

Influence of Fat Crystallization on the Stability of Flocculated Emulsions

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Various degrees of flocculation were induced in a 20 wt % *n*-hexadecane and confectionery-coating fat emulsion by adding xanthan gum (0–0.3 wt %). The emulsions were temperature cycled (40 to –10 to 40 to –10 °C) in a differential scanning calorimeter. The emulsified and de-emulsified fat crystallized at different temperatures, and the ratio of the two enthalpies was used to calculate the proportion of de-emulsified fat and hence the extent of breakdown of the emulsion. The *n*-hexadecane droplets were stable to temperature cycling, whereas the confectionery-coating fat destabilized to a greater or lesser extent. The maximum destabilization of the confectionery-coating fat occurred at those concentrations of xanthan required to induce creaming.

KEYWORDS: Emulsion; crystallization; flocculation; coalescence; partial coalescence

INTRODUCTION

Many foods are oil-in-water emulsions (e.g., milk, cream, ice cream mix, mayonnaise). The dispersed phase may be partially or wholly crystalline which can influence the colloidal stability of the emulsion and hence bulk properties such as texture, stability, and rheology (1–3). Importantly, semicrystalline droplets can destabilize through a mechanism known as partial coalescence. Partial coalescence occurs by penetration of fat crystals from a partially crystalline droplet into another similar droplet. The liquid portions of the two droplets then flow together, but the solid fraction acts as a skeleton to support the distinct shape of the doublet against the Laplace pressure (4). The tendency to partially coalesce depends on the lipid composition, the nature of the surfactant, the solid fat content, and applied shear forces (1, 4–6). As the temperature of food storage corresponds to the melting range of many food fats, it is clear that when temperature fluctuates, the stability of the emulsions to partial coalescence will depend on the phase behavior of the fat.

Crystallization in a finely divided liquid such as emulsion droplets is distinct from crystallization in the bulk (7–9). In a given volume of liquid oil there will be a finite number of potential nucleation sites. Upon freezing a bulk liquid, the crystals will initially form at these impurities (heterogeneous nucleation) then spread throughout the volume. When the liquid is emulsified, each potential nucleus is contained within a very small volume (a single droplet) so that when the emulsion is cooled, heterogeneous nucleation only occurs in those droplets containing an impurity and cannot spread to other droplets. As the original liquid may have been divided into many millions of droplets, the proportion of the total that crystallizes by this

mechanism is insignificant and effectively all of the emulsified liquid crystallizes at a lower temperature through homogeneous nucleation.

We have previously used the difference in freezing point of emulsified and bulk oils to identify some of the factors influencing partial coalescence in emulsions (10). A fixed sample of emulsion was temperature cycled, and the proportion of the fat crystallizing at high temperatures (i.e., nonemulsified fat) increased from zero with the number of cycles and was taken as a measure of the degree of destabilization that had occurred. The speed of the temperature fluctuation and the volume fraction and type of lipid used were shown to affect the degree of destabilization. In the present work we use this method to investigate how flocculation can affect the freeze–thaw stability of similar emulsions.

In a flocculated emulsion the droplets come together for an extended period yet do not merge. Several authors have shown that flocculation can be induced in emulsions by depletion interactions caused by adding a nonadsorbing polymer (11–14). As a result of the finite size of the polymer, it is excluded from the region close to the droplet surfaces. Droplets consequently experience an osmotically based attraction force, which, above a critical value, can lead to flocculation and is frequently associated with creaming. For example, a critical concentration of xanthan gum (0.05 wt %) has been shown to induce creaming, whereas less hydrocolloid created too small an attractive force to induce flocculation and more was too viscous for visible creaming (15).

When semicrystalline droplets flocculate, the interparticle distance is reduced and the duration of encounter is increased, both of which could increase the probability of penetration of fat crystals, and may therefore favor partial coalescence. In the

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present study, this hypothesis was tested by inducing flocculation and subsequently subjecting the emulsions to temperature cycling.

MATERIALS AND METHODS

Materials. A sample of confectionery-coating fat was used in the crystallization studies (Van den Bergh Food Ingredients, CLSP870 cocoa butter substitute "high trans", Joliet, IL). Confectionery-coating fat (CCF) has physical and thermal properties similar to those of cocoa butter, but CCF only has a single stable polymorphic form. Preliminary gas chromatography analysis showed that the fatty acids present in CCF include oleic (48%), palmitic (38%), and stearic (12%). *n*-Hexadecane and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from the Sigma Chemical Company (St. Louis, MO). Xanthan gum was obtained from Kelco (Rahway, NJ).

Sample Preparation. Tween 20 solutions (4 wt %) were prepared in distilled water. Surfactant solution and oil (*n*-hexadecane or CCF) were coarsely mixed in a Polytron blender (Brinkmann Instruments Inc., Westbury, NY) for 30 s so that the final oil content was 40 wt % and the final overall surfactant concentration was 2.4%. The mixture was then recirculated through a twin-stage valve homogenizer (Panda, Niro Saovi, Italy) for 4 min. The homogenization pressures were 6 and 9 MPa for *n*-hexadecane and CCF emulsions, respectively. Throughout the homogenization procedure the oils and emulsions were maintained above their melting points to ensure complete homogenization. The emulsions were characterized using a laser diffraction method (Malvern Mastersizer X, Malvern, Wprcestershire, UK). The internal software of the instrument calculated the particle size distribution of a dilution ($\phi < 0.001$) of the emulsion from the scattering pattern using a presentation code of 0405 (i.e., a relative refractive index of 1.08 and an absorptive index of 0.01). The initial particle size of the emulsions was $d_{32} = 0.67 \pm 0.01 \mu\text{m}$ and there was no discernible change in the droplet size distribution of CCF emulsions over several days if the temperature was high enough to prevent crystallization.

Xanthan gum solutions were prepared in distilled water by stirring them for 24 h to ensure complete solubilization. Equal amounts of emulsion and xanthan gum solutions were mixed on a weight basis to produce samples with a final oil concentration of 19 wt % and a range of hydrocolloid concentrations (0–0.3 wt %). Samples were stored at 40 °C until required for further analysis (<1 day).

Creaming Studies. Portions (~10 g) of the emulsion–Xanthan mixtures were poured into glass tubes, covered, and allowed to stand for 30 h at 40 °C. The total height of the sample (H_s) and the thickness of any cream layer (H_c) were measured and used to calculate a creaming index ($= H_c/H_s \times 100\%$).

Thermal Analysis. Aliquots (~15 mg) of the emulsion–Xanthan mixtures were sealed into differential scanning calorimetry (DSC) pans and temperature cycled from 40 °C to –10 °C to 40 °C and back to –10 °C at 1.5 °C min⁻¹ in a Perkin-Elmer DSC 7 (Norwalk, CT). The pans were gently inverted several times prior to analysis to ensure good mixing of their contents. All samples were run against an empty reference pan and heat flux was recorded as a function of temperature. The DSC was calibrated with an indium standard prior to each experiment. Each experiment was conducted in triplicate.

Previous studies in this laboratory have shown that although these emulsions partially destabilize at slow cooling rates (1.5 °C min⁻¹) they do not when cooled faster (5 °C min⁻¹) (10). Consequently, to study the effect of flocculation on this transition a slow cooling rate was used in the present work.

RESULTS

Assignment of DSC Peaks. Typical cooling–heating thermograms of *n*-hexadecane and CCF emulsions are shown in **Figure 1**. Despite the slow cooling rates the thermograms contained relatively little noise, and peak identification by baseline extrapolation was relatively straightforward. Hexadecane emulsions show a single freezing (exothermic) peak at approximately –1 °C and a single melting (endothermic) peak at 17 °C. These results are typical of those reported in the literature (7). CCF emulsions have a single freezing peak at

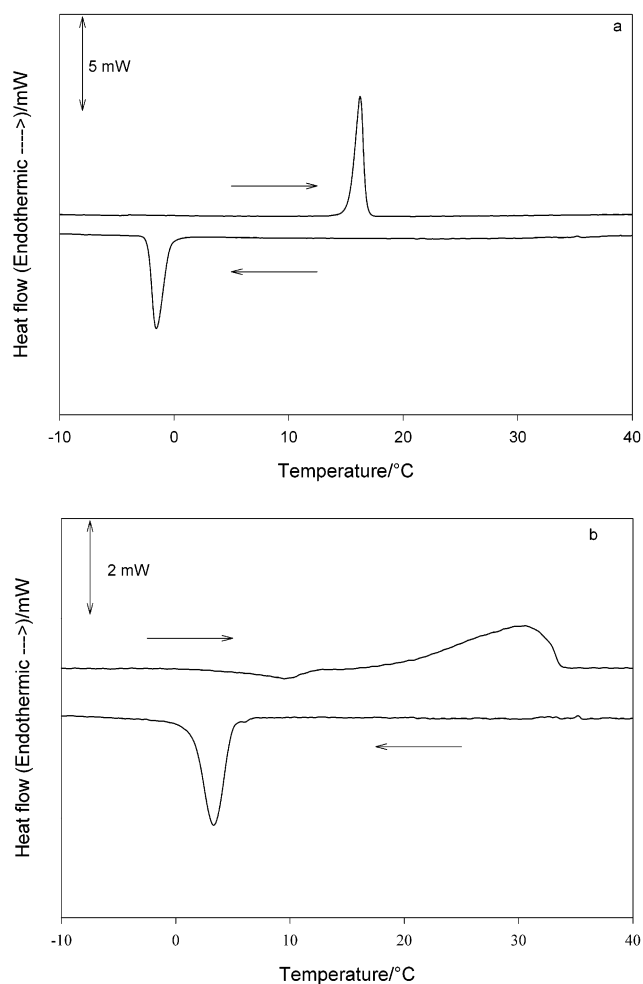


Figure 1. Typical thermograms of (a) emulsified *n*-hexadecane, and (b) emulsified CCF. Samples were cooled from 40 °C to –10 °C then reheated to 40 °C at 1.5 °C min⁻¹.

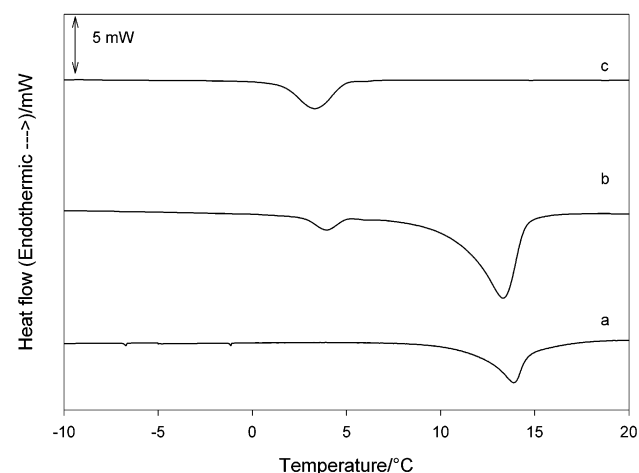


Figure 2. Typical thermograms of (a) bulk CCF, (b) a mixture of emulsified and bulk CCF, and (c) emulsified CCF. Samples were cooled from 40 °C to –10 °C then reheated to 40 °C at 1.5 °C min⁻¹.

~4 °C and a broad melting transition beginning at 10 °C. A sample of bulk CCF shows a similar cooling–heating thermogram (**Figure 2a**) but the freezing peak occurs at 14 °C. When mixtures of emulsified and bulk CCF were contained in the same pan (**Figure 2b**) there was again only one melting peak, but distinct emulsified and nonemulsified freezing peaks were observed. Similar results were established with bulk, emulsified,

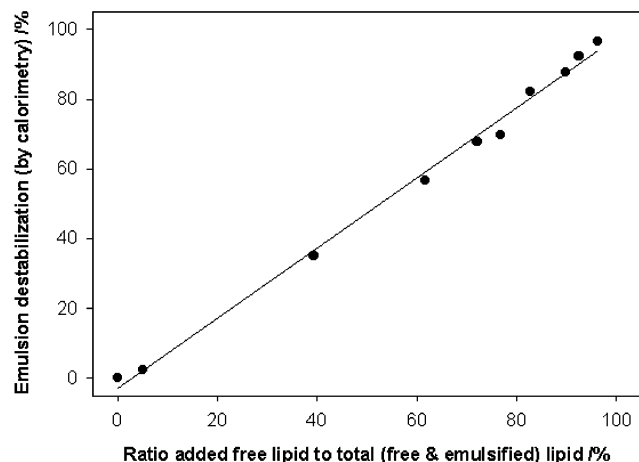


Figure 3. Ratio of the crystallization enthalpy of the high freezing point (~ 14 °C) transition to the total crystallization enthalpy of the fat present as a function of composition for mixtures of emulsified and free CCF.

and a mixture of bulk and emulsified *n*-hexadecane (data not shown). Note that the thermograms do not show a melting or freezing peak due to the water present despite the large degree of undercooling. (In similar experiments we have typically seen the water freeze in the DSC pan at ~ -25 °C.) The heating thermograms (melting profiles) of all subsequent measurements on *n*-hexadecane and CCF emulsions were similar whatever the degree of flocculation/coalescence seen and have been omitted from other figures for clarity.

The higher onset of crystallization in bulk compared to that of emulsified fat (**Figure 2c**) is because the few impurities contained in the pan (dust specks, etc.) are capable of inducing heterogeneous nucleation in bulk fat. The considerable degree of supercooling between the melting peak and the observed freezing peak in emulsions is because the vast majority of the droplets are isolated and impurity-free and so crystal nucleation must proceed via homogeneous nucleation (7, 15). Melting does not require a nucleation event so it occurs close to the thermodynamic melting point.

Verification of Calorimetric Determination of Emulsion Destabilization. The clear distinction of the crystallization peaks of bulk and emulsified oils (**Figure 2**) provides a basis for quantifying the degree of de-emulsification of a system undergoing partial coalescence. A series of DSC pans were prepared, with each containing a different ratio of bulk and emulsified CCF. The pans were cooled as described above, and thermograms similar to those in **Figure 2** were seen in each case. As the proportion of CCF in the emulsified state increased, the magnitude of the peak at 4 °C increased at the expense of the peak at 14 °C. The ratio of nonemulsified to total fat in the pan was proportional to the ratio of the areas of the peaks of nonemulsified (i.e., crystallization enthalpy of the 14 °C peak) to total fat (i.e., sum of the crystallization enthalpies of the 14 and 4 °C peaks) (**Figure 3**). The positions of the peaks did not change as the composition of the system was altered. Therefore, for an unknown system containing a mixture of bulk and emulsified oil, the ratio of enthalpies described above can be used to calculate the extent of emulsion destabilization.

The distinction between bulk and emulsified oil in this work depends on the different crystallization mechanisms acting. Changes in the thermograms on temperature cycling a given sample of emulsion can give a measure of the changing proportions of emulsified and destabilized fat present in the pan as the emulsion breaks down. In preliminary work with these samples we have seen that the transition from largely homo-

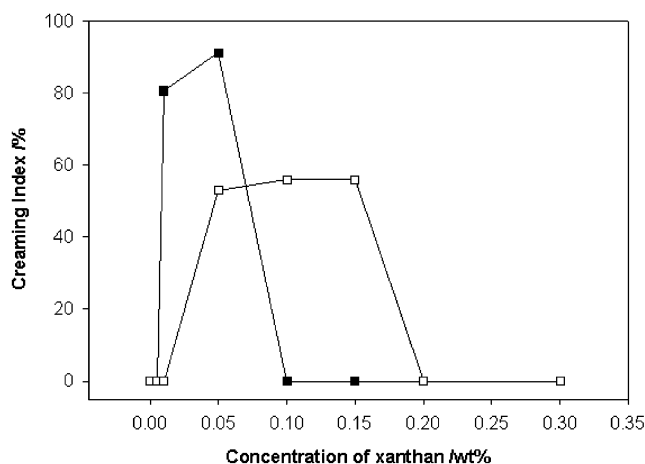


Figure 4. Creaming index as a function of xanthan gum concentration for (□) *n*-hexadecane and (■) CCF emulsions.

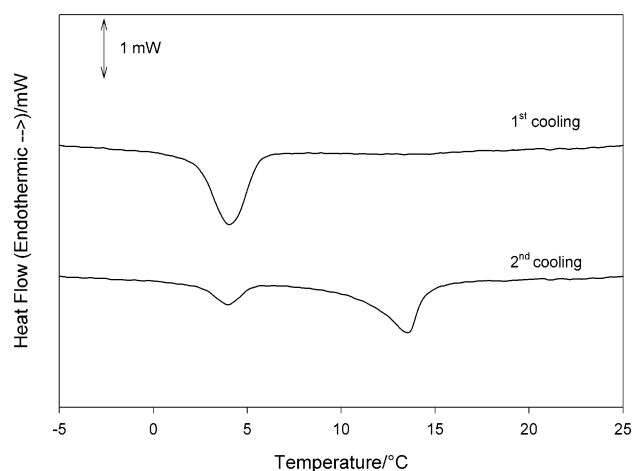


Figure 5. Typical thermogram of a CCF emulsion–xanthan mixture (0.05 wt % xanthan gum). The sample was cooled from 40 °C to -10 °C, reheated to 40 °C, then cooled for a second time to -10 °C at 1.5 °C min^{-1} .

geneous to largely heterogeneous nucleation occurs when the mean particle size was larger than approximately 5 μm . Therefore, when we describe a proportion of the emulsion being destabilized this can be taken to mean that a proportion of the lipid is now present in fat-continuous structures greater than that size.

Creaming Studies. The creaming index for the *n*-hexadecane and CCF emulsions is shown as a function of xanthan concentration in **Figure 4**. *n*-Hexadecane emulsions showed extensive creaming between 0.05 and 0.15 wt % xanthan gum, whereas CCF emulsions creamed between 0.01 and 0.05 wt %. The serum phase of the creamed *n*-hexadecane emulsions was clear, whereas that from the CCF emulsions was slightly turbid indicating some residual fat content. The difference in the creaming behavior of the two types of emulsions may be due to differences in dispersed phase density, floc radius, or floc density.

Temperature Cycling. A typical cooling–heating–cooling thermogram for a CCF emulsion containing xanthan is shown in **Figure 5**. The CCF–xanthan emulsion mixture showed a major peak at 4 °C on first cooling corresponding to the crystallization of the emulsified fat, and on occasion a much smaller peak at 14 °C corresponding to free fat. This suggests that at the start of the experimental run all the fat was in emulsified form. However, on second cooling of the same

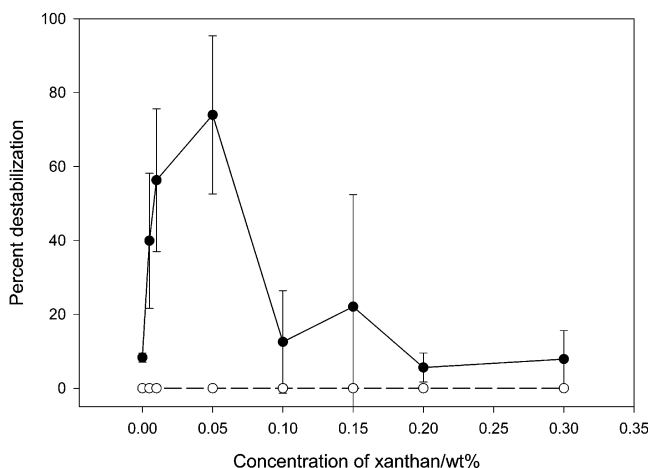


Figure 6. Percentage of fat destabilized after a freeze–thaw cycle (second cooling) as a function of xanthan gum concentration (○) *n*-hexadecane and (●) CCF emulsions.

sample, the peak due to bulk fat was much larger and the peak due to emulsified fat was correspondingly smaller. On the basis of arguments introduced above, this suggests that a portion of the CCF droplets is destabilized by the freeze–thaw cycle, and on second cooling crystallize as bulk oil. In contrast to the CCF samples, *n*-hexadecane–xanthan emulsion mixtures were very stable and did not show any significant destabilization either during first or second cooling (data not shown).

The average destabilization of the CCF emulsions during first cooling was 4% (maximum 9%, minimum <1%) and there was no dependence on xanthan concentration. At least some of the error may be associated with attempting to measure small enthalpies in DSC analysis, but a level of irreproducibility seems to be a feature of this system. The amount of CCF emulsion destabilization on second cooling is plotted against xanthan gum concentration in **Figure 6**. The extent of destabilization approximately followed the tendency to cream, rising to a maximum at intermediate xanthan concentrations (e.g., 75% at

0.05% xanthan) and lower at higher and lower concentrations. The amount of destabilization at xanthan levels 0.005, 0.01, and 0.05 wt % is statistically different ($p < 0.05$) from that at the other levels. The variability in the destabilization data in **Figure 6** is high and may be partly due to the small but variable degree of destabilization on first cooling (~4%). The effects of even a small portion of large droplets (which act as nonemulsified fat) present at the start of the experiment would lead to more rapid rate of subsequent destabilization as the area of contact (between interacting droplets) increases with an increase in droplet size.

Optical Microscopy. In an attempt to understand the mechanism of instability, samples of emulsion were cooled and heated on a thermostated microscope stage (CTS-4ICA with a TS-4 controller, Physitemp Instruments Inc., Clifton, NJ). The droplets were observed under polarized light at 50 \times magnification (Olympus BX40). Images were captured at intervals using a digital camera (Sony 3CCD, PXC-970MD) and imaging software (Pax-It!, Franklin Park, IL). The droplets used in the DSC and light-scattering studies were too small to be observed directly by this method so the coarse emulsion premix from the Polytron blender was used instead (mean droplet diameter $\sim 1 \mu\text{m}$ with many larger droplets). A small amount of xanthan solution was added to this coarse emulsion to induce flocculation. The change in droplet size and the fact we could no longer precisely control heating or cooling rate makes direct quantitative comparison of the micrographs with other results impossible, however some qualitative insight can be gained by considering which emulsion droplets coalesced under which circumstances. Because there was no destabilization observed with DSC in flocculated *n*-hexadecane emulsions, microscopy studies on these emulsions were not performed.

Representative micrographs for CCF droplets are shown in **Figure 7**. This figure shows a single field of view at intervals over a cooling–heating cycle. **Figure 7A** shows the CCF emulsion–xanthan mixture prior to cooling at 40 °C. The field of view shows flocs as well as isolated droplets in the liquid state. The picture shows a static image, but in reality the system

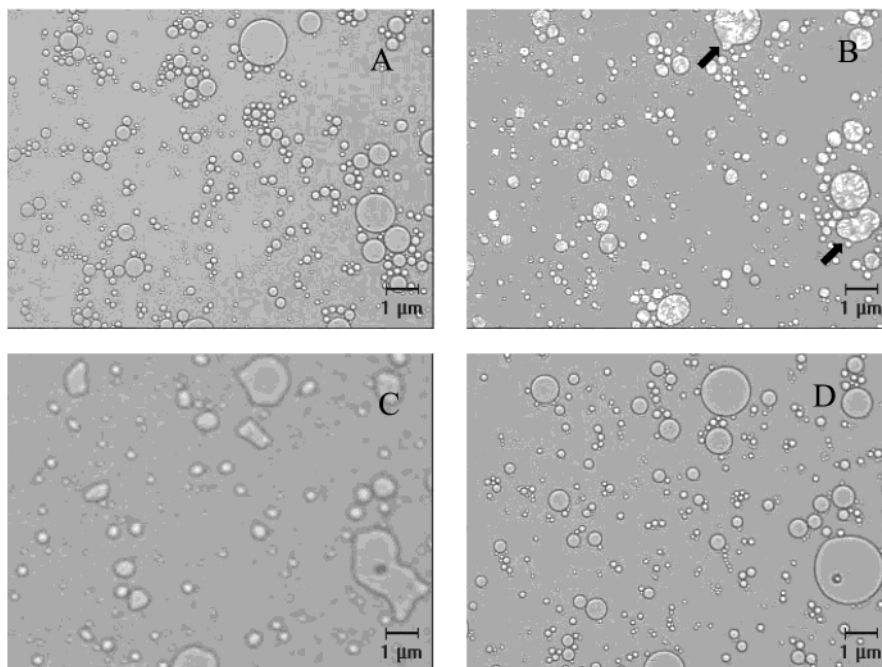


Figure 7. Micrographs of a single field of view of flocculated CCF emulsions during a freeze–thaw cycle: (A) droplets prior to cooling, (B) crystalline droplets and flocs, the arrow shows an aggregate, (C) liquid contents of the droplets in the floc flowing into each other, (D) fully melted droplets attain spherical shape and new flocs are formed. Scale bar is 1 μm .

was very dynamic with the flocs and the individual droplets in constant motion. Upon cooling, the flocs and isolated droplets crystallize as shown in **Figure 7B**. The boundaries of the droplets were no longer spherical and in some cases the flocs formed clumps (arrows in **Figure 7B**) while the isolated droplets remain intact. During the heating cycle, these crystalline particles started to melt, and those droplets that were in close contact with each other either in a floc or otherwise began to coalesce (**Figure 7C**). Interestingly, some, but not all, of the flocculated droplets merge on melting. It can be seen in **Figure 7D** that the merged droplets are larger and new flocs have formed. The results from optical microscopy support the hypothesis that the main mechanism responsible for emulsion destabilization is partial coalescence and that droplets in flocs are more vulnerable to partial coalescence than isolated droplets.

DISCUSSION

The *n*-hexadecane emulsions containing xanthan (at all concentrations) were very stable to the temperature cycling, whereas the CCF emulsion–xanthan mixtures were not. These differences could be attributed to the different crystallization properties of the two emulsions. A pure compound (*n*-hexadecane) cannot exist for a significant amount of time (or temperature range) in a partially crystalline state because its freezing/melting transitions are so sharp that the semisolid droplets required for partial coalescence do not exist. A mixture of compounds that are significantly miscible (such as the triacyl glycerol molecules in CCF) can have an extended freezing and melting temperature range rather than a clear melting point, so there will be more time for semisolid droplets to interact and for partial coalescence to occur. The presence of partially crystalline droplets is a necessary criterion for partial coalescence to occur, and flocculation can only enhance the rate. Alternatively the crystal morphologies of solid CCF and hexadecane in emulsions may be very different and thus have different capacities to promote partial coalescence (4).

The instability of CCF emulsions increases when the droplets are flocculated but decreases when the amount of xanthan increases to the point to limit creaming (**Figures 4 and 6**). The arguments proposed by Dickinson et al. (15) to explain the effect of flocculation on interdroplet nucleation provide some insight into the observed phenomenon. Interdroplet nucleation occurs when one solid droplet initiates crystallization in a second supercooled liquid droplet – presumably caused by a transient collision. Dickinson et al. (15) found that the rate of interdroplet nucleation increased slightly at those levels of xanthan that enhanced creaming and decreased slightly at higher levels. They suggested that flocculation increased the rate of interdroplet nucleation by increasing the duration of collisions, whereas high viscosity (due to higher levels of xanthan) would immobilize the droplets and subsequently retard the collision rate. Since droplet-collision mediated nucleation is related to partial coalescence, it is reasonable to hypothesize that flocculation would have a similar effect here.

At low concentrations of xanthan (<0.01 wt %), there is less flocculation, and so less close droplet interaction necessary for freeze–thaw destabilization. At high concentrations of xanthan (>0.1 wt %), the droplets are flocculated, but the continuous phase viscosity is high and therefore the flocs are immobilized in a gel-network and cannot interact with other semisolid flocs. At intermediate xanthan concentrations (0.01–0.05 wt %) the emulsion is flocculated yet sufficiently mobile for further interactions between flocs leading to fat destabilization.

Finally, emulsion instability is typically expressed in terms of several distinct mechanisms (e.g., flocculation, creaming, and

coalescence). This work provides a striking example of the interdependence of these processes. The added xanthan favors flocculation, which in turn favors creaming and also partial coalescence. To properly understand the shelf life of emulsions it is essential to consider the interactions between destabilization mechanisms.

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