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Effects of pressure, shear, temperature, and their interactions on selected milk quality attributes

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ABSTRACT

The effects of pressure, temperature, shear, and their interactions on selected quality attributes and stability of milk during ultra-shear technology (UST) were investigated. The UST experiments include pressure (400 MPa) treatment of the milk sample preconditioned at 2 different initial temperatures (25°C and 15°C) and subsequently depressurizing it via a shear valve at 2 flow rates (low: 0.15–0.36 g/s; high: 1.11–1.22 g/s). Raw milk, high-pressure processed (HPP; 400 MPa, ~40°C for 0 and 3 min) and thermal treated (72°C for 15 s) milk samples served as the controls. The effect of different process parameters on milk quality attributes were evaluated using particle size, zeta potential, viscosity, pH, creaming, lipase activity, and protein profile. The HPP treatment did not cause apparent particle size reduction but increased the sample viscosity up to 3.08 mPa·s compared with 2.68 mPa·s for raw milk. Moreover, it produced varied effects on creaming and lipase activity depending on hold time. Thermal treatment induced slight reduction in particle size and creaming as compared with raw milk. The UST treatment at 35°C reduced the effective diameter of sample particles from 3,511.76 nm (raw milk) to 291.45 nm. This treatment also showed minimum relative lipase activity (29.93%) and kept milk stable by preventing creaming. The differential effects of pressure, shear, temperature, and their interactions were evident, which would be useful information for equipment developers and food processors interested in developing improved food processes for dairy beverages.

Key words: milk, pressure, shear, temperature, quality

INTRODUCTION

In recent times there has been increasing demand for foods of fresh-like quality that retain nutrients with minimal or no addition of synthetic ingredients such as emulsifiers and preservatives. To satisfy such demand, the food industry adapted alternative food preservation methods such as high-pressure processing (**HPP**). Several pressure-pasteurized products are available in the market, including juices, meat, seafood, salads, and ready-to-eat meals. Commercial success of batch high-pressure technologies for liquid foods led the industry to seek continuous methods of pressure treatment for various commodities (Martínez-Monteagudo et al., 2016).

Ultra-shear technology (UST), or high-pressure homogenization, is a continuous HPP technique that involves pressurization of liquid foods up to 400 MPa and subsequent depressurization by passage through tiny clearance in a shear valve. When the milk sample exits the shear valve, due to significant pressure difference across the valve, the pressure energy is converted into kinetic energy. This kinetic energy is dissipated as heat energy to raise the temperature of the fluid and as heat loss to the surroundings (Martínez-Monteagudo et al., 2016). Remaining kinetic energy is spent on sample physical and structural modifications (mixing, emulsification, dispersion, particle size, enzyme, and microbial reduction) via intense mechanical forces, such as shear, turbulence, or cavitation. Thus, depending upon the product initial temperature and process pressure, UST treatment can result in pasteurization or commercial sterilization effects along with structural modification in the treated liquid.

Fluid milk has been a staple beverage of American life and has been well researched. According to USDA, around 1.72 million metric tons of packaged fluid milk products were shipped in North America by milk handlers in April 2017. Over the years, several studies reported the cumulative effect of ultra-shear treatment on milk quality attributes (Hayes and Kelly, 2003; Datta

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et al., 2005; Hayes et al., 2005; Diels and Michiels, 2006; Pereda et al., 2007). Ultra-shear technology is found to be effective in inactivating various microorganisms including bacterial spores, enzymes, and stabilizing emulsions such as milk and milk-based beverages (Thiebaud et al., 2003; Hayes et al., 2005; Diels and Michiels, 2006; Martínez-Monteagudo et al., 2017). The treatment is also reported to cause changes in the milk protein structure through partial disassociation and coagulation properties in milk (Sandra and Dalgleish, 2007; Zamora et al., 2007; Roach and Harte, 2008). Most of these studies were conducted at pressures below 350 MPa. Moreover, limited efforts were made to separate the contributions of individual process parameters (pressure, heat, and shear) and their interactions on product quality. Thus, the relative importance of different UST process parameters on quality attributes is not well understood. In addition, researchers often subject the fluid food samples to multiple pressure-shear intensity treatments (Diels and Michiels, 2006; Maresca et al., 2011; Ruiz-Espinosa et al., 2012). Although the use of multiple passes was intended to improve the effectiveness of the treatment (Tahiri et al., 2006; Maresca et al., 2011), this may not be practically useful for the industrial food-processing environment. The objective of this research is to evaluate the effects of pressure, shear, temperature, and their interactions during the UST process on selected quality attributes of milk.

MATERIALS AND METHODS

Materials

Raw Milk. Raw cow milk was obtained from The Ohio State University Dairy Farm, Columbus, and transported within 30 min to the OSU Emerging Food Process Technology pilot plant. Using Sprint rapid protein analyzer and SMART Trac II Moisture & Fat Analyzer (CEM Corporation, Matthews, NC), raw milk characteristics were estimated (3.28 ± 0.04% protein, 4.15 ± 0.08% fat, and 12.97 ± 0.06% TS). Immediately upon arrival, the milk was stored refrigerated (<5°C) for a maximum of 1 d before conducting experiments.

Ultra-Shear Technology Laboratory Tester. A custom fabricated benchtop UST laboratory tester (PBI, Easton, MA) capable of attaining 400 MPa pressure was used in the present study (Figure 1). Equipment components include raw product reservoir, pressure generating system, pressure chamber, shear valve, cooling heat exchanger, and processed product reservoir. A 30-mL dispensing syringe was used as the raw product reservoir.

The pressure generating system employed compressed air to move a small plunger to pressurize water (pressure-transmitting fluid). Subsequently, the fluid transmitted the pressure to the milk sample via free-moving piston (called an isolator). The pressurized fluid food is then depressurized via shear valve.

The shear valve consists of a spherical ceramic ball placed over a circular valve seat. The force of the ceramic ball on the valve seat can be adjusted using a micrometer to vary the gap for fluid passage. When the fluid pressure is sufficient to overcome the force provided by the micrometer against the ceramic ball, fluid passes through the gap between the ball and valve seat (Figure 2). The pressure and temperature at various locations were collected using a data acquisition system (PBI).

Methods

UST Treatment. A temperature-preconditioned milk sample was fed into a UST unit, and samples were treated at a pressure of 400 MPa and subsequently decompressed via shear valve. Based on preliminary studies, raw milk samples were preconditioned at initial temperatures of 15 and 25°C to achieve 35 and 65°C process temperature after treatment. A typical pressure process run consists of pressurization of fluid milk (~2.5 to 3 mL) followed by depressurization by passing through the shear valve. Based on preliminary experiments, it was ensured milk sample received certain target pressure and temperature before collection at the exit of the shear valve (see Pressure-Thermal History of UST-Treated Samples section below). The UST process runs were repeated (up to 10 times) to collect the desired sample volume (~30 mL) for various quality analysis. It is to be noted that the product exiting shear valve was not recirculated or passed back into the equipment in the present study. The mass of samples collected during different process runs were recorded to calculate mass flow rate through the shear valve.

Thermal Effects During UST Treatment. The whey proteins in milk are sensitive to temperatures around 65°C, with α-lactalbumin, bovine serum albumin and immunoglobulin G having initial denaturation temperatures of 62, 64 and 72°C respectively (Lee, 1992). On the other hand, at temperatures below 50°C, the thermal effects on product quality and safety often can be assumed to be negligible (Khalil and Villota, 1988). Thus, UST experiments were carried out at 65°C and 35°C to evaluate pressure-thermal-shear effects and pressure-shear effects respectively (Figure 3).

To achieve 65°C process temperature in milk at the exit of shear valve, milk was equilibrated to 25 ± 2°C for 30 min and pressurized up to 400 MPa and sheared under 2 different flow rates; low (0.36 ± 0.11 g/s) and high (1.11 ± 0.12 g/s). A 35°C process temperature

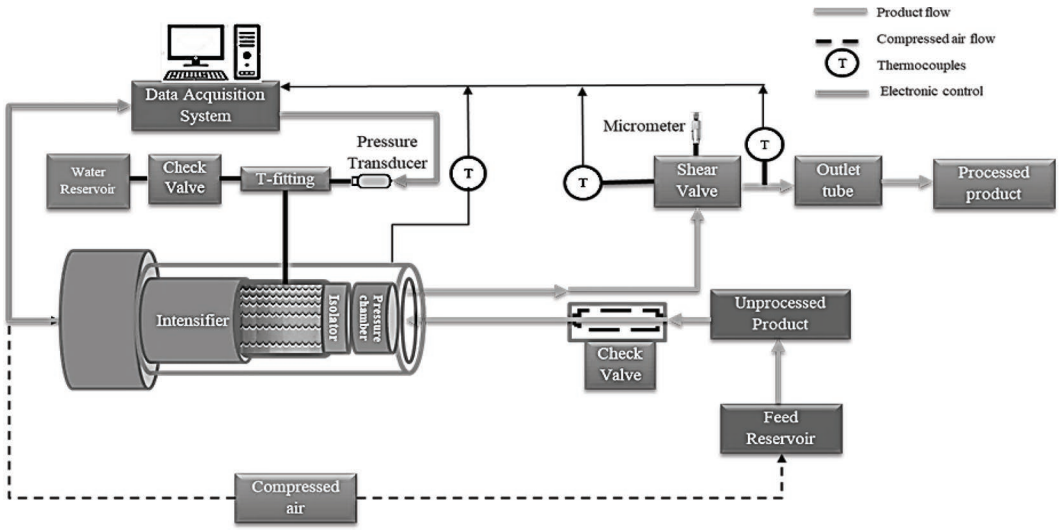

Figure 1. Schematic diagram of bench-scale ultra-shear technology laboratory tester.

was realized by controlling the initial milk temperature and wrapping the shear valve with a flexible cooling pad for removal of heat in the shear valve. Experiments involved equilibrating the milk at 15 ± 2°C for 30 min, pressurizing up to 400 MPa and subsequently shearing under low (0.15 ± 0.01 g/s) and high (1.22 ± 0.20 g/s) flow rates. It is worth noting that this research employed a constant pressure intensifier. The flow rate can be changed by changing the restriction in the valve. This would not change the pressure drop. During the period of discharge, the flow is continuous.

Control Samples. The experiments also used 3 sets of control samples. First, the untreated raw milk served as a control. To investigate the pressure-only (400 MPa) effects, tests were conducted using a batch HPP system. Thermal-only effects were investigated using a laboratory scale thermal pasteurizer. The equipment and experimental details are described below.

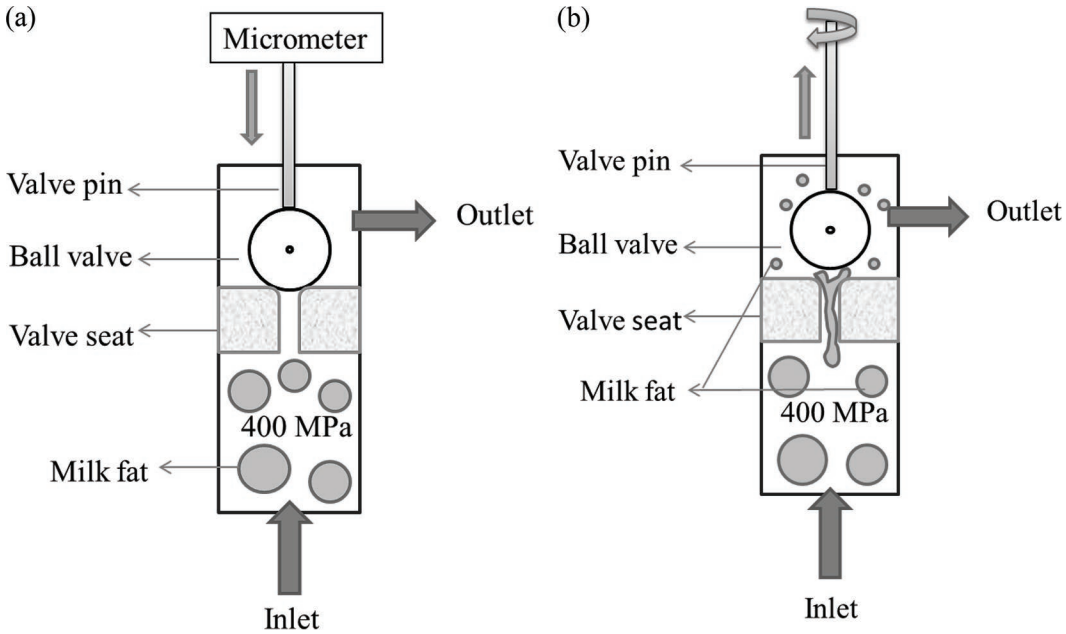

Figure 2. Schematic diagram of operation of shear valve in ultra-shear technology equipment. (a) When the ball valve is in closed position no liquid flow is allowed via the valve seat. (b) When the micrometer is rotated counter-clockwise, the valve pin moves up, gradually opens the ball valve, and allows liquid to exit through the open shear valve.

Pressure Treatment. High-pressure treatment of milk samples was performed using batch HPP equipment (PT1 pressure kinetic testing unit, Avure Technologies, Kent, WA) by adapting the procedure previously published by our laboratory (Dhakal et al., 2016; Balasubramaniam et al., 2016). Briefly, milk (~2.5 mL) pouches at an initial temperature of 25 ± 2°C were pressurized up to 400 MPa for 2 different (0 and 3 min) pressure holding times. The process temperatures for 0 and 3 min were 40.66 ± 0.82°C and 40.97 ± 0.07°C, respectively.

Thermal Treatment. Raw milk was thermally treated at a temperature of 72°C and held for 15 s, followed by cooling to 5°C at a flow rate of 3 L/min, using MicroThermics UHT/HTST Lab-25HV (MicroThermics, Inc., Raleigh, NC) system. This process is analogous to the industrial HTST process, except that the homogenization operation was excluded.

Analysis of Quality Attributes. The quality attributes of UST-treated and various control samples were evaluated using particle size, zeta potential, viscosity, pH, creaming, lipase activity, and protein profile analyses using SDS-PAGE.

Particle Size. The particle size of untreated and treated samples was analyzed by dynamic light scattering technique in a particle size analyzer (NanoBrook, ZetaPALS, Brookhaven, Holtsville, NY). Samples were diluted with ultra-pure water in the ratio of 1:1,000. Milk fat particle refractive index of 1.45–0.01i and dispersant refractive index of 1.33 were used (Matsumiya et al., 2017). Particle size was measured within 1 h after treatments.

Effective diameter (D_{eff}) represents the average size of particles in the sample, which is the hydrodynamic diameter measured by intensity of the light scattered by each particle.

$$D_{eff} = \frac{\Sigma N d^6}{\Sigma N d^5},$$

where N is the number of particles and d is the particle diameter.

The polydispersity index (**PDI**) is a measure of the nonuniformity in the particle size distribution. It is a dimensionless number ranging from 0 to 1.0, with values close to 0 indicating uniform and homogeneous distribution.

The mean diameter by volume ($D_{4,3}$) is the diameter of sphere of equivalent volume for measured particles.

$$D_{4,3} = \frac{\Sigma N d^4}{\Sigma N d^3}$$

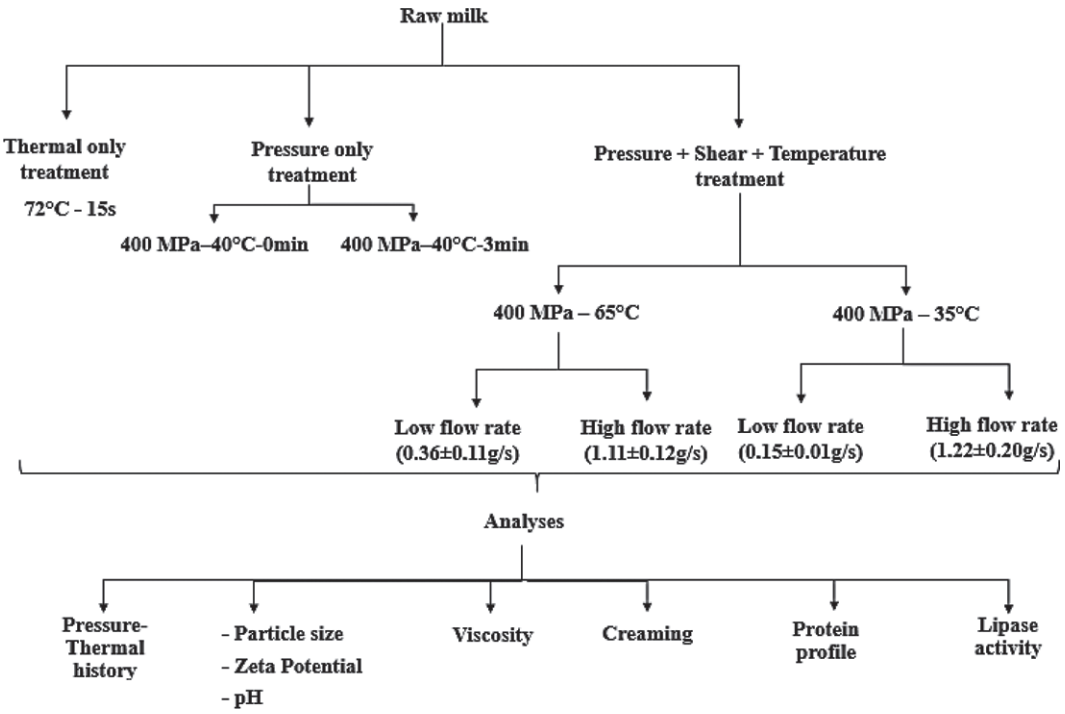

Figure 3. Flowchart summarizing process conditions in raw milk treatments and analysis.

The mean diameter by surface ($D_{3,2}$) is the particle diameter with the same specific surface as that of the whole distribution.

$$D_{3,2} = \frac{\Sigma N d^3}{\Sigma N d^2}$$

The particle size distribution of samples given as differential distribution versus mean diameter by volume was estimated using the BIC Particle Solutions software version 3.5 (Brookhaven Instruments, 2020).

Zeta Potential. Zeta potential measurements of all samples were performed using phase analysis light scattering technique in a zeta potential analyzer (NanoBrook, ZetaPALS, Brookhaven). Samples diluted with ultra-pure water in the ratio of 1:1,000 were used. The electrophoretic mobility of particles was measured using light scattering with a detection angle of 15°. Zeta potential was determined from mobility data using Smoluchowski model.

Viscosity. Viscosities of all samples were measured at 23 ± 2°C using Brookfield viscometer (LV DV2T Extra, Brookfield Engineering Laboratories Inc., Middleboro, MA) with UL adapter. Approximately 16 mL of sample was placed in the sample cup, and the spindle was inserted. Viscosity was measured in m.Pa·s at 60 rpm (shear rate of 73.38 s^{-1}) for 180 s.

pH. The pH of all samples was measured at 23 ± 2°C using a benchtop pH meter (Mettler Toledo, Columbus, OH).

Creaming. To study creaming, the procedure described by Huppertz et al. (2003) was used with modification considering scale of operation. Ten milliliters of milk samples were placed in closed graduated tubes and stored undisturbed at refrigerated temperature <5°C. The volume of cream or fat portion rising to top of the tube was noted to the nearest 0.5 mL for 15 d and expressed as mL of cream/10 mL of milk.

Lipase Activity. The lipase activity in the untreated, HPP-treated, and UST-treated milk samples were determined using the procedure reported by Humbert et al. (1997) with slight modification. Before analysis, an inhibiting mixture and substrate (Humbert et al., 1997) as well as clarifying reagent (Linden et al., 1991) were prepared. For analysis, sample and blank tubes were taken. In sample tubes, 0.5 mL of milk was taken, and 2 mL of 0.05 *M* Tris buffer with pH 7.6 was added. Only the blank tube was added with 0.4 mL of inhibiting mixture. Both tubes were kept at 37°C for 15 min for incubation. The substrate was then added to both tubes, shaken, and kept at 37°C for 10 min for incubation. Then 0.4 mL of inhibiting mixture was mixed with sample tubes only. At last, 2 mL of clarifying reagent was mixed in all tubes and kept at 37°C for 3 to 5 min. The clarified mixtures were taken in cuvette within 15 min to be read with Fisherbrand accuSkan GO UV/Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) absorbance at 420 nm. A standard curve developed using 0 to 40 μmol per assay was used to convert the absorbance values to μmol *p*-nitrophenol and calculate the relative lipase activity (%).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. The SDS-PAGE of raw milk in comparison to HPP- and UST-treated samples was carried out using mini precast gels (4–20% stain-free protein gels, 10 wells) as described by Takagi et al. (2007) with reagents sourced from Bio-Rad Laboratories (Hercules, CA), based on the method described by Marciniak et al. (2018) with slight modification. Twenty microliters of each sample (diluted in PBS buffer) were added to 20 μL of native sample buffer (contains 62.5 m*M* Tris-HCl, pH 6.8, 40% glycerol, 0.01% bromophenol blue). Twenty microliters of this solution was loaded into an individual well. The running buffer was 10× Tris/glycine/SDS buffer. The standards used were SDS-PAGE MW standard-broad range (Bio-Rad Laboratories). Electrophoresis was carried out at 15mA/gel in the mini-protean tetra vertical electrophoresis cell until the tracking dye reached the bottom of the gel. The gel images were then acquired by ChemiDoc Imaging System (Bio-Rad Laboratories) for further analysis.

Protein Aggregation in UST-Treated Samples. To determine the amount of protein denatured or aggregated in the samples treated with UST at different temperatures, a procedure described by Pizzano et al. (2012) was used with slight modification. A 5-mL aliquot of UST-treated milk sample was taken, and the pH of each sample was adjusted to 4.6 by adding 1 N HCl under pH meter. The aliquot was then centrifuged at 4,000 rpm (3,739 × *g*) at 25°C for 15 min in a Sorvall Legend XFR Centrifuge (Thermo Fisher Scientific). The fat portion, if separated on top, was removed manually. The protein content in supernatant was determined in duplicate using Sprint rapid protein analyzer (CEM Corporation).

Statistical Analysis. Statistical significance of the data was analyzed using ANOVA using IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY) statistical analysis package. All process runs and instrumental analyses were done at least in triplicate unless mentioned otherwise. The data were expressed as mean ± SD. Tukey's honest significance difference test was used for comparisons between means. The level of significance was determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Pressure-Thermal History of UST-Treated Samples

During UST treatment, the fluid milk preconditioned at certain initial temperature is pressurized up to 400 MPa in the pressure chamber and passed on to the shear valve. In pressure chamber, the product temperature transiently increased (~3°C/100 MPa) due to adiabatic heating (Rasanayagam et al., 2003). In addition, the temperature of the fluid increases almost instantaneously as it passes through the shear valve (~20°C/100 MPa; Martínez-Monteagudo et al., 2017). This temperature rise during pressure discharge in shear valve is a pure thermodynamic effect that takes place as a result of conversion of potential pressure energy to kinetic energy and subsequently to thermal energy. In theory, the temperature rise in the fluid during discharge is dependent only on the work done (pdV) on the fluid (Martínez-Monteagudo et al., 2016). However, in practical conditions, the temperature rise can vary depending on other factors such as shear valve geometry and flow rate, due to the varying heat loss. For example, when a small volume of fluid is passed at a low flow rate through shear valve with higher mass and longer fluid path, most of the heat generated by shear would be lost to the surrounding environment (e.g., valve body, tubing) resulting in lesser temperature of fluid at shear valve exit, relative to theoretical maximum. On the other hand, in high rate flow condition, the fluid would have lesser ability to lose heat and thus exit at a relatively higher temperature. Therefore, it is essential to understand the pressure-thermal history of the fluid product during UST processing. The pressure-thermal histories of milk samples exiting shear valve at various flow rates during UST treatment are shown in Figures 4 and 5.

Pressure-Thermal History of 65°C UST-Treated Samples

At the beginning of UST experiments at 65°C (Figures 4a and 4b), it can be observed that temperature fell short of target process temperature during the first 2 process runs. The heat energy generated during these process runs were primarily used in warming up the shear valve, which was maintained at ambient temperature. During the third process run, target process tem-

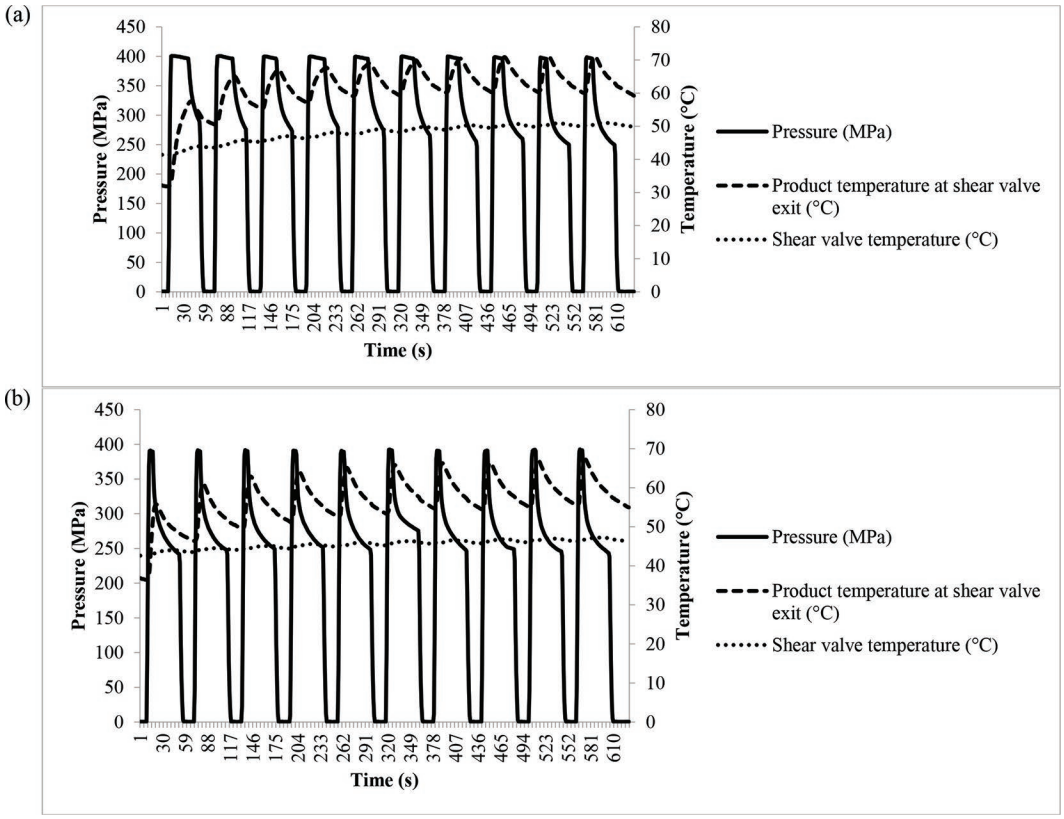

Figure 4. Pressure–temperature history during 65°C ultra-shear technology treatment for 10 process runs at (a) low flow rate and (b) high flow rate.

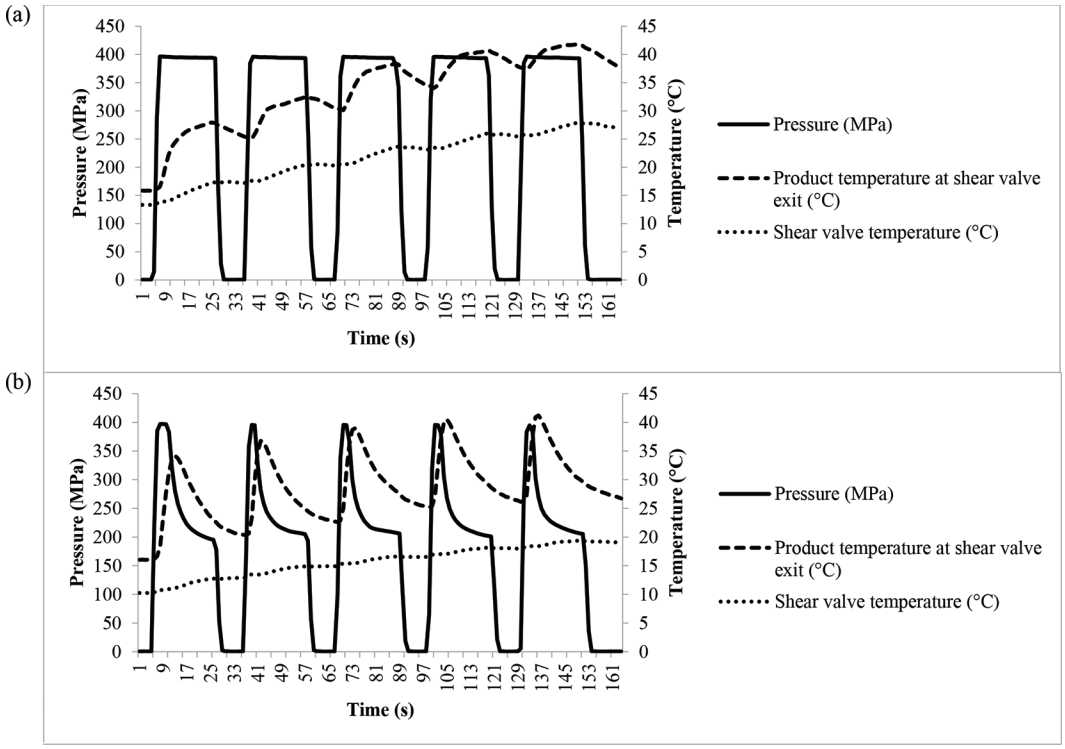

Figure 5. Pressure–temperature history during 35°C ultra-shear technology treatment for 5 process runs at (a) low flow rate and (b) high flow rate.

perature was achieved and appeared to reach a steady state from there on. During 65°C UST treatment, the maximum process temperature at the exit of shear valve at low- and high flow rates were 69.52 ± 1.51°C and 65.48 ± 1.53°C, respectively. Samples for analysis were collected from 3rd through 10th process runs, so that samples were treated under designed process conditions. Assuming milk behaves as water does for initial temperatures of 25°C and 15°C, the temperature rise as a consequence of shearing were theoretically estimated as 26.20°C/100 MPa and 26.25°C/100 MPa, respectively. In the present study, apparent temperature rise in milk samples was 10.12 to 11.13°C/100 MPa during 65°C UST treatment, close to values in earlier studies (Thiebaud et al., 2003; Hayes and Kelly, 2003; Pereda et al., 2007; Martínez-Monteagudo et al., 2017). Cortés-Muñoz et al. (2009) attributed the difference between experimentation and theoretical calculation to heat loss as well as energy expended for particle size reduction.

Pressure-Thermal History of 35°C UST-Treated Samples

During UST treatment at 35°C (Figures 5a and 5b), the maximum process temperature attained during low- and high flow rates were 36.14 ± 5.81°C and 38.24 ± 2.83°C, respectively. The cooling pad acted as a heat sink and prevented excessive thermal exposure on product. At process temperature of 35°C, the highest temperature rise per second of 8.90 ± 3.32°C/s for each process run was observed under high flow rate, due to the higher temperature difference between the shear valve body and product temperature. During 35°C UST experiments, samples were collected from second to fifth process runs. The treatment was restricted to 5 continuous process runs because the sample temperature increased beyond the process temperature after the fifth process run, due to cumulative addition of heat to the shear valve. It is worth noting that the temperature rise per second for each process run for 65°C and 35°C process temperature under high flow rate (5.13 ± 0.78°C/s and 8.90 ± 3.32°C/s) was higher than the low flow rate (1.45 ± 0.48°C/s and 0.40 ± 0.14°C/s). The higher temperature during high flow rate could be attributed to higher power dissipation owing to larger clearance between valve seat and ball valve.

Effect of Various Treatments on Milk Quality Attributes

Milk Particle Size. The influence of pressure, shear, and temperature on the particle size parameters of raw

milk is shown in Table 1. It was observed that the high pressure and subsequent shear in UST treatment caused significant reduction ($P < 0.05$) in the particle size. The UST-treated milk had an average particle diameter of 335.89 nm (65°C UST) and 291.45 nm (35°C UST) compared with diameter of 3,511.76 nm for raw milk samples. Within the experimental conditions, the temperature of UST treatment and magnitude of flow rates did not have significant effect on particle size reduction. Intense physical forces such as shear, turbulence, and elongation stress experienced by milk flowing through the shear valve likely facilitated the particle size reduction. In milk, the lipid portion takes the form of emulsified fat globules enwrapped by surface active milk fat globule membrane (**MFGM**), which maintains fat globule integrity and allows them to remain dispersed (Jiménez-Flores and Brisson, 2008). The physical forces caused disruption of MFGM and reduction of the fat globules size (Lopez et al., 2015).

The PDI of untreated milk was 0.420, which indicated broad distribution of particles. Variations in fat globule size might favor destabilization and fat separation by Ostwald ripening and creaming (Cavazos-Garduño et al., 2016). The UST treatment decreased PDI values to between 0.246 and 0.233 (Table 1). The magnitude of flow rate or the process temperature during UST treatment did not significantly affect the PDI.

The volume-weighted mean diameter ($D_{4,3}$) and surface-weighted mean diameter ($D_{3,2}$) of raw milk were 4,052.62 nm and 1,471.13 nm, respectively. These diameters were similar to those reported by Tobin et al. (2015) for untreated raw milk (4,500 nm and 1,000 nm, respectively). The UST treatment at 35°C under low flow rate reduced these values to 557.02 nm and 403.71 nm, respectively (Table 1). Thiebaud et al. (2003) reported slightly higher $D_{4,3}$ and $D_{3,2}$ values of 1,730 nm and 770 nm, respectively, for milk with 14°C inlet temperature when processed at 300 MPa. This finding might be due to the different experimental apparatus (e.g., model FPG7400 H, Stansted Fluid Power Ltd., Essex, UK) used by Thiebaud et al. (2003), with different shear valve geometry. Earlier research reported that both $D_{4,3}$ and $D_{3,2}$ increase with increasing inlet temperatures (Pereda et al., 2007; Thiebaud et al., 2003; Amador-Espejo et al., 2014). Both D_{10} and D_{90} values reduced from 1,721.93 and 7,177.28 nm in untreated milk up to a minimum of 162.06 and 524.18 nm, respectively, in UST treatment at 35°C under low flow rate (Table 1).

Studies have shown that above certain threshold pressures, the fat globules reaggregate after shear treatment over a period of time. This reaggregation may be due to coalescence of smaller particles into large fat sizes (Pereda et al., 2007; Amador-Espejo et al., 2014).

Table 1. Particle size parameters of milk samples[1]

No.	Treatment[2]	Effective diameter (nm)	Polydispersity index	Mean diameter by volume ($D_{4,3}$, nm)	Mean diameter by surface ($D_{3,2}$, nm)	D_{10} (nm)	D_{50} (nm)	D_{90} (nm)
1	Untreated raw milk	$3,511.76^{a} \pm 357.49$	$0.420^{a} \pm 0.028$	$4,052.62^{a} \pm 242.30$	$1,471.13^{a} \pm 416.01$	$1,721.93^{a} \pm 269.98$	$3,511.76^{a} \pm 357.49$	$7,177.28^{ab} \pm 376.80$
2	Thermal process, 72°C, 15 s	$2,698.75^{b} \pm 456.61$	$0.491^{b} \pm 0.014$	$3,537.20^{a} \pm 236.05$	$505.25^{bc} \pm 136.78$	$1,201.28^{b} \pm 208.88$	$2,698.75^{b} \pm 456.61$	$6,063.33^{b} \pm 1,000.96$
3	HPP, 400 MPa, 40°C, 0 min	$3,467.95^{a} \pm 104.28$	$0.410^{a} \pm 0.045$	$4,052.71^{a} \pm 1,038.04$	$1,636.24^{a} \pm 347.18$	$1,637.16^{a} \pm 31.79$	$3,467.95^{a} \pm 104.28$	$7,352.70^{a} \pm 455.82$
4	HPP, 400 MPa, 40°C, 3 min	$3,441.29^{a} \pm 205.82$	$0.343^{c} \pm 0.008$	$3,507.10^{a} \pm 641.10$	$1,154.22^{ab} \pm 368.58$	$1,721.40^{a} \pm 110.21$	$3,441.29^{a} \pm 205.82$	$6,884.92^{ab} \pm 382.81$
5	UST, 400 MPa, 65°C, low flow rate	$364.88^{c} \pm 6.54$	$0.237^{d} \pm 0.008$	$671.88^{b} \pm 136.83$	$580.63^{bc} \pm 175.03$	$201.96^{c} \pm 4.81$	$364.88^{c} \pm 6.54$	$659.26^{c} \pm 9.68$
6	UST, 400 MPa, 65°C, high flow rate	$335.89^{c} \pm 1.97$	$0.242^{d} \pm 0.004$	$805.93^{b} \pm 107.96$	$409.42^{c} \pm 102.08$	$184.89^{c} \pm 1.11$	$335.89^{c} \pm 1.97$	$610.22^{c} \pm 5.04$
7	UST, 400 MPa, 35°C, low flow rate	$291.45^{c} \pm 30.22$	$0.233^{d} \pm 0.008$	$557.02^{b} \pm 60.63$	$403.71^{c} \pm 72.92$	$162.06^{c} \pm 16.41$	$291.45^{c} \pm 30.22$	$524.18^{c} \pm 55.88$
8	UST, 400 MPa, 35°C, high flow rate	$300.67^{c} \pm 27.90$	$0.246^{d} \pm 0.019$	$716.03^{b} \pm 134.92$	$548.57^{bc} \pm 143.31$	$164.79^{c} \pm 12.72$	$300.67^{c} \pm 27.90$	$548.72^{c} \pm 60.65$

[a–d]Mean values without common superscripts in same column are significantly different ($P < 0.05$).

[1]Values are expressed as mean ± SD. All measurements are in nm, except polydispersity index (dimensionless).

[2]HPP = high-pressure processed; UST = ultra-shear technology. D_{10}, D_{50}, and D_{90} denote the diameter below which 10, 50, or 90% of the volume of particles are found, respectively.

Martínez-Monteagudo et al. (2017) observed that the mean particle size of dairy beverages decreased with increasing pressure from 103 to 160 MPa and gradually increased with increasing pressure from 160 to 288 MPa, thus identifying the threshold pressure at 160 MPa. Earlier, Tornberg (1980) explained that higher intensity of homogenization or greater content of fat could cause coalescence and result in bigger fat globules. Therefore, the fat globule size immediately after shear might have been lesser than observed during analysis in the present study.

Thiebaud et al. (2003) and Datta et al. (2005) reported decreasing fat globule sizes upon homogenization at 200 MPa with increasing outlet temperature. In the present study, the effect of process temperature in UST was insignificant. However, the lowest mean fat globule size of 291.45 nm was noted in UST treatment at 35°C. This finding might emphasize the dominating influence of proteins rather than outlet temperature during UST on the fat globules size reduction. After shear treatment, the MFGM is disrupted and the new smaller fat globule created is covered by a new membrane formed by surface active components, the majority of them being casein micelles (Michalski and Januel, 2006). During UST treatment at 35°C, proteins might have been left intact to cover and stabilize the fat globules.

Batch HPP did not cause significant change in the mean diameter of particles regardless of pressure holding time. This result is consistent with Huppertz et al. (2003) who reported little or no difference between the milk fat globule size in untreated milk and HPP-treated milk at pressures up to 600 MPa at 20°C and pressure holding times up to 30 min. Similar observations were reported by other researchers including Dumay et al. (1996), Ye et al. (2004), and Stratakos et al. (2019).

Thermal process at 72°C for 15 s caused slight reduction in the mean particle size up to 2,698.75 nm (Table 1). Michalski and Januel (2006) stated that heat treatment with no associated homogenization does not change the size of milk fat. On contrary, Stratakos et al. (2019) heat pasteurized raw milk samples at 72 ± 0.5°C for 5 min and reported that pasteurized milk produced significantly ($P < 0.05$) smaller fat globules size (0.32 ± 0.01 μm) compared with untreated raw milk (1.60 ± 0.11 μm). This finding is consistent with the present study. Further, the PDI of milk treated by the thermal process in the present study was the highest at 0.491, which might be attributed to differently sized fat particles in the milk.

Particle Size Distribution. From Figures 6a and 6b, it can be observed that untreated milk showed a multimodal distribution, with 2 major peaks between 3,000 and 4,500 nm and a minor peak around 100 nm. Researchers reported major peak around 3,700 to 3,800 nm corresponding to fat globule and another peak around 100 to 200 nm corresponding to casein micelles in raw milk (Thiebaud et al., 2003; Amador-Espejo et al., 2014; Rodarte et al., 2018). The distribution is also wide, which corroborates the higher PDI (0.420) of the untreated milk samples and implies larger variations among particle sizes.

The UST treatment at temperatures of 65 and 35°C distinctly reduced particle size and shifted peaks toward left in mean diameter scale. The peak was transformed from multimodal and broad to unimodal and narrow distribution, corroborating the lesser PDI values (Table 1). Amador-Espejo et al. (2014) observed conversion from bimodal to monomodal distribution when 200 MPa and initial temperatures ranging from 55 to 85°C were used to homogenize milk. Further, the unimodal distribution and absence of separate peak for casein indicated that UST disrupted the casein into smaller submicelles (Figure 6b). Earlier researches have reported that high pressure and shear action leads to the dissociation of casein to smaller submicelles (Roach and Harte, 2008; Chevalier-Lucia et al., 2011).

The pressure-treated (HPP) samples at 400 MPa for 0 and 3 min showed multimodal and polydisperse distribution with the peaks observed at different diameter ranges. Although HPP for 0 min had a major peak similar to raw milk, HPP for 3 min had a major peak at a lesser diameter than raw milk (Figure 6a). This result can be attributed to disruption of fat globules and subsequent reaggregation of fat particles after HPP treatment (Pereda et al., 2007). These phenomena can be corroborated by presence of a tail after the first major peak in both 0 and 3 min HPP samples.

The distributions of 0- and 3-min pressure holding times also indicated minor peaks at ~450 nm and ~100 nm diameters, respectively, which could be contributed by casein micelles. Literatures suggest that HPP could enable both increase and decrease in casein micelles size and number due to disruption of casein micelle into submicelles by high pressure and the subsequent reassociation phenomena in samples at atmospheric pressure (Huppertz et al., 2006; Broyard and Gaucheron, 2015). Pressure of 250 MPa could cause ~30% increase in size of casein micelle and pressures above 300 MPa could reduce the size by ~50% (Huppertz et al., 2004; Goyal et al., 2018). The effect on milk protein is diverse, based on the different pressures and holding times (Huppertz et al., 2006; Cadesky et al., 2017). The observations indicate that the casein size reduction in UST-treated samples might be caused by shear rather than pressure alone.

The samples treated with thermal process at 72°C for 15 s showed multimodal and polydisperse distribution,

but different than that from untreated milk. Two major peaks between 3,000 and 5,000 nm were noticed, which could be attributed to the fat globules of a different size range. A minor peak was noted around 150 nm, which could be attributed to aggregated casein micelles (Figure 6b). Sauer and Moraru (2012) subjected micellar casein concentrates (**MCC**) at pH < 6.7 to temperatures of 110 and 120°C. The target temperatures were reached in 52 s, and then the MCC was cooled immediately to 20°C by immersion in ice. It was observed that the casein micelles in MCC showed visibly stronger aggregation with increasing temperatures from 110 to 120°C.

Zeta Potential. The zeta potential of untreated raw milk (pH 6.61 ± 0.066) was −47.90 mV (Table 2). The values in our study were higher than values reported by other researchers [−34.1 mV in raw milk with 3.2 to 3.3% fat (Tunick et al., 2016) and −35.5 mV in Friesian cow raw milk (Gallier et al., 2012)]. The relatively higher zeta potential observed in the present study could be due to the composition of milk used, including higher fat content.

Thermally processed (72°C for 15 s) samples had a zeta potential of −51.66 mV that was not significantly different from untreated milk. He et al. (2017) reported that the zeta potential of emulsions prepared with MFGM were only slightly altered by heat, with no significant changes with heat treatment at temperature of 35 to 85°C for 30 min.

Zeta potential of HPP-treated samples at 0 and 3 min were −48.23 mV and −48.74 mV, respectively. In the present study, similar to thermally treated samples, pressure-treated samples did not cause significant difference in zeta potential as compared with the untreated milk sample. This result might be due to dissociation of casein micelles upon compression by high pressure and

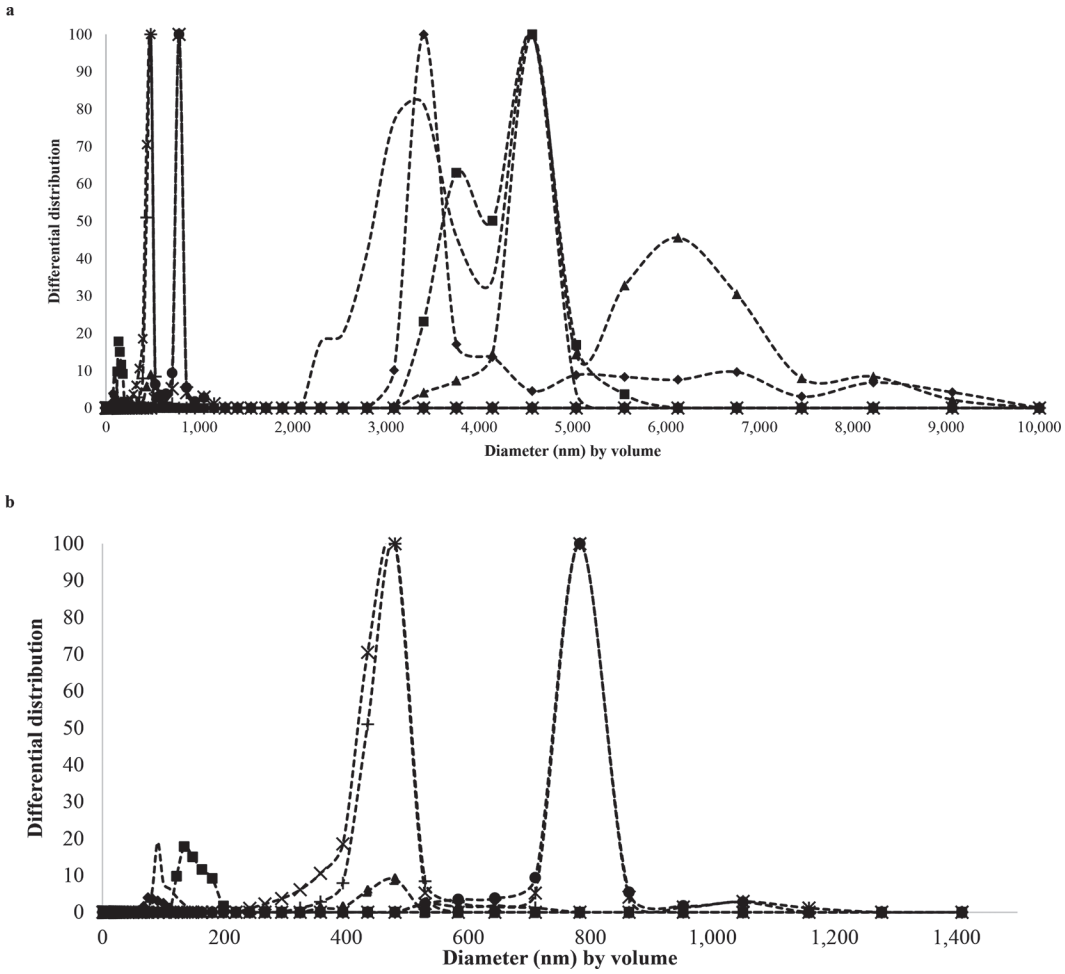

Figure 6. Volume-weighted differential particle size distribution of milk samples (A) from 0 to 10,000 nm and (B) from 0 to 1,400 nm. HPP = high-pressure processed; UST = ultra-shear technology. (– indicates untreated raw milk; ■ indicates thermal process-72°C-15 s; ▲ indicates HPP-400 MPa-40°C-0 min; ◆ indicates HPP-400 MPa-40°C-3 min; × indicates UST-400 MPa-65°C-low flow rate; • indicates UST-400 MPa-65°C-high flow rate; + indicates UST-400 MPa-35°C-low flow rate; * indicates UST-400 MPa-35°C-high flow rate.)

reaggregation of casein micelles after depressurization (Broyard and Gaucheron, 2015).

The zeta potential of UST-treated samples varied from −36.36 to −28.55 mV, which was significantly less than untreated ($P = 0.001$), HPP-treated, and thermally processed samples ($P < 0.05$). During UST treatment, MFGM is disrupted, and a new surface is created on which whey and casein proteins are adsorbed. The differences in casein on the MFGM could have influenced the zeta potential. Meena et al. (2016) homogenized ultra-filtered skim milk at 13.79 MPa and 3.45 MPa and reported zeta potential reduction due to surface modifications in milk protein because of shearing and cavitation. The different temperatures of UST treatment did not elicit significant variation in the zeta potential of samples, eliminating the temperature effect during UST in the studied range. It has been previously reported that magnitude of thermal effects did not significantly alter the zeta potential of milk (Darling and Dickson, 1979). In a similar manner, the magnitude of flow rates and the interaction of temperature and flow rate in UST treatment did not have any influence ($P = 0.138$) on zeta potential. This finding might elucidate equivalent effect of shear in low- and high flow rates, despite the different temperature rise per second (Figures 4 and 5). These observations show the dominating effect of shear on the zeta potential, rather than the high pressure or temperature of UST treatment. Factors such as pH (Meena et al., 2016), casein hydration (Broyard and Gaucheron, 2015), and lipolytic products (Gallier et al., 2012) could also influence the zeta potential. All UST-treated samples exhibited high stability regarding the measured zeta potential, which reiterates the role of fat globule size reduction by high shear in stabilizing the milk against creaming.

pH. The pH of untreated raw milk was 6.61 (Table 2). The pH of samples treated using different processing techniques were not significantly different from untreated samples. In an earlier study, Pereda et al. (2007) homogenized milk with an inlet temperature of 30°C at 300 MPa and observed constant pH until 18 d of storage at 4°C.

Viscosity. The viscosity of untreated milk was 2.680 mPa·s (Table 2). Thermal processing at 72°C for 15 s produced samples with viscosity of 2.747 mPa·s, which was not significantly different from untreated milk and UST-treated samples. The viscosity of milk samples subjected to HPP for 0 min (3.053 mPa·s) was significantly ($P = 0.03$) higher than the untreated samples. Furthermore, viscosity of samples under HPP treatment for 3 min (3.083 mPa·s) were significantly higher than untreated ($P = 0.017$), UST-, and thermally treated samples, indicating the effect of holding time under pressure. Under high pressures above 300 MPa and for pressure holding times over 15 min (Gaucheron et al., 1997; Needs et al., 2000), the casein micelles disintegrate into smaller particles, casein micelle hydration increases, and the viscosity of milk increases (Huppertz et al., 2002; Broyard and Gaucheron, 2015). In the present study, it was also noted that the HPP treatment at 400 MPa for 3 min resulted in no creaming in samples, which might also be attributed to the casein micellar networking and increased viscosity. Viscosity of UST-treated samples varied from 2.710 mPa·s for samples treated at high flow rate at 35°C to 2.970 mPa·s for samples treated at high flow rate at 65°C. It is interesting to note that viscosities of UST-treated samples at 35°C were not significantly different from untreated samples, which might mean that the viscosity increase by HPP was negated by UST through reduction of particle size and protein retention. The temperatures and flow rate interactions in UST treatment did not have significant effect on the viscosity of samples.

Creaming. The creaming of processed milk samples as compared with untreated milk during 15 d of refrigerated storage is shown in Figure 7. The creaming of untreated milk showed a rapid increase from 0 to 1.5 mL/10 mL milk in the first 24 h and subsequently leveled off at 1 mL/10 mL until 15 d. Stokes' law states that the velocity of creaming in milk is correlated di-

Table 2. Zeta potential, pH, and viscosity of milk samples[1]

No.	Treatment[2]	Zeta potential (mV)	pH	Viscosity (mPa·s)
1	Untreated raw milk	$-47.90^{a} \pm 1.66$	$6.61^{ab} \pm 0.066$	$2.680^{a} \pm 0.24$
2	Thermal process, 72°C, 15 s	$-51.66^{a} \pm 1.76$	$6.64^{ab} \pm 0.006$	$2.747^{ab} \pm 0.19$
3	HPP, 400 MPa, 40°C, 0 min	$-48.23^{a} \pm 3.68$	$6.57^{a} \pm 0.006$	$3.053^{c} \pm 0.08$
4	HPP, 400 MPa, 40°C, 3 min	$-48.74^{a} \pm 1.98$	$6.64^{ab} \pm 0.006$	$3.083^{c} \pm 0.07$
5	UST, 400 MPa, 65°C, low flow rate	$-33.81^{b} \pm 2.35$	$6.66^{b} \pm 0.012$	$2.920^{bc} \pm 0.07$
6	UST, 400 MPa, 65°C, high flow rate	$-36.36^{b} \pm 1.37$	$6.66^{b} \pm 0.00$	$2.970^{c} \pm 0.01$
7	UST, 400 MPa, 35°C, low flow rate	$-33.09^{b} \pm 6.72$	$6.59^{ab} \pm 0.035$	$2.740^{ab} \pm 0.04$
8	UST, 400 MPa, 35°C, high flow rate	$-28.55^{b} \pm 1.82$	$6.61^{ab} \pm 0.015$	$2.713^{ab} \pm 0.06$

[a–c]Mean values without common superscripts in same column are significantly different ($P < 0.05$).

[1]Values are expressed as mean ± SD.

[2]HPP = high-pressure processed; UST = ultra-shear technology.

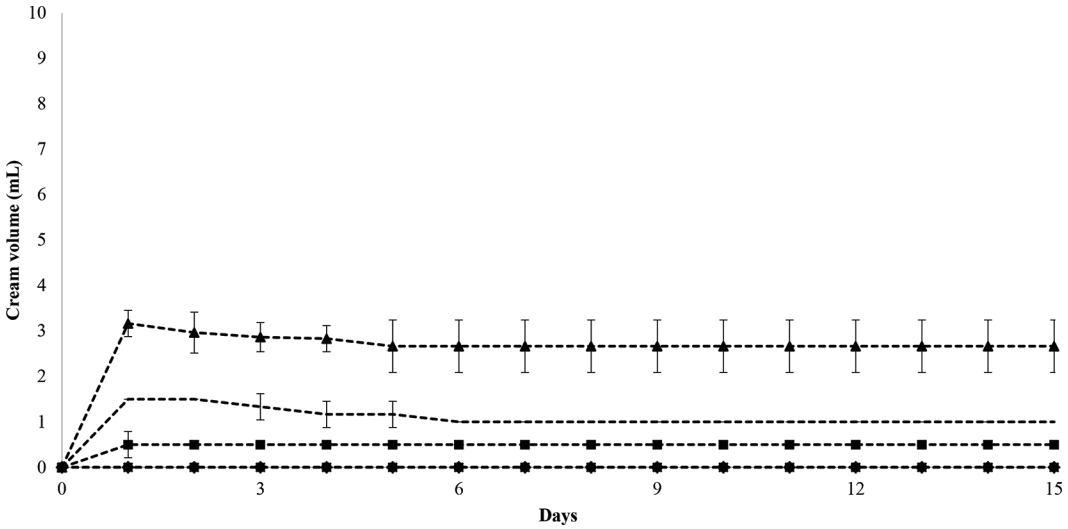

Figure 7. Creaming of milk samples. Data points of all ultra-shear technology–treated samples were identical and therefore the symbols are overlaid. HPP = high-pressure processed; UST = ultra-shear technology. (– indicates untreated raw milk; ■ indicates thermal process-72°C-15 s; ▲ indicates HPP-400 MPa-40°C-0 min; ◆ indicates HPP-400 MPa-40°C-3 min; × indicates UST-400 MPa-65°C-low flow rate; ● indicates UST-400 MPa-65°C-high flow rate; + indicates UST-400 MPa-35°C-low flow rate; * indicates UST-400 MPa-35°C-high flow rate.) Values are means obtained from 3 replicate samples from independent process runs; vertical error bars represent ±SD.

rectly to fat globule size and inversely to viscosity of the dispersed phase. Large fat globules or clusters are more sensitive to creaming effect (Walstra and Jenness, 1984).

The UST-treated samples with smaller particle sizes (Table 1) exhibited no creaming during the studied period. Process temperature and flow rates during UST treatment did not have significant influence on the creaming of samples. Significant reduction in particle size during UST treatment reduced the velocity of fat by multiple degrees and made samples stable. This result is consistent with the earlier observation by Pereda et al. (2007) who exposed fresh raw bovine milk to high-pressure homogenization and reported no creaming in treated milks during refrigerated storage.

Pressure-treated samples at 400 MPa for 0 min showed excessive creaming compared with untreated milk. Such samples showed rapid increase in cream volume up to 3.17 mL/10 mL in 24 h and subsequent leveling off at 2.67 mL/10 mL throughout the storage period (Figure 7). Huppertz et al. (2003) reported a 20% increase in cream volume as compared with untreated milk when treated at 400 MPa at 20°C for 0 min. The increased creaming might be defined by different mechanisms. HPP might have caused aggregation of lipoproteins in the MFGM (Kanno et al., 1998) resulting in clustering of milk fat (Huppertz et al., 2003). Huppertz et al., (2003) noted that the lipoproteins of serum portion of milk, called skim milk membrane (**SMM**), could have associated with the MFGM and/or SMM materials, which might have created SMM networks and facilitated formation of larger milk fat clusters, increasing creaming.

In contrast, pressure treatment at 400 MPa for 3 min did not cause creaming in samples during the studied period. Pressure treatment at above 400 MPa at 20 to 50°C for 5- to 30-min hold times causes denaturation of IgM, which influences its ability to bind with milk fat or SMM and inhibits clustering of fat (Felipe et al., 1997; Huppertz et al., 2003). Further, such treatment might cause disruption of casein micelles, formation of casein aggregates, and protein solubilization to increase the protein associated with fat globules and milk viscosity, thus decreasing creaming (Gervilla et al., 2001; Huppertz et al., 2003, 2011).

Thermal processing at 72°C for 15 s resulted in cream volume of 0.5 mL/10 mL milk in samples throughout storage. It was reported that heating milk above 70°C would presumably denature IgM and reduce creaming considerably (Rowland, 1937). But individual fat globules can rise to the top and exhibit some creaming, because the fat is not homogenized (Huppertz et al., 2003). Pereda et al. (2007) pasteurized raw bovine milk at 90°C for 15 s and observed creaming of <1mL/100 mL during refrigerated storage.

Lipase Activity. The relative lipase activity of UST- and HPP-treated milk samples as compared with untreated milk (100%) is shown in Figure 8. The pressure and subsequent shear action facilitated significant reduction ($P < 0.05$) in lipase activity as compared with untreated and batch-pressure-treated milk. After 65°C UST treatment at low- and high flow rates, the

relative lipase activities of samples were 56.63 ± 3.29 and 60.60 ± 6.36%, respectively, with no significant difference caused by flow rate. Datta et al. (2005) treated raw whole milk with high-pressure homogenization of milk at 200 MPa and increasing outlet temperature from 56 to 80°C and reported greater inactivation of lipase activity at higher outlet temperature with total inactivation happening at temperatures over 71°C.

The relative lipase activities of milk samples UST treated at 35°C at low- and high flow rates were 29.93 ± 9.21 and 31.01 ± 6.93%, respectively, with no significant difference caused by flow rate. The lipase activities of 35°C UST-treated samples were significantly ($P < 0.05$) less than untreated, HPP-treated, and 65°C UST-treated milk. This finding indicated the dominant role of thermal effects of UST treatment on lipase activity. In earlier studies where homogenization was performed at lesser pressures of 200 MPa (Datta et al., 2005) and 17 MPa (Wiking and Dickow, 2013), the lipase activity was observed to be reduced by increasing temperature. In the present study conducted at 400 MPa, the lipase activity of samples UST treated at 35°C were lesser than 65°C UST-treated samples. It is worth noting that, for UST treatment at 35°C, the initial temperature of milk was ∼15°C. The lower initial and process temperatures might have led to relatively less lipase activity. Jandal (1996) reported that cooling caused a decrease in lipase activity in cow milk (3.22 $\mu eqmL^{-1}$ h^{-1}) and a temperature range of 20 to 50°C had only slight influence on lipase activity.

The lipase activity of HPP-treated milk at 400 MPa for 0 and 3 min were 103.14 ± 10.36 and 114.75 ± 8.92%, respectively. Although activity of pressure come-up time (0 min holding) did not significantly differ from untreated milk, the pressure holding of 3 min showed significant ($P = 0.029$) increase over untreated milk. The result indicated the effect of increased hold time under isostatic pressure on lipase activity. This finding is consistent with Pandey and Ramaswamy (2004) who observed that exposure of raw milk to 400 MPa (at 3°C) for no hold time showed an enhancing effect (100%) on lipase and the activity continued increasing with increasing hold time up to 20 min (Pandey and Ramaswamy, 2004).

Lipase is generally unstable to heat treatment (Deeth, 2006). Several researchers have documented that the thermal pasteurization process at 72°C for 15 s can inactivate lipase activity in milk with no or little residual activity (Chandan and Shahani, 1964; Jandal, 1996; Pandey and Ramaswamy, 2004; Deeth, 2006).

It should be noted that lipase was baroresistant (400 MPa at ∼40°C) and the activity further enhanced with increased pressure hold time. However, the shear treatment following the pressure in UST reduced the lipase activity, with reduced activity at a modest process temperature of 35°C.

SDS-PAGE. The changes to the composition of protein fractions in batch HPP- and UST-treated samples as compared with untreated samples was obtained by SDS−PAGE electrophoresis (Figure 9). High-pressure processing at 400 MPa, ∼40°C, at 0 and 3 min hold times had least effect on casein protein fractions of milk samples (Figure 9a). However, the HPP treatment had marked effect on the major whey protein

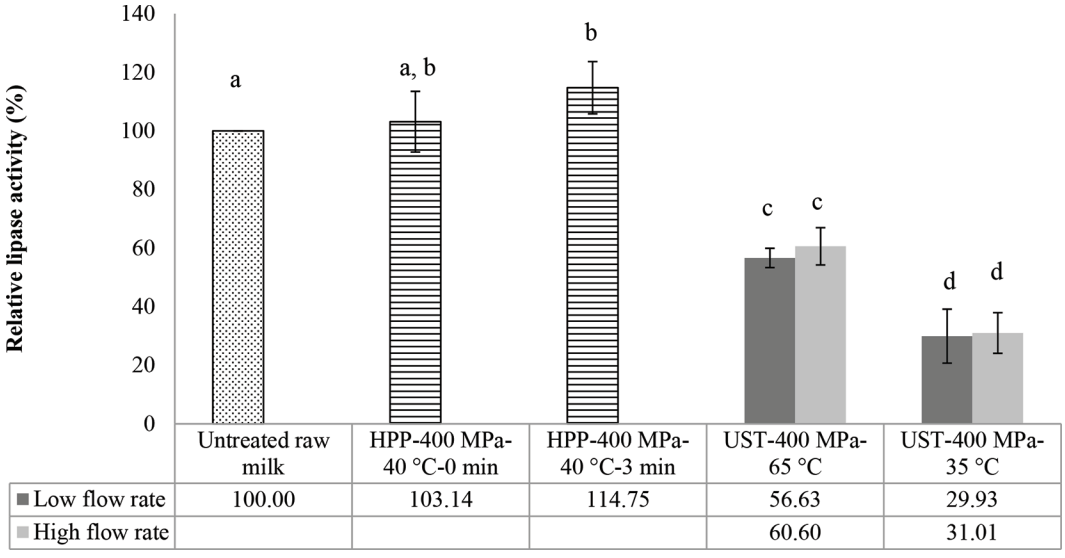

Figure 8. Relative lipase activity of milk samples. HPP = high-pressure processing; UST = ultra-shear technology. Error bars represent ±SD of 3 replicate samples obtained from 3 independent process runs. Labels (a, b, c, and d) above the bars represent statistically significant difference among different treatments ($P < 0.05$).

β-lactoglobulin, with the effect more pronounced for 3 min holding time, as evident by the relatively lesser band intensity. Earlier reports suggest higher sensitivity of β-lactoglobulin to high pressure due to the presence of a free sulfhydryl group and higher resistance of α-lactalbumin to high pressures due to rigid molecular structure and the lack of free sulfhydryl groups (Huppertz et al., 2004). High pressure of 400 MPa at ~20°C results in around 70 to 80% denaturation of β-lactoglobulin with the degree increasing with increasing hold time (Scollard et al., 2000; Huppertz et al., 2004). In contrast, α-lactalbumin in raw milk resists denaturation up to 500 MPa pressure (Garía-Risco et al., 2000). The casein protein fractions appeared to be less influenced under HPP conditions used in the present study. Huppertz et al. (2004) reported that casein micelles are disrupted by pressurization due to solubilization of micellar calcium phosphate and subsequently the casein particles are re-associated upon depressurization. Authors also noted that reassociation might not happen above certain threshold conditions (i.e, pressure >300 MPa for 30 min; Huppertz et al., 2004).

Figures 9b and 9c show the gel patterns of UST-treated samples at 65°C and 35°C, respectively, at different flow rates. When comparing the gel patterns, some interesting differences were observed. In the UST-treated samples at 65°C, the bands of whey proteins; namely, α-lactalbumin and β-lactoglobulin, indicated denaturation as compared with untreated milk. Further, higher molecular weight protein aggregates were noted at the topmost band of gels in 65°C UST-treated samples, corroborating the observation. The denaturation of proteins might be due to the interactive effects of heat, pressure, and shear in UST treatment (Desrumaux and Marcand, 2002; Hayes et al., 2005).

On the other hand, in the UST-treated samples at 35°C, the gel pattern and absence of high molecular weight protein aggregates on top indicated less denaturation in protein fractions (Figure 9c). Removal of thermal effect during 35°C UST treatment by cooling might have minimized protein changes. This effect was also corroborated by the occurrence of more proteins in the supernatant of 35°C UST-treated samples (Figure 10).

Protein Aggregation in UST-Treated Samples. The amount of aggregated protein in the UST-treated samples at 65°C and 35°C as compared with untreated samples are shown in Figure 10. Although untreated milk had 0.927% protein, UST treatment had lesser proteins in the supernatant, indicating relatively higher whey protein denaturation in these samples. It was also interesting to note that samples after 65°C UST treatment showed higher denaturation of whey proteins than samples after 35°C UST treatment. Under 35°C UST treatment at high flow rate the supernatant protein (0.78%) was not significantly different from the untreated sample. Interestingly, the viscosity of 65°C UST-treated samples were slightly higher than 35°C UST-treated samples, which could be attributed to higher whey protein denaturation at higher temperatures (Table 2). Li et al. (2018) observed increased denatured whey protein in ultrapasteurized milk (140°C/2.3 s) as compared with HTST milk (78°C/15s) and the aggregates formed by denatured proteins resulted in increased viscosity in ultrapasteurized milk samples. This

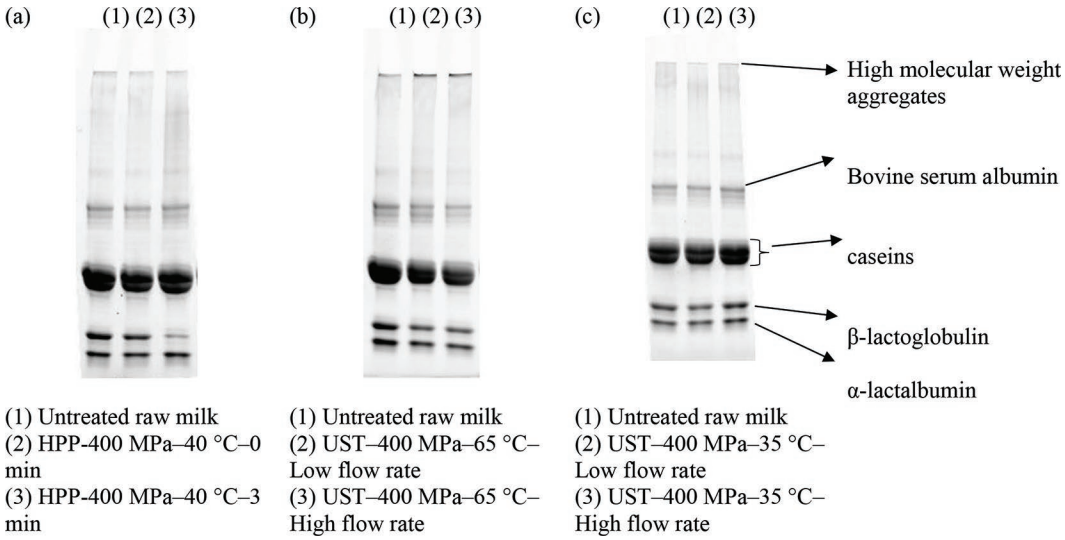

Figure 9. SDS-PAGE analysis of milk samples (the protein fractions are segregated as per molecular weight of the proteins and labeled). HPP = high-pressure processing; UST = ultra-shear technology.

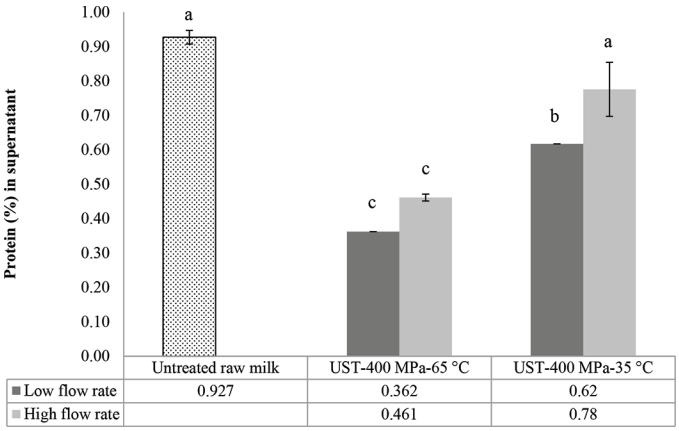

Figure 10. Protein in the supernatant of milk samples. UST = ultra-shear technology. Error bars represent ±SD of 3 replicate samples obtained from 3 independent process runs. Labels (a, b, and c) above the bars represent statistically significant difference among different treatments ($P < 0.05$).

indicated that when the temperature of UST treatment is lesser, the degree of protein denaturation and viscosity in milk could be lesser.

CONCLUSIONS

The pressure-only treatment did not reduce particle size and seemingly increased the viscosity, creaming, and lipase activity of samples as compared with untreated milk. The thermal-only treatment provided a slight reduction in particle size and creaming in milk. The UST treatment, which involved high pressure and subsequent shear action performed at 65 and 35°C, facilitated particle size reduction and eliminated creaming in samples. Within the experimental conditions, process temperature did not have any effect on particle size, zeta potential, viscosity, creaming, or pH. However, UST temperature had marked effect on lipase activity and proteins, with 35°C retaining better protein quality and reducing greater lipase enzyme activity. Therefore, use of milder process temperature in UST is desired for the preservation of milk quality attributes. The flow rates, despite producing different rates of temperature rise, did not exhibit significant difference on most quality attributes studied. This finding could allow flexibility in designing crucial components such as shear valves for dairy beverages. Further, the physical changes and resulting chemical changes at the molecular level, including sensorial and nutritional changes, due to UST need to be studied. The findings revealed the differential effect of pressure, shear, temperature, and their interactions during UST treatment on raw milk quality. This information would be valuable for equipment providers to design shear valves taking into consideration the effect of treatment intensity on the product matrix. By suitable choice of pressure and thermal intensity, UST will also serve as a tool for food processors in introducing homogenized value-added pasteurized or shelf-stable milk protein beverages desired by consumers.

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