCbc}	178.1 (2.47) ^{Ca}	201.7 (4.20) ^{Ad}
0.25 Curdlan	188.6 (1.01) ^{Be}	186.5 (0.55) ^{Cc}	184.4 (2.46) ^{CDb}	182.2 (1.01) ^{Da}	215.7 (6.72) ^{Ac}

Mean value (n=9) in nm and standard deviation shown in parenthesis. Different superscript capital letters in the same line indicate significant differences between different protein concentrations ($P < 0.05$).

Different superscript lower-case letters in the same column indicate significant differences between additions of hydrocolloids ($P < 0.05$).

Table 3 The viscosity (η_{50}) of different protein solutions with/without various concentrations (0.01, 0.05 and 0.25g/100g) of gelatin, κ-carrageenan, LM pectin and curdlan under 15 °C.

Samples (g/100g)	3.0g/100g protein (casein/WPI=2.4/0.6)	4.0g/100g protein (casein/WPI=2.4/1.6)	6.0g/100g protein (casein/WPI=2.4/3.6)	8.0g/100g protein (casein/WPI=2.4/5.6)	10.0g/100g protein (casein/WPI=2.4/7.6)
Control	4.84 (0.02) ^{Eg}	5.27 (0.09) ^{Df}	7.07 (0.27) ^{Ce}	13.91 (0.10) ^{Be}	212.50 (6.12) ^{Aj}
0.01 Gelatin	4.95 (0.03) ^{Ef}	5.35 (0.05) ^{Df}	6.86 (0.36) ^{Ce}	14.69 (0.50) ^{Bd}	290.25 (11.80) ^{Ai}
0.05 Gelatin	5.06 (0.05) ^{Ee}	5.55 (0.13) ^{De}	7.14 (0.13) ^{Ce}	14.52 (0.53) ^{Bde}	361.33 (10.05) ^{Ah}
0.25 Gelatin	7.69 (0.08) ^{Eb}	8.22 (0.13) ^{Da}	12.20 (0.28) ^{Ca}	37.58 (0.60) ^{Ba}	1249.68 (11.76) ^{Ab}
0.01 Carrageenan	5.80 (0.06) ^{Ed}	7.65 (0.08) ^{Db}	11.62 (0.45) ^{Cab}	26.69 (0.98) ^{Bb}	457.85 (4.59) ^{Ag}
0.05 Carrageenan	-	-	-	-	-
0.25 Carrageenan	-	-	-	-	-
0.01 Pectin	5.02 (0.04) ^{Eef}	5.45 (0.05) ^{Def}	7.48 (0.27) ^{Ce}	14.62 (0.99) ^{Bde}	498.70 (10.81) ^{Af}
0.05 Pectin	6.17 (0.11) ^{Ec}	7.25 (0.03) ^{Dc}	9.21 (0.26) ^{Cc}	18.46 (0.59) ^{Bc}	891.45 (11.30) ^{Ac}
0.25 Pectin	-	-	-	-	-
0.01 Curdlan	4.85 (0.04) ^{Eg}	5.53 (0.09) ^{De}	7.14 (0.22) ^{Ce}	14.82 (0.35) ^{Bd}	642.14 (7.73) ^{Ad}
0.05 Curdlan	5.10 (0.04) ^{Ee}	6.38 (0.08) ^{Dd}	8.25 (0.32) ^{Cd}	15.45 (0.49) ^{Bd}	569.22 (6.69) ^{Ae}
0.25 Curdlan	8.03 (0.08) ^{Ea}	8.30 (0.06) ^{Da}	11.7 (0.16) ^{Cb}	27.82 (0.47) ^{Bb}	1799.34 (12.56) ^{Aa}

Mean value (n=3) in mPa·s and standard deviation shown in parenthesis. Different superscript capital letters in the same line indicate significant differences between different protein concentrations ($P < 0.05$). Different superscript lower-case letters in the same column indicate significant differences between additions of hydrocolloids ($P < 0.05$).

Table 4 The viscosity (η_{50}) of different protein solutions with/without various concentrations (0.01, 0.05 and 0.25g/100g) of gelatin, κ-carrageenan, LM pectin and curdlan under 37 °C.

Samples (g/100g)	3.0g/100g protein (casein/WPI=2.4/0.6)	4.0g/100g protein (casein/WPI=2.4/1.6)	6.0g/100g protein (casein/WPI=2.4/3.6)	8.0g/100g protein (casein/WPI=2.4/5.6)	10.0g/100g protein (casein/WPI=2.4/7.6)
Control	3.98 (0.09) ^{Dcd}	4.09 (0.04) ^{De}	4.67 (0.17) ^{Cfg}	7.79 (0.18) ^{Be}	88.71 (4.07) ^{Ag}
0.01 Gelatin	3.96 (0.12) ^{Dcd}	4.02 (0.10) ^{De}	4.49 (0.11) ^{Cg}	7.40 (0.08) ^{Bf}	103.29 (6.29) ^{Af}
0.05 Gelatin	3.90 (0.03) ^{Ed}	4.14 (0.12) ^{Dde}	4.83 (0.10) ^{Cf}	7.60 (0.09) ^{Be}	170.87 (5.73) ^{Ae}
0.25 Gelatin	3.98 (0.02) ^{Ec}	4.16 (0.10) ^{Dde}	5.03 (0.09) ^{Ce}	9.18 (0.27) ^{Bc}	363.65 (6.70) ^{Ab}
0.01 Carrageenan	4.47 (0.03) ^{Eb}	5.12 (0.08) ^{Db}	6.36 (0.18) ^{Cb}	13.17 (0.31) ^{Bb}	176.62 (16.47) ^{Ae}
0.05 Carrageenan	-	-	-	-	-
0.25 Carrageenan	-	-	-	-	-
0.01 Pectin	4.01 (0.16) ^{Dcd}	4.12 (0.05) ^{De}	5.04 (0.01) ^{Ce}	8.10 (0.31) ^{Bde}	218.19 (7.81) ^{Ad}
0.05 Pectin	4.20 (0.03) ^{Ec}	4.52 (0.08) ^{Dc}	5.26 (0.11) ^{Cd}	9.41 (0.16) ^{Bc}	361.60 (11.09) ^{Ab}
0.25 Pectin	-	-	-	-	-
0.01 Curdlan	3.97 (0.05) ^{Dcd}	4.06 (0.09) ^{De}	4.94 (0.21) ^{Cde}	7.97 (0.50) ^{Bde}	251.34 (15.00) ^{Ac}
0.05 Curdlan	4.10 (0.15) ^{Dc}	4.32 (0.11) ^{Dd}	5.69 (0.12) ^{Cc}	8.36 (0.27) ^{Bd}	268.12 (6.65) ^{Ac}
0.25 Curdlan	6.38 (0.11) ^{Da}	5.98 (0.09) ^{Ea}	7.34 (0.17) ^{Ca}	14.93 (0.27) ^{Ba}	1067.05 (14.15) ^{Aa}

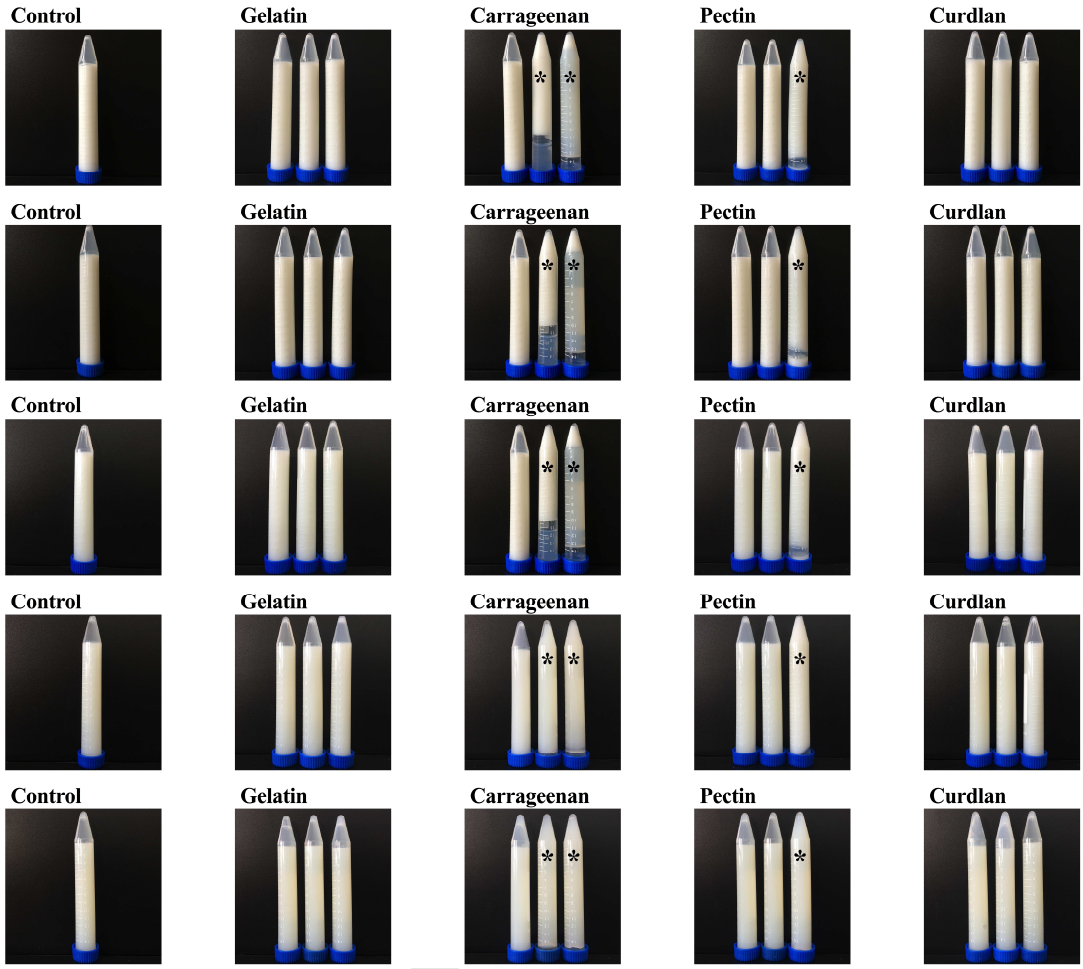
Mean value (n=3) in mPa·s and standard deviation shown in parenthesis. Different superscript capital letters in the same line indicate significant differences between different protein concentrations ($P < 0.05$). Different superscript lower-case letters in the same column indicate significant differences between additions of hydrocolloids ($P < 0.05$).

Table 5 The friction coefficient of 3.0g/100g protein (casein/WPI=2.4/0.6) solution at 37 °C under sliding speed of 0.1, 1 and 10 mm/s.

Samples (g/100g)	0.1 mm/s	1 mm/s	10 mm/s
Control	0.96 (0.004) ^{Aa}	0.89 (0.005) ^{Ba}	0.69 (0.001) ^{Ca}
0.01 Gelatin	0.96 (0.016) ^{Aa}	0.88 (0.009) ^{Bab}	0.68 (0.005) ^{Cb}
0.05 Gelatin	0.97 (0.024) ^{Aa}	0.87 (0.005) ^{Bb}	0.67 (0.001) ^{Cc}
0.25 Gelatin	0.95 (0.034) ^{Aa}	0.87 (0.003) ^{Bb}	0.67 (0.005) ^{Cbc}
0.01 Carrageenan	0.98 (0.057) ^{Aa}	0.84 (0.015) ^{Bc}	0.66 (0.002) ^{Cd}
0.05 Carrageenan	-	-	-
0.25 Carrageenan	-	-	-
0.01 Pectin	0.96 (0.015) ^{Aa}	0.86 (0.011) ^{Bbc}	0.66 (0.002) ^{Cd}
0.05 Pectin	0.95 (0.020) ^{Aa}	0.84 (0.015) ^{Bc}	0.66 (0.001) ^{Cd}
0.25 Pectin	-	-	-
0.01 Curdlan	0.92 (0.012) ^{Aa}	0.81 (0.019) ^{Bcd}	0.64 (0.002) ^{Ce}
0.05 Curdlan	0.92 (0.023) ^{Aab}	0.83 (0.005) ^{Bc}	0.63 (0.004) ^{Cf}
0.25 Curdlan	0.86 (0.044) ^{Ab}	0.78 (0.023) ^{Bd}	0.61 (0.004) ^{Cg}

Mean value (n=3) and standard deviation shown in parenthesis. Different superscript capital letters in the same line indicate significant differences between different sliding speed ($P < 0.05$). Different superscript lower-case letters in the same column indicate significant differences between additions of hydrocolloids ($P < 0.05$).

Fig.1.



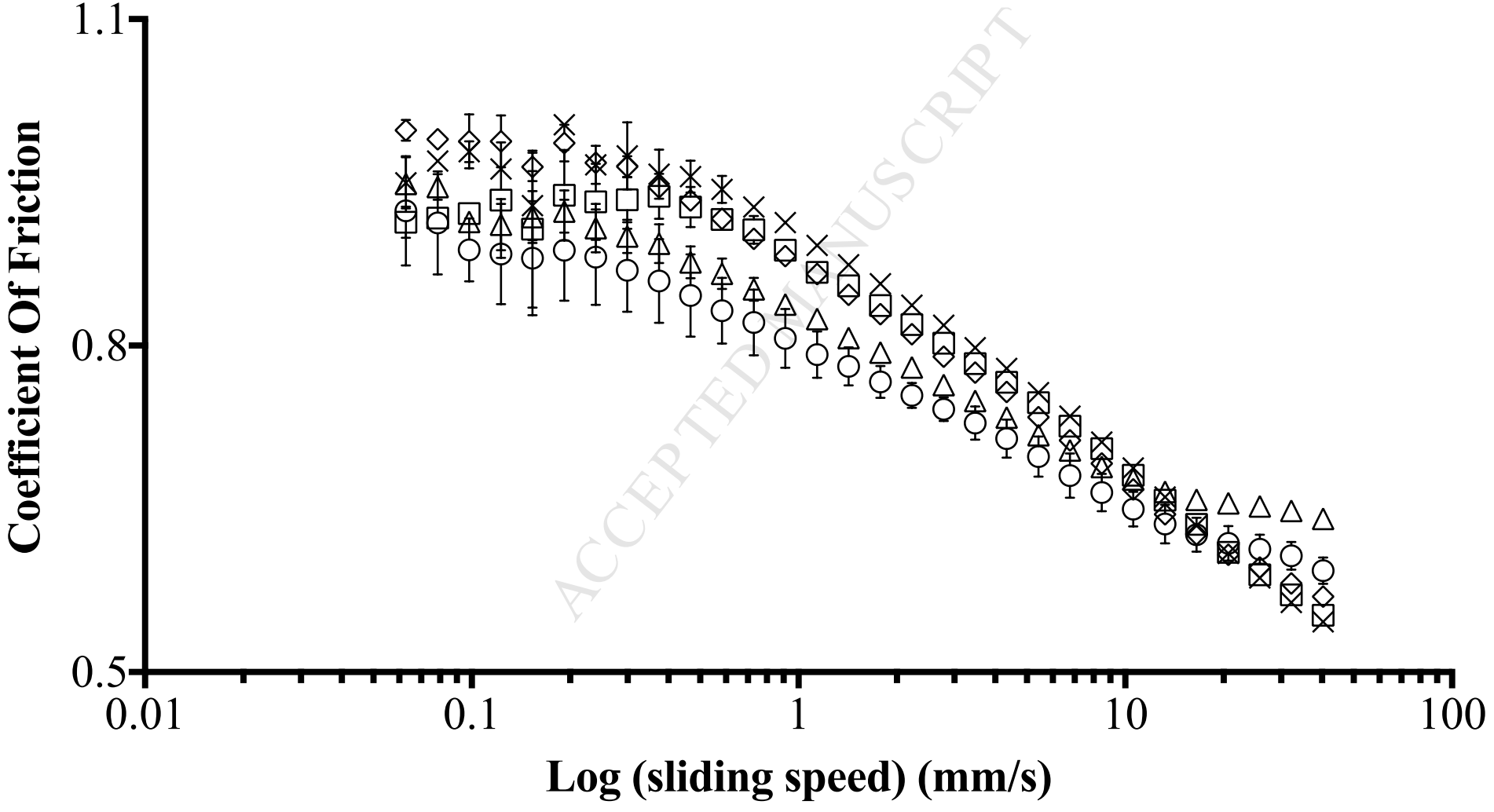
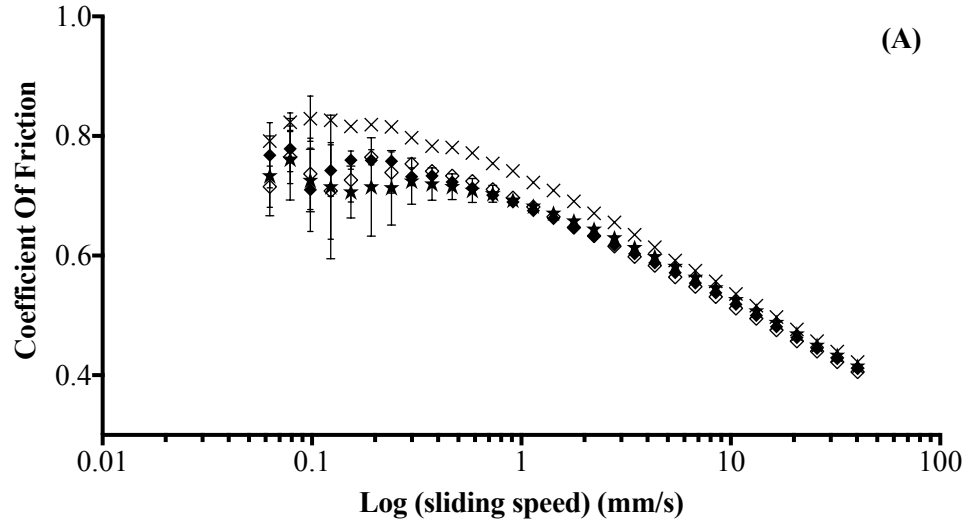
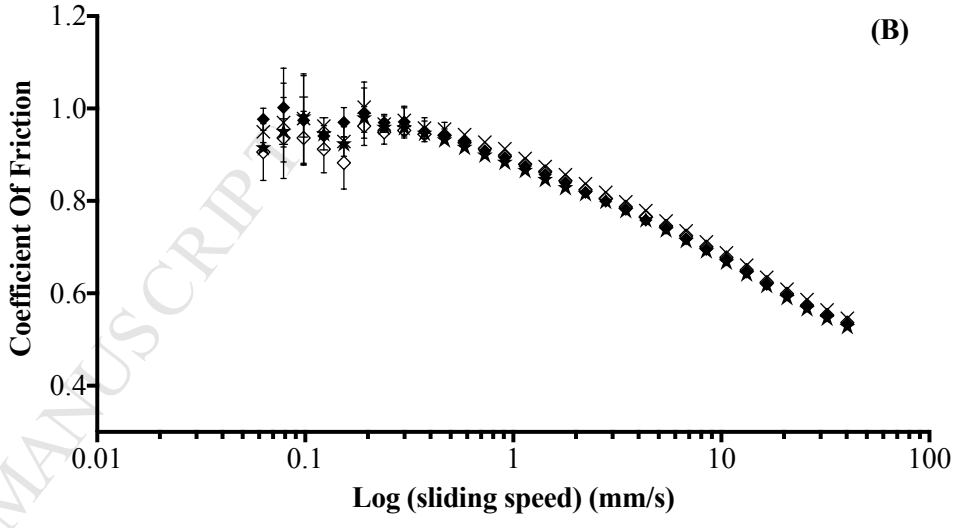
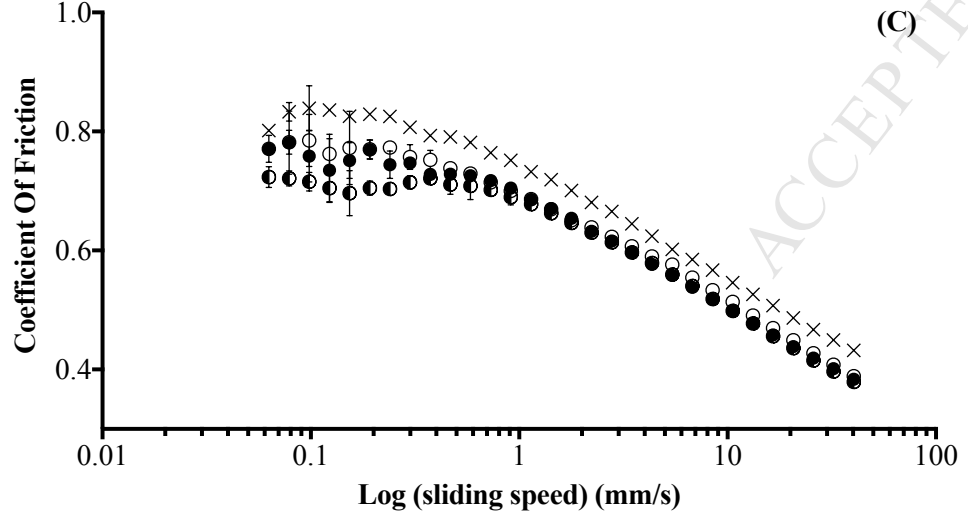


Fig.3.







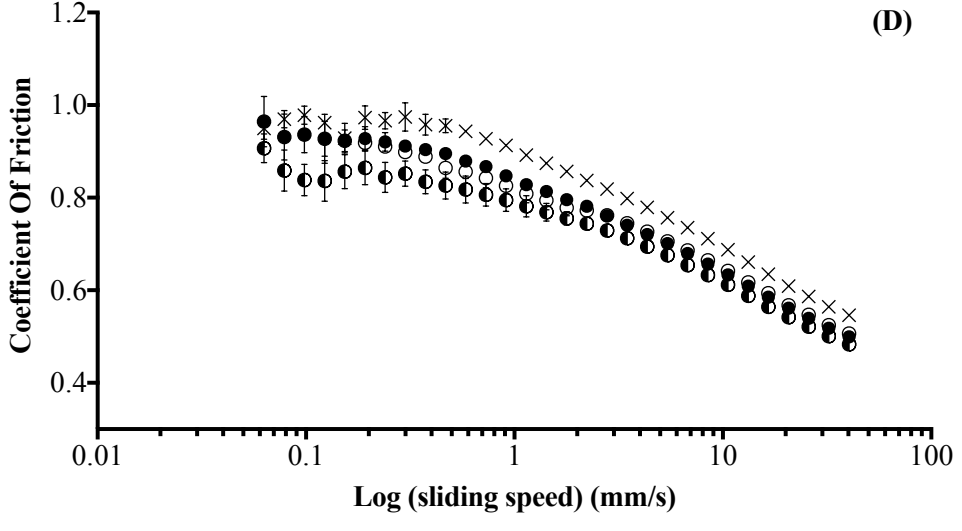
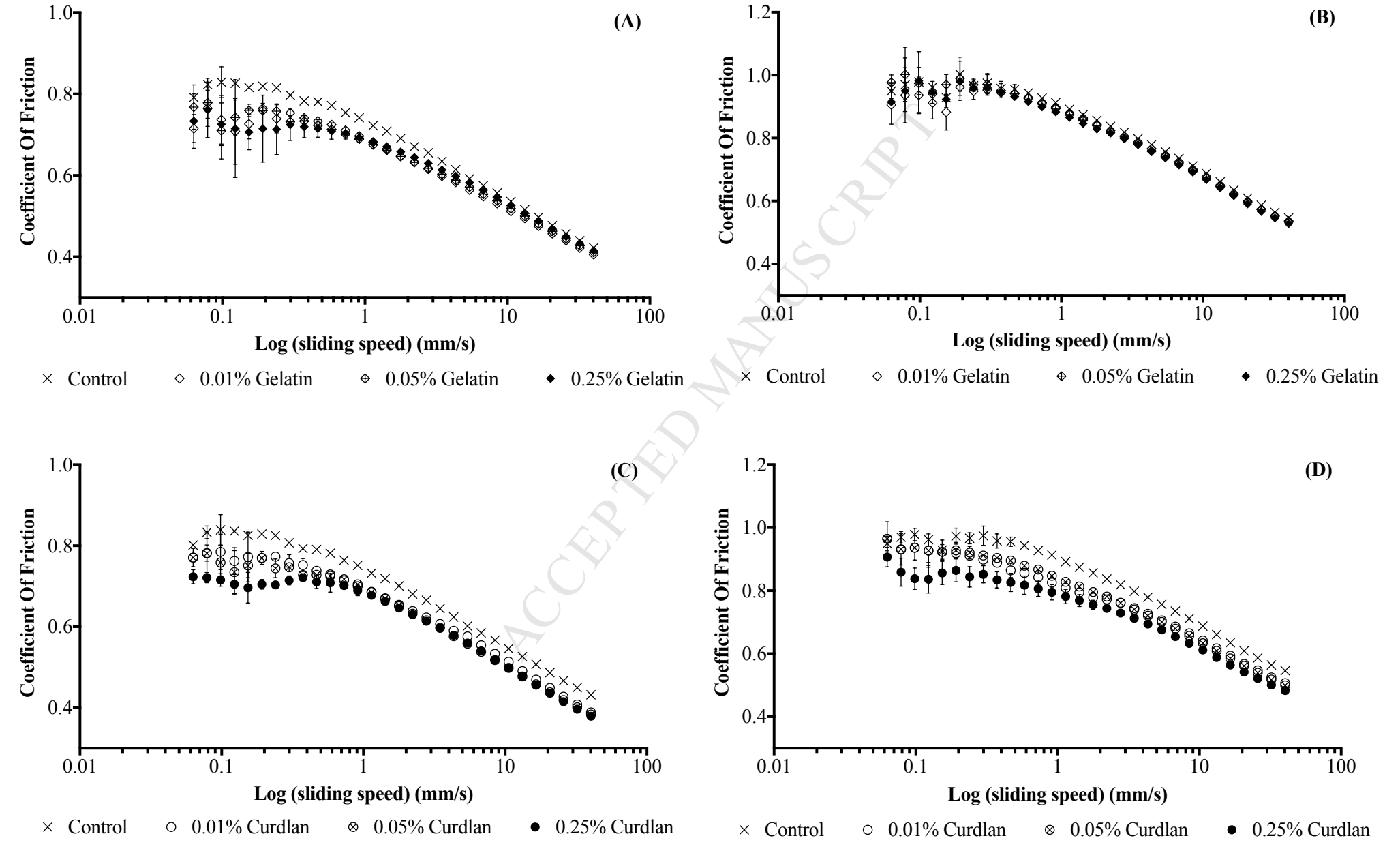


Fig.4.



Highlight

- Addition of WPI improves rheological and tribological property of protein solution
- Hydrocolloids addition improves rheo-tribological property of protein solution
- Protein solution has better lubrication property at low temperature
- Curdlan might be an ideal choice for dairy high protein drinks