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Stability of Whey-Protein-Stabilized Oil-in-Water Emulsions during Chilled Storage and Temperature Cycling

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The stability of heat-treated and/or acidified, partly-crystalline-fat-based, whey-protein-stabilized oil-in-water (o/w) emulsions against partial coalescence was investigated during chilled storage (at 5 °C) and repeated temperature cycling (three times between 5 and 25 °C). Experiments focused on the evolution of firmness and droplet size (using pulsed field gradient NMR and scanning electron microscopy). Besides the effects of denaturation and/or acidification, the influence of the droplet size of the dispersed phase on emulsion stability was investigated also. It was found that heat treatment or acidification before emulsification led to unstable emulsions during temperature cycling, whereas heat treatment after acidification resulted in stable emulsions.

KEYWORDS: o/w emulsion gel; cream cheese; consumer handling; partial coalescence

INTRODUCTION

Oil-in-water (o/w) food emulsions, such as milk, cream, and cream cheese, are usually formed by high-pressure homogenization. During homogenization, the coarse droplets of the preemulsion are stretched and broken up, and protein from the serum phase will adsorb and prevent recoalescence of the newly formed droplets. Thus, a finely dispersed oil phase in a continuous aqueous phase is prepared that is usually (kinetically) stable over longer periods (*1*, *2*).

Unfortunately, however, *usually* does not mean *always*, and conditions exist under which properly prepared emulsions turn out not to be stable at all. This can be either desirable or not: for cream it helps structure formation during whipping (*3*, *4*), but causes thickening during temperature cycling (*5*). Causes are diverse, but frequently involve conditions of transport, storage, or consumer handling. It is the purpose of this paper to pinpoint some of the factors that render emulsions susceptible to instabilities, identify routes by which these might be avoided, and describe a noninvasive technique to characterize some of the microstructural changes taking place.

Although the term emulsion instability covers different phenomena (e.g., flocculation, partial coalescence, coalescence, creaming) (*6*), its meaning will be restricted in the present paper to partial coalescence (sometimes referred to as clumping) only. The presence of partly crystalline fat promotes partial coalescence of the fat droplets, in which neighboring fat droplets partly fuse together, and is an important (but not sufficient) destabilizing factor (*7*, *8*). Flocculation of the emulsion droplets is another factor that promotes partial coalescence.

The study of partial coalescence in complex emulsions requires a well-considered choice of the model system. A typical paper in the field of emulsion science involves a liquid dispersed phase (e.g., mineral or triacylglycerol oil), which generally forms a stable emulsion. Moreover, in the case of protein-stabilized emulsions, preferably neutral and native systems are studied, which tend to be less prone to partial coalescence too. In contrast, heated acidified emulsions prepared with partly crystalline fat, which can serve as model systems for certain cream-cheese-type products, are far less stable and will be studied in the present paper.

A number of studies have addressed partial coalescence, with emphasis on systems stabilized by mixtures of protein and low-molecular-weight emulsifiers (*5*, *9*−*12*), or purely by low-molecular-weight emulsifiers (*13*, *14*). For these systems, it was found that lipid composition (solid fat content, crystal size and shape) and low-molecular-weight emulsifier type were factors affecting the stability of the emulsion against partial coalescence. All of these studies were restricted to neutral emulsions and native proteins, if applicable.

Segall and Goff have addressed the stability of emulsions containing butterfat and only (low amounts of) protein, and investigated the effect of processing (heating before homogenization versus homogenization before heating) and small differences in the pH of the emulsion ($6 \leq pH \leq 8$) (*15*). In these systems, emulsions heated after homogenization ("postheated") and unheated emulsions showed similar stability against partial coalescence during whipping, but emulsions heated before homogenization ("preheated") turned out to be much less stable.

The present paper will build on the work by Segall and Goff and focus too on the behavior of protein-stabilized partly-crystalline-fat-based emulsions without any added low-molecular-weight emulsifiers. Moreover, it addresses emulsions in which the protein content is so high that beforehand destabilization would not be expected to occur. Nevertheless, this study

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will show that such emulsions can be destabilized through reduction of protein functionality by heating and/or acidification, in line with earlier results suggesting that acid emulsions are sensitive to partial coalescence (*16*). In particular, the stability of vegetable-fat-based whey-protein-stabilized emulsions prepared according to various processes will be assessed during chilled storage (at 5 °C) and repeated temperature cycling (three times between 5 and 25 °C), conditions intended to simulate the effects of consumer handling on cream-cheese-type emulsions. Besides the effects of denaturation and/or acidification, also the influence of the droplet size of the dispersed phase on emulsion stability will be investigated.

Partial coalescence can be studied in two ways, in terms of either its microscopic or its macroscopic effects. Both effects will be investigated in the present paper.

Droplet size will be chosen as the microscopic property under investigation. Fat droplet size is known to be related to (partial) coalescence and to textural characteristics of an emulsion gel, and its evolution can be taken as a measure for the long-term stability of the emulsion during chilled storage. Droplet size will be studied by means of pulsed field gradient NMR (pfg-NMR) (*17*, *18*), a technique that was discussed recently by Kiokias et al. as a convenient method to determine droplet sizes in protein-stabilized o/w emulsions (*19*). The absence of any sample pretreatment other than temperature equilibration makes the technique extremely useful to follow changes in the droplet size of various protein-stabilized o/w model emulsions during prolonged storage and temperature cycling. Static light scattering requires extensive sample preparation to break the covalent bonds that form during storage and is therefore not a very suitable alternative for these unstable systems (*16*). Furthermore, the interpretation of static light scattering data depends considerably on the correct choice for the value of the complex refractive index, which may be difficult to obtain independently for these complex model emulsions. The droplet size results will be supplemented with scanning electron microscopy (SEM) imaging.

Changes in the firmness of the emulsion will be studied as a macroscopic effect, as partial coalescence is known to affect firmness in food emulsions (*20*). Firmness will be evaluated in terms of the force required to penetrate a cylindrical rod in the emulsion gel.

As such, this study will be the first to investigate the storage stability of heated acidified emulsions, while systematically comparing the results to emulsions that have experienced simpler processing. The complex nature of these heated acidified emulsions required the application of a novel noninvasive droplet sizing technique. The ultimate aim of this study is to establish to what extent the processing sequence for making these complex heated acidified o/w emulsions can be used to improve the stability of the emulsions against partial coalescence. Such knowledge may help to design products that keep constant properties even under suboptimal storage conditions.

MATERIALS AND METHODS

Ingredients. Model o/w emulsions were prepared from mixtures of 30% lipid phase consisting of either partly crystalline vegetable fat (1:1 mixture of fully hardened coconut oil and fractionated palm oil, 2.4% solid fat content at 25 °C, 14% at 20 °C, 73% at 5 °C) (*19*) or liquid vegetable oil (sunflower oil), 4% of a commercial mostly native whey protein concentrate (Nutrilac QU7560, ex Arla Foods, powder containing 75% protein, ~10% of which is denatured), 0.1% potassium sorbate as a preservative under acidic conditions, and demineralized water. In the recipe, all percentages are weight expressed over total weight of the formulation.

Emulsion Preparation. A premix was prepared at 50 °C. Next, the mix was held at 50 °C and homogenized ("native"), heated to 85 °C and homogenized ("preheated"), or homogenized and heated to 85 °C ("postheated"). Heating and holding steps took 20 min. Homogenization was performed using an APV Lab1000 homogenizer, one stage at 300 bar by default, and is always preceded by turraxing the mixture at 8000 rpm for 2 min. When indicated, the homogenization pressure was varied over the range 0−300 bar, or a second homogenization stage was used at 10 bar. Samples were either kept at pH 6.8 ("neutral") or acidified to pH 4.5 ("acidified") using a 50% citric acid solution in demineralized water. Emulsions were filled in 100 mL tubs (6.5 cm diameter), sealed, and stored at 5 °C until further analysis. The samples cooled in the fridge in 1−2 h.

It should be noted that the choice of materials, processing, and procedures took into account considerations of practical industrial relevance as well.

Emulsion Stability Experiments. In the first part of the investigation, the effect of differences in the processing conditions (especially native/preheated/postheated, neutral/acidified, one-stage/two-stage homogenization, and homogenization pressure) on droplet size, firmness, and protein associated with fat was investigated before temperature cycling for emulsions based on partly crystalline fat.

Subsequently, one-stage-homogenized emulsions (native/preheated/postheated, neutral/acidified) were subjected to prolonged chilled storage at 5 °C. As a reference, a preheated acidified emulsion based on sunflower oil was included as well because emulsions without crystalline fat are known to be stable against partial coalescence. Emulsions were kept for 0, 15, 30, and 45 days at 5 °C in a storage cabinet, after which droplet size and firmness measurements were taken.

Next, the sensitivity of the same one-stage-homogenized emulsions (especially native/preheated/postheated and neutral/acidified) to repeated temperature cycling between 5 and 25 °C was investigated. A sunflower-oil-based preheated acidified emulsion was included as a reference. After 2 weeks of chilled storage at 5 °C, emulsions in 100 mL tubs were placed in a calibrated cabinet at 25 °C for 4 h and subsequently put back at 5 °C for 20 h. This temperature cycling was repeated three times. Droplet size and firmness were measured just before cycling and at the end of each temperature cycle. In specific cases, SEM images of selected samples have been produced to determine whether any changes in the microstructure of the emulsion during cycling could be visualized.

Finally, the effect of the processing conditions (one-stage/two-stage homogenization, homogenization pressure) on droplet size and firmness of preheated acidified emulsions after repeated temperature cycling between 5 and 25 °C was investigated. Again, a sunflower-oil-based preheated acidified emulsion was included as a reference.

Droplet Size Measurements. O/w emulsions were filled to a height of 15 mm in NMR tubes of 10 mm diameter, and thermally equilibrated for 30 min at 20 °C. A restricted diffusion-based droplet size was obtained by means of restricted diffusion through pfg-NMR using a Bruker Minispec MQ20. The details of the technique are discussed elsewhere (*17*−*19*). A measurement yields values for the volume-weighted geometric mean diameter $d_{3,3}$ and the width σ of the droplet size distribution when plotted as a function of the logarithm of the diameter. These parameters can be converted to the surface-weighted mean diameter $d_{3,2}$ using the relation $d_{3,2} = d_{3,3} \exp(-\sigma^2/2)$ (*21*, *22*). Measurements were carried out in triplicate, and averaged results are expressed in terms of surface-weighted mean diameter $d_{3,2}$ values. For clarity, error bars will be shown in bar graphs only.

Determination of Protein Associated with the Dispersed Phase. The amount of protein associated with the fat phase was determined by spinning the emulsion in a high-speed centrifuge and determining the protein/fat ratio in the cream phase from the composition of the serum phase. Freshly prepared emulsion was spun at 18000*g* for 1.5 h at 35 °C (Sorvall RC-5B, refrigerated Super-speed centrifuge). The temperature was chosen to melt out the crystalline fat. The serum phase was removed and analyzed for protein (*23*), and the cream phase was analyzed for moisture (*24*) and fat (*25*) content. In neutral emulsions, this analysis will give the surface coverage of the fat droplets. In addition, the method may collect protein associated with the protein at the interface in acidified emulsions, and aggregates caught in the cream

phase in preheated emulsions. A direct analysis of the cream phase was attempted as well, but was found to give more variable results than the indirect route via the serum phase, as expected.

The amount of protein together with $d_{3,2}$ (m) allowed determination of the surface coverage Γ (mg/m^2), using the relation

$$\Gamma = (c_{total} - (1 - (\rho_{fat}/\rho_{serum})\Phi_{fat})c_{serum})/(6\Phi_{fat}(\rho_{serum}/\rho_{fat})/d_{3,2})$$

with c_{total} as the total protein concentration in the emulsion (mg/m^3), c_{serum} the protein concentration in the serum phase (mg/m^3), Φ_{fat} the weight fraction of fat in the emulsion (−), and ρ_{fat} and ρ_{serum} the densities of the fat and the serum (mg/m^3), respectively [and neglecting second-order terms in $1 - (\rho_{fat}/\rho_{serum})$].

Firmness Measurements. A Stevens-LFRA texture analyzer (ex Stable Micro Systems) was used to take firmness measurements during storage or cycling of the products. Firmness is one of the parameters obtained in the standard texture profile analysis, and indicates the maximum force required to penetrate a rod in a material. A cylindrical probe of 12.7 mm diameter penetrated a sample to a depth of 10 mm at a speed of 2 mm/s, immediately after the sample was taken from a 5 °C storage cabinet. The peak force (by custom expressed in grams; 1 g = 9.81 mN) was recorded, and averaged over triplicate measurements. For clarity, error bars will be shown in bar graphs only.

Firmness represents a yield stress during compression. In principle, it is unrelated to the elastic parameters of the emulsion gel, such as the shear modulus G'. In practice, however, it correlates rather well with G' as long as materials are compared with similar yield deformation.

Scanning Electron Microscopy. Samples were loaded into two aluminum sample disks with a depth of 200 μm. The sample disks were frozen under hyperbaric conditions (2045 bar) using a Leica EM HPF high-pressure freezing device and transferred under liquid nitrogen.

The HPF sample disks were separated under liquid nitrogen to obtain a fractured sample surface. The fractured sample was mounted in a Leica EM UCT cryo-ultramicrotome onto a microtome pin, custom-made to hold the HPF sample disks during the sectioning process. The frozen sample was sectioned at −160 °C at a cutting speed of 0.6 mm/s to create a clean and optimal flat surface. After sectioning the samples were transferred under liquid nitrogen to the Oxford CT1500 cryo preparation unit attached to the JEOL 6304F scanning electron microscope.

The samples were freeze etched under vacuum for 10 min at −95 to −90 °C to remove a little frost from the transfer, coated with 5 nm Au, and imaged under low-dose conditions (3 kV) at −150 °C in the scanning electron microscope.

RESULTS AND DISCUSSION

Effect of Processing on Emulsion Properties before Prolonged Storage or Temperature Cycling. Droplet size decreased as $p^{-0.4}$ with homogenization pressure, as expected (*26*, *27*). Little difference was observed between native and preheated emulsions, between neutral and acidified emulsions, or between one-stage and two-stage homogenization. Deviations from the theoretical −0.6 slope have usually been attributed to recoalescence in the homogenizer (*28*).

Properties such as the amount of protein in the emulsion that is associated with the fat phase and firmness were expected to depend on the presence or absence of an acidification stage, because these properties are affected by the nature of the interactions between proteins. This can be illustrated quite clearly by the amount of protein in the emulsion that is associated with the fat phase.

In native neutral emulsions, protein will associate only with the fat phase when functioning as an emulsifier. **Figure 1** shows that the protein/fat ratio in the cream phase for native neutral and preheated neutral samples depends on the droplet surface in the emulsion and could be described quite well by curves that assume a surface coverage of 5 and 9 mg/m^2, respectively. The surface coverage for the native neutral emulsion, 5 mg/m^2,

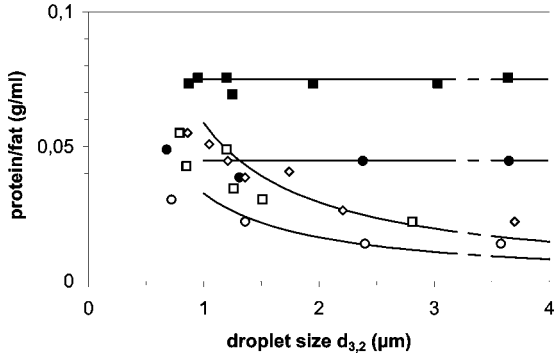

Figure 1. Effect of droplet size (through homogenization pressure) on the amount of protein associated with the fat phase of emulsions prepared with partly crystalline fat: (○) native neutral/one stage; (◇) preheated neutral/one stage; (□) preheated neutral/two stages; (●) native acidified/one stage; (■) preheated acidified/two stages. The curved lines through the data for neutral emulsions represent calculated values based on surface coverage of 5 and 9 mg/m^2, respectively. The straight lines through the data for acid emulsions indicate 0.045 and 0.075 g of protein/mL of fat associated with the fat phase, respectively.

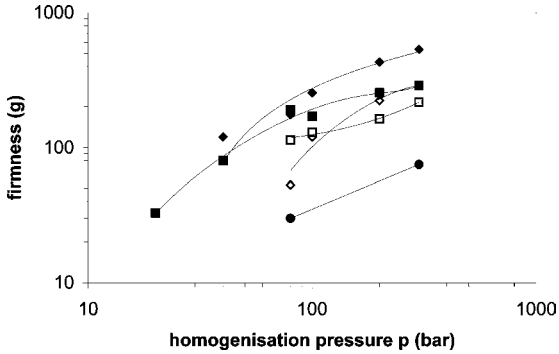

Figure 2. Effect of homogenization pressure on the firmness of emulsions prepared with partly crystalline fat: (◇) preheated neutral/one stage; (□) preheated neutral/two stages; (●) native acidified/one stage; (◆) preheated acidified/one stage; (■) preheated acidified/two stages.

suggested that some protein aggregation took place already as a result of the slight degree of denaturation of the commercial whey protein concentrate used in this study, as the value exceeded the value of 2−3 mg/m^2 typically found in the literature for native whey protein (*10*). This was not investigated further in more detail since the present study did not focus on surface coverage in neutral emulsions as such, but on the much larger differences between the various emulsion preparations.

For acidified emulsions, the situation is quite different. In those systems, denatured protein retained in the serum phase after homogenization interacts and aggregates after acidification with protein adsorbed at the interface. Therefore, the amount of protein associated with the fat phase will hardly be affected by the droplet size in the emulsion, but mostly by the degree of denaturation of the protein. The straight lines in **Figure 1** confirmed this effect, which showed that for native acidified and preheated acidified emulsions 0.045 and 0.075 g of protein/mL of fat was associated with the fat phase, respectively. The theoretical upper limit for the present emulsion is about 0.092 g of protein/mL of fat, if all whey protein denatures and aggregates in clusters associated with the fat phase.

For firmness, clear effects of emulsion processing on physical properties were expected too. This was confirmed by **Figure 2**, which shows the firmness of the emulsion as a function of homogenization pressure. The most obvious effect was a general increase of firmness with homogenization pressure. In addition,

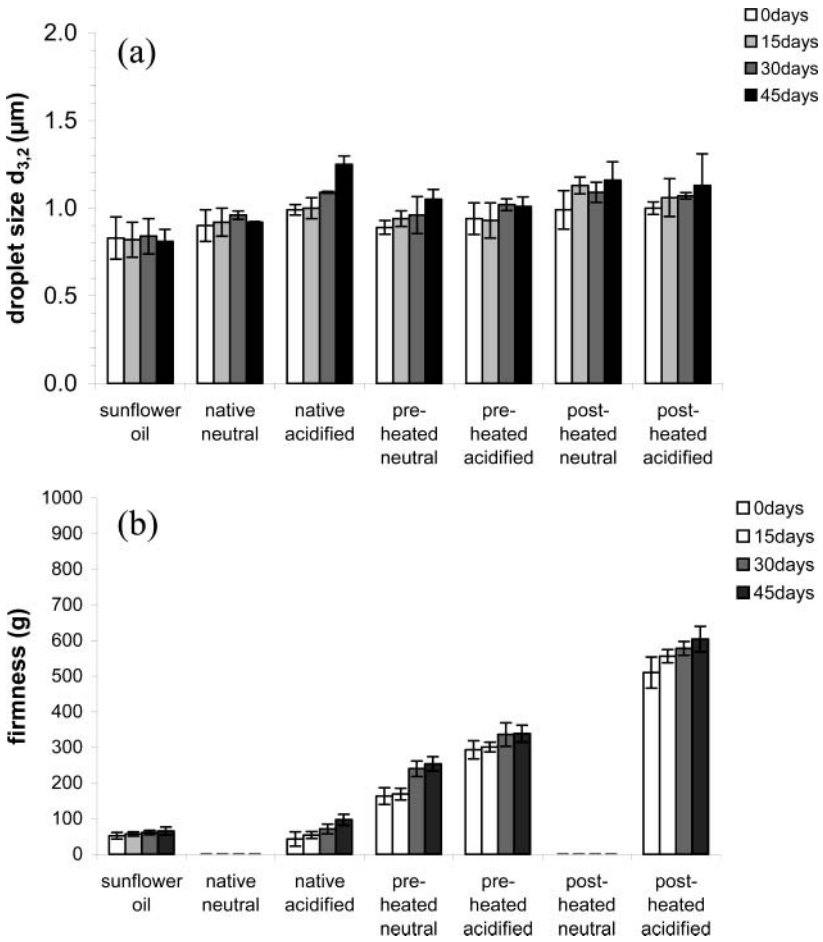

Figure 3. Effect of the duration of chilled storage at 5 °C on (**a**) droplet size and (**b**) firmness, for emulsions based on partly crystalline fat (except one preheated acidified sunflower-oil-based emulsion as a reference). All emulsions were one-stage-homogenized at 300 bar. Missing values refer to liquid products. Results are expressed as average values ± 3 standard deviations.

it was found that native neutral emulsions were completely liquid. Native acidified emulsions formed weak emulsion gels at the highest homogenization pressures. Preheated native emulsions were still firmer. Preheated acidified emulsion gels give the firmest gels.

These effects were not unexpected. For native neutral emulsions, the electrostatic repulsion between the native protein at the interface of the emulsion droplets prevented flocculation of the droplets (and only small amounts of denatured proteins from the commercial WPC were present), and the emulsion droplets themselves provided too little packing volume to achieve a yield stress for the emulsion. Therefore, the emulsion remained fluid. The emulsion droplets in the native acidified emulsion flocculated because the electrostatic repulsion due to the interfacial protein decreases at lower pH, and resulted in network formation. In preheated neutral emulsions covalent bonds formed between emulsion droplets, between protein aggregates, and between aggregates and droplets, resulting in firm emulsion gels. Furthermore, these covalent interactions will develop further during storage (cf. Alting et al. (*29*) for whey protein gels). Finally, combined covalent and physical interactions in preheated acidified emulsions resulted in the firmest emulsion gels.

Furthermore, **Figure 2** shows that two-stage homogenization of preheated emulsions gave firmer gels at low homogenization pressures, but weaker gels at higher homogenization pressures (when the pressure of the second stage of the homogenizer was much lower than at the first stage). Since these emulsions did not differ much in terms of their droplet size, it is concluded that the second stage changed the structure of the emulsion droplet aggregates at higher pressures in some way, e.g., by shearing the aggregates to smaller fragments. A two-stage homogenization process is commonly used in emulsification of neutral emulsions, such as milk, to decluster the emulsion droplets that have been formed during the first stage of emulsification (*28*). For acidified emulsions, in which the droplets interact irrespective of homogenization conditions, it was not obvious a priori that a second homogenization stage would have any effect.

The effect of homogenization pressure was not tested extensively for postheated samples. However, the postheated neutral sample homogenized at 300 bar was fluid (in contrast to the preheated neutral emulsion), whereas the postheated acidified emulsion was much firmer than the preheated acidified sample for this homogenization pressure.

Combining the results of firmness and droplet size, firmness generally increased with decreasing droplet size. This can be explained in terms of the increased surface area at fixed fat content for small droplets and the associated better adhesion between the matrix and the filler in this composite system. Thus, it is clear that the droplets in the emulsion gel are not mere holes, but act to reinforce the structure of the gel.

Emulsion Stability during Chilled Storage. Droplet size during chilled storage at 5 °C was quite stable over a period of 45 days (see **Figure 3a**). There is a slight tendency toward higher droplet sizes $d_{3,2}$ in all systems except the sunflower-oil-based emulsion. For firmness, the qualitative differences between the different processing routes agreed with observation

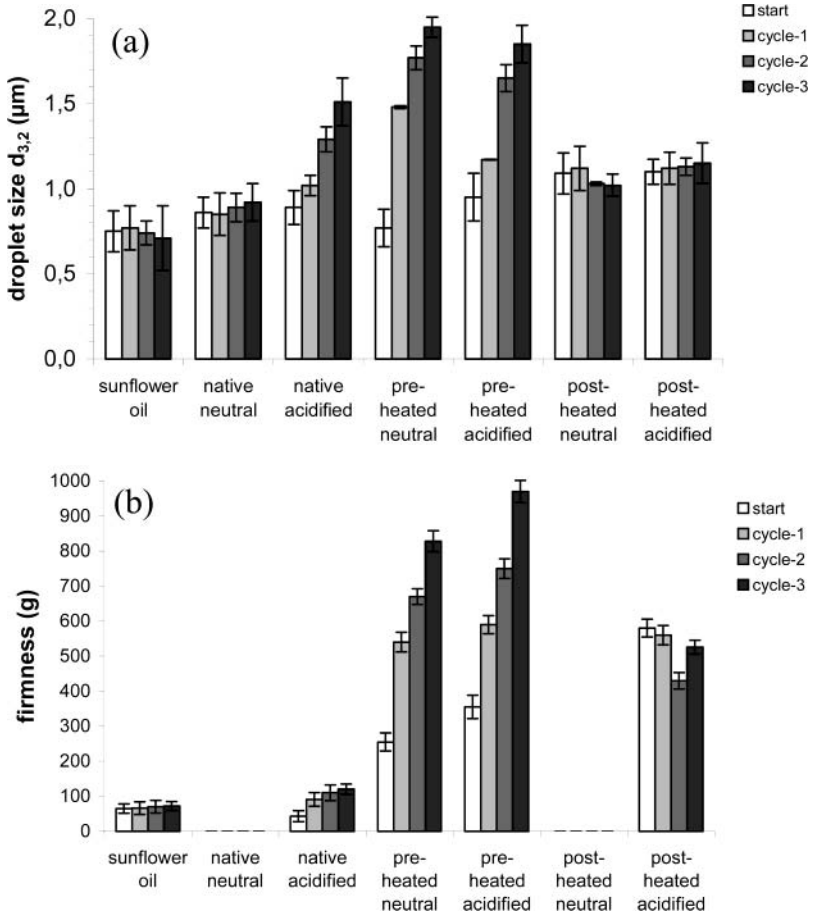

Figure 4. Effect of the number of temperature cycles between 5 and 25 °C on (**a**) droplet size and (**b**) firmness, for emulsions based on partly crystalline fat (except one preheated acidified sunflower-oil-based emulsion as a reference). All emulsions were one-stage-homogenized at 300 bar. Results are expressed as average values ± 3 standard deviations.

in the previous section (see **Figure 3b**). Again, a slight increase in firmness during chilled storage is observed for the products based on crystalline fat.

It was concluded that these emulsions are quite stable during chilled storage at a fixed temperature, independent of the emulsion processing procedure.

Emulsion Stability during Temperature Cycling. Emulsions can be destabilized during temperature cycling as a result of incomplete melting of fat crystals upon heating followed by recrystallization in the presence of seeding crystals during subsequent cooling (*9*). In contrast, such seeding crystals are not present during quiescent cooling in emulsion preparation. During temperature cycling, recrystallizing fat often forms the more thermodynamically favored larger crystals that may locally be able to "invert" the emulsion, although the overall o/w character of the emulsion is retained. The results in **Figure 4** show that temperature cycling indeed destabilized a number of emulsions with respect to droplet size and firmness.

Figure 4a shows that, next to the preheated acidified reference emulsion based on sunflower oil, the native neutral and both postheated samples remained quite stable with respect to droplet size during temperature cycling. For the native acidified and both preheated emulsions, $d_{3,2}$ values steadily increased as cycling proceeded. These changes correlated very well with the variations in firmness, as shown in **Figure 4b**. The firmness of the preheated acidified reference emulsion based on sunflower oil and the postheated acidified emulsion remained quite stable during temperature cycling, whereas the native acidified and both preheated emulsions increased in firmness while temperature cycling progressed. The native neutral and the postheated neutral emulsions were fluid, and therefore, firmness could not be measured.

The changes in droplet size were reflected also in the width σ of the fitted log-normal distribution, despite the relatively large scatter in σ. Before cycling, σ was typically 0.5 ± 0.1 for all emulsions. After three temperature cycles, σ increased to 1.0 ± 0.2 for preheated emulsions (both neutral and acidified), whereas it remained more or less constant for the other emulsions (both neutral and acidified).

The changes for both droplet size and firmness over the three temperature cycles were clearly much bigger than those observed over 45 days of chilled storage. Solid fat content (SFC) measurements in the emulsions suggested that the degree of supercooling did not vary hugely throughout the temperature cycling procedure, thus excluding an increase in crystalline fat as the major cause for the increase in firmness. No relation was established between the amount of protein associated with the fat phase (**Figure 1**) and the stability of the emulsion during temperature cycling.

Since the pfg-NMR droplet sizing technique assumes a log-normal (spherical) droplet size distribution, the above measurements were supplemented by SEM imaging on selected samples. The results for preheated and postheated acidified samples containing partly crystalline fat before and after temperature cycling are shown in **Figure 5**.

Preheated acidified emulsions looked slightly destabilized before temperature cycling, but contained reasonably-well-defined emulsion droplets (**Figure 5a**). After cycling, the

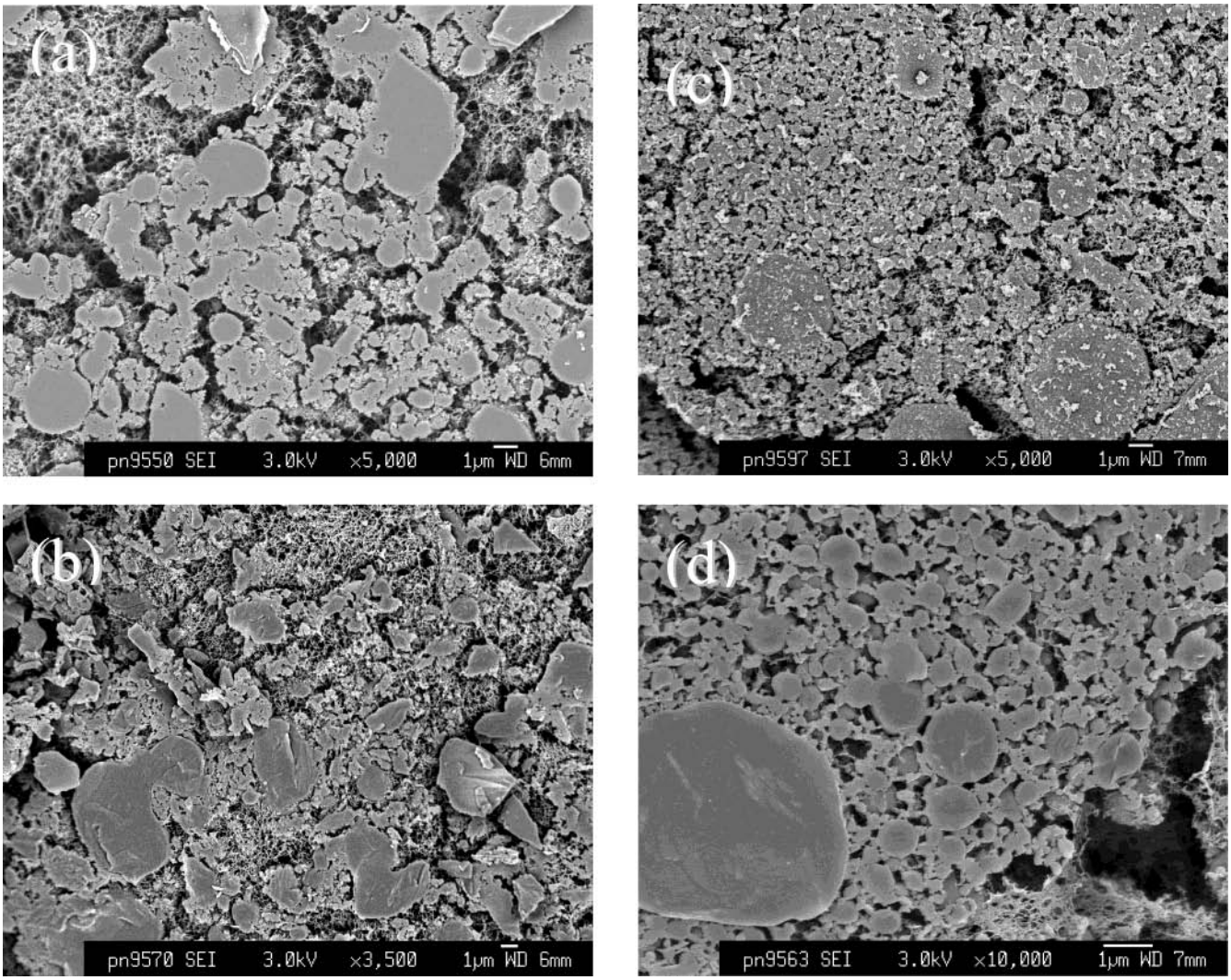

Figure 5. Scanning electron micrographs of emulsions (**a**) preheated acidified before temperature cycling, (**b**) preheated acidified after temperature cycling, (**c**) postheated acidified before temperature cycling, and (**d**) postheated acidified after temperature cycling.

emulsion droplet structure seemed mostly lost (**Figure 5b**), indicating severe destabilization as a result of partial coalescence. This is in line with the pfg-NMR data, though the SEM images for these preheated emulsions imply that parameters obtained by pfg-NMR serve more as an indication of the characteristic dimensions of the dispersed fat phase than as a well-defined oil droplet size distribution because these destabilized emulsions likely do not follow the log-normal distribution anymore.

The situation appeared quite different for the postheated acidified emulsions. Here emulsions both before and after temperature cycling showed well-defined emulsion droplets (**Figure 5c,d**, respectively). The absence of considerable partial coalescence was in agreement with the pfg-NMR droplet size data as well.

More detailed investigations were made on the least stable emulsions, particularly the preheated acidified emulsions. It was already shown that homogenization pressure had a clear effect on droplet size, whereas the number of stages had no clear effect on droplet size. Because the data did show that the second homogenization stage affected firmness, possibly as a result of the structure of the fat droplet aggregates formed during homogenization, the cycling stability of the emulsions was investigated as well.

The results concerning droplet size are plotted in **Figure 6a**, which shows the droplet size for preheated acidified emulsions based on partly crystalline fat prepared at different homogenization pressures. For pressures in the range 0−300 bar, both one-stage and two-stage homogenization was applied. The plot confirms the earlier observation that homogenization pressure was the most important factor determining droplet size, the differences between one-stage and two-stage homogenization being on the order of the scatter in the data. It can be seen that droplet size increased with each temperature cycle, except for the sunflower-oil-based sample. The absolute increase in droplet size was similar for all samples, and therefore, the relative increase was the largest for the emulsions based on the smallest droplets (i.e., high homogenization pressures).

The firmness was monitored for the same samples during cycling. Again, the clear effect of homogenization pressure on firmness is confirmed in **Figure 6b**, especially for one-stage-homogenized emulsions with small droplets. Relative temperature cycling effects seemed more pronounced for two-stage- than for one-stage-homogenized samples.

The relative changes are plotted once more in **Figure 7**, as a function of the initial droplet size $d_{3,2}$. It can be seen that the relative changes in droplet size were largest for the smallest droplet sizes, whereas the effects for firmness were largest for the largest droplet size. One-stage or two-stage homogenization hardly made a difference for the relative change in droplet size, but made a clear difference for the relative changes in firmness. For two-stage homogenization, the relative effects of cycling

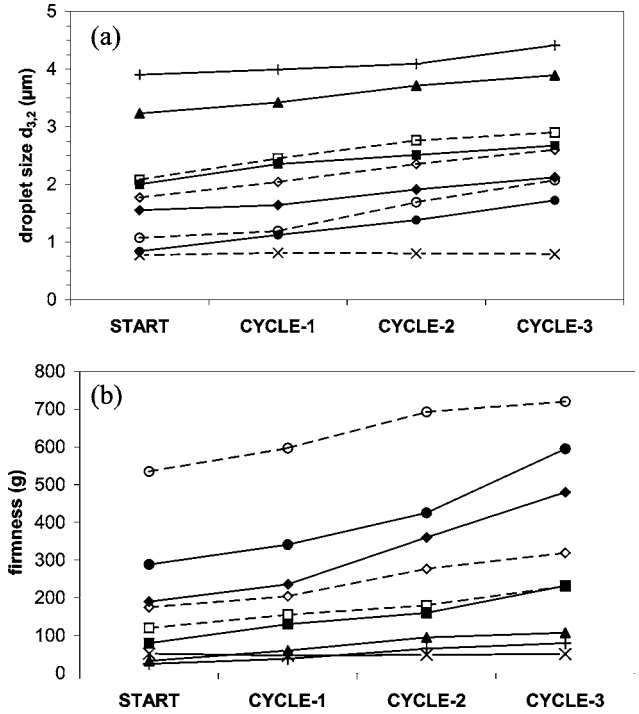

Figure 6. Effect of the number of temperature cycles between 5 and 25 °C on (**a**) droplet size $d_{3,2}$ and (**b**) firmness, for preheated acidified emulsions based on partly crystalline fat prepared with either one-stage (dashed lines) or two-stage homogenization (solid lines): (+) 0 bar/two stages; (▲) 20 bar/two stages; (□) 40 bar/one stage; (■) 40 bar/two stages; (◇) 80 bar/one stage; (◆) 80 bar/two stages; (○) 300 bar/one stage; (●) 300 bar/two stages; (×) one preheated acidified 300 bar/one-stage-homogenized sunflower-oil-based emulsion as a reference.

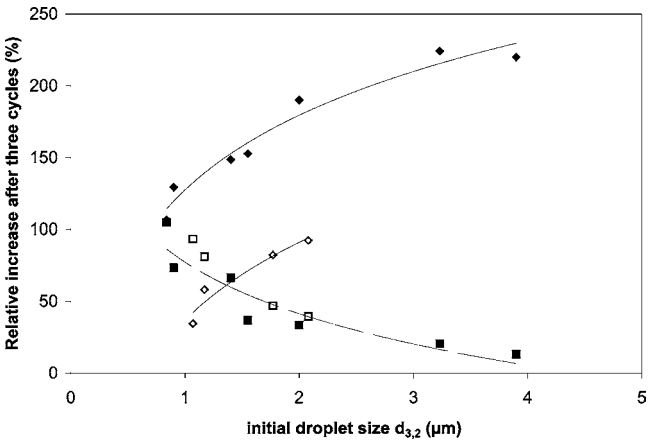

Figure 7. Relative change [(final value − initial value)/(initial value), expressed in percent] of firmness and droplet size $d_{3,2}$ as a function of initial droplet size (varied by homogenization, either one- or two-stage) after three temperature cycles between 5 and 25 °C for preheated acidified emulsions prepared with partly crystalline fat. Firmness: (◇) one stage; (◆) two stages. Droplet size: (□) one stage; (■) two stages.

were much more severe (although the absolute value of the firmness for a two-stage-homogenized sample remains less than for one-stage-homogenized samples).

In agreement with what was previously mentioned, firmness generally decreased with increasing droplet size at each measuring point, as can be seen from the relation between both parameters at the start of cycling in **Figure 8**. **Figure 8**, however, clearly shows a combined increase of droplet size and

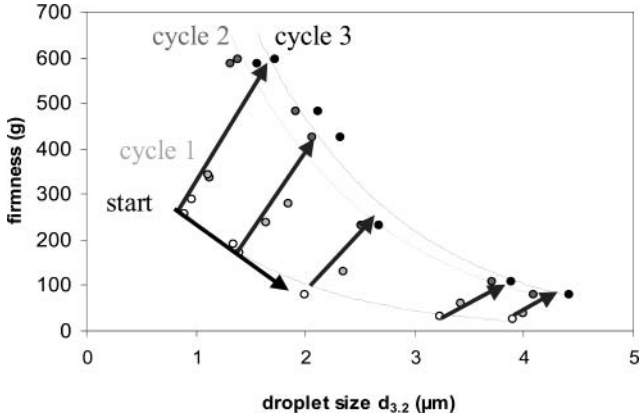

Figure 8. Relation between firmness and droplet size $d_{3,2}$ and its change during three subsequent temperature cycles between 5 and 25 °C, for preheated acidified emulsions prepared with partly crystalline fat and homogenized (two stages). Different initial droplet sizes were obtained by means of homogenization pressure variations in the range between 0 and 300 bar. The arrow pointing down symbolizes the effect of droplet size without cycling, and the arrows pointing upward indicate the effect of repeated temperature cycling.

firmness as a result of temperature cycling. This confirms that firmness during temperature cycling increased due to partial coalescence, in line with the SEM images in **Figure 5**.

The fact that partial coalescence *can* occur in these cream cheese model systems is quite surprising. Beforehand, one would expect the emulsion gels to be rather stable because they contain far more protein than needed to achieve a stable emulsion for neutral emulsions based on native proteins (*3*). The fact that the phenomenon occurs in these preheated acid (low-molecular-weight emulsifier-free) emulsions suggests that partial coalescence in such emulsion gels deserves much closer attention. The pfg-NMR technique applied in this paper, which allows noninvasive droplet size measurements in concentrated emulsions (in contrast with static light scattering, which requires extensive sample preparation (*16*)), will prove to be a very useful tool in such studies.

The present data, however, already identify a number of factors that are important in predicting which emulsion preparations seem more susceptible to partial coalescence than others.

First and foremost, the presence of partly crystalline fat in the emulsions is obviously required (though not sufficient) to have partial coalescence in the current emulsions.

Second, the proximity of the droplets as a result of flocculation is an important factor too.

Third, the presence of sufficient amounts of native whey protein at the moment of homogenization seems to be an overriding factor determining the cycling stability of the emulsion. This explains the stability of the native neutral emulsions. Denaturation of whey protein results in the formation of small aggregates, a process often used in the so-called cold gelation of whey proteins at lower pH (*29*, *30*). These small aggregates are apparently less efficient in stabilizing the emulsion against temperature cycling. Incidentally, it should be noticed that apparently the inhomogeneity of the interfacial layer is crucial, since there is no relation between the amount of protein associated with the fat phase and the stability of the emulsions (see **Figure 1**). Somewhat surprisingly though, the state of the protein does not affect the initial droplet size.

Fourth, the coherence of the interfacial layer will be important as well if the emulsions are kept under conditions under which the droplets will mutually attract (e.g., at low pH) and end up in close proximity. From a comparison between the native

acidified and the postheated acidified emulsions, it can be concluded that a heat treatment after homogenization will improve emulsion stability considerably, most likely because the whey protein has first formed an interfacial layer before it starts to aggregate. Further studies on the mechanical properties of the interface could put this statement on a more quantitative footing.

It is found that droplet size has a clear effect on the degree to which these emulsions suffer from coalescence. In general, smaller droplets lead to bigger absolute changes, though this is not the case if one considers relative changes.

In conclusion, it was demonstrated that partial coalescence may occur during temperature cycling under quiescent conditions in relatively-protein-rich emulsions, which are obviously quite different from the ones containing low-molecular-weight emulsifiers that are traditionally studied in this context. In particular, it was shown that protein-stabilized emulsions in which the protein functionality has been impaired through heat treatment or acidification are sensitive to this type of partial coalescence, even if the total amount of protein in the emulsion is high enough to expect a stable emulsion. However, the sensitivity of the emulsion to partial coalescence is greatly reduced if a stable emulsion was prepared before any heat treatment or acidification.

ABBREVIATIONS USED

NMR, nuclear magnetic resonance; pfg, pulsed field gradient; SEM, scanning electron microscopy; WPC, whey protein concentrate.

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