Ultrasonics Sonochemistry 21 (2014) 2117–2121

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Short Communication

Sonocrystallisation of lactose in concentrated whey

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ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 25 March 2014
Accepted 25 March 2014
Available online 13 April 2014

Keywords:
Ultrasound
Sonocrystallisation
Lactose
Concentrated whey

ABSTRACT

Whey concentrated to 32% lactose was sonicated at 30 °C in a non-contact approach at flow rates of up to 12 L/min. Applied energy density varied from 3 to 16 J/mL at a frequency of 20 kHz. Sonication of whey initiated the rapid formation of a large number of lactose crystals in response to acoustic cavitation which increased the rate of crystallisation. The rate of sonocrystallisation was greater than stirring for approximately 180 min but slowed down between 120 and 180 min as the metastable limit was reached. A second treatment with ultrasound at 120 min delivering an applied energy density of 4 J/mL stimulated further nuclei formation and the rate of crystallisation was maintained for >300 min. Yield on the other hand was limited by the solubility of lactose and could not be improved. The crystal size distribution was narrower than that with stirring and the overall crystal size was smaller.

1. Introduction

Lactose is the most abundant carbohydrate found in milk (4.4–5.2%) and a major constituent of many concentrated and dried milk and whey products. Lactose must first be crystallized before many of these products can be spray dried. Commercial manufacture of whey powder involves concentration of whey, often by evaporation, followed by batch crystallisation which is initiated by rapid cooling or by seeding directly with lactose over many hours (up to 20 h) to ultimately yield up to 80% crystallized lactose [1]. These processes offer limited control and improving the efficiency of crystallisation will benefit the dairy industry [2]. Crystallisation of lactose consists of three phases; the first is supersaturation followed by nucleation (appearance of crystals) and crystal growth. During the crystallisation process it is critical to control crystal purity, shape and size but traditional paddle mixers are known to create non-uniform mixing. Irregularities cause random fluctuations in supersaturation, resulting in uneven and irregular crystal size and growth occasionally forming agglomerates [3,4].

The overall crystallisation process is slow and lactose recovery can be improved. Sonication is known to reduce crystallisation induction times and increase the rate of nucleation in a number of processes including the crystallisation of fats [5] and pharmaceutical lactose [6] in a process known as sonocrystallisation. Sonocrystallisation is most effective when ultrasound is delivered at the nucleation phase [7].

Ultrasonic cavitation can enhance the rate of reaction and facilitate mass transfer in liquid. Studies have shown that sonocrystallisation generally exhibits four characteristics which are not typical of crystallisation without sonication. These are faster primary nucleation, ease of nucleation, initiation of secondary nucleation and production of smaller and purer crystals [8]. Ultrasound in the presence of an anti-solvent such as ethanol was used to increase the yield of lactose crystallisation [7,9–11], in acetone [12] and in glycerine solution [4]. More recently, these characteristics were reported in a simple aqueous system without anti-solvent [13].

Much of the laboratory data reported in literature is based on direct contact sonication. In this approach, a titanium ultrasonic probe was immersed directly into the product. Because the energy density is greatest at the surface of the sonotrode it will cause gradual pitting and degradation. Although the risk associated with such practice is minimal, there is concern that erosion of the sonotrodes may result in product contamination [14]. A non-contact alternative to direct contact sonication exists and this design permits modular implementation and in-line operation. These sonication cells are designed with multiple low power transducers attached to the outer surface of the metal cell, eliminating the need for sonotrodes. Sound waves propagate through the metal surface overcoming sonotrode erosion and improving energy distribution. These generate lower power densities than sonotrodes but efficiently initiate lactose nucleation and have been implemented industrially outside the food industry [6,15].

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http://dx.doi.org/10.1016/j.ultsonch.2014.03.031
1350-4177/

Sonocrystallisation of lactose is known to occur in the presence of anti-solvents and in aqueous solutions but the effect of ultrasound on lactose crystallisation in concentrated whey remains unknown. Since the use of anti-solvent in the manufacture of food grade lactose is unlikely to be feasible at a commercial scale, the aqueous lactose study [13] is probably the most relevant reference publication for industry. In the current study, commercially manufactured whey concentrate was sonicated at pilot scale using non-contact equipment to study the effects on lactose crystallisation.

2. Materials and methods

Concentrated whey was sourced directly from a commercial dairy factory (Northern Victoria, Australia). Whey was concentrated to 32 ± 2% lactose by evaporation at 55 °C. Concentrated whey was then flash cooled to 30 ± 1 °C to initiate lactose crystallisation. Two sonication studies were conducted (Fig. 1).

In the first, sonication was performed with a 20 kHz Sonolab SL250 non-contact sonicator (Prosonix Ltd., Oxford, UK). The processing chamber was 15.4 cm in diameter with a capacity of 2.1 L. Concentrated whey was treated with high intensity low frequency ultrasound at 30 ± 1 °C delivering an applied energy density of 3–16 J/mL in a single pass. The whey concentrate was sampled immediately following the industrial flash cooler and pumped through the SL250 at various flow rates using a peristaltic pump (Masterflex L/S model 7554-95, Illinois, USA). The control solution (T0) was pumped through the ultrasonic rig at the appropriate flow rate without sonication. Three flow rates were explored (0.75, 1.2 and 2 L/min; achieving residence times of 168, 105 and 63 s) at two power settings (100 and 200 W). The applied energy density (J/mL) was calculated as described by Zisu et al. [16] and the corresponding energy densities are shown in Table 1 ($n \geqslant 2$). Sonicated whey (400 g) was transferred to 400 mL glass beakers (6.5 cm diameter) and allowed to crystallize at room temperature (~22 °C) for 60 min.

The magnitude of the experiment was scaled up in the second study based on energy density. A Prosonitron P500 (Prosonix Ltd.) non-contact sonicator was installed in-line with the commercial manufacturing process immediately following the flash cooler. The processing chamber was 15.4 cm in diameter with a capacity of 6.4 L. Concentrated whey (30 ± 1 °C) was diverted to the sonicator at the desired flow rate. Sonication was performed in a single pass at 250–600 W and flow rates of 4–12 L/min (residence time of 96–32 s, respectively) to achieve applied energy densities of 3–15 J/mL. Control samples were passed through the ultrasonic rig at the appropriate flow rate without sonication. Sonicated and control whey (400 g) were then placed in 400 mL glass beakers (6.5 cm diameter) and transferred to a 30 °C water bath. Samples were cooled to 15 °C by lowering the temperature by 2 °C every 30 min (4 °C/h) then holding at temperature for up to 24 h. Whey was stirred continuously during the cooling period and for the entire holding time using an overhead stirrer (RZR 2020, Heidolph Instruments GmbH & Co., Schwabach, Germany) fitted with a 30 mm three paddle operating at 650 rpm.

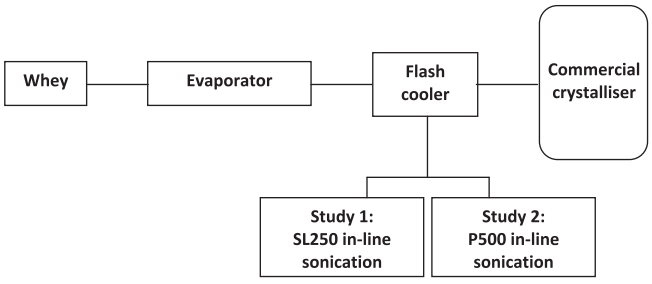

Fig. 1. Process flow diagram for two approaches to sonocrystallisation.

Table 1
Applied energy density (J/mL) delivered by the SL250 at various flow rates and power settings.

Flow rate (mL/min)	Electrical power (W)	
	100 (J/mL)	200 (J/mL)
750	8	16
1200	5	10
2000	3	6

When a second off-line sonication treatment was required, a 1 kW (UIP100hd) 200 mm radial sonotrode (BS2d34SPEC) was used to deliver an applied energy density of 4 J/mL (Hielscher Ultrasonics GmbH, Teltow Germany).

Total dissolved solid (Brix) were measured at 23 °C as an indicator of crystallisation (Refracto 30GS, Mettler Toledo, Schwerzenbach, Switzerland). Samples were frozen immediately and sent to the Dairy Technical Services laboratories (Kensington, Victoria, Australia) for total solids (Test number: MOIS 21 10.00) and lactose by enzyme analysis (Test number: LACT 02 04.93) measurements.

Crystallisation was calculated according to Westergaard [2]:

$$\% \text{ Crystallisation} = \frac{(S_1 - S_2) \times 9500 \times 100}{L \times \text{TS} \times (95 - S_2)} \quad (1)$$

where S_1 = % sugar (Ref. index) of the concentrate direct from the evaporator, S_2 = % sugar (Ref. index) of the crystallized concentrate, L = % lactose and TS = total solids content in %.

Complementary to absorbance, whey solutions were viewed under a light microscope (Olympus BH-2, Tokyo, Japan) fitted with and without a blue light filter at 10× magnification immediately after sonication (T0) and 60 min (T60) of treatment. Whey was also examined after 30 min (T30) in some experiments. Images were captured with a 3.2 mega pixel digital camera (Pro-MicroScan Model DCM310, Oplenic Co., Hangzhou, China) and were used to measure crystal size by Scope Photo image analysis software (Version 3.0, Oplenic Co., Hangzhou, China). The size of crystal was reported as the length of a crystal in the *b* direction (defined by Fries et al. [17]) and the average crystal size was measured as the average size of all the particles viewed under the microscope. The average growth rate of the (0 1 0) face (defined by Michaels and Van Kreveld [18]) was calculated from the slope of the average crystal size as a function of time. Saturated lactose solution was used to dilute the whey when necessary to allow accurate measurement of crystal size.

3. Results and discussion

Crystallisation of lactose in commercially concentrated whey was significantly increased by the application of ultrasound at a low energy density of 3 J/mL and a flow rate of 2 L/min (Fig. 2). A similar observation was made for the various flow rates (0.75, 1.2 and 2 L/min) and power inputs explored (3–16 J/mL) (data not shown). Regardless of the sonication intensity and flow rate, the least number of lactose crystals was observed in the control solutions at T0. A greater number of lactose crystals were present in whey immediately after sonication (T0) at all energy densities (3–16 J/mL). Although some nucleation occurred in the control solution after 30 and 60 min of crystallisation, the number of crystals observed in sonicated solutions was far greater at an equivalent time. Ultrasound generated a large number of nuclei resulting in the growth of many small crystals, differing to the growth of fewer but larger crystals without treatment. Unlike aqueous solutions of reconstituted lactose, a lower energy density

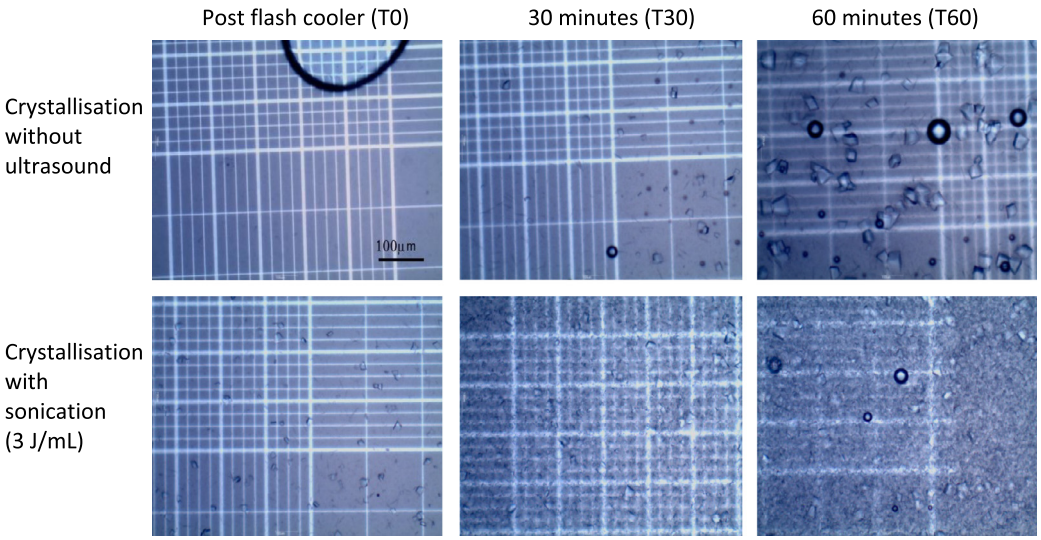

Fig. 2. Concentrated whey viewed under a light microscope at 10× magnification immediately after flash cooling at a flow rate of 2 L/min and after 60 min (scale bar applies to all images).

was sufficient to initiate lactose crystallisation in concentrated whey. In the earlier study of reconstituted lactose (37.5% solids), the minimum applied energy density required to achieve the desired level of lactose crystallisation in aqueous solutions was 75 J/g at a frequency of 20 kHz [13]. The efficiency of sonication in the current study was likely compounded by the higher lactose concentration of concentrated whey. The same study also showed that sonication reduced lactose crystallisation induction times at energy densities of up to 0.15 W g^{-1} and the metastable zone width also reduced but there was no effect on individual crystal growth rate or crystal morphology.

Subsequent scale-up studies confirmed that sonocrystallisation of lactose in concentrated whey was scalable to flow rates of up to 12 L/min at applied energy densities of $\geqslant$3 J/mL. Scale-up potential was only limited by the power of the sonicator. Lower Brix readings indicating a faster rate of crystallisation were obtained for whey sonicated at a flow rate of 11 L/min and an applied energy density of 3.3 J/mL during the first 150 min of crystallisation. The rate of crystallisation slowed at 150 min and the difference between sonication and stirring diminished at $\geqslant$180 min (Fig. 3A). Although the rate of crystallisation is increased by the application of ultrasound which initiates the formation of a large number of crystals, the yield of crystallisation is limited by the solubility of lactose. As the lactose concentration reduced during the crystallisation process, the driving force for nucleation and growth decreased. Consequently the lactose concentration reduces slowly to the solubility value given sufficient time [19]. In crystallisation studies using anti-solvent the yield of crystallisation is significantly increased due to the reduced solubility of lactose in the presence of alcohols [11].

The wide distribution of crystal size depicts the various stages of crystal growth. Acoustic cavitation appeared to generate a larger number of smaller crystals and less secondary nucleation occurred, compared to the control. In unsonicated whey, large crystals formed early and the formation of secondary nuclei widened the crystal size distribution. Ultimately, the crystal size population in sonicated whey was smaller than stirring and its distribution narrower (Fig. 3B and Fig. 4). The average crystal size for sonicated whey was 38.39 ± 10.02 µm and for stirred 57.9 ± 17.71 µm. The relative size distribution (SD/L_{ave}) for sonicated whey was slightly lower than stirred at 0.31 and 0.26, respectively. Initial nucleation was caused by flash cooling with sonication resulting in a second nucleation response which resulted in the formation of a large number of nuclei which reduced the average crystal size. Measurements are supported by microscopy in Fig. 5. Images also confirm the typical "tomahawk" morphology of lactose crystals described by Michaels and Van Kreveld [18].

The intensity of sonication was increased from 3 to 15 J/mL and crystallisation was followed for 24 h (Fig. 6). A typical response was measured, that is, the initial rate of crystallisation was significantly greater in sonicated whey followed by a slower period of crystal growth beyond 150 min. Although the initial lag time slo-

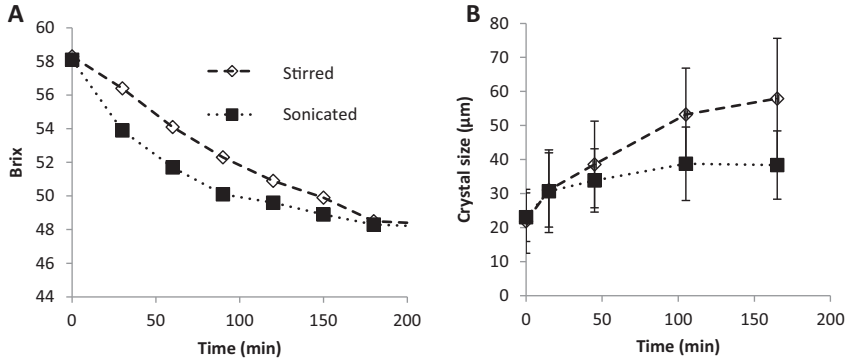

Fig. 3. Change in Brix (A) and crystal size (B) at a flow rate of 11 L/min. Sonication was applied at an energy density of 3.3 J/mL.

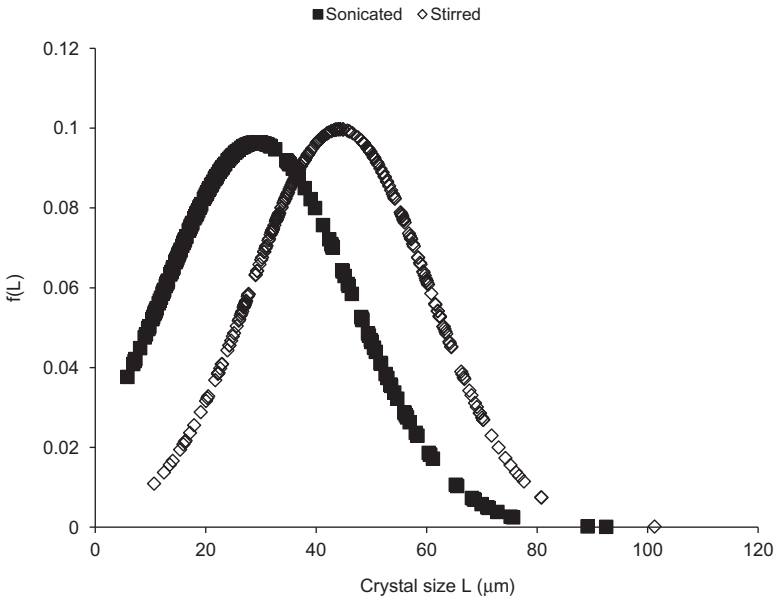

Fig. 4. Normalised crystal size distribution of stirred and sonicated whey at the end of crystallisation.

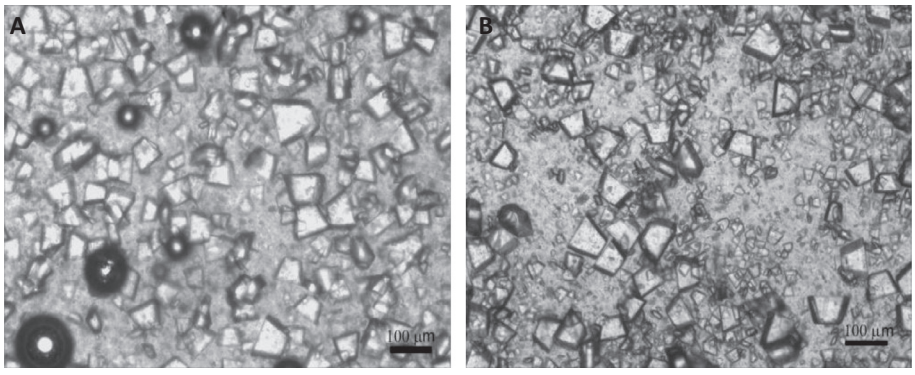

Fig. 5. Crystal morphology and distribution viewed under a light microscope at 10× magnification following stirring (A) and sonication (B).

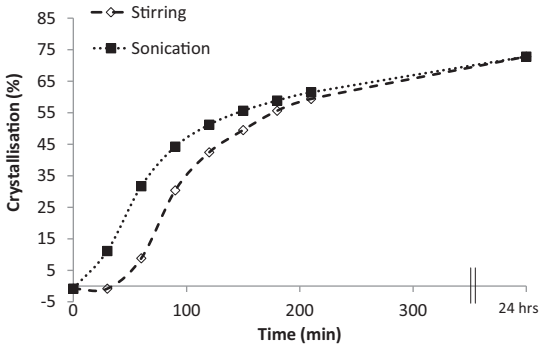

Fig. 6. Crystallisation of stirred and sonicated whey; sonication was delivered at a flow rate of 1 L/min and an applied energy density of 15 J/mL.

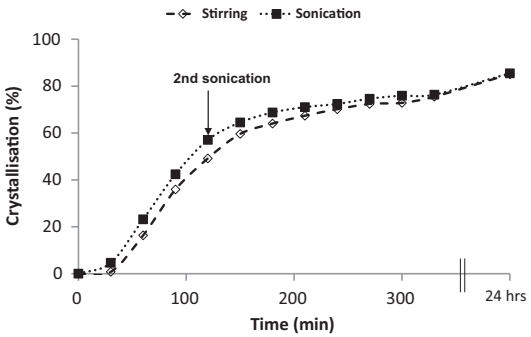

Fig. 7. Crystallisation of stirred and sonicated whey; initial sonication was delivered at a flow rate of 11 L/min and an applied energy density of 6 J/mL. A second treatment of ultrasound was delivered during crystallisation at 120 min and an applied energy density of 4 J/mL.

wed the rate of crystallisation early in stirred whey, it accelerated as the number of crystals increased. Despite applying 5× greater energy density during sonication, the rapid rate of crystallisation could not be maintained beyond 180 min and the two crystallisation curves became similar beyond 220 min. A similar rate of crystallisation was measured for 24 h to yield the same amount of crystallized lactose.

To stimulate further lactose nucleation and maintain the rate of reaction, a second treatment with ultrasound at 4 J/mL was applied at 120 min in the region where the rate of lactose crystallisation begins to slow (Fig. 7). In addition to initial sonication at 6 J/mL, secondary ultrasonic cavitation at 120 min maintained a faster rate

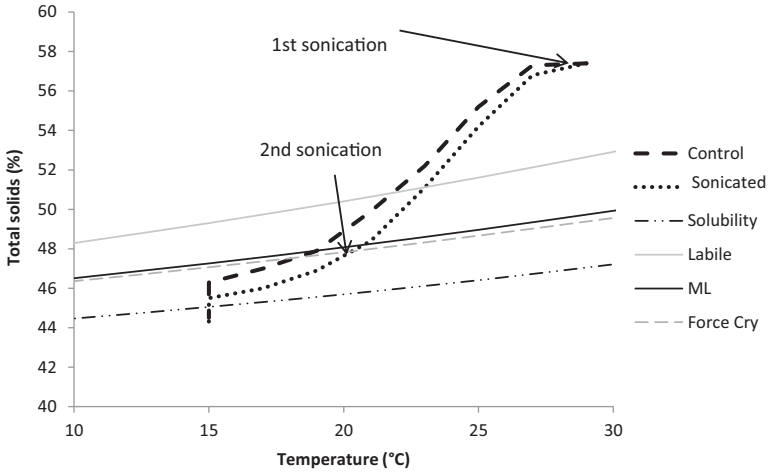

Fig. 8. Crystallisation profile of stirred and sonicated whey showing lactose solubility, forced crystallisation and the labile and metastable zones. Initial sonication was delivered at a flow rate of 11 L/min and an applied energy density of 6 J/mL. The second batch treatment of ultrasound was delivered at an applied energy density of 4 J/mL.

of reaction for twice as long as a single treatment extending beyond 300 min when compared to stirring. Crystallisation slows as it reaches the metastable limit (ML; where spontaneous nucleation is improbable but crystals will grow) and a second sonication treatment nearing this concentration limit seems to improve the rate of crystallisation (Fig. 8). Dincer and co-workers have shown that the impact of ultrasound is more prominent in the intermediate zone when compared to stirring [13]. The solubility, labile zone, metastable zone and forced crystallisation of lactose concentration were converted into total solids content of concentrated whey as described in literature [20–22]. Briefly, these were calculated as follows; Reported Solubility ($C = 10.788\ e^{(0.021T)}$), Secondary Nucleation Threshold SNT ($C_{SNT} = e^{(2.389+0.0287T)}$), Metastable Limit ($C = 17.446\ e^{(0.024T)}$), Forced Crystallisation ($C = 17.152\ e^{(0.0271T)}$) and Super Solubility ($C = 22.308\ e^{(0.0265T)}$) values were expressed as lactose concentration (g/100 g water) as a function of temperature. Using the total solid and lactose contents of the concentrated whey, at each temperature, corresponding solubility, SNT etc., were calculated in terms of total solid assuming only lactose crystallised out of solution.

Although the amount of lactose crystallisation was similar for both treatments at the solubility limit after 24 h (Fig. 7), the result indicates that multiple ultrasonic treatments to create a pulsing effect may yield the maximum amount of crystallized lactose in a shorter time than conventional stirring. Further work is necessary to confirm this hypothesis.

4. Conclusion

Sonication initiates rapid lactose nucleation in concentrated whey but the rate of sonocrystallisation slows after the initial period of accelerated growth. A fast rate of reaction can be maintained for longer by applying a second ultrasonic treatment at the metastable limit to stimulate further nuclei formation. Although the yield of crystallized lactose is limited by the solubility of lactose, the resulting crystals are smaller than conventional stirring and the process delivers greater control of the crystal size distribution.

Acknowledgements

The authors would like to acknowledge Dairy Australia for technology transfer funding and Prosonix Ltd. for supplying the ultrasonic equipment and technical support.

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