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Abstract

Food producers are coming under increasing pressure to reduce fat content of foods. Fat forms a major structuring component in many foods responsible for the desirable texture of foods which are rich in fats. Consumers want healthier foods whilst maintaining desirable sensory properties of these foods and using 'natural' ingredients. In this work we present suspensions of soft gelled protein particles produced by heating induced gelation in shear of proteins. We present egg white fluid gels and compare them with previously characterized WPI fluid gels. Understanding the effects of pH on proteins is important owing to the net charge influencing gelation and gel properties. Soft tribology and rheology were used to investigate textural properties of fluid gels produced and relate these to potential mouthfeel of these systems. Fluid Gels at the IEP were shown to produce aggregated particles of less than 1 µm diameter. These systems produced at the IEP demonstrated greater friction values in the mixed and boundary regimes of lubrication.

Introduction

There is an increasing consumer awareness of health issues associated with diet, due to this there is an increased pressure on manufacturers to reduce the calorie content of foods. There is a desire from consumers for this to be achieved using 'Clean Label' ingredients. Mayonnaises and other emulsion-based products have a high calorific content due to a high percentage of oil. There is demand from consumers for the desirable creamy texture of these emulsions to be maintained, while reducing the calorific content of these products.

Characterizing the desirable creamy textures of emulsions presents challenges owing to the variety of ways in which the texture of foods is perceived in the mouth. The perception of creaminess has been related to thickness, smoothness and slipperiness by Kokini (1987). Thickness of products can be related to the viscosity (Shama and Sherman, 1973), and smoothness has been related to the size of particles. In micro-particulate whey (MPW), it has been shown that particle size is important for the mouth feel of a product. (Singer and Dunn, 1990) showed that particle sizes <0.1 µm have an 'empty' texture, while particle sizes of 0.1–3 µm were perceived as creamy. Particles >3 µm had a gritty sensation. It is noteworthy that the particles discussed in this study are hard particles (Singer and Dunn, 1990).

33 In the latter stages of consumption of 'creamy' products, texture is detected in thin layers between

34 the soft tongue and the rigid palate. In these thin films perception of slipperiness has been shown to

35 correlate with perception of creaminess (Malone et al., 2003). Perceived slipperiness of foods is

36 related to the lubricating properties of foods. This sensation has been correlated to soft tribology

37 measurements (Malone et al., 2003). Thus, tribology may play an important role in predicting

38 perception of texture in high-fat foods.

39 Other studies have used suspensions of soft-gelled hydrocolloid particles were shown to give

40 desirable rheological and tribological properties. Two methods of production for these suspensions

41 of soft gelled particles were presented (Adams et al., 2004, Evans and Haisman, 1980, Frith et al.,

42 2002, Fernández Farrés et al., 2013, Garrec et al., 2013, Holland et al., 2018). The first was a reverse

43 emulsion technique in which hydrocolloid solution was dispersed in oil in a w/o emulsion. This is

44 achieved through the emulsification of a hot hydrocolloid solution, this emulsion is then cooled

45 allowing gelation of the hydrocolloid in the aqueous phase, these gelled particles take on the shape

46 and size of the water droplets. Spherical particles were produced, the size of which can be easily

47 manipulated by the shear rate applied to the emulsion. These droplets were then gelled, washed

48 and re-dispersed; however, this process is time consuming and inefficient. The second method for

49 the production of these soft gelled particles was sheared gelation of hydrocolloids. Suspensions

50 produced by this method have been coined fluid gels. Fluid gels have previously been defined as a

51 suspension of soft gelled particles dispersed in a non-gelled continuous phase. Throughout this

52 paper the term fluid gels is used to describe suspensions of soft gelled particles in a non-gelled

53 continuous phase produced by sheared gelation. This method was shown to produce anisotropic

54 particles. Processing parameters including hydrocolloid concentration, shear environment and rates

55 of cooling can be used to control the properties of these systems. Agar suspensions produced by the

56 reverse emulsion method have been shown to fit the Hertz model by Frith et al. (2002) with a sharp

57 increase in viscosity as the maximum packing fraction of particles is reached. However, for sheared

58 agar fluid gels, an increase in viscosity is observed at considerably lower phase volumes owing to the

59 non-spherical nature of these particles, allowing interparticle interactions (Frith et al., 2002).

60 In recent years, the production of these fluid gel systems from proteins has been investigated

61 (Lazidis et al., 2016, Moakes et al., 2015a). Fluid gels provide a system that has the potential to be

62 controlled through processing parameters (concentration, shear and thermal history). For protein

63 fluid gel production, a heating profile is applied to the protein solution to denature the proteins.

64 However, the use of proteins in acidic products presents new challenges as the gel properties of

65 proteins are influenced by pH (Mudgal et al., 2011, Verheul and Roefs, 1998, Raikos et al., 2007). As

66 the pH is changed, the charges on individual amino acid residues within the polypeptide will change

67   around their individual pK_a. This can lead to protein unfolding due to disruption of intramolecular

68   forces stabilizing the proteins. The net charge of protein molecules has been shown to be important

69   in this, with gels produced away from the isoelectric point giving a fine stranded structure, binding

70   to water, whereas those produced at the isoelectric point (IEP) show a particulate structure with

71   little binding to water (Liu et al., 2010). This is explained by reduced intermolecular repulsion

72   between molecules at the IEP due to the reduced net charge and a reduced affinity for water of less

73   polar molecules. This reduced repulsion increases the rate of aggregation, and thus unfolded protein

74   molecules do not have the chance to order into the fine stranded structures seen away from the IEP

75   (Croguennec et al., 2002).

76   The influence of pH on particle properties around and below their isoelectric point (IEP) is important

77   because the net charge of these proteins will change with reduced pH. The influence of pH on the

78   production of protein fluid gels will be investigated for whey protein isolate (WPI), which was

79   previously characterized (Lazidis et al., 2016), and for egg, which represents a novel system. Egg

80   embodies an exciting functional ingredient for product structuring as it is both natural and easily

81   recognized by consumers.

82   Functionalized proteins offer advantages over more commonly used hydrocolloids owing to their

83   surface active nature. This has been exploited previously for foam stabilization (Lazidis et al., 2016).

84   However, the use of a surface active particulate thickener has not yet been investigated for the

85   production of reduced fat emulsions. The thermo-irreversible nature of protein gelation offers

86   further advantages over fluid gel systems produced using thermoreversible hydrocolloids as these

87   systems will be more resistant to any heat processing post production.

88

Materials and methods

Solution preparation

WPI

Whey protein isolate (WPI) (Davisco) was used. Protein (89.4%), ash (3.0%), Moisture (0.4%) and lactose (0.3%). A stock Solution of 15% w/w was produced by gradual addition of powder to gently stirred distilled water and gently stirred by means of a magnetic stirrer for 12 hours at 6 °C. 0.01% sodium azide was added to this stock solution to prevent bacterial growth. Stock solution was stored in a fridge at 6 °C until use. This stock solution was stored for a maximum of 30 days. Solutions for fluid gel production were prepared from this stock solution by mixing with 2 M acetic acid to the desired pH. These were then made up to the appropriate volume with distilled water to give a final concentration of 12% w/w and further mixed for 10 minutes. Three different pH solutions were prepared pH_{Native} in which the pH was not adjusted of the WPI solution dispersed in distilled water, for WPI this was pH 8. pH 5 this is well documented as the IEP of WPI (Demetriades et al., 1997), and pH 3.5 below the IEP.

Egg white

Chicken eggs were purchased from a supermarket and separated by hand. The egg whites were heated to 65 °C and held at this temperature for 10 minutes while stirring gently. The resultant solution was then passed through a 1 mm sieve. This process removed ovomucoid and ovotransferrin from the egg white; ovotransferrin is the second most prevalent protein in egg white after ovoalbumin. Ovotransferrin was shown to inhibit fluid gel formation. This treated egg white was then stored in a fridge for no longer than a week before use. The treated egg white was mixed with 2 M acetic acid to the desired pH then made up to a final dilution of 75% w/w with distilled water. Three different pH solutions were prepared pH_{Native} in which the pH of the solution was not adjusted, for the treated egg white this pH was 7.5. A pH 4.5 solution was prepared as this is documented as the IEP for Ovoalbumin the main protein in egg white (Stevens, 1991), as for WPI pH 3.5 was used as it is below the IEP of ovoalbumin. Final protein concentration in the egg white sample post heat treatment and dilution was estimated using absorbance at 280nm in line with methods outlined by Ross (1991). The final protein concentration was calculated to be between 6.9 and 7.6% w/w.

Fluid gel preparation

A vane and cup geometry was used on a Malvern Kinexus Rheometer. The solutions were allowed to equilibrate to 40 °C for 10 minutes. A constant shear rate of 500 s^{-1} was applied. Samples were heated while sheared from 40–90 °C at a rate of 2 °Cmin^{-1}. They were then held at 90 °C for 2 minutes while remaining under shear, followed by a cooling step from 90 °C to 5 °C at a rate of 4 °Cmin^{-1}. Samples were then stored in a fridge for 24 hours before testing. The heating and cooling rates used correlate closely with those used previously in pin-stirrers for larger scale production of

125   WPI fluid gels (Lazidis et al., 2016). The hold at 90 °C was used to observe any time dependent

126   effects from heating that continued to occur.

Optical microscopy

128   An optical microscope (Leica Microsystems, UK) was used to directly observe particles produced for

129   fluid gels. Differential interference contrast (DIC) was used to increase the contrast of particles.

130   Samples were diluted with either distilled water or appropriate concentrations of acetic acid to

131   maintain the original pH of samples. Samples were gently inverted 10 times to mix them. A drop of

132   this diluted sample was placed on a slide and covered with a coverslip. 20X and 40X magnification

133   was used to observe the particles.

Particle Sizing

135   Static Light scattering measurements were used to investigate particle size distributions. For this a

136   Malvern Mastersizer 2000 with hydro SM manual small volume dispersion unit (Malvern

137   Instruments, UK) was used. Each repeat consists of 3 measurements, 3 repeats were conducted. A

138   refractive index of 1.456 was used, with a stirrer speed of 800rpm in reverse osmosis water.

Rheology

Viscometry

141   A 40 mm sand-blasted parallel plate geometry was used for this to minimise slip in these

142   experiments. Slip is expected for suspensions due to particle depletion at the shear surfaces.

143   Equilibrium shear experiments were used with up to 2 minutes allowed for samples to reach

144   equilibrium at each shear rate 0.1 s^{-1} – 100 s^{-1}. Samples were tested at 25 °C with a gap of 1 mm.

Amplitude sweeps

146   A frequency of 1Hz at 25 ^{o}C was used for amplitude sweeps. These were obtained using a sand-

147   blasted parallel plate geometry to minimise the effects of slip and repeated 3 times.

Frequency sweeps

149   Quiescent gels were produced by heating and cooling protein solutions on a cone and plate

150   geometry. A cone and plate geometry was used as it applies an even strain across the sample.

151   Solutions were heated from 40 °C to 90 °C, held at 90 °C and then cooled to 5 °C to denature and gel

152   the proteins. These quiescently set gels were then left to stand for 20 minutes before equilibrating

153   to 25 °C and frequency sweeps commencing. A strain of 0.05% was used for these having been

154   determined to be within the LVR (Linear Viscoelastic region) of these quiescent gels.

Determination of ζ-Potential

156   For determination of ζ-potential a Zetasizer (Malvern Instruments, UK) was used. Samples were

157   prepared by dilution 10 times in their respective continuous phase to reduce the particle

158 concentration to a measurable range. The samples were diluted in their own continuous phase to

159 maintain the properties of the systems whilst reducing the concentration of the dispersed phase.

Phase volume

161 To calculate the phase volume of fluid gels produced the elastic modulus of diluted and

162 concentrated fluid gels was observed. Elastic modulus was measured at 1Hz and 1% strain using a

163 1mm gap in a sand blasted plate geometry. Elastic modulus of suspensions of soft particles is

164 expected to plateau at the maximum packing fraction of particles thus from this the phase volume as

165 a function of the maximum packing fraction can be calculated. To reduce the concentration of fluid

166 gels they were diluted in distilled water. To increase the phase volume of fluid gels they were

167 centrifuged and the supernatant removed. Samples were centrifuged at speeds from 500-40,000g

168 for 20 minutes to achieve this.

Tribology

170 An MTM2 (Mini Traction Machine, PCS Instruments, UK) tribometer was used for tribology

171 measurement. This consists of a ball rolling on a disk, normal force, speed and slide–roll ratio (SRR)

172 can be controlled.

173 A mixed sliding and rolling contact was used in this work with an SRR of 50%. SRR can be defined as:

174
$$SRR = \frac{U_{disc} - U_{ball}}{U}$$

175

176 Where U represents the average speed at the contact for each component. A 3N normal force was

177 used. For these experiments, a stainless steel ball-silicone elastomer disk tribopair was used as

178 outlined previously (Mills, 2012). This tribopair and these conditions have been previously shown by

179 Malone et al. (2003) to correlate to mouth feel in the mixed regime of lubrication. In each test

180 Stribeck curves were measured over a speed range of 1–1,000 mms^{-1} with ascending and descending

181 runs repeated three times (6 curves total). Tests were performed at 25 °C. These tests were

182 repeated three times.

Results and discussion

Fluid gel preparation
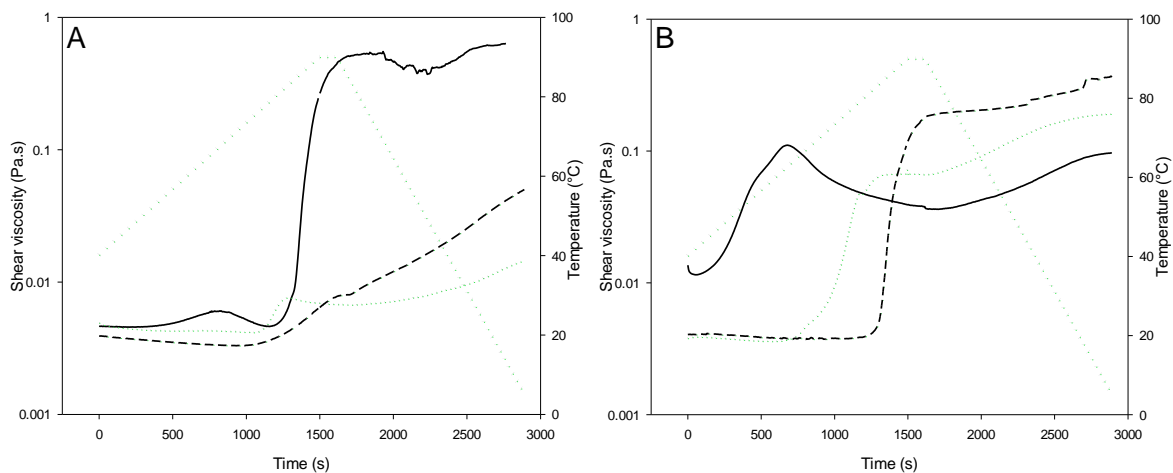
Figure 1. Viscosity profiles during protein fluid production showing sheared gelation of WPI and heat treated egg. A) WPI (pH 3.5 ——; pH 4.9 (IEP) ·····; pH 8 ---; Temperature ····) B) Heat treated egg (pH 3.5 ——; pH 4.5 (IEP) ·····; pH 7.5 ---; Temperature ····). Measurements were made using a cup and vane geometry at 500 s^{-1} while a heating and cooling profile was applied. A heating rate of 2 $^{o}Cmin^{-1}$ was applied, followed by a 2-minute isothermal step, then a cooling rate of 4 $^{o}Cmin^{-1}$ Curves represent an average of three repeats; error bars are not shown for clarity.

A viscosity profile at 500 s^{-1} for protein solutions through fluid gel preparation was produced (Figure 1). This enabled monitoring of the ordering process throughout heating and cooling in order to understand how manipulating protein charge affects ordering. This is due to viscosity being expected to increase as ordering of proteins occurs during heating.

Initially there is little change in viscosity observed with increasing temperature, this is followed by a sharp increase in viscosity for all samples except for WPI pH 8, this sharp increase in viscosity during fluid gel production has been observed previously at the gelling temperature of hydrocolloids (Ellis et al., 2017). This observed increase in viscosity has been attributed to protein aggregation by Lazidis et al. (2016). Particles are expected to form through a nucleation and growth mechanism where by small particles aggregate producing larger particles. The growth of these particles will be limited by break up in the shear field thus final particle size is an equilibrium between particle growth through aggregation due to heat induced gelation and break up due to shear (Norton et al., 1999).

The temperature at which the sharp increase in viscosity (figure 1) attributed to aggregation occurs in egg was shown to decrease with decreasing pH; however, little change in the temperature of aggregation was found for WPI.

WPI pH 3.5 shows a greater rate of increase in viscosity than WPI pH 8, this can be explained by the difference in the rate of gelation. Gelation rate is controlled by two stages, protein denaturation and

209 protein aggregation. Changes in pH will influence aggregation rates, as net charges and charge
210 distribution of the proteins is altered. This will also be influenced by the differences in structure
211 between the egg and WPI.

212 For egg at pH 3.5 a reduction in viscosity with increasing temperature is observed after the initial
213 increase in viscosity. This can be explained by the difference in gelation temperature, because these
214 systems were heated ~40 °C above the gelation temperature. As proteins are further heated above
215 the gelation temperature further denaturation of proteins is expected to occur, as this happens
216 protein-protein interactions will be favoured over protein-water interactions.

217 Both whey and treated egg white produced fluid gels (suspensions of distinct gelled particles in a non-
218 gelled continuous phase) when heated and cooled under shear. Stability of these systems was
219 monitored by eye. Fluid gels produced at pHs away from the IEP for both egg and WPI were stable for
220 a month after production. However, those produced at the IEP of both proteins sediment out over 48
221 hours. In these systems a clear liquid was observed with an opaque white sediment. This will be
222 examined further in the section 'phase volume effects'.

223 The influence of reducing the pH of egg fluid gels produced at pH 7.5 was investigated. For this the pH
224 was reduced form pH 7.5 to 4.5 post production. Within an hour of this pH change a white sediment
225 formed with a clear liquid above it. This sediment could not be easily dispersed. This is explained as
226 due to the reduced net charge of particles upon reduction of pH to the IEP of egg ovalbumin.

227 Particle shape and size determination

228 Light microscopy was used to directly observe particles and to determine particle size and shape
229 (Figure 2). This was important as the contribution of particle morphology to rheology has been shown
230 previously by Wolf et al. (2001). Samples were diluted between 5X and 20X in the appropriate
231 concentration of acetic acid or distilled water to enable observation of individual particles. Both WPI
232 and egg produced distinct particles at all different pHs tested.

233 For both whey and egg at their respective IEPs, the particles appear to be made up of smaller particles
234 that have aggregated together. These smaller particles agreed with observations of small globular
235 particles produced by Lazidis et al. (2016), who showed small individual spherical particles of WPI were
236 produced when fluid gels were prepared at pH 5. However, during their production, a dilution step in
237 shear was used to prevent secondary particle aggregation. It would follow that the large aggregates
238 of apparently smaller spherical particles observed here were produced through secondary
239 aggregation of smaller particles. Lazidis et al. (2016) attributed these smaller particles to reduced
240 electrostatic repulsion between the protein molecules during denaturation and aggregation.
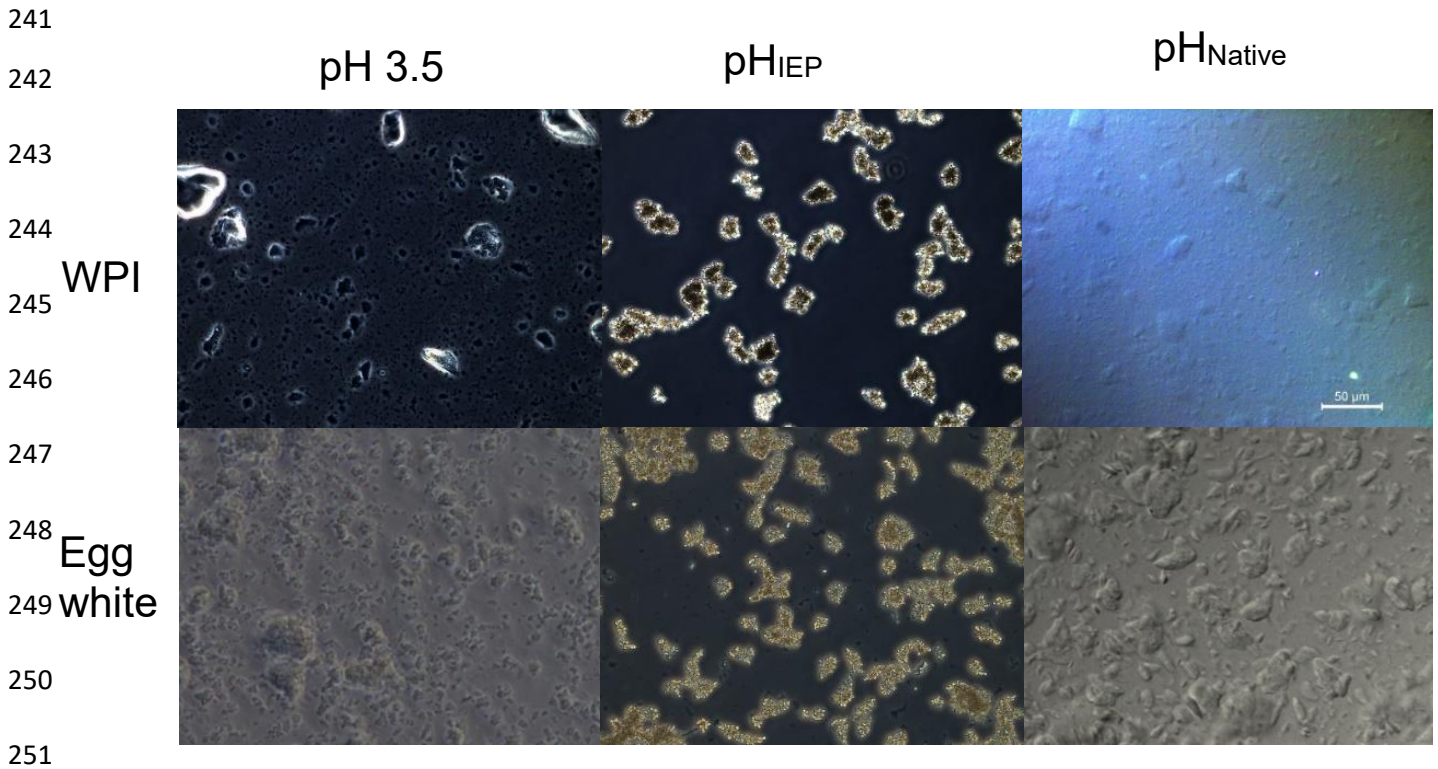
241

242

243

244

245 WPI

246

247

248 Egg

249 white

250

251

252

255   Due to this aggregated appearance of particles further investigation to suggest primary particle

256   sizes. Although these measurements are not directly comparable to the effective particle sizes, they

257   will provide further insight into primary particle sizes. Figure 3 shows static light scattering

258   measurement. For static light scattering measurements particles are dispersed in water with manual

259   dispersion unit, the shear from this is not expected to break up primary particles however will

260   disrupt aggregation.

261   For both egg and WPI fluid gels produced at native pH (pH 7.5 and 8 respectively) produced the

262   largest particles with a broad size distribution. For both egg and WPI particles produced at pH 3.5

263   produced particles <100um.   These broad size distributions produced will be contributed to by

264   inconsistencies in the shear field within the vane geometry, increased shear rates could be used to

265   reduce particle sizes produced, however this was not possible with the setup used due to foaming

266   issues.

267   Both egg and WPI fluid gels produced at their respective IEP show the smallest particle size, with all

268   particles <10 µm. The smaller particles observed at the IEP correlate with a previous suggestion that

269   particles observed at the IEP by microscopy are aggregates of smaller particles.

270   For both egg and WPI at their respective IEP bimodal distributions are observed with peaks at 0.2 µm

271   and 0.9 µm. Smaller particles produced at the IEP of proteins is explained due to the expected

272   dewatered nature of proteins at their IEP where they are expected to have the lowest net charge.

273
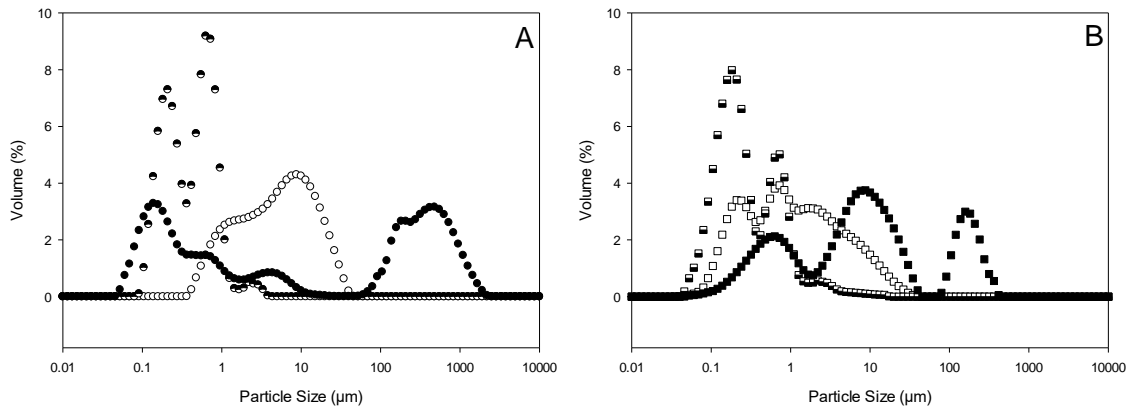
Figure 3. Size distribution of protein fluid gel particles produced at different pH A) WPI (pH 3.5 ○ ; pH
4.9 (IEP) ◑; pH 8 ●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ◩; pH 7.5■). Values represent an average of
three repeats, each repeat consisting of three measurements.

277   Fluid gel rheology

278
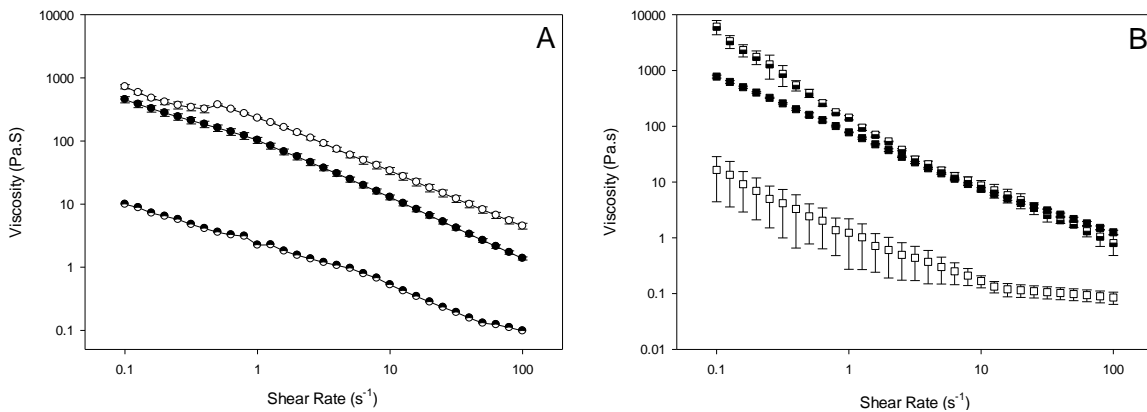
Figure 4. Viscosity profiles of protein fluid gels produced at different pH A) WPI (pH 3.5 ○; pH 4.9 (IEP)
◑; pH 8 ●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ◩; pH 7.5 ■). Measurements were taken at least 24 hours
after production. A sand blasted parallel plate geometry was used to reduce slip, and equilibrium
measurements take. All measurements were undertaken at 25 °C.

283   Shear rate-controlled equilibrium measurements were used to analyse the shear rheology to

284   understand the flow behaviour of these fluid gels. Shear thinning behaviour typical of particle

285   suspension rheology was observed in all fluid gels produced here. The shear thinning nature of

286   suspensions is due to ordering of particles in flow (Krieger and Dougherty, 1959). At low shear rates,

287   when little flow is occurring, particles can interact; as shear rate increases and flow is induced, these

288   interactions break down (Adams et al., 2004).

289   WPI fluid gels produced at the IEP have a much lower viscosity than those produced above and below

290   the IEP. This is in agreement with understanding of WPI gel structures at these pHs. At the IEP, owing

291   to the reduced repulsion between molecules during gelation, aggregation can occur before ordering

292   of the proteins, forming a weaker gel structure. Softer particles will be able to deform and flow past

293   one another more easily than more rigid particles reducing the viscosity of the system. This rapid

294   aggregation leads to a particulate gel structure with limited water binding, for gels produced away

295   from the IEP a fine stranded gel structure is expected as protein molecules order into strands due to

296   the reduced aggregation rate. Particulate gel structures will have a more porous structure with a

297   reduced elastic modulus than those with fine stranded gel structure.

298   For egg a different trend was observed with fluid gels produced at the IEP showing a higher viscosity

299   at low shear rates, however fluid gels produced at pH 7.5 had the highest viscosity at high shear rates.

300   Fluid gels produced at pH 3.5 showed the lowest viscosity at all shear rates. For egg at pH 3.5 a

301   plateauing of viscosity is observed. Suspensions are expected to transition to shear thickening phase

302   as particle jamming occurs this is as at higher shear rates particles do not have time to flow past one

303   another. Egg pH 3.5 is the only sample for which the shear rates tested were high enough for this

304   transition to be observed. This observed difference between the behaviour of egg and WPI is likely

305   due to egg fluid gels produced at the IEP having a phase volume close to the maximum packing fraction

306   this will be shown in figure 7. Suspensions with greater phase volumes will have a higher viscosity due

307   to more particles-particle interactions due to the increased volume occupied by the dispersed phase.

308   ## Particle properties
309   Quiescent gel elastic modulus and zeta potential were used to further probe particle properties. Use

310   of quiescently set gel properties to represent particle properties has been shown previously (Garrec

311   et al., 2013).

312   *Table 1. Zeta potential values of fluid gel particles of egg and whey produced at different pH. For Egg*
313   *pH_{IEP} = 4.5 and pH_{Native} = 7.5. For WPI pH_{IEP} = 4.9 and pH_{Native} = 8.*

	pH 3.5	pH_{IEP}	pH_{Native}
Egg ζ-potential (mV)	10.604 ±0.957	5.697 ±1.599	-18.550 ±3.689
WPI ζ-potential (mV)	11.267 ±1.320	-0.056 ±0.382	-14.950 ±3.200

314

Table 1 shows the zeta potential of fluid gel particles. This is used to investigate the net surface charge of the particles in the fluid gel systems. The net surface charge of protein molecules is expected to be ~0 at its IEP, <0 at pH above the IEP and >0 at pH below the IEP, which is due to protonation and deprotonation of amino acid groups along the protein. Understanding the net charge of particles was important to understand what electrostatic interactions were occurring. This trend was observed for WPI as would be expected. However, for egg, the zeta potential of the particles at the IEP was positive. This can be attributed to the egg consisting of a mixture of proteins, with varying IEPs. Lysozyme would still be present in small quantities within the mixture of proteins with an IEP of 11 (Price et al., 1999), thus lysozyme protein molecules will have a positive charge at all pHs investigated here.

The reduced net charge on the particles may contribute towards the observed sedimentation of fluid gels produced at pH_{IEP}, with reduced electrostatic repulsion between the particles. Formation of aggregates and sedimentation of WPI in solution around pH_{IEP} has been shown (Ju and Kilara, 1998). This aggregation was attributed to the reduced electrostatic repulsion between protein molecules and the reduced the proteins affinity for water. This reduced electrostatic repulsion enabled hydrophobic interactions to dominate, leading to aggregation. This would follow with the reduced net charge shown on particles for fluid gels produced at pH_{IEP}. The contribution of particle charge to instability was further supported by the sedimentation of egg fluid gels produced at native pH then adjusted to pH 4.5 post production. However, the reduced affinity of protein molecules for water at their IEP may also lead to a reduced phase volume for fluid gels produced at pH_{IEP}.
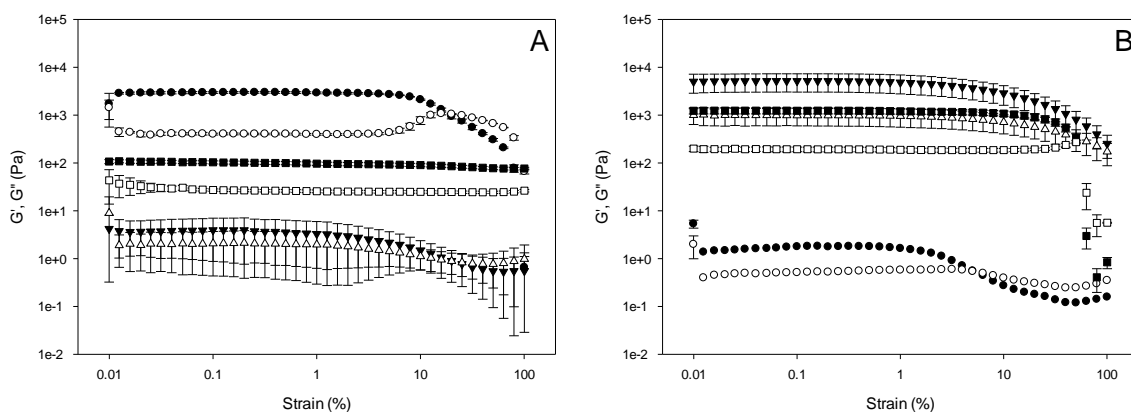
Figure 5. Strain controlled amplitude sweeps for protein fluid gels showing A) WPI (pH 3.5 (G' ●, G" ○); pH 4.9 (IEP) (G' ▼, G" △); pH 8 (G' ■, G" □)) B) Heat treated egg (pH 3.5 (G' ●, G" ○); pH 4.5 (IEP) (G' ▼, G" △); pH 7.5 (G' ■, G" □)). A sand-blasted parallel plate geometry with a frequency of 1 Hz was used to reduce slip. Values represent an average of three repeats with error bars showing one standard deviation of these repeats.

340 In order to observe inter-particle and particle packing properties of these systems, controlled strain

341 mode amplitude sweeps were performed. This is shown through the elastic and viscous components

342 G' and G", respectively these are shown in figure 5.

343 All the fluid gel systems showed a linear viscoelastic region (LVR) in which G' and G" are independent

344 of strain (Figure 5); this was as expected for concentrated suspensions. The observed linear

345 viscoelastic region was typical of solid like behaviour with G' ten times greater than G" and typical for

346 interconnected structures of concentrated suspensions.

347 WPI fluid gels produced at pH 3.5 showed a greater G' in the LVR than whey fluid gels produced at

348 native pH with G' values an order of magnitude higher than those for the fluid gel produced at pH 8.

349 WPI fluid gel produced at the IEP (pH 4.9) showed lower G' values, correlating with the lower G'

350 observed for the quiescent WPI gel produced at pH 4.9. This is because less rigid particles deform

351 more easily.

352 For egg, a different trend in G' in the LVR was observed, with fluid gels produced at the IEP showing

353 the highest values. Egg fluid gels produced at pH 3.5 show G' values three orders of magnitude lower

354 than those produced at native pH. This is expected due to the difference between the gelation

355 temperature of egg at pH 3.5 and the temperature heated to. This favours protein-protein interactions

356 producing dewatered particles giving a sparser network, the voids in this structure will reduce the

357 elastic response of these suspensions. This trend in G' for egg fluid gels correlated with G' values

358 observed for quiescent gel produced at this pH.

359

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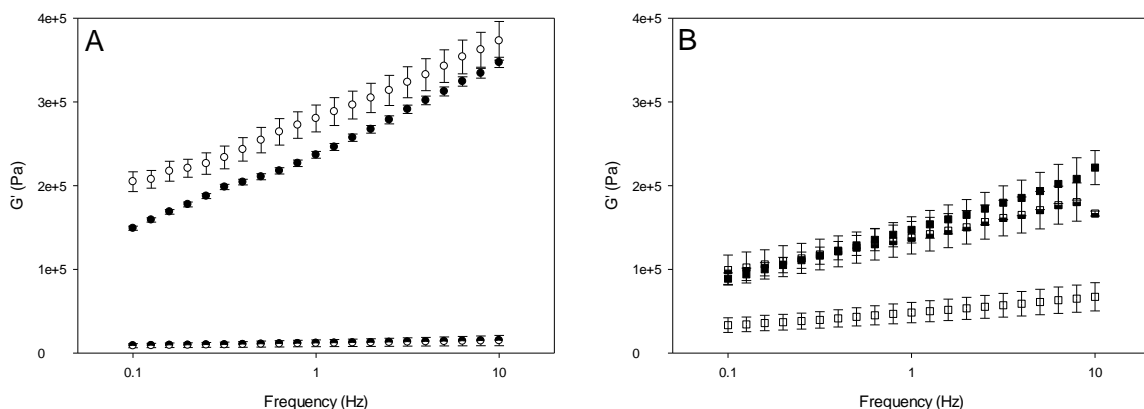
Figure 6. Frequency sweeps of quiescently set protein gels at different pH used to represent particle properties of fluid gels at different pH A) WPI (pH 3.5 ○ ; pH 4.9 (IEP) ◑; pH 8●) B) Egg (pH 3.5□; pH 4.5 (IEP) ◩; pH 7.5 ■). Quiescent gels were set within a cone and plate geometry before the frequency sweeps commenced. 0.05% strain shown to be in the LVR for these gels was used. Values represent three repeats with error bars showing one standard deviation.

366   Quiescent gels were used to represent the properties of the particles in the fluid gel systems.

367   Quiescent gels were produced within the cone and plate geometry prior to testing. All gels tested

368   showed a frequency-dependent nature in the frequency range tested. As frequency increased, the

369   time for energy to dissipate through the system was reduced, evidenced by a higher elastic modulus.

370   The structures of these gels will not be directly observed here as this has been well documented

371   elsewhere (Katsuta et al., 1990, Ould Eleya et al., 2004, Ferry, 1948, Gossett et al., 1984, Handa et al.,

372   1998, Hermansson, 1979, Kiosseoglou, 2003).

373   For egg, little difference was shown in the elastic modulus of the gels produced at pH 7.5 and pH 4.5;

374   however, the gel produced at pH 3.5 had a lower elastic modulus. Egg white gels below the IEP have

375   been shown to have a more porous structure than gels produced at or above the IEP (Handa et al.,

376   1998). This porous structure would give a weak structure and thus a reduced elastic modulus.

377   WPI gels produced at pH 3.5 showed the greatest elastic modulus, and those produced at the IEP (pH

378   4.9) showed the lowest elastic modulus. Notably, quiescent gels produced at the IEP were cloudy in

379   appearance, which is indicative of an aggregated particulate structure (Verheul and Roefs, 1998). This

380   aggregated particulate structure will be dewatered, whereas for gels produced away from the IEP a

381   fine stranded network is expected. This fine stranded network orders water giving a more rigid gel.

382   These differences in gel structure are due to the rate of aggregation during gelation, away from the

383   IEP electrostatic repulsion between molecules reduces the aggregation rate allowing molecules to

384   order into the fine stranded network, however at the IEP when the net charge of the molecules is 0

385   aggregation rates are much higher.

386   For both the whey and egg systems, the quiescent gel elastic moduli showed similar trends to those

387   of their fluid gels, validating the use of quiescent gel modulus to represent particle moduli.

388   Phase volume effects

389   Small amplitude oscillatory measurements were used to observe elastic modulus with changing

390   concentration in order to understand potential contributions of phase volume to the observed

391   properties of fluid gels. Gels produced at the IEP of proteins are expected to produce more dewatered

392   particles, thus occupying a lower phase volume.

393   Figure 7 shows elastic modulus of samples, concentration has been normalized, a concentration of 1

394   is the concentration of the fluid gels when produced with not dilution or concentrating. For all

395   samples that as concentration increases, elastic modulus increases up to a crucial concentration at

396   which elastic modulus plateaus. This has been previously observed for fluid gel particles from whey

397   and agarose (Frith et al., 2002, Moakes et al., 2015b). Adams et al. (2004) explained this plateau as

398   being a result of elastic modulus being dominated by particle modulus once the maximum packing

399 fraction is reached. For monodisperse hard spheres, the maximum packing fraction is 0.62 (Einstein,
400 1906).

401 Egg fluid gels produced at the IEP and native pH were approximately at the maximum packing
402 fraction, as shown by increasing G' up to 1.0 and plateauing at higher concentrations. For egg
403 produced below the IEP, the concentration was below the maximum packing fraction with G'
404 plateauing at 1.35, amounting to an initial phase volume of Φ/Φ_{max} ~0.74. This reduced phase
405 volume for egg fluid gels below the IEP is explained as due to the reduced gelation temperature at
406 pH 3.5 of the egg system. As the egg is heated above the gelation temperature further protein
407 denaturation will lead to the favouring of protein-protein interactions over protein-solvent
408 interactions, reducing the water binding of the gel particles.

409 WPI fluid gels produced below the IEP were approximately at the maximum packing fraction, and
410 those produced below the IEP were above the maximum packing fraction with a phase volume of
411 Φ/Φ_{max} ~1.05. For suspensions of gelled particles the maximum packing fraction can be exceded as
412 particles are deformable. WPI fluid gels produced at the IEP did not plateau below a concentration
413 of 1.75, showing a reduced phase volume (Φ/Φ_{max}< 0.57). This reduced phase volume of fluid gels
414 produced at the IEP can be explained by the reduced affinity of the protein molecules for water at
415 the IEP.

416
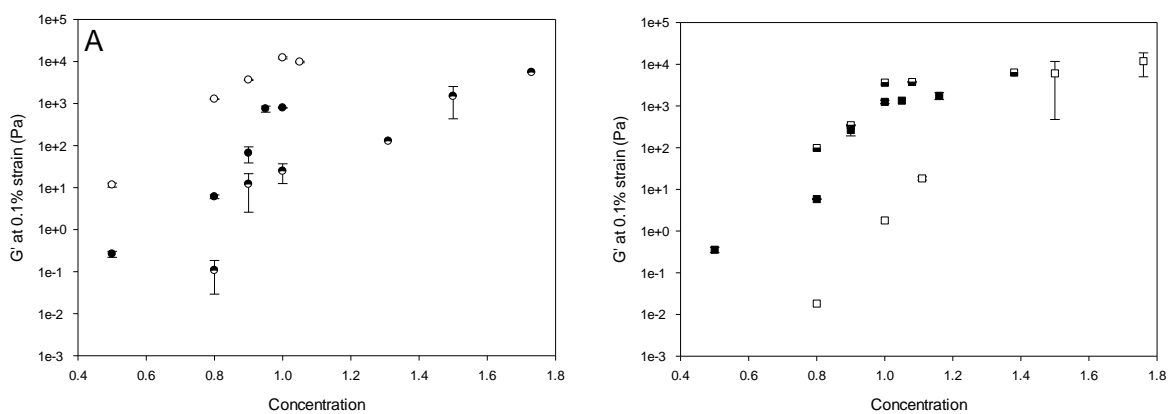
417

418 *Figure 7. Elastic modulus against concentration for protein fluid gels produced at different pH. A) WPI*
419 *(pH 3.5 ○ ; pH 4.9 (IEP) ◓; pH 8●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ◲; pH 7.5■). Concentration has*
420 *been normalized, a concentration of 1 is defined as the concentration of fluid gels post production.*
421 *For concentrations below 1, fluid gels were diluted with the appropriate concentrations of distilled*
422 *water or acetic acid. For concentrations above 1, samples were centrifuged to remove liquid from the*
423 *continuous phase. Values represent an average of three measurements, with error bars representing*
424 *one standard deviation. 0.1% strain was used; this was shown to be within the linear viscoelastic*
425 *region at a frequency of 1 Hz.*

Tribology

The friction and lubrication properties of the fluid gel systems were examined (Figure 8). Tribology was used to evaluate the potential performance of these fluid gel systems for the use in food systems.
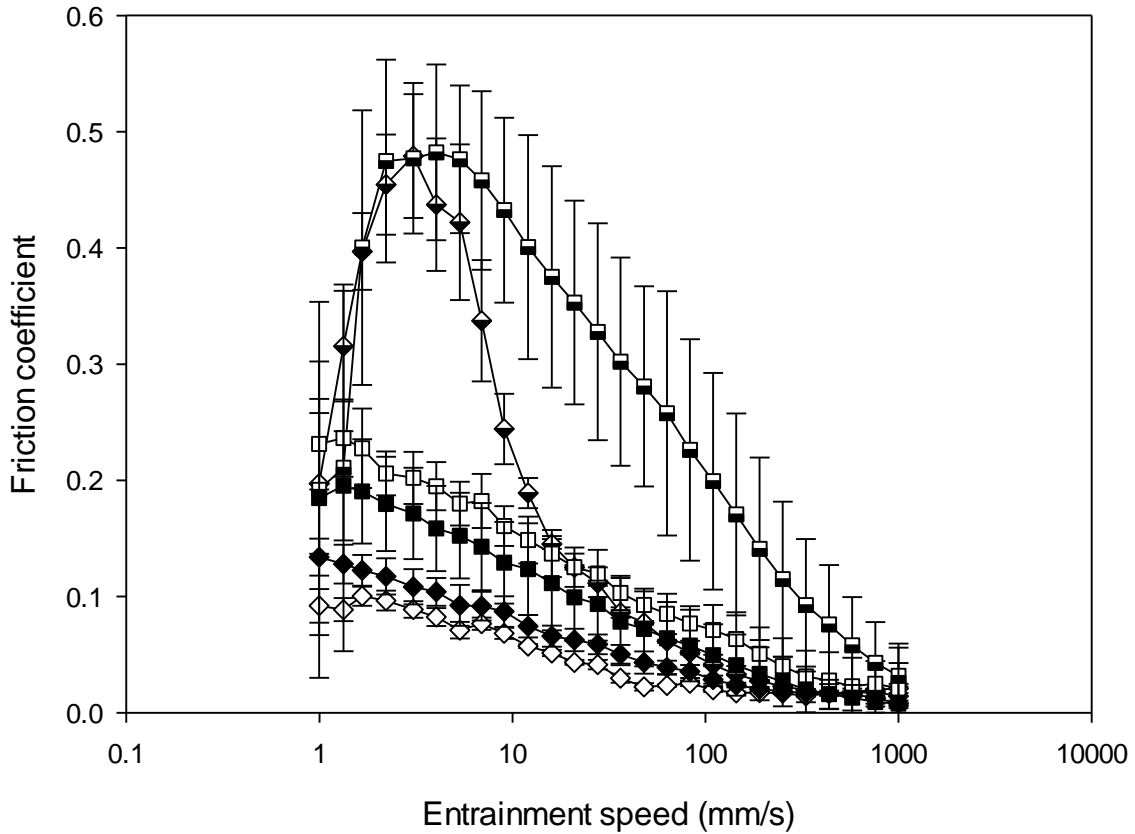
Figure 8. Stribeck curves for egg (pH 3.5 □; pH 4.5 (IEP) ▣; pH 7.5 ■) and WPI (pH 3.5 ◇; pH 4.9 (IEP) ◆; pH 8 ◆). Fluid gels produced at different pH using a stainless steel ball–silicone elastomer disk tribopair at 50% SRR.

WPI fluid gels produced at pH 3.5 were shown to be the most lubricating fluid gel system examined over the speed range tested (Figure 8). For this system, the mixed lubrication regime was observed with friction coefficient decreasing with increasing entrainment speed. For WPI at both pH 3.5 and 8, typical Stribeck behaviour is observed. However, for particles produced at the IEP, a peak in friction coefficient is observed between speeds of 1 mm s^{-1} and 10 mm s^{-1}. Once maximum friction was observed at 5 mm s^{-1}, particles entered the mixed regime and friction decreased rapidly as speed was increased. Above 100 mms^{-1}, friction behaviour is similar to that of particles produced at pH 3.5 and 8.

For egg fluid gels produced at pH 3.5 and 7.5, a peak in friction was observed, but at an entrainment speed of approximately 2 mm s^{-1}. At speeds above 2mm s^{-1} friction coefficient decreased with increasing speed typical of the mixed regime and tends to friction coefficient values similar to WPI fluid gels. As with WPI, for the suspension produced at the IEP, a different behaviour was found. From

446   1 mm s^{-1}, friction coefficient increases as speed increases. A maximum was observed at a similar point

447   to WPI at a speed of approximately 5 mm s^{-1}, with a similar friction coefficient value of ~0.5. As speed

448   increases above 5 mm s^{-1}, friction coefficient decreases at a slower rate than for WPI fluid gels. At

449   1000 mm s^{-1}, all fluid gels show similar friction values.

450   It is clear particles produced at the IEP lubricated different to those produced at other pH.

451   This peak in friction as lubrication transitions from the boundary to the mixed regime has been shown

452   previously for agarose fluid gels of particle size ~100µm greater than the roughness of the surfaces

453   used. This peak may be due to the entrainment of the particles when the gap is smaller than the

454   observed particle size (Gabriele et al., 2010). This observed peak may also be due to aggregation of

455   particles, increasing effective particle size of particles at the IEP.

456   WPI particles were shown to be more rigid than those of egg (Figure 4). Greater lubrication by more

457   rigid particles has been shown previously in Kappa-carrageenan, agar and alginate fluid gel systems

458   (Fernández Farrés et al., 2013, Gabriele et al., 2010, Garrec and Norton, 2013). This is because more

459   rigid particles can support the gap and are deformed less in the contact, reducing the area of contact.

460   This is only true when the particles are softer than the surfaces, as wear will occur within the contact

461   when particle hardness is comparable to the surfaces.

462   Particle rigidity can also explain the difference observed between egg and WPI for fluid gels produced

463   away from the IEP. WPI fluid gels produced below the IEP were shown to have a greater elastic

464   modulus than those produced above the IEP. However, for egg, the opposite trend of particle modulus

465   was observed, with particles produced above the IEP having a greater elastic modulus.

Conclusion

466

467   Fluid gels present a novel solution to reduce the fat content of emulsion-based foods while

468   maintaining the desirable textural properties of their full fat counterparts. Egg white fluid gels have

469   been presented as an interesting ingredient for the production of fluid gels, showing high viscosities

470   and good lubricating properties when produced at pH 7.5 the IEP of egg ovalbumin. WPI fluid gels

471   showed high viscosity and good lubricating properties when produced at pH 3.5 and pH 8. Egg and

472   WPI fluid gels produced at their respective IEP were found to have a poor lubrication. This was

473   explained by the aggregation of particles leading to increased effective particle size for fluid gels at

474   their IEP. Fluid gels produced from WPI at pH 3.5 show the most potential for use as a fat replacer for

475   O/W emulsions from the systems tested. Protein fluid gels offer a nutritionally beneficial functional

476   ingredient offering potential for thickening of products whilst contributing nutritionally increasing the

477   protein content of products.

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