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Egg white protein microgels as aqueous Pickering foam stabilizers: bubble stability and interfacial properties

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Abstract

The aim of this study was to design and characterize aqueous foams stabilized by egg white protein microgels (EWPM) and compare their stability with conventional foams stabilized by egg white protein (EWP). Sub-micron sized EWPM (hydrodynamic diameter = 359 ± 21 nm) were designed using a top-down approach involving the formation of a thermally-crosslinked egg white protein hydrogel (90 ℃/ 30 min, pH 7.0) followed by controlled shearing using jet homogenization (300 bars, two passes). Microstructural evaluation at multiple length scales (confocal laser scanning microscopy and cryogenic scanning electron microscopy) indicated that the EWPM stabilized the aqueous foams via a Pickering-type mechanism. Foamability was higher in EWP-stabilized foams compared to EWPM-stabilized foams, irrespective of the protein concentrations tested (0.5 - 3.0 wt%). However, EWPM-stabilized foams exhibited higher stability to disproportionation over long periods ($p < 0.05$), even though the initial air bubble size was smaller than with EWP. Bubble coalescence experiments also confirmed that the fraction of the coalescence was much lower in EWPM systems as compared to the EWP counterparts. Changes of surface shear viscosities of EWP at the air-water interface indicated that EWP films were more brittle, exhibiting shear thinning during the measurements, whereas the viscosities of the EWPM films were independent of shear after 24 h of ageing. In summary, our study demonstrates for the first time that microgels of EWP have distinct advantages over EWP itself in terms of generating edible foams with ultra-high stability against disproportionation.

Keywords: Foam stability; egg white protein; microgel; disproportionation; interfacial shear viscosity; Pickering foam

1. Introduction

Foam stability is an important subject within food colloids because bubbles are key ingredients in a wide range of food products that include, bread, cakes, ice cream, confectionery and various other whipped products (Curschellas, et al., 2012; Li, et al., 2019). Aqueous foam is defined as a two-phase colloidal structure that is present in a non-equilibrium state containing water as the continuous phase in which gas (usually air) is dispersed as bubbles or gas cells (Drenckhan & Hutzler, 2015). Foams, like emulsions, are characteristically metastable systems, but tend to be even less stable, in that the mean bubble size spontaneously can grow relatively quickly via disproportionation and coalescence over the required lifetime of the product unless careful measures are taken (Guevara, et al., 2013).

The increase in average bubble size is driven mainly by drainage, coarsening and coalescence, in decreasing order of their typical 'rates', unless drainage is completely arrested by solidification of the continuous phase by some means. In foods, proteins are most widely used to stabilize aqueous foams due to their ability to adsorb and unfold at the interface resulting in the formation of viscoelastic interfacial films (Jin, et al., 2017; Sarkar & Singh, 2016) that provide some resistance to bubble disproportionation. This can be improved further, e.g., by the application of high intensity ultrasound to ovalbumin, to produce small protein aggregates (Gharbi & Labbafi, 2019). Low

molecular weight surfactants are good foaming agents and sometimes used in foods, e.g., Tweens (Adhikari, Howes, Wood, & Bhandari, 2009), and monoglycerides (Bos & Vliet, 2001), but generally these give very poor stability to disproportionation because the interfacial films are not as strong and more easily desorb.

In recent years there has been a resurgence of interest in using particles as foam stabilizers (Hu, et al., 2019; Mougel, Bertoncini, Cathala, Chauvet, & Capron, 2019; Ren, et al., 2019), which can therefore be referred to as Pickering foams (Valadbaigi, Ettelaie, Kulak, & Murray, 2019). Pickering foams can have much longer life-times than even protein-stabilized foams because of the high energies (DE) required to remove the particles from the interface once they have adsorbed, the fundamental equation (1) being (Binks, 2002):

$$-\Delta E = \pi r^2 \gamma (1 - |\cos\theta|)^2 \quad (1)$$

where θ is the contact angle in the aqueous phase, r is the radius of the particle (i.e., assumed spherical) and γ is the air-water (A/W) interfacial tension. The long-term challenge has been to find appropriate food-compatible particles that give this Pickering mechanism but, even more recently, protein-based microgel particles have been proposed to fulfill this role (Sarkar, Zhang, Holmes, & Ettelaie, 2019). For example, microgels generated from soy protein (Matsumiya & Murray, 2016), peanut protein (Jiao, Shi, Wang, & Binks, 2018), zein protein (Dai, et al., 2018) and whey protein (Heertje, 2014; Sarkar, et al., 2016b) have shown to give Pickering-type stability of

foams and emulsions. Nevertheless, food microgel research seems to have focused more on emulsions, whilst foams have attracted lesser attention to date (Binks, Muijlwijk, Koman, & Poortinga, 2017).

Egg white protein (EWP) is a classical foaming agent in foods, for example, in meringues and cake batters. However, in large scale manufacturing various polysaccharides (e.g., guar gum, pectin, xanthan gum) are often required to maintain the required overrun due to bubble collapse or shrinkage (Hao, et al., 2016; Majzoobi, Vosooghi Poor, Mesbahi, Jamalian, & Farahnaky, 2017; Ptaszek, et al., 2016). The polysaccharides added, but also the sugars (for sweetness) mainly act by increasing the viscosity of the continuous phase but there are significant demands to improve the stabilization of foams by EWP without sacrificing the appearance of 'clean-label' and/ or reducing the calorific content due to sugars. One exciting strategy is to physically structure EWP into microgels to generate the sort of 'Pickering' particle-stabilized bubbles. This would enable entailing no change in the labelling requirements because the stabilizer is still based on EWP and only requires a physical treatment.

Thus, in order to enhance and broaden the scope of EWP as a foam stabilizer, this study aims to design sub-micron-sized EWP-based microgel particles, via physical treatments, and to characterize the bulk and interfacial foam characteristics of such particle-laden foams and compare their stability with conventional foams stabilized by EWP alone. Besides characterizing the foams produced via microscopic techniques at various length scales (confocal laser scanning microscopy and cryogenic-scanning electron microscopy), we have measured the shrinkage and coalescence of individual

bubbles and related this to the adsorbed film properties via measurements of their interfacial shear rheology.

2. Materials and methods

2.1 Materials

Chicken eggs were purchased from the local supermarket (Tesco Ltd., UK). Sodium dihydrogen phosphate, di-sodium hydrogen phosphate and sodium azide were purchased from Sigma-Aldrich (Dorset, UK). Water purified by treatment with a Milli-Q apparatus (Millipore, Bedford, UK), with a resistivity not less than 18.2 MΩ cm at 25 ℃was used for the preparation of phosphate buffer. The latter was used as the solvent throughout the experiments with addition of 0.02 wt% sodium azide as a bactericide.

2.2 Preparation of samples

2.2.1 Preparation of egg white protein dispersion (EWP)

Egg white was manually extracted from the yolk of freshly purchased eggs manually and then homogenized under magnetic stirring (500 rpm speed) for 2 h, as reported previously (Li, et al., 2019). No further purification of the egg white protein dispersion (EWP) was performed.

2.2.2 Preparation of microgels

Egg white protein microgels (EWPM) were prepared via a top-down approach of preparing of heat-set protein hydrogel followed by controlled shearing using a previous

technique with some modifications (Sarkar, Kanti, Gulotta, Murray, & Zhang, 2017). Briefly, a 6.25 wt% EWP dispersion, obtained by diluting the EWP in 20 mM phosphate buffer (PBS) at pH 7.0 was thermally-crosslinked by heating (quiescent) at 90 ^{o}C for 30 min in a water bath. The gel was then broken up into coarse pieces and passed (twice) through the Leeds Jet homogenizer (University of Leeds, UK) at 300 bar.

2.2.3 Preparation of foams

Different concentrations (0.5 - 3.0 wt% protein) of EWP and EWPM were made up by diluting the aqueous dispersions of protein or microgel particles with 20 mM phosphate buffer at pH 7.0. Approximately 5 mL of these dispersions were placed in 15 mL test tubes, sealed well then shaken by hand for 30 s in order to examine the foamability, foam stability and visible structure of the foams.

2.3 Particle size of microgels

The particle size distribution (PSD) and polydispersity index (PDI) of the EWPM were measured via dynamic light scattering by employing a Zetasizer Nano-ZS (Malvern instruments, Worcestershire UK), using refractive indices of the EWPM and aqueous phase of 1.45 and 1.33, respectively. Measurements were made in triplicate at 25 °C.

2.4 Measurement of foamability and foam stability

Bulk foam stability at room temperature (25 °C ± 3 °C) was monitored via simple measurements of foam height as a function of time, relative to the non-foamed solution

154 height as described elsewhere (Murray, Durga, Yusoff, & Stoyanov, 2011b). In addition,
155 samples of the foam were pipetted from the samples into well slides and examined using
156 an optical microscope (PentaView, Celestron, USA) with 20 × magnification.
157
158 2.5 Bubble disproportionation measurements
159 Bubble disproportionation experiments were conducted in a bubble apparatus
160 (University of Leeds, UK) using methodology developed by Dickinson, Ettelaie,
161 Murray, & Du (2002) and Murray, et al. (2002). Briefly, bubbles were introduced via a
162 specially designed "bubble syringe" into the middle of a stainless steel cell through a
163 hole in the wall of the pressurization chamber (when the piston is clear off the cylinder),
164 and bubbles were allowed to rise to the planar A/W interface at the top of the cell. These
165 bubbles were trapped within the perimeter of circular hole in a paraffin wax-coated
166 mica-sheet floating in the middle of the interface. Bubble size was monitored with an
167 optical microscope and a video camera for at least 9 h. ImageJ image analysis software
168 and Microsoft Office were used to analyze the size of the bubbles at different times
169 from the optical images captured. In order to compare samples, changes in individual
170 bubble sizes versus time and changes in the overall bubble size distribution as a function
171 of time are reported.
172
173 2.6 Bubble coalescence measurement
174 Bubble coalescence experiments were performed in a similar apparatus as mentioned
175 above, where a pressure drop was used to induce and accelerate instability of the foams.

176 The simplified pressure drop apparatus has also been described in detail previously
177 (Murray, Dickinson, Lau, Nelson, & Schmidt, 2005). Briefly, bubbles were injected
178 beneath the A/W interface into the same cell as described in section 2.5. A rubber O-
179 ring and a glass plate seal the top of the steel cell and a pressure drop is induced by
180 withdrawal of the piston whilst the bubbles at the A/W interface are observed. The
181 pressure drop causes the bubbles to expand at the same rate as the pressure drop (which
182 typically was 810.6 mbar), inducing bubble coalescence due to the sudden depletion in
183 adsorbed film coverage. As described previously (Murray, et al., 2011b), coalescence
184 tends to continue for a few seconds after the pressure drop has ceased but then stops
185 and the remaining bubbles are stable to coalescence. (Note that this experiment is
186 designed to induce coalescence in bubbles that are stable to coalescence at constant
187 pressure). The number fraction (F_c) of bubbles that coalesced was then calculated by
188 simple counting of the bubbles in the images before and after the experiment.
189 Measurements were repeated at least eight times and mean values of F_c are reported.
190
191 2.7 Interfacial shear viscosity
192 Apparent surface shear viscosity (η) experiments were conducted using a two-
193 dimensional Couette-type interfacial viscometer. The operating mode and methods
194 have been described in detail previously (Burke, Cox, Petkov, & Murray, 2014; Murray,
195 Dickinson, & Wang, 2009). Briefly, a wire of suitable torsion constant suspends a
196 biconical disk positioned with its edge touching the A/W interface of the sample
197 solution contained in a concentric circular dish. The rheometer was operated in a

198 constant shear-rate mode (Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009) when
199 the surface shear viscosity (η) is calculated from:
200
201 $$\eta = g_f K \theta_i / \omega \quad (2)$$
202
203 where, g_f is the geometric factor of the equipment i.e. $(R_i^{-2}-R_0^{-2}) \cdot (4\pi)^{-1}$, where R_i is the
204 radius of the disk (14.5 mm) and R_0 is the radius of the dish (72.5 mm); ω is the angular
205 velocity of the dish. A fixed value of ω =1.27 × 10^{-3} rad s^{-1} was employed for
206 comparison with other systems. θ_i is the angle of rotation of the disk and K is the torsion
207 constant.
208
209 2.8 Confocal laser scanning microscopy (CLSM)
210 The foams stabilized by EWP or EWPM were observed via a Zeiss LSM 710 confocal
211 microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany), where the EWP or
212 EWPM systems were imaged after mixing with 0.1 mL of 1.0% (w/v) Rhodamine 6G
213 protein stain. The samples were observed at room temperature (25 °C ± 1 °C), using ×
214 63 objective at an excitation wavelength of 543 nm (Sarkar, Arfsten, Golay,
215 Acquistapace, & Heinrich, 2016a).
216
217 2.9 Cryogenic-scanning electron microscopy (cryo-SEM)
218 For cryo-SEM analysis, fresh foams stabilized by EWPM were imaged via an FEI Nova
219 450 SEM (Eindhoven, The Netherlands), as described by other researchers (Binks,

Campbell, Mashinchi, & Piatko, 2015). Briefly, samples were prepared by mounting a small volume of fresh foam onto a copper holder and then placed in liquid nitrogen (-208 °C) where the sample was frozen. Frozen samples were fractured using a sharp blade and sublimed for 3 min at -90 °C, coated with a thin layer of iridium (2 nm) via a Cressington 208 HR sputter coater, then imaged at 3 kV.

2.10 Statistical analysis

Each measurement was conducted at least in triplicate and SPSS version 19.0 was used for statistical analysis of means and standard deviations. Two-way analysis of variance (ANOVA) tests were carried out, and significance differences were defined when the p-value was < 0.05, using Duncan's Multiple Range Test.

3. Results and Discussion

3.1 Characteristics of EWPM

The egg white protein (Li, et al., 2018) formed a thermally cross-linked gel at 6.25 wt% protein (Supplementary Figure S1) which was then used to fabricate the microgel particles via the jet homogenization process. As can be seen from Figure 1a, EWPM had a mean hydrodynamic diameter (D_h) of ~ 359 nm, with a particle size distribution showing the most prominent peak in the region 100-1000 nm and a relatively small peak below 100 nm. The smaller peak probably represents EWP that somehow escaped the microgel formation process. Similar small peaks has been previously observed in the case of whey protein microgel formation (Sarkar, et al., 2017).

242

243 3.2 Microstructures of foams

244 Cryo-SEM images of EWPM are shown in Figure 1b. As seen, the surface of the fresh
245 foams seemed to contain mainly particles characteristic of the larger peak, i.e., 100-
246 1000 nm of Figure 1a. The CLSM images of the foams stabilized by EWP or EWPM
247 are shown in Figure 2. Figure 2a shows representative CLSM image of foams stabilized
248 by EWP. There is not a great deal of brightness (protein-labelled fluorescence) visible
249 around the bubbles. In contrast, with EWPM (Figure 2b) a much brighter and thicker
250 ring can be observed around the bubbles, suggestive of a much thicker adsorbed protein
251 layer, presumably formed of sub-micron EWPM particles, which seem more evident
252 when zooming in on the A/W interface as in Figure 2c and Figure 2d. All in all, particles
253 are clearly evident at the A/W interface of fresh foams at both the length scales (CLSM
254 and Cryo-SEM) that suggest a Pickering-type mechanism of stabilization a Pickering-
255 type stabilization mechanism seems to be clearly taking place in the fresh foams
256 stabilized by EWPM

257

258 3.3 Foaming properties

259 It is known that the method employed for foam production tends to influence the
260 stability properties of aqueous foams (Drenckhan & Saint-Jalmes, 2015; Murray &
261 Ettelaie, 2004). In our experiment, we produced foams by hand-shaking for the same
262 time (30 ± 1 s) allowing a quantitative description in terms of foamability i.e. how much
263 foam is produced and foam stability i.e. how the foam evolves kinetically (Arnould, et

al., 2018; Schmidt, Damgaard, Greve-Poulsen, Larsen, & Hammershøj, 2018). One advantage of hand-shaking is that it is relatively simple but reproducible and can be performed in closed tubes, permitting the evolution of the foam over relatively long times, in this case up to 7 days.

Figure 3 shows the foam volume of EWP (Figure 3a) and EWPM (Figure 3b) dispersions at different protein concentrations as a function of time. (Supplementary Figure S2 showing the corresponding optical microscopic images). Initial foam volumes i.e., foamability at 0 min increased with EWP concentration from 0.5 to 3.0 wt% (Figure 3a). However, initial foamability in case of EWPM was independent of protein concentration ($p > 0.05$) (Figure 3b). With EWPM, a more rapid decrease in foam volume was observed within the first few minutes compared to the EWP-stabilized foams (Figure 2s). Thus, foamability of EWPM was lower than EWP in the shorter time scale. This might be attributed to EWP proteins adsorbing faster to the A/W interface than EWPM by virtue of the smaller size of the former (Ercili-Cura, et al., 2015; Liu, et al., 2019; Tang, 2019). Both EWP and EWPM showed a decrease in foam volume over 7 days (168 h) but at the same protein concentrations, the foam volume with EWPM did not decrease as significantly as with EWP, especially at the higher concentrations (≥ 2.0 wt%, see Figure 3b).

In summary, there seemed to be an advantage in converting EWP to EWP microgels (EWPM) in terms of improving the foam stability, but not necessarily the foamability. This suggest that mixtures of EWP and EWPM might produce an optimal formulation with high enough foamability and foam stability.

Foams are mainly destabilized by disproportionation and coalescence (Rodriguez Patino, Carrera Sanchez, & Rodriguez Nino, 2008). Disproportionation is driven by the differences in the Laplace pressure in the gas bubbles of different sizes (Damodaran, 2005; Wouters, et al., 2018). This results in gas diffusion from the smaller bubbles to the larger ones because the solubility of the gas in the former bubbles is higher than that in the latter ones (Ettelaie, 2003). Disproportionation of individual air bubbles stabilized by EWP or EWPM was followed for up to 9 h; some initial and final bubble images are shown in Figure 4. It is clear that for EWP, more bubbles remained at the end of 9 h only at higher protein concentrations (Figure 4a), whereas for the EWPM systems, the number of bubbles remained higher at all concentrations (Figure 4b). This is despite the fact that the initial sizes of the bubbles in EWPM systems were generally smaller, which is expected to accelerate shrinkage (Ettelaie, 2003).

In order to visualize better the bubble shrinkage, bubble size versus time of individual bubbles in images such as those in Figure 4 are plotted versus time for EWP and EWPM in Figures 5a and 5b, respectively. It should be noted that the diffusion of gas bubbles at the edge of the mica hole and bubbles touching one another during shrinkage will differ from those not touching each other or the mica (Söderberg, Dickinson, & Murray, 2003). Therefore, we have included only bubbles that were not touching in the analysis in Figure 5. For EWP, the bubble shrinkage was relatively rapid, irrespective of the initial size of the bubbles, compared to EWPM. After 9 h virtually most bubbles had disappeared (i.e., diameter = zero). In fact, most bubbles had disappeared after only 360 min regardless of the protein concentration. In other words,

raising the EWP concentration will not necessarily help to increase the foam stability against disproportionation.

With EWPM (Figure 5b), the complete loss of bubbles was significantly less as compared to that in EWP systems. Bubble sizes seemed to plateau out at ca. 75 μm although a few bubbles disappeared at the lowest protein concentration (0.5 wt%). Thus, in general, this confirmed that the foam stability of the EWPM systems was much higher than that of the EWP systems, in this case due to disproportionation. One might speculate that this was due to a stronger and thicker interfacial films formed by the EWPM that persisted towards the end of the shrinking process. This was tested in the subsequent surface shear viscosity measurements, which is discussed later.

Changes in the overall bubble size distribution provides a more useful description of foam stability (Oliveira, et al., 2019), but this is difficult to obtain, except perhaps by X-ray tomography (Solórzano, Pardo-Alonso, de Saja, & Rodríguez-Pérez, 2013). The bubble experiments described so far represent the behaviour of a sort of two-dimensional foam, where at least all the bubbles in one layer are easily visible. The variation in the initial bubble size and the close proximity of neighbouring bubbles means that the shrinkage kinetics are complex, some bubble growing at the expense of other before shrinking later, etc. (Ettelaie & Murray, 2015). Nevertheless, it was interesting to calculate the bubble size distribution in the bubble layer at the planar A/W interface for the different systems at different times.

The bubbles were divided into size classes 100 μm wide and the number % in each size class were calculated and are shown in Figures 6a and 6b, for the EWP and EWPM

systems respectively, at the different protein concentrations. The initial distribution is shown in the left hand side of each panel and the distribution (after 9 h) is shown in the right hand side. For bubbles stabilized by EWP at low protein concentration (0.5 wt%), the initial bubbles sizes ranged from 60 to 430 ± 5 μm. Higher EWP concentrations gave a wider range of bubble sizes, i.e., extending larger bubbles. With time, the bubble size distribution gradually shifted towards smaller diameters for all systems, but at the higher protein concentrations, the final size distribution was wider. With EWPM (Figure 6b) at all concentrations the initial distribution tended to be narrower than with EWP. The distribution shifted to lower sizes and became more narrow after 9 h, there being little difference between 0.5, 1.0 and 2.0 wt%, but 3.0 wt% EWPM definitely seemed to give the most narrow and smallest bubble size distribution. Jakubczyk, et al. (2019) and Parra, Ndoye, Benkhelifa, FlickAlvarez (2018) showed that a narrower bubble size distribution gave a lower degree of disproportionation, but in the 'two-dimensional' foams experiments reported here, every bubble is in contact with the planar W/W interface, i.e., a bubble of effectively infinite curvature, so that there is nothing to prevent diffusion between the two and shrinkage of all bubbles apart from the adsorbed film. Thus, the almost complete cessation of further shrinkage after 100 to 200 min for most bubbles (see Figure 6b) points to the much greater stability to disproportionation of the microgel protein compared to the non-microgel protein.

The other major factor that contributes to foam destabilization is bubble coalescence (Murray, et al., 2005; Murray, Durga, de Groot, Kakoulli, & Stoyanov, 2011a). Coalescence depends on the physical properties of gas phase, liquid phase and

352 bubble characteristics (Yang & Foegeding, 2011). Figure 7 compares the number
353 fraction (F_c) of bubbles that coalesced after the application of the pressure drop (810.6
354 mbar) for the EWP and EWPM systems at different protein concentrations. For both
355 systems, F_c decreased with increasing in protein concentration, as observed previously
356 by (Wouters, et al., 2018), but notable differences were observed between EWP and
357 EWPM. For example, at 0.5 wt% protein, F_c was significantly ($p < 0.05$) greater
358 (approximately 3x higher at 31 ± 5 %) for the EWP-stabilized bubbles compared to its
359 microgel counterpart ($p < 0.05$). At 3.0 wt% protein, Fc decreased to 8.8 ±4.1 % for the
360 EWP system, but this was still significantly ($p < 0.05$) higher (almost 5x higher) than
361 the bubbles stabilized by EWPM. In other words, only 0.5 wt% EWPM was required
362 to give similar stability as 6x higher concentration (3.0 wt%) of EWP, highlighting the
363 higher resistance to coalescence imparted by the microgels.

364 It is tempting to propose that the higher stability of the EWPM systems was due to
365 the presence of the microgel particles at the A/W interface, giving adsorbed films that
366 can greater resist bubble shrinkage due to the higher desorption energy of the adsorbed
367 species and greater overall mechanical strength of the films, even if surface coverage is
368 not complete (Kudryashova & de Jongh, 2008).

369

370 3.4 Interfacial shear rheology

371 In order to obtain more directly some measure of the mechanical properties of the
372 adsorbed films, to see if this agrees with the explanation of the higher stability of the
373 EWPM systems proposed above, measurements of the interfacial shear rheology of the

adsorbed films stabilized by EWP or EWPM were conducted. Interfacial shear rheology is a very sensitive way of monitoring the formation and structuring of the adsorbed protein layers (Felix, Romero, Sanchez, & Guerrero, 2019), that can be related, directly or indirectly, to foam and emulsion stability (Murray, 2002; Murray, 2011; Murray & Dickinson, 1996).

Figure 8 shows measured η values as a function of time at 0.5 wt% and 3 wt% EWP and EWPM. A control experiment with only PBS (20 mM, pH 7.0) was also performed between 0 and 24 h and, as expected, the measured η was zero. Both the protein and particles caused large and rapid increase in the surface shear viscosity from time zero. With 0.5 wt% EWP, η increased to over 6 x 10^3 mN s m^{-1} in the first 95 min, followed by slower decrease to ca. 4.5 x 10^3 mN s m^{-1} in the next 2 h. After leaving overnight, η had increased back again to 7 x 10^3 mN s m^{-1}, but subsequent measurements over the following hour suggested a decrease again. In contrast, the EWPM gave a slower initial increase to around 2.5 x 10^3 mN s m^{-1} in the first 2 h of adsorption, which overnight increased slightly further to around 2.8 x 10^3 mN s m^{-1}, followed by a negligible fall (within experimental error). For 3.0 wt% EWP, η increased rapidly in first 80 min but then, as at 0.5 wt%, decreased again in the next 2 h, this time to ca. 2.3 x 10^3. Similarly, after aging overnight, η appeared to have increased back again to 6 x 10^3 mN s m^{-1}, but started to decrease again in subsequent measurements. Beyond 30 min, all measured η were lower at 3.0 wt% EWP than at 0.5 wt%. With EWPM at 3.0 wt%, there was a similar slower increase in η over the first 30 min, with further steady increase to higher values than for 0.5 wt% EWPM, reaching 3.9 x 10^3 in 4 h. Overnight this steady

increase seemed to have continued, reaching over 10^4 mN s m^{-1} after 1400 min and still increasing, higher than the value measured for EWP at 0.5 wt%. All values for the EWP and EWPM are very high compared to many other proteins (Murray, 2011), i.e., these films are very strong whilst the increases followed but decreases with EWP are reminiscent of stress overshoot and the exhibition of a yield stress of strong films (Martin, Bos, Cohen Stuart, & van Vliet, 2002) when they are continuously measured via such techniques. This results in a final lower steady state stress and apparent η. In the η measurements here the shear was applied intermittently for 10 min and the corresponding shear stress at the end of this period was used to calculate η. This procedure was adopted to try and avoid fracture of the films as they building, which tends to lead to more irreproducible results. All such measured η are apparent, i.e., dependent on the shear rate and shear time, but as long as the same procedure is adopted this allows one to compare the behaviour of the systems qualitatively. Thus, one can probably explain the slower build up in η with EWPM at the same overall protein concentration (0.5 wt%) as due to the slower diffusion and re-arrangement of the microgels at the interface as compared to the proteins themselves, whilst the microgels seems to give an adsorbed layer that is less likely to fracture under stress, possibly due to the greater uniformity and coherence of the packed microgel layer. In addition, the decrease in η with EWP after 24 h indicated the brittle structure of the protein films.

Conclusions

Our findings seem to validate the hypothesis that EWPM stabilizes foams by a Pickering-type mechanism and this is responsible for the long-term stability of aqueous foams. Although higher foam volumes were obtained in egg white protein compared to those stabilized by the egg white protein microgels at the same concentration, the microgel-stabilized foams and bubbles showed better stability to bubble shrinkage (disproportionation) and coalescence due to applied pressure drop. Measurements of interfacial rheology qualitatively seemed to support the idea of the microgels forming a more resilient and uniform adsorbed film, less liable to fracture, although only further measurements at deformation rates corresponding to those occurring during the shrinkage and coalesce can prove this conclusively. Nevertheless, the fundamental insights from this study could pave the way for improved ‘‘surfactant free’’, edible Pickering foam stabilizers for a variety of food and non-food applications (e.g. cosmetics, pharmaceutical, biomedical), where foam stabilization by sustainable natural particles is still an unmet research challenge.

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444 **References**

445 Adhikari, B., Howes, T., Wood, B. J., & Bhandari, B. R. (2009). The effect of low
446 molecular weight surfactants and proteins on surface stickiness of sucrose
447 during powder formation through spray drying. Journal of Food Engineering,
448 94(2), 135-143.

449 Arnould, A., Cousin, F., Salonen, A., Saint-Jalmes, A., Perez, A., & Fameau, A. L.
450 (2018). Controlling foam stability with the ratio of myristic acid to choline
451 hydroxide. Langmuir, 34(37), 11076-11085.

452 Binks, B. P. (2002). Particles as surfactants—similarities and differences. Current
453 Opinion in Colloid & Interface Science, 7, 21-41.

454 Binks, B. P., Campbell, S., Mashinchi, S., & Piatko, M. P. (2015). Dispersion behavior
455 and aqueous foams in mixtures of a vesicle-forming surfactant and edible
456 nanoparticles. Langmuir, 31(10), 2967-2978.

457 Binks, B. P., Muijlwijk, K., Koman, H., & Poortinga, A. T. (2017). Food-grade
458 Pickering stabilisation of foams by in situ hydrophobisation of calcium
459 carbonate particles. Food Hydrocolloids, 63, 585-592.

460 Bos, M. A., & Vliet, T. v. (2001). Interfacial rheological properties of adsorbed protein
461 layers and surfactants a review. Adv Colloid Interface Sci, 97, 437-471.

462 Burke, J., Cox, A., Petkov, J., & Murray, B. S. (2014). Interfacial rheology and stability
463 of air bubbles stabilized by mixtures of hydrophobin and β-casein. Food
464 Hydrocolloids, 34, 119-127.

465 Curschellas, C., Keller, R., Berger, R., Rietzler, U., Fell, D., Butt, H. J., & Limbach, H.

466 J. (2012). Scanning force microscopy as a tool to investigate the properties of
467 polyglycerol ester foams. J Colloid Interface Sci, 374(1), 164-175.
468 Dai, L., Zhan, X., Wei, Y., Sun, C., Mao, L., McClements, D. J., & Gao, Y. (2018).
469 Composite zein - propylene glycol alginate particles prepared using solvent
470 evaporation: Characterization and application as Pickering emulsion stabilizers.
471 Food Hydrocolloids, 85, 281-290.
472 Damodaran, S. (2005). Protein stabilization of emulsions and foams. Journal of Food
473 Science, 70, 54-66.
474 Dickinson, E., Ettelaie, R., Murray, B. S., & Du, Z. (2002). Kinetics of
475 disproportionation of air bubbles beneath a planar air-water interface stabilized
476 by food proteins. J Colloid Interface Sci, 252(1), 202-213.
477 Drenckhan, W., & Hutzler, S. (2015). Structure and energy of liquid foams. Advances
478 in Colloid and Interface Science, 224, 1-16.
479 Drenckhan, W., & Saint-Jalmes, A. (2015). The science of foaming. Advances in
480 Colloid and Interface Science, 222, 228-259.
481 Ercili-Cura, D., Miyamoto, A., Paananen, A., Yoshii, H., Poutanen, K., & Partanen, R.
482 (2015). Adsorption of oat proteins to air–water interface in relation to their
483 colloidal state. Food Hydrocolloids, 44, 183-190.
484 Ettelaie, R. (2003). Computer simulation and modeling of food colloids. Current
485 Opinion in Colloid & Interface Science, 8(4-5), 415-421.
486 Ettelaie, R., & Murray, B. S. (2015). Evolution of bubble size distribution in particle
487 stabilised bubble dispersions: Competition between particle adsorption and

488 dissolution kinetics. Colloids and Surfaces A: Physicochemical and
489 Engineering Aspects, 475, 27-36.
490 Felix, M., Romero, A., Sanchez, C. C., & Guerrero, A. (2019). Modelling the non-linear
491 interfacial shear rheology behaviour of chickpea protein-adsorbed complex
492 oil/water layers. Applied Surface Science, 469, 792-803.
493 Gharbi, N., & Labbafi, M. (2019). Influence of treatment-induced modification of egg
494 white proteins on foaming properties. Food Hydrocolloids, 90, 72-81.
495 Guevara, J. S., Mejia, A. F., Shuai, M., Chang, Y.-W., Mannan, M. S., & Cheng, Z.
496 (2013). Stabilization of Pickering foams by high-aspect-ratio nano-sheets. Soft
497 Matter, 9(4), 1327-1336.
498 Hao, Y., Wang, F., Huang, W., Tang, X., Zou, Q., Li, Z., & Ogawa, A. (2016). Sucrose
499 substitution by polyols in sponge cake and their effects on the foaming and
500 thermal properties of egg protein. Food Hydrocolloids, 57, 153-159.
501 Heertje, I. (2014). Structure and function of food products: A review. Food Structure,
502 1(1), 3-23.
503 Hu, N., Wu, Z., Jin, L., Li, Z., Liu, W., Huang, D., & Yang, C. (2019). Nanoparticle as
504 a novel foam controller for enhanced protein separation from sweet potato
505 starch wastewater. Separation and Purification Technology, 209, 392-400.
506 Jakubczyk, E., Gondek, E., Kamińska-Dwórznicka, A., Samborska, K., Wiktor, A., &
507 Królikowski, K. (2019). A complex approach to assessing properties of aerated
508 agar-fructose gels: Application of acoustic emission technique. Food
509 Hydrocolloids, 91, 66-75.

510 Jiao, B., Shi, A., Wang, Q., & Binks, B. P. (2018). High-internal-phase Pickering
511 emulsions stabilized solely by peanut-protein-isolate microgel particles with
512 multiple potential applications. Angew Chem Int Ed Engl, 57(30), 9274-9278.
513 Jin, Q., Li, X., Cai, Z., Zhang, F., Yadav, M. P., & Zhang, H. (2017). A comparison of
514 corn fiber gum, hydrophobically modified starch, gum arabic and soybean
515 soluble polysaccharide: Interfacial dynamics, viscoelastic response at oil/water
516 interfaces and emulsion stabilization mechanisms. Food Hydrocolloids, 70,
517 329-344.
518 Jourdain, L. S., Schmitt, C., Leser, M. E., Murray, B. S., & Dickinson, E. (2009). Mixed
519 layers of sodium caseinate + dextran sulfate: influence of order of addition to
520 oil-water interface. Langmuir, 25(17), 10026-10037.
521 Kudryashova, E. V., & de Jongh, H. H. (2008). Modulation of the adsorption properties
522 at air-water interfaces of complexes of egg white ovalbumin with pectin by the
523 dielectric constant. J Colloid Interface Sci, 318(2), 430-439.
524 Li, J., Wang, C., Li, X., Su, Y., Yang, Y., & Yu, X. (2018). Effects of pH and NaCl on
525 the physicochemical and interfacial properties of egg white/yolk. Food
526 Bioscience, 23, 115-120.
527 Li, X., Li, J., Chang, C., Wang, C., Zhang, M., Su, Y., & Yang, Y. (2019). Foaming
528 characterization of fresh egg white proteins as a function of different
529 proportions of egg yolk fractions. Food Hydrocolloids, 90, 118-125.
530 Liu, H., Yang, S., Liu, Y., Miao, M., Zhao, Y., Sotto, A., Gao, C., & Shen, J. (2019).
531 Fabricating a pH-responsive membrane through interfacial in-situ assembly of

microgels for water gating and self-cleaning. Journal of Membrane Science, 579, 230-239.

Majzoobi, M., Vosooghi Poor, Z., Mesbahi, G., Jamalian, J., & Farahnaky, A. (2017). Effects of carrot pomace powder and a mixture of pectin and xanthan on the quality of gluten-free batter and cakes. J Texture Stud, 48(6), 616-623.

Martin, A., Bos, M., Cohen Stuart, M., & van Vliet, T. (2002). Stress−strain curves of adsorbed protein layers at the air/water interface measured with surface shear rheology. Langmuir, 18(4), 1238-1243.

Matsumiya, K., & Murray, B. S. (2016). Soybean protein isolate gel particles as foaming and emulsifying agents. Food Hydrocolloids, 60, 206-215.

Mougel, J. B., Bertoncini, P., Cathala, B., Chauvet, O., & Capron, I. (2019). Macroporous hybrid Pickering foams based on carbon nanotubes and cellulose nanocrystals. J Colloid Interface Sci, 544, 78-87.

Murray, B. S. (2002). Interfacial rheology of food emulsifiers and proteins. Current Opinion in Colloid & Interface Science, 7, 426-431.

Murray, B. S. (2011). Rheological properties of protein films. Current Opinion in Colloid & Interface Science, 16(1), 27-35.

Murray, B. S., Campbell, I., Dickinson, E., Maisonneuve, K., Nelson, P. V., & Soderberg, I. (2002). Technique for studying the effects of rapid surface expansion on bubble stability. Langmuir, 18, 5007-5014.

Murray, B. S., & Dickinson, E. (1996). Interfacial rheology and the dynamic properties of adsorbed films of food proteins and surfactants. Food Science and

554 Technology International, 2, 131-145.
555 Murray, B. S., Dickinson, E., Lau, C. K., Nelson, P. V., & Schmidt, E. (2005).
556 Coalescence of protein-stabilized bubbles undergoing expansion at a
557 simultaneously expanding planar air-water interface. Langmuir, 21, 4622-4630.
558 Murray, B. S., Dickinson, E., & Wang, Y. W. (2009). Bubble stability in the presence of
559 oil-in-water emulsion droplets: Influence of surface shear versus dilatational
560 rheology. Food Hydrocolloids, 23(4), 1198-1208.
561 Murray, B. S., Durga, K., de Groot, P. W., Kakoulli, A., & Stoyanov, S. D. (2011a).
562 Preparation and characterization of the foam-stabilizing properties of cellulose-
563 ethyl cellulose complexes for use in foods. J Agric Food Chem, 59(24), 13277-
564 13288.
565 Murray, B. S., Durga, K., Yusoff, A., & Stoyanov, S. D. (2011b). Stabilization of foams
566 and emulsions by mixtures of surface active food-grade particles and proteins.
567 Food Hydrocolloids, 25(4), 627-638.
568 Murray, B. S., & Ettelaie, R. (2004). Foam stability: proteins and nanoparticles. Current
569 Opinion in Colloid & Interface Science, 9(5), 314-320.
570 Oliveira, G. A., Monje-Ramirez, I., Carissimi, E., Rodrigues, R. T., Velasquez-Orta, S.
571 B., Mejía, A. C. C., & Orta Ledesma, M. T. (2019). The effect of bubble size
572 distribution on the release of microalgae proteins by ozone-flotation. Separation
573 and Purification Technology, 211, 340-347.
574 Parra, O. D. H., Ndoye, F.-T., Benkhelifa, H., Flick, D., & Alvarez, G. (2018). Effect of
575 process parameters on ice crystals and air bubbles size distributions of sorbets

576 in a scraped surface heat exchanger. International Journal of Refrigeration, 92,
577 225-234.
578 Ptaszek, P., Kabziński, M., Ptaszek, A., Kaczmarczyk, K., Kruk, J., & Bieńczak, A.
579 (2016). The analysis of the influence of xanthan gum and apple pectins on egg
580 white protein foams using the large amplitude oscillatory shear method. Food
581 Hydrocolloids, 54, 293-301.
582 Ren, J., Ying, W., Zhao, J., Xie, J., Zhou, G., Shi, Y., & Wang, S. (2019). High-strength
583 porous mullite ceramics fabricated from particle-stabilized foams via oppositely
584 charged dispersants and surfactants. Ceramics International, 45(5), 6385-6391.
585 Rodriguez Patino, J. M., Carrera Sanchez, C., & Rodriguez Nino, M. R. (2008).
586 Implications of interfacial characteristics of food foaming agents in foam
587 formulations. Adv Colloid Interface Sci, 140(2), 95-113.
588 Sarkar, A., Arfsten, J., Golay, P. A., Acquistapace, S., & Heinrich, E. (2016a).
589 Microstructure and long-term stability of spray dried emulsions with ultra-high
590 oil content. Food Hydrocolloids, 52, 857-867.
591 Sarkar, A., Kanti, F., Gulotta, A., Murray, B. S., & Zhang, S. (2017). Aqueous
592 lubrication, structure and rheological properties of whey protein microgel
593 particles. Langmuir, 33(51), 14699-14708.
594 Sarkar, A., Murray, B., Holmes, M., Ettelaie, R., Abdalla, A., & Yang, X. (2016b). In
595 vitro digestion of Pickering emulsions stabilized by soft whey protein microgel
596 particles: influence of thermal treatment. Soft Matter, 12(15), 3558-3569.
597 Sarkar, A., & Singh, H. (2016). Emulsions and foams stabilised by milk proteins. In P.

598 L. H. McSweeney & J. A. O'Mahony (Eds.), Advanced Dairy Chemistry:
599 Volume 1B: Proteins: Applied Aspects (pp. 133-153). New York, NY: Springer
600 New York.
601 Sarkar, A., Zhang, S., Holmes, M., & Ettelaie, R. (2019). Colloidal aspects of digestion
602 of Pickering emulsions: Experiments and theoretical models of lipid digestion
603 kinetics. Adv Colloid Interface Sci, 263, 195-211.
604 Schmidt, J. M., Damgaard, H., Greve-Poulsen, M., Larsen, L. B., & Hammershøj, M.
605 (2018). Foam and emulsion properties of potato protein isolate and purified
606 fractions. Food Hydrocolloids, 74, 367-378.
607 Söderberg, I., Dickinson, E., & Murray, B. S. (2003). Coalescence stability of gas
608 bubbles subjected to rapid pressure change at a planar air/water interface.
609 Colloids and Surfaces B: Biointerfaces, 30(3), 237-248.
610 Solórzano, E., Pardo-Alonso, S., de Saja, J. A., & Rodríguez-Pérez, M. A. (2013). Study
611 of aqueous foams evolution by means of X-ray radioscopy. Colloids and
612 Surfaces A: Physicochemical and Engineering Aspects, 438, 159-166.
613 Tang, C.-H. (2019). Nanostructured soy proteins: Fabrication and applications as
614 delivery systems for bioactives (a review). Food Hydrocolloids, 91, 92-116.
615 Valadbaigi, P., Ettelaie, R., Kulak, A. N., & Murray, B. S. (2019). Generation of ultra-
616 stable Pickering microbubbles via poly alkylcyanoacrylates. J Colloid Interface
617 Sci, 536, 618-627.
618 Wouters, A. G. B., Rombouts, I., Fierens, E., Brijs, K., Blecker, C., Delcour, J. A., &
619 Murray, B. S. (2018). Foaming and air-water interfacial characteristics of

620 solutions containing both gluten hydrolysate and egg white protein. Food
621 Hydrocolloids, 77, 176-186.
622 Yang, X., & Foegeding, E. A. (2011). The stability and physical properties of egg white
623 and whey protein foams explained based on microstructure and interfacial
624 properties. Food Hydrocolloids, 25(7), 1687-1701.
625