Author: Leong, Thomas; Johansson, Linda; Juliano, Pablo; Mawson, Raymond; McArthur, Sally; Manasseh, Richard
Title: Design parameters for the separation of fat from natural whole milk in an ultrasonic litre-scale vessel
Year: 2014
Journal: Ultrasonics Sonochemistry
Volume: 21
Issue: 4
Pages: 1289–1298
URL: http://dx.doi.org/10.1016/j.ultsonch.2014.01.007

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PII: S1350-4177(14)00012-1
DOI: http://dx.doi.org/10.1016/j.ultsonch.2014.01.007
Reference: ULTSON 2489

To appear in: *Ultrasonics Sonochemistry*

Received Date: 5 September 2013
Revised Date: 6 January 2014
Accepted Date: 6 January 2014

Please cite this article as: T. Leong, L. Johansson, P. Juliano, R. Mawson, S. McArthur, R. Manasseh, Design parameters for the separation of fat from natural whole milk in an ultrasonic litre-scale vessel, *Ultrasonics Sonochemistry* (2014), doi: http://dx.doi.org/10.1016/j.ultsonch.2014.01.007

Design parameters for the separation of fat from natural whole milk in an ultrasonic litre-scale vessel

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Keywords: ultrasound processing; ultrasound separation; dairy processing; natural whole milk; milk fat globules

Abstract

The separation of milk fat from natural whole milk has been achieved by applying ultrasonic standing waves (1 MHz and/or 2 MHz) in a litre-scale (5 L capacity) batch system. Various design parameters were tested such as power input level, process time, specific energy, transducer-reflector distance and the use of single and dual transducer set-ups. It was found that the efficacy of the treatment depended on the specific energy density input into the system. In this case, a plateau in fat concentration of ~20 % w/v was achieved in the creamed top layer after applying a minimum specific energy of 200 kJ/kg. In addition, the fat separation was enhanced by reducing the transducer reflector distance in the vessel, operating two transducers in a parallel set-up, or by increasing the duration of insonation, resulting in skimmed milk with a fat concentration as low as 1.7 % (w/v) with respect to the unprocessed raw milk after 20 minutes insonation. Dual mode operation with both transducers in parallel as close as 30 mm apart resulted in the fastest creaming and skimming in this study at ~1.6 g fat/min.

Keywords: ultrasound processing; ultrasound separation; dairy processing; raw milk; milk fat globules

1. Introduction

A technique using ultrasound waves to initiate separation of fat globules from a recombined milk-fat emulsion has been recently reported by Juliano et al.[1, 2] Milk fat globules move to the pressure anti-nodes, due to a time-averaged primary radiation force described, for instance, in Yosioka and Kawasima[3]. Enhanced flocculation (reversible combination) or coalescence (irreversible combination) of the fat droplets may occur at these sites. This increases the effective floccule size of the fat globules, and cause a faster rise velocity and hence separation speed[4].

Enhanced creaming of re-emulsified fat orders of magnitude faster relative to natural buoyancy was reported on a litre-scale[1]. On a micro-scale, Grenvall et al.[5] and Johansson et al.[6] showed that acoustic waves could be used to specifically remove fat globules from skim-milk. To the best of our knowledge, no study has yet reported the use of ultrasound to achieve enhanced separation of fat globules from 'natural' whole milk, with particle size distributions and interfacial properties typical of the native product on a litre-scale.

Successful trials using natural whole milk have notably only been reported in smaller-scale experiments[2]. Trials using the same parameters as those reported by Juliano et al.[1] to separate fat from natural whole milk on a litre-scale were unsuccessful (data reported in Thesis by Sandra Temmel, University of Erlangen [7]).

The reason for this is because the model emulsion system investigated by Juliano et al.[1] and natural whole milk are fundamentally very different. Firstly, the particle size distribution of the recombined milk emulsion used by Juliano et al.[1] is significantly different to those found in natural milk. The volume weighted mean diameter, D[4,3], of the initial emulsions used by Juliano et al. were reported to be 23 μm. This is significantly larger than those used found in 'natural' milk, which are typically between 3-4 μm[4]. There are also a significant number of globules in the size range of 10-30 μm found in the recombined emulsions studied (see Fig.2 from Juliano et al.[1]). As noted by Mulder and Walstra[4], even a small number of 'large-globules' present in milk, can make up about 2-3 % of the total fat of the milk sample. Because the occurrence of these large globules in the recombined milk emulsion used by Juliano et al. is high, there is a skew of the percentage of total fat that is represented by large globules in the model system.[1]

In ultrasound separation, the size of the globules plays a significant role in how easily they can be manipulated by the applied ultrasound and also how strongly they are influenced by sedimentation/buoyancy. The primary radiation force scales with the radius to the third power[3].

This means that a globule with a radius of 10 μm is approximately 125 times more strongly influenced by the ultrasound than a globule that is 2 μm in radius. Since a high proportion of the fat is represented by these larger globules, the recombined milk emulsion as studied by Juliano et al.[1] is significantly easier to separate with ultrasound than 'natural' whole milk.

Secondly, the interfacial surface properties of the recombined milk emulsions and 'natural' milk, are completely different. In 'natural' milk, the fat globules are stabilised by a complex membrane layer consisting of primarily a tri-layer of phospholipids and proteins[4]. The nature of this surface prevents coalescence from readily occurring. By contrast, a 'recombined' milk emulsion does not have such a stabilising barrier. Instead, it is surrounded by casein micelles and other milk proteins that self-assemble on the surface[8]. Evidence of significant coalescence was observed upon application of ultrasound in the study by Juliano et al. using the recombined milk emulsion. It is uncertain if coalescence will occur readily in 'natural' milk systems when using high frequency ultrasound for the purpose of separation, but its absence would mean that rapid separation would be more difficult to achieve.

Further intensification of the process is necessary to achieve separation in 'natural' milk systems. Hence, this present study aims to establish the parameters that are suitable for separation of 'natural' whole milk on a litre-scale. Such a study could have significant practical relevance to the dairy industry.

An obvious strategy to speeding up the fat separation rate is to increase the acoustic power input or the frequency. According to the primary radiation force described in [3], this would result in a stronger acoustic force and likely result in more effective fat separation. However, it has been previously noted that achieving effective separation is not so simple as increasing the power, because effects such as acoustic streaming must also be considered[9]. Strong streaming velocities may disrupt the separation effectiveness by preventing globules from collecting at the pressure anti-nodes.

This current study investigates the efficacy of intensifying the specific energy density on the separation of fat from 'natural' milk systems by modifying the vessel geometry, the use of different frequency operation modes and adjusting the duration of ultrasonic insonation.

2. Materials and Methods

2.1.Ultrasonic separation trials

Raw whole bovine milk was sourced directly from the farm (Department of Primary Industries Ellinbank, Australia) and used for separation tests within 24 hours of obtainment. Milk was maintained at refrigerated temperatures during transportation and was stored in a cool-room at approximately 4 °C prior to usage. All trials were performed with reference to an initial starting sample obtained on the day and trials were performed on several days to mitigate the influence of compositional variation. Gentle mixing by stirring was utilised to recombine any natural cream that may have occurred prior to ultrasound processing.

Fully-submersible plate transducers (Sonosys Ultraschallsysteme GmbH, Neuenburg, Germany) of nominal frequency 1 MHz and 2 MHz were available for the separation trials. These transducers are identical in geometry, with dimensions of 160 mm x 160 mm x 30 mm, with an active area of 100 mm x 100 mm. A rectangular reaction vessel (width 201 mm, height 180 mm, length variable, constructed from polycarbonate with a wall thickness of 11.9 mm) was used. The two Sonosys transducers were positioned in parallel, as depicted in Figure 1. The plates themselves acted as the reflector in these trials. A 3.2 mm thick stainless-steel plate was positioned on the rear of the transducer on the liquid side of the vessel to prevent seepage of processed product from within the active side to the non-active side of the container, and vice versa.

For single-plate operation, one plate is operated while the other plate acts as the reflector. For dual-plate operation, both plates are switched on simultaneously. Since only one 1 MHz and one 2 MHz transducer were available, dual-frequency experiments applied greater power to the system as well as applying two frequencies simultaneously.

Milk samples (initial volume 1.6 L) were insonated at 100% nominal power using 1 MHz single (330 W), 2 MHz single (290 W) and 1 and 2 MHz in the dual frequency operation mode (620W). The duration of insonation was varied, with settings of 0, 5, 10, 15 and 20 minutes to evaluate the separation with time (processing was stopped for ~3 minutes to collect samples of ~20 mL by pipette from top and bottom at these times). The vessel geometry here was kept constant with a 45 mm sound source to reflector distance, with height and width of the milk in the vessel of 180 mm and 201 mm respectively.

The effect of vessel geometry was investigated by reducing or increasing the sound source to the reflector distance, while keeping the height and width of the vessel fixed. The distances considered were 30, 45, 85 and 135 mm (alignment accurate to ± 3 mm), corresponding to an initial volume of milk to be processed in the vessel of 1.1, 1.6, 3.1 and 4.9 litres respectively. The operation was with

dual-transducers for all the geometries considered here, whilst the 1 MHz single transducer and the 2 MHz single transducer was operated only for the 2 smallest geometries.

The influence of the power input was further investigated by reducing the nominal power to the transducer to 50 % (179 W) and 25 % (93 W) using the 1 MHz single transducer with the vessel geometry fixed at a distance of 45 mm.

Selected trials were repeated under identical processing conditions on different days. The results with the closest matching initial fat concentrations are shown.

2.2.Sample characterization

Samples were collected from near the bottom and top of the separation vessel using a 10 mL serological pipette. For the top layer, care was taken to extract the sample only from a very thin layer (approximately 2-3 mm depth) from the surface of the container. Approximately 20 mL of sample is collected from top and bottom after the prescribed treatment time.

2.2.1. Fat content

Fat content was analysed using the standard Rose-Gottlieb Method[10] to determine the fat concentration of the milk prior to ultrasound processing, and the top and bottom portions after ultrasound application. 10 mL of sample was digested in 2 mL of 25% ammonia solution with 10 mL of ethanol. The fat was then extracted from the mixture using three subsequent extractions with diethyl ether (VWR, AnalaR NORMAPUR) and petroleum ether (VWR, GPR RECTAPUR) into a pre-weighed spherical flask. The solvent was evaporated by rotary evaporation at 60 °C under vacuum (~0.15 atm), dried at 105 °C for 2 hours and left overnight to dry in a dessicator to remove all water content from the sample before weighing.

2.2.2. Particle size

Particle sizing was performed using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire) with deionised water as the dilutant. A refractive index of 1.46 (with an imaginary refractive index absorbance of 0.001 corresponding to an oil-in-water emulsion) was selected. Optical images were captured using a Leica microscope with a 40X objective.

2.2.3.Zeta-potential

A Zetasizer Nano (Malvern Instruments Ltd., Worcestershire) was used to determine the zeta-potential of the milk fat globules. Phosphate buffer (0.1 M) at a pH of 6.8 was used as the dilutant. Milk was diluted approximately 1:1000 and placed inside a disposable polycarbonate cuvette (ATA scientific, DTS1061). Measurements were repeated a minimum of 10 times per run with a minimum

of 3 runs. Commercially available homogenized full cream milk (Devondale, Australia) was used as a comparison for fully disrupted milk fat globules.

2.3.Vessel characterization

2.3.1. Power and temperature

The nominal power input could be digitally controlled on the Sonosys control unit, ranging from 5 to 100% nominal power. The transducers were operated at 100% nominal power (330 W and 290 W for 1 and 2 MHz respectively) unless otherwise specified. The electrical power draw was determined using a power meter. The energy evolved as heat in the processed milk was determined calorimetrically from temperature measurements using $Q = mC_P\Delta T/t$, where *Cp* is the specific heat capacity for milk, 3.94 kJ/kg.K[11], *m* the mass in kg, ΔT the temperature change in Kelvin, and *t* the time in seconds. The loss of energy due to absorption by the reactor walls and loss of heat to the surroundings by convection is not accounted for in this study, which may contribute to an error of >5% in the actual measurements [12].

2.3.2. Sound pressure

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.

3. Results and discussion

3.1.Vessel characterization and determination of fat surface integrity

A typical change in temperature of the milk during processing with ultrasound operated at 100% nominal power and a reflector/sound source distance of 45 mm, is shown below in Figure 2. At these frequencies, the temperature increase can largely be attributed to absorption of the sound waves as it becomes attenuated through the fluid medium and dissipation of heat from the transducer surface[13].

Calorimetry calculations were used to ascertain approximately how much of the electrical power delivered to the transducer is dissipated as heat in the processed milk. These results are shown in

Table 1. The energy calculations indicate that the energy dissipated as heat in the processed milk using the different modes of operation with either single (1 or 2 MHz frequency) or dual transducers (1 and 2 MHz frequency) are of similar proportion to the energy input from the electrical power draw for a given geometry. The energy efficiency (attributed to heat loss) can be calculated according to Gogate et al.[14] (power dissipated in liquid/electrical power supplied to the system), and ranges between 28-60% for the values in Table 1, depending on the geometry. These efficiencies are within expected levels for ultrasonic transducers operating in bath-type systems[14], indicative of a uniform distribution over a wide area.

The use of dual frequency ultrasound may result in complex interactions in the system which are not known or characterized in this present study. Furthermore, the heterogeneity of milk systems which consists of fat droplets distributed in a liquid matrix, may increase the degree of soundwave reflection, refraction and/or absorption[12]. More detailed assessment of the pressure distributions in such systems would assist in the design of more optimal ultrasonic milk separators in future work.

We make an assumption that the two imposing frequencies may impose constructively, which is confirmed by the maximum pressures measured in the system with dual frequency that are approximately the sum of the maximum pressures during single plate operation. The 2 MHz transducer has a lower maximum pressure compared with the 1 MHz transducer, and is likely due to differences in the pressure distributions resultant from their operation.

It should also be noted that the maximum pressure detected with the hydrophone under these operating conditions appears to be below the transient cavitation threshold predicted by Apfel[15] for water and blood. However, the cavitation that occurs may still disrupt or damage the integrity of the milk fat globules. To confirm the extent of any damage, the apparent zeta-potential of milk (1.6 L) that has been insonated for 20 and 40 minutes at 1 MHz (330 W) was measured and compared to unprocessed whole milk and homogenised full cream milk (Fig. 3).

The zeta-potential gives an indication to the relative proportion of casein micelles and other milk proteins that are at the surface of the milk fat globule[8]. A fully disrupted globule in the case of homogenised full cream milk, is completely surrounded by casein micelles and other milk proteins. In comparison, a milk fat globule in its ‘native’ state will be surrounded by a membrane consisting of a phospholipid tri-layer. The two situations give very different zeta-potential values, in this case -11.2 ± 0.2 mV and -16.2 ± 0.5 mV for unprocessed and homogenized milk respectively. The values obtained here for unprocessed and homogenized milks are similar to those reported by Michalski et al.[8] Values in between the two extremities give a relative indication of the amount of damage

incurred by the milk fat globules during any form of processing. In this case, the zeta-potential of the ultrasound processed samples show almost no change compared to the 'native' sample, with values of -11.4 ±0.2 and -11.5 ± 0.7 mV for 1 MHz processing after 20 and 40 minutes respectively, indicating no significant surface damage by the applied ultrasound.

Although the physical disruption of the milk fat globules under these conditions is shown to be negligible, the influence of other sonochemical effects on the milk components should also be considered. Studies performed by Villamiel and De Jong[16] and Chandrapala et al.[17] have previously reported the influence of power ultrasound on milk systems and their components, namely the fat globules, proteins and enzymes. Of primary concern is the possible formation of amyloid aggregates from the sonication of protein solutions, as reported by Stathopulos et al.[18] However, the recent report by Chandrapala et al.[17] could not find evidence of the formation of β-structures indicative of amyloid structures in milk based systems under the influence of high power ultrasound at 20 kHz frequency.

It is possible that the input of ultrasound in milk leads to slight denaturation of the proteins, such as casein and whey, due to a combination of sonochemical and thermal effects. However, 'natural' milk has many anti-oxidative components such as casein[19], enzymes, vitamins and lactoferrins that limit the extent of redox reactions that may occur[20]. In any case, the industrial processing of milk typically requires pasteurisation of separated milk at high temperatures such that any denaturation of proteins and other milk components by the ultrasound during separation is likely to be negligible in comparison.

3.2.Characterization of controls and insonated milk

Two controls were performed with milk obtained on different days. These controls were set-aside to cream naturally at ambient room conditions, for 15 minutes and 60 minutes respectively. The 15 minute and 60 minute controls had an initial fat concentration determined to be 4.2 % w/v and 4.5 % w/v respectively, which are within a fat content range expected for raw whole milk[4]. The result for these two controls are shown below in Table 2. No major change in fat content of samples drawn from the top and bottom was observed in either of the control samples when left to cream at room conditions (20 °C) for the specified duration.

An untreated sample from the same batch of milk as the 15 minute control was characterised using microscopy (Fig. 4a) and particle sizing (Fig. 4b). A volume from this same batch of milk was

insonated in the experimental vessel using the 1 MHz frequency with a sound source to reflector distance of 45 mm and 100 % (310 W) nominal power for 20 minutes.

Microscope images and particle size distributions of samples from the separated top and bottom after insonation are also shown in Figure 4a and Figure 4b respectively. No evidence for significant coalescence of fat globules can be deduced from either, suggesting that the mechanism for enhanced separation is primarily due to flocculation of the globules into larger entities that can be redispersed. The expected mechanism for this would be due to *agglutination*[4] possibly caused by milk serum immunoglobulins[21] that precipitate onto the surface of the fat globules. The separation therefore proceeds similarly to natural creaming albeit much more rapidly.

The particle size distributions obtained for the milk shows size distributions typical for raw whole milk with a mean diameter in the range of 3 to 4 μm[22]. The distributions of the separated samples also suggest that there is a clear concentrating effect of the larger fat globules in the cream, evidenced by the shift of the curve towards higher particle size (Fig. 4b). Similarly, there is a higher proportion of smaller globules retained in the bottom sample after processing, as these globules are much more difficult to remove by ultrasound as they experience a smaller force according to [3].

3.3.Effects of ultrasound on milk fat separation

3.3.1. Frequency operation mode

In Figure 5, the fat concentration of samples obtained from the top and bottom are plotted as a function of the duration of insonation by ultrasound. The results reported here are for a sound-source to reflector distance of 45 mm unless otherwise stated. Error bars associated with the reported fat concentration from these trials are estimated from systematic error propagation caused by the sampling and fat content analysis. The values obtained for the fat content of the top and bottom layers are estimated to have a relative error of 15% and 5% respectively (maximum absolute error estimated to be ±4% w/v). The relative error of 15% attributed to the fat content of the fat-enriched cream layer is an estimate based on the ability to collect sample from the top of the cream layer. Due to rapid separation, the cream layer is not completely homogenous and the collected samples can sometimes be ‘diluted’ by skim milk that has been entrained with the fat. The bottom sample is assumed to be well-mixed during processing by the fluid motion caused by acoustic streaming.

The dual frequency mode causes the most rapid creaming of fat to the top layer of the vessel (Fig. 5a), and causes the most effective skimming of fat from the bottom layer (Fig. 5b) owing to the

higher power delivery from the use of two transducers (620 W). The depletion rate of fat from the bottom layer is estimated to be ~ 1.6 g fat/min with the dual-transducer operation mode. The 1 MHz single transducer appears to offer slightly faster separation (~1.3 g fat/min) at this geometry compared with the 2 MHz single transducer (~0.8 g fat/min), although the power draw of the 2 MHz transducer is lower (290 W versus 330 W). The lower electrical power draw of the 2 MHz transducer (Table 1) is confirmed by the temperature evolution of the two single transducers in Figure 2, where the gradient of the fitted line for 1 MHz (1.2°C/min) is higher than for 2 MHz (0.95°C/min).

Although there is a steep increase in fat concentration after 10 minutes of insonation using the single 1 MHz and dual 1 and 2 MHz transducers, after 15 minutes of insonation, all three transducer operation modes result in creaming enhancement to the top layer, achieving what appears to be a saturation fat concentration between 15 to 25% (w/v) in a layer approximately 5 to 10 mm thick, which is representative of a thick loose cream layer that is formed rapidly by flocculation[1].

The concentration (% w/v) of fat in the top and bottom samples for the different operation modes performed at 45 mm (Fig. 6a) and 30 mm (Fig. 6b) separation distance are also plotted as a function of the specific energy (calculated as the electrical power consumption per kilogram of fluid, kJ/kg).

Figure 6 confirms that for 1 and/or 2 MHz frequencies, either choice of frequency, achieves similar creaming and skimming if we normalise the energy input with the volume/mass and time. In this case, the advantage of using dual transducers is that a higher rate of energy can be put into the system for the same equivalent time as a single transducer operated at 100% nominal power. This means that a desired fat concentration in either the bottom layer (skim product) or the top layer (cream product) can be achieved comparatively quicker.

The cream formed for the 30 mm separation geometry appears to plateau at a lower fat concentration (approximately 15 % w/v) compared with the 45 mm geometry. However, this top layer fat concentration is reportedly typical for cream formed at conditions that are more optimal for flocculation (pre-heated milks creamed at low temperature[4]). The more rapid flocculation seen here probably results in the cream being more loosely packed with a lower fat content, explaining why the fat concentration of the cream plateaus at approximately 15-20 % w/v rather than 20 – 25 % w/v.

In the case of the 30 mm geometry, a smaller volume (1.1 L) of milk was processed and beyond 10 minutes the temperature of the vessel became high (> 40 °C). It should be noted that very high temperatures can deactivate the agglutination mechanism that may be responsible for enhanced flocculation due to denaturation of the immunoglobulins present in the milk serum[21]. It is

321 therefore possible that increasing the temperature beyond 40 °C may also reduce the efficacy of the
322 ultrasound. However, this does not appear to be a problem in the current operation of the
323 separation system, as the skimming of the bottom product continues to proceed (Fig. 6b) in spite of
324 the higher temperatures. It has been reported by Caplan et al.[21] that temperatures of 76.9 °C (not
325 reached in this current study) are required to completely deactivate the natural creaming
326 mechanism.

327 It may appear surprising that both 1 and 2 MHz single transducer operations achieve similar fat
328 separation with energy input, even though the higher frequency would be expected to generate a
329 stronger acoustic force according to, for instance, Yosioka and Kawasima[3]. This assumption
330 however would require that the transducers to be geometrically identical in terms of their acoustic
331 field distribution and require the separation to be entirely dependent on the rate at which individual
332 globules are moved to the anti-nodal planes. These are not the case, as evident from the different
333 maximal pressures determined by hydrophone measurement for the 1 and 2 MHz (single-mode)
334 transducers in Table 1.

335 It has been claimed by Whitworth et al.[23] that for acoustic separations where ultrasound-assisted
336 flocculation is the mechanism for faster separation, the rate-determining step is not how quickly the
337 globules/particles will move to a banding location (this is usually in the order of 1-2 seconds[24]),
338 but rather how quickly they flocculate and hence rise/precipitate due to gravity owing to the larger
339 floccule size (usually in the order of tens of seconds to minutes). What this suggests is that when
340 operating on a litre-scale such as that considered here, the influence of the frequency for the
341 separation of fat globules in milk (provided that it is sufficiently high enough to influence the fat
342 globules and is not too high such that it is strongly attenuated in the system) is not as critical as
343 other parameters such as the energy density which influences the number and the intensity of
344 possible collision events in the overall system.

345

346 ### 3.3.2. Vessel geometry

347 Using the dual-transducer mode operation, the distance between the 2 plates was varied to 30 mm,
348 45 mm, 85 mm and 135 mm. The influence on the fat content of the collected top and the bottom
349 samples after prescribed durations of insonation can be observed in Figure 7.

350 At the largest geometry investigated (135 mm), the creaming enhancement to the top is the slowest.
351 Another observation for the 135 mm geometry is that the fat depletion in the bottoms does not
352 follow the trend of decreasing fat concentration with time observed for the smaller geometries. A

353 possible explanation for this is that the larger distance between the sound-source and reflector here
354 results in higher intensity acoustic streaming due to more attenuation of the ultrasound over this
355 distance. This can cause high recirculation of separated fat that reduces the efficacy of the
356 separation, similar to observations previously observed in other systems[9].

357 Shortening the distance between the two transducer plates increases the fat separation rate, and it
358 is evident that the separation proceeds most rapidly for the 30 mm plate-to-plate distance. The rate
359 of fat removal from the bottoms is ~1.6 g fat/min. As the processed milk volume is reduced with
360 decreasing plate distance, a higher specific energy is input to the system more quickly if the same
361 power draw (620W) is utilized as for the larger volumes. This higher energy density likely enhances
362 the rate at which globules are flocculated by the agglutinin (immunoglobulins located in the milk
363 serum)[21], possibly by enhancement of the number and the intensity of the globule-globule
364 collisions in the system.

365 A plot for a fixed, dual transducer operation set-up with varying vessel geometries is also plotted as
366 a function of specific energy in Figure 8.

367 Figure 8 suggests that for a fixed nominal power input, the process is scalable by geometry within
368 the investigated range. Of course, these results would be specific to this particular system and
369 transducer type used in this present study. Increasing the vessel geometry further may result in less
370 effective separation due to sound attenuation, which we observe in the results for the 135 mm
371 geometry in Figure 7b.

372

373 ### 3.3.3. Energy input

374

375 The power delivery to the transducers has been kept thus far at 100% nominal output. For the 1
376 MHz single transducer, the output was now lowered to 50% (179 W) and 25% (93 W) to ascertain
377 the effect of power delivery from a given transducer and geometrical setup. The result for the
378 change in fat concentration of the top layer is plotted in Figure 9 as a function of insonation time.

379 As expected, the lower the power input, the slower the rate at which fat is enriched in the top layer
380 resulting in a lower fat concentration. After 20 minutes insonation, fat content at the top layer was
381 determined to be 5.2±0.8 % w/v, 7.1±1.1 % w/v and 13.5±2.0 % w/v at the 25%, 50% and 100%
382 nominal power levels respectively. This would suggest that there is also scalability of fat separation
383 with regards to power and time for a given geometrical volume and transducer. Similar results for

the separation of oil droplets suspended in water have been reported by Nii et al.[25], where the extent of separation was found to be determined by the power input and/or duration of insonation.

As shown earlier in Figure 6 and 8, plotting the fat concentration of separated top and bottom layers as a function of the specific energy provides some interesting insight into how to more effectively and efficiently achieve separation. There appears to be a threshold specific energy input at approximately 100 kJ/kg, where significant creaming and separation begins to occur.

Also interesting is that above 200 kJ/kg of energy input, the creaming efficacy plateaus for the 30 mm and 45 mm geometries considered, suggesting that the saturation concentration of the cream able to be collected after separation by ultrasound is approximately 20-25% w/v, which is the limit for cream layers formed by natural sedimentation[4]. Higher specific energy inputs were not considered with the larger geometries since longer insonation time (>20 minutes) were required.

For the 30 mm separation distance, this plateau is somewhat lower at approximately 12-15% w/v. These concentrations are also lower than for cream separated by use of a centrifuge heated to 40 °C, which can usually achieve upwards of 40% w/v[4]. Cream with a fat content higher than 80% can be generated in the production of anhydrous milk fat by centrifugation[22] (typically at temperatures >90˚C and g-force > 12000). It should however be noted that in the centrifuge, the cream product is collected from between very narrow regions (ie. between stacked disc plates) that enables high-density crowding of the fat globules. In the ultrasound separator, the cream that is collected is possibly resultant from the rapid formation of irregular large floccules and hence has a lower fat crowding concentration. Further evaluation of the flocculation mechanism of the milk fat globules under the influence of ultrasound is suggested in future work to confirm this.

Although the cream formation is limited by a saturation fat content due to the proposed flocculation mechanism, further input of energy causes continued skimming of the bottom fraction indicating that there is continued removal of fat globules from the bulk bottom fraction of the milk by ultrasound. Furthermore, the results suggest scalability with respect to both power and vessel geometry within this investigated parameter space that is simpler than expected. The influence of energy dependent effects such as acoustic streaming must still be considered for larger vessel geometries, and may be the cause of errors in these trials that causes deviation of results from expected trends.

Conclusion

It has been demonstrated that fat separation in natural whole milk on a litre scale using ultrasonic standing wave system is possible. Several important parameters have been evaluated to understand their impact on the ultrasound separation efficacy. It was found that a higher energy density was key to increasing the rate of fat separation, and could be achieved by reducing the vessel geometry or using dual transducers. These observations are useful in providing further insight into suitable design parameters for the scale-up of ultrasonic separation vessels.

Acknowledgements

The authors would like to acknowledge the Australian Research Council (LP110200499) and The Geoffrey-Gardiner Dairy Foundation for providing the funding for this research. The authors would also like to thank Peter Fagan, Amy Logan, Jenny Favaro, Amirtha Puvanenthiran, Manoj Rout and Michael Kelly from CSIRO Animal Food and Health Sciences for their assistance in teaching some of the analytical techniques performed in this study.

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Table captions

Table 1: Determination of energy input to the system from power draw and calorimetry. The pressures reported are the maximum pressure in the system determined using a needle hydrophone.

Table 2: Fat content of control samples

Figure captions

Figure 1: Schematic of the experimental set-up for batch mode operation for raw milk fat separation. The adjustable distances between the two transducer plates considered in the experiments are 30 mm, 45 mm, 85mm and 135 mm.

Figure 2: Temperature change in the vessel with application of 1 MHz (330 W), 2 MHz (290 W) and 1+2 MHz (620 W) dual frequency with a transducer-reflector distance of 45 mm (processing volume of 1.6 L).

Figure 3: Zeta-potential of unprocessed whole milk, ultrasound processed milk and homogenized full cream milk. 1 MHz ultrasound at a nominal power of 330 W was input to a volume of 1.6 L. Homogenized milk was obtained off the shelf from the supermarket.

Figure 4: a) Microscopy images obtained of i) initial milk sample ii) bottom product after 20 minutes insonation and iii) top product after 20 minutes insonation, with 1 MHz and sound source to reflector distance of 45 mm. b) Particle size distribution of the initial milk sample, bottom product after 20 minutes insonation and top product after 20 minutes insonation, with 1 MHz frequency and 45 mm sound source to reflector distance.

Figure 5: Fat content of the a) top and b) bottom samples for different frequency modes of operation. The transducer-reflector distance is kept constant here at 45 mm. Error bars indicate the relative error attributed to the sampling and fat content analysis.

Figure 6: Concentration of fat in the top and bottom for various operating modes of separation for a transducer-reflector separation distance of a) 45 mm and b) 30 mm, plotted as a function of the specific energy density. Error bars indicate the relative error attributed to the sampling and fat content analysis.

Figure 7: Change in fat content (% w/v) of the top (a) and bottom (b) portions as a function of insonation time, for several transducer-reflector distances (30 mm, 45 mm, 85 mm and 135 mm) using the dual separation mode. Error bars indicate the relative error attributed to the sampling and fat content analysis.

Figure 8: Concentration of fat in the top and bottom for the dual transducer operating mode for various separation distances between reflector and transducer, plotted as a function of the specific energy density. Error bars indicate the relative error attributed to the sampling and fat content analysis.

Figure 9: Effect of nominal power delivery to the 1 MHz transducer on the creaming and skimming behaviour using a 45 mm transducer-reflector geometry as a function insonation time. Error bars indicate the relative error attributed to the sampling and fat content analysis.

Table 1: Determination of energy input to the system from power draw and calorimetry. The pressures reported are the maximum pressure in the system determined using a needle hydrophone.

Separation distance (mm)	Frequency operation mode	Electrical power draw (W)	Calorimetric power (W)	Pressure (kPa)
30	1 MHz	332±7	94±9	86±13
30	2 MHZ	288±6	83±8	16±2
30	Dual	620±12	134±13	109±16
45	1 MHz	332±7	138±14	59±9
45	2 MHZ	288±6	106±11	20±3
45	Dual	620±12	214±21	80±12
85	Dual	620±12	363±36	75±11

Table 2: Fat content of control samples

Sample	% Fat w/v	% Enhancement (relative to initial)
Initial control 1	4.2±0.2	
15 min control 1 bottom	4.3±0.2	2±0.1
15 min control 1 top	4.3±0.2	2±0.1
Initial control 2	4.5±0.2	
60 min control 2 bottom	4.6±0.2	2±0.1
60 min control 2 top	4.7±0.2	4±0.2

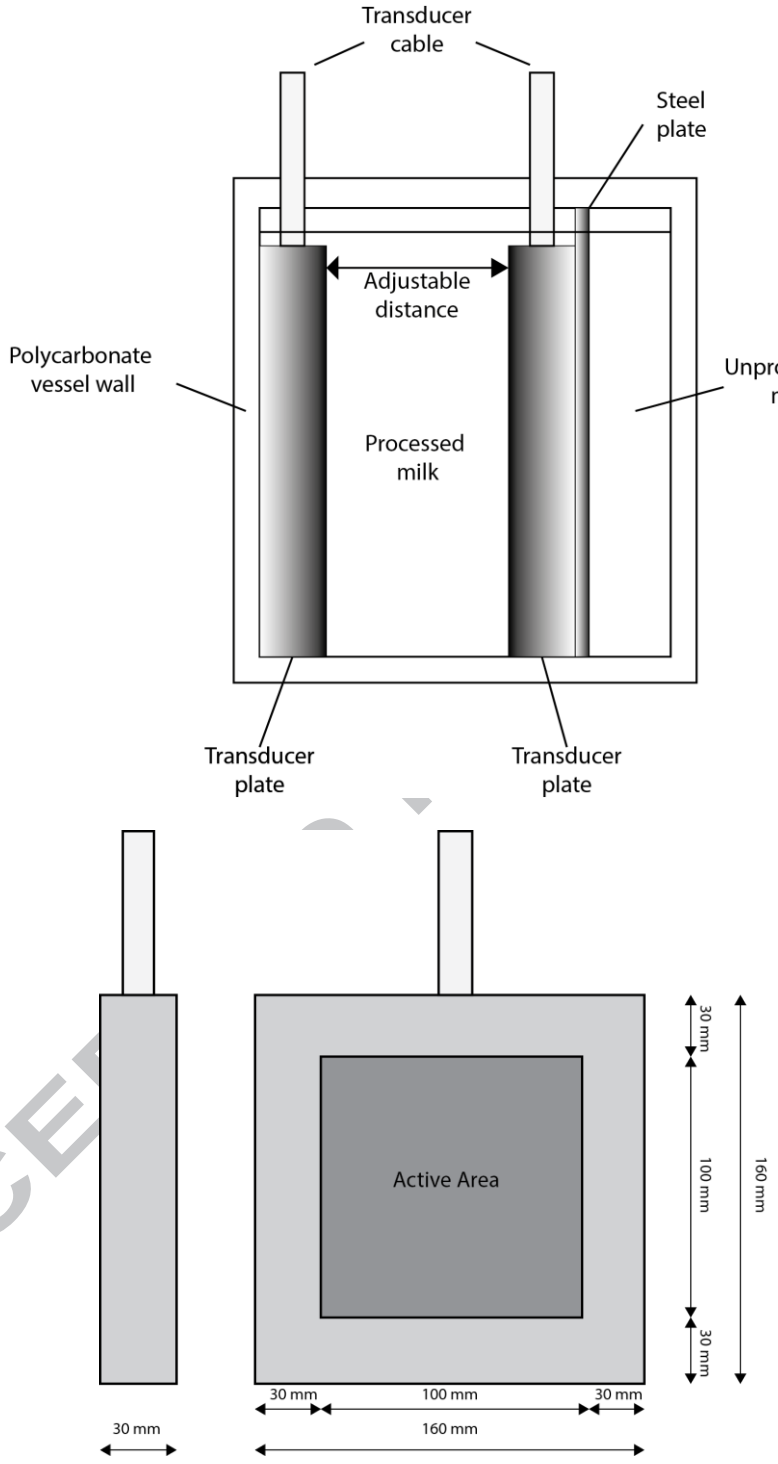

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Figure 2

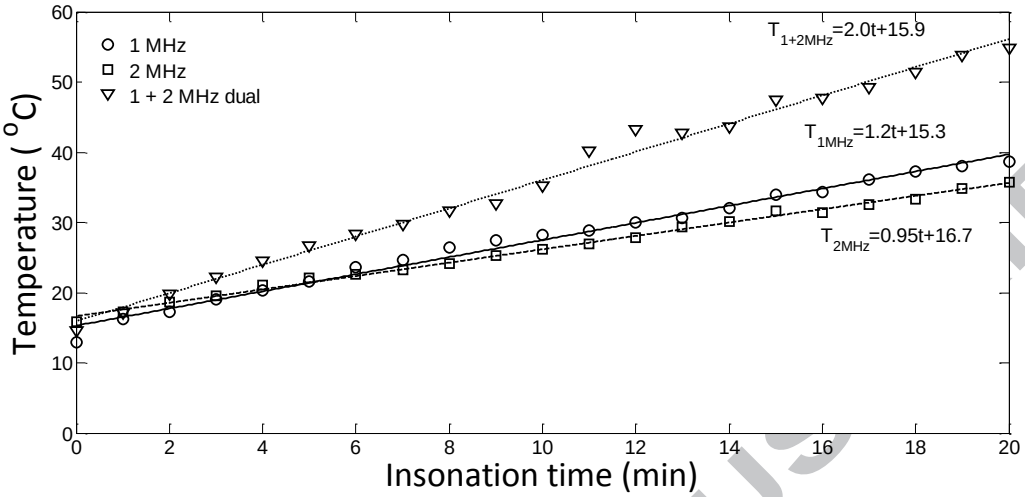

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Figure 3

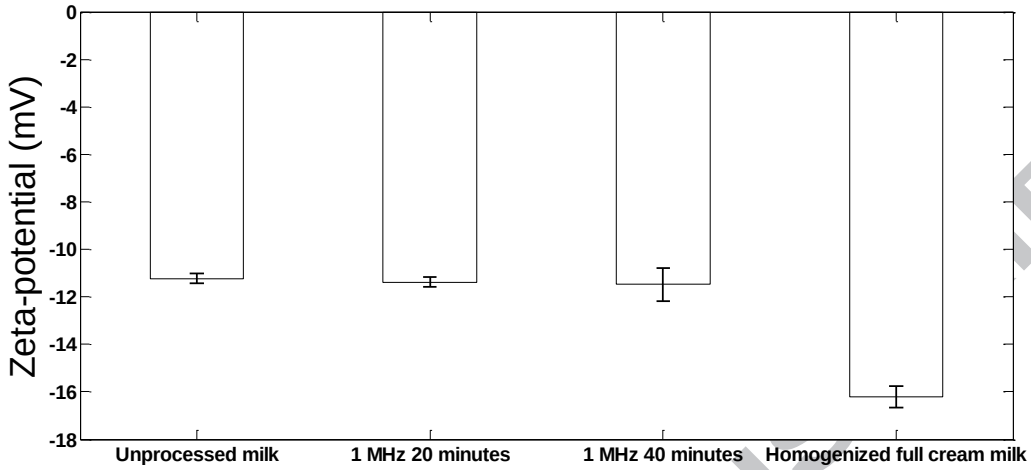

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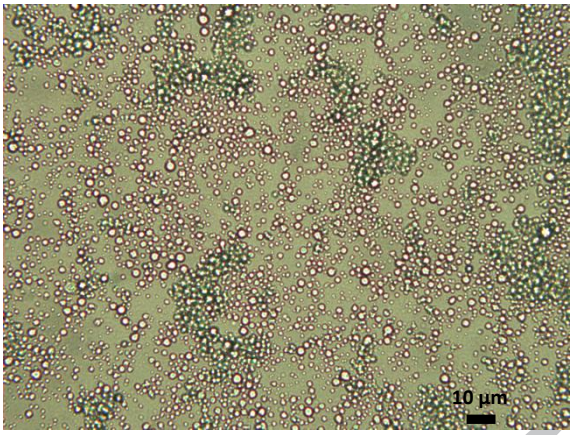
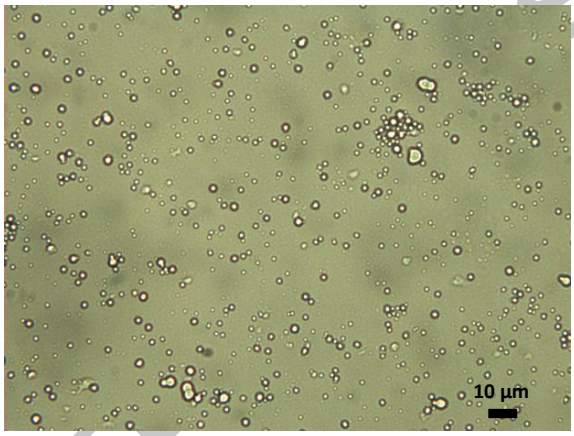
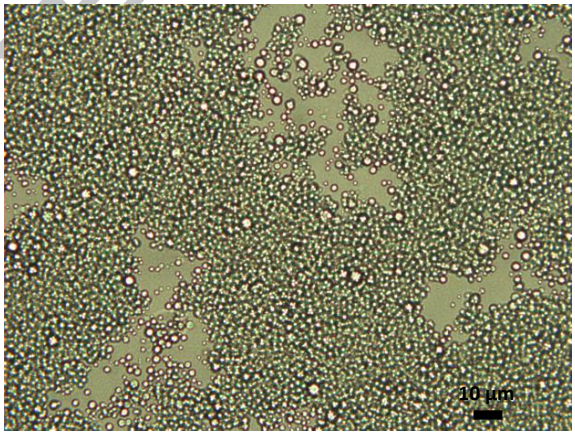
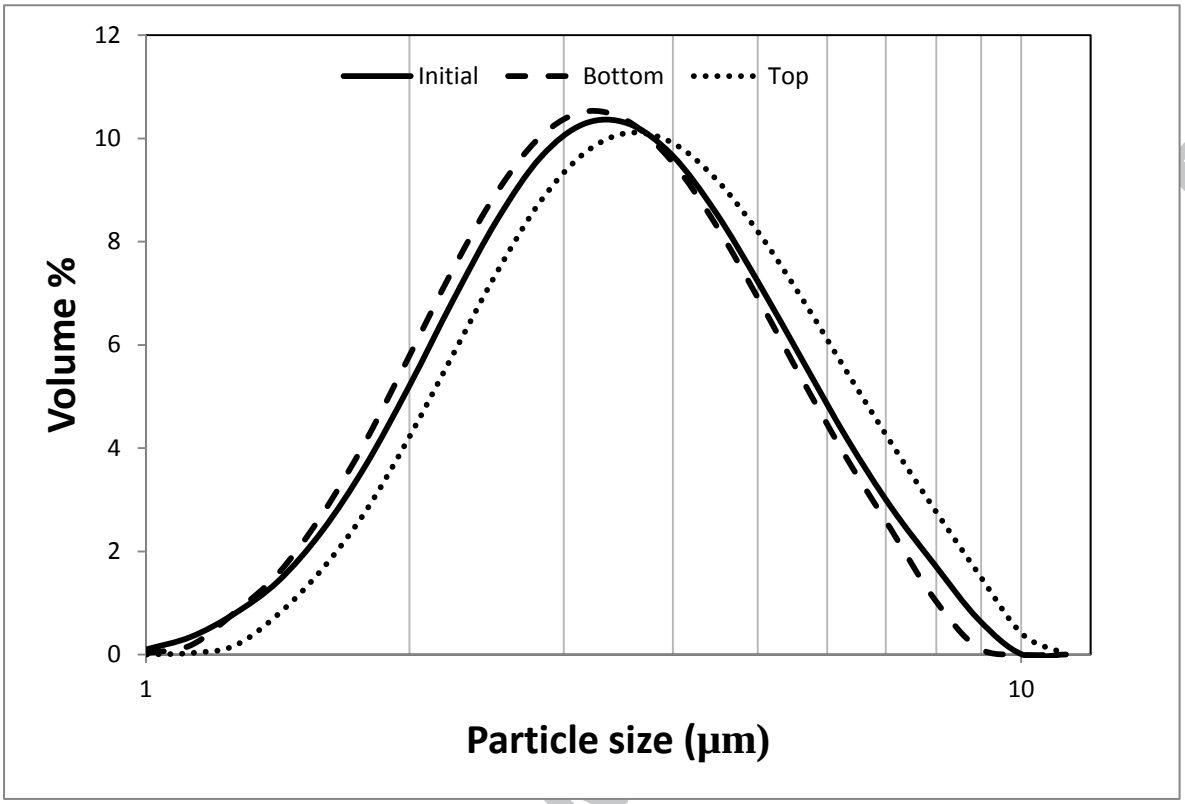
Figure 4

a)i) 

ii) 

iii) 

b)



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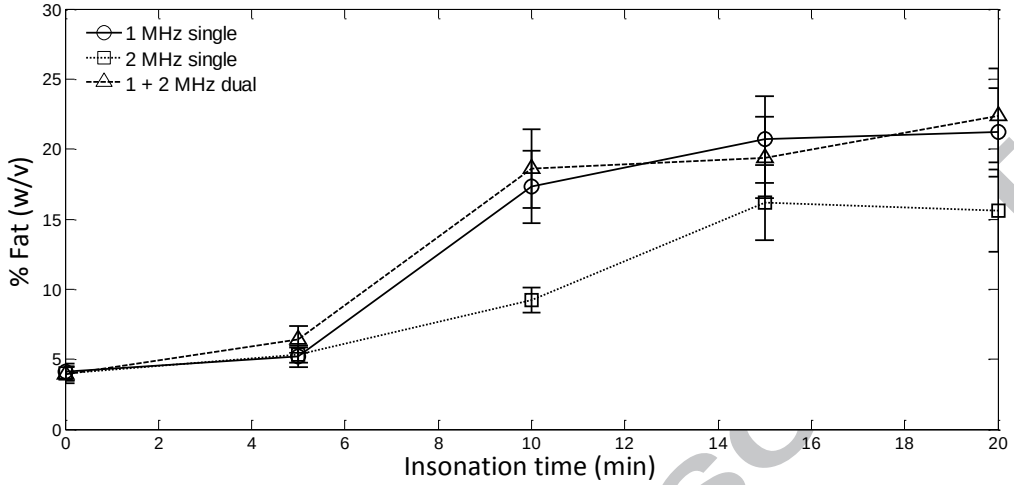
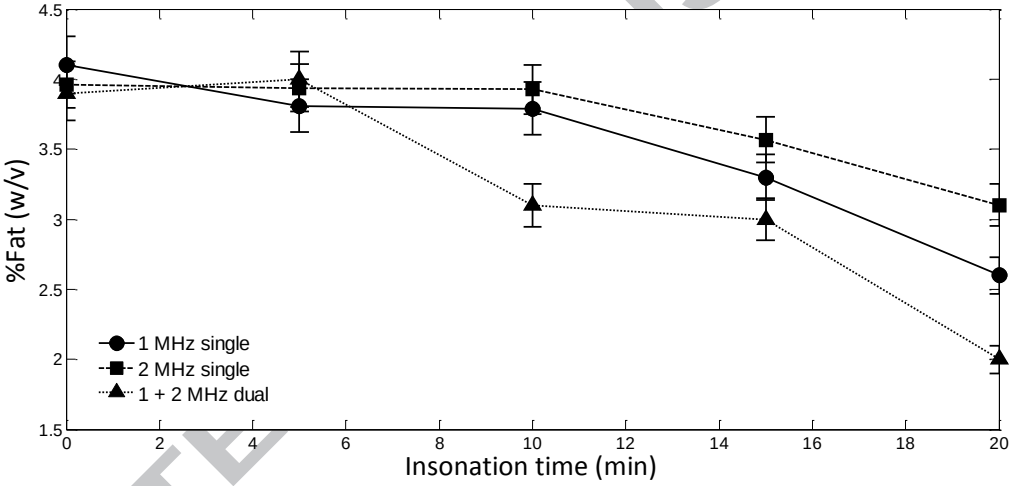


a)



b)

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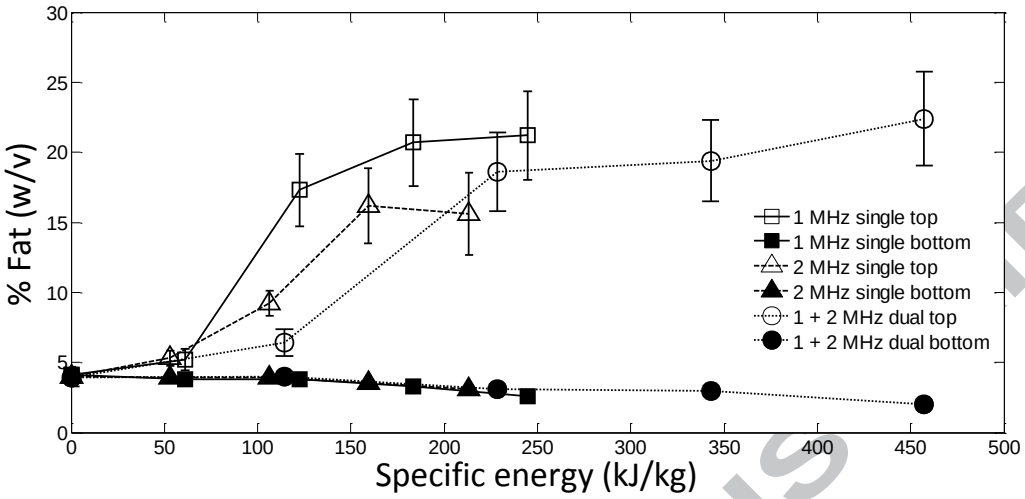
a)



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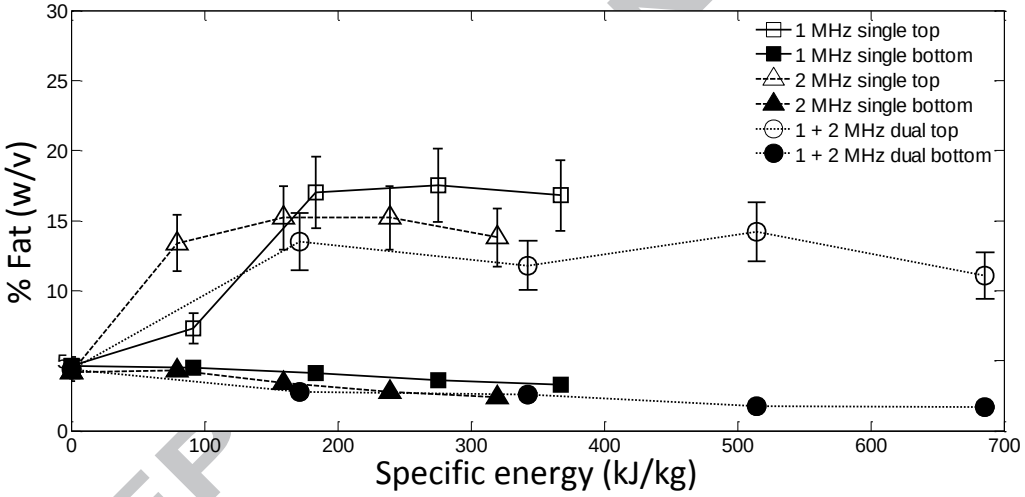

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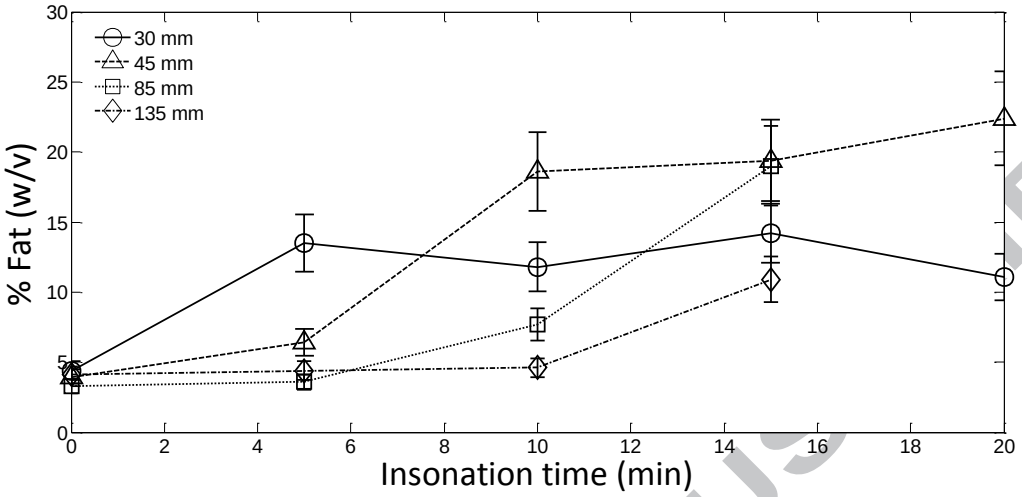
a)



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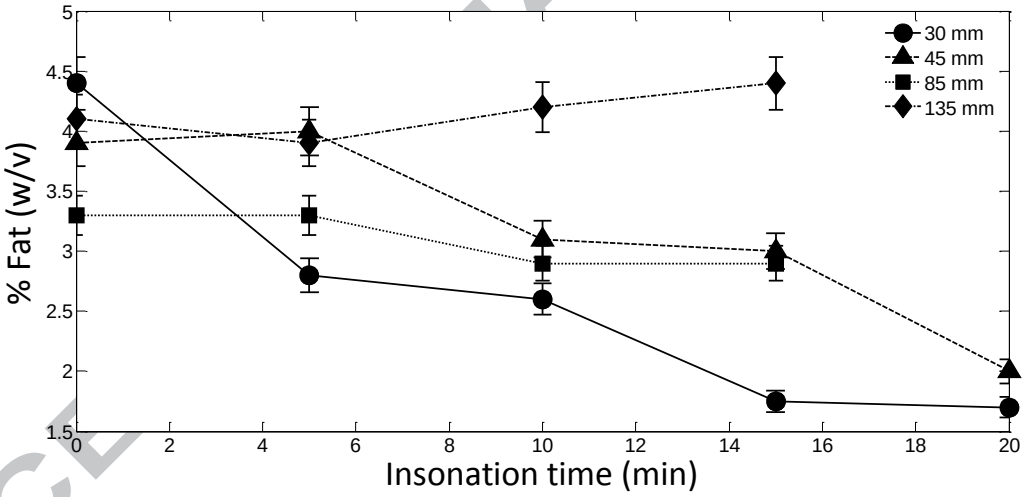

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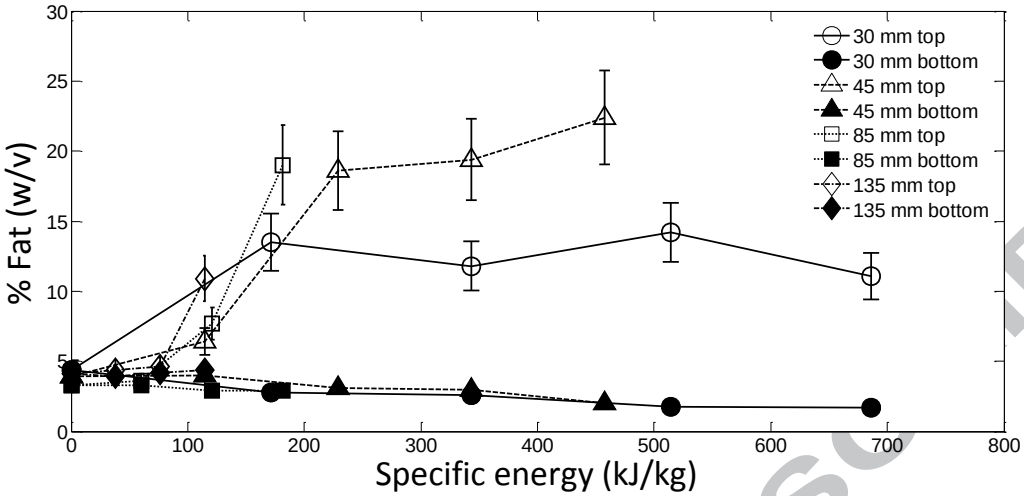
Figure 8



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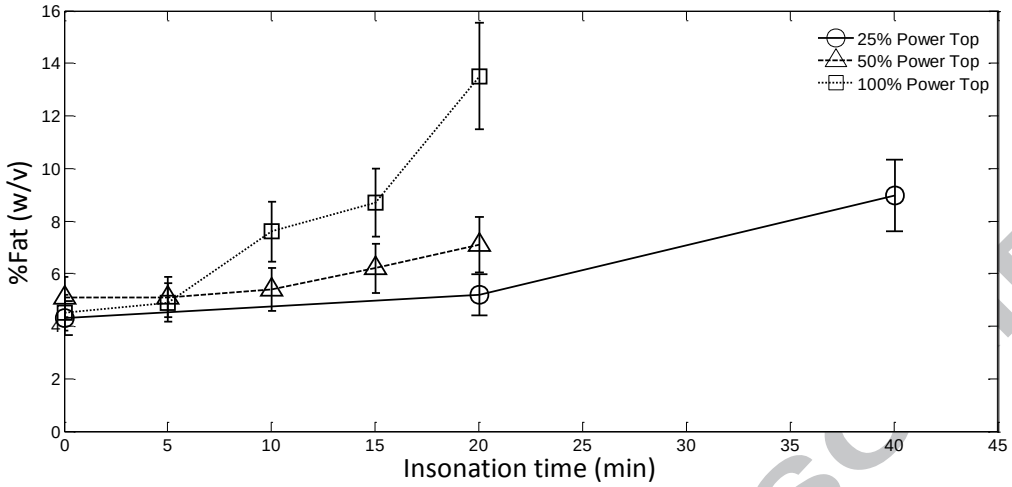

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Design parameters for the separation of fat from natural whole milk in an ultrasonic litre-scale vessel

Research Highlights

► Ultrasonic separation of milk fat from natural whole milk has been demonstrated only in millilitre scale systems previously

►Separation was enhanced in a litre scale ultrasonic reactor holding several transducer arrangements

► The duration of insonation, vessel dimensions, and specific energy input influenced separation

► Ultrasound application at parameters suitable for separation did not disrupt the integrity of fat globules

► This research identifies the key parameters to develop an ultrasonic milk fat separation device