Article:
Murray, BS and Phisarnchananan, N (2016) Whey protein microgel particles as stabilizers of waxy corn starch + locust bean gum water-in-water emulsions. Food Hydrocolloids, 56. pp. 161-169. ISSN 0268-005X

https://doi.org/10.1016/j.foodhyd.2015.11.032

Whey protein microgel particles as stabilizers of waxy corn starch + locust bean gum water-in-water emulsions

Brent S. Murray* and Nataricha Phisarnchananan

Food Colloids Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK

b.s.murray@leeds.ac.uk

* Author for correspondence

Abstract

Food-grade whey protein isolate (WPI) microgel particles were investigated as a particle stabilizer of water-in-water (W/W) emulsions. The microgel particles were produced via the novel method of forcing coarse particles of a pre-formed thermally processed WPI protein gel through a jet homogenizer. The Z-average particle size was 149 ± 89 nm but the particles showed a strong tendency for aggregation when the pH was lowered from pH 7 to 4, when zeta potential also switched from -17 to + 12 mV. The viscoelasticity of suspensions of the particles, measured between 1 and 15 vol.% (0.02 and 3 wt.%) increased with concentration and was also higher at pH 4 than pH 7. However, all the suspensions were only weakly shear thinning, suggesting that they did not form very strong networks. The particles were added (at 1 - 15 vol.%) to a model W/W system consisting of waxy corn starch (S) + locust bean gum (LBG) that normally shows phase separation when the components are mixed at 90 °C then cooled to room temperature (22 to 25 °C). At 10 to 15 vol.% particles and pH 4, visual observation showed striking inhibition of phase separation, for a period of up to 1 year. Confocal laser scanning microscopy suggested that under these conditions extensive aggregation of the microparticles occurred within the starch phase but also possibly at the W/W interface between the starch-rich and gum-rich regions, supporting a Pickering-type mechanism as responsible for the enhanced stabilization of the W/W emulsion by the microgel particles.

Key words: protein microgel, Pickering, phase separation, stabilization

1 Introduction

Food products are complex systems containing many different kinds of ingredients and so mixtures of aqueous biopolymers have been widely studied for many years due to their important role in the food industry (Garnier, Schorsch & Doublier, 1995). Polysaccharides are polydisperse macromolecules that have been extensively used as thickening and texturizing agent. Starch, as the main storage carbohydrate of many plants, is one of the most important and abundant sources of food for humans. In most common starches the percentages of amylose and amylopectin are 20-30% and 70-80% respectively, whilst waxy starches consist of almost exclusively amylopectin, a highly branched, high molecular weight (> 10^6 Daltons) polymer of glucose. Galactomannan gums, such as locust bean gum (LBG),are also very high molecular weight polymers of monosaccharide sugars but their molecular structure is substantially different from that of amylopectin. Such gums consist of a substituted linear mannan backbone with short galactose side chains. Thus, LBG forms highly entangled, viscous solutions that are highly shear thinning at relatively low concentrations, whilst amylopectin forms very weak gels but is a good thickening agent at relatively high concentrations, where the highly branched swollen polymer molecules start to overlap. The very different conformational structures of the amylopectin and LBG molecules means that they have difficulty forming simple mixtures even at relatively low concentrations and this leads to their phase separation.

Albertsson first reported work on the phase separation of aqueous polysaccharides in 1962 and since then there have been numerous studies of the thermodynamic incompatibility of starch and non-starch hydrocolloids (Tolstoguzov, 1986; Alloncle & Doublier, 1991; Kulicke, 1996; Conder-petit, Pfirter &Escher, 1997; Closs et al., 1999; Tolstoguzov, 2006; Frith, 2010, Murray & Phisarnchananan, 2014). Phase separation of mixtures of these polysaccharides (in the absence of particles) has been shown elsewhere (Achayuthakan & Suphantharika, 2008; Ptaszek et al., 2009; Simonet, Garnier & Doublier, 2000) and such mixtures are used in various products and their phase separation is an issue. So study of these systems is of relevance to real products whilst at the same time starch + gum has proved to be a good model system to test ideas of what types of 'surfactants' might be used to stabilize the water-water interface in phase separating aqueous-soluble polymers.

Depending on the relative size and volume fraction of the different polysaccharide-rich phases that form, one can consider such systems as dispersions of one water-rich phase within another, i.e., water-in water (W/W) dispersions (emulsions). Frith (2010) discussed how the detailed microstructure of W/W dispersions could be controlled by solution conditions such as pH, salt, temperature, etc. It is important to understand and be able to control these phase phenomena since excessive phase separation may cause unacceptable changes in the appearance or sensory properties of products in which W/W dispersions exist (Semenova & Dickinson 2010; Firoozmand, Murray and Dickinson, 2012).

This paper builds on previous findings (Murray & Phisarnchananan, 2014) where the phase diagram of a starch + gum system was established and the rheology of the separate gum and starch phases was measured over a range of concentrations and shear rates/frequencies. In addition, it was demonstrated that sub-micron solid, hard (silica) particles possessing a range of surface hydrophobicity, i.e., non-food grade, were largely able to inhibit phase separation over a period of several weeks. In this current work the aim was to extend the idea of 'Pickering' stabilization of W/W systems to a new class of food-grade particle – submicron protein microgel particles.

Pickering emulsions, where solid particles strongly adsorb at the interface between two fluid phases and protect the dispersed phase from coalescing, were largely ignored after their re-discovery by Pickering in 1907 (Chevalier & Bolzinger, 2013). However, in the past decade there has been renewed interest in Pickering stabilization, partly because of the increasingly novel and wide ranging types of nanoparticles and microparticles that are now available. As far as application to foods is concerned, a continuing challenge is to find effective Pickering particles that are acceptable for use in the food industry (Morris, 2011; Dickinson, E. 2012b; Berton-Carabin & Karin Schröen, 2015).
The wetting properties of the particles at the interface (i.e., contact angle) is a key parameter in controlling the effectiveness of the particles as stabilizer and much work has focused on inorganic particles (Binks & Lumsdon, 2000; Binks, Rodrigues, & Frith, 2007; Lopetinsky, Masliyah & Xu, 2006; Yi, Yang, Jiang, Liu & Jiang, 2011) with surface chemistry modification (to increase their hydrophobic nature) or latex particles (Binks, Lumsdon, 2001; Dinsmore, Hsu, Nikolaides, Marquez, Bausch & Weitz, 2002; Paunov, 2003; Firoozmand, Murray & Dickinson, 2009; Du, Glogowski, Emrick, Russell & Dinsmore, 2010) although neither of these are suitable as food-grade ingredients. Particle aggregation to interfaces and its influence on colloidal stabilization has recently been reviewed by Dickinson (2015a)

Herzig et al. (2007) showed that phase separation of an oil/water system (lutidine as the oil phase) can be complete arrested by inclusion of 3 vol.% colloidal surface modified silica particles. In an oil-water system the energy barrier (ΔE) to particle displacement from the interface can reach thousands of k_BT (k_B is the Boltzmann constant and T is the absolute temperature) (Binks & Horozov, 2006; de Folter, van Ruijveb and Velikov 2011; Destribats et al. 2014; Murray & Phisarnchananan 2014). ΔE is given by $\Delta E = \pi r^2 \sigma (1 - |\cos\theta|^2)$, where θ = the three phase contact angle, r= the particles radius and σ = the liquid-liquid interfacial tension. In an oil-water system the interfacial tension is usually at least 1 mN m^{-1}, but with W/W polysaccharide+polysaccharide systems can be extremely low (10^{-4} – 10^{-6} Nm^{-1}, Shum, Varnell & Weitz, 2012) and so the gain in free energy by particles occupying the interface might be expected to be negligible. Nevertheless, Murray and Phisarnchananan (2014)

recently showed that silica particles of varying surface hydrophobicities could apparently inhibit the phase separation of W/W systems consisting of waxy corn starch + LBG or guar gum. Furthermore, Nguyen, Nicolai & Benyahia (2013) have used protein aggregates as particles in controlling phase separation of 'semi-polysaccharide' type W/W system of dextran + poly(ethylene oxide).Protein microgel particles (de Folter et al., 2012, Destribats et al., 2014) are just one type of novel food particle that might be exploited via the Pickering mechanism. Others include chitin nanocrystals (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011), cellulose microparticles (Wege et al., 2008), soy protein particles (Liu & Tang, 2013), modified starch particles (Timgren, Timgren, Rayner, Sjöö & Dejmek, 2011; Murray, 2011; Yusoff & Murray, 2011; Rayner et al., 2012; Tan et al., 2014), flavonoid particles (Luo et al., 2011), solid lipid particles and emulsion droplets (Gupta & Rousseau, 2012; Hanazawa & Murray, 2013, 2014)

Although protein microgel particles cannot really be considered as classic hard particle Pickering stabilizers, particles do not have to be rigid in order to act as good stabilizers, as long as they maintain a size and contact angle sufficient to secure their interfacial attachment as proscribed by eq. (1), so that the term 'Mickering' emulsions has been coined by Schmidt et al. (2011) to describe microgel-particle-stabilized emulsions. In addition, there have been a number of advances recently in the production of truly nanoscale protein aggregate particles of well-defined size or shape (Saglam, Venema, van der Linden & de Vries, R., 2014). Many of these methods rely on heating globular proteins in relatively dilute solution and at extremes of pH, particularly whey protein (Schmitt, et al., 2009, 2010; Schmitt & Ravaine, 2013).

In the work reported here we have opted for forming a thermally processed globular protein gel under more conventional conditions, but reduced this gel to significantly small nanogel/microgel particles through efficient processing through a jet homogenizer. The particles have been tested subsequently as a Pickering stabilizer of a true W/W polysaccharide system that we have studied previously (Murray & Phisarnchananan, 2014), consisting of a waxy corn starch (S) and locust bean gum (LBG). It is hoped that such particles and this method of preparation may form a more practical way of applying the Pickering mechanism to control the stability of W/W emulsions. Applications of protein microgel particles in general have recently been reviewed by Dickinson (2015b).

2 Materials and Methods

2.1 Materials

Gelatinized waxy corn starch (S), product code S9679, and locust bean gum (LBG), product code G0753, were purchased from Sigma-Aldrich (Gillingham, UK). All polysaccharide mixtures were

made up in a pH 7 phosphate buffer consisting of 0.05 mol dm^{-3} KH_2PO_4 + Na_2HPO_4 + 0.05 mol dm^{-3} NaCl. Sodium azide (0.02 wt.%) was also added as a bactericide. The pH was adjusted by adding either 1 mol dm^{-3} NaOH or 1 mol dm^{-3} HCl. Rhodamine B (product code R-6626) and acridine orange hemi (zinc chloride) salt, (product code 158550) were also obtained from Sigma-Aldrich. Water purified by a Milli-Q apparatus (Millipore, Bedford, UK), with a resistivity not less than 18.2 MΩ cm, was used for the preparation of all solutions. Silicone oil AS4 was from Fluka (Gillingham, UK).Powdered whey protein isolate (WPI) was obtained from Fonterra Limited (Auckland, New Zealand).

2.2 Preparation of WPI microgel particle suspensions.

The WPI powder was dispersed at 15 wt.% WPI in 200 ml phosphate buffer pH 7 (mentioned above) and stirred under mild magnetic stirring overnight for a complete solubilization. The WPI solution was transferred to glass bottle with plastic screwed top and heated in a temperature-controlled water bath at 90°C for 30 minutes. It was then cooled down under running water for 15 minute and stored in the refrigerator overnight. The WPI gel was then roughly broken into pieces with a spatula and then the coarse gel fragments were added to the chambers of a jet homogenizer (Burgaud, Dickinson & Nelson, 1990) which were then topped up with buffer. The ratio of the volumes in the two chambers used in the jet homogenizer was 45:55. The fragments were then homogenized at 220 bar. The finer gel fragments obtained were poured in to the larger of the chambers whilst the smaller chamber was filled with buffer and the fragments were homogenized again, but a slightly higher pressure of 300 bar. The volume fraction of microgel particles in this suspension was determined by centrifuging a sample of the suspension in a Beckman Avanti J30i centrifuge using a JA-30.50 rotor at 12000 rpm (approx. 17400 g) until the microparticles sedimented to leave a clear upper aqueous phase. This phase was then carefully removed via a pipette to determine its volume. Before the microparticles were characterized or blended with the starch and gum phases after dilution to the appropriate vol.% with buffer, the suspension sonicated in a Vibra-cell (Sonics&Materials, Newtown USA) for 2 min using 40% amplitude pulses every 2 seconds. (The suspensions also had a notable tendency for foaming and any bubbles that formed during their manipulation were removed via suction through a pipette).

2.3 S + LBG water-in-water emulsion preparation

Stock solutions of starch (7 wt.%) were prepared by dispersing the starch powder in the pH 7 phosphate buffer, followed by heating in an oil bath at 90 °C for 15 minutes with constant stirring, by hand. Stock solutions of gums were prepared by dispersing 2 wt.% LBG in the buffer under the same conditions as for the starch. The LBG solution was then left to cool and centrifuged at 11000 rpm and 25 °C for 1 h in a high speed Beckman Coulter(J2-HS) centrifuge to remove insoluble materials. These

contributed 20 ± 2 wt.% of the original powders. (Panda (2004) has reported that commercial LBG may contain up to 27% impurities). Stock solutions were stored at room temperature before use. The stock solutions were diluted with buffer to the required concentrations based on the soluble part remaining. To prepare mixtures, both stock solutions were heated separately at 90 °C for 5 minutes before blending. Equal volumes of S and LBG phases were blended with up to 10 ml of the WPI microgel particle suspension. Blends were mixed immediately after removal from the oil bath by an Ultra Turrax T25 homogenizer (IKA-Werke GmbH &Co., Staufen Germany) at 24000 rpm for 1 minute, after which the temperature of the samples had fallen to 70 ± 5°C For blends including microgel particles, the particles were added to either the gum or starch phase first. In order to reduce the pH to pH 4, 29 μl of 0.25 M HCl was added during the blending via the Ultra Turrax. For samples intended for confocal microscopy, Rhodamine B (RB) and acridine orange (AO) were added during blending to stain starch phase and particles respectively.

2.4 Particle size distribution and ζ-potential of WPI microgel particles

The particle size distributions of the WPI microgel particles were determined by dynamic light scattering at 25°C using a Zetasizer Nano-ZS (Malvern instruments, Malvern UK) in a PMMA standard disposal cuvette. Particle sizes were measured after diluting samples with phosphate buffer. The refractive index of WPI and the dispersion medium were set at 1.545 (Purwanti, Moerkens, van der Goot & Boom, 2012) and 1.33, respectively. The absorbance of the protein was assumed = 0.001. The Z-average size or cumulant mean was calculated by the autocorrelation function from Zetasizer software.

2.5 Bulk rheology

Bulk shear rheology of the WPI suspension was measured with a Kinexus Rheometer (Malvern Instruments, Worcestershire UK) using the *rSpace* software to control the rheometer, measure and analyze the results. The temperature was set at 25 °C in every experiment. The cone and plate cartridge (CP2/60:PL65) was used in every sample. After placing the sample between the cone and plate the sample was then left to achieve steady state for 5 minutes. Viscosities were measured over a range of shear rates using the shear rate mode in rSpace software. The starting shear rate was 0.1 s^{-1} and the final shear rate 1 s^{-1} the whole range taking 12 minutes in total. In oscillatory mode, the elastic and viscous components G' and G" were measured at 1% strain, in the range 0.1 – 1 Hz, taking 15 min in total for each run. Silicone oil was layered around the edge of the sample to prevent sample evaporation and drying.

193 *2.6 Visual assessment of the W/W emulsion stability*

194 Freshly emulsions were prepared in 75 x 25 mm flat bottom test tubes sealed with plastic cap,
195 stored at room temperature (22 to 25 °C) and photographed periodically.

196 *2.7 Confocal laser scanning microscopy (CLSM)*

197 CLSM of blends was performed using a Leica TCS SP2 confocal laser scanning microscope
198 (Leica Microsystems, Manheim Germany) connected with a Leica Model DM RXE microscope base.
199 The confocal was used with Ar/ArKr (488, 514 nm) and He/Ne (543, 633 nm) laser sources. Laser
200 excitation of the fluorescent samples was at 514 nm (≈29% intensity of laser) for Rhodamine Blue(RB)
201 and 488 nm (≈ 49% intensity of laser) for Acridine Orange(AO). A 20x objective with numerical
202 aperture 0.5 was used to obtain all images, at 1024 x 1024 pixel resolution. 0.5 wt.% of RB and 0.5
203 wt.% AO were dissolved with Millipore water and the solutions were stored in the dark when not being
204 used. For mixtures without WPI particles, 30μl of the RB solution were added per 5 ml of the starch
205 solution before blending with LBG. For polysaccharide mixtures with WPI particles, 30μl of the AO
206 solution were added per 5 ml of gum phase before blending. After blending the mixtures via the Ultra
207 Turrax, samples without added microgel particles were immediately poured into a welled slide 30 mm
208 diameter and 0.3 mm in depth. RB showed preferential staining of the starch whilst the cationic AO
209 showed strong affinity for the WPI microgel particles. Unlabeled areas were therefore assumed to be
210 gum-rich regions. The first image was captured 5 minutes after blending the mixtures. For systems
211 containing microgel particles it was necessary to wait for 20 min for bubbles to rise out of the samples
212 before they could be poured into the welled slide and the cover slip added. The appearance of
213 samples was recorded 0.5 to 24 h after blending. Image analysis was performed using Image J
214 software.

215 **3 Results and discussions**

216 *3.1 Microparticle characterization*

217 The heat-induced WPI gel was broken down into very small fragments by its processing through
218 the jet-homogenizer. The dashed line in Fig. 1 illustrates the size distribution of the microgel particles at
219 pH 7. It can be seen that the smallest dimension in the distribution is *ca*. 250 nm and the largest is
220 about 5 μm. This upper limit was assumed to be aggregates of particles, since Fig. 1 also shows that
221 after sonication for 2 min the distribution was significantly shifted to lower particles sizes: almost no
222 particles were above 1 μm, the Z-average size = 149 nm and the distribution showed a significant tail
223 into the sub-100 nm region. Nevertheless, we resist the temptation to refer to these as 'nanoparticles'.

Fig. 2(a) shows a confocal micrograph of a 5 wt.% suspension of sonicated WPI microgel particles at pH 7, stained with Acridine Orange to highlight protein regions (that appear bright in the images). Not surprisingly, very few particles are visible, given that the above size distribution indicates that most of the particle would be below the resolution on the microscope system used (ca. 0.4 μm) and/or Brownian motion would blur their outlines anyway. Fig. 2(b) illustrates micrographs of the same particles after acidification to pH 4. The formation of large particle aggregates at pH 4 is evident and for this reason it was not possible to obtain good quality particle size distribution data at pH 4 via the Zetasizer (the upper range that the Zetasizer can measure is 6 μm). It was possible, however, to measure the electrophoretic mobility of the WPI particles in dilute suspension. The values obtained were -1.34 and +0.93 at pH 7 and 4, respectively. Assuming a particle size of 150 nm, these mobility values convert, via the Smoluchowski assumption, to corresponding zeta potential values of -17.1 and + 7.4 mV at pH 7 and 4, respectively. WPI mainly consists ofβ-lactoglobulin and α-lactalbumin and the isoelectric point (pI) of these two proteins is in the pH range 4.8 to 5.3 (Fox & McSweeney, 2003) so that charge reversal between pH 7 and 4 was expected. The absolute magnitude of the zeta potential is seen to be lower at pH 4 than at pH 7 and so this passage through zero net charge on acidification probably accounts for the greater preponderance of microgel aggregates at the lower pH.

3.2 Bulk rheology of WPI microgel particles

The intention was to use the WPI microgel particles to try and impart interfacial stability to the phase-separating regions. Therefore, it was also important to establish if the microgels had any significant influence on the rheology of the 'bulk' biopolymer phases. If the microgels caused significant increase in viscosity or gelation of either the starch-rich or gum-rich phases this would also tend to curtail phase separation. The bulk shear viscosity (η) of 1 - 15 vol.% suspensions of the microgel particles was measured at 25 °Cover the shear rate ($d\gamma/dt$) range 0.1 to 1 s^{-1}. The results are shown in shown in Figs. 3 (a) and (b), for pH 7 and 4, respectively. All the WPI microgel dispersions exhibited shear-thinning behavior to some extent, except the 1 vol.% dispersion at pH 7, which within experimental error was practically Newtonian. For the rest of the samples η was adequately fitted by the power law model, i.e.,

$$\eta = K\left(\frac{d\gamma}{dt}\right)^{n-1} \quad (1)$$

The fitting parameters are shown in Table 1 and the curves on Fig. 3 are the fitted power law behaviour. Two observations are relevant. Firstly, that η was higher at pH 4 than at pH 7 at all corresponding vol.% particles, reflecting the greater aggregation of the particles at pH 4. Secondly, none of the samples were strongly shear thinning. This indicates that strong, extensive networks of

256 particles were not formed, nor was the volume fraction of the particles such that they were close
257 packing even at the highest concentration added, i.e., 15 vol.%. The latter also indicates that the
258 particles and aggregates below the resolution of the CLSM probably did not have a high aspect ratio.
259 In any case microgel particles are generally accepted as being quite compressible and the maximum
260 packing fractions that can be reached are generally much higher than for model hard spheres (Stokes,
261 2011). Strongly shear thinning behavior is indicated by much lower magnitude of the flow behavior
262 index *n*, or typically a good fit to the empirical Cross equation:

263 $$\frac{\eta-\eta_\infty}{\eta_0-\eta_\infty} = \frac{1}{1+K\dot{\gamma}^m} \quad (2)$$

264 : whereη_∞ and η_0 are the limiting high and 'zero' shear rate limiting viscosities. Flocculated particle
265 networks or solutions of entangled or weakly cross-linked polymers typically follow the Cross equation.
266 We attempted to fit the data in Fig. 3 to eq. (2) but no convergence was obtained except for the highest
267 viscosity case, i.e., 15 vol.% at pH 4 (which was also by far the most shear thinning at n = –0.27).
268 However, the value of η_0 required to give a good fit was of the order of 10^{10} Pa s, which seems
269 physically unrealistic given the range of the experimentally measured viscosity data.
270 Therefore, when the microgel particles were added to either the starch or LBG phase before the
271 two polymer phases were blended, one might expect some increase in the viscosity of either phase, but
272 nothing very significant. It should be noted that η of the starch and LBG phases before blending were
273 considerably greater than the values measured for the WPI microgel dispersions, e.g., 60 and 42 Pa s
274 at $d\gamma/dt$ = 0.1 s^{-1} for 4 wt.% starch and 0.6 wt.% LBG respectively (Murray & Phisarnchananan, 2014).
275 Thus, any subsequent effect on the phase separation kinetics of including the microgel particles is
276 unlikely to be due to enhanced viscosity or gel formation of either phase

277 *3.3 Macroscopic observations of the effect of particles of W/W emulsions*
278 Two series of mixtures of equal volumes of S + LBG were prepared as described above, in the
279 presence of different vol.% of WPI microgel particles at pH 4 or 7 and observed at regular time
280 intervals. Pure mixtures of S + LBG (i.e., without particles) showed macroscopic phase separation
281 within an hour after mixing and were completely phase separated after 3 days. The mixture formed a
282 more clear LBG-rich phase at the top and a starch-rich phase at the bottom. Fig. 4 shows the
283 appearance of all the mixtures after 1, 3 and 7 days. At pH 7 (Fig. 4(a)) the phase separation appeared
284 to be reduced as the concentration of particles increased, since it was progressively more difficult to
285 observe a more transparent upper phase – for example after 1 day with 10 vol.% particles and with 15
286 vol.% after 7 days . A slight difficulty in discerning phase separation in all the samples was that they
287 also showed increased foam stability as the vol.% particles was increased, so that even after 7 days a

thin layer of bubbles was observed at the top of the tubes. Such prolonged foam stability is unusual for whey proteins but protein in the form of particles, in this case gel microparticles, may also produce enhanced stabilization of bubbles (Schmitt, Bovay & Rouvet, 2014).

Fig. 3(a) shows that the rheology of a 1 vol.% microparticles suspension at pH 7 is essentially Newtonian and this viscosity (ca. 0.02 Pa s) is much lower than the viscosity of the pure starch or gum phase. Nevertheless, Fig. 4(a) shows that after 1 day this low concentration of particles still inhibits phase separation to some extent. Therefore, this slowing down of the phase separation is unlikely to be due to any significant increase in viscosity of either phase due to presence of this low vol.% of particles. The volume fraction of the upper LBG-rich phase decreased as the vol.% of particles increased but after 7 days the differences between the samples had stabilized and the appearance of the mixtures did not significantly change over an additional of observation period of several months. Fig. 4(b) shows the mixtures at pH 4 and overall every sample was more stable to phase separation at pH 4 than at pH 7, at the same time and vol.% particles. With no particles a thin, very clear upper layer formed within 1 day, suggestive of some syneresis, whilst at 5 and 7.5 vol.% particles the mixtures appeared to form a single turbid layer on top of a very clear water-like phase. At 10 and 15 vol.% particles no phase separation was evident after 1 year and the whole sample was completely turbid, although the pH 4 samples appeared to be more optically dense and they seemed to possess less foam.

3.4 Microscopic observations of the effect of particles of on water-in-water emulsions

Fig. 5(a) shows typical confocal micrographs from the S + LBG system, in this case for 2 wt.% starch + 0.3 wt.% LBG 5±2 min after mixing, in the absence of particles. Such a system shows rapid phase separation via spinodal decomposition, as discussed previously (Murray & Phisarnchananan, 2014). In real time the system is quite dynamic with movement and fusion of starch-rich domains (bright areas) and LBG-rich domain (dark areas). Thus, macroscopic phase separation occurs quite readily so that within 24 h (Fig. 5(b)there were only small brighter ‘blobs’ of variable size range (< 10 μm) visible, which are assumed to represent a small fraction of starch remaining within the bulk LBG phase. Figs. 5(c) and 5(d) show representative images of the same system at pH 7 but containing 5 vol.% and 10 vol.% WPI microgel particles, respectively, after 24 h. Compared to without added particles (Fig. 5(b)), Fig. 5(c) shows that 5 vol.% particles seemed to have some effect on the system, since some large starch-rich domains were still visible, although nowhere near as many as just after mixing (e.g., Fig. 5(a)), whilst Fig. 5(d) shows that 10 vol.% particles resulted in considerably more persistence of starch rich domains after 24 h. Furthermore, when the system was acidified to pH 4, Figs. 5(e) and 5(f) show that 5 vol.% and 10 vol.% particles had a dramatic effect on the microstructure

of the system - even after 24 h something like a fine spinodal decomposition structure persisted, although elements of this seemed somewhat aggregated.

Whether there was any definite accumulation of particles at the water-water interface, effecting a Pickering-type stabilization mechanism, was not clear from these images. However, an additional feature of the images with WPI particles present was a greater propensity for the particles(and/or their aggregates) to reside within the starch-rich domains rather than the gum-rich domains. This was the case regardless of whether the particles were deliberately dispersed in the gum phase or the starch phase before blending the two phase together. The propensity for particles to prefer the one phase over another has been noted before: for silica particles and the same starch in a previous paper (Murray & Phisarnchananan, 2014) but also for different particles in completely different bulk phases (Hanazawa & Murray, 2014; Firoozmand et al., 2009). As yet there is no satisfactory explanation for this effect, although the recent review by Dickinson (2015a) indicates the various types of aggregation processes both in the bulk and at the interface that may be involved.

3.5 Image analysis of phase-separating microdomains

Image analysis of a different series of images was used to try and quantify the effects of pH and particle concentration on the phase separation kinetics of the 2 wt.% S + 0.3 wt.% LBG system. Figs. 6(a) and (b) show the extracted characteristic length scale (*L*) as function of time for 5 and 10 vol.% particles at pH 7 and 4, respectively. "*L*" indicates the largest dominant dimension in any direction on the image. It was determined from the two-dimensional fast-Fourier transform of the captured micrographs using Image J software. In the absence of particles, *L* was approximately 60 μm after 5 min (the shortest aging time for which it was possible to obtain any images) and after 0.5 h discrete domains were undetectable because separate layers had started to form in the well of the slide. In the presence of 5 or 10 vol.% WPI particles at pH 7 (Fig. 6(a)), the starch microdomain sizes showed similar trends, with the starch blobs growing to $L > 150$ μm after 0.5 h and continuing to grow to $L > 200$ μm after 24 h. Fig. 6(b) shows the significant effect of acidifying to pH 4. There was a significant increase in the stability of the domain size with both 5 and 10 vol.% added WPI particles, with a relatively small increase in *L* from 35 to 60 μm and 20 to 35 μm, respectively, in the first 24 h. Representative of micrographs of some for the compositions have been included on Fig. 6 to give the reader a better idea of the microstructural differences.

The analysis of the microstructure is therefore consistent with the macroscopic observations (Fig. 4) and the other microscopic observations (Fig. 5), that increasing concentrations of particles seem to inhibit phase separation of the gum + starch system, especially at pH 4 compared to pH 7.

3.6 Bulk rheology of the starch and gum in the presence of WPI microgel particles

From all the above results, it is clear that WPI microgel particles have the ability to inhibit microscopic domain growth and macroscopic phase separation. It is well known that WPI and whey protein microgel particles can form gel networks in a bulk aqueous phase (Vincent & Saunders, 2011; Schmitt, Bovay & Rouvet, 2014) so it is important to test the effect of adding the WPI particles into each domain, in case the inhibition is simply due to a significant increase in the viscosity of either phase. Therefore, WPI microgel particles were dispersed in the separate bulk LBG and starch phases at the different particle concentrations and the bulk rheology measured. Since the major effects of particle addition were at pH 4, these measurement were only conducted at this pH. Fig. 7 shows the bulk viscosity η at a constant shear rate = 0.1 s^{-1}(Fig. 7(a)) plus the storage modulus (G') and loss modulus (G") at 0.1 Hz and 1% strain (Figs. 7(b) and (c), respectively). These low shear conditions were selected so as to be as close as possible to the solutions at rest, whilst still obtaining reproducible results.

Fig. 7 clearly shows that up to 10 vol.% WPI microgel particles added to 4 wt.% S, there was no significant increase in η, G' or G''. In fact, there was a slight decrease in η for particle concentrations below 10 vol.% whilst for 15 vol.% particles η approximately doubled. For G' and G'' the only significant increase also occurred between 10 and 15 vol.%. In contrast, η, G' and G'' remained considerably lower for 0.6 wt.% LBG across the whole range of addition of particles: 0 to 15 vol.%. (Note these two separate concentrations of gum and starch form the same effective final concentrations in the mixtures observed in Figs. 4, 5 and 6 above). Consequently, it seems unlikely that an increase in the viscoelasticity of the gum phase due to the addition of the microgel particles can explain the inhibition of phase separation. It does seem that a significant increase in the viscoelasticity of the starch phase can occur at >10 vol.% microgel particles, probably due to their aggregation in this phase. A likely cause of this might be depletion flocculation of the microgel particles by free polymer (Vincent & Saunders, 2011), in this case the starch molecules. However, since inhibition appears to occur at particle concentrations at and below 10 vol.% particles, plus the fact that the same dynamics occur if the particles are first mixed into the gum phase, an increase in the viscosity of the starch phase due to microgel particle aggregation within this phase similarly cannot explain all the inhibition effects observed. The same conclusion was reached (Murray & Phisarnchananan, 2014) for silica particle addition to the same system, where stabilization by silica particles occurred in particle concentration ranges where no significant increase in bulk phase viscosity occurred due to particle addition.

Detailed measurements of the viscoelasticity of the whole system under going phase separation were not measured, since if phase separation occurs one cannot reproducibly measure and interpret this rheology, since different heights of sample will have different viscoelasticity. However, it is

interesting to speculate how the viscoelasticity of the continuous starch phase might hinder the rise of blobs gum phase within it, or the fall of discontinuous starch blobs within a continuous gum phase. To this end, we have calculated the theoretical creaming velocity (V_s) of spherical blobs of 0.3 wt.% gum phase of nominal diameter = 60 μm rising through a continuous starch phase at [S] = 2 wt.%, from Stokes Law:

$$V_s = \frac{d^2 \Delta\rho g}{18\eta} \quad (3)$$

:where $\Delta\rho$ = the density difference between the starch and gum phase, g = acceleration due to gravity, d = the (gum-rich) blob diameter (assumed spherical) and η = the viscosity of the continuous (starch) phase. It seems reasonable to suppose that this is slower than starch blobs falling gum phase, since the measurements of the individual phases showed that η of the S phase + microgel particles was higher than that of G + particles (see Figure 7). The density of the starch and gum phases were measured as 1.01 and 0.89 g cm^{-3}, respectively. Using the value of η = 0.51 Pa s, measured at the lowest shear rate (0.1 s^{-1}) for the system with 10% microgel particles at pH 7, the calculated creaming velocity is 0.46 μm s^{-1}. Notwithstanding the fact that creaming probably does not follow Stokes law exactly, but will be more hindered, this creaming velocity easily predicts gross visible phase separation in test tubes of the height used (75 mm) since the distance of creaming of such blobs would be 75 mm in less than 2 days. However, systems such as this have not showed any significant separation over 1 year of storage. Consequently, the growth of the gum-rich domains to sizes even as large as this may be assumed to be significantly curtailed by the presence of the microgel particles.

4 Conclusions

Water-in-water (W/W) emulsions formed by mixing waxy corn starch and locust bean gum solutions could be stabilized by addition of whey protein isolate (WPI)microgel particles (size ca. 150 nm) . The stability depended upon the concentration of the particles and pH of the system. Stability was increased with increasing concentration of particles and particularly on lowering the pH from 7 to 4. The particles aggregated at pH 4 and showed a strong preference for the starch domains rather than gum phase under all conditions. At pH 4 extensive aggregation of the particles was observed in the starch phase. However, neither particle aggregation in the starch phase nor any increase in the viscoelasticity of the gum or starch due to the addition of the particles are able to account for the inhibition of phase separation. The individual microgel particles were too small to be discerned at the

W/W interface via confocal microscopy, but in the absence of other evidence, it seems likely that accumulation and aggregation of the protein particles at the W/W interface could account for the enhanced stability, as proposed by Nyguen, Nicolai & Benyahia (2013) for WPI particles of similar size, probably enhanced by their aggregation at the lower pH. In a similar way, Nguyen, Wang, Saunders, Benyahia & Nicolai (2015) have recently shown how the stability of their dextran+PEO W/W system, when stabilized by synthetic cross-linked polymer microgel particles, can be significantly changed by altering the pH or ionic strength and thus the repulsion between the microgel particles at the W/W interface.

5 References

Achayuthakan, P., & Suphantharika, M. (2008). Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. *Carbohydrate Polymers 71*, 9−17.

Albertsson, P. (1962). Partition methods for fractionation of cell particles and macromolecules. *Methods of biochemical analysis*.10, pp.229−262.

Alloncle, M., & Doublier, J. (1991). Viscoelastic properties of maize starch/hydrocolloid pastes and gels. *Food Hydrocolloids*, *5*, 455−467.

Binks, B., & Horozov, T. (2006). Colloidal particles at liquid interfaces: an introduction *In*: B. Binks & T. Horozov, ed. *Colloidal particles at liquid interfaces*. Cambridge: Cambridge University Press, pp. 1−74.

Binks, B., & Lumsdon, S. (2000). Effects of oil type and aqueous phase composition on oil–water mixtures containing particles of intermediate hydrophobicity. *Physical Chemistry & Chemical Physics, 2*, 2959−2967.

Binks B. P., & Lumsdon S. O. 2001. Pickering emulsions stabilized by monodisperse latex particles: effects of particle size. *Langmuir, 17*, 4540–47.

Binks, B. P., Rodrigues, J. A., & Frith, W. J. (2007). Synergistic interaction in emulsions stabilized by a mixture of silica nanoparticles and cationic surfactant. *Langmuir*, *23*, 3626−3636.

Berton-Carabin, C. C., & Karin Schröen, K. (2015) Pickering emulsions for food applications: background, trends, and challenges. *Annual Review of Food Science & Technology, 6*, 12.1–12.35 doi:10.1146/annurev-food-081114-110822.

Burgaud, I., Dickinson, E., & Nelson, P. V. (1990). An improved high pressure homogenizer for making fine emulsions on a small scale. *International Journal of Food Science & Technology, 25*, 39−46.

Cheung Shum, H., Varnell, J., & Weitz, D. A. (2012). Microfluidic fabrication of water-in-water (w/w) jets and emulsions. *Biomicrofluidics*, *6*, 12808−12809

450 Chevalier, Y., & Bolzinger, M. (2013). Emulsions stabilized with solid nanoparticles: Pickering
451 emulsions. *Colloids & Surfaces A: Physicochemical & Engineering Aspects*, *439*, 23–34.
452 Closs, C., Conder-Petit, B., Roberts, I., Tolstoguzov, V., & Escher, F. (1999). Phase separation and
453 rheology of aqueous starch/galactomannan systems. *Carbohydrate Polymers*, *39*, 67–77.
454 Conde-Petit, B., Pfirter, A., & Escher, F. (1997). Influence of xanthan on the rheological properties of
455 aqueous starch-emulsifier systems.*Food Hydrocolloids, 11*, 393–399.
456 deFolter, J., van Ruijven, M., & Velikov, K. (2012). Oil-in-water Pickering emulsions stabilized by
457 colloidal particles from the water-insoluble protein zein. *Soft Matter*, *8*, 6807–??.
458 Destribats, M., Rouvet, M., Gehin-Delval, C., Schmitt, C., & Binks, B. (2014). Emulsions stabilised by
459 whey protein microgel particles: towards food-grade Pickering emulsions. *Soft Matter, 10*, 6941–6954.
460 Dickinson, E. (2012a). Stabilising emulsion-based colloidal structures with mixed food ingredients.
461 *Journal of the Science of Food & Agriculture, 93*, 710–721.
462 Dickinson, E. (2012b). Use of nanoparticles and microparticles in the formation and stabilization of food
463 emulsions. *Trends in Food Science & Technology, 24*, 4–12.
464 Dickinson, E. (2015a). Structuring of colloidal particles at interfaces and the relationship to food
465 emulsion and foam stability. *Journal of Colloid & Interface Science, 449*, 38–45.
466 Dickinson E (2015b). Microgels – An alternative colloidal ingredient for stabilization of food
467 emulsions. *Trends in Food Science & Technology, 43*, 178–188.
468 Dinsmore, A. D., Hsu, M. F., Nikolaides, M. G., Marquez, M., Bausch, A. R., & Weitz, D. A. (2002).
469 Colloidosomes: selectively permeable capsules composed of colloidal particles. *Science, 298*,1006–
470 1009.
471 Du, K., Glogowski, E., Emrick, T., Russell, T. P., & Dinsmore, A. D. (2010). Adsorption energy of nano-
472 and microparticles at liquid–liquid interfaces. *Langmuir, 26*, 12518–12522.
473 Firoozmand, H., Murray, B., & Dickinson, E. (2009). Interfacial structuring in a phase-separating mixed
474 biopolymer solution containing colloidal particles. *Langmu*ir, 25, 1300–1305.
475 Firoozmand, H., Murray, B., & Dickinson, E. (2012). Microstructure and elastic modulus of mixed gels of
476 gelatin+oxidized starch: effect of pH. *Food Hydrocolloids, 26*, 286–292.
477 Fox, P. F., & McSweeney, P. L. H. (Eds.) (2003) *Advanced Dairy Chemistry: Volume 1: Proteins*,
478 Springer US.
479 Frith, W. (2010). Mixed biopolymer aqueous solutions – phase behaviour and rheology. *Advances in*
480 *Colloid & Interface Scienc*e, 161, 48–60.
481 Garnier, C., Schorsch, C., & Doublier, J. (1995). Phase separation in dextran/locust bean gum
482 mixtures. *Carbohydrate Polymers, 28*, 313–317.

Gupta, R., & Rousseau, D. (2012). Surface-active solid lipid nanoparticles as Pickering stabilizers for oil-in-water emulsions. *Food & Function, 3*, 302–3011.

Hanazawa, T., & Murray, B. (2013). Effect of oil droplets and their solid/liquid composition on the phase separation of protein–polysaccharide mixtures. *Langmuir, 29*, 9841–9848.

Hanazawa, T., & Murray, B. 2014. The influence of oil droplets on the phase separation of protein–polysaccharide mixtures. *Food Hydrocolloids, 34*,128–137.

Herzig, E., White, K., Schofield, A., Poon, W., & Clegg, P.(2007). Bicontinuous emulsions stabilized solely by colloidal particles. *Nature Materials, 6*, 966–971.

Kulicke, W., Eidam, D., Kath, F., Kix, M., & Kull, A. (1996). Hydrocolloids and rheology: regulation of visco-elastic characteristics of waxy rice starch in mixtures with galactomannans. *Starch/Stärke, 48*, 105–114.

Liu, F., & Tang, C. (2013). Soy protein nanoparticle aggregates as Pickering stabilizers for oil-in-water emulsions. *Journal of Agricultural & Food Chemi*stry, 61, 8888–8898.

Lopetinsky, R. J. G., Masliyah, J. H., & Xu, Z. (2006). Solids-stabilized emulsions: a review. In B. P. Binks, & T. S. Horozov (Eds.), Colloidal particles at liquid interfaces (pp. 186–224). Cambridge: Cambridge University Press.

Luo, Z., Murray, B., Yusoff, A., Morgan, M., Povey, M., & Day, A. (2011). particle-stabilizing effects of flavonoids at the oil−water interface. *Journal of Agricultural & Food Chemistry, 59*, 2636–2645.

McClements, D., & Keogh, M. (1995). Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. *Journal of the Science of Food & Agriculture, 69*, 7–14.

Morris, V. (2011). Emerging roles of engineered nanomaterials in the food industry. *Trends in Biotechnology, 29*, 509–516.

Murray, B. S., & Phisarnchananan, N. (2014). The effect of nanoparticles on the phase separation of waxy corn starch+locust bean gum or guar gum.*Food Hydrocolloids, 42*, 92–99.

Murray, B. S., Durga, K., Yusoff, A., & Stoyanov, S. (2011). Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. *Food Hydrocolloids, 25*, 627–638.

Nguyen, B., Nicolai, T., & Benyahia, L. (2013). Stabilization of water-in-water emulsions by addition of protein particles. *Langmuir, 29*, 10658–10664.

Nguyen, B. T., Wang, W. K., Saunders, B. R., Benyahia, L., & Nicolai, T. (2015). pH-responsive water-in-water Pickering emulsions. *Langmuir, 31*, 3605–3611.

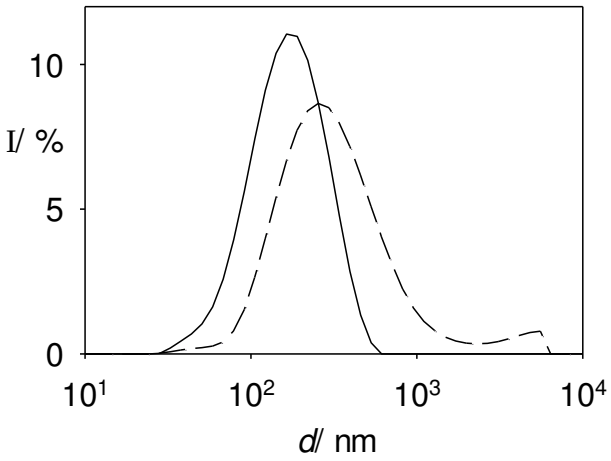
Panda, H. (2004). *The complete technology book on natural products (forest based)*. Delhi: Asia Pacific Business Press.

515 Paunov V. N. (2003). Novel method for determining the three-phase contact angle of colloid particles
516 adsorbed at air-water and oil-water interfaces. *Langmuir, 19*, 7970–7976.
517 Ptaszek, A., Berski, W,. Ptaszek, P., Witczak, T., Repelewicz, U., & Grzesik, M. (2009). Viscoelastic
518 properties of waxy maize starch and selected non starch hydrocolloids gel. *Carbohydrate Polymer*, *76*,
519 567−577.
520 Purwanti, N., Moerkens, A., van der Goot, A., & Boom, R. (2012). Reducing the stiffness of
521 concentrated whey protein isolate (WPI) gels by using WPI microparticles. *Food Hydrocolloids, 26*,
522 240−248.
523 Rayner, M., Sjöö, M., Timgren, A., & Dejmek, P. (2012). Quinoa starch granules as stabilizing particles
524 for production of Pickering emulsions. *Faraday Discussions, 158*, pp.139-155.
525 Saglam, D., Venema, P., van der Linden, E., & de Vries, R. (2014). Design, properties, and
526 applications of protein micro- and nanoparticles. *Current Opinion in Colloid and Interface Science, 19*,
527 428–437.
528 Semenova, M., & Dickinson, E. (2010). *Biopolymers in food colloids*. Leiden: Brill.
529 Schmidt, S., Liu, T., Rütten, S., Phan, K.-H., Möller, M., & Ritchering, W. (2011). Influence of microgel
530 architecture and oil polarity on stabilization of emulsions by stimuli-sensitive coreeshell poly(N-
531 isopropylcrylamide-co-methacrylic acid) microgels: Mickering versus Pickering behaviour? *Langmuir,*
532 *27*, 9801–9806.
533 Schmitt, C., Bovay, C., Vuilliomenet, A.-M., Rouvet, M., Bovetto, L., Barbar, R., & WSanchez C. (2009).
534 Multiscale characterization of individualized β-lactoglobulin microgels formed upon heat treatment
535 under narrow pH range conditions. *Langmuir, 25*, 7899–7909.
536 Schmitt, C., Moitzi, C., Bovay, C., Rouvet, M., Bovetto, L., Donato, L., Leser, M. E., Schurtenberger, P.,
537 & Stradnerb, A. (2010). Internal structure and colloidal behaviour of covalent whey protein microgels
538 obtained by heat treatment. *Soft Matter, 6*, 4876–4884.
539 Schmitt, V., & Ravaine, V. (2013). Surface compaction versus stretching in Pickering emulsions
540 stabilized by microgels. *Current Opinion in Colloid and Interface Science, 18*, 532–541.
541 Schmitt, C., Bovay, C., & Rouvet, M. (2014). Bulk self-aggregation drives foam stabilization properties
542 of whey protein microgels. Food Hydrocolloids, 42, 139–148
543 Simonet, F., Garnier, C., & Doublier, J.L. (2000). Partition of proteins in the aqueous guar/dextran two-
544 phase system. *Carbohydrate Polymer*, *14*, 591−600.
545 Stokes, J. R. (2011). *Rheology of industrially relevant microgels*. In A. Ferndanez Nieves, H. Wyss, J.
546 Mattsson, & D. A. Weitz (Eds.), Microgel suspensions. Weinheim: Wiley–VCH, Chap. 13.

547 Tan, Y., Xu, K., Niu, C., Liu, C., Li, Y., Wang, P., & Binks, B. P. (2014). Triglyceride–water emulsions
548 stabilised by starch-based nanoparticles. *Food Hydrocolloids, 36*, 70−75.
549 Timgren, A., Rayner, M., Sjöö, M., & Dejmek, P. (2011). Starch particles for food based Pickering
550 emulsions. *Procedia Food Science, 1*, 95−103.
551 Tolstoguzov, V. (1986). Functional properties of food macromolecules *In*: J. Mitchell & D. Ledward, ed.
552 London: Elsevier Applied Science, p. 385.
553 Tolstoguzov, V. (2006). Phase behavior in mixed polysaccharide systems *In*: M. Alistair et al., ed. *Food*
554 *polysaccharides and their application*. Florida: CRC Press Inc., pp. 589-620.
555 Tzoumaki, M., Moschakis, T., Kiosseoglou, V., & Biliaderis, C. (2011). Oil-in-water emulsions stabilized
556 by chitin nanocrystal particles. *Food Hydrocolloids,* 25, 1521-1529.
557 Vincent, B., & Saunders, B. R. (2011). Interactions and colloid stability of microgel particles. In A.
558 Ferndanez Nieves, H. Wyss, J. Mattsson, & D. A. Weitz (Eds.), Microgel suspensions. Weinheim:
559 Wiley–VCH, Chap. 6.,
560 Wege, H., Kim, S., Paunov, V., Zhong, Q., & Velev, O. (2008). Long-term stabilization of foams and
561 emulsions with in-situ formed microparticles from hydrophobic cellulose. *Langmuir*, 24, 9245-9253.
562 Yi, C. L., Yang, Y. Q., Jiang, J. Q., Liu, X. Y., & Jiang, M. (2011). Research and application of particle
563 emulsifiers. *Progress in Chemistry, 23*, 65−79.
564 Yusoff, A., & Murray, B. S. (2011). Modified starch granules as particle-stabilizers of oil-in-water
565 emulsions. *Food Hydrocolloids, 25*, 42−55.
566

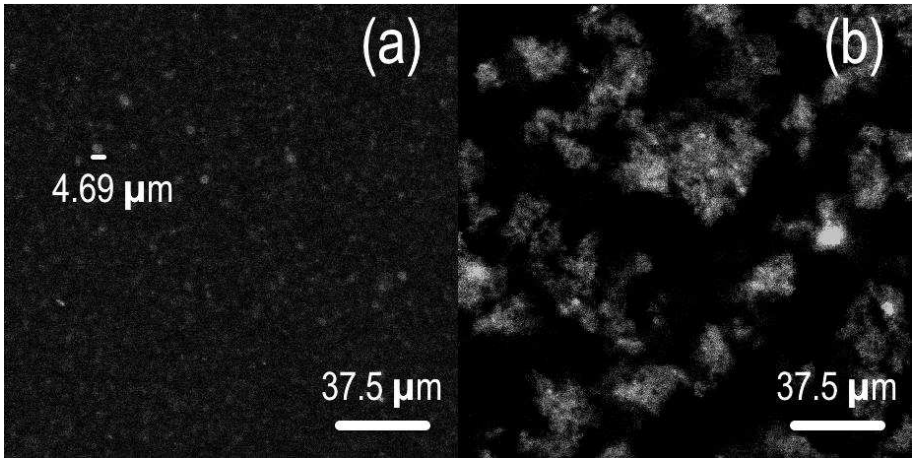
567 Figure 1

568



569

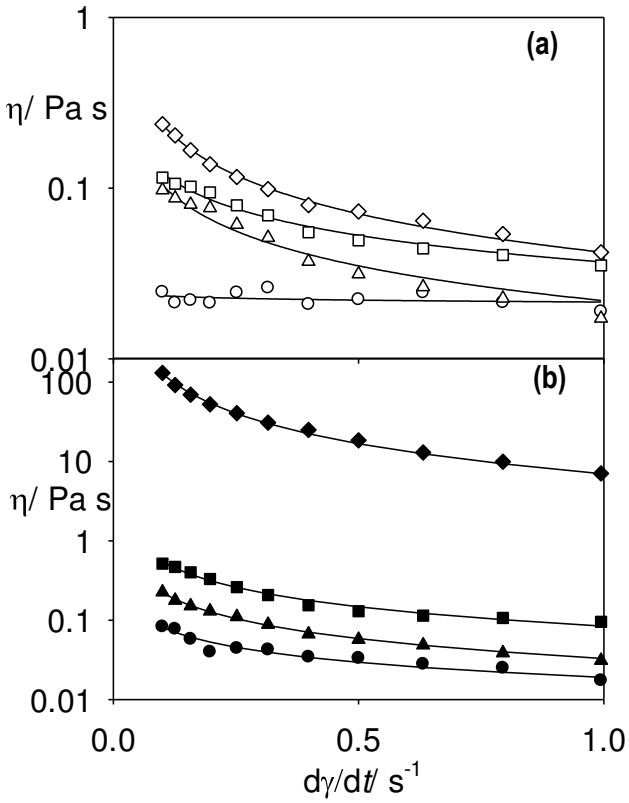
570 Figure 2



571

572

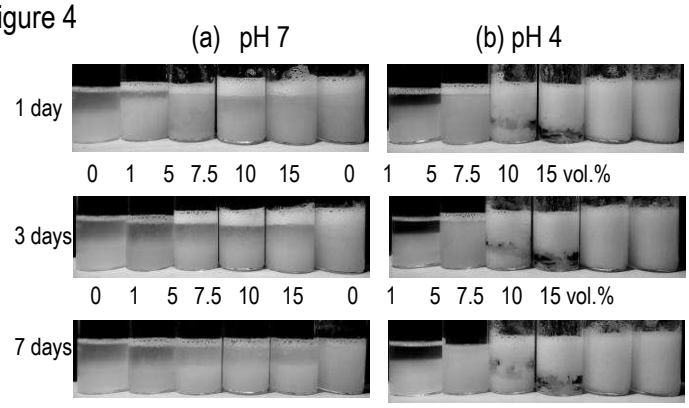
573 Figure 3



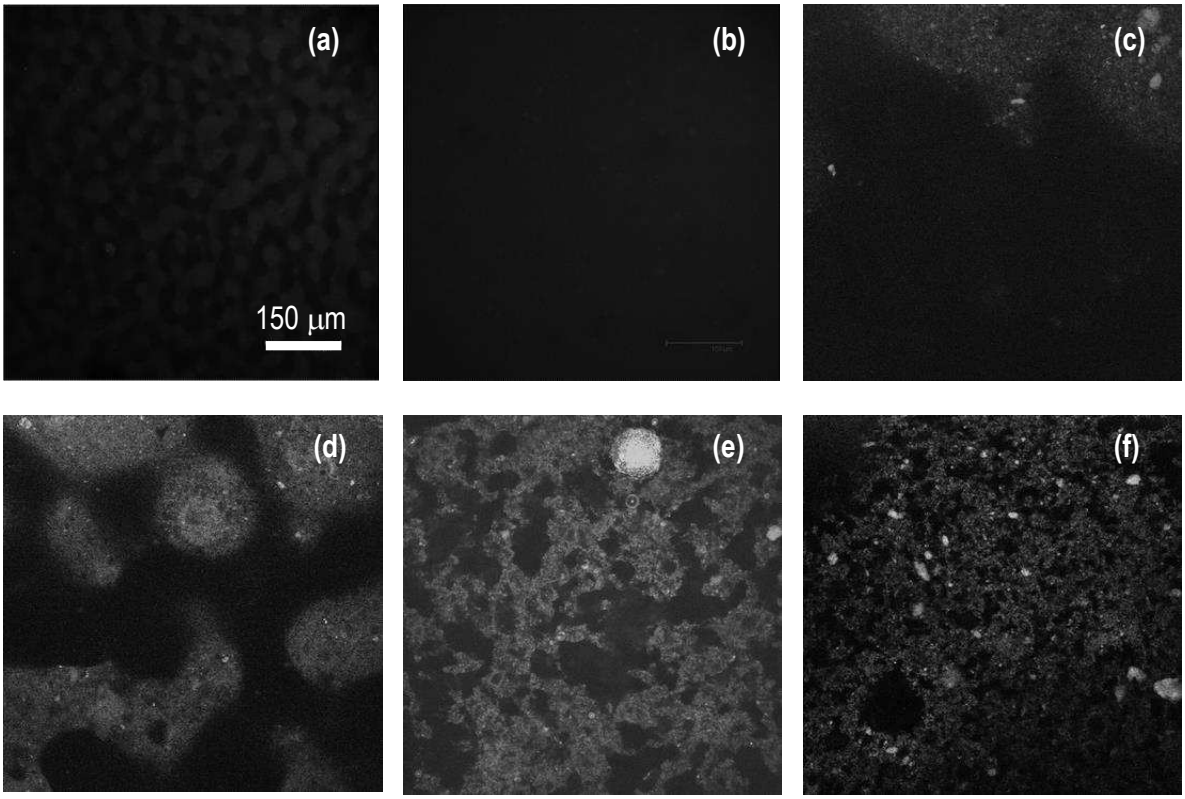
574

575

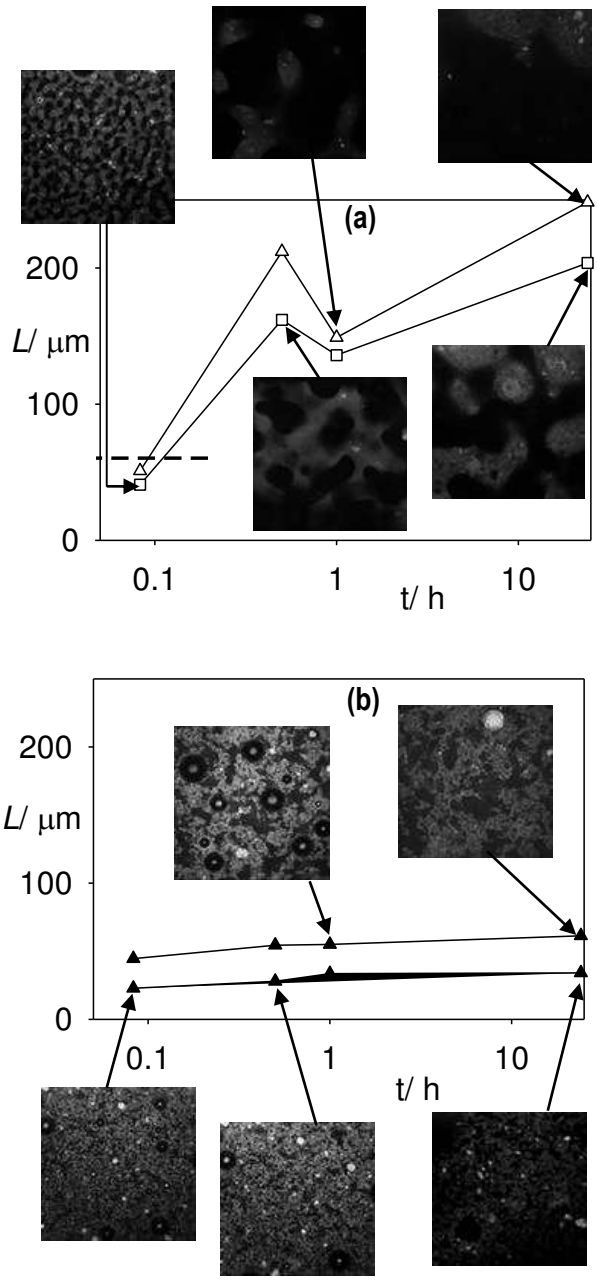
Figure 4


(a) pH 7
(b) pH 4
1 day
0 1 5 7.5 10 15 0 1 5 7.5 10 15 vol.%
3 days
0 1 5 7.5 10 15 0 1 5 7.5 10 15 vol.%
7 days

591 Figure 5
592
593
594

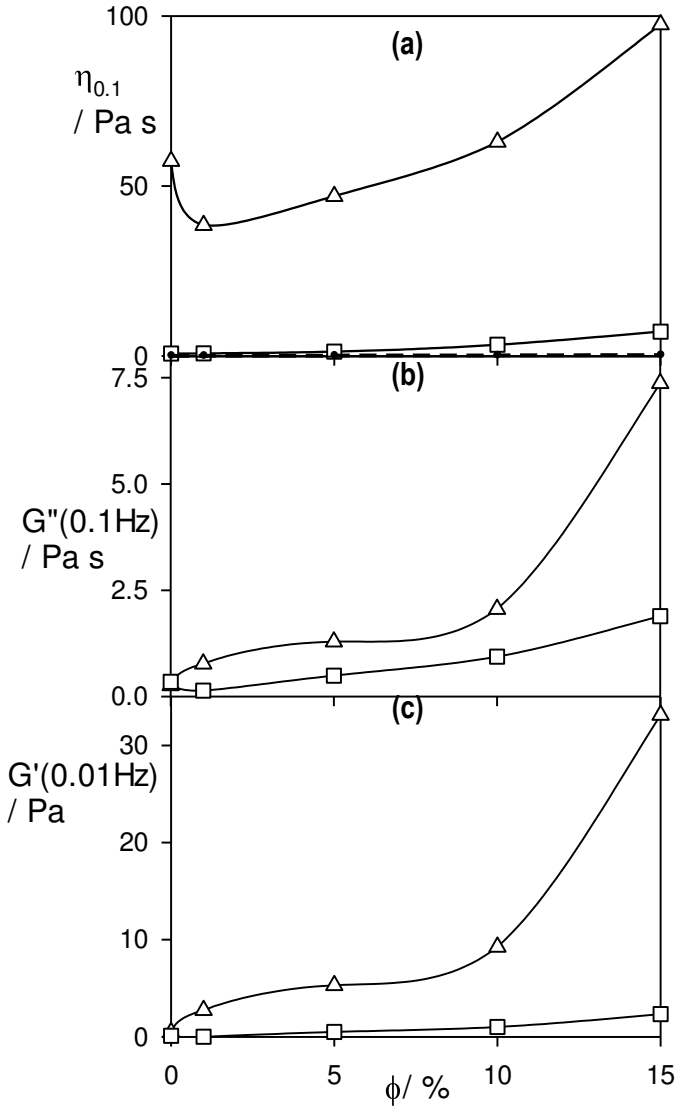


595 Figure 6
596
597



598 Figure 7

599



600

601

602

603 **Table 1**.

vol.%	*K*	*n*	P_K	P_m	*R*
pH 7					
1	0.022	0.96	<0.0001	0.37	0.3066
5	0.022	0.32	<0.0001	<0.0001	0.9823
10	0.037	0.48	<0.0001	<0.0001	0.9872
15	0.042	0.25	<0.0001	<0.0001	0.9973
pH 4					
1	0.019	0.37	<0.0001	<0.0001	0.9680
5	0.033	0.17	<0.0001	<0.0001	0.9982
10	0.0841	0.19	<0.0001	<0.0001	0.9934
15	6.96	-0.27	<0.0001	<0.0001	0.9985

604

605

606 **Figure & Table Captions**

607 **Fig.1**. Size distribution of WPI microgel particles. Intensity (I) versus particle size (*d*): before sonication
608 (- - -); after sonication (—).

609 **Fig. 2**. CLSM micrographs of 5vol.% suspensions of WPI microgel particles at: (a) pH7; (b) pH4. Bright
610 regions are WPI microgel particles, dark regions are background aqueous phase.

611 **Fig. 3.** Viscosity (η) versus shear rate ($d\gamma/dt$) for WPI microgel particle suspensions at: (a) pH 7; (b) pH
612 4; for1 vol.%(○,●); 5 vol.%(△,▲); 10 vol.%(□,■) and 15 vol.%(◇,◆) particles. The curves show the
613 fitted power law behaviour according to the parameters shown in Table 1.

614 **Fig. 4.** Appearance of W/W emulsions at 1, 3 and 7 days formed by mixtures containing 2 wt.% starch
615 + 0.3 wt.% LBG, with 0 to 15 vol.% added WPI microgel particles at: (a) pH7; (b) pH 4.

616 **Fig.5.** Representative confocal micrographs of mixtures containing 2wt.% starch + 0.3 wt.% LBG in the
617 absence and presence WPI microgel particles: (a) no particles, age 5min; (b) no particles, age 24 h; (c)
618 5 vol.% WPI particles, pH 7, age 24 h; (d) 10 vol.% WPI particles, pH 7, age 24 h; (e) 5 vol.% WPI
619 particles, pH4, age 24 h; (f) 10 vol.% WPI particles, pH4, age 24 h

620 **Fig.6.** Characteristic length scale, *L*, versus time since mixing for 2 wt.% starch + 0.3 wt.% LBG at:(a)
621 pH 7; (b) pH 4; for 5 vol.% (△) and 10 vol.% (□) added WPI microgel particles. Representative
622 micrographs are shown for various systems and times as indicated by the arrows. The dashed line
623 shows $L \approx 60$ μm after 5min the absence of particles.

624 **Fig.7**(a) Viscosity at shear rate 0.1 s^{-1}; ($\eta_{0.1}$); (b) storage modulus (G') measured at 0.1 Hz and 0.01
625 strain; (G") loss modulus measured at 0.1 Hz and 0.01 strain: versus vol.%(ϕ) of WPI microgel particles
626 at pH 4 added to individual solutions of: 4 wt.% starch (△); 0.6 wt.% LBG(□).

627 **Table 1**. Fitting parameters of power law model (eq. 1) to viscosity of WPI microgel suspensions of
628 different concentrations(vol.%), as shown in Figure 3. P_K and P_m are *P* values for fitted *K* and *n* values,
629 respectively, and *R* is the global goodness of it.

630

631