Dispersed Phase Destabilization in Table Spreads

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ABSTRACT: Commercially available butter, regular-fat margarine, and a fat-reduced margarine (38% fat w/w) were stored between 10 and 35°C for up to 4 d to elaborate on the relationship between droplet size and solid fat content (SFC) that exists in these spreads. At 10°C, the mean volume-weighted droplet size for butter was 4.22 ± 0.40 µm followed by margarine (6.22 ± 0.10 µm) and fat-reduced margarine (12.62 ± 0.28 µm). At higher temperatures, as a result of decreasing SFC, the mean droplet size increased as did the droplet size distribution, leading to eventual coalescence and destabilization in all spreads. In butter, the critical SFC was ~9%, whereas in margarine notable coalescence occurred at ~5% SFC. The fat-reduced margarine destabilized at lower temperatures than the other spreads (~20°C vs. ~30°C), at an SFC of ~6.5%. In these spreads, two different mechanisms influenced dispersed phase stability: (i) steric stabilization against coalescence *via* fat crystals located at the droplet interface, known as Pickering stabilization, and (ii) stabilization against droplet sedimentation (and droplet encounters) due to the presence of the fat crystal network.

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KEY WORDS: Dispersed phase, fat crystal network, interface, Pickering stabilization, table spreads.

Table spreads are multiphase colloidal systems consisting of an aqueous phase dispersed as fine droplets (typically 1–20 µm) within a continuous oil phase and fat crystal network. In butter and margarine, the aqueous phase (16% w/w) usually consists of water and salt, and in the case of margarine, milk protein (usually buttermilk or whey) and preservatives are dispersed in an oil and fat network (80% w/w total). The stability of table spreads may depend on two mechanisms: (i) Pickering stabilization, whereby interfacially absorbed colloidal particles sterically stabilize dispersed droplets (1), and (ii) the presence of a fat crystal network that physically "locks" the water droplets in place within the spread matrix, thereby preventing droplets from migrating, flocculating, coalescing, and eventually creaming (2). This is in contrast to fat-reduced spreads (≤40% w/w fat), where the kinetic stability of the dispersed phase, although still dependent on the presence of a fat crystal network, also relies on the composition of the aqueous phase. Thickeners such as gelatin or polysaccharide gums are often added to fat-reduced spreads to hinder droplet–droplet coalescence by increasing the viscosity of the dispersed phase. Furthermore, surfactants such as MAG are added to such systems to increase stability.

Few studies on the differing mechanisms involved in table spread stability have been reported in the literature. Borwankar *et al.* (3) and Borwankar and Buliga (4) examined the emulsion properties of low-fat spreads and margarines and the relationship between rheology and meltability. Heertje (5) investigated the relationship between structure and functionality in numerous spreads, commenting on the differences in network structures between spreads of differing compositions and processing conditions. Conspicuously lacking, however, is a thorough examination of the destabilization mechanisms of the dispersed phase in edible spreads. The purpose of this study was to investigate the role that solid fat content (SFC) plays in the stability of butter, margarine, and a fat-reduced margarine (FRM) and to provide insight into the mechanism(s) involved in the destabilization of the dispersed phase in these spreads.

EXPERIMENTAL PROCEDURES

Materials. Butter and margarine (both 80% fat w/w) as well as an FRM (38% fat w/w) were purchased from a local grocery store and stored in a refrigerator at 5°C until required. SFC of the spreads was evaluated without first removing the water phase. Reported SFC are relative to the proportion of fat in the spread. SFC and dispersed-phase droplet size distributions in the spreads were determined using a Bruker Minispec Mq pulsed nuclear magnetic resonance (pNMR) unit (Bruker Canada, Milton, ON, Canada) equipped with a pulsed gradient field (PGF) unit allowing unimodal characterization of emulsion droplet size distribution in these products. The field gradient strength was initially calibrated with $CuSO_4$-doped water ($D = 1.31 \times 10^{-9}$ m^2/s at 5°C). Diffusion (D) of the water molecules is usually taken as being equal to the diffusion coefficient of the neat liquid (pure water for water-in-oil emulsions). However, the interactions of water molecules with multiple chemical species (e.g., surfactants, salts, protein, thickeners) can strongly affect diffusional properties (6,7). In the presence of solutes, the true self-diffusion coefficient will be lower than in the neat liquid. Therefore, in this study, D was taken as the bulk molecular diffusion of the aqueous phase of the individual spreads separated *via* centrifugation. SFC and droplet size measurements were taken at 1, 2, 4, 6, 12, 24, 48, and 96 h at each storage temperature, and at other times within this range, specific to each spread.

The principle of droplet size measurements using pNMR is based on the restricted diffusion of water molecules in

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emulsified systems and is thoroughly described elsewhere (8–11). In the present study, the volume-weighted mean droplet diameter (d_{33}) was determined for samples tempered at 10–35°C in 5–10°C increments for up to 4 d. Where necessary, experiments were also conducted at specific temperatures (e.g., 32°C for butter) to pinpoint key temperatures in emulsion destabilization. The temperature of the NMR sample chamber was maintained by a refrigerated water bath.

Microscopy. Differential interference contrast (DIC) microscopy and polarized light microscopy (PLM) were used to examine the dispersed phase and crystal morphology of the spreads. Samples were placed on viewing slides (Fisher, St. Louis, MO), upon which a cover slip (Fisher) was gently placed. A 63× water-immersion objective was used for visual examination using an inverted Zeiss Axioplan 2 microscope (Zeiss, Toronto, Canada). Images were captured with a Q-Imaging CCD camera and analyzed using Northern Eclipse software (version 6.0; Empix Imaging, Burlington, ON, Canada). Samples were temperature-controlled using a cold stage (TS60 stage with STC200 controller; Instec, Boulder, CO). Numerous images were acquired, and each image represented a typical field.

Statistical analyses. Triplicate analyses were performed on all SFC and droplet size measurements. ANOVA and *t*-tests were performed, and statistical differences were considered significant at $P = 0.05$. Errors bars represent the SD.

RESULTS AND DISCUSSION

Figure 1 represents the relative contribution to the dispersed-phase volume by the aqueous droplets, based on their diameters. Integration of this frequency distribution leads to the sum distribution. The d_{33} is the volume-weighted average droplet diameter whereby the two halves of the dispersed-phase volume are contained in droplets above and below this diameter. The three spreads consisted of divergent droplet size distributions at refrigerator temperature (10°C) ($P < 0.05$). Butter had the lowest d_{33} (4.22 ± 0.40 μm), followed by margarine (6.22 ± 0.10 μm) and FRM (12.62 ± 0.28 μm). Both the butter and margarine consisted of narrow droplet size distributions, whereas that of the FRM was much wider. Figure 2 shows the evolution in d_{33} in the spreads as a function of temperature. Values shown follow 24 h of storage at each temperature. Between 10 and 25°C (the temperature range where these spreads are most often consumed), there was little evolution in the mean d_{33} in margarine and FRM ($P > 0.05$) and a significant increase in the mean d_{33} in the butter ($P < 0.05$). Above 25°C, the mean d_{33} increased gradually in butter and sharply in margarine. Butter was fully destabilized at 35°C, whereas margarine was not. Each spread underwent a different SFC vs. temperature evolution (Fig. 3). Between refrigerator (10°C) and room temperature (25°C), the SFC in butter decreased from 46 to 13%. This drop is usually attributed to the melting of the low-melting and medium-melting fractions

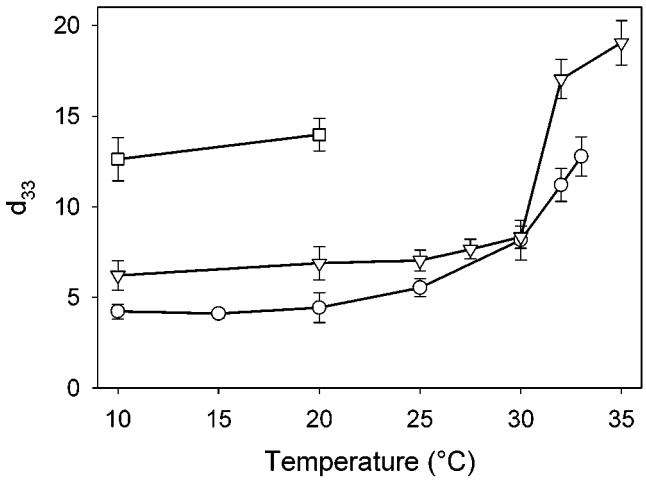

FIG. 2. Volume-weighted mean droplet size (d_{33}) in butter (○), margarine (▽), and a fat-reduced spread (□) following 24 h of storage at 10–35°C. Error bars represent SD.

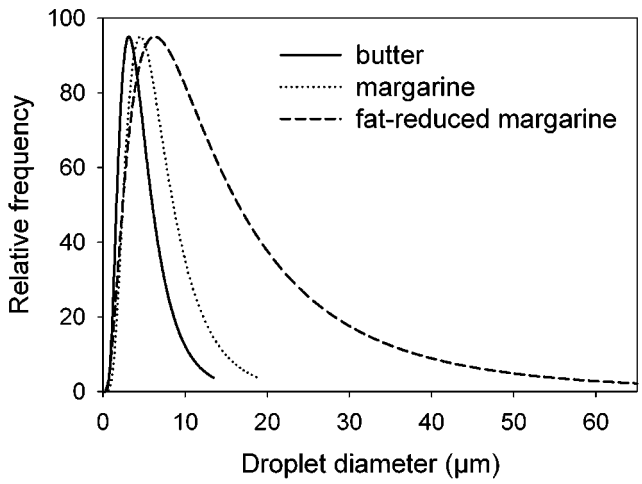

FIG. 1. Droplet size distributions in butter, margarine, and a fat-reduced margarine at 10°C.

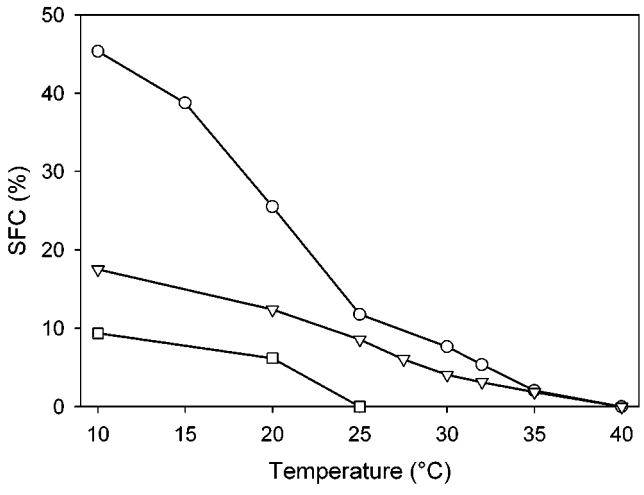

FIG. 3. Solid fat content (SFC) in butter (○), margarine (▽), and a fat-reduced spread (□) following 24 h of storage at 1–35°C.

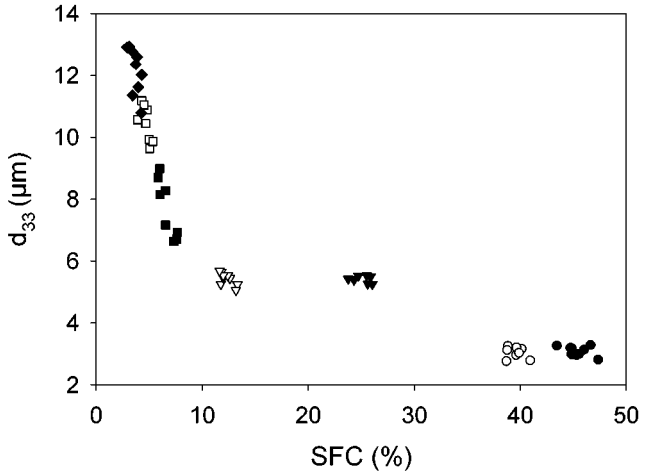

FIG. 4. d_{33} of the dispersed phase in butter as a function of SFC following storage at 10–33°C for up to 4 d. 10°C (●); 15°C (○); 20°C (▼); 25°C (▽); 30°C (■); 32°C (□); 33°C (◆). For abbreviations see Figures 2 and 3.

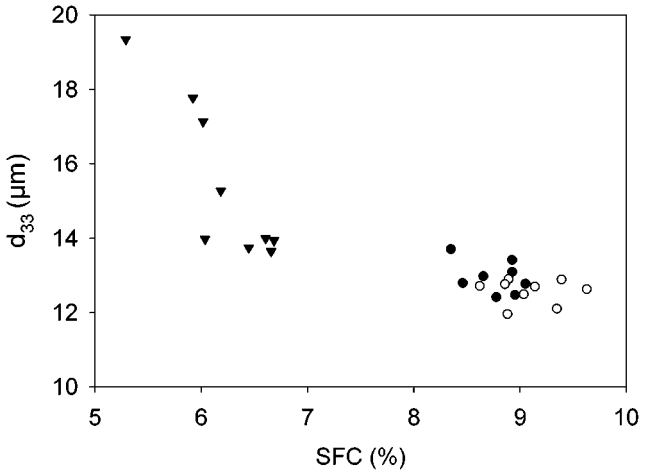

FIG. 6. d_{33} of the dispersed phase in the fat-reduced margarine as a function of SFC following storage at 5–20°C for up to 4 d. 5°C (○); 10°C (●); 20°C (▼). For abbreviations see Figures 2 and 3.

in milkfat. The SFC of the margarine and FRM, by contrast, dropped by ~8%, with the latter being fully melted at 25°C.

Germane to elucidating the mechanism(s) involved in the destabilization of the dispersed phase in the spreads is the relationship between SFC and d_{33} (Figs. 4–6). Complete destabilization was defined as the increase in d_{33}, leading to the inability to detect individual droplets *via* PGF-pNMR. This resulted from pronounced coalescence and/or sedimentation of the dispersed aqueous phase at longer storage times and higher temperatures. In this respect, each spread behaved differently. In the case of butter (Fig. 4), there were three distinct regions in the SFC vs. d_{33} relationship. At SFC above ~35%, the mean droplet size in butter was unaffected by any change in SFC ($P > 0.05$). This corresponded to samples stored at 10 and 15°C. SFC at these two temperatures were significantly different ($P < 0.05$). Another plateau was evident for SFC between ~10 and 35% (which corresponded to butter stored at 20–25°C), where d_{33} values were higher than at 10 or 15°C. SFC were significantly different ($P < 0.05$), whereas d_{33} values were not ($P > 0.05$). At SFC of <9%, droplet sizes continued to increase as a function of storage time and temperature. This was defined as the critical SFC. By contrast, the critical SFC for margarine was ~5% (Fig. 5). The d_{33} values for samples stored at 10–25°C were unaffected by the changes in SFC ($P > 0.05$). When stored at temperatures resulting in SFC below this critical value, d_{33} values increased upon storage and complete emulsion destabilization was gradually reached. For example, samples stored at 35°C underwent extensive coalescence, d_{33} values increasing from ~9 to ~19 μm within a 96-h period (corresponding SFC ~2%). The critical SFC for the FRM was ~6.5% (Fig. 6), an SFC situated between those of the critical butter and critical margarine values. FRM samples stored at 20°C or above eventually underwent complete phase separation.

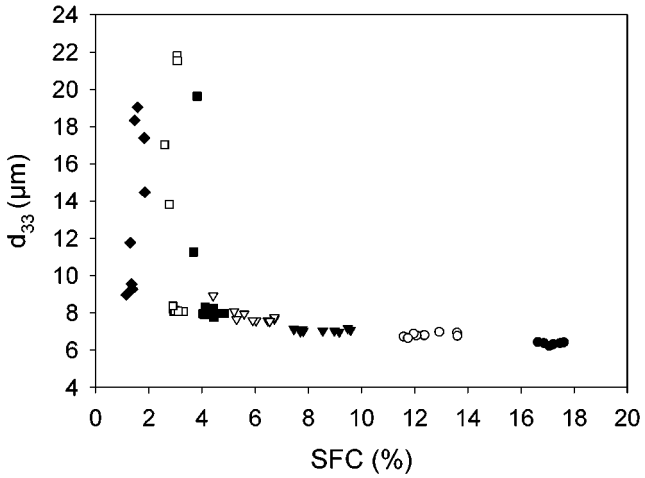

FIG. 5. d_{33} of the dispersed phase in margarine as a function of SFC following storage at 10–35°C for up to 4 d. 10°C (●); 20°C (○); 25°C (▼); 27.5°C (▽); 30°C (■); 32°C (□); 35°C (◆). For abbreviations see Figures 2 and 3.

Factors controlling coalescence in spreads. Dispersed-phase stability arising from the presence of fat crystals in these spreads was two-pronged and depended on the presence of (i) a fat crystal network and (ii) surface-active crystals at the interface. Within fat crystal networks in spreads, flocculated fat crystals enmesh droplets. Seminal work by Lucassen-Reynders (12) demonstrated that the energy content of the bonds in a fat crystal network could be deemed an energy barrier against the free diffusion of crystals away from the network and toward an interface. Once formed, droplets were kept separate from one another due to the presence of networked interstitial crystals between droplets. Second, in all three spreads, there was the possibility that droplet-stabilizing surface-active crystals existed, particularly at higher temperatures. These would result from (i) crystallization of TAG or MAG, (ii) incorporation of polar/charged species within fat crystal lattices, or (iii) adsorption of noncrystallizing species to crystal surfaces (e.g., lecithin, milk protein) rendering them polar. Besides the potential surface activity of crystals in the spreads, an effective protective layer would

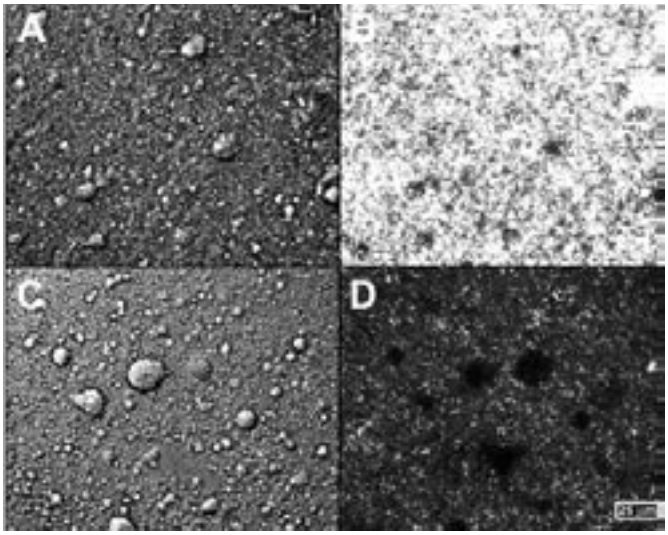

FIG. 7. Differential interference contrast (DIC) and polarized light microscopy (PLM) photomicrographs of butter. (A) DIC at 10°C; (B) PLM at 10°C; (C) DIC at 30°C; (D) PLM at 30°C. The bar represents 25 µm.

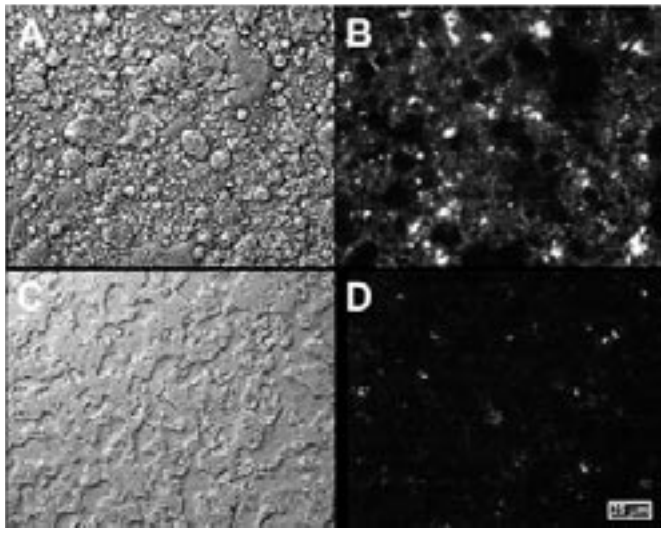

FIG. 9. DIC and PLM photomicrographs of fat-reduced margarine. (A) DIC at 10°C; (B) PLM at 10°C; (C) DIC at 30°C; (D) PLM at 30°C. The bar represents 25 µm. For abbreviations see Figure 7.

necessitate that crystals be much smaller than the droplet. Larger crystals (e.g., similar in size to the dispersed droplets) would not successfully stabilize droplets. Based on Figures 7–9, excessive crystal size was not a concern, except perhaps in the FRM. Furthermore, surface-active crystals should be optimally wetted, ideally with contact angles between the crystal, oil, and aqueous phases close to 90°C (13). Wetting behavior is highly dependent on crystal composition and the presence of added surfactants or proteins. The last key ingredient is a sufficient crystal concentration at the interface. In the presence of few crystals, as would be encountered at higher temperatures, the ability of the emulsion droplets in these spreads to resist coalescence *via* Pickering stabilization would largely depend on the properties of the interface and how the immediate vicinity of the interface is populated. A highly viscous and rigid interfacial film resulting from a substantial number of crystals (at least sufficient for monolayer coverage) at the interface would retard coalescence by slowing the rate of film drainage and eventual rupture of the droplets, thereby promoting the kinetic stability of the system (14). Interfacial rheology would also affect the displacement energy of crystals away from the interface during droplet coalescence, particularly as droplets approach one another (15).

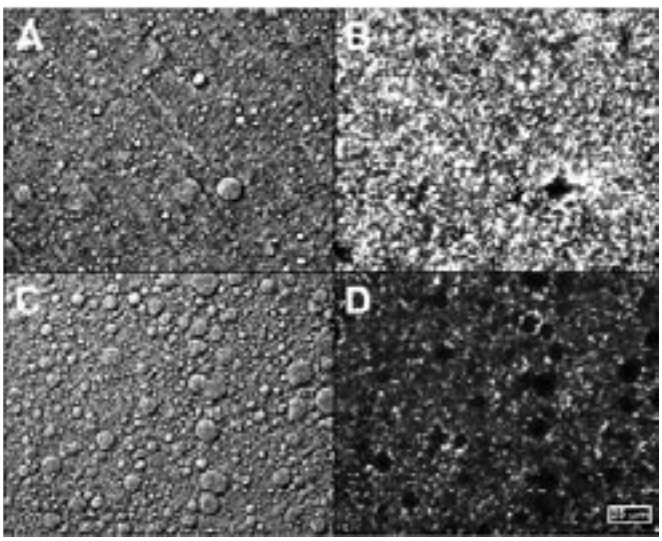

FIG. 8. DIC and PLM photomicrographs of margarine. (A) DIC at 10°C; (B) PLM at 10°C; (C) DIC at 30°C; (D) PLM at 30°C. The bar represents 25 µm. For abbreviations see Figure 7.

To clarify the mechanism(s) of spread stabilization, DIC and PLM images were captured at 10 and 30°C. At 10°C, butter consisted of small, well-dispersed droplets (Fig. 7A). The polarized light image of the same field demonstrated a densely packed fat crystal mass (corresponding to an SFC of ~46%). The corresponding PLM image shows dark regions where the aqueous droplets and/or liquid fat are located (Fig. 7B). Most crystals appeared to be platelets measuring 1–3 µm in length. In certain instances, droplets were either fully covered by fat crystals or were fat globules that had not coalesced and phase-inverted during butter production. Using electron microscopy, Heertje (5) determined that this phenomenon was not unusual in butter-making. Increasing the storage temperature to 30°C for 1 h helped to determine how fat crystals stabilized the dispersed phase and whether fat crystals were surface-active. At 30°C, droplets were larger than at 10°C and more polydisperse (Fig. 7C). The presence of fat crystals was not visibly higher at the periphery of the droplets compared with the continuous phase, although images did reveal some crystals associated with droplet interfaces (Fig. 7D). DIC images of margarine at 10°C showed a dispersion similar to that found in butter (Fig. 8A). Given its lower SFC (~18%), however, the fat crystal network structure in margarine was less dense than in butter, although fat crystal size and morphology

were similar (Fig. 8B). Increasing the storage temperature to 30°C led to gradual destabilization of the margarine emulsion with the mean droplet size substantially higher, although droplets were not as polydisperse as in butter (Fig. 8C). The estimated SFC for this system at 30°C was 4%, yielding a much sparser fat crystal network than at 10°C. Furthermore, contrary to butter, a greater polarized light intensity was visible around the droplets (Fig. 8D) compared with the bulk, indicative of surface-active and partially wetted crystals. Heertje (5) noted similar findings on the microstructure of butter and margarine. In margarine, the microstructure consisted of crystalline shells surrounding water droplets as well as a continuous fat crystal network. Conversely, in butter, there were few crystalline shells around water droplets. Rather, its microstructure consisted of a mottled crystal network incorporating interglobular crystals and fat globules that had survived the churning process. Finally, in the FRM, a polydisperse droplet size distribution was visible at 10°C (Fig. 9A). The accompanying PLM image (Fig. 9B) revealed a heterogeneous crystal mass (the SFC of this systems was ~9%) consisting of birefringent layers around many of the droplets accompanied by single crystals and spherulitic crystal agglomerates, measuring up to 15 μm in size. This was in contrast to butter and margarine, where agglomerates were not prevalent. Given their presence at the periphery of the droplets, these agglomerates appeared responsible for the stability of the FRM. Images at 30°C indicated that the system was fully destabilized, containing no droplets and little, if any, solid fat (Figs. 9C and 9D). Once the single crystals and spherulites surrounding the dispersed droplets melted, any remaining stabilizing effect would have resulted from the presence of surface-active species and/or aqueous phase thickeners, although this was not examined in great detail.

In conclusion, dispersed phase stability in these spreads relies on the presence of a fat crystal network as well as surface-active crystals. In butter, the fat crystal network stabilizes the spread with few interfacial crystals playing a role. In margarine and FRM, while the fat crystal network is still important, interfacial crystals are present and assist in stabilization of the dispersed phase, especially at higher temperature.

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