Temperature effects on the ultrasonic separation of fat from natural whole milk

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Abstract

This study showed that temperature influences the rate of separation of fat from natural whole milk during application of ultrasonic standing waves. In this study, natural whole milk was sonicated at 600 kHz (583 W/L) or 1 MHz (311 W/L) with a starting bulk temperature of 5 °C, 25 °C, or 40 °C. Comparisons on separation efficiency were performed with and without sonication. Sonication using 1 MHz for 5 min at 25 °C was shown to be more effective for fat separation than the other conditions tested with and without ultrasound, resulting in a relative change from 3.5 ± 0.06 % (w/v) fat initially, of -52.3 ± 2.3 % (reduction to 1.6 ± 0.07 % (w/v) fat) in the skimmed milk layer and 184.8 ± 33.2 % (increase to 9.9 ± 1.0 %(w/v) fat) in the top layer, at an average skimming rate of ~5 g fat/min. A shift in the volume weighted mean diameter (D[4,3]) of the milk samples obtained from the top and bottom of between 8 and 10 % relative to an initial sample D[4,3] value of 4.5 ± 0.06 µm was also achieved under these conditions. In general, faster fat separation was seen in natural milk when natural creaming occurred at room temperature and this separation trend was enhanced after the application of high frequency ultrasound.

1. Introduction

Ultrasonic separation has been identified as a technology suitable for initiating separations for a range of different applications and is an area of growing interest[1]. The technique using ultrasound waves to initiate separation of fat globules from milk has been recently reported by Juliano et al.[2, 3] using a recombined emulsion and Leong et al.[4] using natural milk. Milk fat separation rates many times faster than natural creaming have been reported using ultrasound.

In the absence of ultrasound, the natural creaming of fat from milk has been shown to be more effective at room temperatures (i.e., ~15 °C) at extended creaming times, compared with when it is maintained at cool temperatures (~4°C)[5]. The rising speed (terminal velocity) of an individual fat globule of diameter *d* can be calculated by[6]:

$$v = \frac{g(\rho_m - \rho_p)d^2}{18\eta_p} \quad (1)$$

where ρ is the density, η is the viscosity of the milk medium and g is the gravitational acceleration. The subscripts *p* and *m* refer to the fat globules and the surrounding medium respectively.

Natural separation at very high temperatures however(>77 °C) is impeded due to the denaturation of immunoglobulins in the milk which promote flocculation of fat globules into larger entities[7]. In regards to the temperature at which industrial milk fat separation normally occurs by centrifugation, there is no set standard (personal communication), with dairy plants reportedly using a range of temperatures ranging from cool (~15-20 °C) to hot temperatures (40-55°C) dependent on the desired product to be manufactured.

The temperature influences the physical properties of milk fat globules in the milk medium. Milk fat exists largely as a liquid above 40 °C and generally as a solid below – 40 °C[8]. At intermediate temperatures such as at room temperature, it exists as a mixture of crystals and liquid fat. This is because the milk fat is composed of many component triglycerides that melt over a considerable temperature range[8]. With the application of ultrasound, the manipulation of the fat globules is dependent on what is known as the primary acoustic radiation force, detailed for example in Yosioka and Kawasima[9] for a standing wave field. The primary acoustic radiation force is proportional to the size of the fat globules, the frequency of the ultrasound applied, and also the material properties of the milk and fat globules. The properties of importance are the density and compressibility of the

fat globules and their surrounding medium, and their influence is defined by the acoustic contrast factor, ϕ , calculated using[9]:

$$\phi = \frac{5\rho_p - 2\rho_m}{2\rho_p - \rho_m} - \frac{\beta_p}{\beta_m} \quad (2)$$

where β is the compressibility.

These properties are temperature dependent, and the influence of temperature on the ultrasonically enhanced separation of milk fat, in particular 'natural' whole milk, has not yet been examined. In the study by Leong et al.[4], carried at 1 MHz (67-300W/L), no temperature control was considered. It was observed that the input of ultrasound to the milk system resulted in heat generation over time, causing the overall system to increase in temperature. Studies by Juliano et al.[2, 3] demonstrated the enhanced ultrasonic-assisted fat separation using reconstituted milk fat emulsions at 400 kHz, 1 MHz, and 2 MHz (~35 W/L per transducer used). These studies were performed with an initial temperature of 35 °C, on the supposition that the higher proportion of liquid milk fat at these temperatures would be more readily separable by ultrasound.

A possible advantage of ultrasonic separation, is that high frequency ultrasound has capability to initiate 'gentle' separation of fat globules. During high frequency separation, the physical effects of unstable cavitation are negligible[10] and therefore will not affect the integrity of milk fat globules. Application of ultrasound using 1 MHz at similar power levels considered in this study has shown no evidence of damage to the structural integrity of the fat globules previously by zeta potential and visual inspection[4]. Even though the cavitational yield due to transient collapse peaks between 600 kHz and 1 MHz, decreasing at higher frequencies[11, 12], free radicals formed are unlikely to affect the structural integrity of such globules, although modification of other components in the bulk medium may occur[13-15].

The aim of the study is to establish the effect of temperature on ultrasonic-assisted fat separation at selected temperature and frequencies.

2. Materials and Methods

2.1. Ultrasonic separation trials

A similar protocol as reported by Leong et al.[4] using raw whole bovine milk sourced from the farm (Department of Primary Industries Ellinbank, Australia) has been employed.

Fully-submersible plate transducers (Sonosys Ultraschallsysteme GmbH, Neuenburg, Germany) of nominal frequency 600 kHz and 1 MHz were available for the separation trials. The transducers were positioned inside a stainless steel box with dimensions 182 x 242 x 62 mm and wall thickness 1 mm. The transducers were positioned such that the non-active side of the transducer was firmly placed against one side of the stainless steel wall. A gap between the active side of the transducer and the wall of the stainless steel vessel of ~30 mm (alignment ± 3 mm) was used for all trials in this study (Figure 1).

The transducers were operated at 100% nominal power (700 W and 343 W for 600 kHz and 1 MHz, respectively). The electrical power draw was determined using a power meter. The temperature of the processed milk was monitored every minute using a thermocouple positioned near the side wall of the separation vessel.

Sound pressure levels were determined using a needle hydrophone (model HNC-1000, Onda Corp., Sunnyvale, USA). The hydrophone was positioned at various locations across the container to measure the maximal pressure in the vessel. A minimum of 10 values were recorded to determine the maximum pressure.

Milk was transported from the farm at 5 °C and then stored at 5 °C in a cool room for trials to be carried out the next day. The volume of milk processed was 1.2 L using the 600 kHz, and 1.1 L using the 1 MHz (a slightly larger volume was required to be processed for 600 kHz due to its higher surface area). Milk was placed in the stainless steel ultrasound reactor vessel at three starting temperatures for each selected frequency: (a) 5 °C (directly from the cool room); (b) 25 °C and (c) 40 °C. Trials at the lowest temperature consisted of placing the ultrasonic reactor vessel in an ice-water bath to maintain milk temperature below <20 °C during sonication; in this case more of the milk fat was present in a solid state. Higher temperature trials were started by placing the ultrasound reactor vessel inside a thermo-regulated heating bath (Ratek TH2 Thermoregulator). Constant gentle stirring of the milk was performed during the preheating step inside the reactor up to each target temperature (several minutes required). The starting temperature of 25 °C was selected to represent ambient room conditions (an intermediate temperature for semi-liquid milk fat), and the 40 °C was selected so that the milk fat was mostly liquid.

The ultrasound was switched on once the milk reached the desired preheating temperature, and applied continuously for 5 minutes. Controls where no ultrasound was applied were performed inside the vessel using the exact same set-up as that used during processing. All trials were performed in duplicate with milk obtained on the same day. Trials were repeated with milk from

different days to mitigate the influence of natural variation. A minimum of four replicates was performed for all trials under identical processing conditions. The error bars calculated are from the standard error across replicated trials unless otherwise stated.

2.2. Sample characterization

Samples were collected from the fat-enriched top and fat-depleted bottoms after processing and characterized using the same procedure as reported by Leong et al.[4]

Fat content was analysed using the standard Rose-Gottlieb Method[16] to determine the fat concentration of the milk prior to ultrasound processing, and the top and bottom portions after ultrasound application.

The particle size distribution and the associated volume weighted mean diameter, D[4,3], was determined for all samples before and after processing by ultrasound using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire) with deionised water as the dilutant. Further details of the analysis can be found in Leong et al.[4]. The D[4,3] value represents the mean particle size of the samples, weighted by the total volume of the fat droplets. The purpose of this measurement was to provide an indication of the selectivity of ultrasound under different conditions (frequency and temperature) to manipulate different sized fat globules in a sample of natural whole milk to the fat enriched top or fat depleted bottoms.

Zeta potential was determined using a Zetasizer Nano (Malvern Instruments Ltd., Worcestershire). Phosphate buffer (0.1 M) at a pH of 6.8 was used as the dilutant. Further details can be found in Leong et al.[4]

2.3. Statistical analysis

The statistical significance of the results was evaluated using a General Linear Model by ANOVA (Matlab 2012c, MathsWorks Inc.) for a confidence threshold of $p<0.05$. The error bars reported, unless otherwise stated, are the standard error across a minimum of 4 replicated trials.

3. Results and discussion

3.1. Ultrasonic reactor vessel characterization

The electrical power draw of the transducers during operation are measured to be 700 W (3.08 W/cm^2) and 343 W (3.43 W/cm^2) for the 600 kHz and 1 MHz transducer respectively. The maximum

pressure level detected within the ultrasound field of the vessel is reported to be 84.7 ± 21.1 kPa and 75.8 ± 8.2 kPa for the 600 kHz and 1 MHz frequencies respectively. The calorimetric values have been determined previously using similar set-ups[4] and show that the energy evolved as heat is proportional to the electrical power draw for these transducers.

Inside a standing wave field, there are regions of intense pressure (antinodes) and regions of minimal pressure (nodes). The milk fat globules move to the pressure antinodes by the primary radiation force described, for example, in Yosioka and Kawasima[9]. At these pressure levels, no structural damage to the surface of the fat globules was reported when using 1 MHz ultrasound based on zeta-potential measurements[4]. No damage to the fat globules is detected by zeta-potential measurements when using 600 kHz at the power settings investigated in this study (20 minutes sonication, 583 W/L). A zeta-potential value of -11.2 ± 0.6 mV was measured, which when compared with values for natural milk, -11.2 ± 0.2 mV, and homogenized milk, -16.2 ± 0.5 mV, indicate no significant change in the surface properties[17].

At frequencies between 400 kHz to 1 MHz, the yield of free-radicals is predicted to be in a peak range in aqueous systems[11, 12]. However, a recent study into the use of either 600 kHz and 1 MHz frequencies in milk based systems using similar levels of power input and with similar pressure levels, have shown that the oxidation of lipids in cheddar cheese whey was not significant owing to the application of ultrasound at these frequencies[18].

The temperature increase during sonication is shown in Figure 2. The maximum temperature rise over 5 minutes processing observed in all of the trials does not exceed 15 °C. The temperatures during sonication hence range from 5-18 °C, 25-40 °C and 40-52 °C. These ranges are within the typical conditions used for centrifugal separation in industrial dairy manufacture (personal communication).

The temperature of the medium may influence the ultrasound propagation and other system conditions in several ways. The speed of sound, which influences the wavelength of a standing wave system, increases with temperature [19], peaking at approximately 1550 m/s at 74 °C. This means that the number of anti-nodes (and hence sites at which fat globules may collect at) becomes less with increasing temperature. However, given that there are many wavelengths and hence antinodal sites (~24 using 600 kHz, ~40 using 1 MHz) within the system and the temperature rise is <15 °C, the change in sound speed will at most increase/decrease the number of antinodes by 1 to 2 sites for the given geometry, and is not expected to play a significant role in the separation.

175 Furthermore, an increase in the bulk temperature may modify the sonochemical yield of radicals
176 since the temperature can alter the gas solubility and vapour pressure that affects the ease of
177 cavitational events as well as the final collapse intensities[10]. Higher temperatures for example, can
178 reduce the solubility of gas and lower the potential yield of sonochemical entities[20]. For ultrasonic
179 separation applications, a decline in sonochemical yield is likely to be beneficial since the interest is
180 not usually to cause any change to the separated products. Nevertheless, in milk systems as
181 mentioned above, recent results suggest that the sonochemical oxidation of lipid components at
182 similar operating conditions is insignificant[18], likely due to the presence of antioxidant compounds
183 in milk such as casein[14], vitamins, enzymes and lactoferrin that can act as radical scavengers[21].

184 ## 3.2. Fat concentrations

185 ### 3.2.1. Natural creaming

186 The change in fat content of the collected samples relative to the initial samples from the trials
187 performed at various preheating temperatures with no ultrasound application (control) are shown in
188 Figure 3. At the temperatures considered, the change in fat content is small relative to the initial
189 without ultrasound application. The creaming capacity in the absence of ultrasound appears to be
190 highest when the milk is preheated to 25 °C for the time frames investigated in this study (5
191 minutes). At 25 °C, the milk fat changes from 3.4 ± 0.07 % (w/v) fat initially to 3.3 ± 0.06 % (w/v) in
192 the bottom (-3.4 ± 0.3 % relative change) and 3.9 ± 0.09 % (w/v) fat in the top (13.6 ± 0.4 % relative
193 change).

194 In the absence of ultrasound, Stokes' law (Eq. 1) can be used to predict the rising speed of individual
195 fat globules. An important factor, independent of temperature, is the particle size distribution.
196 Larger particles rise faster, and hence result in faster observed creaming rates if they are present in
197 greater numbers in the milk. Milk fat globules can flocculate into larger entities when they come into
198 contact, further enhancing their rise speed and hence creaming rate. The flocculation behaviour
199 when milk creams naturally is facilitated by mechanisms that are dependent on the temperature[8].

200 Natural creaming of fat by gravity separation is influenced by the agglutination process, which at the
201 same time is influenced by the bulk temperature in the milk. Agglutination is the process whereby
202 immunoglobulins present in the milk will promote the flocculation of globules as they come into
203 contact with one another, enhancing their effective size. At low temperature, the agglutinin will be
204 attached to fat globules, whereas at high temperature, it will be in the medium[8]. Hence,
205 separation may become less effective with higher temperatures as the agglutinin detaches from the

surface of globules and possibly even denatures if high enough in temperature[7]. It has been shown by Caplan et al.[7] that temperatures in excess of 77 °C impair the ability of fat to separate naturally by gravity separation.

The data for density and viscosity parameters across a range of temperatures from Mulder and Walstra [8] is shown in Table 1. As can be gauged from the values in Table 1, it is expected that fat separation will occur faster with increasing temperature. Higher temperatures will increase the terminal velocity (fat globule rise speed) and hence rate of separation of milk fat, largely because the density difference between the fat and the surrounding medium increases with temperature. This behaviour is well known and has recently been reported by Ma and Barbano[5], although at different temperatures (4 °C and 15 °C) to our study and much longer separation times (>2 hours). The separation at 40 °C in the absence of ultrasound is not as effective compared with 25 °C for the time frames considered in this present study, likely due to the agglutinin being moved from the surface of the fat globules to the bulk phase at higher temperature.

3.2.2. Ultrasonic assisted creaming

The application of ultrasound (Figure 3) results in significantly improved fat separation ($p<0.05$) compared with the controls at the preheated temperatures of 25 °C and 40 °C, for both 600 kHz and 1 MHz frequency, after 5 minutes sonication. Interestingly, at 5 °C (without preheating) only the 600 kHz frequency ultrasound resulted in significantly more effective separation compared with the control for the sample obtained from the top. A change from initially 3.4 ± 0.02 % (w/v) fat to 3.3 % (w/v) fat) in the skim layer (-5.1 ± 1.2 % relative change) and increase to 6.0 ± 1.4 % (w/v) fat in the top layer (122 ± 46% relative change) is observed when applying 600 kHz ultrasound, compared with a change from 3.58 ± 0.07 % (w/v) fat to 3.56 ± 0.02 % (w/v) fat in the bottom (-0.6 ±1.0% relative change) and increase to 3.61 ± 0.04 % (w/v) fat in the top (1.0 ±0.6% relative change) when using 1 MHz ultrasound at 5 °C. A likely reason for this is due to the higher energy input rate of the 600 kHz (700 W) compared to the 1 MHz (343 W) which, from Figure 2, causes a higher temperature increase.

As reported by Leong et al.[4], when raw milk with a starting temperature of ~4-8 °C was treated with ultrasound, a threshold specific energy input of 100 kJ/kg of energy was required when using 1 MHz ultrasound, before observation of rapid fat separation was observed. By comparison, the specific energy input to the system in the low temperature experiments is 93 kJ/kg and 175 kJ/kg after 5 minutes for the 1 MHz and 600 kHz frequencies respectively. Hence, a possible reason to

237 explain this observation is because the threshold specific energy is reached sooner with application
238 of 600 kHz due to the higher energy input of the transducer.

239 With preheating to 25 °C, the 1 MHz frequency ultrasound appears to offer more rapid fat depletion
240 of the bottoms at a rate of ~5.0 g/min from 3.5 ± 0.06 % (w/v) fat to a concentration of 1.6 % (w/v)
241 and enrichment of fat to a cream layer with a concentration of 9.9 % (w/v) fat, after 5 minutes
242 processing. This corresponds to a relative change of -52.8 ± 2.3 % and 184.9 ± 33.2 % in the bottom
243 and top respectively. The bottom fraction separated by 600 kHz ultrasound was also significantly
244 improved ($P<0.05$) compared to the control by preheating to 25 °C, although less so compared to the
245 1 MHz ultrasound. In this case, a change from 3.4 ± 0.004 % (w/v) fat initially to 2.9 ± 0.16 % (w/v)
246 fat (-15.1 ± 4.7 % relative change) in the bottom was observed. The top fraction observed an
247 increase in fat to 4.3 ± 0.42 % (w/v) fat (26.4 ± 12.6 % relative change) which is not significant
248 ($p<0.05$) relative to the control).

249 Preheating the milk to 40 °C also improved the separation rate of fat from milk compared with the
250 sonication at low temperature for both the ultrasound frequencies considered. However, the rate at
251 which the separation occurred was (significantly, $P<0.05$) less compared with preheating to 25 °C
252 when using the 1 MHz frequency ultrasound.

253

254 Values of the acoustic contrast factor calculated for milk using Eq. 2 at various temperatures from
255 available density data reported by Mulder and Walstra[8] are shown in Table 1. At temperatures
256 above 40 °C, the milk fat also becomes mostly liquid meaning that the fat globules become slightly
257 more compressible relative to its surroundings. The increase in density difference and
258 compressibility also increase the magnitude of the acoustic contrast factor as per Eq. 2. If we
259 compare the values for 5 °C (2.72, -0.289) and 40 °C (12.41, -0.406), we can observe that there is an
260 increase in the magnitude by 4.5 and 1.4 times for the density and acoustic contrast factor,
261 respectively. This means that the primary radiation force will, according to theory, increase at higher
262 temperature, therefore promoting faster separation. The results obtained are interesting because,
263 despite this, the fat separation is not significantly more effective at the elevated preheating
264 temperature of 40 °C compared with preheating to 25 °C.

265 ## 3.3. Particle size distributions

266 The volume weighted mean diameter (D[4,3]) of controls (non-insonated) samples collected before
267 the 5 min period was 4.4 ± 0.03 µm, 4.5 ± 0.06 µm and 4.3 ± 0.07 µm for 5 °C, 25 °C and 40 °C

268 respectively, and did not change significantly ($P>0.05$) when held for 5 minutes at 5 °C and 40 °C
269 (Fig. 4). However, particle size significantly changed ($P<0.05$) in milk held at 25 °C after 5 min (Fig. 4)
270 to 4.62 ± 0.05 μm for sample collected at the top. This again confirms that the natural separation
271 rate for the time frames considered (5 minutes) in this study is fastest when the milk was preheated
272 to 25 °C.

273 Ultrasonic treatment at 5 °C with either 600 kHz or 1 MHz transducers, showed small change
274 (significant relative to control, $p<0.05$) after 5 min sonication (Fig. 4). For 600 kHz ultrasound, mean
275 D[4,3] values changed from 4.5 ± 0.15 μm initially to 4.4 ± 0.19 μm in the bottom and 4.7 ± 0.09 μm
276 in the top, whilst for 1 MHz ultrasound values changed from 4.36 ± 0.03 μm initially to 4.39 ±0.04 in
277 the bottom and 4.44 ± 0.11 μm in the top.

278 Ultrasound separation with preheating to either 25 °C or 40 °C however, lead to a larger shift to the
279 D[4,3] values of the top and bottom products compared with the initial product after 5 minutes
280 sonication (Fig. 4). This is again most prominent for the 1 MHz frequency after preheating to 25 °C;
281 under these conditions, a statistically significant shift of the D[4,3] from 4.5 ± 0.06 μm to 4.0 ± 0.05
282 μm in the samples taken from the bottom (10.8 ± 0.7 % relative change) and 4.9 ± 0.08 μm in the
283 top product (8.3 ± 1.0 % relative change) is observed. With preheating to 40 °C, the 1 MHz frequency
284 application results in a change from 4.5 ± 0.07 μm to 4.1 ± 0.07 μm in the bottom (-8.4 ± 1.6 %
285 relative change) and 4.8 ± 0.12 μm in the top (6.0 ± 1.5 % relative change).

286 Application of 600 kHz ultrasound after preheating milk to 25 °C resulted in a D[4,3] change from 4.6
287 ± 0.02 μm initially to 4.4 ± 0.04 μm in the bottom (-5.0 ± 0.9 % relative change) and 4.7 ± 0.09 μm in
288 the top (3.0 ± 1.6 % relative change), which are significantly less ($p<0.05$) compared with separation
289 using 1 MHz ultrasound at the same preheating temperature. With milk preheated to 40 °C, D[4,3]
290 values changed from 4.4 ± 0.08 μm initially to 4.2 ± 0.13 μm in the bottom (-6.0 ± 1.2 % relative
291 change) to 4.5 ± 0.02 μm in the top (2.4 ± 1.4 % relative change).

292 A comparison of the particle size distributions measured for the control and processed samples after
293 25 °C preheating is shown in Figure 5. As can be seen, the 1 MHz ultrasound is able to achieve high
294 differentiation in the particle size distributions to the top and bottom samples after processing for 5
295 minutes. By comparison, the lower frequency 600 kHz is not as effective even though the power
296 draw (and specific energy input) over the 5 minute duration is approximately double the 1 MHz
297 frequency. The likely reason for this is because the lower frequency ultrasound generates a smaller
298 acoustic force, and hence is not as efficient or as selective in moving the fat globules to the pressure
299 anti-nodes under these conditions. These results indicate that the 1 MHz ultrasound (with

preheating to 25 °C) studied resulted in the most rapid and hence selective removal of the fat globules after ultrasound application that leads to skimmed samples with proportionally higher amounts of small fat globules, and fat enriched samples with proportionally higher amounts of large fat globules. The control where no ultrasound is applied sees no observable change.

3.4. Role of agglutination

The observation that ultrasonic treatment of the milk at temperatures >40 °C is less effective for separation compared with preheating to 25 °C is interesting. A possible reason for this is the role of the agglutinin in the milk fat separation process. The immunoglobulins present in the milk, are known to aid the flocculation process by providing a means for globules which have collided to stick together more strongly[7]. The ultrasound increases the probability of fat globules colliding since they concentrate and accumulate in the anti-nodal planes of the standing wave field. At higher temperature, the influence of the agglutinin in the flocculation process decreases. As noted by Mulder and Walstra[8], the agglutinin is moved to the medium when temperatures are increased.

The role of agglutinin should be most prominent under cold conditions, where it remains attached to the surface of the fat globules. However, this study has shown that the ultrasound-assisted separation proceeds slowest when milk is initially at 5°C, except for when 600 kHz frequency ultrasound is applied.

4. Conclusion

The preheating of milk to 25 °C or 40 °C prior to ultrasound separation is beneficial to improving the efficiency of the separation process. The ability to reduce the time for which milk is exposed to ultrasound separation by operation at an 'optimal' temperature is important for industrial application as it reduces the required residence time of milk inside the ultrasound separation reactor, enabling potentially higher throughput. In this study, it was found that preheating the milk to 25 °C and applying 1 MHz frequency ultrasound resulted in the most rapid and synergistic fat separation than the separation without ultrasound. The 1 MHz ultrasound was also found to be significantly more effective at causing fat separation from the milk compared with the 600 kHz except for when no preheating was employed. It is speculated that effects of temperature may include alterations to globule ductility as the transition from solid to liquid progresses with temperature, in turn affecting agglutination. It is also possible that sonochemical modifications to

330 the immunoglobulins may occur. Further investigation to test hypotheses based on these
331 speculations would be warranted in future studies.

332

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336 # Table captions
337 Table 1: Density and viscosity parameters as a function of temperature obtained from Mulder and
338 Walstra[8]. The data for ρ_p are estimates assuming super cooling in fat crystallization.

339

340 # Figure captions
341 Figure 1: a) Photograph of experimental set-up used for experiments b) Schematic diagram of
342 experimental set-up.

343 Figure 2: Temperature change of the processed milk with time for a) 600 kHz and b) 1 MHz
344 frequency ultrasound processing. Error bars are the standard deviation from a minimum of 4
345 experimental trials.

346 Figure 3: Percentage change in fat concentration relative to initial sample after 5 minutes without
347 ultrasound processing (control) and with ultrasound processing using 600 kHz and 1 MHz frequency
348 for a) top and b) bottom samples. Error bars are the standard error of a minimum of 4 replicated
349 trials.

350 Figure 4: Percentage change in volume weighted mean diameters (D[4,3]) relative to initial sample of
351 milk for collected samples of initial and 5 minutes ultrasound processed samples from the a) top and
352 b) bottom fractions of the milk for no preheating (5 °C sample), preheating to 25 °C and preheating
353 to 40 °C. The error bars are the standard error of a minimum of 4 trials replicated under identical
354 conditions.

355 Figure 5: Particle size distributions with 25 °C preheating prior to 5 minutes of ultrasonic processing
356 for a) control (no ultrasound) b) 1 MHz ultrasound and c) 600 kHz ultrasound.

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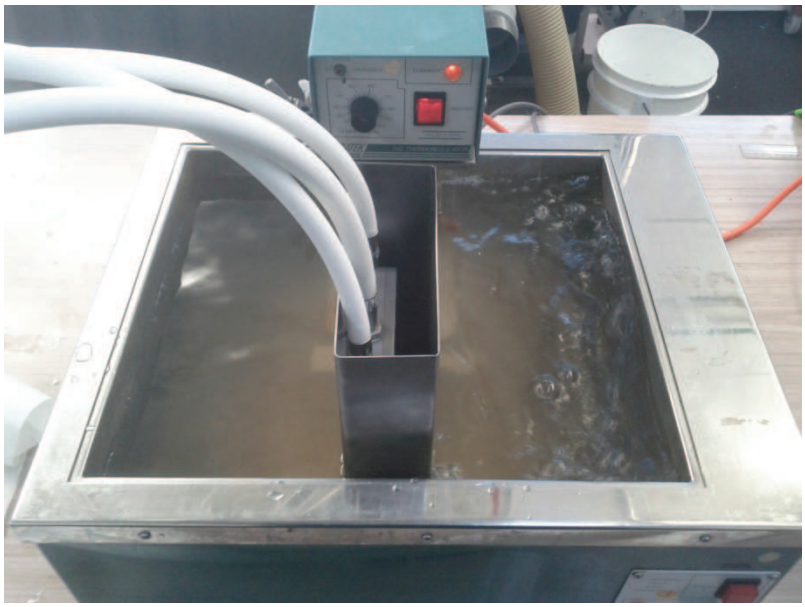
Table 1

Table 1: Density and viscosity parameters as a function of temperature obtained from Mulder and Walstra[8]. The data for ρ_p are estimates assuming super cooling in fat crystallization.

T (°C)	ρ_m (kg/m^3)	ρ_p (kg/m^3)	η_m (poise)	$\frac{(\rho_m - \rho_p)}{\eta_m}$	β_m (m^2/N)	β_p (m^2/N)	ϕ
5	1.0359	0.959	0.0283	2.72	4.40 x 10^{10}	5.32 x 10^{10}	-0.289
10	1.0352	0.951	0.0235	3.58	4.40 x 10^{10}	5.36 x 10^{10}	-0.306
15	1.0344	0.938	0.0199	4.84	4.40 x 10^{10}	5.44 x 10^{10}	-0.335
20	1.0333	0.916	0.0168	6.98	4.41 x 10^{10}	5.57 x 10^{10}	-0.387
25	1.0319	0.912	0.0144	8.33	4.41 x 10^{10}	5.59 x 10^{10}	-0.394
30	1.0300	0.909	0.0126	9.60	4.42 x 10^{10}	5.61 x 10^{10}	-0.397
40	1.0261	0.902	0.0100	12.41	4.44 x 10^{10}	5.66 x 10^{10}	-0.406
50	1.0198	0.895	0.0082	15.22	4.46 x 10^{10}	5.70 x 10^{10}	-0.410
60	1.0166	0.889	0.0069	18.49	4.48 x 10^{10}	5.74 x 10^{10}	-0.418

Figure 1

a)



b)

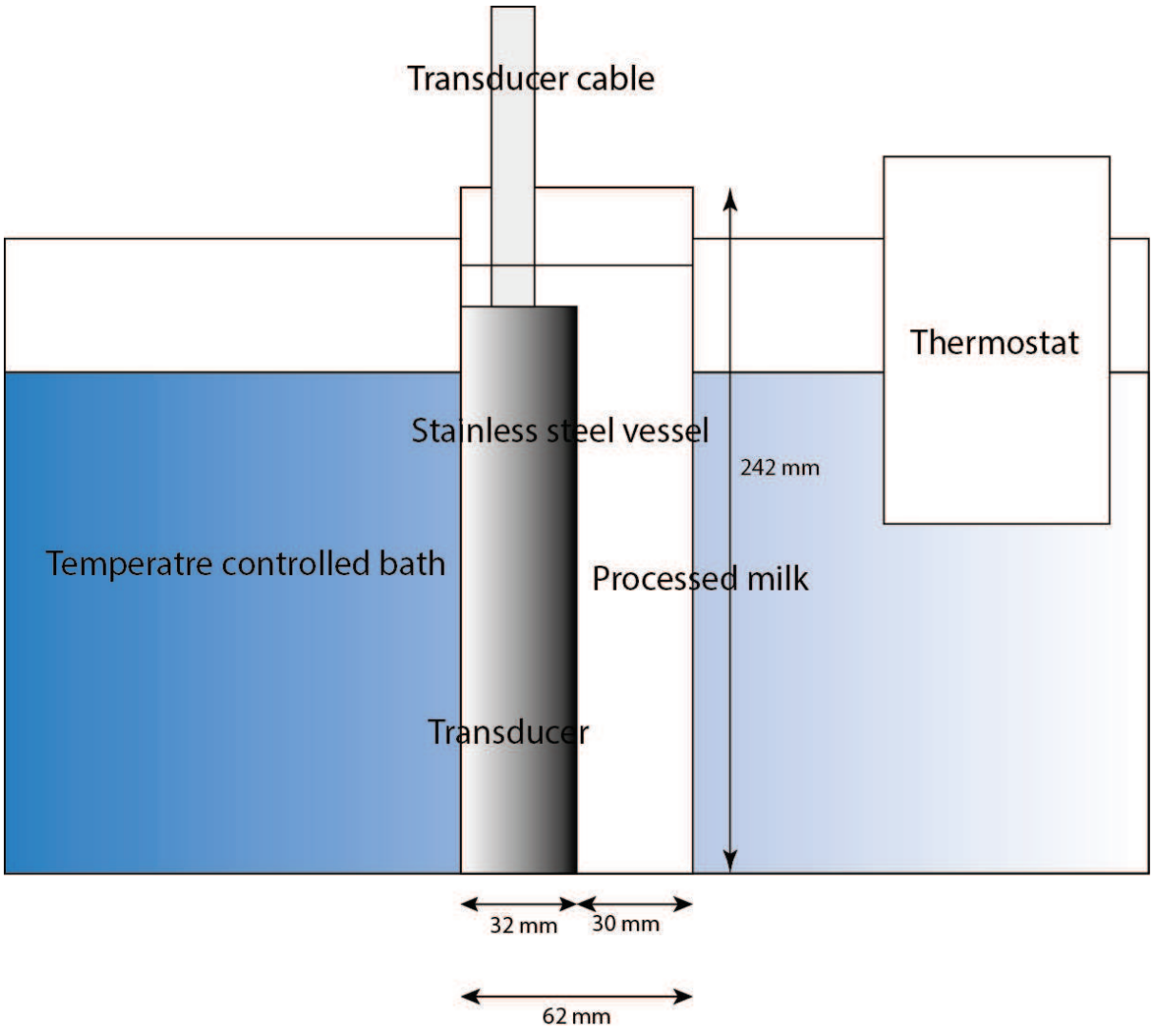
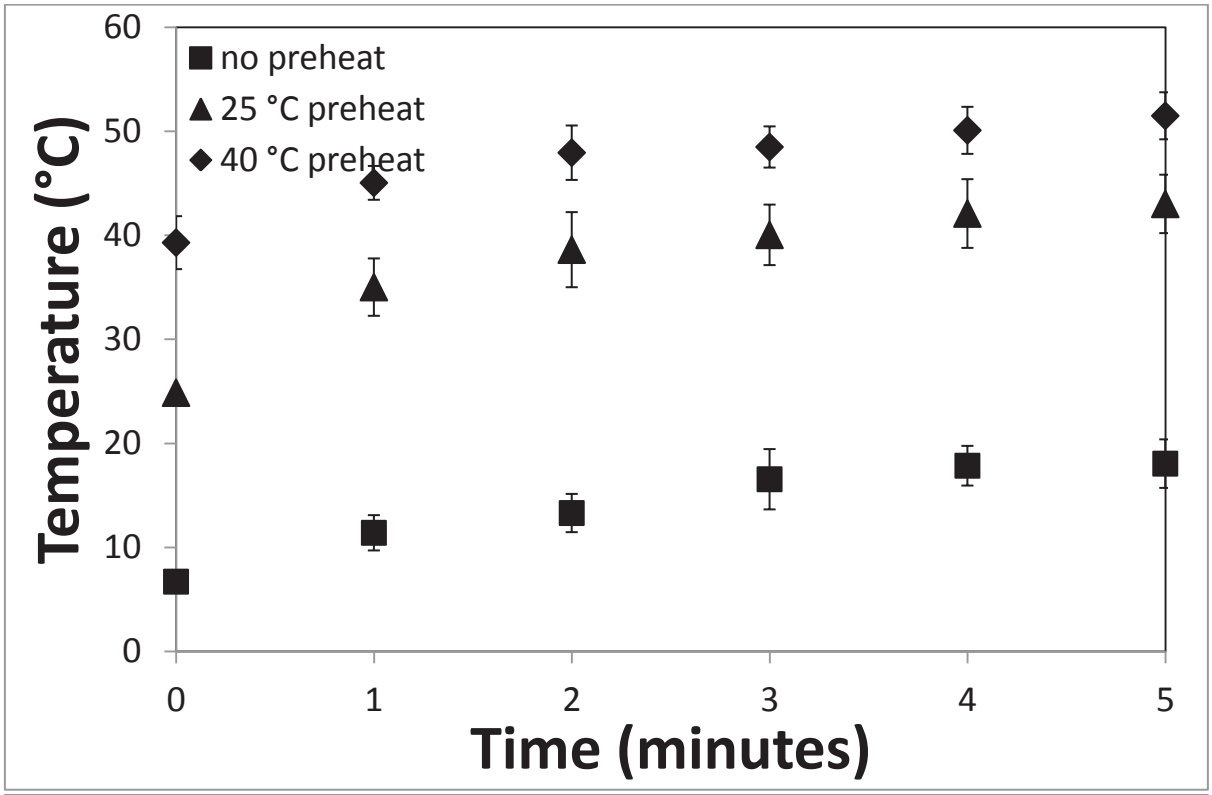
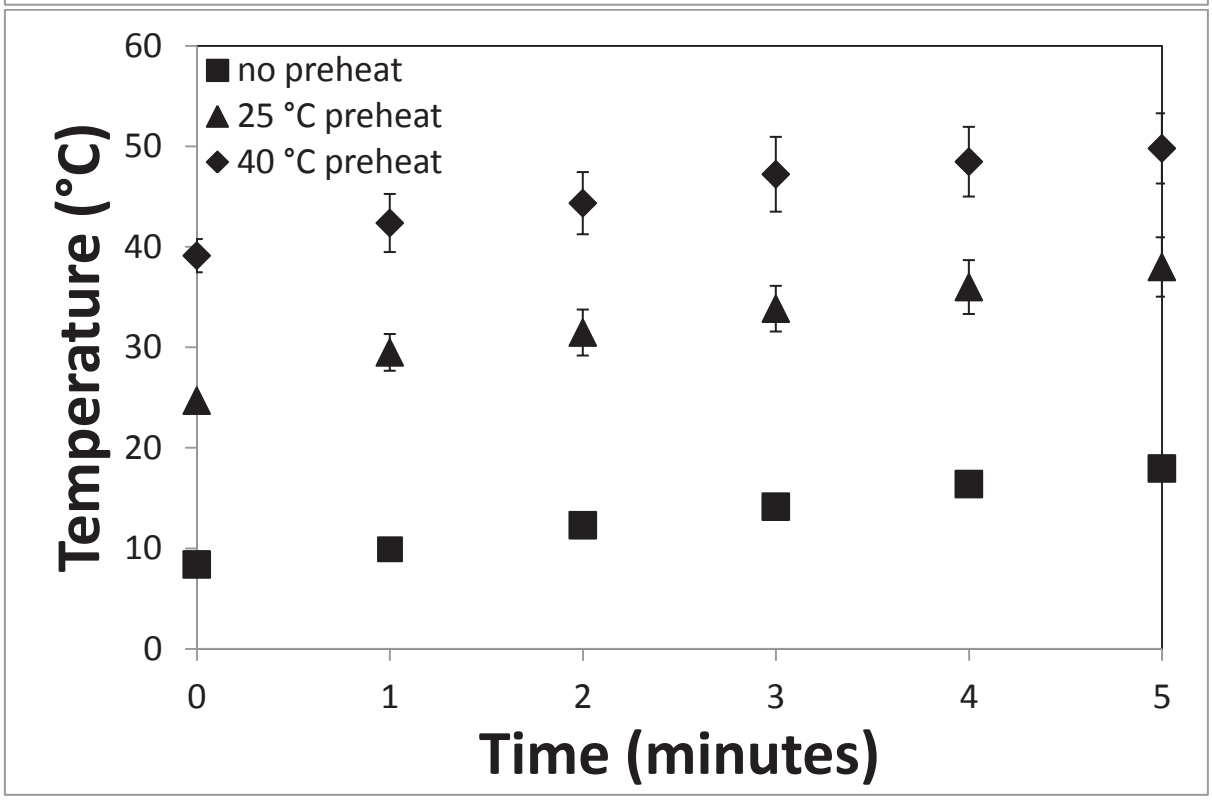


Figure 1: a) Photograph of experimental set-up used for experiments b) Schematic diagram of experimental set-up.

Figure 2



a)



b)

Figure 2: Temperature change of the processed milk with time for a) 600 kHz and b) 1 MHz frequency ultrasound processing. Error bars are the standard deviation from a minimum of 4 experimental trials.

Figure 3

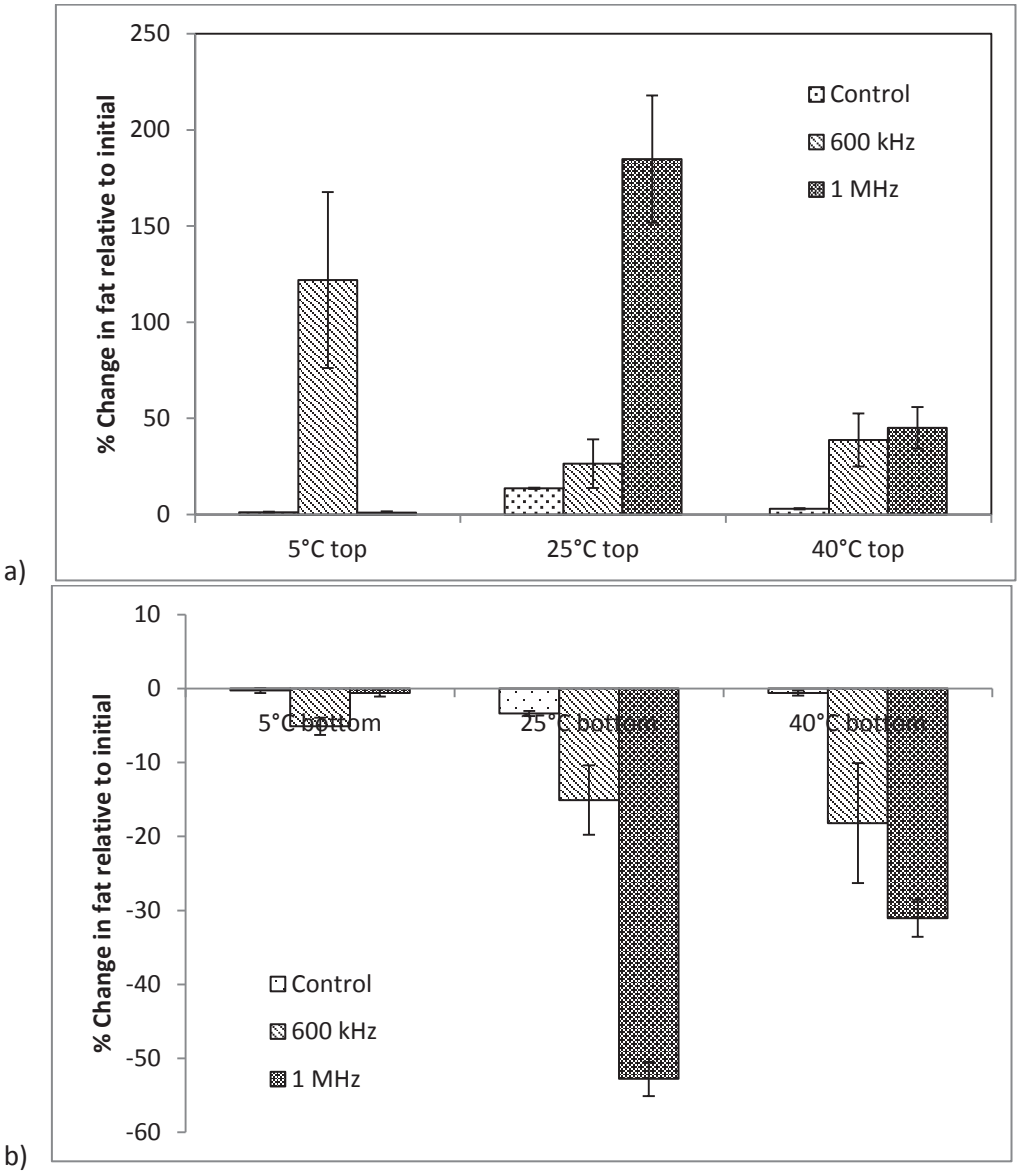


Figure 3: Percentage change in fat concentration relative to initial sample after 5 minutes without ultrasound processing (control) and with ultrasound processing using 600 kHz and 1 MHz frequency for a) top and b) bottom samples. Error bars are the standard error of a minimum of 4 replicated trials.

Figure 4

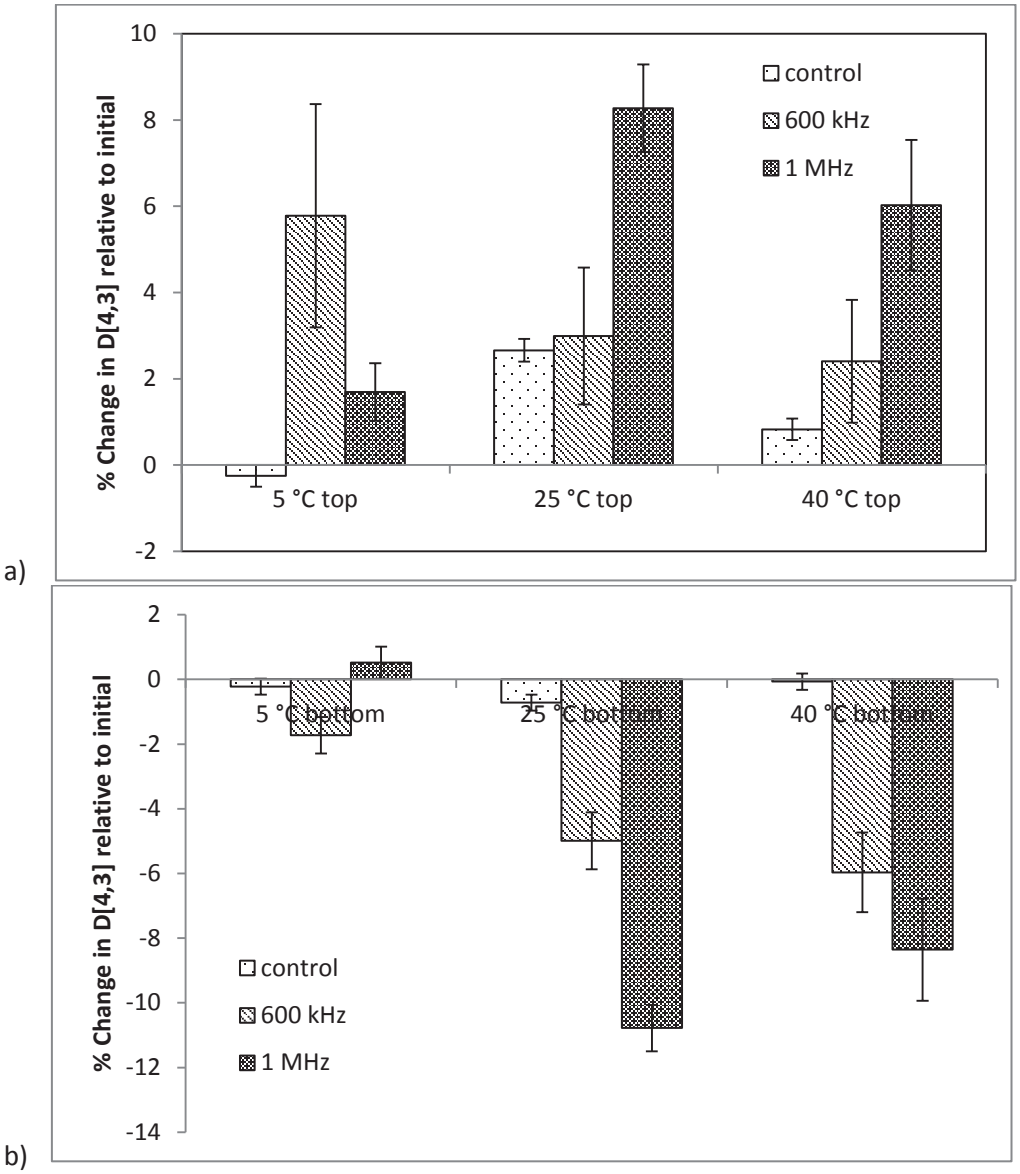


Figure 4: Percentage change in volume weighted mean diameters (D[4,3]) relative to initial sample of milk for collected samples of initial and 5 minutes ultrasound processed samples from the a) top and b) bottom fractions of the milk for no preheating (5 °C sample), preheating to 25 °C and preheating to 40 °C. The error bars are the standard error of a minimum of 4 trials replicated under identical conditions.

Figure 5

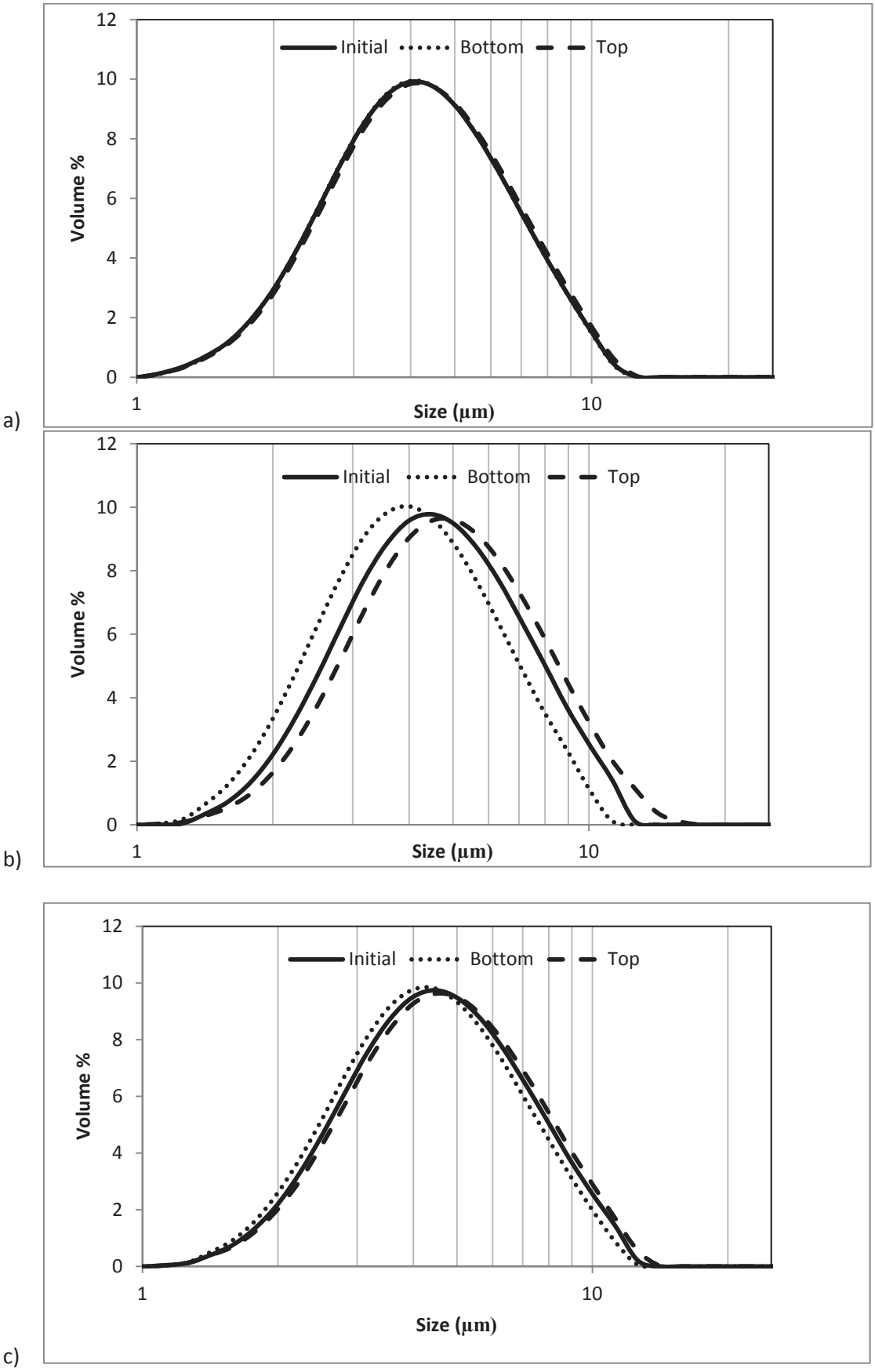


Figure 5: Particle size distributions with 25 °C preheating prior to 5 minutes of ultrasonic processing for a) control (no ultrasound) b) 1 MHz ultrasound and c) 600 kHz ultrasound.