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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).

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 related to the lubricating properties of foods. This sensation has been correlated to soft tribology measurements (Malone et al., 2003). Thus, tribology may play an important role in predicting perception of texture in high-fat foods.

Other studies have used suspensions of soft-gelled hydrocolloid particles were shown to give desirable rheological and tribological properties. Two methods of production for these suspensions of soft gelled particles were presented (Adams et al., 2004, Evans and Haisman, 1980, Frith et al., 2002, Fernández Farrés et al., 2013, Garrec et al., 2013, Holland et al., 2018). The first was a reverse emulsion technique in which hydrocolloid solution was dispersed in oil in a w/o emulsion. This is achieved through the emulsification of a hot hydrocolloid solution, this emulsion is then cooled allowing gelation of the hydrocolloid in the aqueous phase, these gelled particles take on the shape and size of the water droplets. Spherical particles were produced, the size of which can be easily manipulated by the shear rate applied to the emulsion. These droplets were then gelled, washed and re-dispersed; however, this process is time consuming and inefficient. The second method for the production of these soft gelled particles was sheared gelation of hydrocolloids. Suspensions produced by this method have been coined fluid gels. Fluid gels have previously been defined as a suspension of soft gelled particles dispersed in a non-gelled continuous phase. Throughout this 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 particles. Processing parameters including hydrocolloid concentration, shear environment and rates of cooling can be used to control the properties of these systems. Agar suspensions produced by the reverse emulsion method have been shown to fit the Hertz model by Frith et al. (2002) with a sharp increase in viscosity as the maximum packing fraction of particles is reached. However, for sheared agar fluid gels, an increase in viscosity is observed at considerably lower phase volumes owing to the 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 around their individual pK_a. This can lead to protein unfolding due to disruption of intramolecular forces stabilizing the proteins. The net charge of protein molecules has been shown to be important in this, with gels produced away from the isoelectric point giving a fine stranded structure, binding to water, whereas those produced at the isoelectric point (IEP) show a particulate structure with little binding to water (Liu et al., 2010). This is explained by reduced intermolecular repulsion between molecules at the IEP due to the reduced net charge and a reduced affinity for water of less polar molecules. This reduced repulsion increases the rate of aggregation, and thus unfolded protein molecules do not have the chance to order into the fine stranded structures seen away from the IEP (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.

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

WPI fluid gels (Lazidis et al., 2016). The hold at 90 °C was used to observe any time dependent effects from heating that continued to occur.

Optical microscopy

An optical microscope (Leica Microsystems, UK) was used to directly observe particles produced for fluid gels. Differential interference contrast (DIC) was used to increase the contrast of particles. Samples were diluted with either distilled water or appropriate concentrations of acetic acid to 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 was used to observe the particles.

Particle Sizing

Static Light scattering measurements were used to investigate particle size distributions. For this a Malvern Mastersizer 2000 with hydro SM manual small volume dispersion unit (Malvern 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.

Rheology

Viscometry

A 40 mm sand-blasted parallel plate geometry was used for this to minimise slip in these experiments. Slip is expected for suspensions due to particle depletion at the shear surfaces. Equilibrium shear experiments were used with up to 2 minutes allowed for samples to reach 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

A frequency of 1Hz at 25 °C was used for amplitude sweeps. These were obtained using a sand-blasted parallel plate geometry to minimise the effects of slip and repeated 3 times.

Frequency sweeps

Quiescent gels were produced by heating and cooling protein solutions on a cone and plate geometry. A cone and plate geometry was used as it applies an even strain across the sample. 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 determined to be within the LVR (Linear Viscoelastic region) of these quiescent gels.

Determination of ζ-Potential

For determination of ζ-potential a Zetasizer (Malvern Instruments, UK) was used. Samples were prepared by dilution 10 times in their respective continuous phase to reduce the particle concentration to a measurable range. The samples were diluted in their own continuous phase to maintain the properties of the systems whilst reducing the concentration of the dispersed phase.

Phase volume

To calculate the phase volume of fluid gels produced the elastic modulus of diluted and concentrated fluid gels was observed. Elastic modulus was measured at 1Hz and 1% strain using a 1mm gap in a sand blasted plate geometry. Elastic modulus of suspensions of soft particles is 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 gels they were diluted in distilled water. To increase the phase volume of fluid gels they were centrifuged and the supernatant removed. Samples were centrifuged at speeds from 500-40,000g for 20 minutes to achieve this.

Tribology

An MTM2 (Mini Traction Machine, PCS Instruments, UK) tribometer was used for tribology measurement. This consists of a ball rolling on a disk, normal force, speed and slide–roll ratio (SRR) can be controlled.

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

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

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 ^{o}C. These tests were repeated three times.

Results and discussion

Fluid gel preparation

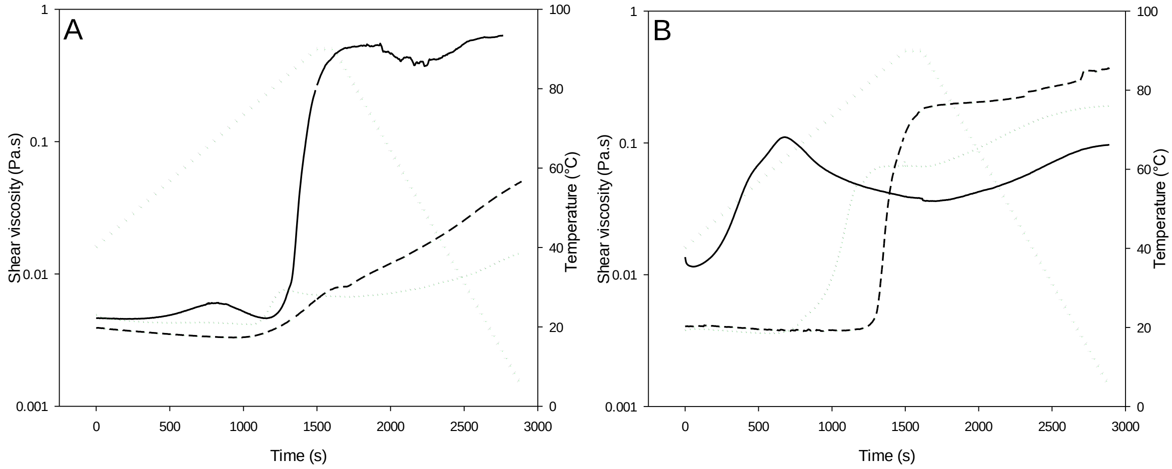

Figure 1. Viscosity profiles during protein fluid production showing sheared gelation of WPI and heat treated egg. A) WPI (pH 3.5 ——; pH 4.9 (IEP) ·····; pH 8 - - - -; Temperature ····) B) Heat treated egg (pH 3.5 ——; pH 4.5 (IEP) ·····; pH 7.5 - - - -; Temperature ····). Measurements were made using 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 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 non-gelled 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.

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.

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

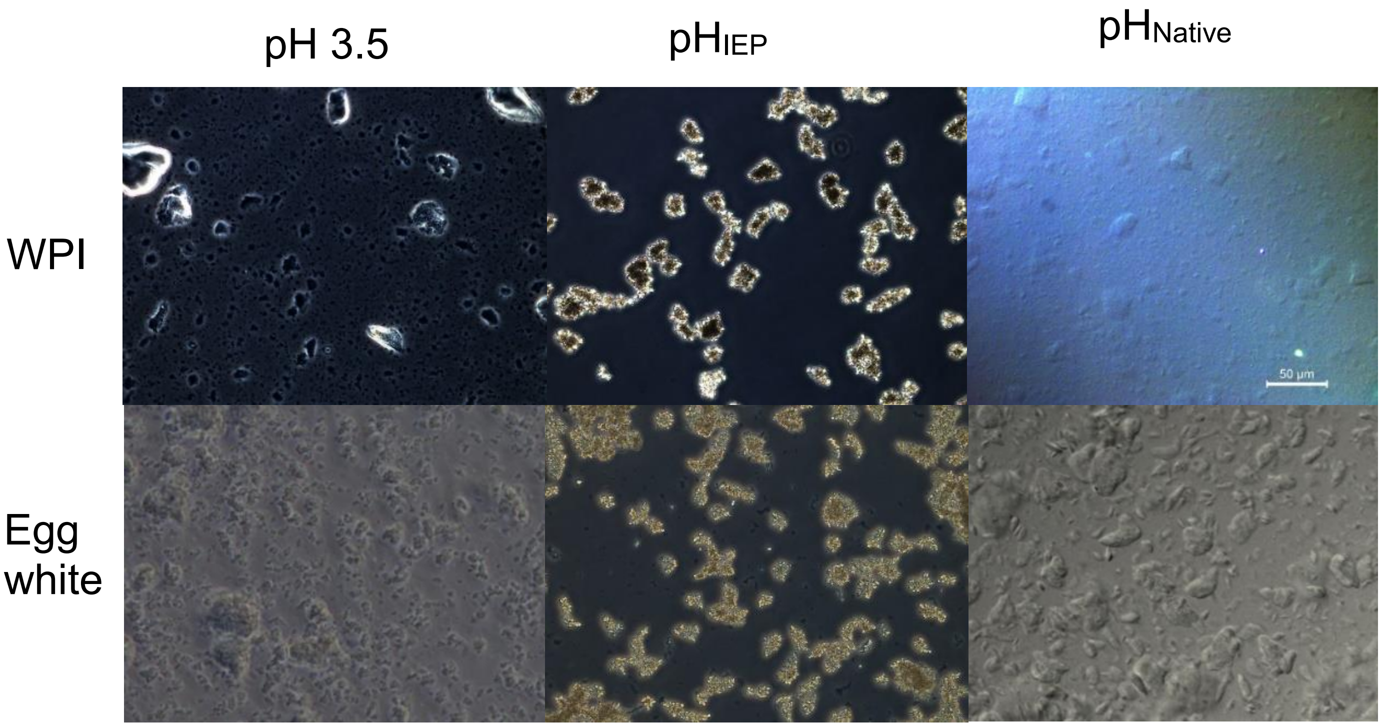

Figure 2. Micrographs at 20 time magnification of fluid gel particles of egg and WPI produced at different pH showing fluid gels diluted in distilled water or appropriate concentrations of acetic to maintain the appropriate pH. This shows particle shapes and sizes. The scale bar represents 50 µm. For egg, pH_{IEP} = 4.5 and pH_{Native} = 7.5. For WPI, pH_{IEP} = 4.9 and pH_{Native} = 8.

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

For both egg and WPI fluid gels produced at native pH (pH 7.5 and 8 respectively) produced the largest particles with a broad size distribution. For both egg and WPI particles produced at pH 3.5 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 reduce particle sizes produced, however this was not possible with the setup used due to foaming issues.

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.

For both egg and WPI at their respective IEP bimodal distributions are observed with peaks at 0.2 µm and 0.9 µm. Smaller particles produced at the IEP of proteins is explained due to the expected dewatered nature of proteins at their IEP where they are expected to have the lowest net charge.

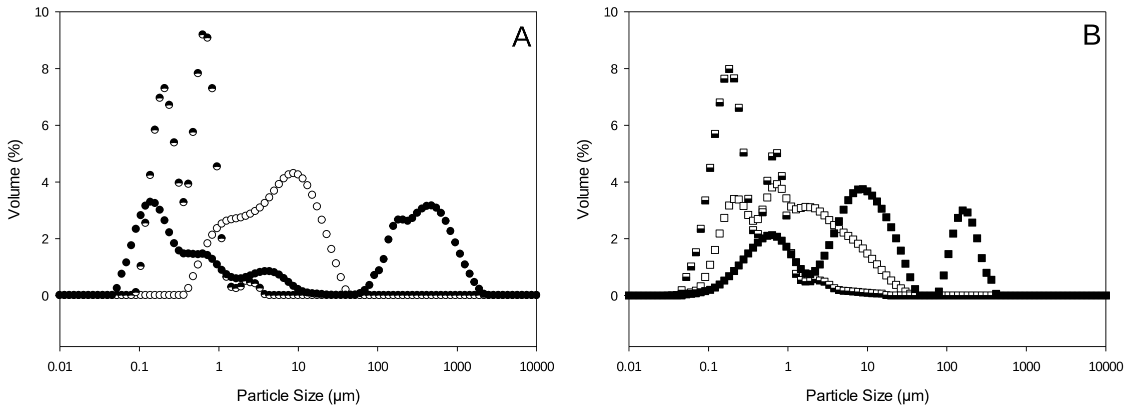

Figure 3. Size distribution of protein fluid gel particles produced at different pH A) WPI (pH 3.5 ○ ; pH 4.9 (IEP) ◒; pH 8 ●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ⬓; pH 7.5■). Values represent an average of three repeats, each repeat consisting of three measurements.

Fluid gel rheology

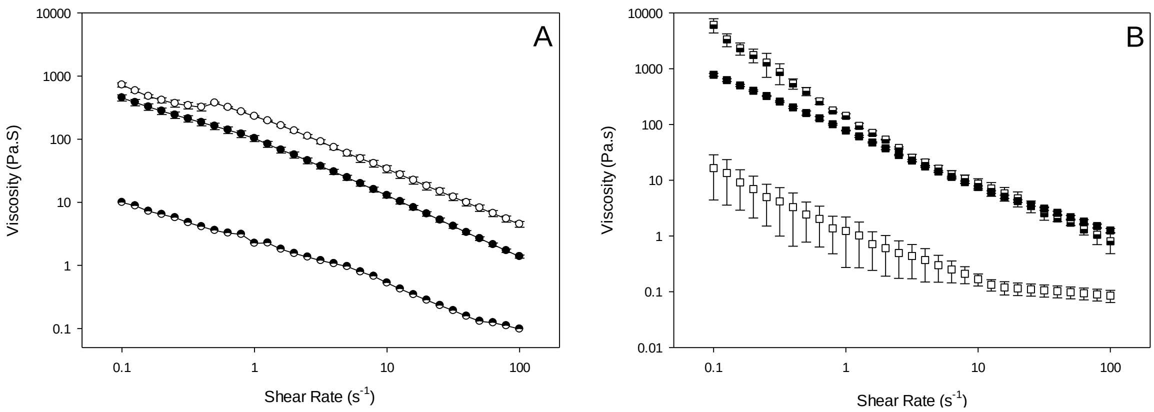

Figure 4. Viscosity profiles of protein fluid gels produced at different pH A) WPI (pH 3.5 ○; pH 4.9 (IEP) ◒; pH 8 ●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ⬓; pH 7.5 ■). Measurements were taken at least 24 hours after production. A sand blasted parallel plate geometry was used to reduce slip, and equilibrium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

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).

WPI fluid gels produced at the IEP have a much lower viscosity than those produced above and below the IEP. This is in agreement with understanding of WPI gel structures at these pHs. At the IEP, owing to the reduced repulsion between molecules during gelation, aggregation can occur before ordering of the proteins, forming a weaker gel structure. Softer particles will be able to deform and flow past one another more easily than more rigid particles reducing the viscosity of the system. This rapid aggregation leads to a particulate gel structure with limited water binding, for gels produced away from the IEP a fine stranded gel structure is expected as protein molecules order into strands due to the reduced aggregation rate. Particulate gel structures will have a more porous structure with a reduced elastic modulus than those with fine stranded gel structure.

For egg a different trend was observed with fluid gels produced at the IEP showing a higher viscosity at low shear rates, however fluid gels produced at pH 7.5 had the highest viscosity at high shear rates. Fluid gels produced at pH 3.5 showed the lowest viscosity at all shear rates. For egg at pH 3.5 a plateauing of viscosity is observed. Suspensions are expected to transition to shear thickening phase as particle jamming occurs this is as at higher shear rates particles do not have time to flow past one another. Egg pH 3.5 is the only sample for which the shear rates tested were high enough for this transition to be observed. This observed difference between the behaviour of egg and WPI is likely due to egg fluid gels produced at the IEP having a phase volume close to the maximum packing fraction this will be shown in figure 7. Suspensions with greater phase volumes will have a higher viscosity due to more particles-particle interactions due to the increased volume occupied by the dispersed phase.

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.

	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

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}.

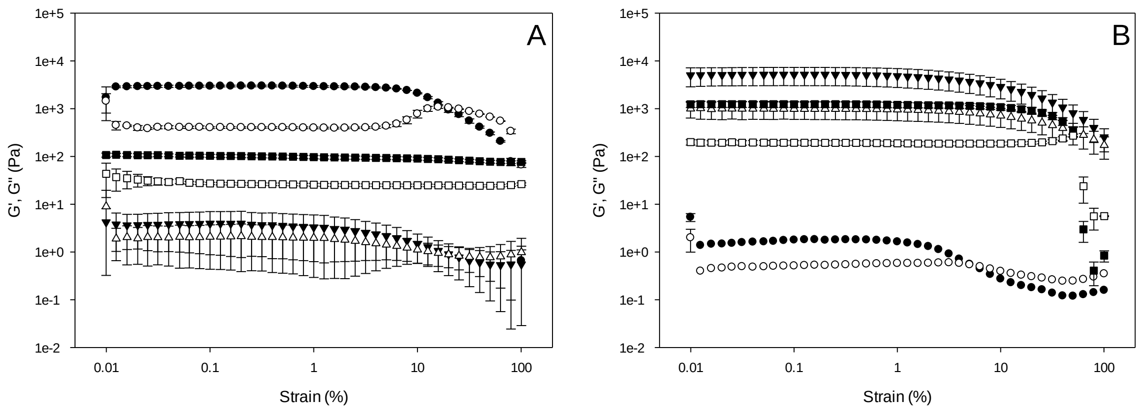

Figure 5. Strain controlled amplitude sweeps for protein fluid gels showing A) WPI (pH 3.5 (G′ ●, G″ ○); pH 4.9 (IEP) (G′ ▼, G″ △); pH 8 (G′ ■, G″ □)) B) Heat treated egg (pH 3.5 (G′ ●, G″ ○); pH 4.5 (IEP) (G′ ▼, G″ △); pH 7.5 (G′ ■, G″ □)). A sand-blasted parallel plate geometry with a frequency of I Hz was used to reduce slip. Values represent an average of three repeats with error bars showing one standard 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.

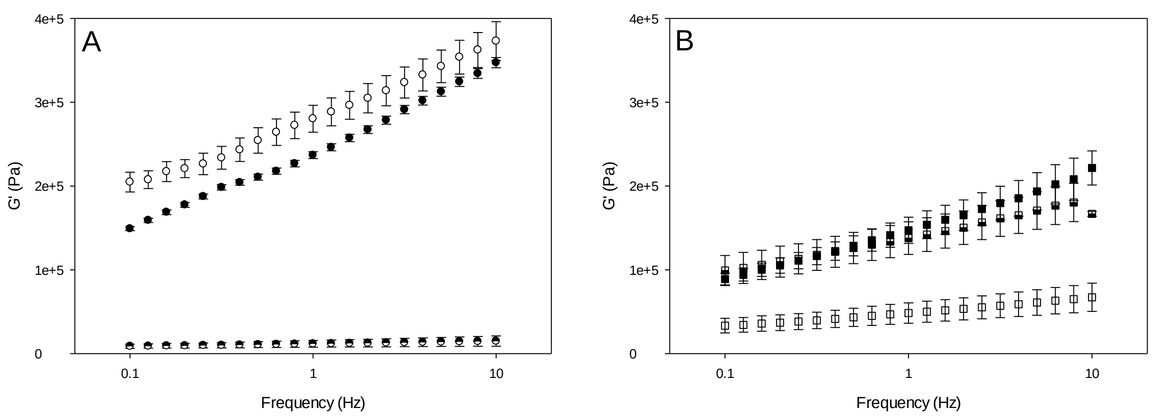

Figure 6. Frequency sweeps of quiescently set protein gels at different pH used to represent particle properties of fluid gels at different pH A) WPI (pH 3.5 ○ ; pH 4.9 (IEP) ◒; pH 8●) B) Egg (pH 3.5□; pH 4.5 (IEP) ⬓; pH 7.5 ■). Quiescent gels were set within a cone and plate geometry before the frequency 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.

WPI gels produced at pH 3.5 showed the greatest elastic modulus, and those produced at the IEP (pH 4.9) showed the lowest elastic modulus. Notably, quiescent gels produced at the IEP were cloudy in appearance, which is indicative of an aggregated particulate structure (Verheul and Roefs, 1998). This aggregated particulate structure will be dewatered, whereas for gels produced away from the IEP a fine stranded network is expected. This fine stranded network orders water giving a more rigid gel. These differences in gel structure are due to the rate of aggregation during gelation, away from the IEP electrostatic repulsion between molecules reduces the aggregation rate allowing molecules to order into the fine stranded network, however at the IEP when the net charge of the molecules is 0 aggregation rates are much higher.

For both the whey and egg systems, the quiescent gel elastic moduli showed similar trends to those of their fluid gels, validating the use of quiescent gel modulus to represent particle moduli.

Phase volume effects

Small amplitude oscillatory measurements were used to observe elastic modulus with changing concentration in order to understand potential contributions of phase volume to the observed properties of fluid gels. Gels produced at the IEP of proteins are expected to produce more dewatered particles, thus occupying a lower phase volume.

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

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

WPI fluid gels produced below the IEP were approximately at the maximum packing fraction, and those produced below the IEP were above the maximum packing fraction with a phase volume of Φ/Φ_{max} ~1.05. For suspensions of gelled particles the maximum packing fraction can be exceded as 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 produced at the IEP can be explained by the reduced affinity of the protein molecules for water at the IEP.

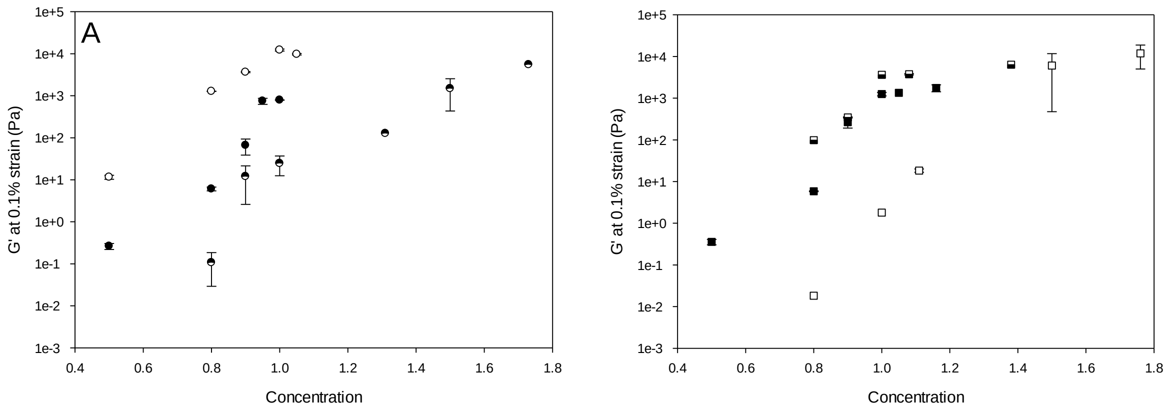

Figure 7. Elastic modulus against concentration for protein fluid gels produced at different pH. A) WPI (pH 3.5 ○ ; pH 4.9 (IEP) ◒; pH 8●) B) Egg (pH 3.5 □; pH 4.5 (IEP) ⬓; pH 7.5■). Concentration has been normalized, a concentration of 1 is defined as the concentration of fluid gels post production.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

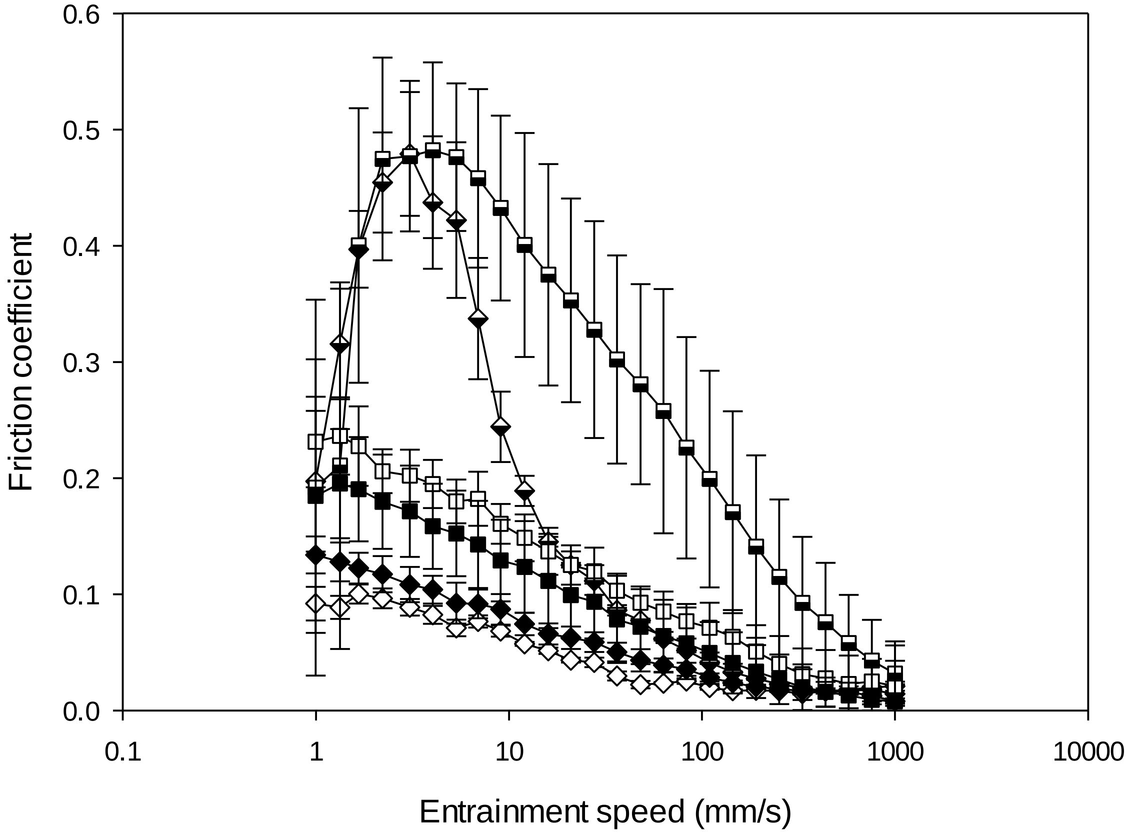

Figure 8. Stribeck curves for egg (pH 3.5 □; pH 4.5 (IEP) ⬓; pH 7.5 ■) and WPI (pH 3.5 ◇; pH 4.9 (IEP) ⬙; pH 8 ◆). Fluid gels produced at different pH using a stainless steel ball–silicone elastomer disk tribopair at 50% SRR.

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

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.

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.

Conclusion

Fluid gels present a novel solution to reduce the fat content of emulsion-based foods while maintaining the desirable textural properties of their full fat counterparts. Egg white fluid gels have been presented as an interesting ingredient for the production of fluid gels, showing high viscosities and good lubricating properties when produced at pH 7.5 the IEP of egg ovalbumin. WPI fluid gels showed high viscosity and good lubricating properties when produced at pH 3.5 and pH 8. Egg and WPI fluid gels produced at their respective IEP were found to have a poor lubrication. This was explained by the aggregation of particles leading to increased effective particle size for fluid gels at their IEP. Fluid gels produced from WPI at pH 3.5 show the most potential for use as a fat replacer for O/W emulsions from the systems tested. Protein fluid gels offer a nutritionally beneficial functional ingredient offering potential for thickening of products whilst contributing nutritionally increasing the protein content of products.

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