



Ultrasonically enhanced fractionation of milk fat in a litre-scale prototype vessel



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ABSTRACT

The ultrasonic fractionation of milk fat in whole milk to fractions with distinct particle size distributions was demonstrated using a stage-based ultrasound-enhanced gravity separation protocol. Firstly, a single stage ultrasound gravity separation was characterised after various sonication durations (5–20 min) with a mass balance, where defined volume partitions were removed across the height of the separation vessel to determine the fat content and size distribution of fat droplets. Subsequent trials using ultrasound-enhanced gravity separation were carried out in three consecutive stages. Each stage consisted of 5 min sonication, with single and dual transducer configurations at 1 MHz and 2 MHz, followed by aliquot collection for particle size characterisation of the formed layers located at the bottom and top of the vessel. After each sonication stage, gentle removal of the separated fat layer located at the top was performed.

Results indicated that ultrasound promoted the formation of a gradient of vertically increasing fat concentration and particle size across the height of the separation vessel, which became more pronounced with extended sonication time. Ultrasound-enhanced fractionation provided fat enriched fractions located at the top of the vessel of up to $13 \pm 1\%$ (w/v) with larger globules present in the particle size distributions. In contrast, semi-skim milk fractions located at the bottom of the vessel as low as $1.2 \pm 0.01\%$ (w/v) could be produced, containing proportionally smaller sized fat globules. Particle size differentiation was enhanced at higher ultrasound energy input (up to 347 W/L). In particular, dual transducer after three-stage operation at maximum energy input provided highest mean particle size differentiation with up to 0.9 μm reduction in the semi-skim fractions. Higher frequency ultrasound at 2 MHz was more effective in manipulating smaller sized fat globules retained in the later stages of skimming than 1 MHz. While 2 MHz ultrasound removed $59 \pm 2\%$ of the fat contained in the initial sample, only $47 \pm 2\%$ was removed with 1 MHz after 3 ultrasound-assisted fractionation stages.

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1. Introduction

There has been recent interest in the ‘fractionation’ of milk fat globules (MFGs) into defined particle size distributions for the production of dairy products with more desirable properties [1–3]. Notably, milk with higher proportions of small MFGs have been reported to produce cheeses with superior taste and texture [1] and also be advantageous in increasing cheese yields [2]. Milk or cream with proportionally larger fat globules have also been shown to be desirable in the production of butter [1]. A recent

study by Logan et al. [4] has shown that small and large MFGs influence the gelling properties of milk for cheese making.

The use of ultrasonics to increase gravity separation velocity by size flocculation is expected to provide fat globule size specificity to its separated milk fractions. Higher frequency ultrasound induces high acoustic radiation forces [5], making its application potentially more amenable to influence smaller sized globules. Although the ultrasound-assisted fat separation by use of different frequencies and arrangements have been reported demonstrating enhancement of larger fat globules in the cream fraction [6,7], a more targeted study for ultrasound to assist the fractionation of milk fat in whole milk into size fractions has not yet been performed.

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The usual process for fat separation at industrial scale is by centrifugation, which involves spinning of the milk at high speeds to apply high g-forces [8]. An industrial-sized centrifuge commonly used to achieve rapid separation of fat has limitations in its ability to fractionate milk fat globules. The g-forces imparted by centrifugal acceleration are also difficult to control; either sequential operation by variations in rotational speed, or carefully timed extraction of fractions demanding a much more complex centrifuge, would be needed to specifically remove fat globules of particular sizes. Timmen and Patton [9] have shown the possibility of achieving changes in the mean particle sizes of the cream and skim by centrifugation ($\sim 300\times g$ acceleration, 15 min), although the volumes reported (20 mL tubes) are small compared with industrial operation. Logan et al. [4] have also reported MFG fractionation by differential centrifugation using several stages of centrifugation. The total centrifugation time required for such a process was reported to be ~ 75 min.

Some manufacturers of traditional hard cheeses in regions like Northern Italy, famed for products such as Parmesan Reggiano and Romano cheeses, take advantage of the fractionation achieved during gravity separation of the cream in shallow vats [10,11]. As larger globules rise quicker according to Stokes' Law [12], the semi-skimmed milk ($\sim 2\%$ fat milk) retained in the bottom will have a particle size distribution containing a proportionally higher amount of smaller fat globules. It has been reported that the size distribution produced from natural gravitational separation aids the flavour development of the cheese [10] and provides a smoother texture and mouth-feel. However, natural gravitational separation requires long wait times (several hours) which may be problematic for food safety in the context of dairy production.

The use of membrane filtration has been identified as one possible technology to achieve size-specific fractionation of milk fat globules [13,14]. It has been shown that milk with distinct fat globule distributions [13] can be produced. Membrane separation systems however are prone to fouling and blockage over time [14], which eventually limits the throughput and selectivity of the process. The high pumping pressures, required to minimise fouling and maximise milk flux, may also contribute to damage of the milk fat globule membrane-coating [15]. Milk fat globules with disrupted membrane coatings are undesirable for cheese-making. The milk proteins (casein micelles, casein sub-units and whey proteins) that self-assemble on the free milk fat surface [16] to replace the milk fat globule membrane, consequently increase the strength of intermolecular interactions between the fat globules and the surrounding protein network. This can result in negative consequences during cheese making such as poor syneresis (i.e., low liquid expulsion from the cheese gel) that leads to cheeses with higher moisture content and formation of cheese curds with a coarse and brittle structure [17]. Membrane filtration can provide milk fractions with small fat globules and discrete distributions when operating with a milk flux of ~ 400 L/h per m^2 , although the low amount of fat retained (<1 g fat/1 kg milk) makes such fractions of low commercial value [13].

The premise of the ultrasound separation technology is its potential to facilitate rapid fat separation with high specificity to globule size, yet causing little to no damage to its membrane-coating surface. Recent work has shown the ability of ultrasound in standing wave fields to initiate rapid separation of fat globules in recombined milk fat emulsions [6,18] and natural whole milk [7,19]. These studies have shown that ultrasound at frequencies greater than 600 kHz and energy levels up to 583 W/L do not disrupt the physical integrity of the milk fat globules [19]. Juliano et al. [20] has shown that only when providing prolonged sonication (>20 min) or high specific energies (>230 kJ/kg) will the flavour-related volatile profile of the milk be significantly modified at these frequencies. Another study by

Torkamani et al. [21] showed no significant oxidation by ultrasound on pasteurised cheddar cheese whey. Johansson et al. have recently used sonochemiluminescence to show that 2 MHz ultrasound operated at similar energy levels to 1 MHz ultrasound, results in significantly lower cavitation [22].

The aim of the present study is to ascertain the ability of ultrasound to achieve rapid fat globule fractionation of milk samples into streams with defined particle size distributions for potential use in the manufacture of novel dairy products or the recreation of traditional products at higher process speeds compared to natural creaming. The speed of the process and specificity of the fat globule distributions obtained will be compared with existing gravity separation methods previously reported in the literature. Furthermore, the expected fat yields distributed across the height of the separation vessel will be presented.

2. Materials and methods

2.1. Ultrasonic processing

Raw whole bovine milk ($3.7 \pm 0.2\%$ w/v) sourced directly from a farm (Department of Primary Industries and Environments, Ellinbank, Australia) was used for all trials. All trials were performed in duplicate with milk obtained on the same day. Note that due to natural variation, the initial particle size distributions and fat content of milks used in the trials differ from day to day. Trials were repeated with raw whole milk on a second day to mitigate the influence of natural variation, and a minimum of 4 processing replicates were performed unless otherwise specified. Comparisons are made by normalising the change in either fat content or particle size to the initial starting values.

The ultrasonic milk fractionation prototype device, a stainless steel vessel (Fig. 1(a)) with a wall thickness of 1.5 mm, was used for these trials. An initial processing volume of 1.8 L was used for all trials unless otherwise stated. The milk was placed within an ultrasound processing region with dimensions detailed in Fig. 1(b).

Milk was preheated inside a thermo-regulated heating bath (Ratek TH2 Thermoregulator). Constant gentle stirring of the milk was performed during the preheating step. A preheating temperature of 25°C was used for all trials based on findings from Leong et al. [19]. Water with an initial temperature of $\sim 25^\circ\text{C}$ was positioned in the side chambers to act as a thermal mass and reduce the rate of temperature increase of the processed milk.

Fully-submersible plate transducers (Sonosys Ultraschallsysteme GmbH, Neuenburg, Germany) of nominal frequency 1 MHz and 2 MHz were available for the separation trials. The dimensions of the transducers were identical, with a total surface face area of $160\text{ mm} \times 160\text{ mm}$ (active area of $100\text{ mm} \times 100\text{ mm}$) (see Fig. 1(c)). The transducers were fixed into position facing each other, as depicted in Fig. 1(a). The transducers were operated at either 50% or 100% nominal power unless otherwise specified. 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.

Maximum sound pressure levels in the system were previously determined using a needle hydrophone (model HNC-1000, Onda Corp., Sunnyvale, USA) and reported by Leong et al. [7,19] using a similarly sized vessel. Pressure distributions and penetration distances with the transducers used in this study have been previously characterised [23,24].

2.2. Single stage fractionation protocol

In the first set of experiments, a single stage batch sonication process using dual 2 MHz ultrasound (330 W/L) was employed to

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