Protein-Stabilized Emulsions and Whipped Emulsions: Aggregation and Rheological Aspects

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ABSTRACT

By exploiting the combined gelling and stabilizing properties of the milk protein casein, creamy foam structures can be made by whipping air into a matrix of flocculated protein-coated emulsion droplets. Acidified sodium caseinate-stabilized emulsions based on liquid triglyceride oil give rise to elastic foams of low rigidity and high apparent fracture strain. Replacing all-liquid droplets with all-solid emulsion droplets (crystalline *n*-eicosane) produces a brittle foam of shear modulus similar to that of traditional whipped dairy cream. Addition of emulsifier (LACTEM) affects interdroplet interactions during whipping, leading to a fracture strain characteristic of traditional whipped cream, even in systems with a high proportion of all-liquid droplets.

INTRODUCTION

A common procedure for producing an aerated dairy system is through the shear-induced destabilization of an emulsion during whipping.[1,2] In generic terms, this multiphase system can be regarded as a particle-stabilized foam.[3] The emulsified dairy fat is semi-crystalline, and the gas bubbles incorporated during whipping are stabilized by aggregated fat globules. This type of shear-induced aggregation is known as 'clumping' or partial coalescence.[4,5] It is caused by the crystalline fat from one milk fat globule rupturing the adsorbed layer of another under the influence of the externally applied shear forces. On shearing emulsions in the absence of air under conditions of gradually increasing applied stress, the onset of partial coalescence is associated with a dramatic jump in viscosity[6] and substantial fat particle structuring.[7]

Traditional whipped cream can be made by whipping dairy cream (~ 35% fat) at 5 °C for 2–3 minutes in a kitchen mixer at moderately high speed until it develops characteristic 'stiff peaks'. Fully whipped cream is a soft elastic solid that can support its own weight (shear modulus ~ 5 kPa) and is easy to 'shape'. Additionally, it has a brittle ('short') rheology, with a low yield strain (< 0.1%), and it flows easily in the mouth with a smooth creamy texture. The main physical destabilization mechanism is syneresis.[8] This typically leads to extensive separation of serum after a few hours.

Question 1: Is it possible to prepare an aerated emulsion possessing the texture of traditional whipped cream, but without the structure being stabilized by clumping of partially crystalline fat droplets? To try to answer this question, we have been investigating the stabilization of model aerated systems *via* bridging aggregation of protein-coated emulsion droplets containing a completely liquid triglyceride oil.[9] The underlying concept of the method is that the lowering of the pH towards the isoelectric point of the protein (sodium caseinate) leads to less steric and electrostatic stabilization, enhanced protein–protein interactions, and formation of an emulsion gel structure.[10–12] Using this concept, we can make an aerated caseinate-stabilized emulsion (pH ~ 5, gas content similar to whipped cream) based on a liquid oil (groundnut oil) having good stability with respect to serum separation. However, the appearance and texture more resemble a gelled dessert (*i.e.*, a mousse) rather than traditional whipped cream.[9] That is, the model system is characterized by a polymer gel-like rheology, *i.e.*, with rubber-like elasticity, a relatively low shear modulus, and a high fracture strain.

Question 2: Is the difference in rheology between traditional whipped cream and our aerated acidified caseinate-based emulsion mainly due to the liquid-like character of our emulsion droplets. In experiments designed to answer this question,[13] we replaced the all-liquid droplets (groundnut oil) with all-solid droplets (*n*-eicosane). This generated a considerably more rigid and brittle foam, *i.e.*, more similar to whipped dairy cream. Moreover, unlike the partially coalesced globules of whipped cream, the aerated *n*-eicosane emulsions contained the stabilizing globules as clearly well separate entities, as observed by scanning electron microscopy (SEM). Therefore it is suggested[13] that the solid-like internal character of the dispersed droplets, accompanied by the presence of less 'soft' droplet–droplet interactions, is essential for the support of 'whipped-cream-like' mechanical stresses within the network of droplets in the aerated acidified caseinate-stabilized emulsion.

This report focuses on the influence of a low-molecular-weight oil-soluble surfactant on the properties of these aerated acidified protein-stabilized emulsions. The emulsifier is called LACTEM (lactic acid esters of monoglycerides) which is commonly used in the food industry as a commercial 'whipping aid' for dairy and non-dairy creams.[14] The objective is to explore the feasibility of using emulsifier addition to assist in the preparation of aerated acidified caseinate-stabilized emulsions containing a high proportion of all-liquid droplets and possessing a 'short', rigid, whipped-cream-like texture.

Full details concerning the materials and methodology used in this research have been described in full elsewhere.[9,13,15] Emphasis here is on the key results and conclusions.

BASIC EMULSION PROPERTIES

Samples of the oil-in-water emulsions (2 wt% caseinate, 30 vol% groundnut oil or *n*-eicosane) were prepared with different LACTEM contents (0.25, 0.5, 0.75 and 1.0 wt%) by high-pressure homogenization. To ensure complete droplet crystallization, the emulsions containing *n*-eicosane (melting point 37 °C) were prepared at 60 °C and subsequently rapidly cooled to 5 °C. The mean droplet diameter of the freshly prepared emulsion containing 0.25 wt% LACTEM was d_{32} = 0.4 μm ± 0.05 μm as determined by static multi-angle light scattering (Malvern Mastersizer). This was the same d_{32} value as found for equivalent emulsions without added emulsifier. For higher concentrations of LACTEM, the droplet-size distributions showed increasing evidence of flocculation, probably due to protein bridging; these flocs could be redispersed with excess sodium dodecyl sulfate (SDS). Groundnut oil emulsions with ≥ 1 wt% LACTEM were visibly unstable, as were *n*-eicosane emulsions containing ≥ 0.75 wt% LACTEM. This was manifest as 'oiling off' in the groundnut oil emulsions, and a clumped 'cottage-cheese-like' texture in the *n*-eicosane emulsions.[15]

EMULSION ACIDIFICATION

We first consider the change in rheology of the emulsion systems during acidification in the absence of air incorporation. The emulsion samples were slowly acidified by hydrolysis of glucono-δ-lactone (GDL). The acidulant level was such as to reduce the pH from 7 to 5 in around 2 hours (see Fig. 1) and to give a 'final' value of pH ≈ 4 after around 6 hours.

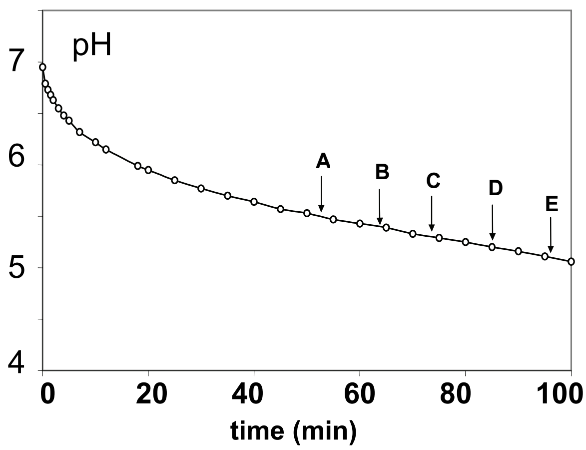

Figure 1. Change in pH of emulsions as a function of acidification time.[9] The labels show different times at which whipping was begun to produce the overrun values shown in Fig. 3.

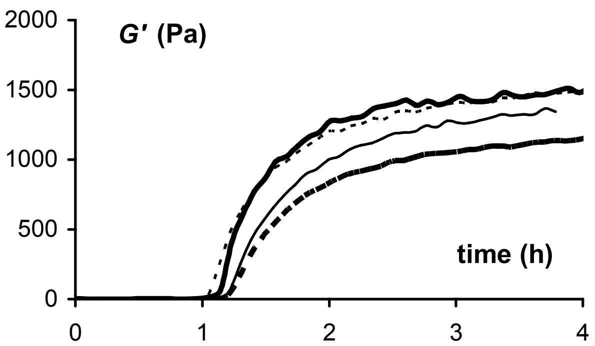

Figure 2. Time-dependent storage modulus *G'* (25 °C, 1 Hz) of caseinate-stabilized emulsions (30 vol% groundnut oil, 2 wt % protein) acidified with 0.6 wt% GDL and with various LACTEM concentrations: 0 wt% (thick solid line); 0.25 wt% (thin dashed line); 0.5 wt% (thin solid line); 1 wt% (thick dashed line).[15]

Oscillatory viscoelasticity measurements were carried out in the concentric cylinder cell of the Bohlin CVO Rheometer as a function of the time following addition of GDL. Fig. 2 indicates the time-dependent storage modulus *G'* (at 1 Hz) of groundnut oil emulsions (2 wt% sodium caseinate, 30 vol% oil) containing 0, 0.25, 0.5 or 1.0 wt% LACTEM.[15] With higher emulsifier content, there is a slight increase in gelation time and also a moderate decrease in the developing gel rigidity (after 3–4 h). This behaviour is similar to that previously reported[16] for the effect of addition of a low concentration of the oil-soluble emulsifier, Span 20 (sorbitan monolaurate), on the acid-induced gelation of a sodium caseinate-stabilized emulsion. The reduction in modulus on addition of surfactant may be attributed to competitive protein displacement from the oil–water interface leading to a change in the droplet character from active to inactive filler particles.[17]

OVERRUN OF AERATED EMULSIONS

According to convention, the 'overrun' is defined as the gas-to-liquid volume ratio expressed as a percentage. Fig. 3 shows the overrun as a function of whipping time for a sodium caseinate-stabilized emulsion (30 vol% groundnut oil, 2 wt% protein) which was gradually acidified using GDL (0.6 wt%). Whipping was done with a standard kitchen whisk beater used under controlled conditions. The overrun reaches a maximum at pH ~ 5.1–5.2, which is roughly the stage when the foam no longer drips from the whisk beater.

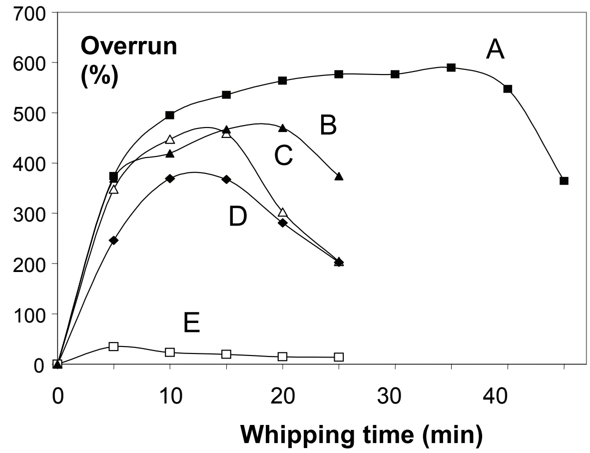

Figure 3. Overrun against time for whipping of sodium caseinate-stabilized emulsion (30 vol% groundnut oil, 2 wt% protein, 0.6 wt% GDL) started at different stages of acidification: A, ■, pH = 5.50; B, ▲, pH = 5.40; C, Δ, pH = 5.30; D, ♦, pH = 5.20; E, □, pH = 5.10.[9] The labels A–E correspond to the same acidification times as indicated in Fig. 1.

The curves in Fig. 3 refer to experiments started at different pH values as indicated by the labels A–E in Fig. 1. The higher the pH at which whipping starts, the greater is the overrun. If whipping begins at a pH further from the protein's p*I*, there is a greater opportunity for air incorporation as the system is acidified. But if whipping begins later, at a pH where the aggregated emulsion gel network is already formed, then less air can be incorporated, and so the final overrun achieved is lower. Prolonged whipping after the foam has gelled causes a substantial loss of overrun as the network structure capable of holding the air is broken down into fragments by the mechanical agitation of the mixer. These compact dispersed fragments do not have the capability for stabilizing freshly incorporated bubbles.[9]

We now consider the effect of emulsifier addition on whipping of acidified emulsions (2 wt% protein, 30 vol% groundnut oil). It has been found[15] that, in contrast to the high-volume foams produced from the emulsions without emulsifier present, there is only a narrow pH range available for dispersing air into emulsions containing 0.5 wt% LACTEM. Significant incorporation of air was possible only in the pH range 5.2–5.1, and the maximum overrun achieved (~150%) was considerably lower than that recorded in the absence of emulsifier (300–600%) (see Fig. 3). This negative effect of LACTEM on the foaming capacity of protein-based systems has also been noted elsewhere.[18] Presumably the emulsifier is disrupting the interfacial protein layer. And consequently the density of protein–protein interactions at the surface of the oil droplets is being reduced, with implications for the amount of air the system can hold.

It has been established[15] that LACTEM addition causes no irreversible aggregation during whipping. Droplet redispersion with SDS following aeration/acidification gave the same mean diameter d_{32} (and size distribution) as was measured with the Mastersizer for the original emulsion.

RHEOLOGY OF AERATED SYSTEMS

Systems with all-liquid droplets

Oscillatory rheology tests (1 Hz, 25 °C) were carried out on the aerated acidified emulsions using the Bohlin CVO rheometer with a parallel plate geometry. To minimize effects of wall slip, the stainless steel plates were coated with sandpaper. Fig. 4 shows that the presence of 1 wt% LACTEM in the acidified emulsion system (2 wt% sodium caseinate, 30 vol% groundnut oil) causes only a very slight decrease in foam stiffness. This small reduction in G' is consistent with the effect of LACTEM on the viscoelasticity of the emulsion gels in the absence of whipping (see Fig. 2).

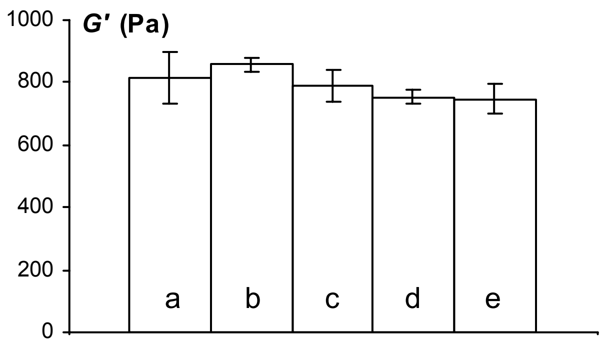

Figure 4. Storage modulus G' (25 °C, 1 Hz) of aerated acidified caseinate-stabilized emulsions (2 wt% protein, 30 vol% groundnut oil, 0.6 wt% GDL, pH 5.0) containing various concentrations of LACTEM: (a) none; (b) 0.25 wt%; (c) 0.5 wt%; (d) 0.75 wt%; (e) 1.0 wt%. The overrun for each system was 120% (± 10%).[15]

To characterize foam large-deformation rheological behaviour, we have followed the complex shear modulus, defined as $G^* = (G'^2 + G''^2)^{1/2}$, as a function of the shear oscillatory strain amplitude at 1 Hz. Above some critical yield strain, the measured moduli are no longer independent of the stress/strain. The reduction in G^* is interpreted as the breakage of internal bonds within the material, which then leads to fracture/flow behaviour on the macroscopic scale. Fig. 5 presents the strain dependence of the normalized complex modulus G^*/G^*_0 for the systems with and without LACTEM (0.5 wt%), together with data for the chosen reference system — normal whipped dairy cream.[9]

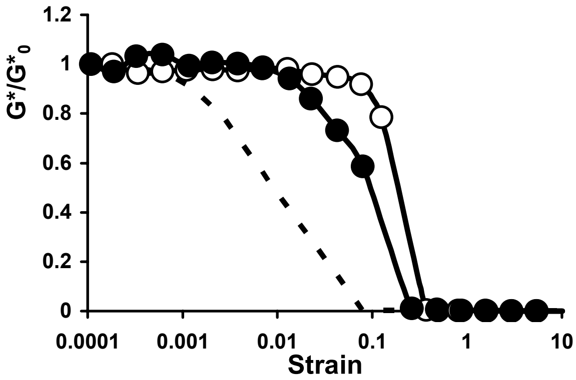

Figure 5. Strain-dependent normalized complex modulus G^*/G_0^* (25 °C, 1 Hz) for whipped cream and aerated acidified caseinate-stabilized emulsions (2 wt% protein, 30 vol% groundnut oil, pH 5.0, overrun ~ 120%): —○—, without emulsifier; —•—, 0.5 wt% LACTEM; – – –, whipped cream.[15]

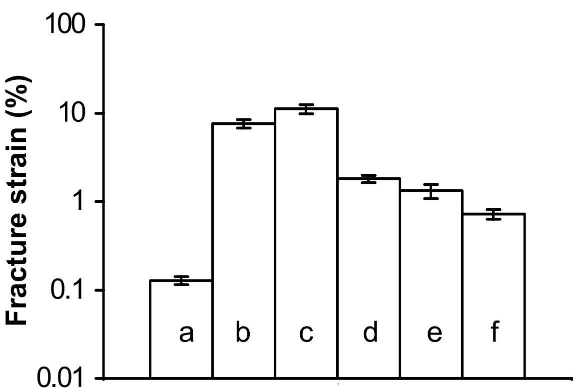

Figure 6. Apparent fracture strain for whipped cream and range of aerated acidified caseinate-stabilized emulsions (2 wt% protein, 30 vol% groundnut oil, variable content of LACTEM, overrun ~ 120%): (a) whipped cream; (b) no added emulsifier; (c) 0.25 wt%; (d) 0.5 wt%; (e) 0.75 wt%; (f) 1.0 wt%.[15]

We define an 'apparent fracture strain' as the percentage strain at which there is a difference of ≥ 10% from the limiting low-strain value of $G^*/G^*_0 = 1$. The solid-like structure of normal whipped cream becomes disrupted at the very low strain of ~ 0.001 (*i.e.* ~ 0.1%), indicating a brittle network composed of strong short-ranged bonds. The behaviour resembles that of a classical particle gel system,[19] whereas the acidified caseinate-stabilized emulsion foam behaves more like a traditional polymer gel (with fracture strain ~ 0.1, *i.e.* ~ 10%).

Fig. 6 shows the apparent fracture strain as a function of emulsifier content. Addition of ≥ 0.5 wt% LACTEM produces substantial lowering of the fracture strain; the value (~ 1–2%) lies much closer to that of normal whipped cream, although it is still 10 times higher. The 'shortening' of the texture may be associated with the emulsifier competing successfully with protein for adsorption at the oil–water interface and so eliminating the extensible protein network connections.

Systems with all-solid droplets

Aerated acidified emulsions composed entirely of all-solid droplets (*n*-eicosane) exhibit quite different rheology from those composed of all-liquid oil droplets.[13] Fig. 7 shows that the addition of 0.5 or 0.75 wt% emulsifier produces a substantial increase in G' of the aerated *n*-eicosane emulsion. The value is 2–3 times that of whipped cream.

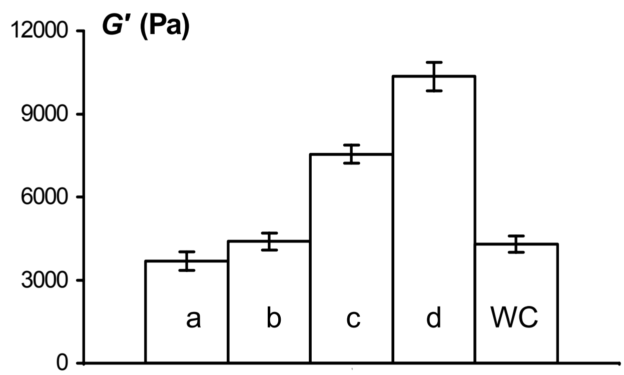

Figure 7. Effect of LACTEM content on storage modulus G' (25 °C, 1 Hz) of aerated acidified caseinate-stabilized emulsions (2 wt% protein, 30 vol% *n*-eicosane, 0.6 wt% GDL, pH 5.0, overrun ~ 120%): (a) no emulsifier; (b) 0.25 wt%; (c) 0.5 wt%; (d) 0.75 wt%. WC = normal whipped cream.[15]

It is postulated[15] that the displacement of sodium caseinate from the surface of the solid hydrocarbon droplets causes some bare patches to appear in the adsorbed protein layer. This facilitates formation of direct crystal–crystal contacts between adjacent droplets under the influence of the high local stresses that arise during the prolonged shearing action of whipping. The much higher modulus for the systems containing ≥ 0.5 wt% LACTEM can be attributed to the important contribution of crystal–crystal bonding within the microstructure. As this obviously does not happen with all-liquid droplets, the measured change in elastic modulus with emulsifier addition in the groundnut oil system is small (Fig. 4).

We obtained evidence[15] from the particle sizing (Mastersizer) measurements of some irreversible aggregation of solid *n*-eicosane emulsion droplets redispersed from the foam with SDS. The appearance of large entities (10–100 μm) in the particle-size distribution indicates strong aggregation caused by direct bonding involving the internal regions of solid emulsion droplets.

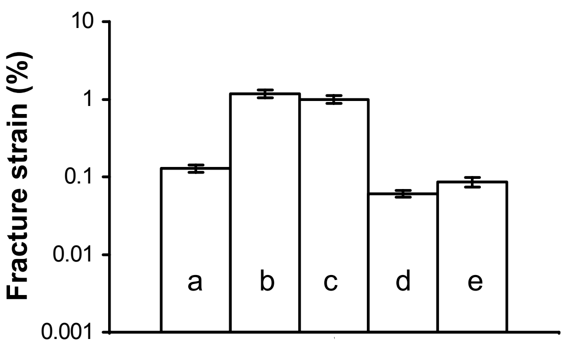

Figure 8. Apparent fracture strain for whipped cream and aerated acidified caseinate-stabilized emulsions (2 wt% protein, 30 vol% *n*-eicosane, 0–0.75 wt% LACTEM, overrun ~ 120%): (a) whipped cream; (b) no added emulsifier; (c) 0.25 wt%; (d) 0.5 wt%; (e) 0.75 wt%.[15]

Fig. 8 indicates that the presence of 0.5 wt% emulsifier in the aerated acidified *n*-eicosane emulsion leads to a reduction in the yield (fracture) strain to a value similar to that of whipped cream. Again, this seems consistent with formation of an aggregated particle-type gel structure with short-range particle–particle interactions.

<u>Systems with all-liquid + all-solid droplets</u>

In the systems without added emulsifier, we found[13] that the partial replacement of all-liquid droplets by all-solid ones had a significant influence on foam rheology only at high replacement fractions (≥ 75–80%). Fig. 9 shows *G'* of the foam (120% overrun) against the proportion of *n*-eicosane droplets in emulsions of constant total volume fraction (30 vol%) containing all-solid + all-liquid droplets. With 0.5 wt% LACTEM, the system containing 25% all-solid droplets exhibited similar stiffness to the equivalent emulsifier-free system containing 75% all-solid droplets. And the system with 50% all-solid droplets and 0.5 wt% LACTEM gave a modulus value that matched closely the one of the 90% emulsifier-free system.

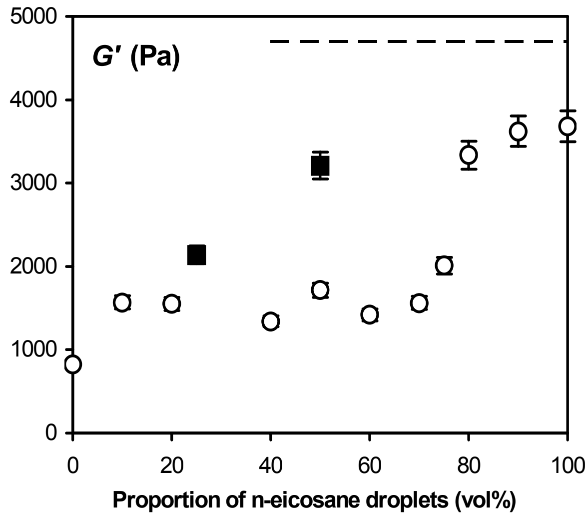

Figure 9. Effect of added LACTEM on aerated acidified emulsions (2 wt% protein, 30 vol% total dispersed phase, 0.6 wt% GDL, pH 5.0) containing *n*-eicosane droplets + groundnut oil droplets.[13,15] Storage modulus *G'* (25 °C, 1 Hz) is plotted against proportion of solid droplets in dispersed phase: ○, no emulsifier; ■, 0.5 wt% emulsifier. The horizontal dashed line denotes the typical modulus of whipped dairy cream.

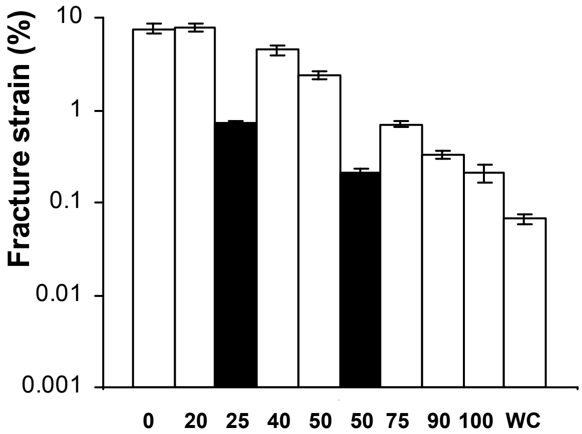

Figure 10. Influence of LACTEM addition on apparent fracture strain of aerated acidified emulsions (2 wt% protein, 30 vol% dispersed phase, 0.6 wt% GDL, pH 5.0) containing mixed *n*-eicosane droplets + groundnut oil droplets.[13,15] The numbers underneath the columns indicate the percentages of solid (*n*-eicosane) droplets for systems without any emulsifier present (white columns) and with 0.5 wt% emulsifier (black columns). WC = normal whipped cream.

Fig. 10 compares the fracture strains of the aerated systems containing 0.5 wt% emulsifier with those of the equivalent systems of different compositions of all-solid + all-liquid droplets but with no emulsifier added. The LACTEM-containing system with 25% all-solid droplets has a similar fracture strain to the emulsifier-free system with 75% all-solid droplets. The LACTEM-containing system with 50% *n*-eicosane droplets has a fracture strain that matches the emulsifier-free system with 100% *n*-eicosane droplets. The mechanism of emulsifier action in increasing the brittle character of the texture probably involves protein being displaced from the emulsion droplet surface. This would then generate the formation of inflexible crystal–crystal interdroplet linkages, instead of the elastic polymer-bridging linkages that predominate in the absence of emulsifier.

CONCLUSIONS

The incorporation of a food-grade oil-soluble surfactant (LACTEM) was found to produce 'shorter' textures in model aerated systems based on acidified protein-stabilized emulsions. At the higher studied LACTEM concentrations, foams that were even more rigid and brittle than whipped cream could be produced from aeration/acidification of *n*-eicosane emulsions, although this was accompanied by irreversible aggregation. Incorporation of a low content of LACTEM into mixed emulsions containing various ratios of all-solid and all-liquid droplets has enabled us to reduce the proportion of all-solid droplets required to mimic whipped cream rheology. Further improvements in the drainage stability of this same aerated system can be achieved by including a small amount of hydrocolloid such as pectin.[13]

The underlying mechanism behind these textural improvements is suggested to lie in the propensity of the oil-soluble emulsifier to displace caseinate partially from the oil–water interface, with consequences for the orthokinetic stabilizing properties of the residual adsorbed layer. This competitive displacement has been confirmed by protein surface coverage experiments.[15]

To reproduce the textural characteristics of traditional whipped cream, the presence of aggregated solid emulsion droplets seems essential to impart the necessary structural rigidity. The surfactant interfacial action is required to displace some adsorbed protein, thereby inducing strong interdroplet crystal–crystal interactions during whipping, and conferring the desired large-deformation foam rheology. This study has highlighted the importance of solid crystalline fat, even when partial coalescence does not occur. Nevertheless, it has been established that the amount of solid fat can be significantly reduced by incorporating a low level of emulsifier in aerated emulsions containing a mixture of all-solid + all-liquid droplets.

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