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DOI: 10.1016/j.foodhyd.2020.106108

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Document Version Peer reviewed version

Citation for published version (Harvard):

Young, PW, Mills, TB & Norton, IT 2021, 'Influence of pH on fluid gels produced from egg and whey protein isolate', *Food Hydrocolloids*, vol. 111, 106108. https://doi.org/10.1016/j.foodhyd.2020.106108

Link to publication on Research at Birmingham portal

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Influence of pH on fluid gels produced from egg and whey protein isolate

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5 Abstract

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4

6 Food producers are coming under increasing pressure to reduce fat content of foods. Fat forms a 7 major structuring component in many foods responsible for the desirable texture of foods which are 8 rich in fats. Consumers want healthier foods whilst maintaining desirable sensory properties of these 9 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 10 11 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 12 13 and rheology were used to investigate textural properties of fluid gels produced and relate these to 14 potential mouthfeel of these systems. Fluid Gels at the IEP were shown to produce aggregated 15 particles of less than 1 µm diameter. These systems produced at the IEP demonstrated greater 16 friction values in the mixed and boundary regimes of lubrication.

17 Introduction

18 There is an increasing consumer awareness of health issues associated with diet, due to this there is

19 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
- 22 demand from consumers for the desirable creamy texture of these emulsions to be maintained,
- 23 while reducing the calorific content of these products.

24 Characterizing the desirable creamy textures of emulsions presents challenges owing to the variety 25 of ways in which the texture of foods is perceived in the mouth. The perception of creaminess has 26 been related to thickness, smoothness and slipperiness by Kokini (1987). Thickness of products can 27 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 28 29 the mouth feel of a product. (Singer and Dunn, 1990) showed that particle sizes $<0.1 \ \mu m$ have an 30 'empty' texture, while particle sizes of 0.1–3 μ m were perceived as creamy. Particles >3 μ m had a 31 gritty sensation. It is noteworthy that the particles discussed in this study are hard particles (Singer 32 and Dunn, 1990).

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

- the soft tongue and the rigid palate. In these thin films perception of slipperiness has been shown to
 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 continuous phase produced by sheared gelation. This method was shown to produce anisotropic 53 particles. Processing parameters including hydrocolloid concentration, shear environment and rates 54 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).

In recent years, the production of these fluid gel systems from proteins has been investigated
(Lazidis et al., 2016, Moakes et al., 2015a). Fluid gels provide a system that has the potential to be
controlled through processing parameters (concentration, shear and thermal history). For protein
fluid gel production, a heating profile is applied to the protein solution to denature the proteins.
However, the use of proteins in acidic products presents new challenges as the gel properties of
proteins are influenced by pH (Mudgal et al., 2011, Verheul and Roefs, 1998, Raikos et al., 2007). As
the pH is changed, the charges on individual amino acid residues within the polypeptide will change

67 around their individual pKa. 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).

The influence of pH on particle properties around and below their isoelectric point (IEP) is important
because the net charge of these proteins will change with reduced pH. The influence of pH on the
production of protein fluid gels will be investigated for whey protein isolate (WPI), which was
previously characterized (Lazidis et al., 2016), and for egg, which represents a novel system. Egg
embodies an exciting functional ingredient for product structuring as it is both natural and easily
recognized by consumers.

Functionalized proteins offer advantages over more commonly used hydrocolloids owing to their surface active nature. This has been exploited previously for foam stabilization (Lazidis et al., 2016). However, the use of a surface active particulate thickener has not yet been investigated for the production of reduced fat emulsions. The thermo-irreversible nature of protein gelation offers further advantages over fluid gel systems produced using thermoreversible hydrocolloids as these systems will be more resistant to any heat processing post production.

89 Materials and methods

90 Solution preparation

91 WPI

92 Whey protein isolate (WPI) (Davisco) was used. Protein (89.4%), ash (3.0%), Moisture (0.4%) and 93 lactose (0.3%). A stock Solution of 15% w/w was produced by gradual addition of powder to gently 94 stirred distilled water and gently stirred by means of a magnetic stirrer for 12 hours at 6 °C. 0.01% 95 sodium azide was added to this stock solution to prevent bacterial growth. Stock solution was stored 96 in a fridge at 6 °C until use. This stock solution was stored for a maximum of 30 days. Solutions for 97 fluid gel production were prepared from this stock solution by mixing with 2 M acetic acid to the 98 desired pH. These were then made up to the appropriate volume with distilled water to give a final 99 concentration of 12% w/w and further mixed for 10 minutes. Three different pH solutions were 100 prepared pH_{Native} in which the pH was not adjusted of the WPI solution dispersed in distilled water, 101 for WPI this was pH 8. pH 5 this is well documented as the IEP of WPI (Demetriades et al., 1997), and 102 pH 3.5 below the IEP.

103 Egg white

- 104 Chicken eggs were purchased from a supermarket and separated by hand. The egg whites were
- 105 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
- 107 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
- 109 was then stored in a fridge for no longer than a week before use. The treated egg white was mixed
- 110 with 2 M acetic acid to the desired pH then made up to a final dilution of 75% w/w with distilled
- 111 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
- 114 **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
- 116 methods outlined by Ross (1991). The final protein concentration was calculated to be between 6.9
- 117 and 7.6% w/w.

118 Fluid gel preparation

- 119 A vane and cup geometry was used on a Malvern Kinexus Rheometer. The solutions were allowed to
- 120 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
- 122 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
- 124 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.

127 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
- this diluted sample was placed on a slide and covered with a coverslip. 20X and 40X magnification
- 133 was used to observe the particles.

134 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
- refractive index of 1.456 was used, with a stirrer speed of 800rpm in reverse osmosis water.

139 Rheology

140 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
- equilibrium at each shear rate $0.1 \text{ s}^{-1} 100 \text{ s}^{-1}$. Samples were tested at 25 °C with a gap of 1 mm.

145 *Amplitude sweeps*

- 146 A frequency of 1Hz at 25 °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.

148 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
- the proteins. These quiescently set gels were then left to stand for 20 minutes before equilibrating
- 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.

155 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.

160 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
- 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.

169 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

Where U represents the average speed at the contact for each component. A 3N normal force was used. For these experiments, a stainless steel ball-silicone elastomer disk tribopair was used as outlined previously (Mills, 2012). This tribopair and these conditions have been previously shown by Malone et al. (2003) to correlate to mouth feel in the mixed regime of lubrication. In each test Stribeck curves were measured over a speed range of 1–1,000 mms⁻¹ with ascending and descending runs repeated three times (6 curves total). Tests were performed at 25 °C. These tests were repeated three times.

183 Results and discussion

184 Fluid gel preparation

185



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 °Cmin⁻¹ was
applied, followed by a 2-minute isothermal step, then a cooling rate of 4 °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.

196 Initially there is little change in viscosity observed with increasing temperature, this is followed by a 197 sharp increase in viscosity for all samples except for WPI pH 8, this sharp increase in viscosity during 198 fluid gel production has been observed previously at the gelling temperature of hydrocolloids (Ellis et 199 al., 2017). This observed increase in viscosity has been attributed to protein aggregation by Lazidis et 200 al. (2016). Particles are expected to form through a nucleation and growth mechanism where by small 201 particles aggregate producing larger particles. The growth of these particles will be limited by break 202 up in the shear field thus final particle size is an equilibrium between particle growth through 203 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 thedifference in the rate of gelation. Gelation rate is controlled by two stages, protein denaturation and

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

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

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

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

227 Particle shape and size determination

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





Figure 2. Micrographs at 20 time magnification of fluid gel particles of egg and WPI produced at different pH **25**:*showing fluid gels diluted in distilled water or appropriate concentrations of acetic to maintain the appropriate pH.*

This shows particle shapes and sizes. The scale bar represents 50 μ m. For egg, pH_{IEP} = 4.5 and pH_{Native} = 7.5. For WPI, 25 pH_{IEP} = 4.9 and pH_{Native} = 8.

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

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

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

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
- particles <10 µm. The smaller particles observed at the IEP correlate with a previous suggestion that
 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
- and 0.9 μ m. 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.



- 274 Figure 3. Size distribution of protein fluid gel particles produced at different pH A) WPI (pH 3.5 \bigcirc ; pH
- **275** 4.9 (IEP) ⊖; pH 8 ●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ⊒; pH 7.5 ■). Values represent an average of
- 276 three repeats, each repeat consisting of three measurements.



277 Fluid gel rheology

273



Shear rate-controlled equilibrium measurements were used to analyse the shear rheology to understand the flow behaviour of these fluid gels. Shear thinning behaviour typical of particle suspension rheology was observed in all fluid gels produced here. The shear thinning nature of suspensions is due to ordering of particles in flow (Krieger and Dougherty, 1959). At low shear rates,
when little flow is occurring, particles can interact; as shear rate increases and flow is induced, these
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

Quiescent gel elastic modulus and zeta potential were used to further probe particle properties. Use
of quiescently set gel properties to represent particle properties has been shown previously (Garrec
et al., 2013).

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

	рН 3.5	рН _{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

Table 1 shows the zeta potential of fluid gel particles. This is used to investigate the net surface charge 315 316 of the particles in the fluid gel systems. The net surface charge of protein molecules is expected to be 317 \sim 0 at its IEP, <0 at pH above the IEP and >0 at pH below the IEP, which is due to protonation and 318 deprotonation of amino acid groups along the protein. Understanding the net charge of particles was 319 important to understand what electrostatic interactions were occurring. This trend was observed for 320 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 321 322 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. 323

324 The reduced net charge on the particles may contribute towards the observed sedimentation of fluid 325 gels produced at pHIEP, with reduced electrostatic repulsion between the particles. Formation of 326 aggregates and sedimentation of WPI in solution around pH_{IEP} has been shown (Ju and Kilara, 1998). 327 This aggregation was attributed to the reduced electrostatic repulsion between protein molecules and 328 the reduced the proteins affinity for water. This reduced electrostatic repulsion enabled hydrophobic 329 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 330 was further supported by the sedimentation of egg fluid gels produced at native pH then adjusted to 331 332 pH 4.5 post production. However, the reduced affinity of protein molecules for water at their IEP may 333 also lead to a reduced phase volume for fluid gels produced at pHIEP.



Figure 5. Strain controlled amplitude sweeps for protein fluid gels showing A) WPI (pH 3.5 (G' \bullet , G" 336 \bigcirc); pH 4.9 (IEP) (G' \bigtriangledown , G" \triangle); pH 8 (G' \blacksquare , G" \square)) B) Heat treated egg (pH 3.5 (G' \bullet , G" \bigcirc); pH 4.5 (IEP) 337 (G' \checkmark , G" \triangle); pH 7.5 (G' \blacksquare , G" \square)). A sand-blasted parallel plate geometry with a frequency of I Hz was 338 used to reduce slip. Values represent an average of three repeats with error bars showing one standard 339 deviation of these repeats.

In order to observe inter-particle and particle packing properties of these systems, controlled strain
 mode amplitude sweeps were performed. This is shown through the elastic and viscous components
 G' and G", respectively these are shown in figure 5.

All the fluid gel systems showed a linear viscoelastic region (LVR) in which G' and G" are independent of strain (Figure 5); this was as expected for concentrated suspensions. The observed linear viscoelastic region was typical of solid like behaviour with G' ten times greater than G" and typical for interconnected structures of concentrated suspensions.

WPI fluid gels produced at pH 3.5 showed a greater G' in the LVR than whey fluid gels produced at native pH with G' values an order of magnitude higher than those for the fluid gel produced at pH 8. WPI fluid gel produced at the IEP (pH 4.9) showed lower G' values, correlating with the lower G' observed for the quiescent WPI gel produced at pH 4.9. This is because less rigid particles deform more easily.

For egg, a different trend in G' in the LVR was observed, with fluid gels produced at the IEP showing the highest values. Egg fluid gels produced at pH 3.5 show G' values three orders of magnitude lower than those produced at native pH. This is expected due to the difference between the gelation temperature of egg at pH 3.5 and the temperature heated to. This favours protein-protein interactions producing dewatered particles giving a sparser network, the voids in this structure will reduce the elastic response of these suspensions. This trend in G' for egg fluid gels correlated with G' values observed for quiescent gel produced at this pH.





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.

Quiescent gels were used to represent the properties of the particles in the fluid gel systems. Quiescent gels were produced within the cone and plate geometry prior to testing. All gels tested showed a frequency-dependent nature in the frequency range tested. As frequency increased, the time for energy to dissipate through the system was reduced, evidenced by a higher elastic modulus. The structures of these gels will not be directly observed here as this has been well documented elsewhere (Katsuta et al., 1990, Ould Eleya et al., 2004, Ferry, 1948, Gossett et al., 1984, Handa et al., 1998, Hermansson, 1979, Kiosseoglou, 2003).

For egg, little difference was shown in the elastic modulus of the gels produced at pH 7.5 and pH 4.5; however, the gel produced at pH 3.5 had a lower elastic modulus. Egg white gels below the IEP have been shown to have a more porous structure than gels produced at or above the IEP (Handa et al., 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.

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

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
- and agarose (Frith et al., 2002, Moakes et al., 2015b). Adams et al. (2004) explained this plateau as
- being a result of elastic modulus being dominated by particle modulus once the maximum packing

fraction is reached. For monodisperse hard spheres, the maximum packing fraction is 0.62 (Einstein,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 $\Phi/\Phi_{max} \sim 0.74$. 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 exceeded as

412 particles are deformable. WPI fluid gels produced at the IEP did not plateau below a concentration

- of 1.75, showing a reduced phase volume ($\Phi/\Phi_{max} < 0.57$). 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





426 Tribology

427 The friction and lubrication properties of the fluid gel systems were examined (Figure 8). Tribology

- 428 was used to evaluate the potential performance of these fluid gel systems for the use in food
- 429 systems.

430



431 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) 432 ◊; pH 8 ♦). Fluid gels produced at different pH using a stainless steel ball–silicone elastomer disk 433 tribopair at 50% SRR.

434 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 435 436 with friction coefficient decreasing with increasing entrainment speed. For WPI at both pH 3.5 and 8, 437 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 438 439 observed at 5 mm s⁻¹, particles entered the mixed regime and friction decreased rapidly as speed was 440 increased. Above 100 mms⁻¹, friction behaviour is similar to that of particles produced at pH 3.5 and 441 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

- 1 mm s⁻¹, friction coefficient increases as speed increases. A maximum was observed at a similar point
 to WPI at a speed of approximately 5 mm s⁻¹, with a similar friction coefficient value of ~0.5. As speed
 increases above 5 mm s⁻¹, friction coefficient decreases at a slower rate than for WPI fluid gels. At
 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.

This peak in friction as lubrication transitions from the boundary to the mixed regime has been shown previously for agarose fluid gels of particle size ~100µm greater than the roughness of the surfaces used. This peak may be due to the entrainment of the particles when the gap is smaller than the observed particle size (Gabriele et al., 2010). This observed peak may also be due to aggregation of particles, increasing effective particle size of particles at the IEP.

WPI particles were shown to be more rigid than those of egg (Figure 4). Greater lubrication by more rigid particles has been shown previously in Kappa-carrageenan, agar and alginate fluid gel systems (Fernández Farrés et al., 2013, Gabriele et al., 2010, Garrec and Norton, 2013). This is because more rigid particles can support the gap and are deformed less in the contact, reducing the area of contact. This is only true when the particles are softer than the surfaces, as wear will occur within the contact when particle hardness is comparable to the surfaces.

Particle rigidity can also explain the difference observed between egg and WPI for fluid gels produced
away from the IEP. WPI fluid gels produced below the IEP were shown to have a greater elastic
modulus than those produced above the IEP. However, for egg, the opposite trend of particle modulus
was observed, with particles produced above the IEP having a greater elastic modulus.

466 Conclusion

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.

478 Acknowledgements

479 The authors thank Bakkavor Foods Ltd for financial support throughout this project.

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