Food-grade Pickering stabilisation of foams by *in situ* hydrophobisation of calcium carbonate particles

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Abstract

The aim of this study was to investigate the possibility of stabilising foam bubbles in water by adsorption of calcium carbonate ($CaCO_3$) particles. Because $CaCO_3$ is hydrophilic and not surface-active, particles were hydrophobised *in situ* with several emulsifiers. The used emulsifiers were food-grade and negatively charged at the pH employed. The effect of particle addition on foamability and foam stability of solutions containing either β-lactoglobulin, sodium caseinate, Quillaja, sodium dodecanoate (SD) and sodium stearoyl-2-lactylate (SSL) was studied. It was found that the ability of the emulsifiers to induce surface activity such that the particles are able to adsorb to the air-water interface is related to their structure. The structure needs to consist of a well-defined hydrophobic part and a charged part. Large emulsifiers with a complex structure, such as β-lactoglobulin, sodium caseinate and Quillaja, were able to partially hydrophobise the particle but were not able to act synergistically with the particles to increase the foam stability. Low molecular weight emulsifiers, however, consisting of a single tail with one charged group, such as SD and SSL, adsorbed at the particle surface rendering the particles partially hydrophobic such that they adsorb to the air-water interface. In a subsequent investigation, the pH was changed to a value typical for food products (pH 6-7) and the addition of milk salts on the foamability and foam stability was assessed. Based on these results, the use of food-grade $CaCO_3$ particles hydrophobised *in situ* with food-grade surfactants (SD or SSL) to prepare ultra-stable aqueous foams is demonstrated.

1. Introduction

Aqueous foam is an air in water dispersion. Energy is needed to generate bubbles in the liquid, for example by agitation with a homogeniser or by hand shaking. Because of the thermodynamic instability of foams, ingredients are needed to stabilise the foam. Surfactants and proteins are generally used for this purpose. Through the formation of a surfactant monolayer at the interface with the hydrophobic part directed to the air phase and with the hydrophilic group in the water phase, the interfacial tension is decreased which provides an increased stability against coalescence of bubbles. Drainage of liquid in between the bubbles can be reduced by the formation of interfacial tension gradients which is called the Gibbs-Marangoni effect (Walstra, 2003). However, because of the relatively high free energy of the air-water interface and the resulting high Laplace pressure difference between the inside and outside of the bubbles, it is difficult to completely stabilize foams against disproportionation, coalescence and liquid drainage (Dickinson, 2010).

Through the adsorption of small solid particles to the interface, the stability of foams can be dramatically improved; this is called Pickering stabilisation (Pickering, 1907). Small solid particles can be used for stabilising foams and emulsions, as reviewed by Binks *et al*. (Aveyard, Binks, & Clint, 2003; Binks, 2002) and by Dickinson (2010, 2011). The contact angle, set by a balance of the solid-air, solid-water and air-water interfacial tensions, determines whether and how the particle adsorbs to the air-water interface. When the contact angle measured into water is smaller than 90°, the particle is partially hydrophobic and is thus only partly wetted by the water phase (Gonzenbach, 2006). In contrast to surfactants, particles are practically irreversibly adsorbed because the energy needed to remove the particles from an air-water surface (ΔG) can be several orders of magnitude larger than the thermal energy. It has been shown that adsorption of particles to the air-water interface can completely stabilise the bubble against coalescence and disproportionation (Binks & Horozov, 2005).

Air bubbles in water can be stabilised by partially hydrophobic particles. Examples are polymer microrods (Alargova, Warhadpande, Paunov, & Velev, 2004), hydrophobised silica nanoparticles (Binks, et al., 2005), polystyrene latex microparticles (Fujii, Iddon, Ryan, & Armes, 2006), particles from hydrophobic cellulose (Wege, Kim, Paunov, Zhong, & Velev, 2008) and ethyl cellulose particles (Jin, et al., 2012). The use of many of these particles in food is inhibited because they are not approved for use in food (*e.g.*, hydrophobised silica and polystyrene) or the production of the particles involves the use of solvents (*e.g.*, ethylcellulose particles). On the contrary, a wide range of hydrophilic particles that can be used in food are available (*e.g.*, hydrophilic silica and calcium carbonate). In order to use hydrophilic particles to stabilise bubbles, these particles have to be partially hydrophobised by *ex situ* chemical modification or *in situ* modification with surfactants. Examples of particle-surfactant combinations used for the stabilisation of aqueous foams are crystalline sodium chloride particles with cetyltrimethylammonium bromide (CTAB) (Vijayaraghavan, Nikolov, & Wasan, 2006); Laponite clay particles with either hexylamine (Liu, Zhang, Sun, & Xu, 2009), alkylammonium bromides (Liu, Zhang, Sun, & Xu, 2010), CTAB (S. Zhang, Lan, Liu, Xu, & Sun, 2008), or dodecyltetraoxyethylene ether, $C_{12}E_4$ (S. Zhang, Sun, Dong, Li, & Xu, 2008); alumina particles with short chain carboxylic acids (Gonzenbach, Studart, Tervoort, & Gauckler, 2006a, 2006b, 2007); silica particles with n-amylamine (Arriaga, et al., 2012); and calcium carbonate ($CaCO_3$) particles with sodium dodecyl sulphate (SDS) (Cui, Cui, Cui, Chen, & Binks, 2010). However, none of these systems are allowed in food. There are several reviews on food-grade particles for emulsion stabilisation (Berton-Carabin & Schroën, 2015; Dickinson, 2011; Rayner, et al., 2014). Examples of food-grade options are protein particles (*e.g.* soy, egg, corn) and polysaccharide particles (*e.g.* cellulose, chitin, starch). However, only few studied have been done on Pickering stabilisation of foam systems.

85 The aim of this study is to obtain Pickering stabilisation of aqueous food foams, therefore
86 both particles and surfactants should be food-grade. From all the particles studied previously
87 in foams, only cellulose and calcium carbonate particles are food-grade. Calcium carbonate
88 particles were selected as particles to be used in this study because of their successful use with
89 SDS in the stabilisation of foam (Cui, et al., 2010). Cui and co-workers studied the use of
90 calcium carbonate particles in combination with three different surfactants to increase the
91 foam stability. They found no synergistic effect on foam stability for $CaCO_3$ particles with the
92 cationic CTAB or with the nonionic surfactant OP-10. This indicates that the $CaCO_3$ particles
93 cannot be surface activated by these cationic or nonionic surfactants. Addition of particles had
94 a synergistic effect on the foam stability of anionic SDS surfactant. However, because SDS is
95 not food-grade, food-grade alternatives need to be found that have the same synergistic effect
96 on foam stability with $CaCO_3$ particles. The use of such a food-grade alternative in
97 combination with $CaCO_3$ particles was studied recently by Binks *et al.* (2015) and they found
98 that this combination yields ultra-stable foams.

99 In this study five different emulsifiers that are negatively charged at the relevant pH were
100 selected; β-lactoglobulin (β-lg), sodium caseinate (NaCas), Quillaja*,* sodium stearoyl 2-
101 lactylate (SSL) and sodium dodecanoate (SD). β-lactoglobulin is a dairy protein present in
102 milk serum. It is a globular protein with its isoelectric point at pH 5.2 (Walstra, Wouters, &
103 Geurts, 2005). Sodium caseinate is made by acidification of casein micelles. It is a random
104 coil protein mixture consisting of α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein and γ-casein of
105 which the α_{s1} and β caseins are most abundant and most surface-active (Fennema, 1996). The
106 caseins contain 35-45 % apolar amino acids which make them relatively hydrophobic and
107 their isoelectric point is at pH 4.6 (Walstra, et al., 2005). Quillaja is an extract from the inner
108 bark of the tree *Quillaja saponaria* and contains a high concentration of saponins. The
109 properties of Quillaja are variable; the type used in this research is negatively charged at

neutral pH because of the presence of a carboxylic acid group with a pK_a value of 3.5 (Y. Yang, Leser, Sher, & McClements, 2013). Sodium stearoyl lactylate is an anionic surfactant consisting of an ester of stearic acid and lactic acid (Kurukji, Pichot, Spyropoulos, & Norton, 2013). Sodium dodecanoate is an anionic surfactant consisting of a sodium carboxylate with a C_{12} chain (Cui, Cui, Zhu, & Binks, 2011). The potential of the emulsifiers to hydrophobise calcium carbonate particles was investigated by measuring the effect of particle addition on the foamability and foam stability of the different foaming agents. As a next step towards practical application, the effect of lowering the pH to around 7 and the addition of salts was studied.

2. Experimental

2.1. Materials

Precipitated calcium carbonate particles ($CaCO_3$) from Tianli Construction Material Co. Ltd. were used. Water was first passed through a reverse osmosis unit and then through a Milli-Q reagent water system. The emulsifiers used were: β-lactoglobulin (β-lg) from DOMO (Hiprotal 35) with 35% protein and a molecular weight of 1.84×10^4 g mol^{-1} (Kontopidis, Holt, & Sawyer, 2004); sodium caseinate (NaCas) from DMV International (EM7) with 90% protein and an average molecular weight of 2.33×10^4 g mol^{-1} (Fennema, 1996); Quillaja from National Starch (Q-Naturale) with 90% saponins, 20% dry matter and a molecular weight of 1.65×10^3 g mol^{-1} (Mitra & Dungan, 1997); sodium stearoyl-2-lactylate (SSL) $C_{24}H_{34}NaO_6$ from Kerry Ingredients (Admul SSL 2012) of 100 % purity with a molecular weight of 4.52×10^2 g mol^{-1} and sodium dodecanoate (SD) $C_{12}H_{23}NaO_2$ from Sigma-Aldrich with a purity of > 99% and a molecular weight of 222 g mol^{-1}. The critical aggregation (CAC) and micelle (CMC) concentrations of the proteins and surfactants are given in Table 1.

Simulated milk ultra-filtrate (SMUF) was made from three solutions and stored up to a week at 5 °C. The solutions were stored for a maximum of 6 months at 5 °C. 20 mL of each solution was diluted to 500 mL with water before use. Solution 1: 39.5 g KH_2PO_4 (≥ 98%), 30.0 g $C_6H_5K_3O_7.H_2O$ (≥ 99%), 44.8 g $C_6H_5Na_3O_7.2H_2O$ (≥ 99%) and 4.5 g K_2SO_4 (99.5%) were dissolved in Milli-Q water and made to 500 mL. Solution 2: 7.5 g K_2CO_3 (≥ 99%) and 15.0 g KCl (99.5%) were dissolved in Milli-Q water and made to 500 mL. Solution 3: 33.0 g $CaCl_2.2H_2O$ and 16.3 g $MgCl_2.6H_2O$ (≥ 99%) were dissolved in Milli-Q water and made to 500 mL. All the salts were from Sigma-Aldrich except calcium chloride (99%) which was from Fisher Scientific.

2.2. Size distribution of particles

1 wt.% of $CaCO_3$ particles was dispersed in Milli-Q water with a high intensity ultrasonic processor (Vibra-cell VC100) with a 20 kV CV18 ultrasonic probe of 2.3 mm diameter for 2 min at 15 W (Sonic & Materials). The sample was diluted in a Hydro 2000SM sample dispersion unit and the particle size distribution was measured using a Malvern Mastersizer 2000 instrument.

2.3. Preparation of surfactant and protein solutions

Stock solutions of the emulsifiers were made fresh daily with Milli-Q water at room temperature (23 °C); samples were magnetically stirred until dissolved. The SSL stock solution was heated at 60 ºC for 30 min in a water bath connected to a Grant GD120 thermostat and was allowed to cool to room temperature prior to use. The desired concentrations were prepared using the stock solutions and Milli-Q water.

2.4. Preparation of dispersions

Calcium carbonate particles were added to the surfactant or protein solution. Particles were dispersed with a high intensity ultrasonic processor (Vibra-cell VC100) with a 20 kV

158 CV18 ultrasonic probe of 2.3 mm diameter for 2 min at 15 W (Sonic & Materials). Unless
159 stated otherwise, the pH of the samples was not modified. For pH modifications, sodium
160 hydroxide and hydrochloric acid were used. After addition of base or acid, the sample was
161 stirred for 30 min after which the pH was checked and modified again if necessary. For the
162 SMUF-containing samples, calcium carbonate particles were added to surfactant and protein
163 solutions that were twice more concentrated. After preparation of the dispersion, SMUF and
164 surfactant solutions were mixed in a 1:1 ratio. The pH was measured with a pH meter from
165 Hanna instruments (HI2210).

166 ### 2.5. Aeration

167 10 mL of aqueous sample was aerated in a 50 mL graduated cylinder for 2 min using a
168 homogeniser (IKA, Ultra-Turrax T25 digital) with an 18 mm diameter dispersion tool (IKA, S
169 25 N–18 G) at 13,000 rpm. Directly after aeration, the cylinders were sealed with parafilm.
170 Immediately after aeration and 24 hr after, the liquid and foam height were recorded and a
171 photograph was taken (Panasonic DMC-FS3). The error in foam volumes is ± 1 cm^3.

172 ### 2.6. Zeta potential measurement

173 The zeta potential of particle dispersions was measured five min after mixing the samples
174 with a Nano-ZS Zetasizer (Malvern Instruments) at 25 °C. When measuring the zeta potential
175 of $CaCO_3$ particles as a function of pH, the pH was adjusted 30 min before measurement
176 using HCl or NaOH.

177 ### 2.7. Emission inductively coupled plasma (ICP) spectrometry

178 The dissolution of the $CaCO_3$ particles under various conditions was measured with
179 emission inductively coupled plasma spectrometry. 1 wt. % of $CaCO_3$ particles was dispersed
180 in Milli-Q water or SMUF. The pH was kept constant or adjusted to the desired value.
181 Dispersions were left overnight to let the particles settle. The supernatant was filtered using a

0.45 μm filter to remove any remaining particles. A digest of the $CaCO_3$ particles was prepared by dissolving the particles in nitric acid such that all calcium was dissolved. A calibration was made with 10, 20, 40 and 60 ppm prepared from a certified 1000 ppm calibration standard. The samples were measured at wavelengths of 318, 316, 393 (recommended emission wavelength for calcium) and 397 nm on a Perkin Elmer Optima 5300DV emission ICP instrument.

2.8. Contact angle measurement

450 mg FC10 calcium carbonate particles (FordaCal range, diameter = 2.8 μm, Minelco) was compressed to a disk (diameter, 13 mm; thickness, 2 mm) in a steel die with diameter 13 mm using a hydraulic press (Research and Industrial Instrument Co.) with a pressure of $8.0 \cdot 10^8$ N m^{-2}. Subsequently, contact angles were measured using a Krüss DSA Mk 10 apparatus. The advancing contact angle in air was measured by placing 10 μL aqueous solution on the surface of the disk with a microsyringe. Thereafter, the receding contact angle was measured by withdrawing 3 μL from the drop. Three different readings were taken from which the average was determined. The results obtained are given in Table 1.

2.9. Optical microscopy

Optical microscope images of foams were taken using an Olympus BX51 optical microscope with a 12-bit Olympus DP70 camera at room temperature directly after aeration. A sample of foam was placed on a microscope slide.

3. Results and discussion

3.1. Effect of emulsifiers on zeta potential of calcium carbonate particles

The used $CaCO_3$ particles consisted of primary particles with a diameter of around 150 nm which were agglomerated into aggregates of several microns (Figure 1). The natural pH of

the particles dispersed in water was 9.6 with a corresponding zeta-potential of +2.5 mV; particles thus had a slight positive charge at this pH. The isoelectric point of the particles was determined to be just above pH 10 (Figure 2). The particles alone were not surface-active as no stable bubbles were formed upon aeration of a 1 wt.% particle dispersion at pH 6, 9.6 or 11. Upon addition of the emulsifiers, the zeta-potential of particle dispersions decreased from a slightly positive value to negative values upon increasing the emulsifier concentration, and this transition was always at a concentration below the CAC/CMC (Figure 3 and Table 1). The emulsifiers were all negatively charged at pH 9.6. The zeta-potential decrease can be attributed to the adsorption of monomeric emulsifier *via* electrostatic interactions with the calcium carbonate surface.

3.2. Foams prepared with emulsifier alone and in particle-emulsifier mixtures

As expected, the foamability of the three protein foams generally increased with the bulk concentration. An example of the foaming behaviour for these proteins with and without particles is given in Figure 4 for NaCas. Foaming results for β-lg (Figure S1) and Quillaja (Figure S2) were comparable to those of NaCas. The decrease in foamability at high concentrations seen for Quillaja is due to the high viscosity of these solutions hindering air incorporation. Without particles, foams of NaCas and β-lg collapsed within one day whilst those of Quillaja are more stable. It must be reminded that foams of the first two proteins were made at concentrations below the respective CAC whereas half of those made with Quillaja were above the CAC; it may be that aggregates formed in water in the latter case aid stability by blocking Plateau borders between bubbles. On addition of 1 wt.% $CaCO_3$ particles, the initial foam volume was reduced for all emulsifiers at low concentrations and was either increased slightly for β-lg or reduced slightly for NaCas and Quillaja at higher concentrations. Microscopy images of foams produced from particle-protein mixtures revealed perfectly spherical bubbles with smooth interfaces (insets Figure 4 and Figure S1).

Since it has frequently been observed that bubbles stabilised by adsorbed particles typically have a slightly textured and buckled surface (Cui, et al., 2010), this indicates that the particles did not adsorb to the bubble surface in the presence of these surface-active materials.

By contrast, the foamability and foam stability data for SSL with and without added particles displays different behaviour (Figure 5). For foams stabilised by SSL alone, the foamability increases only above the CAC and a large increase in foam volume is observed from 10 mM. These foams were stable for at least 1 day. Maltese crosses were visible in cross-polarised microscopy images of 30 mM SSL dispersions (Figure S3) due to the formation of bilayer vesicles which is in line with the findings of Bezelgues *et al*. (2008) and Binks *et al*. (2015). With added $CaCO_3$, the foam volume passes through a maximum around 0.2-0.6 mM after which it falls close to zero before rising again. Foams at higher concentration (≥ 20 mM) were very coarse compared with those at lower concentration (0.2–0.6 mM) while the foam volume was similar. In the first region, particles stabilise the foam because of partial surface hydrophobisation resulting from SSL adsorption to particle surfaces. With increasing SSL concentration a bilayer is formed on particle surfaces which renders the particle hydrophilic again and prevents their adsorption to the air-water interface (Binks, Kirkland, & Rodrigues, 2008). The increasing foam volume at higher concentrations is due to the influence of free SSL molecules, *i.e*. those not adsorbed to particle surfaces. Foams were very stable in the presence of particles losing little volume within a day.

Addition of particles also had a synergistic effect on foamability and foam stability of SD–containing foams (Figure 6). Without particles, the initial foam volume increased with concentration to a plateau just above the CMC. The produced foam, however, was very unstable and most of the foams collapsed within 30 min. When $CaCO_3$ particles were added, the initial foam volume increased between 0.03 and 6 mM SD and was significantly higher than in the absence of particles. The produced foams were quite coarse after 24 hr; the best

255 foam with limited decay was obtained with 0.1 mM SD. The roughness of bubble interfaces
256 observed with microscopy indicated that particles were adsorbed at the interface (inset Figure
257 6). This supports the claim that the enhanced foamability and foam stability is due to *in situ*
258 hydrophobisation of $CaCO_3$ particles.

259 For both SSL and SD, the increase in foam volume in the presence of particles started at a
260 concentration where the zeta potential became negative (*i.e.* 0.01 mM SSL and 0.06 mM SD).
261 This is not in line with the observations of Cui *et al.* (2010) who reported the highest foam
262 volumes for $CaCO_3$ particles in combination with SDS at concentrations where the zeta
263 potential was still positive and upon further increasing the concentration the zeta potential
264 became negative and the foamability decreased. It is however in line with the findings of
265 Binks *et al.* (2015) who studied a similar system with SSL and $CaCO_3$ particles; they reported
266 a maximum in foamability at 30 mM SSL under which conditions the particles were also
267 negatively charged. Cui *et al*. (2010) explain their results by the fact that at SDS
268 concentrations where the zeta potential becomes negative, a bilayer of SDS adsorbs at the
269 particle surface which decreases the hydrophobicity of the particles such that the particles
270 adsorb less at the bubble surface. For SSL a similar effect would be expected. However, the
271 expected loss at SSL concentrations where the zeta potential becomes negative may be
272 overcompensated by the fact that at these concentrations SSL forms lamellar structures that
273 have excellent foaming characteristics.

274 Foams produced with 1 wt.% $CaCO_3$ and 0.3 mM SSL or 0.1 mM SD were stable for at
275 least 3 weeks (Figure 7). In the foams containing SSL and particles, non-adsorbed particles
276 sedimented within a day and some bubbles collapsed in the first week. The foams containing
277 SD and particles were more stable and bubble collapse was very limited.

3.3. Surface activation of $CaCO_3$ particles

As shown in section 3.1, all the emulsifiers modified the zeta potential of the calcium carbonate particles implying adsorption onto their surfaces. Advancing and receding contact angles of aqueous emulsifier solutions on the particle surface in air were measured at the emulsifier concentration with the highest foam stability in the presence of particles (Table 2). The contact angle of water at the particle surface confirms that particles were hydrophilic. When using the emulsifier solutions the contact angles increased but remained below 90° from which we can conclude that particles were partially hydrophobised with all emulsifiers. In section 3.2 it was shown that only SSL and SD activated the particle surfaces such that particles could adsorb to the air-water interface. The group consisting of β-lg, NaCas and Quillaja adsorbed to the particle surface and rendered them partially hydrophobic, but particles were not able to adsorb to the air-water interface. The difference between the two groups can possibly be explained by the structure of the emulsifier and their adsorption to the particle surface. SSL and SD have a single chain with a negatively charged head group and a molecular weight below 500 g mol^{-1}. The other three emulsifiers have a complex structure with multiple charged groups and a relatively high molecular weight (> 1500 g mol^{-1}).

β-casein, which is the main protein present in sodium caseinate, is a random coil protein consisting of two negatively charged regions which can adsorb to the positively charged particle surface but the positively charged hydrophobic tail will remain in solution (Evers, Andersson, Lund, & Skepö, 2012). β-lactoglobulin is a globular protein with positively and negatively charged patches (Saikia, Saha, & Das, 2014). Quillaja has a negatively charged carboxylic acid group, that is most likely responsible for adsorption to the $CaCO_3$ surface, and various hydrophobic groups among which is quillaic acid (Y. Yang, et al., 2013). The hydrophobic groups of sodium caseinate, β-lactoglobulin, and Quillaja are larger and more hydrophilic than the tails of SSL or SD. Adsorption of sodium caseinate-, β-lactoglobulin-,

303 and Quillaja-hydrophobised particles at the air-water interface may thus be less energetically
304 favourable compared with the energy gain associated with the adsorption of particles
305 hydrophobised with SSL or SD because the presence of larger and more hydrophilic tails in
306 the air phase is less favoured.

307 ### 3.4. Effect of particle concentration

308 We have also investigated the influence of increasing particle concentration. At a fixed
309 surfactant:particle ratio of 0.3:1 for SD and 0.1:1 for SSL, the foam volume increased with
310 increasing particle concentration (Figure 8) and foams were very stable over one day. The
311 average bubble diameter decreased from ~ 5 mm to below 1 mm upon increasing the particle
312 concentration.

313 ### 3.5. Foaming in a food-grade system

314 Food products such as dispersions or emulsions are generally of acidic to neutral pH.
315 Therefore, we also performed experiments at a pH around 6 and compared those with the
316 above work at around pH 9.6 for systems containing SSL or SD (Figure 5, Figure 6 and
317 Figure 9). When the pH of the dispersion was changed to 6 before aeration, the stable foams
318 obtained earlier at 0.3 mM SSL disappeared and instead there was no maximum in
319 foamability and these foams became very coarse within 24 hr. In the case of SD almost no
320 foam at all was obtained when the pH was changed to 6 at any concentration. We established
321 that $CaCO_3$ particles partially dissolve upon decreasing the pH (Table 3). At the natural pH of
322 9.6, 0.1% of the particles dissolved whilst at a pH of 6 almost 50% of the particles dissolved.
323 In order to investigate the influence of calcium that was released when particles dissolve,
324 calcium chloride ($CaCl_2.2H_2O$) was added to solutions of SSL and SD with and without
325 $CaCO_3$ particles and the dispersions were aerated afterwards (Figure 9C). The amount added
326 was comparable to the calcium concentration in the solution at pH 6. In both samples
327 precipitates were observed before foaming, which indicated the formation of calcium-

surfactant complexes. In the case of SSL at 0.3 mM, addition of calcium decreased the foam volume with and without particles slightly. For 0.1 mM SD, addition of calcium decreased the initial foam volume drastically in systems with and without particles.

Because of the divalent charge of calcium, calcium is able to form a precipitate by binding two negatively charged surfactant molecules. It has been shown that dodecanoate forms a precipitate with calcium ions (Acosta, Scamehorn, & Christian, 2003). To our knowledge, no literature is available on the effect of these precipitates on foaming. Calcium stearoyl lactylate is used as an emulsifier in the food industry but there is no literature available on its foaming properties. Calcium soap complexes, however, can act as anti-foaming agents through the formation of soap-particle-bridges between foam films (H. Zhang, Miller, Garrett, & Raney, 2004). Whether the calcium soap complexes do indeed have a destabilising effect depends on the type of surfactant (W. Yang & Yang, 2010). Apparently the formation of calcium-dodecanoate precipitate acts as an anti-foaming agent which inhibits foaming completely. The calcium-stearoyl lactylate precipitate does not inhibit foaming to a large extent so these precipitates were not very effective anti-foaming agents. From this we can conclude that the large decrease in foaming ability of SD and SSL dispersions with particles at pH 6 was because of different mechanisms. Sodium dodecanoate formed a complex with the dissolved calcium ions from the $CaCO_3$ particles that acted as an anti-foaming agent which prevented the formation of a foam. Sodium stearoyl lactylate also formed a calcium complex but this complex was not a very effective anti-foaming agent. As a result of the dissolution of half of the particles, not enough particles remained to stabilise the foam. In addition, the SSL-calcium complexes might not be able to adsorb to the remaining $CaCO_3$ particles to surface activate them.

A possible food application could be to use the particles to increase the foam stability of a milk product. Milk contains different salts that may influence the foaming behaviour. The

353 effect of these salts on SD-$CaCO_3$ and SSL-$CaCO_3$ foams was therefore tested by dispersing
354 the particles and surfactants in simulated milk ultra-filtrate (SMUF). SMUF resembles the
355 salts present in milk serum and is therefore a good model system (Walstra, et al., 2005). The
356 pH of SMUF was 6.7 and remained such after adding $CaCO_3$ particles. This pH equals the pH
357 of milk and is relevant for many other food systems and hence no pH modification was
358 applied. When the $CaCO_3$ dispersion was modified to a pH of 6.7 in pure water, 9.7% of the
359 particles dissolved, whereas at the same pH in the presence of milk salts only 0.8% of the
360 particles dissolved (Table 3). Addition of milk salts inhibited $CaCO_3$ particle dissolution
361 which agrees with the findings of Kannelopoulou and Koutsoukos (2003). In the presence of
362 milk salts no precipitates were observed in the mixed dispersions of surfactants and particles.
363 One would expect that calcium soap complexes would be formed with the available salts. The
364 absence of precipitate formation between surfactants and calcium or magnesium cations
365 present in SMUF can be explained by the low calcium ion activity in highly concentrated salt
366 solutions (Walstra, et al., 2005). As a result, good foaming was achieved from dispersions of
367 particles and surfactants in SMUF (Figure 9D).

368 ## 4. Conclusions

369 Hydrophilic particles like $CaCO_3$ first need to be partially hydrophobised before they can
370 adsorb at a fluid interface. Here we show that Pickering stabilisation with hydrophilic $CaCO_3$
371 particles was only possible when a certain type of emulsifier was used. Large anionic
372 emulsifiers with a complex structure and multiple charged groups, such as β-lactoglobulin,
373 sodium caseinate and Quillaja, had a different effect on foaming with $CaCO_3$ particles than
374 low molecular weight anionic surfactants that consist of a single hydrophobic chain with a
375 charged head group, such as sodium dodecanoate and sodium stearoyl lactylate. Although
376 both groups of emulsifiers adsorbed at particle surfaces and rendered the particles partially

hydrophobic, particles were only surface activated with the low molecular weight surfactants. An explanation could be that the adsorption energy of particles with high molecular weight emulsifiers was too high in order to enable the particles to adsorb at the air-water interface. Thus, *in-situ* hydrophobisation of a hydrophilic particle is only possible with a low molecular weight emulsifier that has a distinct charged part and a distinct hydrophobic part.

When the pH of the dispersions was lowered to a pH value relevant to food products (pH 6-7) before foaming, a decrease in foamability was observed. At these pH values a fraction of the particles was dissolved. Therefore, less particles were present to stabilise the foam but also calcium ions were released which formed calcium-soap complexes. It was found that calcium-dodecanoate complexes act as a very efficient anti-foaming agent. In milk products, and possibly other foods containing a significant level of salts, this will however not be a problem because the salts inhibit particle dissolution.

In order to stabilise foams in food products, $CaCO_3$ particles can be used when their surface is hydrophobised *in situ* with small surfactants like SSL or SD. Using this system at a pH typical for food products is only possible in the presence of salts such as those in milk products. However, when using such particles in milk products, proteins are also present which may influence the surface activation of the particles. Such proteins include β-lactoglobulin and β-casein which on their own do not surface activate particles. A future investigation will be needed to explore whether proteins enhance or inhibit the surface activation of particles by surfactants.

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511

6. Figures and tables

Table 1. Critical micelle concentration or critical aggregation concentration of the used surfactants and proteins at 25 °C.

Surface-active agent	CAC or CMC	Reference
β-lactoglobulin	1 wt.%/500 μM	(Álvarez Gómez & Rodríguez Patino, 2006)
Sodium caseinate	0.016 wt.%/900 μM	(Abascal & Gracia-Fadrique, 2009)
Quillaja	150 μM	(Stanimirova, et al., 2011)
Sodium stearoyl-2-lactylate	0.1 mM	(Binks, et al., 2015)
Sodium dodecanoate	5.9 mM	(Cui, et al., 2011)

Table 2. Advancing and receding contact angles with the standard deviation measured in air on the surface of disks of FC10 FordaCal particles at room temperature.

Liquid	Advancing contact angle (°)	Receding contact angle (°)
water	18 ± 1	< 5
20 μM β-lactoglobulin	64 ± 1	44 ± 1
30 μM sodium caseinate	76 ± 2	51 ± 1
60 μM Quillaja	55 ± 1	32 ± 1
0.3 mM sodium stearoyl-2-lactylate	72 ± 1	45 ± 1
30 μM sodium caseinate	76 ± 2	51 ± 1

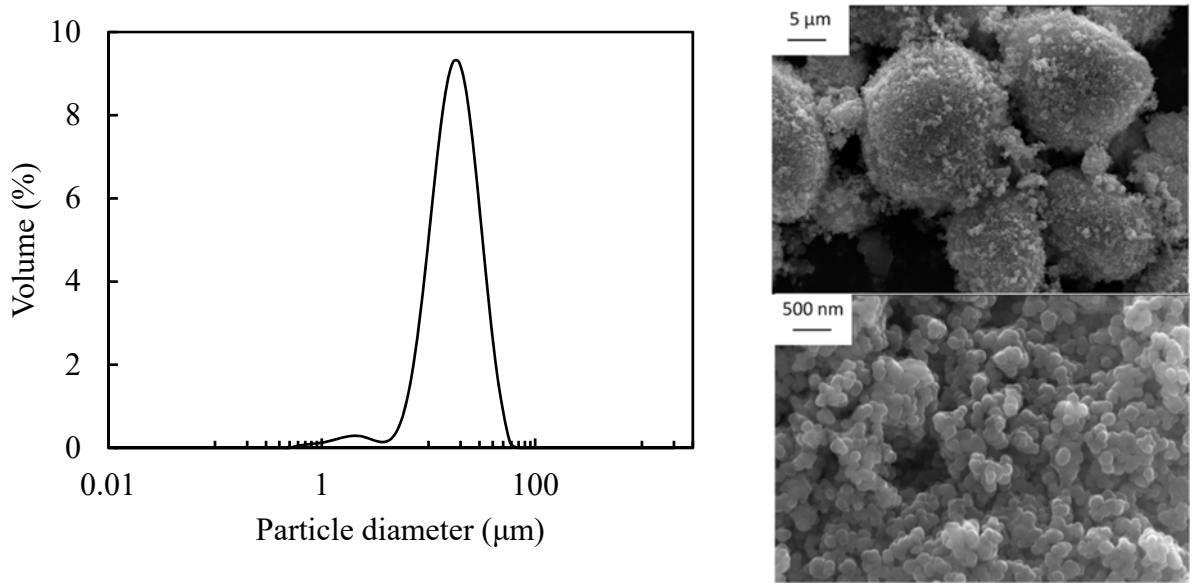

Figure 1. Particle diameter distribution of a 1 wt. % $CaCO_3$ dispersion (left) and SEM images of dry $CaCO_3$ nanoparticles (right).

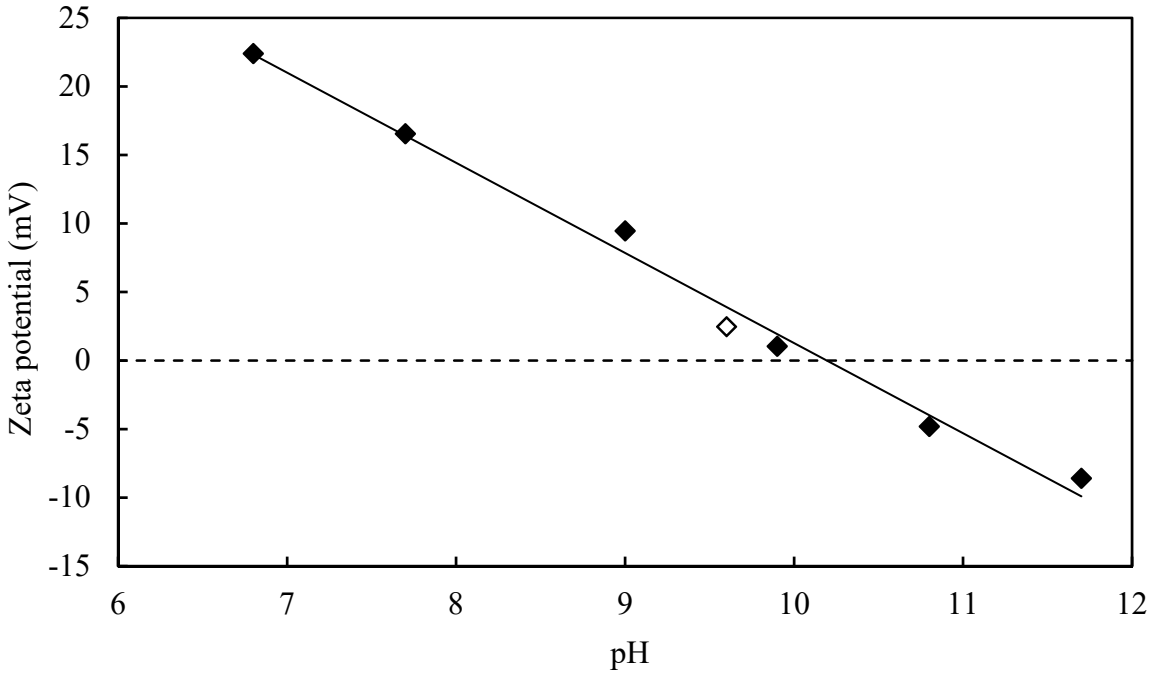

Figure 2. Zeta potential of 1 wt.% $CaCO_3$ particles dispersed in water after pH modification (♦) and at natural pH (◊).

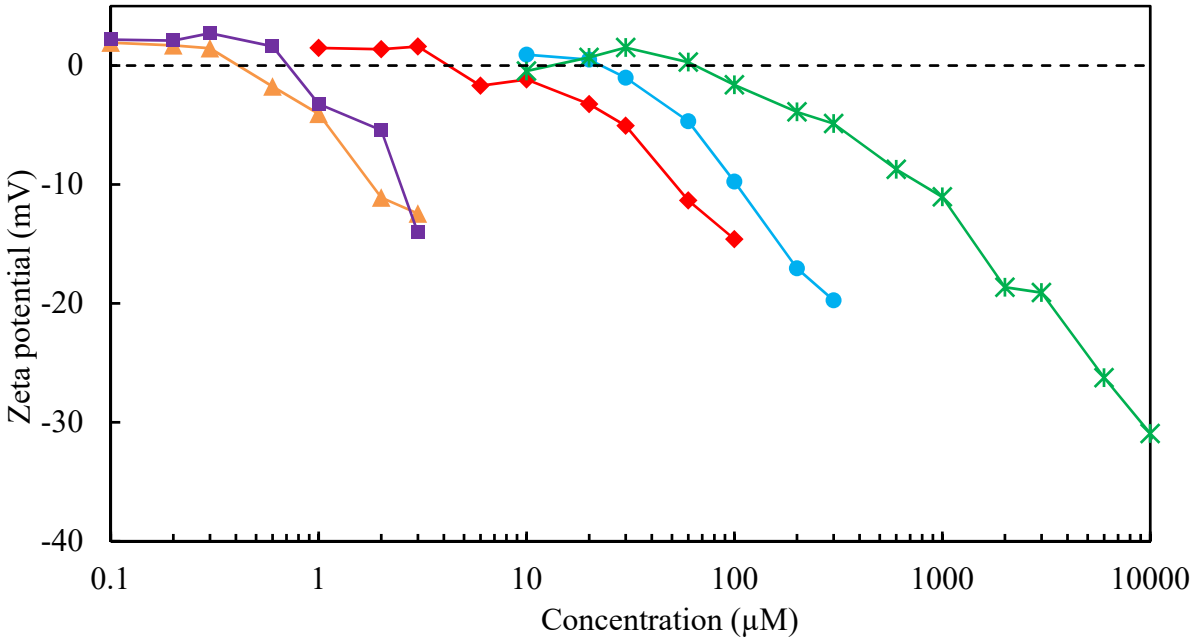

Figure 3. Zeta potential of 1 wt.% $CaCO_3$ particles as a function of β-lactoglobulin (▲), sodium caseinate (■), Quillaja (♦), sodium stearoyl lactylate (●) and sodium dodecanoate (*) concentration.

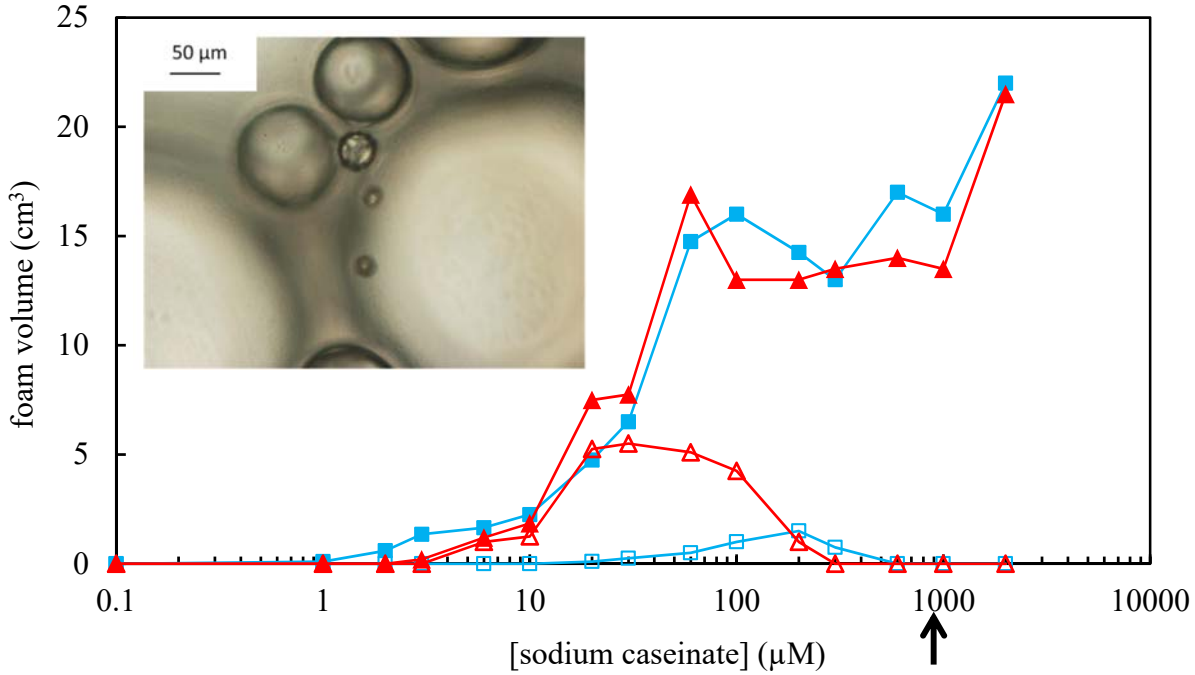

Figure 4. Volume of sodium caseinate foam without particles immediately (■) and 24 hours (□) after aeration, and of sodium caseinate foam with 1 wt. % $CaCO_3$ particles immediately (▲) and 24 hours (Δ) after aeration. The arrow indicates the CAC. Inset: Microscopy image of foam stabilised by 30 µM sodium caseinate with 1 wt.% $CaCO_3$ particles immediately after aeration.

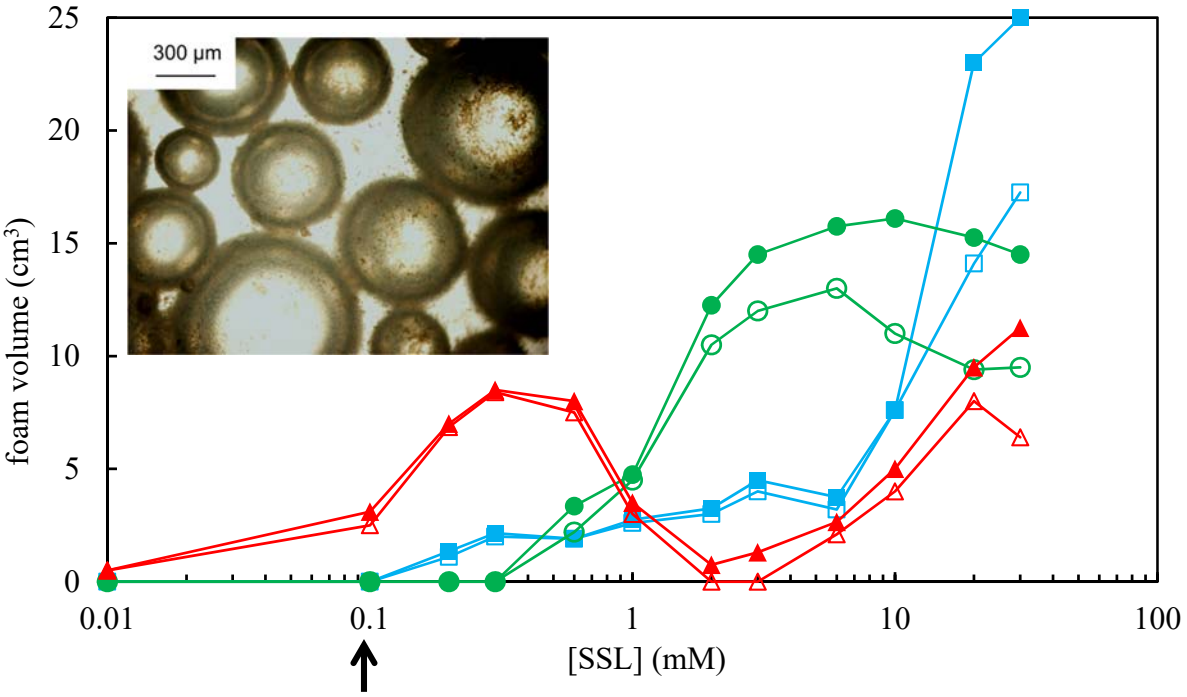

Figure 5. Volume of SSL foam without particles immediately (■) and 24 hr (□) after aeration, and of SSL foam with 1 wt. % $CaCO_3$ particles immediately (▲) and 24 hr (Δ) after aeration, and of SSL foam with 1 wt. % $CaCO_3$ particles at pH 6 immediately (●) and 24 hr (○) after aeration. The arrow indicates the CAC. Inset: Microscopy image of foam stabilised by 0.3 mM SSL with 1 wt.% $CaCO_3$ particles immediately after aeration.

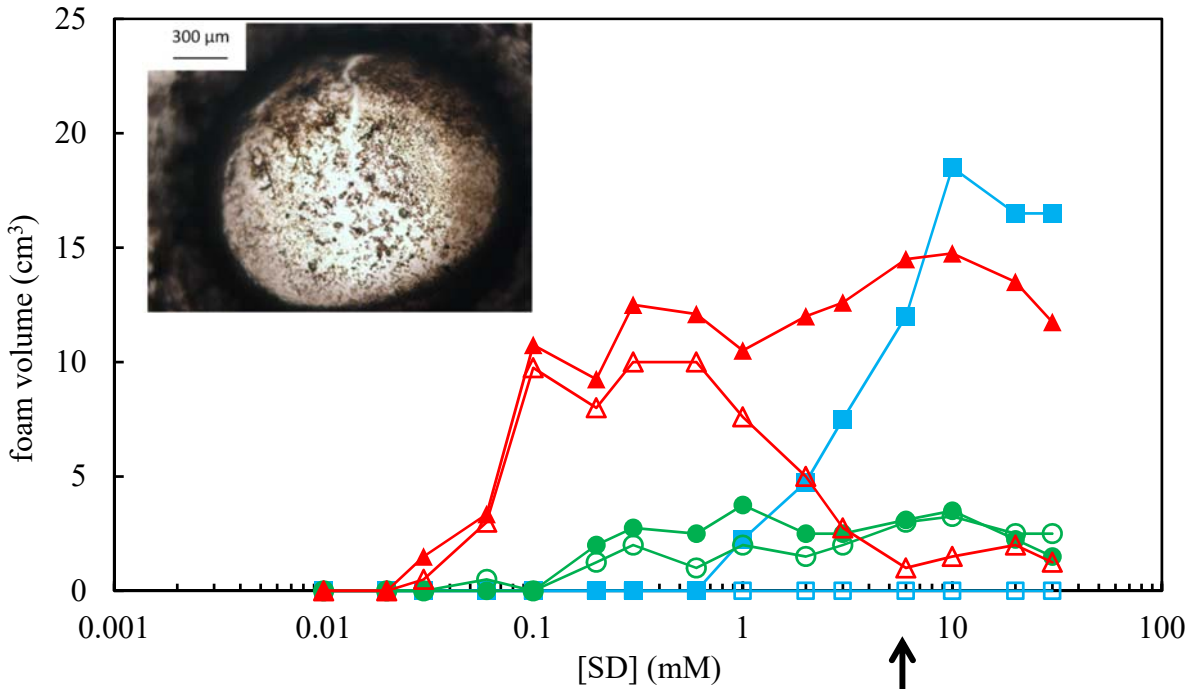

Figure 6. Volume of foam stabilised by SD without particles immediately (■) and 24 hours (□) after aeration, and of foams of SD with 1 wt. % $CaCO_3$ particles immediately (▲) and 24 hours (Δ) after aeration, and of foams of SD with 1 wt. % $CaCO_3$ particles at pH 6 immediately (●) and 24 hours (○) after aeration as a function of SD concentration. The arrow indicates the CMC. Inset: Microscopy image of foam stabilised by 0.1 mM SD with 1 wt.% $CaCO_3$ particles immediately after aeration.

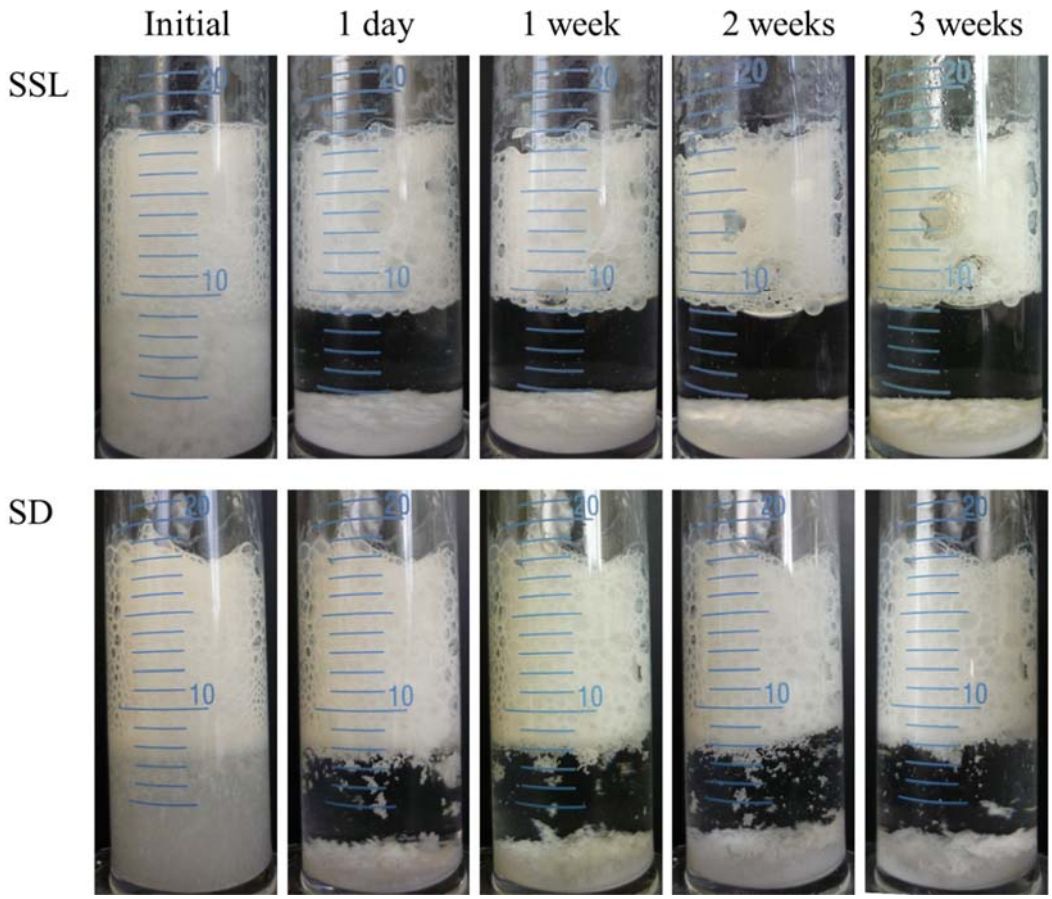

Figure 7. Photographs of vessels containing foams of 0.3 mM SSL or 0.1 mM SD with 1 wt.% $CaCO_3$ particles at different times after aeration.

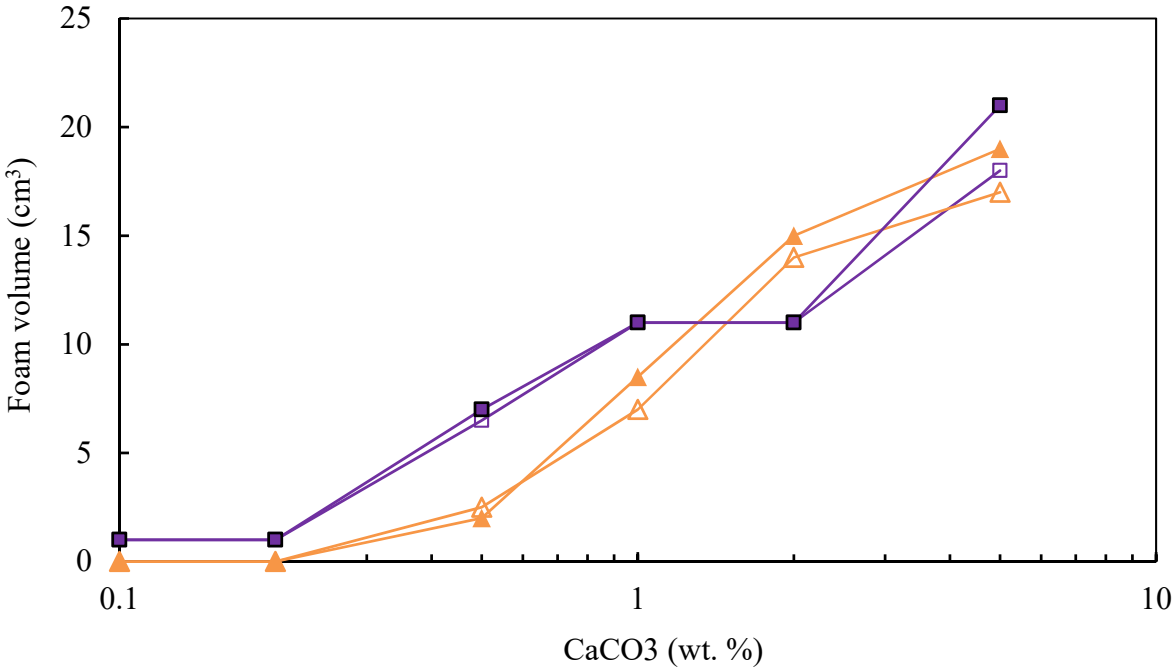

Figure 8. Volume of SD-$CaCO_3$ foams immediately (▲) and 24 hours (△) after aeration and of SSL-$CaCO_3$ foams immediately (■) and 24 hr (□) after aeration as a function of the particle concentration at a fixed surfactant:particle ratio of 0.3:1 and 0.1:1 respectively.

Table 3. Data on the dissolution of $CaCO_3$ particles and the calcium content in simulated milk ultra-filtrate.

Sample	pH	Calcium in solution (%)	Particles dissolved (%)
1 wt. % $CaCO_3$	9.6	0.0003	0.1
1 wt. % $CaCO_3$	8	0.003	0.8
1 wt. % $CaCO_3$	7	0.019	5.2
1 wt. % $CaCO_3$	6.7	0.035	9.7
1 wt. % $CaCO_3$	6	0.180	49.0
SMUF	6.7	0.036	-
SMUF + 1 wt. % $CaCO_3$	6.7	0.039	0.8
0.18 wt. % Ca^{2+}	7.4	0.175	-
0.18 wt. % Ca^{2+} + 1 wt. % $CaCO_3$	7.2	0.174	0.0

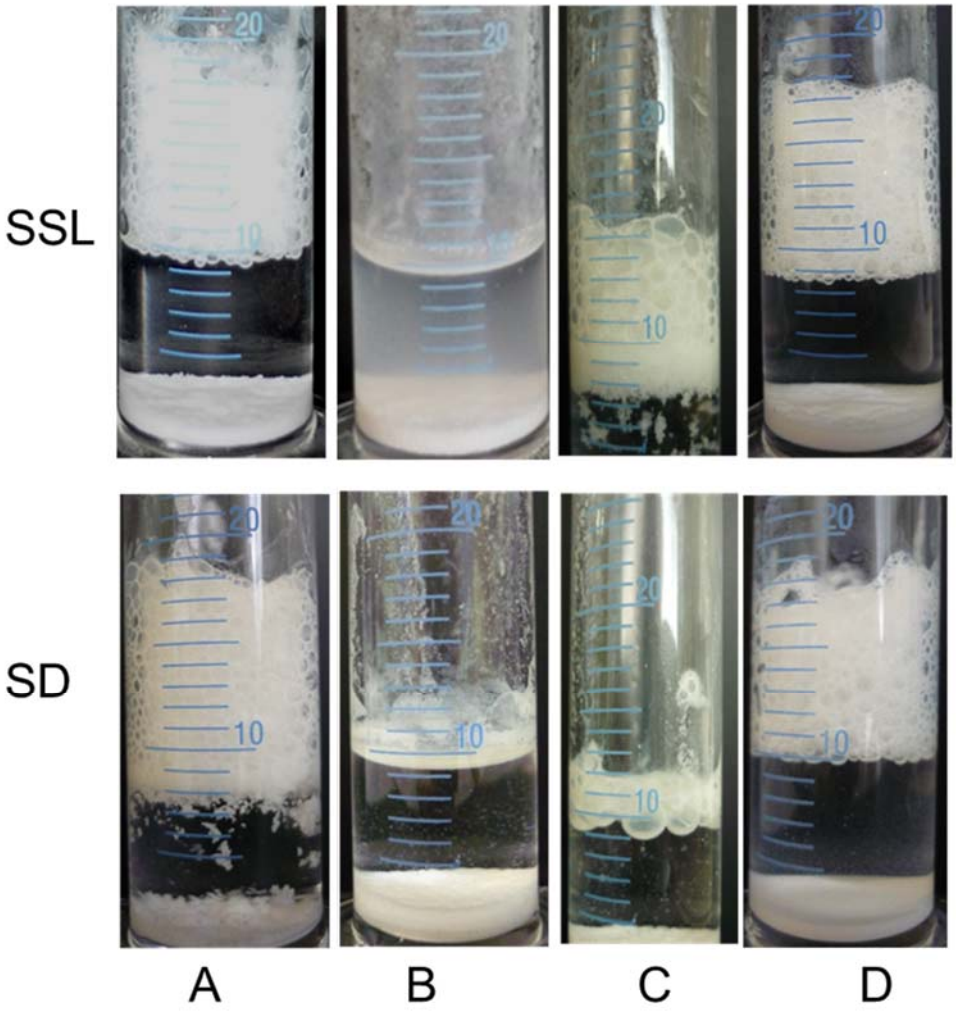

Figure 9. Photographs of vessels containing either 0.3 mM SSL or 0.1 mM SD-containing foams with 1 wt.% $CaCO_3$ particles taken 24 hr after aeration. The surfactants and particles were dispersed in: (A) water pH = 9.6, (B) water which was adjusted to pH 6 after dispersion, (C) water with 45 mM $CaCl_2.H_2O$ and (D) SMUF.

Supporting information

Food-grade Pickering stabilisation of foams via *in situ* hydrophobisation of calcium carbonate particles

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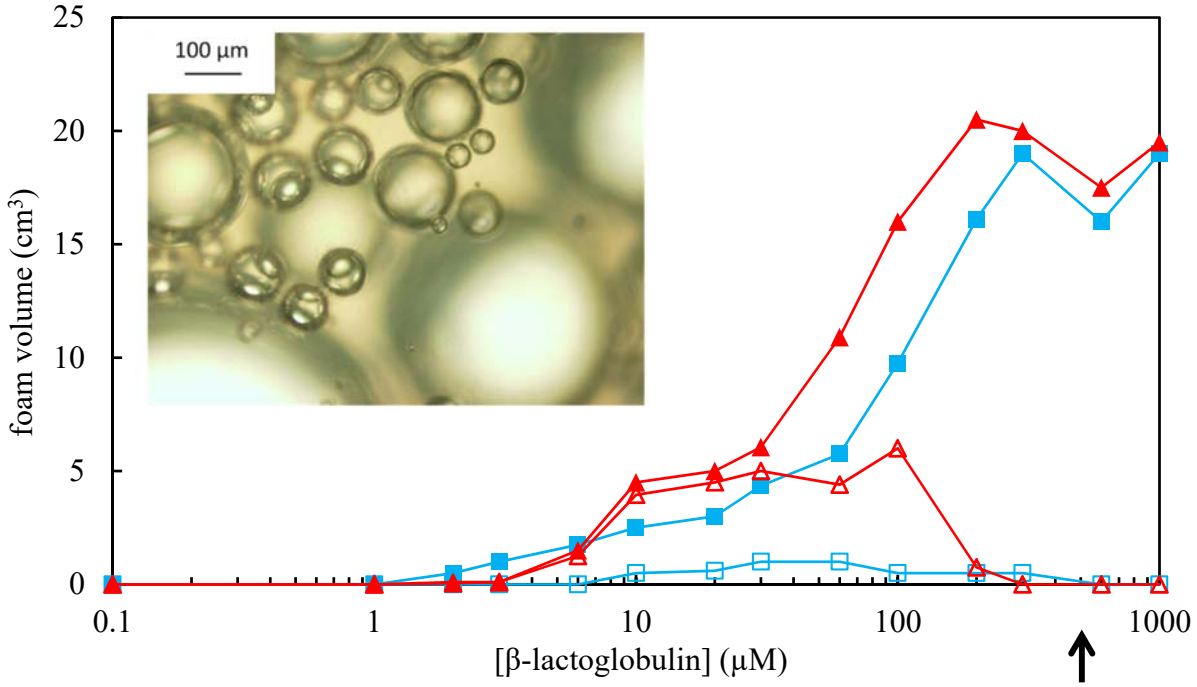

Figure S1. Volume of foam stabilised by β-lactoglobulin without particles immediately (■) and 24 hours (□) after aeration, and of foams of β-lactoglobulin with 1 wt. % $CaCO_3$ particles immediately (▲) and 24 hours (Δ) after aeration as a function of β-lactoglobulin concentration. The arrow indicates the CAC. Inset: Microscopy image of foam stabilised by 20 µM β-lactoglobulin with 1 wt.% $CaCO_3$ particles immediately after aeration.

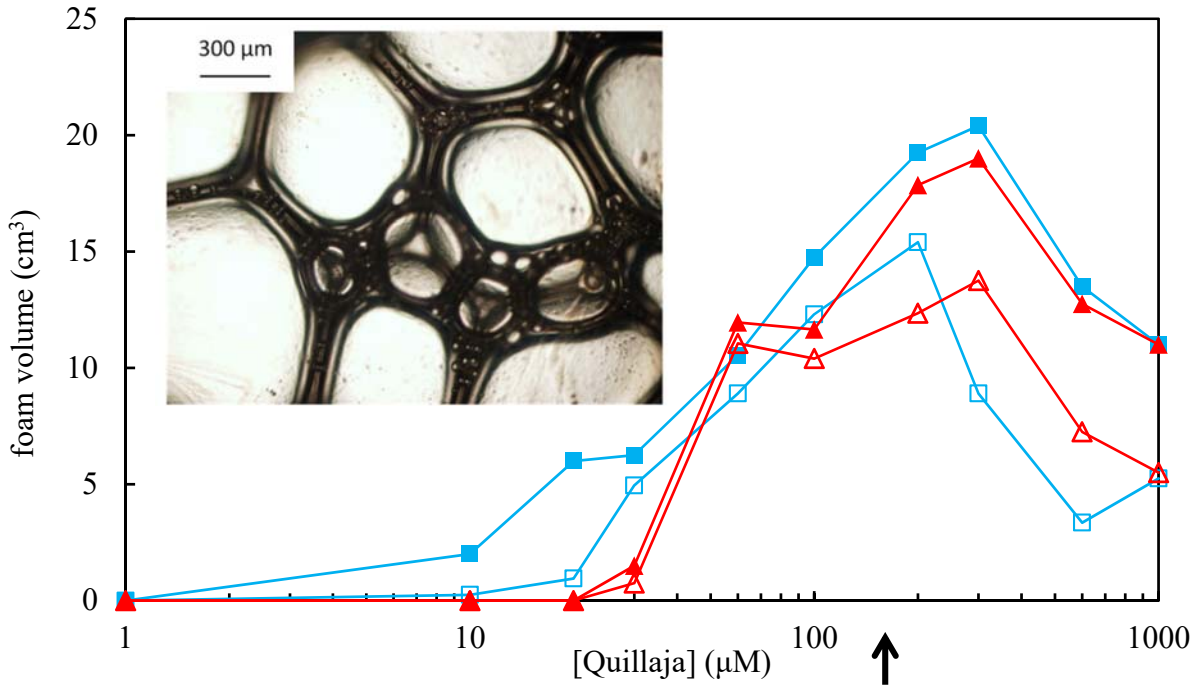

Figure S2. Volume of foam stabilised by Quillaja without particles immediately (■) and 24 hours (□) after aeration, and of foams of Quillaja with 1 wt. % $CaCO_3$ particles immediately (▲) and 24 hours (Δ) after aeration as a function of Quillaja concentration. The arrow indicates the CAC. Inset: Microscopy image of foam stabilised by 600 µM Quillaja with 1 wt.% $CaCO_3$ particles immediately after aeration.

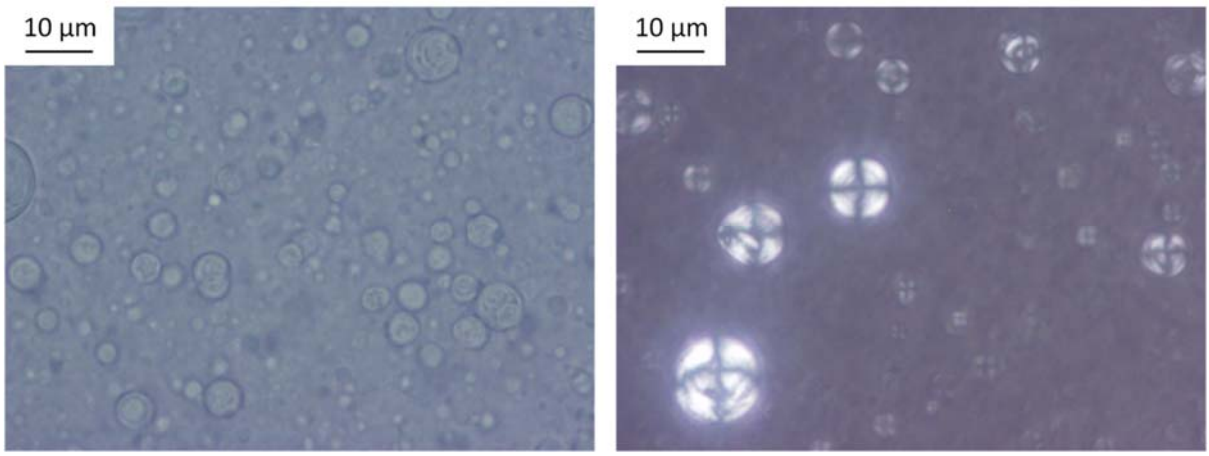

Figure S3. Microscopy images of 30 mM SSL dispersion with normal light (left) and cross-polarised light (right).