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Combination of egg white protein and microgels to stabilize foams: impact of processing treatments

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Abstract

The aim of this study was to compare the properties of foams stabilized by egg white protein (EWP) and egg white protein microgels (EWPM), and combinations thereof (EWP+EWPM; 1.0+0, 0.7+0.3, 0.5+0.5, 0.3+0.7 or 0+1.0, wt%) without or with processing treatments (freezing at –20 °C/30 min, oven-heating at 80 °C/30 min, or microwaving at 700 W/15 s). In order to provide a twofold benefit of high initial foam volume (dictated by rapid adsorption of EWP) and high foam stability (governed by Pickering stabilization by EWPM), various ratios of EWP+EWPM on foam stabilization were investigated. The EWP+EWPM systems generated similar initial foam volumes as compared to that prepared solely with EWP ($p>0.05$), and foams generated with increasing ratios of EWPM+EWP showed higher stability to bubble shrinkage and coalescence at longer time scales (9 h). Confocal images revealed that EWPM were preferentially located at the air/water interface with the increasing EWPM+EWP ratio, suggesting pure Pickering stabilization at 1.0 wt% EWPM. Plateauing of bubble size at *ca*. 75 μm occurred only at EWPM> 0.5 wt% for EWP+EWPM=1.0 wt% total protein, when stability became EWPM-dominated. Frozen foams showed the most stable bubbles, irrespective of the systems ($p>0.05$). The combination of EWP+EWPM significantly improved the stability of the bubbles during oven-heating as compared to the EWP-stabilized counterparts ($p<0.05$), whilst the EWPM system was most stable during microwaving. These results suggest that microgels could be used to formulate food foams with enhanced stability to processing conditions, whereas a combination with protein can improve the initial foamability.

Keywords: Foam stability; egg white protein; microgel; bubble shrinkage; disproportionation; food processing

1. Introduction

Aqueous foams are dispersions of a high volume fraction of gas bubbles in a small volume of liquid stabilized by surfactants, proteins or small particles (Ashok Bhakta and Ruckenstein, 1997; Briceno-Ahumada and Langevin, 2017; Karthick et al., 2019). Despite the presence of these stabilizing species, foams are still characteristically metastable systems, tending to destabilize *via* disproportionation and coalescence within the required lifetime of a product (Dickinson, 2010; Horozov, 2008; Murray et al., 2002). In foods, proteins are widely used to stabilize aqueous foams to make various foamed products, such as cakes, meringue, soufflés, mousse, whipped cream, *etc.*, due to the ability of the proteins to adsorb and unfold at the air-water (A-W) interface, resulting in the formation of viscoelastic interfacial films (Sarkar and Singh, 2016) that provide some kinetic resistance to bubble disproportionation and coalescence. Besides protein monolayers, there is burgeoning interest in using Pickering stabilization, *i.e.* stabilization of bubbles by solid particles (Lesov et al., 2017; Li et al., 2019b). Adsorbed particles can enhance the stability of foams against disproportionation and coalescence for longer periods by virtue of the ultra-high desorption energies of the particles once adsorbed (Binks et al., 2017). In this domain, food-grade microgels prepared from proteins have raised interest in providing Pickering-type stabilization of foams and/or emulsions, such as soy protein- (Matsumiya and Murray, 2016), zein- (Dai et al., 2018)

and whey protein-based microgels (Araiza-Calahorra and Sarkar, 2019; Sedaghat Doost et al., 2019), and so on. Although considerable research attention has been given to Pickering emulsions in the food literature (Araiza-Calahorra et al., 2018; Murray, 2019; Sarkar et al., 2019), not as much emphasis has been placed on designing particle-stabilized food foams (Binks et al., 2017) and hence this needs further attention.

Egg white protein (EWP) is the classic foaming agent used in a wide variety of foods to generate high foamability. But EWP on its own is unable to provide longer-term stability against bubble shrinkage or collapse. One strategy to improve foam stability can be to physically structure EWP into egg white protein microgels (EWPM) to generate 'Pickering' particle-stabilized bubbles (Li et al., 2019c). Therefore, EWPM prepared by physical treatment from EWP itself could be taken as a kind of "clean" stabilizing materials, however EWPM suffers from relatively poor foamability. One alternative approach is to investigate the combination of EWP and EWPM, which might not only result in optimized foaming properties, but would also be cheaper and more sustainable compared to using EWPM alone, the latter requiring thermal processing and homogenization for preparation. As far as we are aware, to date such combinations have not been investigated in the literature.

In addition to the foaming properties of different systems under ambient conditions, there is an unresolved research challenge of retaining bubbles and prevention of textural deterioration of aerated foods during food processing. Such processing varies from low temperatures (e.g., freezing) to high temperatures (e.g., oven- or microwave-heating) (De Vries et al., 2018; Misra et al., 2017). As reported in the literature (Assegehegn et

al., 2019), bubble morphology and size distribution in aerated foods can change significantly during freezing processes. For instance, Wang et al. (2014) reported that freezing-associated rearrangement of the conformation of γ-gliadin resulted in ~ 26% loss of foam volume upon frozen storage (frozen at −35 °C for 12 h and stored at −18 °C for 45 days). Conventional oven-heating is another common treatment in food processing for aerated systems. For instance, significant liquid drainage and bubble coalescence can occur, especially for making breads and cakes (Campbell et al., 2016; Deleu et al., 2019; Lambrecht et al., 2018), where heat treatments may range from 80 to 190 °C for 30 min to 2 h (Hesso et al., 2015; Marston et al., 2016; Sahagún et al., 2018). Also, microwave processing has been reported to toughen the texture of bread-like products, due to bubble coalescence and disproportionation (Uzzan et al., 2007). However, so far we believe that there has been no investigation of the stability of foams stabilized by a combination of proteins and particles when subjected to various food processing conditions.

Given the context, the objective of this study was to compare the foaming properties of combinations of EWP and EWPM without or with being subjected to different food processing treatments (freezing, oven-heating and microwave cooking). In theory a combination of EWP and EWPM might be ideal since the EWP should reach the A-W interface rapidly and provide good foamability, whilst the more slowly adsorbing EWPM would provide longer term foam stability by providing a more robust, particle-dominated interfacial film capable of protecting against the disrupting effects of processing.

2. Materials and methods

2.1 Materials

Fresh chicken eggs were purchased from a local supermarket (Tesco Ltd., UK). Di-sodium hydrogen phosphate, sodium dihydrogen phosphate, Rhodamine 6G and sodium azide were purchased from Sigma-Aldrich (Dorset, UK). Milli-Q water with a resistivity of not less than 18.2 MΩ cm at 25 °C (Milli-Q apparatus, Millipore, Bedford, UK) was used to prepare 20 mM phosphate buffer at pH 7 as the aqueous phase, and 0.02 wt% sodium azide was added as a bactericide. Multiwell chambered microscope slides with coverslips were used to observe foams via light microscopy, purchased from Life Technologies Corporation (Invitrogen, USA).

2.2 *Preparation of samples*

2.2.1 Preparation of egg white protein dispersion (EWP)

Egg white was extracted from the freshly purchased eggs, by manually separating it from the yolks and then homogenized under magnetic stirring (500 rpm speed) for 2 h, as reported previously (Li et al., 2019a). No further purification of the egg white protein dispersion (EWP) was performed and EWP contained 12.5 wt% protein (Li et al., 2019a).

2.2.2 Preparation of egg white protein microgels (EWPM)

Sub-micron sized egg white protein microgel (EWPM) particles (Li et al., 2019c) were prepared using a top-down approach of heat-set gel formation followed by shearing into

133 microscopic gel particles based on previous methods (Sarkar et al., 2017a; Sarkar et al.,
134 2016b). The z-average hydrodynamic diameter the EWPM dispersion was ~ 350 nm
135 (see supplementary Figure S1), measured using dynamic light scattering (*DLS*). Note
136 that this was the size measured for the sample diluted into the *DLS* cell, as is the usual
137 procedure, to avoid multiple scattering, so that this should be taken as the primary
138 particle size, since it was obvious from confocal imaging, etc. (see later), that at the
139 higher concentrations used for foaming the EWPM had a tendency to aggregate. Briefly,
140 a EWP dispersion (6.25 wt% protein) was obtained by diluting EWP in 20 mM
141 phosphate buffer at pH 7.0, followed by formation of a thermally cross-linked
142 proteinaceous hydrogel by heating the aqueous dispersion of EWP at 90 ^{o}C for 30 min.
143 The hydrogel was broken down into coarse gel particles using a hand blender (HB724,
144 Kenwood) and then EWPM particles were created by passing the macroscopic gel
145 pieces twice through a high-pressure two-chamber homogenizer (Leeds Jet
146 homogenizer, University of Leeds, UK) at 300 bar.
147
148 *2.2.3 Preparation of mixed dispersions of EWP and EWPM*
149 EWP (protein concentration 1.0 wt%) and EWPM (protein concentration 1.0 wt%) on
150 their own and their mixtures at different w/w ratios (EWP: EWPM = 7:3, 1:1 and 3:7),
151 keeping the total protein concentration constant at 1.0 wt% were prepared at pH 7.0 (20
152 mM phosphate buffer as described in section 2.1).
153
154 *2.2.4 Preparation of foams*

155 Exactly 5 mL (total protein concentration is 1.0 wt%) of the above-mentioned EWP +
156 EWPM dispersions were collected separately in 15 mL test tube, sealed well and
157 manually shaken for 30 s in order to examine the foam volume for foamability and to
158 determine the foam stability of the samples as a function of storage time.
159
160 2.3 *Foaming properties*
161 Changes in heights of the foams were measured as a function of time to determine
162 stability of foams. The initial height was used as a measure of foamability. Foam
163 volumes of sample were calculated relative to an equal volume of pure water at room
164 temperature (25 ± 1 °C).
165
166 2.4 *Bubble disproportionation measurement*
167 Bubble disproportionation experiments were conducted in a bubble apparatus
168 (University of Leeds, UK) using a methodology developed by Dickinson et al. (2002).
169 Briefly, bubbles stabilized by EWP or EWPM on their own or their mixtures at different
170 w/w ratios (EWP: EWPM = 7:3, 1:1 and 3:7) were introduced into the cell filled with
171 the same mixture *via* a specially designed "bubble syringe" into the middle of a stainless
172 steel cell through a hole in the wall of the pressurization chamber (when the piston is
173 clear off the cylinder), and bubbles were allowed to rise to the planar A-W interface at
174 the top of the cell. These bubbles were trapped within the perimeter of a circular hole
175 in a paraffin wax coated mica sheet floating in the middle of the planar A-W interface.
176 Bubble size was monitored with an optical microscope and a video camera for at least

9 h. Microsoft Office and ImageJ were used to analyze the real size of the bubbles. To compare the samples, changes in individual bubble size versus time and changes in the overall bubble size distribution as a function of time are reported.

2.5 *Bubble coalescence measurement*

Bubble coalescence experiments were performed in the apparatus as mentioned above, where a pressure drop was used to induce and accelerate instability of the foams (Murray et al., 2005). Briefly, bubbles were injected beneath the A-W interface as for the disproportionation experiments, then the piston was moved down to fill the adjoining cylinder and a glass plate was used to seal the top of the cell. By withdrawing the piston to a predetermined distance at a specific speed, the pressure in the system falls and the bubbles at the interface expand, inducing a proportion of them to coalesce, due to the relatively sudden depletion in the adsorbed film coverage. The time for the pressure to drop to its full extent in these experiments was 22 s. Note that in this experiment the concentration of stabilizer used was high enough so that coalescence under quiescent conditions was negligible (over at least 10 min) and a short time (< 5 s) after the pressure had stopped decreasing no further coalescence occurred. The remaining bubbles were stable to coalescence (over at least the next 10 min) so that the number fraction (F_c) of bubbles that coalesced could be determined from the images before and shortly after the pressure drop. The experiments were repeated at least eight times and mean values of F_c are reported. This type of 'accelerated coalescence' experiment has been shown to be a very useful and highly discriminating method of

199 measuring differences between foamed systems that under constant pressure typically
200 exhibit very little coalescence, or differences in coalescence, over several h (Ettelaie et
201 al., 2003).

202 It should be noted that in the above measurements (and those that follow below)
203 the EWPM dispersion was diluted to a maximum concentration of 1 wt% protein (i.e.,
204 in the EWP + EWPM = 0 + 1.0 wt% system). However, this means that the actual
205 microgel particle concentration was still 16 wt%, since the original gel from which the
206 EWPM particles were formed was 6.25 wt% protein (see Methods above). Since
207 microgel dispersions can have very high viscosities at high weight (volume) fractions
208 (Sarkar et al., 2017a), we performed some measurements (not shown) of the viscosity
209 of 1.0 wt% EWPM, 1.0 wt% EWP and an equal mixture of 0.5 wt% EWPM and 0.5
210 wt% EWP over the shear rate range 10^{-3} to 10^{3} s^{-1}. Although all these three systems
211 were shear thinning to a certain extent, there were no differences between them in terms
212 of their bulk viscosities at any shear rate in this range (data not shown). Thus any
213 differences in foam stability of these systems cannot be attributed to differences in the
214 bulk viscosity of the continuous phase, but rather the adsorption and interfacial
215 properties of the EWP and EWPM.

216

217 2.6 *Confocal laser scanning microscopy (CLSM)*

218 Foams stabilized by combinations of EWP and EWPM at different ratios were observed
219 using a Zeiss LSM 700 confocal microscope (Carl Zeiss MicroImaging GmbH, Jena,
220 Germany), where foam samples were imaged after mixing with 0.1 mL of 1.0% (w/v)

Rhodamine 6G protein stain. In order to immobilize bubbles, xanthan gum solution (0.1 wt%) was added in the aqueous phase. The samples were observed at room temperature (25 ± 1 °C), using × 63 objective at an excitation wavelength of 543 nm (Sarkar et al., 2016a). Images were recorded at a resolution 1024 × 1024 pixels.

2.7 *Interfacial shear viscosity (η_i)*

A two-dimensional Couette-type interfacial viscometer, which has been described in detail many times previously (Burke et al., 2014; Murray et al., 2009; Sarkar et al., 2017b), was used to measure the surface shear viscosity of three representative sample systems *i.e.* EWP + EWPM, 1.0 + 0, 0.5 + 0.5 and 0 + 1.0, wt%. Briefly, a wire of suitable torsion constant suspends a biconical disk positioned with its edge touching the A-W interface of the sample solution contained in a concentric circular dish. The rheometer was operated in a constant shear-rate mode (Jourdain et al., 2009), and the surface shear viscosity, η_i, is given by the following equation:

$$\eta_i = g_f K \theta_i / \omega \quad (1)$$

where, K is the torsion constant of the wire; θ_i is the angle of rotation of the disk; 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 radius of the disk (14.5 mm) and R_0 is the radius of the dish (72.5 mm); ω is the angular velocity of the dish. A fixed value of $\omega = 1.27 \times 10^{-3}$ rad s^{-1} was used to compare with measurements made on the other systems at the same shear rate.

2.8 *Food processing treatments*

Fresh foams generated after homogenization were immediately transferred into the Multiwell chambered cells and sealed with a cover slip. Three food processing conditions typical of those employed in various aerated products were applied to the three systems EWP + EWPM = 1.0 + 0, 0.5 + 0.5 and 0 + 1.0 wt%. These were heating the foams in an oven at 80 °C for 30 min, heating in a microwave oven (700 W for 15 s, at 2450 Hz) and freezing (–20 °C for 30 min) followed by thawing at room temperature for 2 min. The processed foams were observed *via* a Nikon SMZ-2T stereomicroscope (Nikon, Japan). The experiments were repeated at least five times and mean values of the number percentage of bubbles surviving the processing conditions were assessed by counting the number of bubbles before and after subjecting to the processing treatments.

2.9 *Statistical analysis*

All experiments were conducted at least in triplicate. SPSS 19.0 package was used for statistical analysis and results are presented as the means and standard deviations of these measurements unless mentioned otherwise. One-way analysis of variance (ANOVA) tests were carried out, and significant differences between means were considered when the p-value was < 0.05, as obtained using Tukey's Multiple Comparison Test.

3. Results and discussions

265 *3.1 Foam volume*

266 In our work, foams were produced by a simple and reproducible method: hand-shaking
267 for the same time (30 ± 1 s), allowing a quantitative description in terms of foamability
268 (*i.e.*, how much foam is produced) and foam stability (*i.e.*, how the foam evolves
269 kinetically) (Schmidt et al., 2018). Results of the foam volume for mixtures (total
270 protein concentration = 1.0 wt%) of EWP and EWPM at different ratios as a function
271 of time are shown in Figure 1a, and their initial foam volumes are shown in Figure 1b.
272 (supplementary Figure S2 shows the corresponding optical microscopic images.)
273 Combination of EWP and EWPM resulted in different degrees of foam stability based
274 on the foam volume as a function of time. All the mixtures showed a decrease in foam
275 volume over 7 days (*i.e.*, 168 h) but the foams with a higher proportion of EWPM (i.e.,
276 EWPM = 0.3 to 0.7 wt%) did not decrease in volume as significantly as those with
277 higher proportions of EWP (e.g., EWP + EWPM = 0.7 + 0.3 wt%, $p < 0.05$).
278 Especially for the foams stabilized solely by EWPM (EWP + EWPM = 0 + 1.0 wt%),
279 the foam volume after 30 min did not decrease any further, in agreement with our
280 previous work (Li et al., 2019c)

281 Comparing the initial foam volumes (*i.e.*, foamability at 0 min, Figure 1b), samples
282 containing any EWPM showed significantly lower volume ($p < 0.05$) as compared to
283 those containing only EWP, whereas 0.3, 0.5 and 0.7 wt% EWP improved the
284 foamability of EWPM slightly, but significantly ($p < 0.05$). This can be attributed to
285 the longer time-scales for particles to adsorb to the A-W interface due to their much
286 larger size (100 nm to several μm) compared to the size of the constituent EWP proteins,

287 the latter being only a few nm (Ravera et al., 2006). This larger size will slow down
288 mass transport of EWPM to the interface and possibly also increase the time taken for
289 them to adopt an orientation favourable for adsorption. In addition, microgel particles,
290 consisting of already unfolded and cross-linked protein, may take longer than individual
291 protein molecules to re-arrange and unfold at the interface once they are anchored there.
292 All this decreases the capacity for rapid bubble stabilization, i.e., foamability.
293
294 *3.2 CLSM observations*
295 Confocal images and schematic representation of the fresh foams stabilized by EWP
296 and EWPM are shown in Figure 2. No brightness (*i.e.*, protein-labeled fluorescence)
297 could be observed around the bubbles that were solely stabilized by EWP. With
298 increasing concentration of EWPM, a uniform fluorescent ring that gradually increased
299 in thickness could be observed around the bubbles, suggestive of increasing adsorbed
300 amounts of the larger EWPM particles. This particle coating was apparently at its
301 thickest when the bubbles were solely stabilized by EWPM, i.e., with 'pure' Pickering-
302 type stabilization. We propose that in these systems the A-W interface ranges from a
303 protein-dominated interface to a microgel-dominated one (particularly when the
304 concentration of EWPM > EWP), EWPM giving higher foam stability. Thus, the
305 increasing foam stability of the mixed EWP+EWPM systems with increasing
306 concentration of EWPM observed in Figure 1a is not surprising, due to increasing
307 proportion of microgel particles at the A-W interface.
308

309 *3.3 Bubble coalescence*

310 Foams are mainly destabilized by coalescence and disproportionation (Foegeding et al.,
311 2017; Rodriguez Patino et al., 2008). Coalescence depends on the physical properties
312 of the gas and liquid phases, the bubble size and the adsorbed film properties, and
313 occurs on rupturing of the thin liquid film between two adjacent bubbles (Yang and
314 Foegeding, 2011). Figure 3 compares the number fraction (F_c) of bubbles that coalesced
315 after the application of the pressure drop (810 mbar) for mixtures with different
316 proportions of EWP and EWPM. F_c decreased from approximately 28% to 10% with
317 increasing proportions of EWPM in the mixtures ($p < 0.05$). Even addition of a
318 relatively small proportion of microgel (EWP + EWPM = 0.7 + 0.3 wt%) made the
319 bubbles less prone to coalescence as compared to the ones solely stabilized by EWP
320 alone ($p < 0.05$) (Figure 3). As the proportion of EWPM increased, a larger number of
321 bubbles could be observed in Figure S2 for the different combinations. The thicker
322 microgel particle layers (Figure 2) are expected to give mechanically stronger
323 interfacial films and prevent the close approach of bubbles necessary for coalescence
324 (Kudryashova and de Jongh, 2008). On the other hand, although the ultra-high
325 detachment energies of such particles will prevent their removal, this cannot stop the
326 expansion of the bubbles due to the pressure drop. Therefore, either the microgel
327 particle layer re-arranges fast enough to maintain a strong, coherent and thick enough
328 layer, or possibly additional microgel particles adsorb on the time-scale (22 s) of the
329 expansion. Since additional adsorption is apparently not fast enough in the case of EWP
330 alone to give the same or lower F_c as with EWPM, the latter possibility is perhaps less

likely.

To illustrate more clearly the coalescence events and the differences between EWP, EWPM and their combinations, videos of three representative systems (EWP + EWPM, 1.0 + 0, 0.5 + 0.5 and 0 + 1.0, wt%) are supplied in the supplementary information (supplementary videos S1, S2 and S3, respectively).

3.4 Bubble disproportionation

Disproportionation is the main factor that contributes to foam destabilization in the long term, driven by the differences in Laplace pressure of bubbles of different size. It is clearly evident that for bubbles with just EWP (*i.e.*, EWP + EWPM = 1.0 + 0 wt%), fewer bubbles remained at longer times (Figure 4), while combinations of EWP and EWPM resulted in more bubbles remaining visible at the end of 9 h. Initial and final bubble size distributions are shown on the left and right hand sides of the images, respectively. The number of bubbles remaining after 9 h in the pure EWPM system was lower than that with the mixture of EWP + EWPM = 0.5 + 0.5 or 0.3 + 0.7 wt% ($p > 0.05$). This might have been a result of the initial injection state with the pure EWPM system, where there seemed to be a greater tendency for the injected bubbles to cluster together, which will accelerate mass transfer of gas between adjacent bubbles. On the other hand, it is now well known (Murray and Ettelaie, 2004) that in order to stabilize bubbles completely against shrinkage by the Pickering mechanism, a delicate balance has to be achieved between (i) the rate of bubble shrinkage, (ii) the rate of co-adsorption of desorbable foaming agents (in this case EWP) and (iii) the rate of adsorption of non-

desorbable particles (in this case EWPM). Small (e.g., less than 50 μm diameter) air bubbles without a complete enough adsorbed particle layer shrink very rapidly. Thus, some EWP adsorption, which will be faster than EWMP microgel adsorption, may help to stabilize bubbles initially to some extent until a high enough interfacial coverage by EWPM is reached. Mixtures may therefore, in the end, be better than the pure (EWPM) system in stabilizing against disproportionation.

It is noteworthy that, the final bubble size distribution shifted towards smaller diameters (from 0 to 100 μm) for systems with a higher proportion of EWPM. Thus, the systems stabilized solely by EWPM seemed to result in the narrowest bubble size distribution with the smallest-sized bubbles, again suggesting full stability was only achieved in the later stages of shrinkage with the pure particle system. Jakubczyk et al. (2019) and Parra et al. (2018) showed that a narrower bubble size distribution gave a lower degree of disproportionation, but every bubble, regardless of whether or not it is 'touching' its neighbours or the edge of the mica hole is included in the data shown in the left and right side in Figure 4, so that again differences in the degree of clustering will also affect the final size distribution (Söderberg et al., 2003). Bubbles touching each other influence their mutual shrinkage kinetics (Ettelaie et al., 2003). The clustering, number and exact size distribution of the bubbles injected is very difficult to control; indeed the tendency for clustering may also be a function of the type of stabilizer. However, it should be noted that we have excluded such bubbles from the quantitative analysis in Figure 5 (see below), all of which were at least 2 bubble diameters from their neighbours or the edge of the mica hole.

375 In order to quantify better the foam stability and thus obtain a better understanding
376 of relative contributions of the EWP and EWPM in the mixed systems, shrinkage
377 kinetics of bubbles are shown in Figure 5, measured from images like those in Figure
378 4. It is therefore even more clear from Figure 5 that, in the absence or in the presence
379 of a small (0.3 wt%) proportion of EWPM, all bubbles showed dramatic shrinkage as a
380 function of time, irrespective of their initial size. In the experimental window of 9 h,
381 bubble shrinkage did not seem to slow down at all for the pure EWP-stabilized foams.
382 For equal concentrations (0.5 wt%) of EWP + EWPM the shrinkage rates definitely
383 seemed to be decreasing after *ca*. 100 min for most bubbles, whilst for EWP + EWPM
384 = 0.3 + 0.7 wt% all bubble sizes reached a plateau relatively quickly, when one might
385 suppose that the interfaces were now dominated more by EWPM over EWP.
386 Interestingly, the pure EWPM-stabilized system seemed to take slightly longer (approx.
387 540 min) before all the bubble shrinkage seemed to cease. This may again point to the
388 slight advantage in having a mixture of desorbable (EWP) and non-desorbable (EWPM)
389 material at the start of the shrinkage process, as discussed above in connection with the
390 data in Figure 4.
391 Overall, it is seen that the addition of microgels contributed to a delay in the
392 shrinking (disproportionation) process as compared to bubbles stabilized solely by
393 EWP, where the bubbles disappeared relatively rapidly owing to the lack of a
394 permanent and rigid interfacial film (Kudryashova and de Jongh, 2008).
395
396 *3.5 Interfacial shear rheology*

To understand the mechanical properties of the adsorbed films and to check if this agrees with the explanation of the higher stability of systems containing higher proportions of EWPM as proposed above, measurements of the interfacial shear viscosities (η_i) of the adsorbed films stabilized solely by EWP or EWPM and one representative mixture (0.5 wt% EWP + 0.5 wt% EWPM) were measured as a function of time. The results are shown in Figure 6, including a control experiment with just buffer, where between 0 and 24 h, as expected, $\eta_i = 0$.

In Figure 6a, the system with pure EWP (1.0 wt%) exhibited a large and rapid increase in the surface shear viscosity in the first 80 min, to $4.7 \pm 0.2 \times 10^3$ mN s m^{-1}, which was attributed to the rapid adsorption of EWP on the A-W interface. This was then followed by a decrease to *ca*. 3.5×10^3 mN s m^{-1} in the next 2 h during the continued measurement. This is indicative of a brittle nature of the films, which yield to some extent as a result of repeated measurement, whilst at the same time further EWP adsorption is tending to 'heal' these breakages and further raise η_i. Thus, after leaving undisturbed overnight, η_i for this pure EWP sample had increased to *ca*. 6.3×10^3 mN s m^{-1}, but on further measurements this was followed by a decrease to 3.4×10^3 mN s m^{-1}. The brittle structure of EWP protein films is in line with previous results (Li et al., 2019c). We have zoomed in on the last 30 min around 1400 min to more clearly differentiate the samples in Figure 6b.

For the mixed EWP + EWPM system very interesting behaviour was observed. The initial rise in perfectly matched ($p > 0.05$) that for 1.0 wt% EWP alone, corroborating the rapid adsorption of EWP as evidenced in Figure 1b. However, beyond

419 the time (80 min) when the value for EWP alone started to decrease, η_i for the mixture
420 continued to increase until a relatively stable value of 5.5 ± 0.2 x 10^3 mN s m^{-1} was
421 reached. This value had not decreased the next day (i.e., after 1400 min). The pure (1.0
422 wt%) EWPM system gave a slower rate of initial increase of η_i, reaching 1.5 ± 0.2 x
423 10^3 mN s m^{-1} in 80 min (cf. 4.7 x 10^3 mN s m^{-1} for 1 wt% EWP alone) but rather than
424 decreasing (as for EWP) after this time, η_i showed a further steady increase in first 4 h
425 of adsorption, to 3.1 ± 0.2 x 10^3 mN s m^{-1}. Overnight this had increased further to
426 around 5.8 x 10^3 mN s m^{-1} and was apparently still increasing (Figure 6b).
427 The interfacial viscosity results therefore seemed to confirm the explanation of
428 many of the foam stability results, in terms of slower adsorption of EWPM compared
429 to EWP, but the latter ultimately forming more mechanically strong films in the
430 mixtures, particularly at longer times of adsorption, where the microgels are assumed
431 to dominate the adsorbed interfacial film. All values for these three systems are very
432 high compared to many other proteins (Murray, 2011), *i.e.*, these films are very strong
433 whilst the increases followed by decreases with EWP are reminiscent of stress
434 overshoot and the exhibition of a yield stress of strong films (Martin et al., 2002) when
435 they are continuously measured *via* such techniques.
436
437 *3.6 Foam stability after food processing treatments*
438 In order to compare the effect of the different processing treatments (freezing, oven
439 heating and microwaving) on foam stability, three representative samples (EWP +
440 EWPM, 1.0 + 0, 0.5 + 0.5 and 0 + 1.0, wt%, respectively) were investigated using

441 stereomicroscopy images of the samples sealed within the Multiwell slides (Figure 7a).
442 Irrespective of the stabilizer, bubble sizes showed an increase in the following order of
443 treatments: frozen < oven-heating < microwave, i.e., microwaving produced the largest
444 increase in bubble sizes. As reported elsewhere (Carvalho et al., 2017), freeze-drying
445 often maintains a very aerated structure. Altan (2014) showed that the bulk density of
446 puffed grains decreases significantly with microwave puffing and food pellets have
447 been reported to expand from 30 mm in length and 3 mm in diameter to 50-60 mm in
448 length and 6 mm in diameter after microwave treatment (Gutiérrez et al., 2017). This is
449 line with the high localized temperatures with microwave heating, that will lead to large
450 degrees of gas expansion (Lopez-Gil et al., 2015). It is noteworthy in Figure 7a that
451 more bubbles and smaller bubbles were present after processing when the EWP was
452 combined with EWPM, with the possible exception of the frozen EWP-stabilized
453 system. This appeared to contain more small bubbles after freezing, but this may have
454 been due to greater disproportionation of the original bubbles during the freeze-thaw
455 process in the absence of EWPM.

456 In Figure 7b we have attempted to quantify the changes more accurately by
457 counting the number % of fresh bubbles surviving the processing treatments. It can
458 clearly be seen that indeed the number of bubbles surviving freezing increased as the
459 EWPM concentration increased, although this was not statistically significant. It is
460 confirmed that oven heating caused more bubble loss than freezing, but 0.5 wt% EWP
461 + 0.5 wt% EWPM and 1.0 w.t% EWPM gave greater stability than no EWPM. One
462 should also note here that during oven heating the EWP will denature (Deleu et al.,

2016) which would influence its foamability and foam stability. However, EWPM has been already denatured during the thermal processing step in the preparation of these microgel particles, and therefore it makes sense that oven-heating might have less influence on the foaming behavior of systems containing EWPM.

Similarly, the microwave heating is the most destructive process, but the system stabilized by 1.0 wt% EWPM was significantly (3 ×) more stable than the system stabilized by the equal mixture or just EWP. This highlights the ability of the protein microgel particles alone to help stabilize the foams in the microwave treatment, which was not achieved by the combination.

Conclusions

Properties of aqueous foams stabilized by mixtures of protein (EWP) and protein microgel particles (EWPM) have been examined. The results highlight that increasing the proportion of EWPM gives rise to greater long-term foam stability, via the EWPM providing a Pickering-type stabilization mechanism (*i.e.*, Pickering foams). Foam stability and interfacial rheology experiments support the hypothesis that an optimum combination of EWP + EWPM (approximately an equal mixture at 1.0 wt% protein overall) not only provides higher foam stability against disproportionation but also high initial foam volume. In such mixtures we propose that the EWP rapidly adsorbs at the A-W interface whilst the EWPM co-adsorbs or adsorbs later but remains irreversibly attached to the interface, whereas the EWP may detach or remain synergistically co-adsorbed with the EWPM. The combination of EWP with EWPM also provided better

stability to foams during oven-heating as compared to the ones solely stabilized by EWP. However, the combination could not provide better stability as compared to the Pickering foams solely stabilized by the microgel counterparts under the microwaving conditions.

In summary, the fundamental insights of this study could pave a way for improving the initial foamability and foam stability of egg white protein, by combining the original protein with microgel particles made from it, to generate new kinds of superior food foams.

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