Effect of high-pressure-jet processing on the physiochemical properties of low-fat ice cream mix

Grace L. Voronin,1 Robert Roberts,1 Tara L. Felix,2 John N. Coupland,1 and Federico M. Harte1*

1Department of Food Science, The Pennsylvania State University, University Park 16802
2Department of Animal Science, The Pennsylvania State University, University Park 16802

ABSTRACT

The objective of this study was to use high-pressure-jet (HPJ) processing to produce functional properties in a low-fat (4.5% fat) ice cream mix similar to those seen when emulsifiers are used. Ice cream mix or serum (nonfat portion of the ice cream mix) were subjected to 200 or 400 MPa HPJ processing and compared with a non-HPJ-treated control. A similar non-HPJ-treated formulation but containing polysorbate 80 (0.075% wt/wt) was also used as a control. The mix samples were characterized in terms of their particle size, density, flow properties, stability, crystallization kinetics, and fat–protein interactions. The sample from the mix subjected to 400 MPa HPJ processing (HPJ-M-400) had increased consistency coefficient (5°C; 228 ± 102.7 mPa·s) and particle size (D[4,3]; 16.0 ± 2.5 μm) compared with the non-HPJ-treated control sample, with viscosity and particle size (volume-moment mean diameter, D[4,3]) values of 7.5 ± 0.4 mPa·s and 0.50 ± 0.1 μm, respectively. These differences were attributed to an increase in casein–fat interactions and casein–casein interactions caused by the 400 MPa HPJ treatment, which were observed using confocal scanning laser microscopy and inferred from an increase in protein and fat concentrations in the sediment after ultracentrifugation. Interestingly, the density of HPJ-M-400 was also lower (0.79 ± 0.17 g/mL) than that of the control (1.04 ± 0.00 g/mL) because bubbles were trapped within these complexes. The large casein–fat complexes formed in the HPJ-M-400 sample also appeared to act as steric barriers that slowed ice crystal growth during quiescent freezing. The alterations in physicochemical properties and apparent ice crystal growth induced by the 400 MPa treatment of low-fat ice cream mix have many potential applications, including clean-label confections. Key words: high-pressure jet, ice cream, emulsifier, clean label

INTRODUCTION

High-pressure technology has been used in a variety of dairy systems to achieve microbial and enzymatic destruction (Mussa and Ramaswamy, 1997; Hayes et al., 2005; Yang et al., 2012) without heat-induced quality loss (Pereda et al., 2007; Huang et al., 2014) or to encourage changes in physicochemical properties. High hydrostatic pressure (HHHP) is a batch pressurization process with unique effects on both casein micelles and whey proteins. In brief, whey proteins (β-LG and α-LA) vary in their sensitivity to pressure-induced denaturation, with β-LG being more sensitive (20.2% native β-LG vs. ~100% native α-LA remaining after a 400 MPa treatment for 10 min at 20°C; Huppertz et al., 2004). Casein micelles reportedly disintegrate at pressures between 250 and 310 MPa (25°C, in seconds), decreasing in size and altering the native micellar structure (Harte et al., 2007).

High-pressure homogenization (HPH) uses a continuous system that is similar to a traditional 2-stage homogenizer with a ball-seat or needle-seat valve but operating at higher pressures (up to 400 MPa). These operating conditions induce strong shear and cavitation, which causes collision, temperature, and turbulence-dependent alterations in milk components. As seen in HHHP, β-LG is still highly sensitive to pressure-induced denaturation during HPH (>150 MPa at 45°C for 20 s; Hayes et al., 2005). Roach and Harte (2008) reported an increase in micelle size with HPH treatment of >250 MPa and argued that this was due to a disruption and subsequent reaggregation of casein micelles at pressure release. Hayes et al. (2005) demonstrated decreased average fat globule size with HPH treatment (100–200 MPa), which allowed for an increase in cream stability over conventional commercial homogenization.

High-pressure-jet (HPJ) technology, a more recent advancement in high-pressure processing, uses a positive-displacement, high-pressure pump coupled with a diamond, sapphire, or ruby nozzle. This continuous system allows for pressures up to 600 MPa, which exit the flow-restrictive nozzle at extreme speeds (~Mach 4.
or 1,372 m/s at 600 MPa), creating an aerosol (Mohan et al., 2016). This aerosol is immediately sprayed into a countercurrent heat exchanger, which collects and cools the fluid back into a liquid stream. Although the flow profile at the HPJ restriction nozzle has not been thoroughly investigated, this restriction likely causes laminar elongational flow similar to jet dispersers and orifice valves in which fluid is stretched by shear stress (McClements, 2015). One disadvantage of current HPJ is the relatively low flow rate (<25 L/min) of water-jets, which is not compatible with the large-scale needs in the dairy industry. This limitation will be lifted if HPJ demonstrate unique advantages for the processing of liquid foods, as previously occurred with the now-mature HHP.

After HPJ processing at ≥300 MPa, skim and whole milks showed enhanced foaming ability as well as increased particle size and apparent viscosity (Mohan et al., 2016; Hettiarachchi et al., 2018; Tran et al., 2018). For skim milk, these alterations were attributed to a disruption in casein micellar structure followed by a subsequent reassociation that resulted in larger, more disperse protein aggregates and greater availability of surface-active casein monomers. In addition, the formation of casein–fat complexes was observed in whole milk (Tran et al., 2018).

Previous studies involving HPJ technology have demonstrated novel industrial applications, but more complex and concentrated dairy systems including ice cream mix have yet to be investigated. The most basic ice cream mix consists of a matrix of fat, milk SNF (lactose, casein and whey proteins, and minerals), sweetener, and water (Goff and Hartel, 2013b). Typically, emulsifiers (e.g., mono- and diglycerides, polysorbate 80) are added to induce the formation of a destabilized fat matrix, which contributes to ice cream stability and mouthfeel (Amador et al., 2017). Hydrocolloids are often included to contribute to a viscous barrier to bind water and slow the development of iciness (Flores and Goff, 1999). However, with a current trend toward cleaner and simpler ingredient labels, the use of emulsifier and hydrocolloid ingredients becomes a concern for both the industry and the consumer. The use of high pressure in ice cream processing has shown potential for replacing nondairy emulsifiers and hydrocolloids. High hydrostatic pressure was shown to increase the apparent viscosity and shear thinning behavior of ice cream mixes with increasing pressure, and no change in fat globule size was seen (Huppertz et al., 2011). However, the frozen ice cream made from the HHP-treated mixes (400 MPa, 5 min, 20°C) melted more slowly.

The objectives of this study were to characterize the effects of HPJ processing on the microstructure and functional properties of low-fat ice cream mix. By applying HPJ treatment (≥300 MPa; i.e., the pressure above which Hettiarachchi et al. (2018) saw changes in casein structure in skim milk) to ice cream mix, we hypothesize that changes in micellar structure and casein–fat interactions will lead to increased particle size and apparent viscosity in ice cream mix. We expect that these changes in physical properties will also function to slow ice crystal growth and ice cream mix separation, similar to the functions performed by commercial nondairy emulsifiers and hydrocolloids. The ultimate goal is to functionally replace nondairy emulsifiers and hydrocolloids in ice cream mix by using novel processing interventions, including HPJ.

MATERIALS AND METHODS

Ice Cream Mix Processing

Pasteurized skim milk, skim milk powder, sugar, and unsalted butter were purchased from the Penn State Berkey Creamery (University Park, PA). Low-fat ice cream mixes were formulated based on a standard formulation of 4.5% fat, 11.5% milk SNF, and 13% sugar either with 0.075% polysorbate 80 (C-P80) or without emulsifier (C-0). To prepare the ice cream serum, skim milk was heated to 43°C before skim milk powder was added. Either before or after HPJ processing (see below), sugar and melted butter were added to the serum to achieve the final formulation. Batches of samples were heated to 71°C for 30 min (i.e., low temperature, long time pasteurization) under continuous stirring at a moderate speed, being careful not to incorporate air. The pasteurized samples were then homogenized (Gaulin, Lake Mills, WI: 13.8 MPa in the first stage, 3.4 MPa in the second stage) and immediately cooled to <4°C and stored (<3 d) until HPJ treatment (see below).

HPJ Processing

Either just the serum or the entire formulation were HPJ processed at 200 or 400 MPa, leading to 4 treatments (where S indicates serum, M indicates mix, and 200 and 400 indicate level of pressure): HPJ-S-200, HPJ-S-400, HPJ-M-200, and HPJ-M-400, and 2 non-HPJ-treated controls (C-0 and C-P80). Samples were kept at approximately 4°C for 24 h before being HPJ processed using a Hyperjet 94i-S pump system (Flow Internationals Corp., Kent, WA). The sample pressures (200 or 400 MPa) were maintained by a hydraulic accumulator before exiting through a 10-μm diamond nozzle. At 200 and 400 MPa, the flow rate of
the samples through the HPJ was 1.1 and 1.6 L/min, respectively. Importantly for this study, at the top of the heat exchanger is a venting tube that opens to the surrounding air, which creates a venturi-like effect and allows air incorporation into the product at higher flow rates and pressures. Immediately after exiting the system, a tube-in-tube heat exchanger cooled the sample with concurrent water at approximately 2°C to achieve outlet sample temperatures <30°C. The mixes were then allowed to age for at least 24 h before analysis. Sodium azide (0.04%) was added to the mixes to prevent microbial growth, and samples were either stored at 4°C until required for most analyses or frozen until required for fat and protein analysis.

Ice Cream Mix Physical Characterization

Mix Composition. Total solids and fat contents of ice cream mixes were determined with a calibrated semiautomated moisture and fat analyzer (Smart Trac-II, CEM Corp., Matthews, NC).

Density. Density was determined by accurately weighing aliquots (5 mL) of mix. Each sample was gently inverted 3 times before extracting a sample for analysis to ensure the volume used for measurement was representative of the entire mix. The analysis was conducted in triplicate.

Particle Size. Particle size was measured by static light scattering using a method modified from Warren and Hartel (2018). Samples were diluted in water before scattering methods using the obscuration values provided by the instrument (Mastersizer 3000, Malvern Instruments Ltd., Malvern, UK). The particle refractive index and absorbance were set at 1.47 (milk fat) and 0.01, respectively, and the continuous phase was assumed to have the same optical properties as water.

Rheology. Rheological properties were determined by developing a flow curve using a Discovery HR-3 rheometer (TA Instruments, New Castle, DE) operating at a shear rate range of 1 to 100 s⁻¹ at 5°C with a double-wall concentric cylinder geometry (inside cup diameter = 40.02 mm, outside cup diameter = 44.82 mm, inside bob diameter = 40.77 mm, outside bob diameter = 43.88 mm, inner cylinder height = 60.02 mm). Flow curves were modeled using a Power law model:

\[ \sigma = K(\dot{\gamma})^n \]  

where \( \sigma \) is the shear stress, \( K \) is the consistency coefficient, \( \dot{\gamma} \) is the shear rate, and \( n \) is the flow behavior index (Steffe, 1996).

Ice Cream Mix Composition

Ultracentrifugation. Mix samples (20 mL) were centrifuged at 100,000 × g for 30 min at 4°C (Optima XPN-80 ultracentrifuge, Beckman Coulter, Indianapolis, IN; fitted with a 50.2Ti rotor). The centrifuged samples separated into 3 layers (top, middle, and bottom). The top and bottom layers were manually separated using a spatula and freeze-dried for subsequent fat and protein analysis. The middle section was not retained for analysis because it was difficult to cleanly separate from the other layers. This procedure was modified from Tran et al. (2018).

Protein Determination. Following ultracentrifugation and freeze drying, protein was determined as nitrogen using a combustion method (Chang and Zhang, 2017) following a procedure modified from Tran et al. (2018). Aliquots of dried sample (~0.10 g) were weighed, placed into a tin foil cap, and deposited into the instrument (Leco FP528, Leco Corp., St. Joseph, MI) for combustion. A nitrogen conversion factor of 6.38 was used to determine protein content from measured nitrogen.

Crude Fat Analysis. Following ultracentrifugation and freeze drying, samples (0.1–0.3 g) were weighed into previously weighed filter bags and heat-sealed. Fat was extracted with petroleum ether at 90°C for 60 min using an automated fat extractor (Method 2, Ankom XT15, Ankom Technology, Macedon, NY). Following extraction, samples were dried at 105°C for 30 min. After drying, when the samples reached room temperature, the samples were reweighed. Based on the weight following ether extraction, the crude fat percentage was calculated as follows:

\[ \text{crude fat}(\%) = \left[ \frac{\text{sample weight} - \text{weight after extraction}}{\text{sample weight}} \right] \times 100. \]  

This method was validated by Tran et al. (2018).

Total Fat Analysis. Total fat analysis, including a hydrolysis step, was conducted after preliminary studies found that fat extraction was insufficient in some samples when using the crude fat extraction method. Filter bags (XT4 bags, Ankom Technology) were weighed and labeled with a solvent-resistant marker. Diatomaceous earth (~0.6 g) was added to the filter bags followed by approximately 0.15 to 0.30 g of each sample. Additional diatomaceous earth (~0.6 g) was added to cover the sample. The filter bags were sealed and submerged in 3 M HCl, which was heated to and held at 90°C for 1 h. After hydrolysis, the bags were removed and washed...
with distilled water for 20 min. The samples were dried in an oven (105°C) for 3 h, cooled to room temperature (in a desiccant pouch), and reweighed. Two blanks (filled with diatomaceous earth, but no sample) underwent the same procedure and were used as a calibration mechanism. The sample bags were then subjected to the crude fat analysis procedure detailed above. Based on the weight following fat extraction, the total fat percentage was calculated as follows:

\[
\text{total fat } \% = \frac{\text{Sw after drying} - \left[ \text{Sw after extraction} + \left( B_i - B_f \right) \right]}{\text{original Sw}} \times 100, \tag{3}
\]

where \text{Sw} is the sample weight, \( B_i \) is the dry weight of the filter bag and diatomaceous earth, and \( B_f \) is the weight of the filter bag and diatomaceous earth after extraction.

Ice Cream Mix Functional Characterization

**Stability.** Phase separation was measured from optical backscattering measurements (\(\lambda = 880\) nm) as a function of height in a column of the sample (15 mL) at intervals over 24 h (Turbiscan Lab Expert, Toulouse, France). The software (TurbiSoft Lab, version 2.2) then calculated the Turbiscan stability index (TSI; i.e., the combined difference in backscattering intensity at all heights divided by the total sample height) as an overall stability measurement.

**Crystallization.** Sample freezing was measured by optical microscopy using a method modified from Regand and Goff (2003). A small volume (10 \(\mu\)L) of mix was placed on a glass slide and covered with a coverslip and cooled to \(-50^\circ\)C using a Linkam LTS 350 controlled-temperature stage (Linkam Scientific Instruments, Tadworth, UK), warmed to \(-6^\circ\)C and held for 10 min, warmed to \(-3.5^\circ\)C and held for 10 min, and finally cooled to \(-6^\circ\)C. All heating and cooling rates were \(5^\circ\)C/min. The temperature cycle (\(-6^\circ\)C to \(-3.5^\circ\)C with 10-min hold at each) were repeated 3 times. Images were taken immediately when the temperature reached \(-3.5^\circ\)C during each cycle using an Olympus BX41 light microscope (Olympus Corp., Center Valley, PA).

Images were analyzed using ImageJ software (Schneider et al., 2012). Briefly, images were converted to binary and inverted (inverting light and dark figures), a watershed was applied to segment the image into individual ice crystals, and to reduce noise only particles with areas >100 \(\mu\)m² and circularity >0.8 were measured. In this procedure, crystals that were merging were considered separate entities and measured as such.

Microstructure Characterization

**Confocal Scanning Laser Microscopy.** Each ice cream mix (2 mL) was dyed with 10 \(\mu\)L of fluorescein isothiocyanate (0.1% wt/vol in acetone) and Nile red (0.01% wt/vol in ethanol). The dyes were added to the mixes 24 h before observation under an Olympus Fluoview 1000 confocal microscope (Olympus Corp.) at excitation wavelengths of 488 and 633 nm for the blue and red fluorescent probes, respectively. The fluorescing images were overlaid, and the location of the fat and protein was determined. This method was modified from He et al. (2019).

**Cryogenic Electron Microscopy.** Non-HPJ-treated serum and 400 MPa-treated serum were observed under cryogenic electron microscopy following methods described by Hettiarachchi et al. (2018). Briefly, the samples were diluted 30-fold with protein-free serum. Then, 3 mL of each sample was placed onto a glow-discharged perforated carbon film supported on a 200-mesh copper grid (Ted Pella, Redding, CA). The grids were vitrified by submersion in liquid ethane and transferred into a single-tilt cryoholder under liquid nitrogen. The samples were then observed using an FEI Tecnai 12 BioTwin electron microscope (120 kV; FEI Company, Hillsboro, OR), and images were selected from approximately 20 images taken with a Gatan Orius SC 1000 CCD camera (Gatan Inc., Pleasanton, CA).

Statistical Analyses

Most samples (C-0, C-P80, HPJ-S-200, HPJ-S-400, HPJ-M-200, and HPJ-M-400) were produced in quadruplicate, but 1 replicate was rejected from C-P80, HPJ-S-200, and HPJ-M-400 due to accidental sample dilution. All procedures were completed in a randomized order. Minitab software (version 18.1; State College, PA) was used to conduct 1-way ANOVA with Tukey’s test applied for mean comparisons and significance of treatment designated at \(P < 0.05\).

RESULTS AND DISCUSSION

The characterization of the low-fat ice cream mix samples is reported in 3 categories: physical characterization, structural characterization, and functional characterization. In the first section, density, rheology, and particle size of the ice cream mixes are described and a physical model is introduced. In the next section, the microstructure of the samples is evaluated using...
an ultracentrifugation procedure and confocal scanning laser microscopy. Finally, in the last section, mix stability and ice crystal growth kinetics are described.

**Physical Characterization of Mixes**

The average fat and TS contents for all treatments were 4.53 ± 0.25% and 28.8 ± 1.2%, respectively. These values were not far from the targeted fat content (4.5%) and TS content (29%). Ice cream mix density is generally dependent only on mix composition (Goff and Hartel, 2013a), so it was expected that no differences would be seen here; indeed, most samples (C-0, C-P80, HPJ-S-200, HPJ-S-400, and HPJ-M-200) had similar densities (1.03 g/mL; Table 1). However, the HPJ-M-400 sample had a significantly \((P < 0.05)\) lower density (0.79 ± 0.17 g/mL). It was noted that the air vent immediately after the restriction nozzle of the HPJ enabled air to be sucked into the system. For the HPJ-M-400 sample, this air was incorporated with the sample and contributed to this decreased density. These measurements were made 96 h after HPJ treatment, so for this sample the foam formed was relatively stable; indeed, pulling vacuum over the sample failed to increase the measured density.

Flow behavior indices and consistency coefficients for all samples are presented in Figure 1. All samples were statistically similar with Newtonian-like flow behavior except for the HPJ-M-400 sample, which exhibited a significantly \((P < 0.05)\) decreased flow behavior index \((0.778 ± 0.034)\) at 5°C and a significantly \((P < 0.05)\) increased consistency coefficient \((228 ± 103\) mPa·s) at 5°C.

Apparent particle size in the mix samples was measured by static light scattering (Table 1). Most mixes (C-0, HPJ-S-200, HPJ-S-400, and HPJ-M-200) showed a monomodal distribution with volume-moment mean diameter \((D^{[4,3]}_3) < 0.50 \mu m\) (Table 1), presumably due to the homogenized lipid droplets. The mix containing emulsifier (C-P80) had a bimodal distribution and \(D^{[4,3]}_3\) of 0.814 ± 0.369 \(\mu m\), which may reflect enhanced partial coalescence in the lipid droplets (Warren and Hartel, 2018). However, the HPJ-M-400 sample was again unique, with a bimodal distribution containing very large particles (1 to >200 \(\mu m\)). A 400 MPa HPJ treatment of skim milk has been shown to result in a bimodal size distribution as the casein micelles are disrupted, dispersed, and reaggregated to form large particles (Hettiarachchi et al., 2018).

In density, rheology, and particle size measurements, the HPJ-M-400 sample emerged as an outlier. Based on previous literature regarding the high-pressure treatment of dairy systems (Hayes and Kelly, 2003; Harte et al., 2007; Roach and Harte, 2008; Hettiarachchi et al., 2018), the large particles developed in the HPJ-M-400 sample likely involved the disruption of milk fat globules and casein micelles at the HPJ restriction nozzle followed by subsequent reaggregation. We suggest that the reaggregation of casein and milk fat formed large fat–protein complexes, which trapped air. A model (Figure 2) detailing the proposed HPJ-induced effects on casein micelles and fat globules was constructed in an attempt to describe these results. Considering this model, the large alterations in the rheological properties of the HPJ-M-400 sample was likely due to the entrapment of air, the presence of large particles, or a combination of these 2 features. Large flocculated particles typically have shear thinning behavior as moderate shear causes the flocs to elongate and align with the shear field (McClements, 2015). As shear is further increased, the flocs can fracture, causing a reduction in apparent viscosity as their effective volume fraction is decreased, similar to what was seen in the shear thinning profile of the HPJ-M-400 sample.

It is interesting that the HPJ-M-400 sample had anomalous density, rheology, and particle size, whereas

---

### Table 1. Particle size and density (5°C) of low-fat ice cream mix (C-0), low-fat ice cream mix with polysorbate 80 (C-P80), and low-fat ice cream mix prepared in different combinations using a high-pressure jet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bimodal</th>
<th>Particles &gt;1 (\mu m) (%)</th>
<th>(D^{[50]}) ((\mu m))</th>
<th>(D^{[4,3]}) ((\mu m))</th>
<th>Span</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-0</td>
<td>No</td>
<td>4.44</td>
<td>0.441 ± 0.069(\text{b})</td>
<td>0.498 ± 0.089(\text{a})</td>
<td>1.287 ± 0.103(\text{a})</td>
<td>1.040 ± 0.003(\text{a})</td>
</tr>
<tr>
<td>C-P80</td>
<td>Yes</td>
<td>8.80</td>
<td>0.402 ± 0.022(\text{b})</td>
<td>0.814 ± 0.369(\text{a})</td>
<td>1.452 ± 0.193(\text{a})</td>
<td>1.037 ± 0.001(\text{a})</td>
</tr>
<tr>
<td>HPJ-S-200</td>
<td>No</td>
<td>3.48</td>
<td>0.439 ± 0.010(\text{b})</td>
<td>0.497 ± 0.023(\text{b})</td>
<td>1.280 ± 0.111(\text{a})</td>
<td>1.027 ± 0.004(\text{a})</td>
</tr>
<tr>
<td>HPJ-S-400</td>
<td>No</td>
<td>2.78</td>
<td>0.456 ± 0.010(\text{b})</td>
<td>0.498 ± 0.012(\text{b})</td>
<td>1.180 ± 0.052(\text{b})</td>
<td>1.031 ± 0.005(\text{b})</td>
</tr>
<tr>
<td>HPJ-M-200</td>
<td>No</td>
<td>3.96</td>
<td>0.421 ± 0.057(\text{b})</td>
<td>0.466 ± 0.074(\text{b})</td>
<td>1.288 ± 0.095(\text{b})</td>
<td>1.039 ± 0.002(\text{b})</td>
</tr>
<tr>
<td>HPJ-M-400</td>
<td>Yes</td>
<td>62.38</td>
<td>10.287 ± 2.190(\text{b})</td>
<td>16.000 ± 2.458(\text{b})</td>
<td>3.609 ± 1.370(\text{b})</td>
<td>0.794 ± 0.171(\text{b})</td>
</tr>
</tbody>
</table>

\(\text{a}\) Different superscripts within a column reflect significant differences at \(P < 0.05\) (Tukey’s test).

\(\text{b}\) Either just the serum or the entire formulation were high-pressure-jet (HPJ) processed at 200 or 400 MPa, leading to 4 treatments (where S indicates serum, M indicates mix, and 200 and 400 indicate level of pressure): HPJ-S-200, HPJ-S-400, HPJ-M-200, and HPJ-M-400.

\(\text{c}\) Values are presented as mean ± SD for triplicate (C-P80, HPJ-S-200, HPJ-M-400) or quadruplicate (C-0, HPJ-S-400, HPJ-M-200) samples. \(D^{[50]}\) = cumulative 50% point of diameter.

\(\text{d}\) \(D^{[4,3]}\) = volume-moment mean diameter.
the HPJ-S-400 sample did not. We suggest that the HPJ treatment of the serum may have created large particles capable of increasing the viscosity and trapping air bubbles, but they were broken down when passing through the conventional homogenizer as part of mix manufacture. Indeed, particle size analysis of the HPJ-S-400 sample (Supplemental Figure S1, https://doi.org/10.3168/jds.2019-17814) and cryogenic electron microscopy of the HPJ-S-400 sample (Supplemental Figure S2, https://doi.org/10.3168/jds.2019-17814) taken before homogenization showed the presence of large particles.

**Structural Characterization of Mixes**

**Ultracentrifugation.** Conventionally processed whole milk separates into 3 distinct layers during ultracentrifugation, with the supernatant and precipitate layers comprising predominantly fat and protein, respectively. However, Tran et al. (2018) showed that, with an increasing HPJ pressure, more of the fat co-sedimented with the protein at the bottom of the tube. This was attributed to the complexation of fat and protein during the HPJ treatment of whole milk (≥250 MPa) to produce complexes that sedimented during centrifugation.

Using the same ultracentrifugation procedure as Tran et al. (2018; 100,000 × g, 30 min, 4°C), the low-fat ice cream mix samples in this work also separated into 3 distinct layers: the top and bottom layers were both solid, and the center remained liquid. The top and bottom layers were extracted, freeze-dried, and weighed (Figure 3a, b). The fat and protein contents of the DM from the top and bottom layers are shown in Figure 3c, d and Figure 3e, f, respectively. Interestingly, the crude fat (Soxhlet-based) extraction used by Tran et al. (2018) was not enough to extract the fat from the HPJ-M-400 treatment and gave a much lower apparent fat content than that seen in the other samples (see explanation below). A limitation of the Soxhlet procedure is the inability to extract fat that is chemically bound to protein (Ellefson, 2017). For this reason, an additional hydrolysis step was used, and the crude and total (with hydrolysis) fat contents are compared in Figure 3c, d.

Similar to what was seen in the physical characterization results, the HPJ-M-400 sample appeared as a clear outlier following the proximate analysis of the ultracentrifuged samples. The HPJ-M-400 sample had a significantly ($P < 0.05$) greater precipitate mass (1.75 ± 0.66 g) than the other samples (<0.78 g; Figure 3b), less total fat ($P < 0.05$) in the top portion (0.13 ± 0.08 g; Figure 3c), and more total fat ($P < 0.05$) in the bottom portion (0.26 ± 0.10 g; Figure 3d) compared with the other samples. The HPJ-M-400 sample also had significantly ($P < 0.05$) more precipitated protein (0.55 ± 0.17 g) than all of the other samples except the HPJ-S-400 sample (0.37 ± 0.11 g).

In addition to the fat and protein co-precipitating in the HPJ-M-400 sample, the HPJ-M-400 sample was the only sample that experienced variation between the fat extracted by the crude fat and total fat extraction methods, with approximately 2.5-fold more fat extracted in the total fat extraction method. This variation indicates a complex resistance to crude solvent extraction, which was likely caused by a strong interaction between protein and fat. Other samples presented much greater extractability with the crude fat extraction method, with minor discrepancies likely due to a small portion of the lipids bound to carbohydrates or proteins present in the sample (Ellefson, 2017).
In summary, the precipitate of HPJ-M-400 comprised both fat (23.0%) and protein (26.6%), unlike the other treatments, which had predominately (>44%) protein precipitates. The fat in the HPJ-M-400 sample required a hydrolysis step for sufficient extraction, which reflects extensive interactions between the fat and protein in this sample. As suggested by Tran et al. (2018) and in agreement with the proposed model (Figure 2), this fat and protein co-precipitation can likely be attributed to the complexation of fat and casein caused by the HPJ treatment at 400 MPa.

Confocal Scanning Laser Microscopy. Confocal scanning laser microscopy was used to visualize the microstructure of the mixes. The confocal scanning laser microscopy images (Figure 4) of the C-0, C-P80, and HPJ-M-400 samples support what was seen after ultracentrifugation and what was proposed in the original model (Figure 2).

The control (C-0) sample had well-dispersed fat and protein (Figure 4a, b), whereas the control with emulsifier (C-P80) sample had well-dispersed components and some small destabilized fat aggregates (Figure 4c, d), which is consistent with particle size results (Table 1). In contrast, the HPJ-M-400 samples had large aggregates consisting of both fat and protein (Figure 4e, f). These large aggregates were also measured in particle size analysis (Table 1), are responsible for the fat–protein precipitate following ultracentrifugation (Figure 3d, f), and are seen with light microscopy during ice crystallization. Furthermore, small bubbles stabilized within the network of these unique complexes were observed under confocal scanning laser microscopy and

**Figure 2.** Model detailing the proposed effect of high-pressure-jet (HPJ) processing (400 MPa) on casein micelles and fat globules in the sample reservoir (1), hydraulic pump (2), restriction nozzle (3), and product output (4). The HPJ schematic was modified from Tran et al. (2018). May not be to scale.
are presumably responsible for the lower density of the HPJ-M-400 sample compared with the other samples (Table 1).

**Functional Characterization of Mixes**

**Stability.** It is important that ice cream mix is stable to creaming and sedimentation, so ice cream mix stability was investigated using a Turbiscan instrument (Figure 5). Most samples (C-0, HPJ-S-200, HPJ-S-400, and HPJ-M-200) were similarly stable to creaming, with destabilization rates of <0.012 TSI/min; however, significantly (*P* < 0.05) more rapid separation was seen in the HPJ-M-400 sample, with a destabilization rate of 0.042 TSI/min. We hypothesize that this anomalous separation of the HPJ-M-400 sample was due to the presence of large particles with entrapped air (described above; Figure 2) that were less dense than the serum phase. The control sample with emulsifier (C-P80) was also slightly more prone to creaming, with a destabilization rate of 0.019 TSI/min, presumably due to the presence of more partially coalesced fat.

**Ice Crystallization Kinetics.** Representative images of the ice crystallization process are presented from one sample replicate (Figure 6; Supplemental Figure S3; [https://doi.org/10.3168/jds.2019-17814]). Images

---

**Figure 3.** Total dry solids (a, b), fat (c, d), and protein (e, f) determination of the top and bottom sections of ultracentrifuged low-fat ice cream mix (C-0), low-fat ice cream mix with polysorbate 80 (C-P80), low-fat ice cream mix prepared with 200 MPa and 400 MPa high-pressure-jet (HPJ)-treated serum (HPJ-S-200, HPJ-S-400), and low-fat ice cream mix HPJ treated at 200 MPa and 400 MPa (HPJ-M-200, HPJ-M-400). Error bars represent SD of triplicate (C-P80, HPJ-S-200, HPJ-M-200) or quadruplicate (C-0, HPJ-S-400, HPJ-M-200) samples. Values assigned different letters (a–c) are significantly different (*P* < 0.05).
of the samples after the crystallization procedure are also presented with fat particle size distributions overlaid to orient these images to the particles measured using light scattering (Figure 6j–l). In most samples (C-0, C-P80, HPJ-S-200, HPJ-S-400, and HPJ-M-200) ice crystals melted slightly at −3.5°C, allowing for the free migration of liquid water and gradual growth in ice crystal size over temperature fluctuation cycles. However, ice crystallization in the HPJ-M-400 samples again showed marked differences. Ice crystals in the HPJ-M-400 sample appeared to stay in one position on the slide during the freezing process, as their movement was hindered by the surrounding fat–protein agglomerate dispersion. This kept some crystals relatively small, whereas other crystals appeared larger because the small crystals in the immediate proximity (i.e., not blocked by fat–protein aggregates) were able to merge together with the temperature fluctuations. When the ice cream was fully melted, gaps where the crystals had been were seen in the network of fat–protein aggregates (Figure 6l).

Quantitative analysis of these images was used to show the growth in ice crystal size over time (Supplemental Table S1, https://doi.org/10.3168/jds.2019-17814). However, this quantitative analysis of ice crystal size involved segmentation of ice crystals at merge points; therefore, the visual differences described above (Figure 6), specifically the merging of crystals, more accurately depict crystallization kinetics.

Although the freezing process used here is not representative of the dynamic freezing process in ice cream manufacture, the fat–protein complexes in the HPJ-M-400 sample seem to physically block moisture migration and ice crystal growth in a quiescently frozen system, which could translate to an increased resistance to heat shock.
CONCLUSIONS

The utilization of HPJ technology to alter the physiochemical properties of a low-fat ice cream mix was demonstrated by this study. High-pressure-jet processing (400 MPa) was found to increase the particle size, non-Newtonian behavior, and consistency coefficient of ice cream mix compared with a non-HPJ-treated control. These physical alterations were attributed to the formation of HPJ-induced fat–protein complexes, which were visualized by confocal scanning laser microscopy and quantified by proximate analysis after ultracentrifugation. These fat–protein complexes also appeared to slow ice crystal growth by acting as steric barriers between ice crystals. However, these large complexes also separated from the mix over time, causing a reduction in ice cream mix stability. The enhanced physiochemical properties created by a 400 MPa HPJ treatment of low-fat ice cream mix have potential for commercial applications (e.g., clean label products, novel frozen confections); however, the novel structures formed with this technology have yet to be analyzed in a realistic ice cream manufacturing process. That is, the freezing procedure in this experiment resembled a quiescent freezing instead of the agitated freezing required for ice cream production. Ongoing research in our laboratory will investigate the effect of dynamic freezing on a similar HPJ-treated low-fat (6% fat) ice cream mix.

ACKNOWLEDGMENTS

We are grateful to Gabriel Somarriba (Zamorano University, Tegucigalpa, Honduras) for his assistance with ice cream mix preparation and Charith Hettiarachchi (The Pennsylvania State University, University Park) for his guidance and microscopy assistance.
This project was partially funded by USDA National Institute of Food and Agriculture (Washington, DC) Federal Appropriations under project PEN04565 and accession number 1002916. The authors have not stated any conflicts of interest.

REFERENCES

Hayes, M. G., and A. L. Kelly. 2003. High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other

Figure 6. Brightfield images of low-fat ice cream mix (left), low-fat ice cream mix with polysorbate 80 (middle), and low-fat ice cream mix high-pressure-jet treated at 400 MPa (right) at the first (a, b, c), second (d, e, f), and third (g, h, i) cycles as well as when melted (j, k, l). Also included are fat particle size distributions of the mixes. Scale bars represent 100 μm.


**ORCIDS**

Grace L. Voronin • https://orcid.org/0000-0001-7620-5844

Tara L. Felix • https://orcid.org/0000-0001-6263-180X

Federico M. Harte • https://orcid.org/0000-0001-6822-0083