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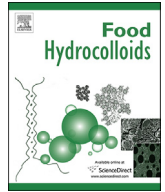
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Whey protein fluid gels for the stabilisation of foams

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ABSTRACT

The ability of whey protein fluid gels to produce very stable foams was demonstrated. These systems were prepared by heat induced gelation within the turbulent flow field of a pin stirrer at pH 5 and 8. The effect of pH and final protein concentration on the morphology of the particles, the bulk, interfacial and rheological properties and finally the foaming properties of their aqueous suspensions were investigated. Whey protein fluid gels, when produced close to the isoelectric point, consist of small spherical protein aggregates without significant functionality. Micrographs taken suggest that the protein aggregates created have the ability to adsorb at the air/water interface. Nevertheless, the lack of further increase in interfacial viscosity or elasticity indicates that either the adsorption is easily reversible or that it is only partial due to lack of material available to provide complete coverage. By increasing the pH of these systems the protein entities present acquire a negative charge, which causes an increase to both the bulk and interfacial viscoelasticity and increase of the stability of foams. The proposed mechanism is that during foaming, the smaller and mobile protein entities diffuse fast to the interface and provide the necessary interfacial tension reduction to facilitate foam formation. Subsequently, the larger protein particles fill the free space between the air bubbles and increase the local bulk viscosity, which improves foam stability mainly by preventing drainage. Whey protein fluid gels were able to create the same amount of foam as non-treated whey proteins but with substantially increased stability.

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1. Introduction

Aeration of foods is a process that is used both in traditional food products like bread and whipped cream but also in contemporary ones such as aerated chocolate, hot beverages and gourmet dishes. The presence of air, usually in the form of bubbles, throughout the volume of food provides a unique texture, which is frequently associated with luxury and high quality. Furthermore, when aeration is used effectively it can reduce the caloric value and the cost of foods by introducing an ingredient that costs nothing and has no nutritional value.

Aerated materials where the gas, in most cases atmospheric air, is distributed throughout an aqueous continuous phase are colloidal dispersions known as foams. These are thermodynamically unstable systems that generally have significantly shorter lifetime when compared to other colloidal dispersions such as emulsions (hours or even minutes compared to months) (Walstra,

2003). The main reason for the high instability lies to the fact that the interface between the dispersed and continuous phase, also called film, is larger in size when looking at bubbles in contrast to emulsion droplets. Moreover, the interfacial tension on the air–water interface (surface tension) is larger compared to the one between oil and water by a factor of 5 (Dickinson, 2010). The main mechanisms responsible for destabilising foams are drainage, disproportionation (the equivalent of Ostwald ripening in emulsions) and coalescence (Kinsella, 1981). In practice, these mechanisms reinforce each other commencing a snowball effect towards the breakdown of the foam structure. For instance, the larger bubbles formed due to disproportionation increase the size of the films which accelerate drainage and escalate instability (Walstra, 2003).

Foams are stabilised by amphiphilic entities, which can adsorb at the air/water interface and reduce the surface tension. Food foams are usually stabilised by high molecular weight surfactants or particles. Milk and egg proteins are very common ingredients present in foods that contain bubbles. Particle stabilised foams on the other hand are only present in whipped cream and ice cream where the foam structure is stabilised by a particulate matrix of

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either partially coalesced fat or ice crystals or both (Dickinson & Murray, 2006). Particles have shown an increased potential in producing ultra stable foams but in most of the cases the material used is non food (Dickinson, 2010). Trying to fulfil this need for finding food grade materials than can produce particles with foam stabilising properties, several studies have focused on plant carbohydrate materials (starches and cellulose derivatives) in combination with surfactants like proteins. These systems have demonstrated potential (Murray, Durga, Yusoff, & Stoyanov, 2011). Particles present in foams can provide stability whether they adsorb on the interface or remain on the continuous phase. In case of adsorption, they form rigid films that are stable against drainage and disproportionation, which is evident from an increase of the interfacial elasticity and viscosity. When there is no adsorption the particles either go through a percolation process and create a gel-like network or act as corks reducing the drainage of the continuous phase due to gravity (Rullier, Novales, & Axelos, 2008).

As the recent consumer trend demands foods to be 'natural' and 'wholesome' accompanied by a 'clean label', there is a need of producing particles from readily available food ingredients (Brockman & Beeren, 2011). A solution lies on the ability of several proteins to produce aggregates and gels when a set of conditions are met. In this study the heat denaturation of whey proteins is being explored as a mechanism to create micron sized aggregates or gel particles that will entail the necessary surface properties to adsorb on the air/water interface and produce very stable foams. Similar systems containing discrete gel protein particles created by freeze drying quiescently gelled whey protein isolate (WPI) suspensions prepared at a range of pH (5–8) has shown prominent results (Lazidis et al., 2014). The whey protein gel particles when rehydrated were able to produce foams with an increased stability by up to an order of magnitude compared to native proteins at the same concentration. That method allowed a significant proportion of whey proteins to remain in their native form and potentially in a form of soluble aggregates. It has been demonstrated in the past that the foaming properties of mixtures of soluble proteins with insoluble aggregates have enhanced foaming properties (Zhu & Damodaran, 1994).

This study focuses on exploring a process of producing gel micro particles that has a potential in future upscaling for manufacture through an industrially applicable process. The ultimate goal is to produce micro particle suspensions that will be in a state which will allow transport through a pump to a drying operation in order to achieve a powder formulation. The route chosen was the implementation of the well established method of producing fluid gels by subjecting a polymer solution to gelling conditions (pH, ionic strength, concentration, temperature) while being under a shear field (Norton, Jarvis, & Foster, 1999). In this case WPI solutions were heated at the critical gelation temperature while subjecting them to the turbulent flow field of a pin stirrer device. In this context, the effect of processing conditions (heating and mixing rate) along with the effect of environmental conditions (pH) on the physicochemical characteristics and foaming properties of the WPI gel particulates were assessed.

2. Materials and methods

2.1. Manufacture of WPI fluid gels

WPI BiPro (Davisco, MN, US) suspensions were prepared at 12 wt% protein concentration using reverse osmosis (RO) water and 0.001 wt% sodium azide (NaN_3) (Sigma Aldrich, Dorset, UK) to prevent microbiological growth. The suspensions were left stirring overnight at 4 °C to fully hydrate. The pH of the solutions was then adjusted to the desired value using 5M NaOH or 5M HCl (Sigma

Aldrich, Dorset, UK). For creating the fluid gels the solutions were pre-heated to 40 °C and then fed through a peristaltic pump to a series of two jacketed pin stirrers of well defined geometries (Gabriele, 2011). The first pin stirrer device was set to 80 °C and 2000 rpm rotation speed. The speed of the feed pump was adjusted in order to provide a retention time inside the pin stirrer corresponding to a heating rate of 2 °C min^{-1} . The outlet of the first pin stirrer was connected to an identical second pin stirrer rotating at 2000 rpm and set to a temperature of 5 °C that was used for cooling and diluting the primary fluid gel. For diluting the primary fluid gel RO water at room temperature (25 °C) was fed through a second inlet of the second pin stirrer at a rate that would allow the final dilution of the initial 12 wt% to 5, 3 and 1 wt% which represented more relevant concentrations to the study of foaming. The final fluid gel was stored in glass bottles at 4 °C until further characterisation.

2.2. Preparation of foams and determination of foam stability

Foams were prepared with two different methods, gas sparging and mechanical whipping. When foam stability and rate of drainage was assessed, foams were prepared by bubbling using a method adapted from literature (Waniska & Kinsella, 1979). Foam was created inside a clear acrylic circular column (75 mm internal diameter and 500 mm in height) by air sparging at a rate of 3 l min^{-1} and pressure of 2 bars through the bottom of the column where a porosity 3 (15–40 μm pores) glass sintered plate is located. A sample of 150 ml suspension was placed in the column and then air sparging was initialised until the production of a foam head of 20 cm. The reduction of the height of the foam head was then recorded with a CCD camera and the foam half-life was later calculated. All measurements were carried out in four replicates.

For all other measurements, foams were produced by mechanical whipping using a commercial milk frother with a spiral impeller of 10 mm diameter rotating at approximately 2000 rpm. For the means of foam production, 20 ml of sample was placed in a 100 ml glass beaker and whipped for 30 s. All measurements were performed at 25 °C unless otherwise stated.

2.3. Determination of foam overrun

The foaming ability of the systems studied was investigated by measuring the amount of air that was able to be incorporated after foaming by mechanical whipping. This was done by weighing equal volumes of the original dispersion and the corresponding foam and calculating the overrun through Eq. (1). The experiments were repeated in a set of five replicates.

$$\% \text{ overrun} = \frac{m_{\text{dispersion}} - m_{\text{foam}}}{m_{\text{foam}}} \times 100 \quad (1)$$

2.4. Determination of foam drainage from conductivity data

A conductivity column was developed in house based on the concept from (Barigou, Deshpande, & Wiggers, 2001) where the conductivity was measured at different heights of the foam across the side and the centre of the column. Alternating current (AC) in a square waveform with a frequency of 1 kHz and a max voltage of 10 V was fed through the volume of the foam and the drop of the root mean square value of the AC voltage (AC_{RMS}) was monitored. A calibration curve (Fig. 1) of different AC_{RMS} values of known liquid fractions of a foam were plotted and a two parameter logarithmic

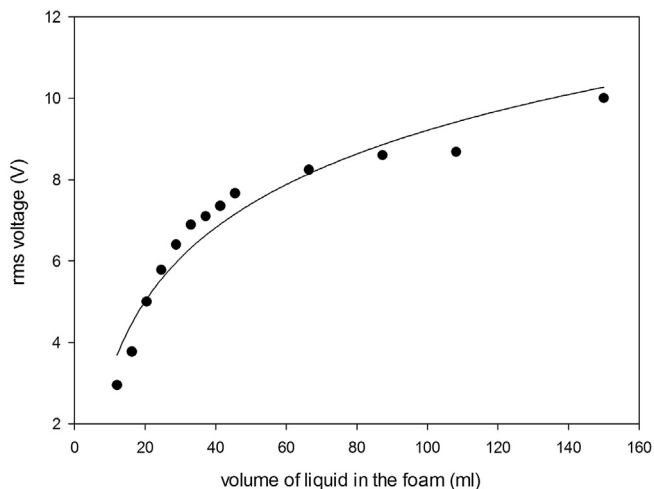


Fig. 1. Voltage measured through the foam corresponding to the liquid fraction of the foam. Data was fitted with a logarithmic equation with a R^2 value of 0.94.

equation was fitted which was used to calculate the volume of liquid of foams according to Equation (2).

$$AC_{RMS} = 2.609 \times \ln V_{liq} - 2.806 \quad (2)$$

where V_{liq} is the volume of the liquid in the foam and AC_{RMS} is the voltage square mean reading through the whole volume of the foam.

2.5. Measurement of particle size

The particle size distributions of the gel particles present in the fluid gels were determined by laser diffraction after dispersing the powders in water at a concentration of 0.25 wt% and using a Malvern Mastersizer 2000 with a Hydro SM manual small volume dispersion unit attached (Malvern Instruments, UK). The scattered intensity as a function of the angle was transformed into size distribution using the Mie theory and the relevant refractive index for the protein (1.456) was used. All size measurements were carried out in three replicates.

2.6. Determination of ζ -potential

The ζ -potential was determined using a Zetasizer (Malvern Instruments, UK) equipped with an automatic titration unit that allowed the determination of ζ -potential over a wide range of pH (2–12). The titrator used NaOH and HCl of 0.5 and 1M to increase and decrease the pH accordingly.

2.7. Characterisation of rheological properties of the fluid gels

Viscosity and rheological measurements were performed using a Kinexus rheometer (Malvern, UK). Flow curves of the WPI fluid gels were obtained by using roughened parallel plates with a diameter of 60 mm and applying a range of shear rates (0.01–100 s^{-1}) while measuring the viscosity.

The rheological properties of the bulk phase were determined by oscillatory rheology by applying a strain controlled frequency sweep at a strain rate within the linear viscoelastic region of the samples as defined by an amplitude sweep performed beforehand using a similar sample. The oscillations were performed using the same parallel plate geometry. All rheological measurements were carried out in three replicates.

2.8. Surface tension measurements

The Wilhelmy plate method was used to measure the static surface tension of the solution using a K100 Tensiometer from Kruss GmbH (Hamburg, Germany). The plate was allowed to equilibrate for 1200 s while the surface tension was being recorded. All measurements were carried out in three replicates.

2.9. Interfacial dilational rheology

The dynamic air/water (a/w) surface elasticity and viscosity were measured using a pendant-drop Sinterface PAT1 tensiometer (Sinterface, Berlin, Germany). A droplet of the liquid sample with an area of 20 mm^2 was formed automatically at the tip of a syringe driven by a motor plunger within a thermostatically controlled glass cuvette set to 20 ± 0.5 °C. The image of the drop was captured and digitised by a CCD camera. The interfacial tension ($\sigma_{a/w}$) was calculated by analysing the profile of the drop and fitting it to the Laplace equation. After allowing 1000 s to reach equilibrium sinusoidal oscillations of the interface occurred by injecting and extracting volume into and from the drop while the response in interfacial tension was recorded. The relative amplitude ($\Delta A/A$) of the oscillations was set to 5% in order to stay within the linear viscoelastic region and the frequency ranged from 0.01 to 0.2 Hz while 0.01 Hz was the frequency chosen as the one relevant to foams (Schmitt, Bovay, & Rouvet, 2014). The dilatational parameters were calculated through a Fourier transformation algorithm implemented in the software package. The dilatational elasticity and viscosity were calculated from Equations (3) and (4).

$$|E| = A \times \frac{\Delta\sigma_{a/w}}{\Delta A} = E' + iE'' \quad (3)$$

$$\eta_d = \frac{E''}{\omega} \quad (4)$$

where A is the area of the drop (mm^2), $\sigma_{a/w}$ the air/water interfacial tension ($mN m^{-1}$), E' the dilatational elasticity ($mN m^{-1}$), E'' the loss dilatational modulus ($mN m^{-1}$), η_d the dilatational viscosity ($mN s m^{-1}$) and ω the frequency (Hz). All experiments were duplicated.

2.10. Imaging microstructure by confocal laser scanning microscopy (CLSM)

The microstructure of the protein gel particles in the foams were visualised at 20 °C using a confocal scanning laser microscope Leica TCS SPE (Heidelberg, Germany) equipped with laser operating at a wavelength of 532 nm. The WPI fluid gel particles were stained with 0.01 wt% rhodamine B (Sigma Aldrich, Dorset, UK) that was diluted in the protein suspensions prior to the fluid gel manufacturing.

2.11. Statistical analysis

All the data of the replicated measurements of the study that are plotted in the present publication include the average of the measurement accompanied by error bars that consist of the standard deviation (SD) of the mean. In the case where mean values of an observation are compared between samples the data have been subjected to analysis of variance (one-way ANOVA) in order to determine significant differences between the samples. Data were checked for following normal distribution and equality of variance prior to carrying out the ANOVA. Finally, Tukey's test for pairwise comparison of means was used in order to determine the trends of

the samples that exhibited different behaviour. The level of significance of $p < 0.05$ was chosen. The statistical analysis was performed using Minitab 17 statistical package (Minitab Inc., PA, US).

3. Results and discussion

3.1. Preparation and characterisation of whey protein fluid gels

Whey proteins upon heating undergo an irreversible denaturation, which takes place in a two-step mechanism. First the globular protein species present in whey (predominately β -lactoglobulin and α -lactalbumin) unfold, exposing many of their active groups. On a second step, these exposed groups form covalent bonds causing the proteins to aggregate and form a 3-dimensional network (Mulvihill & Donovan, 1987). When heat denaturation occurs at pH higher or lower than the isoelectric point the resulting aggregates have a fibrillar shape and appear clear. On the other hand when denaturation happens close to the isoelectric point (around 4.9 for whey) the aggregates have a more compact spherical shape and appear opaque (Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007). The presence of electrostatic interactions therefore, seems to be detrimental for the structure of these aggregates. In order for whey proteins to form a gel, a set of conditions has to be met. For the given environmental conditions (pH and ionic strength) there is a critical protein concentration (C_g) and a critical temperature (T_g) needed in order to form a gel. Generally both C_g and T_g increase while moving away from the isoelectric point. At pH 8 the critical concentration is approximately 12 wt% and the critical temperature is 80 °C, when heat is provided with a rate of 1 °C min⁻¹ (Stading & Hermansson, 1990).

During the heat gelation of whey proteins under shear, nuclei are formed as a consequence of the disruption to the secondary structure, which leads to the formation of oligomers. These nuclei grow through a series of weak interactions and covalent bridging up to an aggregate size, which is limited by the magnitude of the shear applied. Subsequently, the disruption of the shear intra particle interactions can cause bridging mainly due to disulphide bonds, which lead to the formation of a continuous network. A way of manipulating the size of the aggregates formed is by choosing the magnitude of the applied shear and the heating rate. It has been shown that at large shear rates (higher than 600 s⁻¹) the size of the aggregates is limited by the shear whilst at lower shear rates the size of the aggregates is generally larger but can be retained by choosing lower heating rates (Moakes, Sullo, & Norton, 2015).

The kinetics of the fluid gel formation can be monitored by applying a uniform shear within the geometry of a rheometer while a heating profile is being administered. In this study the viscosity profile during the formation of whey protein fluid gels at a protein concentration of 12 wt% at pH 5 and pH 8 with a heating rate of 3 °C min⁻¹ were monitored within the flow field of a vane geometry (Fig. 2). For both systems there is a notable increase in viscosity when the temperature reaches 80 °C which indicates the beginning of gelation. This increase continues until it reaches a maximum, suggesting the end of gelation. Both the maximum viscosity (0.9 Pa s) and the time needed for it to be attained seems to be independent of the pH. Upon cooling, the fluid gels made at pH 8 retain their high viscosity whilst the fluid gels made at pH 5 show a dramatic drop in viscosity. The high viscosity sustained at pH 8, even though the shear environment is still present, suggests that the structure and interaction between the aggregates created remain unchanged. On the other hand, the drop in viscosity at pH 5 indicates that the size of the aggregates continues to decrease

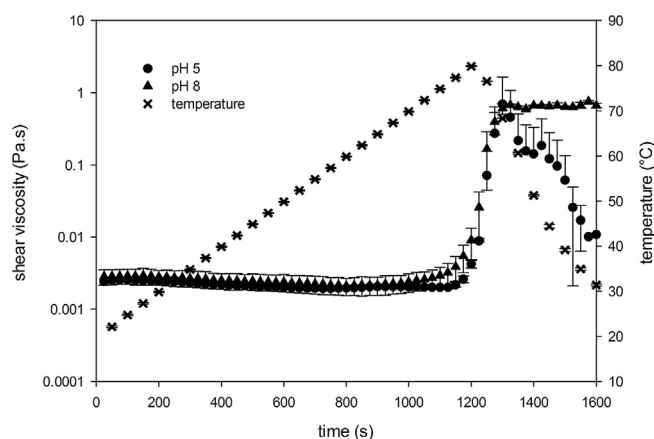


Fig. 2. Typical viscosity curve during the production of whey protein fluid gels at 12 wt %.

under shear, as there are limited interactions between the aggregates.

The fact that the gelation starts and ends at the same time at the given concentration and heating rate for the systems at the two different pH values implies that the conditions for gelling are the same and therefore the two systems can be created under the same conditions. When fluid gels were prepared inside the geometry of the rheometer, the sample was exposed to the atmosphere allowing the incorporation of air and limited foaming occurred during the preparation of the fluid gel which to an extent added to the increase of the values of viscosity. This phenomenon was more profound in the fluid gel samples made at pH 8 which can have also partially contributed to the retention of the initial viscosity. Finally the fluid gels made at pH 8 tend to post-order during cold storage and form a non-flowable gel. This is believed to happen due the larger number of available –SH groups in gel structures created at elevated pH values which can form S–S bridges over time and cause the system to form a continuous network (Klemaszewski & Kinsella, 1991). Post ordering was prevented by diluting the fluid gels at the desired concentrations (1, 3 and 5 wt%) right after their production. The reduction in the packing of the gel particles did not promote the formation of significant amount of disulphide bridges and the fluid gels remained flowable without their viscosity increasing over time.

The main purpose of this study was to create WPI fluid gels in a continuous manner. In terms with this, the production of fluid gels was done as described in Section 2.1 in a pin stirrer device for both systems at pH 5 and pH 8. The production set up did not allow the application of as wide a range of shear or heating rates as could have been achieved within a rheometer. According to a series of computational fluid dynamic (CFD) calculations carried out on a pin stirrer device with the same geometry the maximum shear rate is behind the rotating pins at a rotational speed of 2000 rpm and is in the order of 200 s⁻¹ (Gabriele, 2011). The effect of the rotation speed of the pin stirrer (500–2000 rpm) and the heating rate (2–6 °C min⁻¹) within the limits allowed by the system did not have a significant impact on the size of the particles present in the fluid gels (data not shown here).

The size of the gel particles obtained from this production method is significantly dependent on the pH at which the fluid gels were made as shown in Fig. 3. The size of the aggregates at pH 5 is significantly smaller with a $D_{3,2}$ around 10 μ m, whilst the samples produced at pH 8 consisted of larger sized aggregates of approximately 150 μ m. This is due to the fact that at pH 5 the fluid gels exist as compact spherical aggregates with a structure at the particle

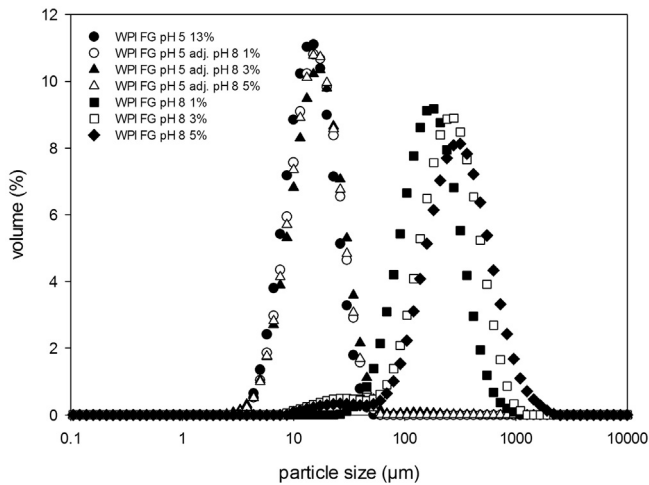


Fig. 3. Particle size distributions of protein gel particles present in the fluid gel samples.

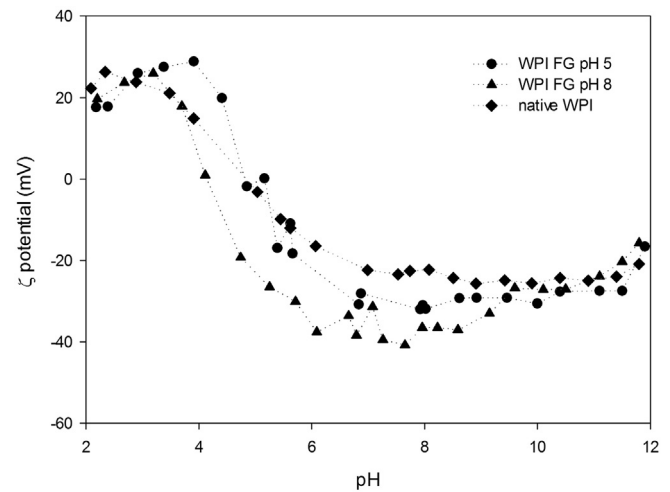


Fig. 5. ζ -potential of the samples over a range of pH.

level resembling that of quiescent gels made at the same conditions in the absence of shear (Fig. 4a) (Schmitt et al., 2014). On the other hand, at pH 8 whey proteins tend to create fibrillar aggregates which when formed in the presence of shear seem to create larger but still spherical structures as shown in Fig. 4c. The transparency of the fluid gels produced at pH 8 is caused by the similarity of the refractive index of the gel particles with that of the continuous aqueous phase which makes distinguishing them microscopically, but also in terms of dynamic laser scattering, difficult. No increase in size was observed after the adjustment of the pH of the fluid gels that were prepared at pH 5 to 8. The properties of these pH-adjusted samples have also been investigated in this study and are referred to as pH 5 adj. to 8 throughout this work.

Proteins are charged species and many of their functional properties depend on the level of charge and the electrostatic interactions caused as a result of this. The effect, therefore, of the pH on the charge in terms of ζ -potential of these systems is of

importance and was investigated. The ζ -potential over a wide range of pH values (pH 2–12) of dilute WPI fluid gel dispersions made at pH 5 and pH 8 and compared with native WPI showed a shift to the original isoelectric point (4.8) (Fig. 5). The fluid gels made at pH 8 show a lower isoelectric point closer to 4.1 whilst the fluid gels made at pH 5 show an isoelectric point similar to that of native proteins. Moreover, differences in ζ -potential behaviour can be observed at the pH values between pH 5 and 8. The fluid gels made at pH 8 show higher levels of charge than the ones prepared at pH 5 and the native proteins. This difference is not as prominent at pH higher than 8 and lower than 4. The shown differences in ζ -potential suggest also changes to the magnitude of the electrostatic repulsions between particles in these systems, even at the same pH. This dependence of the pH at which the fluid gels were made on the electric charge of the system can be due to the difference in the amount of free non-aggregated proteins still present in the system following the heating step. Recent work (unpublished) by the authors has shown that the amount of non-aggregated proteins in the

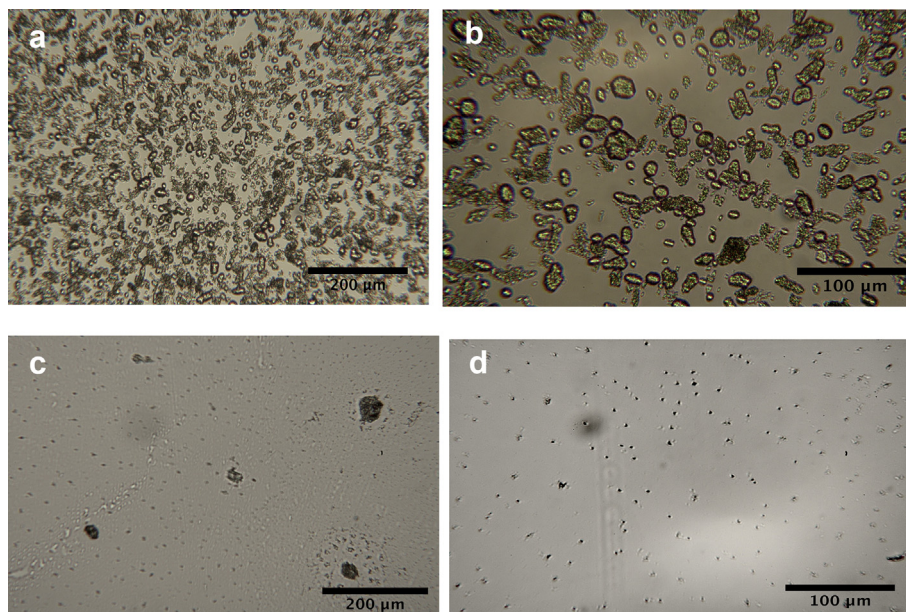


Fig. 4. Optical microscopy images of WPI Fluid Gels made at pH 5 (a) and (b) and pH 8 (c) and (d).

system is higher when the gelation takes place at pH 5 compared to pH 8.

3.2. Interfacial properties of WPI fluid gels

The functional properties of proteins also depend on their ability to adsorb at surfaces/interfaces and lower the surface/interfacial tension of the system stabilising the newly formed interface. This behaviour is important when looking at colloidal systems such as foams, where the surface tension (SFT) of the system is a good indicator of the ability of the surfactants, in this case proteins, to adsorb on the interface during foaming. When statistically comparing the SFT values (Table 1) of the samples it seems that the samples made at pH 5 and then readjusted to 8 and the heat treated WPI have significantly lower values which is also dependant on the concentration. Whilst the samples made at pH 8 and at pH 5 have higher values. It is worth mentioning that the difference between the lowest (heated WPI 5 wt%) and highest (WPI FG pH 5 1 wt%) mean is less than 4 mN/m². Therefore there might be statistical differences between the samples but these are in reality small. This indicates that the ability of the protein systems to lower the surface tension remains unaffected by exposure to heat during fluid gel production. This is probably due to the presence of a significant amount of non-aggregated proteins, which rapidly adsorb and saturate the surface across all samples studied, but also due to the ability of the formed protein aggregates to adsorb on the a/w interface. The WPI fluid gels contain enough protein material that can diffuse fast and adsorb on the interface lowering the surface tension and allowing the formation of a foam.

Colloidal particles have shown to stabilise foams due to their ability to adsorb on the air/water interface and form a strong film (Dickinson & Murray, 2006). The strength of this film provides an energy barrier that prevents the diffusion of gas between different sized bubbles (disproportionation). During disproportionation the bubbles tend to shrink and in order for that to happen the bubbles have to work against the interfacial elasticity and viscosity which can suppress shrinkage (Murray & Ettelaie, 2004). When particles adsorb at the air/water interface the resulting increase in interfacial elasticity and viscosity reduces significantly the diffusion of gas between bubbles of different size and therefore disproportionation. The interfacial rheological properties of the systems studied here were therefore investigated using pendant drop tensiometry. The interfacial elasticity and viscosity values of native and heated WPI were also measured for means of comparison as shown on Table 1.

The conventional condition that bubbles are stable against disproportionation is the Gibbs stability criterion in Equation (5) (Martinez et al., 2008). The surface elasticity and viscosity values

of the control systems (native and pre-heated WPI) show a maximum at 3 wt% concentration indicating that at this level the packing at the interface is optimum giving the highest film strength. The WPI fluid gels produced at pH 5 showed a statistically significant decrease in both surface elasticity and viscosity compared to the control systems indicating that the Gibbs criterion is not met.

$$E' > \frac{\sigma_{a/w}}{2} \quad (5)$$

When the pH of the same fluid gels was adjusted to 8 then both the surface elasticity and viscosity increased significantly (statistically not different to the control systems for the 5 wt% concentration). The fluid gels produced at pH 8 had higher values of interfacial elasticity and viscosity than the ones at pH 5 and statistically similar to the ones when the pH was readjusted from 5 to 8. This implies that both electrostatic repulsions and size affect the interfacial rheological properties of these systems. More specifically the interfacial elasticity increases enough to fulfil that $E' > \sigma_{a/w}/2$, implying that disproportionation can be significantly limited. The smaller gel particles produced at pH 5 when provided with a charge by increasing the pH to 8 can produce films that have high elasticity and viscosity but not higher than the ones of native or heat treated proteins. This is either due to weak adsorption of these particles on the interface or lack of complete interfacial coverage.

3.3. Bulk rheological properties

The viscosity of the bulk phase affects the mobility of the continuous phase around the foam bubbles and therefore influences the rate of foam drainage (Yang & Foegeding, 2011). Investigating the effect of the pH on the viscosities of the different WPI fluid gels and comparing with those of native WPI at the same concentrations revealed an overall increase in the fluid gel viscosity, especially at the higher concentrations studied (3 and 5 wt%).

All samples show a shear thinning behaviour that suggests the presence of interactions and structural formation, which eventually disrupted by shear (Fig. 6a). The fluid gels made at pH 5 show the lowest viscosity values amongst the fluid gel samples but when their pH is adjusted to 8 there is a clear increase in shear viscosity. This indicates that the introduction of pH-induced electrostatic repulsions amongst the protein species and fluid gel particles is causing the viscosity to increase. The fluid gels produced at pH 8 have the significantly highest viscosity especially at the 5 wt% concentration studied (Fig. 6b). This is most certainly due to the

Table 1
Interfacial properties of fluid gels and native WPI samples.

Sample	Protein content	Surface tension after 1200 s (mN m ⁻¹)	Interfacial elasticity E' (mN m ⁻¹)	Interfacial viscosity η_d (mN m ⁻¹ s)	Foam overrun (%)
Native WPI	1 wt%	–	41.8 ± 7.5	329.7 ± 47.2	197.5 ± 11.3
	3 wt%	–	82.5 ± 2.1	516.3 ± 9.5	205.9 ± 19.9
	5 wt%	–	67.4 ± 9.9	454.3 ± 35.0	198.9 ± 8.3
WPI heated to 80 °C	1 wt%	49.4 ± 0.2	69.3 ± 7.2	440.7 ± 52.2	–
	3 wt%	50.5 ± 0.3	74.6 ± 7.4	476.0 ± 111.7	–
	5 wt%	48.3 ± 0.2	54.5 ± 1.6	337.1 ± 3.0	–
pH 5	1 wt%	52.9 ± 0.2	13.7 ± 0.9	48.4 ± 7.7	231.0 ± 14.0
	3 wt%	52.6 ± 0.6	7.0 ± 2.7	10.7 ± 9.4	235.2 ± 34.8
	5 wt%	51.1 ± 0.6	8.9 ± 2.7	41.7 ± 24.3	212.8 ± 8.6
pH 5 adj pH 8	1 wt%	51.6 ± 0.6	18.9 ± 0.3	142.5 ± 60.0	183.4 ± 16.9
	3 wt%	50.2 ± 0.2	59.4 ± 3.2	381.0 ± 41.7	207.8 ± 16.4
	5 wt%	49.2 ± 0.3	55.3 ± 10.2	434.3 ± 37.3	197.5 ± 23.6
pH 8	1 wt%	51.4 ± 0.4	51.3 ± 8.7	382.5 ± 41.0	240.2 ± 33.2
	3 wt%	51.8 ± 0.1	46.3 ± 4.5	266.5 ± 14.6	201.0 ± 20.6
	5 wt%	51.7 ± 0.2	65.9 ± 9.3	310.2 ± 75.7	241.5 ± 25.5

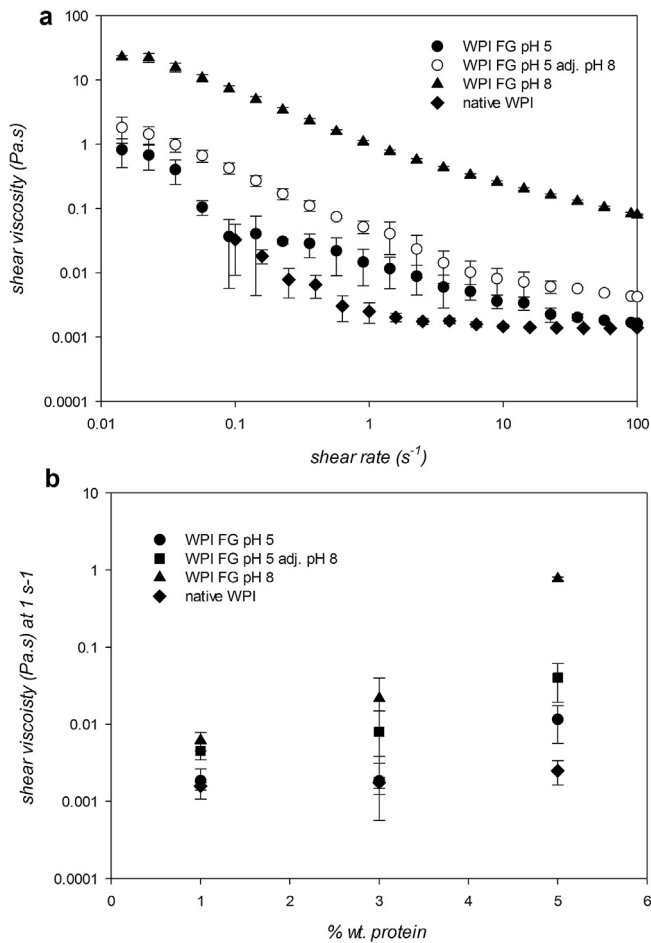


Fig. 6. Flow curves of fluid gels and native WPI at 5 wt% over shear rate (a) and viscosity over protein concentration at 1 s⁻¹ (b).

larger particles present in combination with the higher magnitude of electrostatic forces as suggested by the higher ζ -potential values.

The liquid/solid behaviour of the WPI fluid gels has been studied through measurements of the storage and loss moduli from the viscoelastic response to oscillations within the linear viscoelastic region (Fig. 7). The WPI fluid gels produced at pH 5 exhibit significantly low values for both storage and loss moduli showing a liquid behaviour. When the pH of the same sample was increased to pH 8 both elasticity and viscosity increased. The elasticity values increased for all concentrations and a weak gel behaviour could be observed for the sample at 5 wt% at the lower frequencies were both G' and G'' are close to each other and remain parallel. The fluid gels produced at pH 8 showed intermediate values for both G' and G'' across all concentrations studied but not statistically different from the ones made at pH 5 and then adjusted to 8. Additionally, at 5 wt% an elastic solid to viscous liquid transition at high frequencies was observed as suggested by a cross over of the G' and G'' curves. The combination of aggregate size and level of electrostatic repulsions appear to affect the elasticity of WPI fluid gels indicating that both the pH at which they are made but present plays an important role in their viscoelastic response.

3.4. Foaming properties of WPI fluid gels

The functionality of WPI fluid gel systems in terms of producing and stabilising foams is presented here. The ability of the fluid gels to incorporate sufficient amount of air and produce foams was

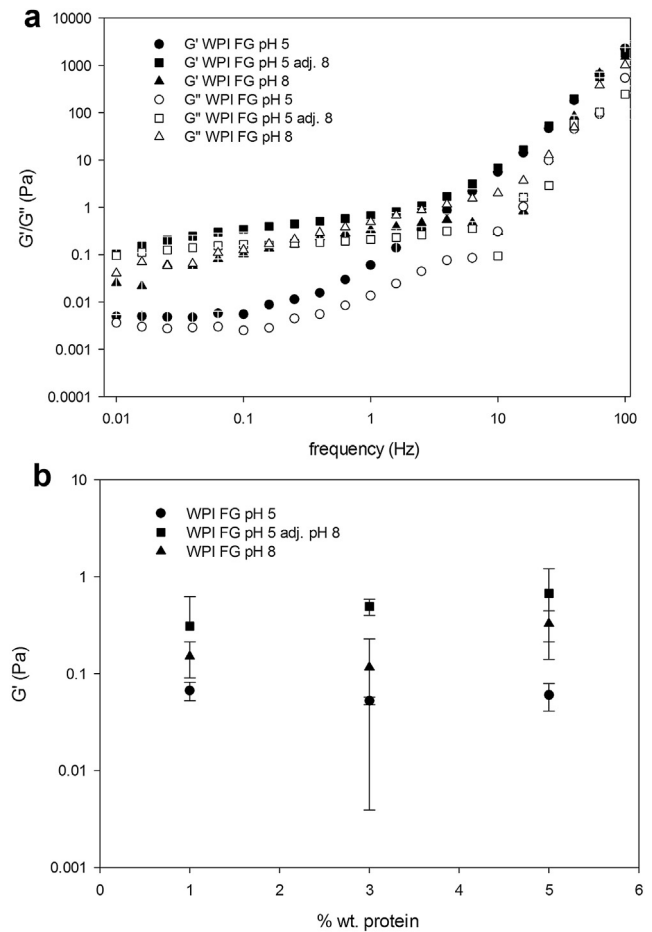


Fig. 7. Storage (G') and loss (G'') moduli over frequency (error bars were omitted for means of clarity) at 5 wt% (a) and storage moduli (G') at 1 Hz over protein concentration (b).

assessed in terms of overrun (Table 1). All fluid gel samples were able to produce foams with very similar overrun to the ones made by native proteins. The statistical evaluation has shown that the fluid gel samples made at pH 8 and pH 5 produced foams with the highest overrun values amongst the samples studied but still with

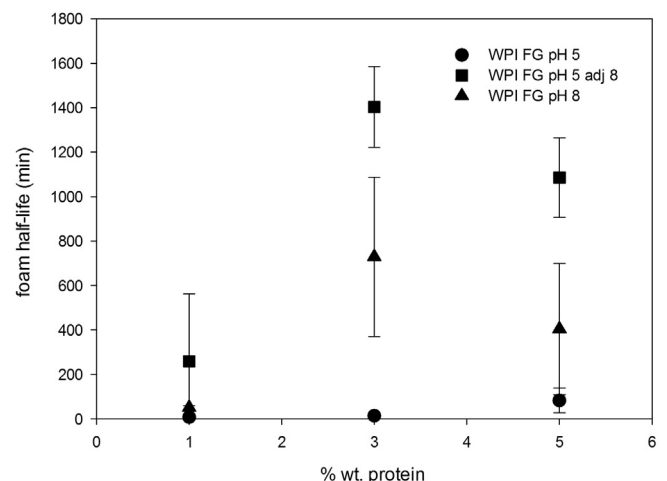


Fig. 8. Half life of the different systems over protein concentration.

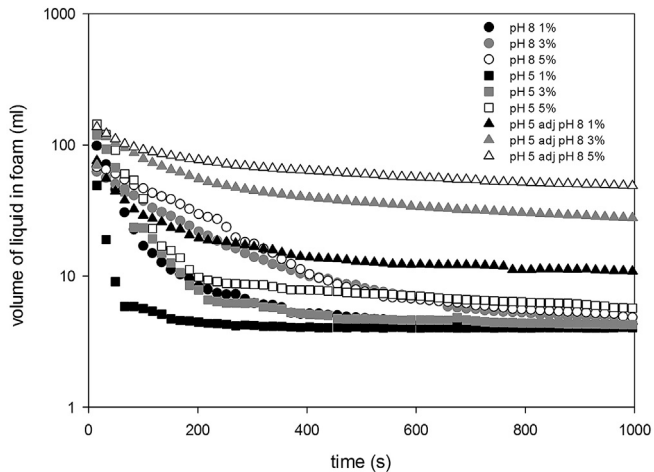


Fig. 9. Variation of volume of liquid present in foams stabilised by different WPI fluid gel systems at different concentrations over time.

values close to the native proteins. The ability of the system to incorporate air was not hindered as it is common with particle containing systems (Murray & Ettelaie, 2004). The overrun results are in agreement with the surface tension data (Table 1) indicating that for all fluid gel systems the controlling factor is the sufficient amount of soluble proteins that can diffuse fast to the newly formed interface and stabilise it during the incorporation of air to the system.

The stability of the foams produced by the WPI fluid gel systems was evaluated in terms of foam half-life (Fig. 8). The fluid gel

systems showed similar foam stabilising behaviour to systems containing dried gel protein particles and an overall significant increase in foam stability compared to native WPI was observed for all samples (Lazidis et al., 2014). Whilst the error bars representing the standard deviation between at least 4 replicates appear quite large due to the nature of the measurement, the foam stability of the systems can be illustrated. The fluid gels produced at pH 5 while only moderately increase the stability of protein foams at their intrinsic pH showed statistically significant increase in foam stability once their pH was increased to pH 8 especially at 3 and 5 wt% concentrations, the highest amongst all samples. Fluid gels produced at pH 8 were also able to produce foams with increased stability (compared to those stabilised by native proteins) but did not perform as well as the fluid gels of smaller aggregate size (formed at pH 5 and adjusted to pH 8) even though the charge was smaller as indicated by the ζ -potential data. The importance of size was therefore highlighted as the most important factor affecting foam stability followed by the existence and the extent of electrostatic repulsions. Foam stability displayed a maximum value for the 3 wt% protein concentration fluid gels showing that further increase in protein concentration had limited effect within the range studied.

The effect of the pH at which the WPI fluid gels were formed and their concentration on the rate of drainage of the foams these stabilised was also investigated. The rate of drainage was determined by measuring the electric conductivity of the foam and calculating the amount of water present in the foam over time (Section 2.4). In all samples the majority of liquid drainage took place within the first 10 min after foam formation. As shown in Fig. 9, the liquid drainage is affected by both the pH at which the

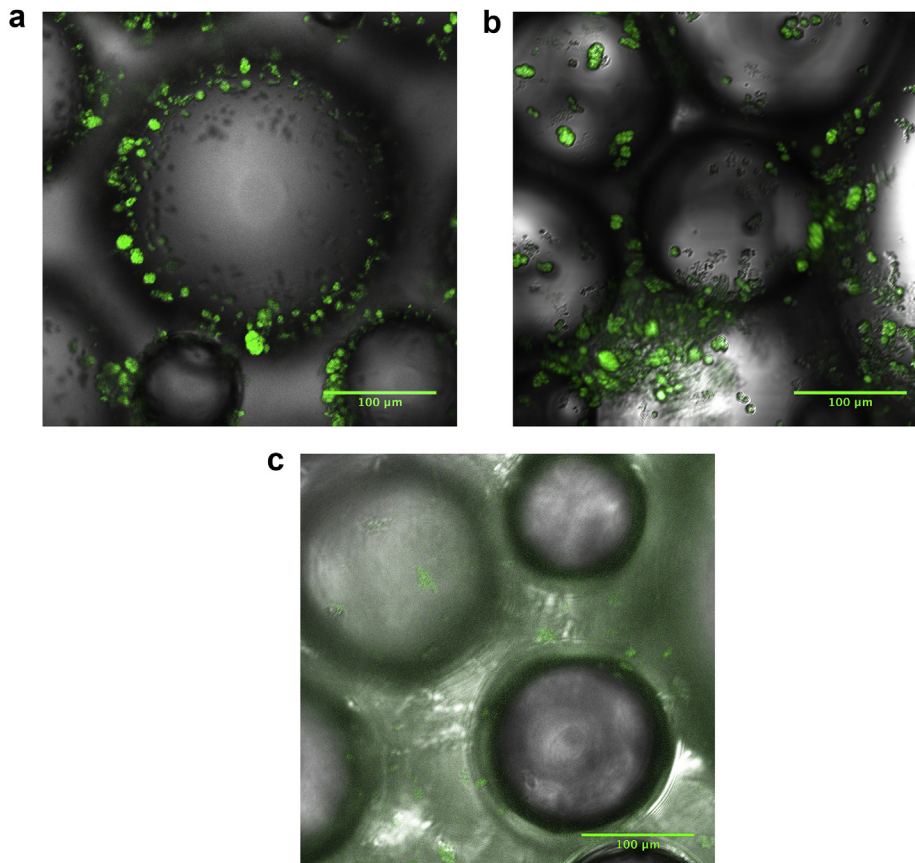


Fig. 10. Confocal images of WPI Fluid Gels made at pH 5 (a) pH 5 and adjusted to pH 8 (b) and pH 8 (c), protein aggregates are stained with a fluorescent probe and appear green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluid gel system was formed and then adjusted at, and by the overall protein concentration. Foam drainage behaviour follows closely that observed from foam half-life data. The fastest drainage was observed in the foams stabilised by WPI fluid gels formed at pH 5 and aerated at their intrinsic pH. On the other hand when the fluid gel pH was increased to pH 8 (following formation and before aeration) drainage was significantly reduced especially for the higher protein concentration fluid gels studied. This indicates that while non-charged small spherical fluid gels although they do position themselves around air bubbles do not prevent the flow of the continuous phase due to gravity. When the same fluid gel structures are adjusted to pH 8 and the aerated, drainage is reduced potentially by the increase in the local (around the air bubbles) rheology as demonstrated through the oscillatory rheology data. Foams stabilised by fluid gels made at pH 8 showed a moderate rate of drainage probably due to their larger size which potentially causes them to be excluded from the spaces between the bubbles which did not seem to be the case with the smaller aggregates.

The microstructure of foams stabilised by the different WPI fluid gels structures were also visualised by confocal laser scanning microscopy (CLSM). Images taken from foams stabilised by these systems show that in the case of the fluid gels formed at pH 5 (Fig. 10a) were the aggregates are smaller and spherical seem to occupy some of the space around the bubbles and potentially adsorb on the air water interface and partially cover it. The partial coverage of the bubbles was also present after increasing the pH of those systems to 8 (Fig. 10b) indicating that it is not dependent on the presence of charge. In the case of the foams stabilised by the fluid gels formed at pH 8 there seems to be exclusion of the particulates from the space between the air bubbles.

4. Conclusions

WPI fluid gels produced by thermally treating WPI under shear show interesting foaming properties which are dependent on both the pH at which these structures were originally formed but also the pH at which they were aerated.

Fluid gels made at pH 5 whilst not having any specific foaming functionality at their intrinsic pH, when adjusted and aerated at pH 8, demonstrated an enhanced foaming capacity. This is due to what is proposed as an enhancement to the electrostatic inter-particle interactions promoted by the change to the pH environment. This is supported by both the ζ -potential measurements but also by the presented interfacial elasticity and viscosity data. The small aggregates formed at pH 5 seem to be able to fill effectively the spaces between the bubbles and reduce the rate of liquid drainage by the particles acting as corks anchored on the interface and increase the local viscosity around the bubbles improving the stability of foams.

Fluid gels formed at pH 8, while having stronger charge and higher bulk viscosity and elasticity were able to produce less viscoelastic films probably due to the larger size of the aggregates present, which limited their ability to pack which lead to exclusion from the spaces around the air bubbles. This resulted in producing stable foams compared to native proteins by reducing the rate of liquid drainage but not to the same extent as the fluid gels made at pH 5 and aerated after adjustment to pH 8.

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