fluids

MDPI

Article

An Experimental Study on Human Milk Rheology: Behavior Changes from External Factors

Diana Alatalo * and Fatemeh Hassanipour *

Department of Mechanical Engineering, The University of Texas at Dallas, 800 W. Campbell Rd, Richardson, TX 75080, USA

* Correspondence: diana.alatalo@utdallas.edu (D.A); fatemeh@utdallas.edu (F.H.)

Received: 29 February 2020; Accepted: 18 March 2020; Published: 27 March 2020

Abstract: The influence of external factors, including temperature, storage, aging, time, and shear rate, on the general rheological behavior of raw human milk is investigated. Rotational and oscillatory experiments were performed. Human milk showed non-Newtonian, shear-thinning, thixotropic behavior with both yield and flow stresses. Storage and aging increased milk density and decreased viscosity. In general, increases in temperature lowered density and viscosity with periods of inconsistent behavior noted between 6–16 °C and over 40 °C. Non-homogeneous breakdown between the yield and flow stresses was found which, when coupled with thixotropy, helps identify the source of nutrient losses during tube feeding.

Keywords: human milk; tube feeding; breastfeeding; viscosity; complex modulus; density

1. Introduction

Milk is a species-specific bio-fluid produced in the mammary gland and traditionally fed directly to young at the breast. In humans, exclusive breastfeeding is recommended for the first 6 months of life with continued breastfeeding until 1 year of age or longer with introduction of complementary foods [1]. When infants cannot feed directly at the breast, mothers express their milk to be fed by artificial methods, such as gastric tubes, cups, or bottles [2]. Storage and feeding methods can result in nutrient losses in expressed human milk [3–5] which indicates that rheological changes in milk occur during storage and negatively impact flow. The main aim of this work is to explore rheological behavior of human milk and how external factors impact that behavior.

Most rheological studies on mammalian milk occur with dairy animals, predominately bovine. Studies often formed relationships between specific components in milk (i.e., fat), viscosity, and some external factor. External factors known to impact viscosity include pressure, temperature, pH, pasteurization, and homogenization [6–17]. Regression equations have been developed for different applications and are presented in Table 1. These equations show that the basic rheological behavior of bovine milk in regards to temperature appears to be consistent; milk viscosity decreases as temperature increases. This decrease is dependent on whether or not the proteins have begun to denature [10] and the concentration of proteins and fats. Difficulty comparing the studies lies in the lack of details, which are summarized in Appendix A.

Modern studies use homogenized and pasteurized milk or milk products and assume Newtonian behavior. However, an early work [6] found that raw bovine milk, both skim and whole, is a shear-thinning non-Newtonian fluid with greater variability at low pressures. Repeated trials of milk through capillary tubes at constant pressure resulted in lowering whole milk viscosity with repeated runs (thixotropic) but no change in skim milk viscosity. The decrease in whole milk viscosity with repeated experiments was even more evident as the age of the milk increased. Repeated trials with homogenized whole milk produced results similar to skim milk with viscosity remaining constant

during each run. The authors attributed the decrease in viscosity in raw whole milk from repeated runs through a capillary to clumps of fat globules breaking up. They further investigated the effect of aging on raw skim milk viscosity since previous studies found aging to increase the viscosity of raw whole milk. Their results found that refrigeration of skim milk increased viscosity but freezing initially decreased viscosity which later increased with longer freeze times. The authors concluded that bovine milk viscosity was dependent on shearing force, age, method of storage, and mechanical agitation (for raw whole milk). All testing occurred at 25 °C, below the melting temperature of bovine milk fat [18].

Table 1. Regression equations for bovine viscosity and density.

Author	Regression Equation and Nomenclature
Snoeren et al. [10]	$\mu = \mu_{ref}[1 + \frac{1.25(\phi_c+\phi_{nw}+\phi_{dw})}{1-\frac{\phi_c+\phi_{nw}+\phi_{dw}}{\phi_{max}}}]^2$ $\mu_{ref}(cP)$: viscosity of medium ϕ_c: volume fractions of casein ϕ_{nw}: volume fractions of native whey protein ϕ_{dw}: volume fractions of denatured whey protein ϕ_{max}: maximum volume fractions of all protein
Jebson and Chen [11]	$ln\ \mu = 3.911 + 0.0202(\frac{S-482.5}{0.85}) - 0.1291(\frac{T-52.5}{7.5})$ S: solids content (g/kg) T: temperature (°K)
Phipps [13]	$log_{10}\ \mu = [1.2876 + 11.07 \times 10^{-4}T_C][F + F^{\frac{5}{3}}] + \frac{0.7687\times10^3}{T_K} - 2.4370$ F: fat content (%) T_C: temperature (°C) T_K: temperature (°K)
Bakshi and Smith [12]	$ln\ \mu = -8.9 + 0.1F + \frac{2721.5}{T}$ $\rho = 0.3T - 0.03T^2 - 0.7F + 1034.5$ F: fat content (%) T: temperature (°K)

Human milk rheological studies are limited. An early work by Blair noted shear-thinning behavior but determined the decrease to be so minor that milk could be classified as Newtonian [19]. Blair's work was limited to shear rates above 100 s^{-1} but provided the basis for a major study on viscosity of raw human milk by Waller et al. Assuming that raw human milk was Newtonian, Waller et al. explored the kinematic viscosity of human milk during the first 10 days of lactation, when the composition of milk, particularly proteins, changes the most [20]. They tested the samples at 37 °C when milk fat was liquid [18]. Kinematic viscosity significantly decreased during the first 10 days postpartum which corresponded to the drop in total nitrogen content. A linear relationship between the log of kinematic viscosity, ν, and total nitrogen content, c, was determined and expressed as $\log\ \nu = 0.65c - 0.07$.

Waller et al. [20] further investigated the decrease in kinematic viscosity by exploring the relationship between casein and globulin, two known protein nitrogens, which undergoes a significant change during the first 14 days of lactation before becoming almost constant. This work demonstrated a relationship between human milk content and viscosity yet is difficult to compare with other works. Handling of samples prior to testing was inconsistent as some samples were fresh while others were previously refrigerated, and time between collection and testing varied. Also, density differences were not disclosed nor discussed.

A few more recent studies examined human milk viscosity with most research assuming Newtonian flow behavior [21–26]. Almeida et al. studied human milk viscosity in regards to aging as it pertains to clinical treatment of infants with dysphagia and determined no significant changes occurred when previously frozen human milk was reheated and then maintained at 37 °C over 9 h [21]. A subsequent study in regards to managing infant dysphagia tested previously frozen human milk samples from 2 donors at 25 °C from 1–1000 s^{-1} and found shear-thinning with greater variability in viscosity when compared with infant formula [23]. Another study concerning lipid digestion by infants

noted shear-thinning using a logarithmic shear rate sweep from 0.5–500 s^{-1} at 37 °C on previously refrigerated human milk [22]. In a separate test conducted at an arbitrarily chosen 20 s^{-1}, they dropped milk pH from 6.5 to 4.0 with no changes in viscosity. The authors did not show the results of the viscosity testing nor was density mentioned.

In light of the importance of human milk to the health and development of infants, this work aims to explore the general rheological behavior of human milk in response to temperature, storage (refrigeration/freezing), aging (constant body temperature), time (thixotropy), and shear rate with additional consideration of the intra-individual milk content variations between breasts of the same woman (inter-breast) and over the course of a single expression of milk by pump or infant suckling (intra-feed). Particular attention is given to low shear rates and body temperature, as experienced in tube feeding [4,5,27,28].

2. Materials and Methods

2.1. Recruitment and Milk Collection

A total of 8 participants were recruited and provided informed consent at different points in the study (for further details concerning recruitment and milk collection procedures, please refer to [29]). The Institutional Review Board of University of Texas at Tyler approved the study (IRB 15–10). Participants #1–6 expressed simultaneously from both left (L) and right (R) breasts continuously until milk flow stopped using double electric vacuum pumps. Participants who expressed larger volumes had 20 mL separated from total volume for testing unless participant opted to donate the entire expression (Participants #2, #5, & #6). Participants #7–8 expressed approximately 15 mL at the beginning of their expression/nursing (hereafter referred to as foremilk (F)) and additional milk at the end of their expression/nursing (hereafter referred to as hindmilk (H)). Participant #8 expressed using a double electric vacuum pump while Participant #7 hand expressed before and after breastfeeding her infant only from the suckled breast. In total 15 raw human milk samples were obtained ranging in volume from 5 mL to 52 mL. Macronutrient content was calculated as outlined in Appendix B. All fresh samples were tested within 5 h of expression with most tests beginning within 30 min. Any volume not aliquoted for fresh testing was immediately refrigerated/frozen as a whole (not in aliquots). Refrigerated samples were held at 4 °C for up to 5 days while frozen samples were held at −20 °C for 3 months until thawed in a warm water bath between 30–40 °C. After thawing, samples were held at 4 °C for up to 5 days.

2.2. Experimental Methods

The procedures for density and viscosity testing are detailed by Alatalo and Hassanipour [29] with an overview provided below. Density (ρ) was measured at temperature equilibrium with an Anton-Paar DMA 4500M density meter with an accuracy rating of ± 0.00005 g cm^{-3} and ± 0.03 °C. To study the effect of storage, density was calculated at a single temperature for Participants #1–6 within 10 min after expression and compared with the results of density after freezing and thawing. The density of the foremilk and hindmilk of Participant #8 was tested the same day as expressed and after refrigeration at 4 °C to determine the effect of time of expression (hereafter referred to as intra-feed variations) and storage by refrigeration. Testing parameters for experiments on the density meter are outlined in Table 2.

Table 2. Density Testing Details.

Parameter	Test # 1	Test # 2
Temperature Range	3–50 °C	36–41 °C
Tested Sample(s)	#1–6 *	#8 **
Total Data Points	40	6
Measured At	Sample Equilibrium	
Recording Frequency	Every °C	
Sample Volume	1.3 mL	

* After freezing; ** Fresh.

Viscosity experiments were performed using an Anton-Paar MCR-302 rheometer. Shear-dependent rotational experiments used a cone-plate measuring system with Peltier temperature controlled plate and hood. The cone measured 49.9384 mm in diameter with a 50 µm truncation and 0.49° angle. Since the focus of this study is on low shear rates and sample availability was limited, the cone-plate measuring system allowed for more experiments as testing required loading volumes of 0.4 mL trimmed to 0.29 mL. The test parameters were initially determined by testing whole raw bovine milk purchased from Lavon Farms, Plano, Texas, USA, and are outlined in Table 3. Two separate shear rate ranges were tested. For both tests, samples were brought to 37 °C and held at that temperature for 4 min before applying shear. Body temperature was chosen due to the practice of warming infant feeds to body temperature, particularly in fragile and preterm infants [27,28]. Three separate temperature sweep ranges were tested using a linear ramp profile of 1 °C every minute. All tests occurred with a 4 min pre-shear of 50 s^{-1} at the initial temperature for the individual sweep. The viscosity was read after the pre-shear and compared with the first data reading of the sweep. This comparison was made to ensure that temperature was the only variable affecting the viscosity readings during the sweep. The shear rate of 50 s^{-1} was chosen based on the shear rate point used by the National Dysphagia Diet for classification of food thickness and to allow for comparison with results published by Frazier et al. [23]. The loop test followed the same 4 min rest period at 37 °C as the shear rate tests.

Oscillatory experiments on the Anton-Paar MCR-302 were performed using a double gap cylinder measuring system with Peltier temperature control. The use of a double-gap cylinder measuring system requires greater sample volume, 4 mL, but significantly increases the available shear area enabling uniform shear conditions on both the inner and outer walls and detects lower torques better compared to other measuring systems [30]. The bob effective length, inner diameter and outer diameter are 40, 24.66 and 26.66 mm respectively. The cup inner diameter is 23.826 mm and outer diameter is 27.592 mm. Samples from Participants #2, #5, and #6 from both left and right breasts were heated to 37 °C and held at that temperature for 4 min before applying shear strain. The oscillating shear strain was applied using a logarithmic ramp from 0.01% to 1000% with a constant angular frequency of 5 rad s^{-1} which approximates infant suckling [31]. The duration of the test was set by the device. Testing parameters for all experiments on the rheometer are summarized in Table 3.

The complex shear modulus, $G^{\star}$, was broken down into its two components: (1) G'—the storage modulus that characterizes the elastic behavior—and (2) G''—the loss modulus that characterizes the viscous behavior. The limit of the linear viscoelastic (LVE) region, γ_L, was used to determine the yield point, τ_y, for each sample. For consistency between samples, γ_L was determined at the highest G' value before the slope of the curve became negative. The flow point, τ_f, was determined from the crossover points for G' and G'' where viscous behavior begins to dominate. While some authors associate the crossover stress and strain as the yield point, this association is inaccurate since G' and G'' are only valid in the LVE region [32]. The final characteristic of raw human milk considered in this study was the flow transition index (FTI). The FTI is the ratio of τ_y to τ_f and describes the transition behavior of the milk from the LVE region until flow begins.

Table 3. Viscosity Testing Details.

Parameter	Shear Rate Sweep		Temperature Sweep			Shear Rate Loop	Amplitude Sweep
	Test #1	Test #2	Test #1	Test #2	Test #3		
Temperature(s)	37 °C		0–50 °C	29–45 °C	36–43 °C	37 °C	37 °C
Sample(s)	#1–6 *, 8 **	#1–6 *, 8 **	#1–6 *, 8 ***	#1–6 *	#7 **	#8 ***	#2 *, 5 *, 6 *
# Data Points	100	40	51	33	8	400	25
Measured At	Linear Ramp 1–100 s^{-1}	Linear Ramp 0.01–20 s^{-1}	50 s^{-1}	50 s^{-1}	50 s^{-1}	1–200–1 s^{-1}	0.01–1000% at ω = 5 rad s^{-1}
Point Density	1 $\dot{\gamma}^{-1}$	2 $\dot{\gamma}^{-1}$	1 °C 60 s^{-1}	1 °C 30 s^{-1}	1 °C 60 s^{-1}	1 $\dot{\gamma}^{-1}$	6 $decade^{-1}$
Recording Frequency	Constant Every 2 s	Linear Ramp 10–1 s	Constant Every 60 s	Constant Every 30 s	Constant Every 60 s	Constant Every 1 s	Set By Device
Volume	0.29 mL	0.29 mL	0.29 mL	0.29 mL	0.29 mL	0.29 mL	4.0 mL
Test Time	440 s	460 s	55 min	21 min	12 min	640 s	Varied

* After freezing only; ** Fresh & After refrigeration; ***After refrigeration only.

2.3. Uncertainty Analysis

Limited sample volume often restricts the number of times experiments can be repeated. When dealing with a biofluid, the composition from an individual is in constant flux and would require either pooling samples or limiting experiments to single-samples (no repetition). One of the goals of this work is to explore the breadth of rheological behavior of human milk normally found in nature. In keeping with that purpose and the limited sample sizes noted in Section 2.1, repetition of experiments was minimized. Since density is assumed to be incompressible, the accuracy of the meter was considered sufficient for uncertainty. To estimate the uncertainty of rheological single-sample experiments, the Kline-McClintock method [33] was employed and described herein.

The Anton-Paar MCR-302 rheometer has torque resolution of 0.1 nNm, angle resolution (determined from displacement by optical encoder) of 10 nrad, temperature resolution of 0.1 °C, and time constant of 5 ms. Viscosity measurements for cone-plate system are calculated from McKennell [34]:

$$\mu = \frac{3\alpha T}{2\pi r^3 \omega} \tag{1}$$

where α is cone angle, T is measured torque, r is cone radius, and ω is rotational speed. The uncertainty of the cone geometry is $\pm 0.005^\circ$ for angle and ± 0.00005 mm for diameter. Using Kline-McClintock [33] analysis, the uncertainty for the cone-plate measuring system are determined using:

$$w_\mu = \sqrt{\left(\frac{3\times 10^{-10}\alpha}{2\pi r^3\omega}\right)^2 + \left(\frac{10^{-3}T}{24r^3\omega}\right)^2 + \left(\frac{-1.5\times 10^{-8}\alpha T}{\pi r^3\omega^2}\right)^2 + \left(\frac{-7.5\times 10^{-8}\alpha T}{\pi r^4\omega}\right)^2} \tag{2}$$

with $r = 0.0249692$ m and $\alpha = 0.49^\circ$ for all rotational tests. The vast majority of w_μ remained under 1.03% with less than 10 data points having a higher uncertainty at $\dot{\gamma} \leq 1\ \mathrm{s}^{-1}$. The maximum uncertainty was 8.20% for w_μ = 0.3 mPa s at $\dot{\gamma}$ = 0.01 s^{-1}.

3. Results

3.1. Milk Content

The macronutrient content of milk samples, presented in Table 4, was assumed to match reference values from clinical studies. These values do not account for the natural variations found between breasts of the same participant [35] but provide reasonable expected values for evaluation of data. Further discussion of these values is provided in Appendix B.

Table 4. Estimated Macronutrient Content.

Participant	Month of Lactation	Carbohydrates (g/100 mL)	Proteins (g/100 mL)	Fats (g/100 mL)
#1	9.0	7.01	0.80	4.11
#2	16.0	6.80	0.97	5.23
#3	12.5	6.91	0.90	4.67
#4	12.25	6.91	0.89	4.63
#5	3.5	7.18	0.66	3.23
#6	4.25	7.16	0.68	3.35
#7F	8.0	7.04	0.78	3.17
#7H	8.0	7.04	0.78	6.61
#8F	1.0	7.26	0.60	2.25
#8H	1.0	7.26	0.60	4.81

3.2. Flow Behavior of Human Milk

Human milk demonstrates shear-thinning non-Newtonian behavior in response to increasing shear rates (see Figure 1a) as reported regarding human milk by [22,23]. While the slope of the curve decreases at higher shear rates, viscosity never becomes constant over the range of shear rates tested. Conversely, as shear rate approaches 0 s^{-1}, viscosity approaches infinity. This flow behavior is especially evident in the Shear Rate Sweep Test #2 results in Figure 1b, which began at 0.01 s^{-1} and provides greater detail of rheological behavior at or near zero. The standard deviation (SD) for milk viscosity was largest at lower shear rates (±683.6 mPa s at 0.01 s^{-1}) and consistently decreased as shear rate increased (±0.7 mPa s at 100 s^{-1}). By 61 s^{-1}, the standard deviation was ≤1 mPa s. While milk with the lowest viscosity values fell within the expected range of standard deviation, the highest viscosity milk remained outside the standard deviation range for the entire sweep. Some milk demonstrated an oscillatory viscosity pattern at low shear rates, generally between 10–60 s^{-1}, that was obscured when calculating the mean viscosity for the samples [29]. Two likely sources for these viscosity patterns are deformation of fat globules, which usually range in size from 1–10 µm, and the breakdown of casein micelles (comprising approximately 13% of total protein) into smaller micelles [36] similar to breaking up red cell aggregation in blood [37].

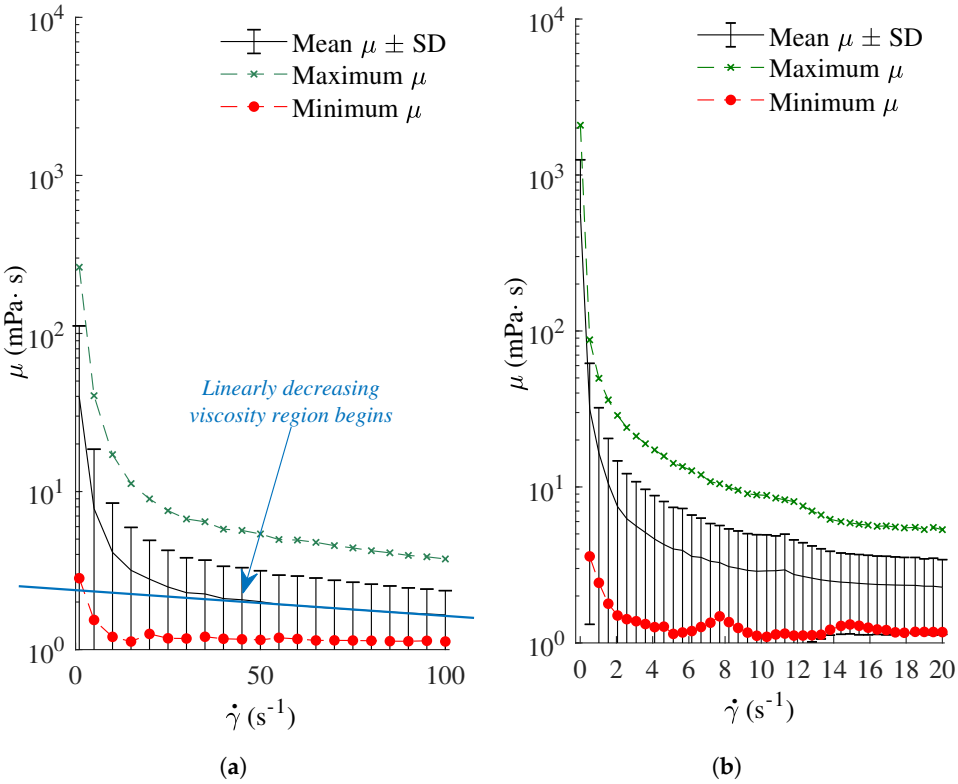

Figure 1. Shear-thinning flow behavior of human milk. Raw human milk demonstrates shear-thinning flow behavior with μ decreasing as $\dot{\gamma}$ increases. The maximum and minimum μ values for each Shear Rate Sweep Test are presented to show the range of data in comparison with the mean and SD. (**a**) Shear Rate Sweep Test #1 (1–100 s^{-1}) shows a continuously decreasing slope that never reaches Newtonian. (**b**) Shear Rate Sweep Test #2 highlights $\lim_{\dot{\gamma}\to 0} \mu \to \infty$.

To determine the presence of time dependence for the viscosity, the Shear Rate Loop Test from 1 s^{-1} to 200 s^{-1} and back to 1 s^{-1} was performed on the 5-day post-refrigerated milk from Participant #8. The result of each sample was similar with a maximum uncertainty of 0.22 mPa s calculated from Equation (2) (see Figure 2) and confirmed human milk to be a thixotropic fluid.

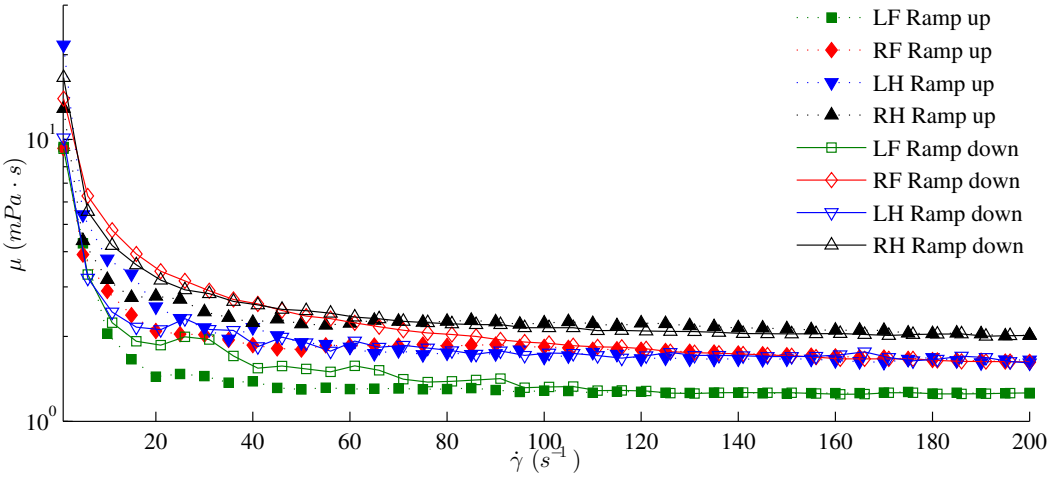

Figure 2. Evidence of time dependence for human milk flow properties. Shear Rate Loop Test demonstrates time dependence for human milk for both foremilk and hindmilk. Viscosity of milk from the right breast ended higher while milk from the left breast ended lower and shows inter-breast variation (see Section 3.4).

Rheological rotational tests where viscosity approaches infinity as shear rate approaches 0 s^{-1} indicate the existence of a yield stress that defines a plastic fluid. This behavior was seen in every Shear Rate Sweep Test for human milk, which suggests that raw human milk has a yield point (i.e., plastic fluid) and firm texture when at rest. The Amplitude Sweep Test with controlled shear strain allowed for calculation of shear stress, phase shift, complex modulus, storage modulus, and loss modulus that confirmed the viscoelastic behavior of raw human milk, particularly as it pertains to infant suckling behavior. All samples had G′ values greater than G″ in the LVE region as shown in Figure 3a for Participant #6, although the differences between G′ and G″ were small (0.2936 ± 0.5200 Pa). This relationship classifies raw human milk as a viscoelastic solid (gel-like). Since G′ increased linearly for some samples in the LVE region, such as Sample #6L in Figure 3a, the yield point was confirmed using the stress-strain curve as shown in Figure 4. The increase in G′ within the LVE region indicates a strengthening of structure under small amplitudes. Increases in intermolecular crosslinking, aggregation, and particle size of proteins within raw milk has been found in response to increases in shear rate below 500 s^{-1} which likely accounts for the increase in G′ [38]. The mean γ_L was 1.10 ± 0.76%.

The shear stress necessary to begin breakdown of structure and initiate flow, τ_y, varied between samples with a mean value of 13.903 ± 26.901 mPa showing a wide range of expected values. Inter-breast differences ranged from a factor of 5 to a factor of 100 with each participant having milk from one breast that required <1 mPa to initiate yielding (intra-individual variations discussed further in Section 3.4). Homogeneous flow begins at τ_f when G′ crosses G″, as shown in Figure 3a for Participant #6, allowing for viscous dominated flow behavior. The mean τ_f was 24.28 ± 35.68 mPa. Both τ_y and τ_f can be seen in Figure 3b. The mean difference between τ_y and τ_f for each sample was 10.38 ± 10.01 mPa. FTI values, which describe the transition behavior of milk from the LVE region τ_f, ranged from 1.39 to 22.17 with mean FTI of 6.34 ± 8.08. A summary of these points are provided in Table 5.

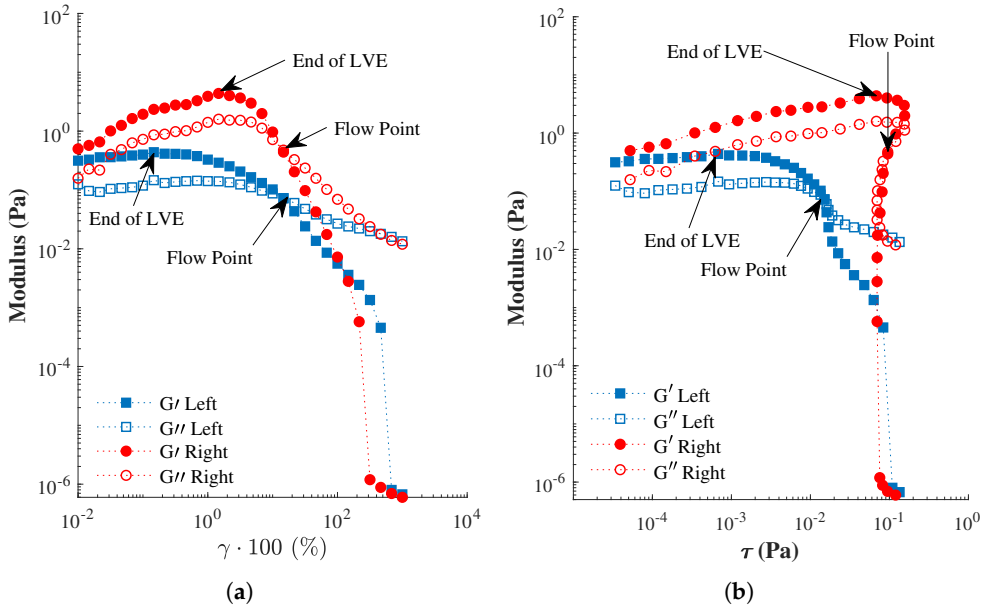

Figure 3. The viscoelastic behavior of human milk represented by milk from Participant #6. (**a**) The deformation at the limit of LVE, γ_y, and crossover of G′ and G″. (**b**) The corresponding shear stress required for yield and flow of human milk.

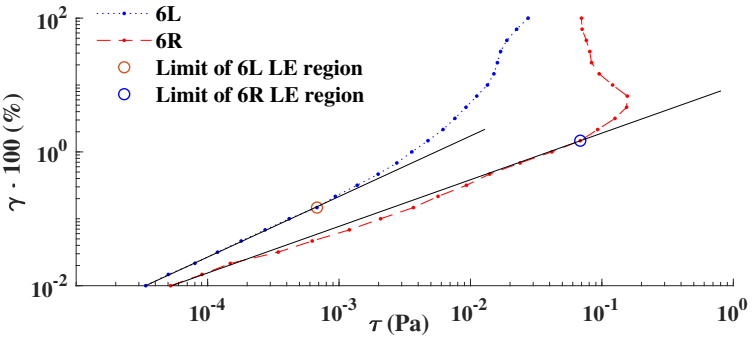

Figure 4. Determining the yield point by finding the limit of the linear-elastic (LE) region. When a straight line is fitted to the linear region of the stress-strain curve, where the Law of Elasticity applies, the final point before the yield exceeds the line corresponds to the end of the LVE shown in Figure 3.

Table 5. Limit of LVE region (γ_L), yield point (τ_y), flow point (τ_f), and flow transition index (FTI) values for six samples.

Sample	Density (g cm^{-3})	γ_L (%)	τ_y (mPa)	τ_f (mPa)	FTI
2R	1.02432	1.47	8.428	22.37	2.65
2L	1.02656	0.32	0.220	1.68	7.63
5R	1.03002	1.00	0.891	2.00	2.24
5L	1.02604	2.16	4.766	9.26	1.94
6R	1.02210	1.47	68.433	95.30	1.39
6L	1.02795	0.15	0.680	15.07	22.17
mean ± SD		1.10 ± 0.76	13.903 ± 26.901	24.28 ± 35.68	6.34 ± 8.08

3.3. Effect of Temperature on Human Milk Density and Viscosity

The effect of temperature on density for the first 12 samples post freezing, expressed as specific gravity (ρ_{milk}/ρ_{water}) in Figure 5, shows a decrease as the temperature increases. The density decrease was approximately 0.02 g cm^{-3} over 47 °C for each sample, although the decrease is not linear. The slopes

for specific gravity curves decrease above 20 °C and indicate that at lower temperatures volume changes of components within the milk exert a larger influence on density than at higher temperatures.

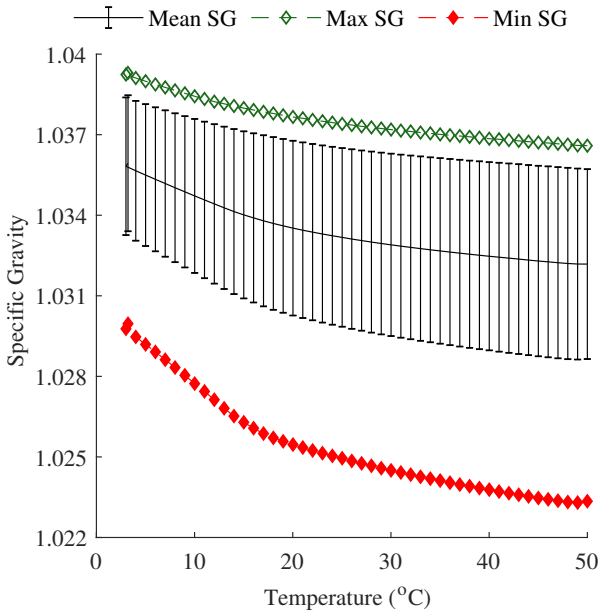

Figure 5. Human milk density response to temperature. Specific gravity (SG) (ρ_{milk}/ρ_{water}) shows greater changes in lower temperature range. The maximum and minimum SG values both fall outside the expected standard deviation (SD) range shown in the figure.

Temperature influences on viscosity were detected by Temperature Sweep Tests #1 and #2. The pre-shear viscosity value was compared to the first viscosity recorded during the actual temperature sweep with only 2 pre-shear viscosity values varying greater than 1 mPa s compared to the first data point in the sweep. These results indicated that 4 min was sufficient time for the milk particles to orient themselves and to isolate the effect of temperature on the viscosity. Temperatures greater than 50 °C were avoided to prevent denaturization of proteins that would alter viscosity [14]. In general an increase in temperature decreased the viscosity. However as shown in Figure 6a, some samples demonstrated an increase in viscosity between 6–16 °C before returning to anticipated behavior resulting in the mean temperature rise seen between 8–11 °C. This range corresponds to the slope change in specific gravity seen in Figure 5. All the samples that were tested to 50 °C demonstrated an increase in viscosity beginning as early as 41 °C for some samples and all samples by 46 °C. Temperature Sweep Test #2 used a new loading of sample from Participants #1–6 over a smaller temperature range (29–45 °C) and faster recording frequency to shorten the total testing time and ensure that viscosity increases above 40 °C were not related to sample drying due to the long test time in Temperature Sweep Test #1. The results from both temperature sweeps were averaged over normal milk reheating temperature ranges and presented in Figure 6b.

A linear approximation of each individual Temperature Sweep Test #1 and #2 result for Samples #1–6 was completed in MATLAB using the "fit" function and polynomial model "poly1". The mean ± SD slope from 36–40 °C was −0.11941 ± 0.13916. All but one sample had a negative slope indicating a decrease in viscosity as temperature increased. The slope for Temperature Sweep Test #2 (29–45 °C) result for Sample #6L was positive (0.06988) which prompted a second test with a new loading that also resulted in a positive slope (0.1078). Both the density and specific gravity slopes over that same temperature range were unremarkable for Sample #6L. The slope for Temperature Sweep Test #1 (0–50 °C) for Sample #6L was negative (−0.07364) as expected. The only noted differences between the tests were in point density and recording frequency. The possibility exists that Temperature Sweep Test #2 for Sample #6L with repetition contained experimental errors since the slopes were inconsistent

with all other results, although the positive slopes were so small that the resultant viscosity values, which increase with temperature increase, fail to be significant.

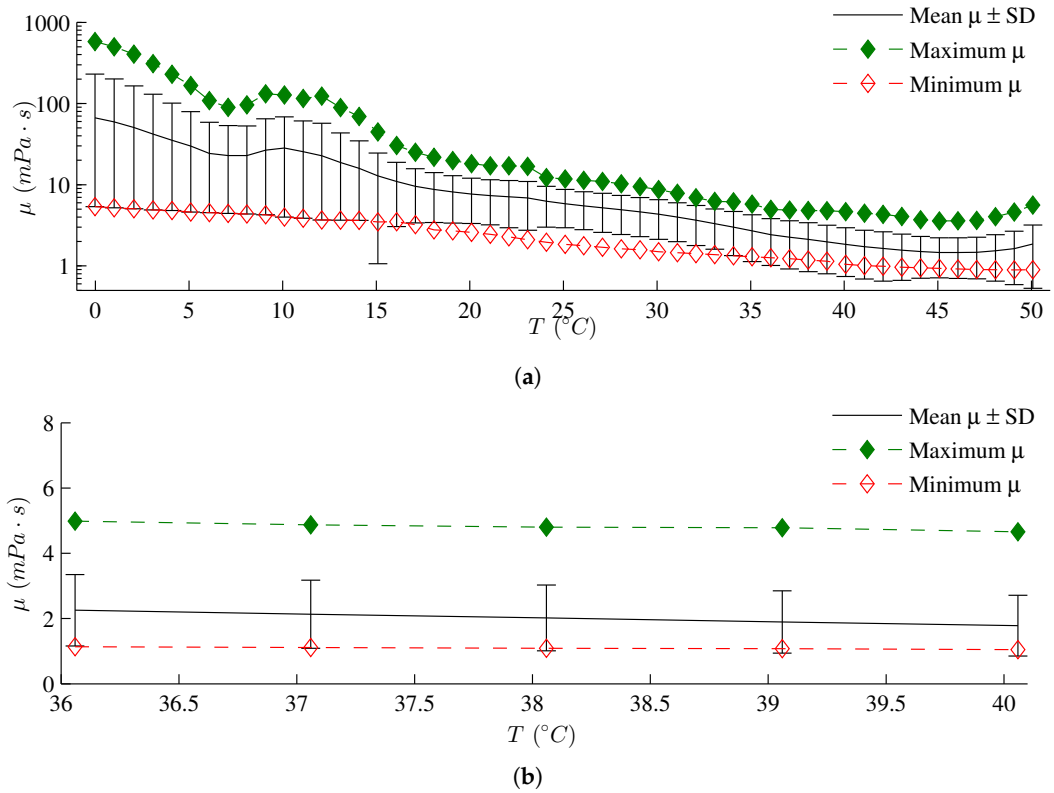

Figure 6. Human milk viscosity response to temperature at 50 s^{-1}. Human milk viscosity generally decreased as temperature increased. The highest (maximum) and lowest (minimum) viscosity values are presented to show the range of recorded data with maximum viscosity falling outside the expected value range of SD. (**a**) Unexpected increases in mean and maximum viscosity began at 8 °C and 46 °C. (**b**) While temperature decreased viscosity over normal reheating temperature ranges, the decrease was minimal.

3.4. Changes in Density and Viscosity Associated with Intra-Individual Human Milk Variations

Inter-breast density varied among participants and likely stems from the known inter-breast variations in milk composition, which can be significant [35] (further discussion in Appendix B). At 37 °C the difference between breasts ranged from as high as 0.00730 g cm^{-3} for Participant #1 to as low as 0.00011 g cm^{-3} for Participant #4. The mean difference was 0.00289 g cm^{-3} with details for each participant shown in Table 6.

Table 6. Human milk, after thawing from the same participant but different breasts, shows differences in ρ (g cm^{-3}) at 37 °C.

Participant	Right Breast	Left Breast	Difference
1	1.02443	1.01713	0.00730
2	1.02432	1.02656	0.00224
3	1.02583	1.02883	0.00300
4	1.02750	1.02761	0.00011
5	1.03002	1.02604	0.00398
6	1.02210	1.02795	0.00585
Mean ± SD			0.00289 ± 0.00269

The mean±SD density of fresh milk foremilk and hindmilk for Participant #8 is shown Figure 7 and reflects intra-feed variations that correspond to intra-feed variations in fat seen in Table 4. Milk reheating practices and temperatures can vary [28], so the small temperature range around 37 °C in Figure 7 highlights the degree of change in density with small fluctuations in temperature. All fresh samples were held at 37 °C until time of testing. The difference between fresh foremilk and hindmilk density in Figure 7 averaged 0.00489 g cm^{-3} in the right breast and 0.00464 g cm^{-3} in the left over the temperature range tested.

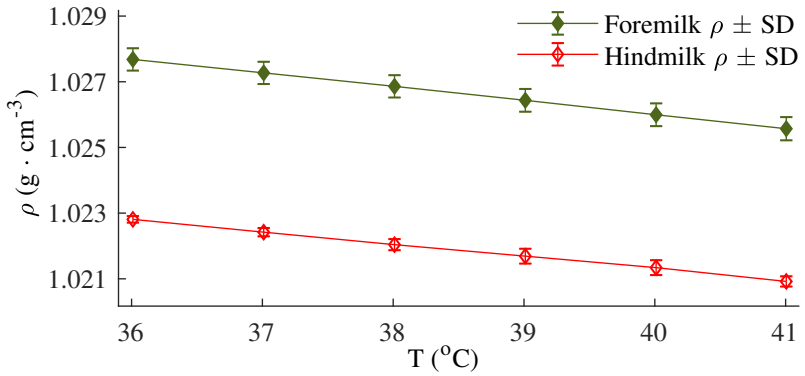

Figure 7. Density Test #2 for samples from Participant #8 for intra-feed (foremilk/hindmilk) and inter-breast (shown with SD caps) variations tested fresh. While higher fat content lowers density, inter-breast differences are minimal, particularly for hindmilk.

Temperature Sweep Tests #1 and #3 for Samples #8 and #7, respectively, show viscosity differences (Figures 8 and 9) correspond to intra-feed variations in fat content with hindmilk consistently having higher viscosity compared to foremilk from the same breast and feed. Hindmilk viscosity increased when the temperature increased beginning at 41 °C for Sample #7H (Figure 8) and at 44 °C and 47 °C for Samples #8LH and #8RH, respectively, (Figure 9a). Foremilk failed to show any increase in viscosity suggesting that the increase is associated with higher fat content, particularly since the highest fat content milk, Sample #7H, also showed the earliest increase in viscosity at 39.5 °C. This finding merits further investigation considering possible implications for infant feeding. A closer look at viscosity over reheating temperature range showed hindmilk was higher than foremilk (see Figure 9b) with the Sample #8R hindmilk twice the viscosity value of either Sample #8 foremilk sample.

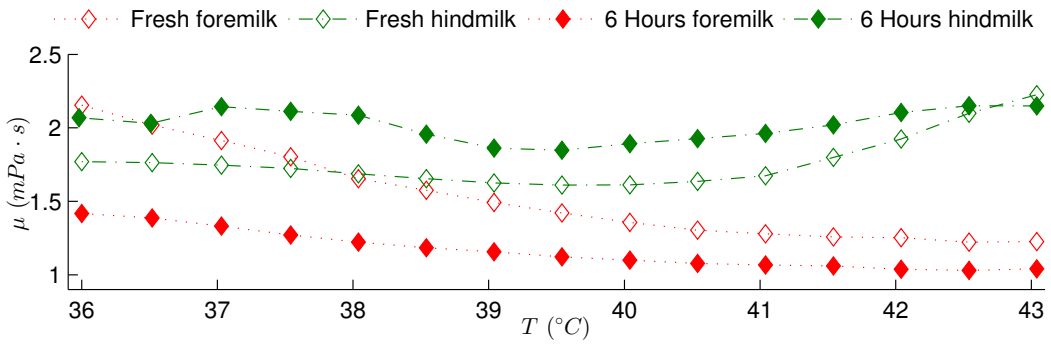

Figure 8. Temperature Sweep Test #3 at $\dot{\gamma}$ = 50 s^{-1} shows intra-feed variations in viscosity that relate to composition. Hindmilk consistently has higher viscosity compared to foremilk from the same breast and expression (intra-feed variations). When the samples were aged at 37 °C for 6 h (filled diamonds), the foremilk viscosity decreased slightly while hindmilk increased (see Section 3.5). Samples from Participant #7.

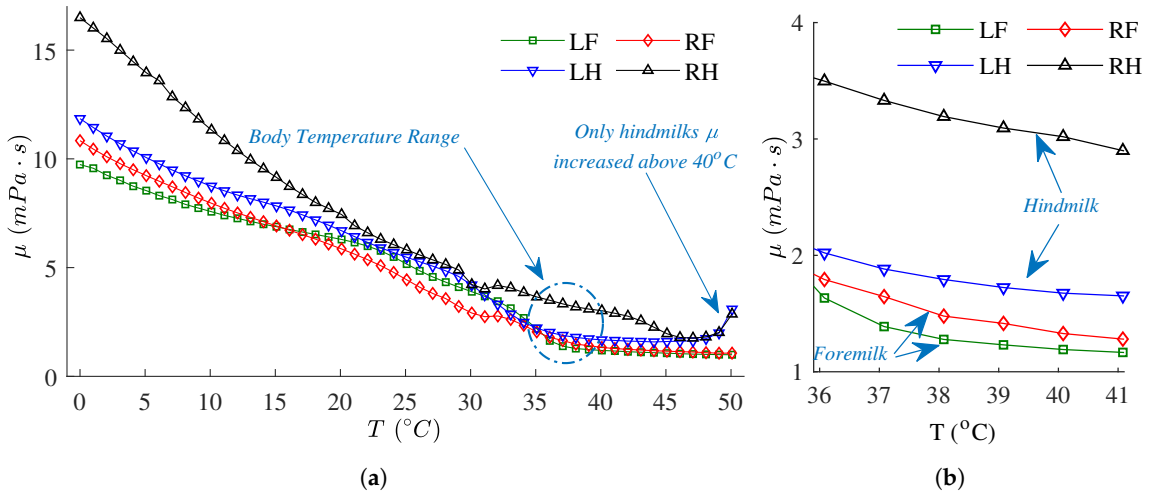

Figure 9. Temperature Sweep Test #1 for samples from Participant #8 at $\dot{\gamma}$ = 50 s^{-1} after 5 days refrigeration. (**a**) Temperature decreased viscosity for both foremilk and hindmilk until 45 °C when hindmilk began to increase, (**b**) A closer look over body temperature range shows a steady decrease in viscosity in response to temperature.

Shear Rate Sweep Tests #1 and #2 for samples from Participant #8 showed intra-feed variations particularly at low shear rates <10 s^{-1}. At $\dot{\gamma}$ = 0.01 s^{-1}, fresh foremilk viscosity exceeded fresh hindmilk viscosity by more than one order of magnitude, which can be seen in Figure 10a. At that same shear rate the inter-breast viscosity variations for foremilk (right breast dominated) exceeded the differences for hindmilk (left breast dominated). As shear rate approached 100 s^{-1}, the intra-feed variations did not remain consistent. As seen in Figure 10b, fresh foremilk from the right breast dominated all other samples yet viscosity for fresh foremilk from the left breast was lower than other samples, including previously refrigerated ones (for further discussion regarding storage effects, see Section 3.5). These results clearly show the influence of proteins at low shear rates on milk rheology similar to research on raw skim bovine milk [6].

Inter-breast and intra-feed thixotropic viscosity variations appeared in Shear Rate Loop Test. A comparison of start and end viscosities at 1 s^{-1} for the loop test shows that milk from the left breast decreased viscosity while milk from the right breast increased viscosity. The percentage of increase or decrease is presented in Table 7. The differences in fat content between foremilk and hindmilk do not appear to be a factor in determining whether viscosity increases or decreases with time.

Table 7. Start and end viscosities at 1 s^{-1} for the loop test.

Sample	Initial μ (mPa s)	Final μ (mPa s)	Increase/Decrease (%)
Left Foremilk	9.4136	9.2705	−1.52
Right Foremilk	9.3010	13.9690	+50.19
Left Hindmilk	21.6380	10.1380	−53.15
Right Hindmilk	12.8800	16.6060	+28.93

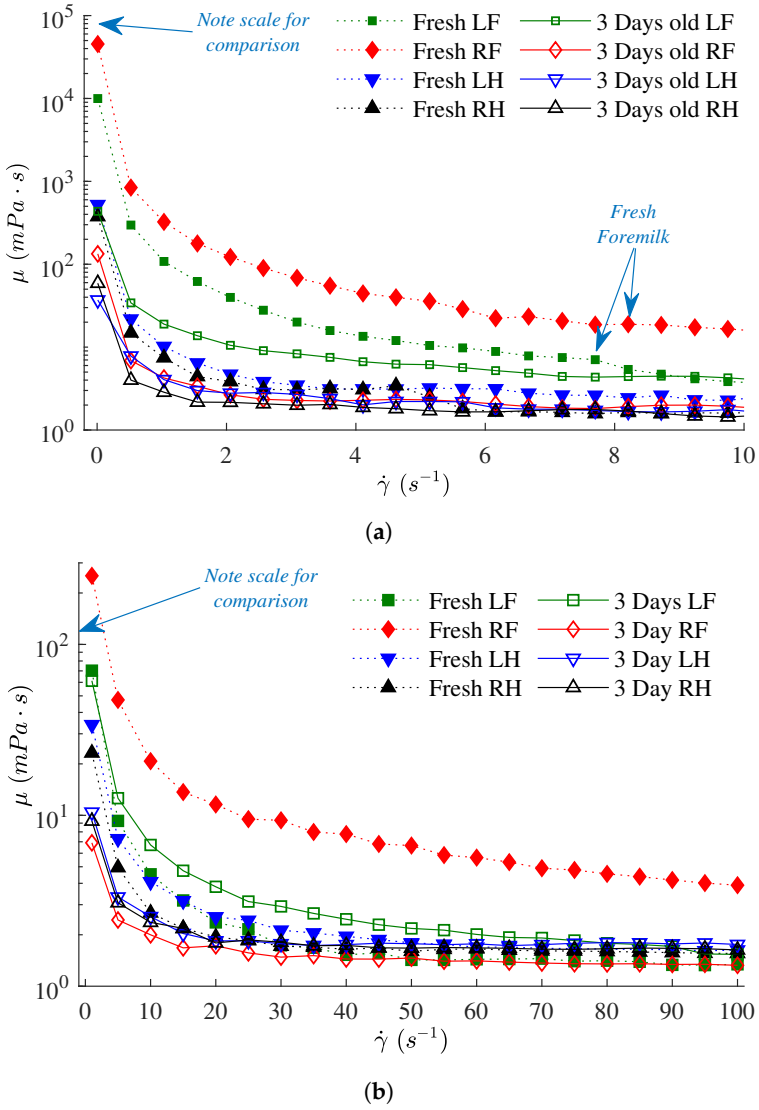

Figure 10. Shear Rate Sweep Tests #2 (Figure 10a) and #1 (Figure 10b) results for milk from Participant #8 show both inter-breast and intra-feed variations in viscosity. Effect of 3 days of refrigeration on milk viscosity associated with intra-individual variations is also seen (discussed in Section 3.5). (**a**) The low shear rates experienced during the beginning and end of a suck cycle show higher viscosity in fresh foremilk (lower fat) compared to hindmilk (higher fat). Storage by refrigeration lowered viscosity of each sample until crossover for foremilk at 8 s^{-1} and hindmilk at 6 s^{-1}. Inter-breast variations were greater for fresh foremilk than fresh hindmilk and for fresh milk compared to stored milk. (**b**) The starting viscosity (at $\dot{\gamma} = 1\ s^{-1}$) for all fresh samples was higher than after 3 days of refrigeration but that pattern did not continue as shear rate increased.

3.5. The Effect of Storage & Aging on Human Milk Density and Viscosity

Density measurements for both fresh and previously frozen milk are shown in Table 8. The average density for fresh milk, after removing the outlier Samples 3R and 3L, was 0.964 g cm^{-3} with a standard deviation of 0.007 g cm^{-3}. The source of deviation for fresh Samples 3R and 3L is assumed to be due to experimental error since they were tested at the same time. A 95% confidence interval for both fresh and thawed density mean was calculated using a *t* distribution. The results show an increasing in density for thawed milk (average 6.8%) when compared with fresh milk measurements at the same temperature, with the samples having the lowest fresh density increasing the most.

Table 8. Human milk density increased in response to storage at −20 °C for 3 months. Fresh samples were tested within 20 min of expression in a single reading at the milk temperature at time of arrival to equipment. The thawed values for comparison were recorded in Density Test #1. The fresh results for milk from Participant #3 were outliers due to assumed experimental error and removed from these test results.

Sample	Temperature (°C)	Fresh ρ (g cm^{-3})	Thawed ρ (g cm^{-3})
1R	26.4	0.970	1.029
1L	24.8	0.964	1.022
2R	25.8	0.967	1.028
2L	26.7	0.964	1.030
4R	26.0	0.972	1.031
4L	27.0	0.969	1.031
5R	24.8	0.952	1.034
5L	25.4	0.962	1.030
6R	26.2	0.968	1.026
6L	26.0	0.952	1.032
Mean ± SD	25.9 ± 0.74	0.964 ± 0.007	1.029 ± 0.003
95% Confidence Interval		$0.959 \leq \rho_{mean} \leq 0.969$	$1.008 \leq \rho_{mean} \leq 1.050$

Patterns in viscosity changes due to storage by refrigeration and aging were determined with milk from Participants #7 and #8. Participant #7's milk was tested at a constant shear rate of 50 s^{-1} over a small temperature range representative of milk reheating temperatures both fresh and after aging at a constant 37 °C for 6 h similar to what [21] performed. Aging effects can be seen in Figure 8. Hindmilk viscosity increased with aging while foremilk viscosity decreased.

Participant #8's milk samples were held at 37 °C and not initially tested until 5 h after collection. Due to this delay, data regarding changes due to aging at 37 °C were not obtained. All samples from Participant #8 were tested at 5 h after expression and after 3 days refrigeration at 4 °C using Shear Rate Sweep Tests #1 and #2. For the fresh and 3 days post-refrigeration tests, each foremilk and hindmilk sample was tested twice with a new loading and then averaged. The results in Figure 10a show that the fresh milk samples tested considerably higher at very low shear rates (Shear Rate Sweep Test #2) compared to post-refrigeration samples. This pattern does not continue for all samples as shear rate increases. Figure 10b shows that at 1 s^{-1} milk viscosity was greater when fresh than post-refrigeration, yet by 100 s^{-1} post-refrigeration samples were greater for all but right breast foremilk, which maintained a significant difference between fresh and post-refrigeration results throughout testing.

4. Discussion

To date there is very little rheological data pertaining to raw human milk, which is not surprising considering early works determined human milk to be approximately Newtonian [19] and few engineering models of breastfeeding have been produced [39–41]. In this study, freshly expressed human milk was tested to determine general behavior patterns and alterations in density and viscosity in response to environmental factors, storage, aging, and intra-individual milk content variations.

Density is frequently used by clinicians and in research studies when estimating milk intake (mass or volume) for infants by assuming density is close to 1 g cm^{-3} [42–44]. Since human milk is a dynamic biofluid with over 100 components that show intra-feed variations during each breastfeed [35,36,45–51] and factors such as temperature and storage affect density, understanding the range of variation can help ensure more accurate estimations of milk intake. The results of this study show that in the clinical setting where test-weighing is used to estimate infant intake, this approximation is sufficient considering infant scale accuracy is often ±1 g or more. Inter-breast differences in milk density existed

and likely originated from composition differences [35,46]. The influence of intra-feed composition changes on density are evident when comparing foremilk and hindmilk. Since fat concentration varies the most over the course of a single expression/feed while lactose and protein remain fairly constant [47,52], the differences in density between foremilk and hindmilk are primarily due to fat fluctuations with higher fat content decreasing density.

Human milk flow behavior demonstrated consistent patterns of variation with regards to shear rate, temperature, storage, and aging when variations in macronutrient content was considered, although its thixotropic behavior, similar to blood [53], provided large range of expected values for some experiments. The Shear Rate Loop Test results were inconsistent in pattern with inter-breast variations since milk viscosity from one breast increased and from the other breast decreased as shear ramped down. The test was performed on post-refrigerated milk, so the effect of dissolved gases, that may be present in fresh milk, were minimized. However, chemical reactions and composition changes during storage (i.e., bacterial growth) could affect viscosity and be reflected in tests for thixotropy. In bovine milk studies, thixotropy was noted only in whole not skim milk [6] which suggests that the human milk fat globule has an associated relaxation time after deformation under shear. Since thixotropic behavior remained after storage, the fat globules maintained elasticity, although whether elasticity alters during storage is unknown but likely because lipolysis of human milk lipids occurs when milk is stored at any temperature above −70 °C [54].

The viscosity curve as a function of shear rate clearly shows non-Newtonian, shear-thinning flow behavior. As shear rate approaches 0 s^{-1}, viscosity approaches infinity indicating that raw human milk has a yield point and firm texture at rest, which was confirmed with oscillatory tests. The viscosity curve of individual samples showed an oscillatory behavior beginning around 20 s^{-1} (see [29]) that smoothed as shear rate increased. The oscillations are likely due to changes in orientation, shape, and size of various milk particles. The decrease in slope at higher shear rates likely accounts for the many researchers who assumed Newtonian behavior for milk. However, currently there is insufficient research on human milk to define at what shear rate Newtonian behavior begins. Since milk is often infused at slow rates in narrow tubes, the non-Newtonian flow behavior is significant especially at low shear rates.

The non-Newtonian flow behavior at low shear rates also pertains to the oscillatory nature of suckling that constantly varies the pressure profile exerted upon milk when fed from artificial nipples. From the oscillatory testing, inter-breast variations in yield and flow points were determined. The disparities in yield and flow points indicate large differences in the structural strengths or gel strengths of the milk between breasts. This gel-like structure likely helps keep particles from settling when the milk is at rest. The decrease of the G′ curves after the LVE region begins the nonlinear viscoelastic regime [32] and indicates a slow decline in structural strength, which demonstrates a smoother, more homogeneous flow behavior. A gradual rise in G″ after the LVE region occurred in 4 of the 6 samples tested and indicates that deformation energy was transformed into friction heat due to internal viscous friction while elastic behavior dominated. The high standard deviation shows the range of shear stress required to complete the necessary structural breakdown to initiate viscous dominated behavior.

Samples with FTI closer to 1 had a greater tendency for brittle fracturing or non-homogeneous breakdown to occur past the yield point. Sample 6R had the FTI closest to 1 and, as seen in Figure 3a, showed the steepest curve upon leaving the LVE region and had the highest G′ value. So 6R experienced a large amount of internal viscous friction as it transitioned from elastic dominated to viscous dominated flow behavior. Other samples had FTI close to 1 with steep strain curves upon leaving the LVE region, so non-homogeneous breakdown appears common and may be correlated to fat content. Fat content is the only component with large intra-feed variations where pressure forces at the breast vary from multiple sources, like alveolar contractions and suckling. In tube feeding, fat is the component with the largest losses with significant protein losses also reported [4,5]. The loss of nutrients during tube feeding is likely due to non-homogeneous breakdown whereby a shear stress

between the yield point and flow point is exerted causing a lower fat milk to flow. The apparent wall shear stress, ($\dot{\gamma}_a$), for tube feedings can vary from low (2.6 s^{-1}) to high (1697.7 s^{-1}) depending on flow rate and tube diameter (see Appendix C). Even though certain feeding parameters result in a high $\dot{\gamma}_a$, fat losses of over 50% are reported [5], which indicate pressure within the tube is insufficient to achieve homogeneous flow. Additionally, the thixotropic behavior of human milk adds an additional factor that likely contributes to feeding problems and requires further study.

The current recommendation for feeding preterm infants human milk is to warm milk to body temperature with the ideal temperature range of 35.5–37.2 °C although reported prefeed temperatures vary greatly (21.8–46.4 °C) [55]. Of particular interest in this study was the temperature range between 36–40 °C that immediately surrounds the normal body temperature. The mean density change between 36–40 °C was -0.0017 g cm^{-3} and viscosity decreased with fresh foremilk decreasing more compared to fresh hindmilk. However, higher temperatures showed areas of unusual flow behavior that could impact infant feeding. As noted in Section 3, viscosity began increasing for some samples as early as 41 °C and in all samples by 46 °C. The higher fat, hindmilk samples' viscosities began increasing at lower temperatures than their foremilk counterparts. Since feeding hindmilk to preterm infants is common in hospitals [48], small variations in heat have less impact on improving flow in tubing, but temperatures above 40 °C should be avoided.

The impact of storage on density and viscosity also suggested interesting changes in milk content. Past research tested milk after storage [18], but for the fresh samples in Table 8, density increased due to storage. Density for pure water is 0.9970–0.9965 g cm^{-3} for 25–27°, so freshly expressed milk density appears to be lower than water. One reason could be due to chemical changes [7,9] that altered the attraction between molecules and increased volume. Alternatively, milk may contain dissolved gases. The source of gases in human milk could be in vivo, being diffused from blood, or introduced during expression by vacuum pump. If the source is in vivo, then human milk is compressible, which theory requires further testing. Regardless of the source, with storage dissolved gases would be released leading to an increase in density. Dissolved gases would also impact viscosity behavior in fresh milk at low shears causing a higher resistance to flow and possible slip effects. This study found that refrigeration appears to decrease viscosity compared to fresh, particularly at low shear rates, which is supported by previous findings on bovine milk [6].

Lastly, the intra-feed variations associated with foremilk and hindmilk provided insight into the influence of fat content on milk viscosity. The hindmilk samples with higher fat content had lower viscosities than foremilk samples at low shear rates as shown in Figure 10a. This trend was also seen when the samples were tested over a higher shear sweep in Figure 10b. However, with the exception of fresh right-side foremilk, no extreme differences were seen at 100 s^{-1}. While the number of samples is insufficient to make absolute statements, the results demonstrate previously unreported flow behaviors. Since intra-feed protein concentration is stable, foremilk contains a higher protein to fat ratio. The protein composition during early lactation was found to heavily influence viscosity [20]. These results indicate that the protein content may have a stronger influence on viscosity at lower shear rates than previously thought and is supported by [38]. While testing for the milk yield point was not conducted on these samples, the extremely high viscosity values at 0.01 s^{-1} for the foremilk likely denotes higher internal cohesion forces when the protein to fat ratio is higher by allowing for formation of larger casein micelles that require greater shear stress to breakdown into smaller micelles to achieve flow.

5. Conclusions

Raw human milk flow properties vary with respect to temperature, storage, aging, time, and shear rate. Density of fresh milk when newly expressed may be lower than water due to dissolved gases that are released into the atmosphere during storage. A clear non-Newtonian flow behavior was found with large variations in viscosity, especially at low shear rates. The structural strength of milk varied with many samples showing non-homogeneous breakdown during the transition from yielding

to flowing. This flow behavior can explain why tube feeding results in nutrient losses. The results highlight the need for sufficient pressure application to achieve homogeneous flow.

Author Contributions: Conceptualization, D.A. and F.H.; Methodology, D.A.; Software, D.A.; Validation, D.A.; Formal Analysis, D.A.; Investigation, D.A.; Resources, D.A. and F.H.; Data Curation, D.A.; Writing—Original Draft Preparation, D.A.; Writing—Review and Editing, D.A. and F.H.; Visualization, D.A.; Supervision, F.H.; Project Administration, D.A. and F.H.; Funding Acquisition, D.A. and F.H. All authors have read and agreed to the published version of the manuscript.

Funding: This material is based upon work supported by the National Science Foundation under Grant No.1454334 and 1707063, National Science Foundation Graduate Research Fellowship Program under Grant No.1746053, and Eugene McDermott Graduate Fellowship No.201701.

Acknowledgments: The authors thank Jimi Francis, University of Texas at Tyler, for her assistance with recruitment and collection, Maxine Quitaro and Anton-Paar for use of equipment, and all the mothers who donated to this study. Some preliminary findings were presented in 2016 ASME IMECE Young Engineer Paper Contest sponsored by the Fluids Engineering Division [29].

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

As the dairy industry grew, the need to model the relationship between viscosity and factors such as content and temperature led to the development of a series of regression equations. The usefulness of each equation depends on the application for which it was developed. Snoeren et al. found a relationship between the voluminosity of bovine milk proteins and the dynamic viscosity of commercial skim milk [10]. They showed that viscosity of heat-treated skim milk is a function of the volume fractions of casein, native whey protein, denatured whey protein, and the viscosity of the medium. Based on Bateman and Sharp's research on raw bovine milk [6], Snoeren et al. should have considered the shear rates used in testing due to the presence of native whey protein, which is found in raw bovine milk, yet no mention of shear rate is provided. Additionally, no data concerning the temperature of the milk used by Snoeren et al. was provided, even though studies show that temperature affects the viscosity of skim milk [14].

The effect of temperature and fat content on milk viscosity was investigated by Jebson and Chen [11]. They noted that whole milk has a higher solid content and higher viscosity in comparison to skim milk. In their research on the evaporation of bovine whole milk, for concentrates of solids content (larger that 450 g/kg), they adapted a relation for viscosity as a function of temperature and concentrate total solid. Similarly, Phipps [13] examined the relationship between viscosity, bovine cream fat content up to 50%, and temperature variations of 40 °C to 80 °C. He determined a regression equation for viscosity for creams with fat content (F) of less than 40%, as a function of temperature and fat content. Neither research considered shear rate in their experiments or equations.

More recent work with raw bovine milk was completed by Bakshi and Smith [12]. They performed several experiments on bovine milk to find a regression equation for the experimental value of viscosity based on the variable parameters of fat content and temperature. They noted that homogenized milk has a higher viscosity than raw milk which they attributed to the fine, dispersed state of the fat when homogenized. The temperature range tested was 0 °C to 30 °C and fat content range was 0.1% to 30%. Bakshi and Smith found the viscosities of skim milk and whole milk at 30 °C, to be about the same, approximately 1.25 mPa s. They also found that at lower temperatures, the effect of fat percentage on viscosity is greater. Similarly, they found a relationship between density, temperature, and fat content. All experimental work was performed at temperatures when milk fats are solid [18]. No regression equations were determined for raw milk nor was shear rate disclosed.

Appendix B

Human milk content varies in response to multiple factors. However, prior clinical studies provide some general guidelines that allow for estimation of content, which were used in this study.

Determining macronutrient content. Equations (A1)–(A3) determined by [49] based on month of lactation (n) to approximate macronutrient content (g/dL) are applicable under the following condition: the breast is fully emptied between the hours of 08:00 and 14:00. Participants #1–6 all met this requirement, so Equations (A1)–(A3) were used to calculate macronutrient content reported in the main text with limitations described later in this Appendix. For Participants #7–8, Equations (A1)–(A3) were used to calculate macronutrient content of their entire breast content and then modified to account for the normal variations found in foremilk and hindmilk based on percentages provided by [48]. The procedure for these modifications is outlined herein.

$$Carbohydrate = 7.2915 - 0.0309\, n \tag{A1}$$

$$True\ Protein = 0.5732 + 0.0258\, n \tag{A2}$$

$$Fat = 2.673 + 0.1597\, n \tag{A3}$$

Adjusting macronutrient content in foremilk and hindmilk. Over the course of a single feed, researchers [35,50] found that, between foremilk and hindmilk, protein levels remain the same, glucose decreases, and fat significantly increases 2–3 fold. Participants #7–8 both expressed milk after 14:00, however, since protein and carbohydrate content remains fairly constant throughout the day, only fat content was increased to account for the time of day [51]. Based on the aforementioned works, the assumption was made to exclude the changes in glucose since the molecular size is negligible when compared to fat globules, and macronutrient values were only adjusted for the fat content using the ratios found in [48] where foremilk has 82% and hindmilk has 161% of fat compared to composite or whole expression milk. Thus Equations (A1) and (A2) were employed to calculate carbohydrates and proteins, respectively. For fat, a composite value was found using Equation (A3), converted to g/L, and then used to calculate creamatocrit (%) using Equation (A4) [56]. The resulting creamatocrit value was then adjusted to foremilk or hindmilk based on [48] and converted back to g/dL.

$$Creamatocrit = 0.146 \times Fat + 0.59 \tag{A4}$$

Limitations of Equations (A1)–(A3). These equations do not take into consideration the natural variations between mothers nor the normal content differences between the breasts of the same mother, particularly fat. The differences between breasts for macronutrients involve fats and carbohydrates, primarily glucose. Work by [35] found significant fat variations in 60% of participants with results equally split between which breast expressed higher fat milk. The percent difference between breasts for those participants ranged from 25–72% with a mean difference of 46%. The significant fat differences may have stemmed from time differences between when each breast was last fed from. Glucose also showed significant differences in 60% of participants with 50% showing higher glucose in milk from the left breast and 10% with higher glucose in right breast milk.

Appendix C

Various tubes and feeding rates are used in medicine with huge losses in fat and protein reported [4,5]. Since human milk viscosity shows high dependence on shear rate, the apparent wall shear rate should be maximized to ease milk flow. Methods for tube feeding include continuous drip and bolus feed with a syringe over a specified time period. Calculating the apparent wall shear rate ($\dot{\gamma}_a$) for tube feeding of human milk can be approximated by using the equation

$$\dot{\gamma}_a = \frac{4Q}{\pi r^3} \tag{A5}$$

for a Newtonian fluid flow through a circular duct where Q is the volumetric flow rate and r is the radius of the tube [57]. Using tube diameters [5] and feeding rates [48,58] reported in literature in combination with Equation (A5), a table of approximate apparent wall shear rates for bolus feeds is calculated in Table A1.

Table A1. Approximations of $\dot{\gamma}_a$ in Bolus Feeds over 20 min every 2 h.

Tube Inner Diameter (mm)	Infant Weight (g)	Feed Volume (mL kg^{-1} day^{-1})	$\dot{\gamma}_a$ (s^{-1})
0.5	1000	100	565.9
0.5	1000	200	1131.8
0.5	1500	100	848.8
0.5	1500	200	1697.7
3.0	1000	100	2.6
3.0	1000	200	5.2
3.0	1500	100	3.9
3.0	1500	200	7.9

References

1. Eidelman, A.I.; Schanler, R.J.; Johnston, M.; Landers, S.; Noble, L.; Szucs, K.; Viehmann, L. Breastfeeding and the Use of Human Milk. *Pediatrics* **2012**, *129*, e827–e841. [CrossRef]
2. World Health Organization. *Infant and Young Child Feeding: Model Chapter for Textbooks For Medical Students and Allied Health Professionals*; World Health Association: Geneva, Switzerland, 2009.
3. Vieira, A.A.; Soares, F.V.M.; Pimenta, H.P.; Abranches, A.D.; Moreira, M.E.L. Analysis of the influence of pasteurization, freezing/thawing, and offer processes on human milk's macronutrient concentrations. *Early Hum. Dev.* **2011**, *87*, 577–580. [CrossRef] [PubMed]
4. Stocks, R.; Davies, D.; Allen, F.; Sewell, D. Loss of Breast Milk Nutrients during Tube Feeding. *Arch. Dis. Child.* **1985**, *60*, 164–166. [CrossRef]
5. Jarjour, J.; Juarez, A.; Kocak, D.; Liu, N.; Tabata, M.; Hawthorne, K.; Ramos, R.; Abrams, S. A novel approach to improving fat delivery in neonatal enteral feeding. *Nutrients* **2015**, *7*, 5051–5064. [CrossRef] [PubMed]
6. Bateman, G.; Sharp, P. A Study of the Apparent Viscosity of Milk as influenced by some Physical Factors. *J. Agric. Res.* **1928**, *36*, 647–674.
7. Bienvenue, A.; Jiménez-Flores, R.; Singh, H. Rheological properties of concentrated skim milk: Importance of soluble minerals in the changes in viscosity during storage. *J. Dairy Sci.* **2003**, *86*, 3813–3821. [CrossRef]
8. Trinh, B.; Haisman, D.; Trinh, K.T. Rheological characterisation of age thickening with special reference to milk concentrates. *J. Dairy Res.* **2007**, *74*, 106–115. [CrossRef]
9. Vélez-Ruiz, J.; Barbosa-Cánovas, G. Rheological properties of concentrated milk as a function of concentration, temperature and storage time. *J. Food Eng.* **1998**, *35*, 177–190. [CrossRef]
10. Snoeren, T.; Damman, A.; Klok, H. *The Viscosity of Skim-Milk Concentrate*; Zuivelzicht: Ede, The Netherlands, 1981.
11. Jebson, R.S.; Chen, H. Performances of falling film evaporators on whole milk and a comparison with performance on skim milk. *J. Dairy Res.* **1997**, *64*, 57–67. [CrossRef]
12. Bakshi, A.; Smith, D. Effect of fat content and temperature on viscosity in relation to pumping requirements of fluid milk products. *J. Dairy Sci.* **1984**, *67*, 1157–1160. [CrossRef]
13. Phipps, L. The interrelationship of the viscosity, fat content and temperature of cream between 40 and 80 °C. *J. Dairy Res.* **1969**, *36*, 417–426. [CrossRef]
14. Whitaker, R.; Sherman, J.; Sharp, P.F. Effect of temperature on the viscosity of skimmilk. *J. Dairy Sci.* **1927**, *10*, 361–371. [CrossRef]
15. Magee, H.E.; Harvey, D. Studies on the Effect of Heat on Milk: Some Physico-Chemical Changes induced in Milk by Heat. *Biochem. J.* **1926**, *20*, 873.
16. Evenson, O.L.; Ferris, L.W. The viscosity of natural and remade milk. *J. Dairy Sci.* **1924**, *7*, 174–188. [CrossRef]
17. Zhao, D.b.; Bai, Y.h.; Niu, Y.w. Composition and characteristics of Chinese Bactrian camel milk. *Small Ruminant Res.* **2015**, *127*, 58–67. [CrossRef]

18. Macy, I.G.; Kelly, H.J.; Sloan, R.E. *The Composition of Milks. A Compilation of the Comparative Composition and Properties of Human, Cow, and Goat Milk, Colostrum, and Transitional Milk*; National Research Council: Washington, DC, USA, 1953.
19. Blair, G.W.S. The determination of the viscosity of human milks and the prenatal secretions. *Biochem. J.* **1941**, *35*, 267. [CrossRef]
20. Waller, H.; Aschaffenburg, R.; Grant, M.W. The viscosity, protein distribution, and 'gold number' of the antenatal and postnatal secretions of the human mammary gland. *Biochem. J.* **1941**, *35*, 272. [CrossRef]
21. de Almeida, M.B.d.M.; de Almeida, J.A.G.; Moreira, M.E.L.; Novak, F.R. Adequacy of human milk viscosity to respond to infants with dysphagia: Experimental study. *J. Appl. Oral Sci.* **2011**, *19*, 554–559. [CrossRef]
22. Fondaco, D.; AlHasawi, F.; Lan, Y.; Ben-Elazar, S.; Connolly, K.; Rogers, M. Biophysical Aspects of Lipid Digestion in Human Breast Milk and Similac^{TM} Infant Formulas. *Food Biophys.* **2015**, *10*, 282–291. [CrossRef]
23. Frazier, J.; Chestnut, A.H.; Jackson, A.; Barbon, C.E.; Steele, C.M.; Pickler, L. Understanding the viscosity of liquids used in infant dysphagia management. *Dysphagia* **2016**, *31*, 672–679. [CrossRef]
24. Laogun, A. Effect of temperature on the RF dielectric properties of human breast milk. *Phys. Med. Biol.* **1986**, *31*, 893. [CrossRef]
25. Laogun, A. Dielectric properties of mammalian breast milk at radiofrequencies. *Phys. Med. Biol.* **1986**, *31*, 555. [CrossRef]
26. McDaniel, M.; Barker, E.; Lederer, C. Sensory characterization of human milk. *J. Dairy Sci.* **1989**, *72*, 1149–1158. [CrossRef]
27. Bransburg-Zabary, S.; Virozub, A.; Mimouni, F.B. Human milk warming temperatures using a simulation of currently available storage and warming methods. *PLoS ONE* **2015**, *10*, e0128806. [CrossRef]
28. Dumm, M.; Hamms, M.; Sutton, J.; Ryan-Wenger, N. NICU breast milk warming practices and the physiological effects of breast milk feeding temperatures on preterm infants. *Adv. Neonat. Care* **2013**, *13*, 279–287. [CrossRef]
29. Alatalo, D.; Hassanipour, F. An Experimental Study on Human Milk Viscosity. In Proceedings of the ASME 2016 International Mechanical Engineering Congress and Exposition, Phoenix, AZ, USA, 11–17 November 2016.
30. Mezger, T.G. *The Rheology Handbook: For Users of Rotational and Oscillatory Rheometers*, 4th ed.; Vincentz Network GmbH & Co. KG: Hanover, Germany, 2014.
31. Cadwell, K.; Turner-Maffei, C. *The Lactation Counselor Certificate Training Course Notebook*, 2017–2018 ed.; Healthy Children Project, Inc.: East Sandwich, MA, USA, 2017.
32. Fernandes, R.R.; Andrade, D.E.; Franco, A.T.; Negrão, C.O. The yielding and the linear-to-nonlinear viscoelastic transition of an elastoviscoplastic material. *J. Rheol.* **2017**, *61*, 893–903. [CrossRef]
33. Kline, S.J.; McClintock, F.A. Describing Uncertainties in Single-Sample Experiments. *Mech. Eng.* **1953**, *75*, 3–8.
34. McKennell, R. Cone-plate viscometer. *Anal. Chem.* **1956**, *28*, 1710–1714. [CrossRef]
35. Neville, M.C.; Keller, R.P.; Seacat, J.; Casey, C.E.; Allen, J.C.; Archer, P. Studies on human lactation. I. Within-feed and between-breast variation in selected components of human milk. *Am. J. Clin. Nutr.* **1984**, *40*, 635–646. [CrossRef]
36. Andreas, N.J.; Kampmann, B.; Le-Doare, K.M. Human breast milk: A review on its composition and bioactivity. *Early Human Dev.* **2015**, *91*, 629–635. [CrossRef]
37. Merrill, E.; Gilliland, E.; Cokelet, G.; Shin, H.; Britten, A.; Wells, R., Jr. Rheology of human blood, near and at zero flow: effects of temperature and hematocrit level. *Biophys. J.* **1963**, *3*, 199–213. [CrossRef]
38. Mediwaththe, A.T.M. Impact of Heating and Shearing on Native Milk Proteins in Raw Milk. Ph.D. Thesis, Victoria University, Footscray, Australia, 2017.
39. Elad, D.; Kozlovsky, P.; Blum, O.; Laine, A.F.; Po, M.J.; Botzer, E.; Dollberg, S.; Zelicovich, M.; Sira, L.B. Biomechanics of milk extraction during breast-feeding. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5230–5235. [CrossRef]
40. Mortazavi, S.N.; Geddes, D.; Hassanipour, F. Lactation in the Human Breast From a Fluid Dynamics Point of View. *J. Biomech. Eng.* **2017**, *139*, 011009. [CrossRef]
41. Azarnoosh, J.; Hassanipour, F. Fluid-structure interaction modeling of lactating breast. *J. Biomech.* **2020**, *103*, 109640. [CrossRef]

42. Arthur, P.; Hartmann, P.; Smith, M. Measurement of the milk intake of breast-fed infants. *J. Pediatr. Gastr. Nutr.* **1987**, *6*, 758–763. [CrossRef]
43. Daly, S.; Owens, R.A.; Hartmann, P.E. The short-term synthesis and infant-regulated removal of milk in lactating women. *Exp. Physiol. Transl. Integr.* **1993**, *78*, 209–220. [CrossRef]
44. Lucas, A.; Lucas, P.; Baum, J. Pattern of milk flow in breast-fed infants. *Lancet* **1979**, *314*, 57–58. [CrossRef]
45. Lucas, A.; Gibbs, J.; Baum, J. The biology of human drip breast milk. *Early Hum. Dev.* **1978**, *2*, 351–361. [CrossRef]
46. Mitoulas, L.R.; Kent, J.C.; Cox, D.B.; Owens, R.A.; Sherriff, J.L.; Hartmann, P.E. Variation in fat, lactose and protein in human milk over 24h and throughout the first year of lactation. *Br. J. Nutr.* **2002**, *88*, 29–37. [CrossRef]
47. Mizuno, K.; Nishida, Y.; Taki, M.; Murase, M.; Mukai, Y.; Itabashi, K.; Debari, K.; Iiyama, A. Is increased fat content of hindmilk due to the size or the number of milk fat globules? *Int. Breastfeed. J.* **2009**, *4*, 1. [CrossRef]
48. Ogechi, A.A.; William, O.; Fidelia, B.T. Hindmilk and weight gain in preterm very low-birthweight infants. *Pediatr. Int.* **2007**, *49*, 156–160. [CrossRef] [PubMed]
49. Czosnykowska-Łukacka, M.; Królak-Olejnik, B.; Orczyk-Pawiłowicz, M. Breast Milk Macronutrient Components in Prolonged Lactation. *Nutrients* **2018**, *10*, 1893. [CrossRef] [PubMed]
50. Saarela, T.; Kokkonen, J.; Koivisto, M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatr.* **2005**, *94*, 1176–1181. [CrossRef] [PubMed]
51. Demmelmair, H.; Koletzko, B. Lipids in human milk. *Best Pract. Res. Clin. Endocrinol. Metab.* **2018**, *32*, 57–68. [CrossRef] [PubMed]
52. Woolridge, M.; Fisher, C. Colic, "overfeeding", and symptoms of lactose malabsorption in the breast-fed baby: A possible artifact of feed management? *Lancet* **1988**, *332*, 382–384. [CrossRef]
53. Apostolidis, A.J.; Armstrong, M.J.; Beris, A.N. Modeling of human blood rheology in transient shear flows. *J. Rheol.* **2015**, *59*, 275–298. [CrossRef]
54. Jensen, R.G. Lipids in human milk. *Lipids* **1999**, *34*, 1243–1271. [CrossRef]
55. Jones, F. *Best Practice for Expressing, Storing and Handling Human Milk in Hospitals, Homes and Child Care Settings*, 4th ed.; Human Milk Banking Association of North America, Inc.: Fort Worth, TX, USA, 2019.
56. Lucas, A.; Gibbs, J.; Lyster, R.; Baum, J. Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk. *Br. Med. J.* **1978**, *1*, 1018–1020. [CrossRef]
57. Son, Y. Determination of shear viscosity and shear rate from pressure drop and flow rate relationship in a rectangular channel. *Polymer* **2007**, *48*, 632–637. [CrossRef]
58. Schanler, R.J.; Shulman, R.J.; Lau, C.; Smith, E.; Heitkemper, M.M. Feeding strategies for premature infants: Randomized trial of gastrointestinal priming and tube-feeding method. *Pediatrics* **1999**, *103*, 434–439. [CrossRef]