Effects of ultrasound on the fermentation profile of fermented milk products incorporated with lactic acid bacteria

A.M.N.L. Abesinghe, N. Islam, J.K. Vidanarachchi, S. Prakash, K.F.S.T. Silva, M.A. Karim

PII: S0958-6946(18)30258-9

DOI: https://doi.org/10.1016/j.idairyj.2018.10.006

Reference: INDA 4411

To appear in: *International Dairy Journal*

Received Date: 28 March 2018

Revised Date: 30 October 2018

Accepted Date: 30 October 2018

Please cite this article as: Abesinghe, A.M.N.L., Islam, N., Vidanarachchi, J.K., Prakash, S., Silva, K.F.S.T., Karim, M.A., Effects of ultrasound on the fermentation profile of fermented milk products incorporated with lactic acid bacteria, *International Dairy Journal*, https://doi.org/10.1016/j.idairyj.2018.10.006.

Effects of ultrasound on the fermentation profile of fermented milk products incorporated with lactic acid bacteria

A. M. N. L. Abesinghe [a,d], N. Islam [a], J. K. Vidanarachchi [c], S. Prakash [b], K. F. S. T. Silva [c], M. A. Karim [a]*

[a] *Queensland University of Technology, Australia*
[b] *The University of Queensland, Australia*
[c] *University of Peradeniya, Sri Lanka*
[d] *Uva Wellassa University of Sri Lanka*

*Corresponding author. Tel.: +61 7 3138 6879
E-mail address: azharul.karim@qut.edu.au (M. A. Karim)

ABSTRACT

Ultrasonic processing of fermented milk products has created much interest in current research on dairy products. This has been employed in cultured milk products to enhance the emulsification of milk fat and to intensify the fermentation process. Benefits including remarkable product stability, reduced processing time and enhanced quality are being recorded. Ultrasound (US) altered the colour and flavour profile of milk; however, the effect of US-induced fermentation on the synthesis of flavour compounds in milk has not been reported in the literature. This review paper presents a comprehensive scenario on the impact of power US on the fermentation profile and quality of ultrasonically processed dairy products. A theoretical background on US and details of its effect on the metabolic performance of lactic acid bacteria are presented. Finally, it describes how the quality attributes of fermented milk gels are modified due to the intensification of the fermentation process with US.

Contents

1. Introduction

Ultrasound (US) refers to sound waves above a frequency of 20,000 Hz, which are not detectable by the human ear, and can be divided into three main categories based on frequency range: (i) power US (20–100 kHz); (iii) high-frequency US (20 kHz – 2 MHz) and (iii) diagnostic (1–10 MHz) (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Martini, 2013b).

Power US has energy intensities between 10 and 1000 W cm^{-2}. When power US travels through a medium, it causes significant physical and chemical changes through a phenomenon called “acoustic cavitation” that induces the formation of cavities (Martini, 2013a). This has been widely employed in the food industry for technologies such as drying, deforming, microbial inactivation and emulsification (Charoux, Ojha, O'Donnell, Cardoni, & Tiwari, 2017; Kumar, Karim, & Joardder, 2014). The application of power US in emulsification/homogenisation and microbial inactivation in milk has been extensively reviewed by Awad et al. (2012), Chemat and Khan (2011) and Paniwnyk (2017) and, therefore, outside of the focus of this paper.

Intensification of milk fermentation using power US is another area of interest in the dairy industry. Fermentation is the most time- and resource-consuming stage during the manufacture of cultured milk products. Numerous research studies have revealed that power US can enhance the fermentation rate of lactic acid bacteria (LAB) by modifying their metabolism while improving the quality characters such as water holding capacity (WHC), texture profile and syneresis of fermented milk gels (Riener, Noci, Cronin, Morgan, & Lyng, 2010; Sfakianakis, Topakas, & Tzia, 2015; Shershenkov & Suchkova, 2015). However, the application of power US in dairy fermentation has not yet been adequately reviewed in the literature. While a recent review by Ojha, Mason, O’Donnell, Kerry, and Tiwari (2017) revealed some avenues of applying US in milk fermentation, the objective of this review is to provide a comprehensive analysis of recent studies on power US towards improving the overall fermentation profile of dairy products.

2. Ultrasound apparatus for fermentation experiments and acoustic cavitation

The major components of a US generation system are an electrical power generator, transducer(s), and an emitter (Bermúdez-Aguirre, Mobbs, & Barbosa-Cánovas, 2011); the electrical generator supplies the required energy to run the transducer at a certain frequency. The US transducer consists of a piezoelectric material that converts electrical oscillations into mechanical vibrations of a similar frequency. The major function of the emitter is to discharge the US wave from the transducer into the medium. Moreover, the transducer can also amplify the ultrasonic vibrations.

Ultrasonication devices are classified as either direct (US probe) and indirect types (US bath) as shown in Fig. 1. In the direct type, acoustic energy is directly dissipated from the transducer to the sample and this is approximately 100 times higher than the energy intensity of indirect sonication (Marcela, Silvana, Fabiana, Renata, & Lisiane, 2018). In this system, a horn is attached to the transducer to amplify the signal and bring it to the sample. The tip of the horn, often a separate attachable device known as a sonotrode, radiates the ultrasonic waves into the sample. The higher cavitational intensity acquired for less volume makes probe sonicators more appropriate for laboratory scale operation than bath sonicators. In the case of indirect mode, US is introduced to the sample indirectly through one or more transducers that are attached to the walls or at the bottom of a vessel. US energy is indirectly dissipated from the transducer to the sample through a coupling fluid, most often water (Sancheti & Gogate, 2017).

When US waves pass through a liquid medium it creates a series of compression (positive pressure) and expansion cycles (negative pressure). During the negative pressure cycle, gaseous impurities in the liquid medium such as pre-existing bubbles that are coated with contaminants, solid particles with trapped gases or tiny crevices in the walls of the vessel lead to the disruption of the liquid medium and nucleation to form gas bubbles (Leong, Ashokkumar, & Kentish, 2016). These bubbles start to grow in size due to rectified diffusion and bubble-bubble coalescences.

Rectified diffusion is the uneven transfer of mass through the air/liquid boundary during the rarefaction and compression phase of the sound wave cycle (Church, 1988). There are two major causes for this uneven mass transfer, namely “area effect” and “shell effect” (Leong et al., 2016). The “area effect” means that the bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas and solvent vapour into the bubbles, but these are not fully expelled during the subsequent compression phase where the surface area is comparatively smaller. The “shell effect” refers to the increase in the thickness of liquid shell that covers the bubble upon contraction, whereas the thickness reduces during the expansion phase. The concentration gradient of gas is low when the bubble has a thick mass transfer boundary layer and vice versa and this results in a net accumulation of mass into the bubble. Once the US energy provided is not adequate enough to retain the vapour phase inside the bubble, the local pressure declines to some point below the saturated vapour pressure of the liquid. As a result, a rapid condensation occurs and the condensed molecules collide violently, creating shock waves and generating very high temperature (Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013; Huang et al., 2017). The implosion of cavitation bubbles generates an excessive amount of heat and the temperatures within the bubbles that could go up to 750–6000 K within a short period of time (Ashokkumar, 2011).

The creation, expansion and implosive collapse of micro-bubbles in ultrasonically irradiated liquids is known as acoustic cavitation (Torley & Bhandari, 2007). If cavitation occurs close to a firm surface, the bubbles may break asymmetrically and create fast-moving liquid jets that may create localised surface damage. There are several physical effects generated in the medium during the oscillation and implosion of cavitation bubbles such as shock waves, shear forces, micro-jets, turbulence, etc. (Bermúdez-Aguirre et al., 2011; Louisnard & González-García, 2011). Depending on the conditions used such as amplitude, temperature, pressure, and the composition of the medium, several mechanisms can be activated including increase of the temperature, surface instability, generation of agitation and friction, increase of mass transfer, generation of free radicals and disruption of cell materials (Ashokkumar, 2011; Martini, 2013b; Salazar, Chávez, Turó, & García-Hernández, 2009).

3. Application of power ultrasound in lactic fermentation of milk

Application of both low power ultrasound (LPU) and power US in fermentation has been reported in the literature. LPU has power intensities below 1 Wcm^{-2} and is commonly used for non-destructive analysis in the food industry to characterise food components, often on quality assurance lines and to monitor fermentation processes (Novoa-Díaz et al., 2014) and is not a focus for this review paper. On the other hand, PU (with power intensities above 10 Wcm^{-2}) alone (sonication) or in combination with external pressure (manosonication), heat (thermosonication) or both pressure and heat (manothermosonication) has been reported to influence the lactic fermentation in cows' milk, soy milk and sweet whey and is outlined in Table 1.

4. Effect of power ultrasound on fermentation time

Reducing the fermentation time in cultured dairy products by US is one of the most promising approaches that has been identified previously in the literature (Barukčić, Jakopović, Herceg, Karlović, & Božanić, 2015; Nguyen, Lee, & Zhou, 2009; Riener et al., 2010; Sfakianakis et al., 2015; Shimada, Ohdaira, & Masuzawa, 2004; Wu, Hulbert, & Mount, 2001). For yoghurt, fermentation time is defined as the interval between the time of addition of cultures and the time at which the pH of the yoghurt reaches pH 4.7 (Puvanenthiran, Williams, & Augustin, 2002). Reduction of the fermentation time helps decrease production time and cost. This can also be used to improve the consistency and the texture of the milk gels. Shorter fermentation time is reported to reduce the extent of rearrangements within the yoghurt gel network that are caused by electrostatic repulsions and the dissolution of colloidal calcium phosphate crosslinks. As a result, whey separation and formation of large pores are decreased compared with longer fermentation times (Peng, 2010).

It was observed that the application of US (20 KHz, 180 W, 270 W and 450 W) for 8 min to a mixture of Jersey and Holstein milk (sample size 150 mL) after inoculation with yoghurt cultures followed by the fermentation reduced the fermentation time by 30 min in set type yoghurt (Wu et al., 2001). Similarly, Dolatowski, Stadnik, and Stasiak (2007) reported a reduction of set yoghurt production time up to 40% with the use of US. Further, the sonication of reconstituted skimmed milk (15%, w/v) inoculated with *Bifidobacterium* sp. at 20 KHz and 100 W for 15 min that was followed by the fermentation at 37 °C reduced the fermentation time by 11–26% (Nguyen et al., 2009). More recently, the fermentation of reconstituted sweet whey (6% of the dry matter) by a US treated culture of *Lactobacillus acidophilus* with 84 W for 150 s was reported to reduce fermentation time by 30 min (Barukčić et al., 2015). In contrast, a few authors have reported that ultrasonication led to a reduction or total elimination of the lag phase of the growth curve of lactic acid bacteria (LAB) in milk without influencing the total duration of fermentation. Sfakianakis et al. (2015) observed a complete disappearance of the lag-phase of the lactic acid bacteria during the fermentation of pre-sonicated skimmed bovine milk (fat: 0.1% w/w, SNF: 14% w/w) with power US (750 W at 500 mL sample volume, 1500 kWm^{-3}; 10 min) without affecting the total fermentation time. Moreover, sonication of raw skim milk (fat content: 0.1%) during the fermentation using an ultrasonic water bath (45 kHz, 200 W, 17 kWm^{-3}) significantly reduced the pH during the lag phase compared with the untreated sample without affecting the duration of fermentation process (Nöbel et al., 2016b).

Apparently, the effect of US on fermentation time may rely on process parameters such as acoustic intensity, frequency, treatment duration, the point of application (before inoculation or after inoculation) and the composition of milk. In an initial investigation, Shimada et al. (2004) found that the fermentation time of a kefir culture (time at which the pH reaches 4.5) was shortened exponentially when the sonication frequency was increased from 28 kHz to 200 kHz during fermentation. Consequently, authors suggested that ultrasonic waves promoted the fermentation process under conditions where cavitation was not generated, and was suppressed when cavitation occurred. However, the influence of factors such as different milk composition, starter culture used and process parameters on fermentation kinetics have not been reported in the literature to date.

Several mechanisms are proposed to describe the role of power US in inducing the fermentation process. Some authors suggested that PU can improve membrane permeability of starter bacteria, so allowing the release of intracellular enzymes such as β-galactosidase (EC 3.2.1.23) from the cell (Ewe, Abdullah, Bhat, Karim, & Liong, 2012; Nguyen et al., 2009; Wang & Sakakibara, 1997; Wu et al., 2001). Another mechanism, proposed by Shimada et al. (2004) and Piyasena, Mohareb, and McKellar (2003), is that a slight local temperature rise due to the heat derived from ultrasonic absorption may activate the lactic bacteria and shorten the fermentation time. Moreover, Pitt and Ross (2003) suggested that US may accelerate the supply of oxygen and nutrients for microorganisms and increase the discharge of waste products from the cells, thus enhancing microbial cell growth. A different mechanism was hypothesised by Nguyen et al. (2009), who demonstrated that the stimulatory effect of fermentation was due to the leakage of some cellular contents such as β-galactosidase, complex photolytic systems and some growth factors from the ruptured bacterial cells under sonication.

5. Effects of ultrasound on cell membrane permeability

Sonoporation describes the progressive opening of the cell membrane due to micro-bubble cavitation upon US exposure of cells (Lentacker, De Cock, Deckers, De Smedt, & Moonen, 2014; Maciulevičius et al., 2016). The micro-bubbles create micro-streaming and/or liquid jets (Maciulevičius et al., 2016), which generate a strong shear force that breaks the chemical bonds in the cell membranes (Tabatabaie & Mortazavi, 2008), puncture cell surfaces and create cell membrane pores (membrane permeabilisation). To date, there have been several mechanisms proposed to understand the interaction of micro-bubbles with cell membranes that leads to sonoporation such as: (i) push and pull effect of micro-bubble, (ii) micro-streaming (liquid flow around micro bubbles) that tears the lipid membrane, and (iii) penetration of micro bubbles into a cell. The recent literature reported that relatively small oscillation amplitude at lower US intensities exhibited higher impact on the cell membrane, compared with non-adhered micro-bubbles (Lentacker et al., 2014).

Furthermore, it has now been suggested that, apart from this mechanical stress, some chemical effects induced by US are also responsible for pore formation. For example, stable micro-bubble oscillations can induce the formation of free radicals and molecular products such as H_2O_2 (Gao, Hemar, Ashokkumar, Paturel, & Lewis, 2014a; Gao, Lewis, Ashokkumar, & Hemar, 2014b), which play a vital role in lipid bilayer relocation and membrane disruption through lipid peroxidation. Furthermore, it was also revealed that peroxidation of membrane lipids (Ewe et al., 2012; Lentacker et al., 2014) and conformational unfolding of proteins that are located on the surface of the cell membrane increase membrane fluidity and membrane permeabilisation upon US treatment (Ewe et al., 2012). From the available literature, it is clear that a low level of sonoporation can be used to improve the permeability of cell membranes, resulting in improved mass transfer of substrates across the microbial cell membrane and efficient removal of by-products of cellular metabolism, which eventually improves microbial growth (Ojha et al., 2017). However, to achieve the desired level of cell permeabilisation and to avoid cell death, ultrasound process parameters must be precisely quantified and controlled, because an excessive level of sonoporation can lead to a leakage of cellular content because of the physical disruption and eventually lead to cell death (Ojha et al., 2017).

Using microscopy, the effect of power US (20 kHz, 30 min) on cell wall permeability of lactic acid bacteria has been investigated by several researchers (Cameron, McMaster, & Britz, 2008; Shershenkov & Suchkova, 2015; Tabatabaie & Mortazavi, 2008). LAB that were exposed to US treatment showed both pore formation and cellular damage (Ewe et al., 2012). Three types of micro-damage, namely micro-cracks, micro-voids and ruptures, have been identified in cell membranes of LAB (Tabatabaie & Mortazavi, 2008). An in-depth analysis of the effect of power US (20 KHz) on the extent of structural damage of *Lb. acidophilus* was performed using transmission electron microscopy (TEM) by Cameron et al. (2008) as shown in Fig. 2. It was demonstrated that an US treatment of 5 min leads to both external and internal cell damage to *Lb. acidophilus* where the cell terminus had been trimmed and a low number of liposome-like vesicles were presented inside the cells.

Moreover, flow cytometric analysis revealed that US increased both membrane permeability and fluidity of LAB (Ewe et al., 2012). These changes may result from emulsification of cell membrane lipids (lipid peroxidation) due to intracellular cavitation or associated air bubbles. Therefore, it can be suggested that the coagulation time of milk is shortened by US as pore formation in bacterial cell membranes increases cell membrane permeabilisation and enhances the cellular transport of metabolites. However, it was observed that the changes associated with the bacterial cell membrane were more prominent with increasing treatment amplitudes and treatment durations (Ewe et al., 2012). Therefore, the optimum conditions for such ultrasonication parameters should be carefully determined before applying sonication to the fermented dairy products.

6. Effect of ultrasound on growth and cell viability of lactic acid bacteria during fermentation

Depending on the intensity and the duration of sonication, US has shown both acceleration and inhibition effects on proliferation and viability of microbial cells. Application of US (25 kHz, 160 W for 10 min) increased the cell biomass and fibrinolytic enzyme production in *Bacillus sphaericus* due to de-agglomeration of cell clusters and improvement of nutrient utilisation (Avhad & Rathod, 2015). Similarly, Wang, Shi, Zhou, Yu, and Yang (2003) observed an increased proliferation ability of *Saccharomyces cerevisiae* upon US treatment due to enhanced membrane permeability. Lanchun et al. (2003) found that US treatment of *S. cerevisiae* during the lag phase and exponential phase enhanced cell growth and proliferation by overcoming the mass transfer limitations with the generation of strong convection through micro-streaming. Moreover, Dahroud et al. (2016) showed that US treatment at 60% amplitude for 15 s increased the logarithmic phase duration and growth of *Lactobacillus casei* subsp. *casei* in MRS broth (Fig. 3).

The inhibition effect is due to unrepairable cellular injuries such as breaking and shearing of the microbial cell wall when exposed to intense US. Gao et al., (2014b) suggested that this was mainly due to the mechanical forces and the pressure changes generated through the violent collapse of micro-bubbles within the microbial cells (intracellular cavitation) that eventually resulted in a cell death (Piyasena et al., 2003). Similarly, this can damage the cytoplasmic membrane, which results in the leakage of intracellular contents and coarseness of the cell membrane by the deposition of cell debris on the surface of other cells (Huang et al., 2017). The intensity of US and the duration of the sonication should therefore be carefully selected for application in probiotic dairy products where the viable cell count (VCC) is a critical parameter in determining the shelf-life. The growth and viability of LAB under various ultrasonication conditions, observed by different researchers are summarised in Table 2.

An inhibitory effect on the VCC of lactobacilli was observed by Wang and Sakakibara (1997) during continuous sonication (200 kHz, 17.2 kW m $^{-2}$) within the fermentation period. Interestingly, sonicated fermentation did not affect the proliferation ability of the lactobacilli cells that survived and the cell counts rose when fermentation continued under static conditions. However, the initial reduction of VCC may result in a slower acidification during the fermentation process, leading to extended fermentation time.

Some research findings revealed that the frequency and/or power of ultrasonication that exerts a lethal effect towards microbial cells is dependent on the type of microorganism; different strains have a different response to US (Huang et al., 2017). Therefore, it can be expected that US may affect the viability of different lactic acid bacteria to different extents. Though the effectiveness of ultrasonication on cell viability can be simply assessed through enumeration of microbes before and after treatment, differences in US parameters used in previous studies make comparison of results difficult. Additionally, there are several other variables that influence the effect of US on growth and viability of microorganisms such as process parameters (temperature, amplitude, pressure and duration of sonication) and the physical and biological properties of the microorganism (growth phase, size, capsule thickness), etc. (Gao et al., 2014b; Puvanenthiran et al., 2002; Vercet, Oria, Marquina, Crelier, & Lopez-Buesa, 2002). Similarly, volume of food being processed and the properties of the food, such as composition, viscosity and size of particulates, may influence both the stimulation and inactivation effects of US on microorganisms (Piyasena et al., 2003); this warrants further investigation. There is, however, another important factor, i.e., the level of inoculation, which determines the effectiveness of sonicated fermentation; inoculum rates different from those used in commercial manufacturing might produce different results during sonicated fermentation, but this is not reported in the literature.

7. Effect of ultrasound on enzyme activity

β-Galactosidase (β-gal, β-D-galactoside galactohydrolase or lactase) is the major intracellular enzyme possessed by LAB to catalyse the hydrolysis of β-D-galactoside to galactose (Hermanson, 2013). Several authors found that US accelerated the activity of β-galactosidase in the LAB (Ewe et al., 2012; Nguyen et al., 2009; Wang, Sakakibara, Kondoh, & Suzuki, 1996). This stimulation activity may be due to the collective effects of US such as: (i) enhanced membrane permeabilisation of LAB causing the release of intracellular enzymes into the substrate network (Ewe et al., 2012; Wang & Sakakibara, 1997), (ii) reduction of the activation energy of the enzymes (Delgado-Povedano & de Castro, 2015) and (iii) alteration of the characteristics of the enzyme and the substrate that may enhance the exposure of active sites of membrane-bound enzymes to substrates (Ewe et al., 2012; Huang et al., 2017).

Alteration of the enzyme structure upon US treatment was observed by Ma et al. (2011) with free cellulase where the α-helix structure was partially deformed and the random coil content and the number of surface tryptophan residues were increased upon US treatment (24 kHz, 15 W, 10 min). It might be assumed that the changes to the unique structure of the enzyme and/or the substrate should reduce the activity of the enzyme owing to failure in forming specific enzyme-substrate complexes. However, some contrasting results were achieved with cellulase where the enzyme activity was increased by 18.17% with US treatment compared with untreated cellulase (Wang et al., 2012). Similar findings with respect to increased enzyme activity were reported by Huang et al. (2017) where the degree of hydrolysis of US treated rice proteins was improved due to significant changes to the microstructure of the substrate. Although it was proposed that US with suitable intensity and frequency improves efficiency of enzymolysis due to sonochemistry effects such as cavitation, oscillation and magnetostrictive effects on the molecular conformation of enzymes and substrates, further experiments are warranted to elucidate the exact mechanism behind the acceleration of affinity between the enzyme and the substrate upon sonication.

It has been claimed that process parameters such as duration of sonication and amplitude have different influence towards activity of intracellular and extracellular enzymes (Nguyen et al., 2009). Bacterial cells treated with increased amplitude US for shorter duration (1 min) showed significantly higher intracellular enzyme activities, whereas higher amplitude and longer duration (3 min) were favourable with respect to activity of extracellular enzymes. This was due to an increase in lipid peroxidation by higher amplitude and longer duration of US treatment which eventually enhanced membrane permeability. In contrast, prolonged exposure to sonication (30 min) reduced the activity of β-galactosidase in *B. longum* possibly due to decreased cell viability (Nguyen et al., 2009).

Moreover, it was observed that the effect of US process parameters on enzyme activity varied with the particular strain of LAB used. This strain-dependent effect upon sonicated fermentation was assumed to be influenced by survival rate, the inherent ability of the LAB strain to produce β-galactosidase and growth phase. The effect of US on different strains of the LAB was exhibited by Nguyen et al. (2009) where *Bifidobacterium breve* and *Bifidobacterium* *infantis* were more resistant to US and showed higher fermentation rate, even though they had lower enzyme activity. Wang and Sakakibara (1997) reported similar findings in that *Lactobacillus delbrueckii* subsp. *bulgaricus* showed higher β-galactosidase activity (1.5 unit; where 1 unit of β-galactosidase activity was defined as the amount of the enzyme that liberated 1 µmol *o*-nitrophenol from *o*-nitrophenyl-β-D-galactopyranoside per cm^3 of sample per min) compared with *Lb. acidophilus* (0.05 unit) upon sonicated fermentation (200 kHz, 17.2 kW m^{-2}). Further, they revealed the release of β-galactosidase under sonicated fermentation was prominent in *Lb. delbrueckii subsp. bulgaricus* during the exponential phase of growth where cell division is active.

Additionally, the activity of β-galactosidase was dependent on several other process conditions such as pH, temperature, ionic strength and presence of inhibitors. Stability of β-galactosidase was optimum at pH 6.0–7.0 for the LAB (Wang & Sakakibara, 1997; Wang et al., 1996). When the pH varied from this optimal range, there was a significant drop in enzyme activity. Wang et al. (1996) observed that the activity of extracellular β-galactosidase decreased by 90% and 57% when the pH changed from 6.5 to 5.5 and from 7 to 8, respectively. However, it was reported that the intracellular β-galactosidase was comparatively more resistant due to the protective mechanism of the bacterial cell membrane, which isolates the internal content of the microbial cell from the external environment. Further, this favourable pH range for the optimum activity of β-galactosidase was influenced by some other variables such as temperature and presence of ions. At 25 °C, the enzyme was relatively stable at all pH levels, whereas, at higher temperatures (51 and 56 °C), β-galactosidase was stable only at pH 6 and 7. Presence of cations such as Na^+ and K^+ affect the stability and activity of β-galactosidase differently. Na^+ acts as a strong inhibitor of the β-galactosidase enzyme where lactose was the substrate. Compared with

Na$^+$, the stability of β-galactosidase was higher with the presence of K$^+$ (Kreft & Jelen, 2000). Apparently, sonication enhanced the β-galactosidase activity of LAB and the maximum activity of β-galactosidase could be achieved if sonicated fermentation was carried out under optimum conditions.

8. Effect of ultrasound on lactose metabolism

High-intensity US was used to accelerate lactose hydrolysis in milk through the modification of metabolic performance of LAB (Dahroud et al., 2016; Kreft & Jelen, 2000; Nguyen et al., 2009; Toba, Hayasaka, Taguchi, & Adachi, 1990; Wang et al., 1996; Wang & Sakakibara, 1997). Several authors reported that US accelerated both consumption of lactose and production of glucose, galactose and oligosaccharides, and the effect was improved with prolonged sonication. Lactose consumption by *Bifidobacterium* sp. and *Lactobacillus* sp. was enhanced 2–4 times compared with non-sonicated samples (Nguyen, Lee, & Zhou, 2012; Toba et al., 1990; Wang et al., 1996). Moreover, it was observed that consumption of lactose was notable when sonication was initiated at the beginning of fermentation. In contrast, lactose consumption by non-sonicated cultures started at a later (exponential phase) stage of growth. However, the inoculum levels of the LAB differed between experiments, ranging from 3% to 5% and hence the effect of initial concentration of the LAB cells on the lactose metabolism upon sonication was not adequately explained. It was assumed that sonication accelerated lactose consumption by extracellular β-galactosidase released by sonoporation (Nguyen et al., 2012). US accelerates both hydrolysis and transfer reactions of lactose metabolism, where more simple sugars such as glucose and galactose are available for the bacteria. Further, availability of partially prehydrolysed lactose, in return, may enhance the growth of LAB (O'Leary & Woychik, 1976). There may be some other process parameters such as pH, temperature and the presence of inhibitors, etc., which affect the enzyme activity and thus the rate of lactose metabolism. Even though sonication resulted in the highest levels of extracellular β-galactosidase activity, lactose metabolism was low at pH 4.7 (Wang & Sakakibara, 1997). However, the degree of lactose hydrolysis increased by 13.2% when fermentation was carried out at controlled pH.

Several authors showed that enhanced lactose hydrolysis upon sonicated fermentation depended on bacterial strains used. For an example, degrees of lactose hydrolysis with *Lb. delbrueckii* subsp. *bulgaricus* (39.9%) and *Lactobacillus helveticus* (35%) were higher than *Lb. delbrueckii* subsp. *lactis* (38.1%) and *Lb. acidophilus* (19.6%) under same conditions (Wang & Sakakibara, 1997). Comparable findings were reported by Nguyen et al. (2012) who showed that lactose consumption by different *Bifidobacterium* sp. were significantly different. This could be explained by the fact that different LAB strains have different inherent abilities to hydrolyse lactose since they have various degrees of trans-galactosylation activities and survival rates.

Moreover, US can be used to enhance production efficiency of hydrolysed lactose milk, which is suited to lactose-intolerant individuals. The application of periodic sonication (sonication and static incubation) under pH controlled conditions have reportedly reduced the lactose content of milk inoculated with *Lb. delbrueckii* subsp. *bulgaricus* (B-6 and B-5b) and *Lb. helveticus* (LH-17) by up to 71–76%, whereas lactose hydrolysis in non-sonicated milk was only up to 39–51% (Toba et al., 1990; Wang & Sakakibara, 1997). Therefore, the development and implementation of continuous sonication techniques during fermentation may help produce lactose-hydrolysed fermented milk under industrial scale.

9. Effect of ultrasound on texture and sensory attributes of fermented dairy products

Fermented milk gels should have a smooth and uniform texture without defects such as weak body, wheying-off and lumpiness (Lucey & Singh, 1997). US can influence the sensory properties of fermented milk products either negatively or positively. US treatment before inoculation improved textural characteristics of fermented products whereas, sonication during fermentation caused textural defects as summarised in Table 3 and further discussed below in subsections 9.1 to 9.4.

9.1. Formation of visible particles

Lumpiness (the presence of large protein aggregates) adversely affects the texture of fermented milk products. This occurs due to high incubation temperature, extreme whey protein to casein ratio and certain types of starter bacteria (Lucey & Singh, 1997). Sonication during fermentation was also reported to induce the formation of lumps (d > 0.9 mm) in stirred yoghurt (Körzendörfer, Nöbel, & Hinrichs, 2017; Nöbel, Protte, Körzendörfer, Hitzmann, & Hinrichs, 2016a; Nöbel et al., 2016b). Two possible mechanisms demonstrated for this are (i) lower zeta potential associated with low pH conditions (below 5.4) may enhance the formation of new bonds and (ii) the disruption of casein-whey protein complexes that exposes thiol-groups in whey proteins may enhance cluster formation (Körzendörfer et al., 2017; Nöbel et al., 2016b). According to the observations made by Nöbel et al. (2016b), sonication of a stirred yoghurt sample during fermentation (pH 5.4–5.3) using US (40 KHz, 17 kW m^{-3}, 5 min) increased the size of large visible particle from 1.25 mm to 1.65 mm. Additionally, the number of particles per

100 g was increased from 506 to 2360 over the same pH range. These colloidal particles within the yoghurt gel structure were felt as soft grains and were broken up by subsequent low pressure. The oscillations themselves may induce particle formation as demonstrated by Körzendörfer, Temme, Schlücker, Hinrichs, and Nöbel (2018) who observed lumpiness in set yoghurts along with the vibrations (25–1005 Hz) during the gelation, probably due to the increase in collision probability of aggregating milk proteins.

Sonication-induced lumpiness in fermented milk gels was influenced by several other conditions such as pH, dry matter (DM) content and the type of starter culture used (Körzendörfer et al., 2017). Moreover, sonication-induced lumpiness was observed only within the pH range of 5.4 to 5.1 which is known as the “critical pH range” (Nöbel et al., 2016b). Over this range, the whey proteins attached to the surface of casein micelles reach their isoelectric point, resulting in lump formation. However, sonication may cause reversible interaction within particles above pH 5.4 and casein micelles were not affected by sonication below pH 5.1 since they may already be stabilised within the gel network. Fig. 4 illustrates the macroscopic transmission images of stirred yoghurt gels sonicated at 40 KHz and energy density of 17 kW m^{-3} for 5 min under different pH values during fermentation.

However, stirred-milk gels with low DM content were more susceptible to sonication-induced lump formation, whereas milk gels with DM content of more than 14.2% were not affected by sonication under any pH condition tested (Nöbel et al., 2016a). Therefore, fermented gels produced from sheep and buffalo milk, which have higher dry matter content compared with cow milk, might give different results on sonication-induced lumpiness, but this has not been reported to date. In addition, Körzendörfer et al. (2017) observed that LAB with high levels of exopolysaccharide production reduced the formation of large particles. This may be due to the attachment of exopolysaccharides to casein particles that makes an incompatibility between the exopolysaccharides and casein-modified gel structure, and thus behave as spacers to reduce the lump formation (Körzendörfer et al. (2017).

9.2. *Whey separation and syneresis*

Whey separation can be defined as the presence of whey (milk serum) on the surface of acid milk gels mainly due to the shrinkage of the gel (syneresis) (Lucey, 2004). Conditions that result in whey separation in cultured products are high incubation temperature, extreme whey protein to casein ratio, low solids content and physical mishandling of the products. In addition, fermented gels produced from milk with a high number of larger fat globules, such as buffalo milk, showed porous gel network and thus excessive whey separation (Nguyen, Ong, Kentish, & Gras, 2015).

Sonication improved WHC and reduced the syneresis of set yoghurts and fermented beverages. Wu et al. (2001) observed a prominent increase in WHC when the cow milk was treated with US (20 kHz, 225–450 W) for 6–8 min at 15 °C compared with the yoghurt obtained through conventional homogenisation. Comparable findings were reported by Erkaya et al. (2015) who showed that the thermosonication (60–80 °C, 35 KHz, 1–5 min) of a fermented beverage called “Ayran” on the day following that of production reduced serum liberation by 31% compared with heat treatment at 90 °C for 1 min. This was further verified by Vercet et al. (2002) using manothermosonication (117 μm amplitude, 20 kHz frequency, and 2 kg cm^{-2} pressure) of cow milk for the production of set yoghurts; syneresis was reduced by 14.8% compared with the control that was thermised at 60 °C for 15 s and homogenised.

The effect of US over conventional homogenisation on whey separation and syneresis may be due to sonochemistry effects, mainly towards the milkfat globule (MFG) and milk proteins. US improves WHC through strong cavitation and results in a greater rupturing of the MFG compared with conventional pressure milk homogenisation that subsequently increased the surface area of MFG and the associations with the caseins. Moreover, US causes modifications to the structure of both β-lactoglobulin and α-lactalbumin, which are the major whey proteins in bovine milk. Chandrapala, Zisu, Kentish, and Ashokkumar (2012) reported that whey proteins are unfolded into monomeric units due to partial cleavage of intermolecular hydrophobic interactions either reversibly or irreversibly depending on the intensity of the US treatment. Shanmugam, Chandrapala, and Ashokkumar (2012) observed that these partially denatured whey proteins were aggregated among themselves or with other free caseins, mainly κ-caseins, to form aggregates upon US treatment at 20 kHz and 20 W for up to 60 min. These soluble aggregates further interacted with casein micelles to form micellar aggregates by thiol-disulphide exchange reactions between the denatured whey proteins and the κ-caseins of the micelles. The significant increase in the surface area of MFG upon sonication enhanced the association of modified whey proteins and casein micelle with the MFG membrane (Nguyen & Anema, 2017). As a result, thiol groups and the hydrophobic regions of amino acids are exposed toward water molecules in the surrounding environment. This enhanced the WHC of the milk proteins and serum liberation was reduced. Nevertheless, pasteurisation and other intense heat treatments that were often accompanied with milk before or after the US treatment may cause considerable changes to the serum proteins and thus alter the WHC; this is poorly described in the literature.

However, both prolonged sonication and mechanical disturbances during gel formation has been reported to have a negative impact on gel formation and WHC (Körzendörfer et al., 2017, 2018; Zhao et al., 2014). Moreover, prolonged sonication led to dissociation of whey proteins from micellar aggregates (Shanmugam, Chandrapala, & Ashokkumar, 2012). Similarly, prolonged sonication (20 KHz, 20 W, for 30 min) reduced the size of MFG where the surface available for aggregation was further decreased, which resulted in a weak gel network with greater syneresis (Zhao et al., 2014). Moreover, it was reported that low frequency vibrations (1000 Hz) during the early stages of gelation results in considerable loss of structure and a weak body, leading to further occurring of syneresis (Körzendörfer et al., 2018).

9.3. Texture

Textural properties are typically related to the structure of the milk gel. Structure of set-yoghurt is established through crosslinking of κ-casein on the surface of casein micelles with denatured whey proteins, mostly β-lactoglobulin, which entraps the MFG and milk serum (Lucey, 2004). Shear stress and the temperature rise during sonication resulting in a significant modification in the physicochemical properties of macromolecules such as milk fat and protein and thus alter the consistency and textural properties of fermented milk products. Sonication reportedly has a significant reduction in the size of MFG and proteins compared with pressure homogenisation; Nguyen and Anema (2017) observed a decline of the diameter of MFG from 375 nm to 200 nm during the first 5 min of the US treatment (22.5 kHz and 50 W) of bovine milk (18 g). Moreover, Nguyen and Anema (2010) reported a reduction in the size of casein micelles by about 10–20 nm during the sonication of skimmed milk at 60–70 °C for 5 min due to the solubilisation of κ-casein and denaturation of whey proteins. Therefore, it is anticipated that the structure of milk gels, which greatly relies on the nature of MFG and the denaturation and aggregation state of proteins, and thus the textural properties of milk gels, will be affected upon US treatment (Ahmed, Ramaswamy, Kasapis, & Boye, 2009).

Several researchers have found that high amplitude sonication applied either before or after inoculation of starter cultures significantly increases the viscosity and firmness of set yoghurt (Nguyen & Anema, 2010; Riener et al., 2010; Sfakianakis et al., 2015). This was mainly due to the homogenisation of MFG and denaturation of serum proteins by US treatment (Abbas et al., 2013; Nguyen & Anema, 2017). The substantial reduction of the size of MFG may facilitate the integration of fat into the protein network, while their increased surface area by more than 50% favours the crosslinking between fat and unfolds the peptide chains of whey proteins and subsequent formation of whey-whey and whey-casein aggregates, during gel formation (Nguyen & Anema, 2017; Shanmugam et al., 2012). It can be assumed that the formation of soluble aggregate between denatured whey proteins and casein micelles leads to an increase in viscosity. Moreover, denatured whey proteins have reduced repulsive charges and therefore, easily aggregate. These denatured whey proteins associated with casein micelles may act as bridging material between casein micelles and thus firmer yoghurt gels were formed easily. This effect is conventionally achieved by heating the milk before fermentation to higher temperature such as 90 °C for 5–10 min.

Similarly, manothermosonication was reported to increase the viscosity and firmness of set-gels (Vercet et al., 2002). This might be due to some modification to the MFG membrane upon manothermosonication where the interactions in between MFG and/or casein micelles were enhanced. However, based on their findings, Nguyen and Anema (2010) concluded that most of the benefit from US treatment over the modification of texture properties was due to the heat generated, and non-thermal effects of sonication resulted in minor improvements over conventional heating. A contradictory observation was made by Riener et al. (2010) who indicated that a different kind of molecular interaction may occur during gelation of thermosonicated milk rather than the denaturation of whey proteins and this was responsible for the viscosity modification compared with conventional heat treatment. This hypothesis was further confirmed by the subsequent findings of the same author that thermosonication of 200 mL full-fat milk for 10 min at 400 W led to more whey protein denaturation compared with heating at 90 °C for 10 min (52.2% versus 28.1%).

Furthermore, US homogenisation showed considerably different impact towards the texture of set-gels compared with conventional pressure milk homogenisation. Sfakianakis et al. (2015) observed a significant increase of the final viscosity of set yoghurts with US homogenisation (20 KHz, 562 and 750 W, and 500 mL) compared with two-stage pressure milk homogenisation (30 and 5 MPa). They suggested that US treatment caused whey proteins to denature and both self-aggregate and aggregate with casein micelles and form insoluble high molecular weight material, whereas no significant change in the soluble protein content was observed with pressure homogenisation. Apparently, the US treated milk sample was exposed to a strong heating as sonication itself increased the temperature up to 87 °C in addition to the subsequent heating to 80 °C for 20 min compared with pressure homogenisation that had only the latter heat treatment. This extensive heating of US treated milk may result in comparatively higher denaturation of proteins and was not described by the authors.

Scanning electron microscopic analysis revealed that the set-gels produced from thermosonicated milk (45 °C, 10 min, frequency 24 kHz) showed a honeycomb-like structure where casein micelles were more interconnected and the pores were larger compared with the untreated milk gels (Riener et al., 2010). As a result, the gel texture and viscosity were improved in ultrasonicated milk gel sample. Untreated milk gels showed highly cross-linked network structure and few pores were interspaced throughout the gel structure. However, ultrasonication during gelation reduced the strength of stirred-milk gels and Körzendörfer et al. (2017) observed a reduction in 28% of the maximum force required to puncture the gel. Accordingly, it can be concluded that US was an alternative to homogenisation and heat treatment in yoghurt production, modifying the textural properties of yoghurts mainly through modifications to MFG and milk proteins. However, the degree of the modifications to fat and protein were significantly different as a result of US compared with the conventional method, possibly due to the sonochemistry effects associated with the US.

9.4. Sensory attributes

Effect of thermosonication on the colour of Ayran was recently investigated by Erkaya, Başlar, Şengül, and Ertugay (2015). It was found that fermentation of Ayran followed by thermosonication at 80 °C for 5 min caused a slight reduction in L* value (lightness in Lab colour space) compared with heat treatment for 1 min at 90 °C. Significant loss of L* in Ayran may be due to the acceleration of non-enzymatic browning and the structural changes in milk proteins due to heat and low pH conditions. However, the b* (colour opponents blue–yellow in Lab colour space) value was significantly increased when the duration and temperature of thermosonication increased. However, they have not reported the influence on other sensory attributes such as the flavour of the product.

Similarly, several authors reported that US alters the sensory quality of fresh milk (Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Marchesini et al., 2012, 2015).

A recent study was conducted by Marchesini et al. (2015) on the generation of volatile compounds in US treated milk; it was found that ultrasonication of 100 mL milk under 24 kHz and 160.4 J s^{-1} power intensity for more than 100 s led to the production of volatile compounds, mainly, dodecanoic acid, octanoic acid, δ-dodecalactone and decanoic acid methyl ester. These compounds were responsible for the metallic, burnt, rubbery and sharp off-flavours in milk upon sonication. Hence, it was suggested that ultrasonication beyond 100 s was not appropriate for milk that is intended for direct consumption. Comparable results were reported by Riener, Noci, Cronin, Morgan, and Lyng (2009) and Chouliara et al. (2010), showing that ultrasonicated pasteurised milk resulted in a ‘‘rubbery’’ odour and “burnt” and “foreign” off-taste. However, Vercet et al. (2002) founded that this offensive “cooked” flavour distinguished during manothermosonication of milk, was not detectable when the milk was fermented into set-yoghurts. This might be due to the masking of “cooked” flavour by the flavour compounds generated through fermentation. As yet, the impact of ultrasound assisted fermentation on the synthesis of flavour compounds by LAB has not been reported in the literature.

10. Assessment of realistic conditions used for ultrasonication of fermented dairy products

US has numerous applications in the dairy industry, such as particle size reduction, monitoring of the fermentation process, reduction of the fermentation time, etc. Thus, the appropriate frequency, amplitude and exposure time of the US treatments should be carefully determined for each unique application. The frequency of US could be easily controlled in acoustic experiments since the US apparatus generates vibration at the set frequency. In comparison, the intensity of US is difficult to control during experiments because the milk particles close to the emitter of the sonicator typically have greater pressure oscillations compared with the particles further away as energy is dissipated as heat. Moreover, this effect is enhanced by the bulk mixing of the particles during cavitation, resulting in an uneven exposure of particles to US. Hence, it was suggested that the amount of particle mixing should be considered together with the intensity and exposure time in US treatments (Leong, Martin, & Ashokkumar, 2018). Similarly, the acoustic energy intensity is reported differently in the experiments in the literature. Some sonicators displayed the energy intensity (total energy drawn by the ultrasonic device per unit volume of material processed in J mL^{-1}) whereas, in others, it was calculated using the amplitude of US, the surface area of the emitter and the treatment time. However, a particular energy density can be attained by treating the sample for a long time with a low level of amplitude or short time duration using high level of amplitude. This may bring about different extents of physical and chemical changes in the milk and thereby variation in chemical alterations or degradation in the fermentation milieu. Moreover, the chemical and physical effects of US depend on the properties of the medium. The viscosity and the density of the medium greatly affect the speed and the intensity of the pressure (Leong et al., 2018). Therefore, compositional variation among the milk samples used for the US experiments may have a considerable impact on the results obtained.

11. Feasibility of using ultrasound technology in industrial-scale production processes

The effectiveness of US to enhance or replace different food processes such as emulsification, homogenisation, extraction, crystallisation, freezing, meat tenderisation, dewatering, low temperature pasteurisation, deforming, activation and inactivation of enzymes, particle size reduction and viscosity alteration have been investigated by several authors (Welti-Chanes, Morales-de la Peña, Jacobo-Velázquez, & Martín-Belloso, 2017). A recent approach was to enrich plant foods with bioactive compounds by the induction of stress conditions using US (Del Rosario Cuéllar-Villarreal et al., 2016).

Advantages of high-powered US over conventional processes are higher product yields, shorter processing times and improved product characteristics (Patist & Bates, 2008). However, the main technological limitations that makes the scaling-up of laboratory applications of US in to industrial scale is the increase of the US horn diameter without reducing the vibration amplitude (Kiss et al., 2018). In industrial applications, a larger horn diameter is preferred to produce a larger cavitation zone. However, recent findings on “Barbell horns” shed light upon the scaling-up of US devices where the diameter of the horn and the amplification of US were simultaneously improved without any undesirable effect on the product quality (Peshkovsky, 2017).

In addition, overheating of transducers during continuous processing and poor uniformity are other restrictions. This limitation can be overcome by using an appropriately designed reactor chamber that guarantees the direction of the liquid to be treated through the cavitation zone without bypassing. Moreover, a suitable temperature control and/or cooling system should be installed to the reactor chamber. Peshkovsky (2017) suggested that process efficiency of scaled-up US processors could be enhanced by mounting several US devices in a series or two Barbell horns on to a common reactor chamber.

However, there are several unsettled scale-up challenges, such as irregular cavitation field distribution during the installation of transducers on curved surfaces that may be essential for distillation columns (Kiss et al., 2018). The employment of US technology to the food industry still faces considerable challenges mainly due to the limitations in conventional US processes that have partly been resolved with the invention of the Barbell horn. Nevertheless, further improvements with precise construction procedures and methods may accelerate the adoption of US in the commercial setting.

12. Summary and future perspectives

US technology has been employed in dairy streams to intensify fermented milk product processing by reducing the processing time, minimising ingredient and additive requirements and lowering the resources required. Production of acid milk gels having good gel strength, smooth body and texture and little or no syneresis without using hydrocolloid stabilisers is a challenging task in the industry. Use of US has proved to be a good alternative for stabilisers in fermented milk gels. Further, US treatment minimised the requirement of milk solids that are usually incorporated into the raw milk to strengthen the yoghurt gel. Moreover, US treatment has been reported to shorten the fermentation time of milk through enhancing the metabolic activity of LAB. Meanwhile, it was noted that different bacterial species showed different responses to the US treatment. For example, *Streptococcus* sp. form longer chains than *Lactobacillus* sp. under US influence. Therefore, it is important to re-define optimum growth conditions such as temperature and inoculation rates for the US treated LAB starter cultures for fermented milk products; this needs further investigation. Moreover, power US may be a useful tool to overcome most of the inherent defects associated with buffalo yoghurt, which is significantly more thixotropic and exhibits greater syneresis and poorer structural stability than that made from bovine milk. However, this could be achieved if the process parameters of sonication such as frequency, acoustic intensity and pressure are carefully selected. Hence, the optimisation of sonication parameters to get desirable gelation and fermentation kinetics warrant further studies.

Acknowledgements

This work was supported by the University Grant Commission, Sri Lanka (grant number: UGC/DRIC/QUT2016/UWU/01), Queensland University of Technology, Australia and Queensland Government Advanced Queensland Fellowship (AQF).

References

Abbas, S., Hayat, K., Karangwa, E., Bashari, M., & Zhang, X. (2013). An overview of ultrasound-assisted food-grade nanoemulsions. *Food Engineering Reviews, 5*, 139–157.

Ahmed, J., Ramaswamy, H. S., Kasapis, S., & Boye, J. I. (2009). Ultrasound processing: Rheological and functional properties of food. In K. Muthukumarappan, B. K. Tiwari, C. P. O'Donnell, & P. J. Cullen (Eds.), *Novel food processing: effects on rheological and functional properties* (pp. 85–98). Boca Raton, FL, USA: CRC Press.

Ashokkumar, M. (2011). The characterization of acoustic cavitation bubbles – An overview. *Ultrasonics Sonochemistry, 18*, 864–872.

Avhad, D. N., & Rathod, V. K. (2015). Ultrasound assisted production of a fibrinolytic enzyme in a bioreactor. *Ultrasonics Sonochemistry, 22*, 257–264.

Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International, 48,* 410–427.

Barukčić, I., Jakopović, K. L., Herceg, Z., Karlović, S., & Božanić, R. (2015). Influence of high intensity ultrasound on microbial reduction, physico-chemical characteristics and fermentation of sweet whey. *Innovative Food Science & Emerging Technologies, 27,* 94–101.

Bermúdez-Aguirre, D., Mobbs, T., & Barbosa-Cánovas, G. V. (2011). Ultrasound applications in food processing. In H. Feng, G. Barbosa-Canovas & J. Weiss (Eds.), *Ultrasound technologies for food and bioprocessing* (pp. 65–105) New York, NY, USA: Springer.

Cameron, M., McMaster, L. D., & Britz, T. J. (2008). Electron microscopic analysis of dairy microbes inactivated by ultrasound. *Ultrasonics Sonochemistry, 15,* 960–964.

Chandrapala, J., Zisu, B., Kentish, S., & Ashokkumar, M. (2012). The effects of high-intensity ultrasound on the structural and functional properties of α-lactalbumin, β-lactoglobulin and their mixtures. *Food Research International, 48,* 940–943.

Charoux, C. M., Ojha, K. S., O'Donnell, C. P., Cardoni, A., & Tiwari, B. K. (2017). Applications of airborne ultrasonic technology in the food industry. *Journal of Food Engineering, 208,* 28–36.

Chemat, F., & Khan, M. K. (2011). Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics Sonochemistry, 18,* 813–835.

Chouliara, E., Georgogianni, K. G., Kanellopoulou, N., & Kontominas, M. G. (2010). Effect of ultrasonication on microbiological, chemical and sensory properties of raw, thermized and pasteurized milk. *International Dairy Journal, 20,* 307–313.

Church, C. C. (1988). Prediction of rectified diffusion during nonlinear bubble pulsations at biomedical frequencies. *Journal of the Acoustical Society of America, 83*, 2210–2217.

Dahroud, B. D., Mokarram, R. R., Khiabani, M. S., Hamishehkar, H., Bialvaei, A. Z., Yousefi, M., et al. (2016). Low intensity ultrasound increases the fermentation efficiency of *Lactobacillus casei* subsp. *casei* ATTC 39392. *International Journal of Biological Macromolecules, 86*, 462–467.

Del Rosario Cuéllar-Villarreal, M., Ortega-Hernández, E., Becerra-Moreno, A., Welti-Chanes, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2016). Effects of ultrasound treatment and storage time on the extractability and biosynthesis of nutraceuticals in carrot (*Daucus carota*). *Postharvest Biology and Technology*, 119, 18–26.

Delgado-Povedano, M., & de Castro, M. L. (2015). A review on enzyme and ultrasound: a controversial but fruitful relationship. *Analytica chimica acta, 889*, 1–21.

Dolatowski, Z. J., Stadnik, J., & Stasiak, D. (2007). Applications of ultrasound in food technology. *Acta Scientiarum Polonorum Technologia Alimentaria, 6*, 88–99.

Durnikin, D., Silantyeva, M., & Ereshchenko, O. (2016). Ultrasound-enhanced cell production of lactic and propionic acid bacteria under submerged cultivation for industrial purposes. *Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6*, 287–293.

Erkaya, T., Başlar, M., Şengül, M., & Ertugay, M. F. (2015). Effect of thermosonication on physicochemical, microbiological and sensorial characteristics of ayran during storage. *Ultrasonics Sonochemistry, 23,* 406–412.

Ewe, J., Abdullah, W.W., Bhat, R., Karim, A., & Liong, M. (2012). Enhanced growth of lactobacilli and bioconversion of isoflavones in biotin-supplemented soymilk upon ultrasound-treatment. *Ultrasonics Sonochemistry, 19*, 160–173.

Gao, S., Hemar, Y., Ashokkumar, M., Paturel, S., & Lewis, G. D. (2014a). Inactivation of bacteria and yeast using high-frequency ultrasound treatment. *Water Research*, *60*, 93–104.

Gao, S., Lewis, G. D., Ashokkumar, M., & Hemar, Y. (2014b). Inactivation of microorganisms by low-frequency high-power ultrasound: Effect of growth phase and capsule properties of the bacteria. *Ultrasonics Sonochemistry, 21,* 446–453.

Hermanson, G. T. (2013). Enzyme modification and conjugation. In *Bioconjugate techniques* (3rd edn., pp. 951–957). Boston, MA, USA: Academic Press.

Huang, G., Chen, S., Dai, C., Sun, L., Sun, W., Tang, Y., et al. (2017). Effects of ultrasound on microbial growth and enzyme activity. *Ultrasonics Sonochemistry, 37,* 144–149.

Kiss, A. A., Geertman, R., Wierschem, M., Skiborowski, M., Gielen, B., Jordens, J., et al. (2018). Ultrasound-assisted emerging technologies for chemical processes. *Journal of Chemical Technology & Biotechnology, 93*, 1219–1227.

Körzendörfer, A., Nöbel, S., & Hinrichs, J. (2017). Particle formation induced by sonication during yogurt fermentation–Impact of exopolysaccharide-producing starter cultures on physical properties. *Food Research International*, *97*, 170–177.

Körzendörfer, A., Temme, P., Schlücker, E., Hinrichs, J., & Nöbel, S. (2018). Vibration-induced particle formation during yogurt fermentation—Effect of frequency and amplitude. *Journal of Dairy Science*, *101*, 3866–3877.

Kreft, M. E., & Jelen, P. (2000). Stability and activity of β-galactosidase in sonicated cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 as affected by temperature and ionic environments. *Journal of Food Science, 65*, 1364–1368.

Kumar, C., Karim, M. A., & Joardder, M. U. (2014). Intermittent drying of food products: A critical review. *Journal of Food Engineering*, *121*, 48–57.

Lanchun, S., Bochu, W., Zhiming, L., Chuanren, D., Chuanyun, D., & Sakanishi, A. (2003). The research into the influence of low-intensity ultrasonic on the growth of *S. cerevisiae*. *Colloids and Surfaces B: Biointerfaces*, *30*, 43–49.

Lentacker, I., De Cock, I., Deckers, R., De Smedt, S., & Moonen, C. (2014). Understanding ultrasound induced sonoporation: definitions and underlying mechanisms. *Advanced Drug Delivery Reviews, 72*, 49–64.

Leong, T., Ashokkumar, M., & Kentish, S. (2016). The growth of bubbles in an acoustic field by rectified diffusion. In M. Ashokkumar, F. Cavalieri, F. Chemat, K. Okitsu, A. Sanmbandam, K. Yasui et al. (eds.), *Handbook of ultrasonics and sonochemistry* (pp. 69–98). Singapore: Springer

Leong, T. S. H., Martin, G. J. O., & Ashokkumar, M. (2018). Ultrasonic food processing. In A. Proctor (Ed.), *Alternatives to conventional food processing* (Vol. 53, pp. 316–354). Cambridge, UK: Royal Society of Chemistry.

Louisnard, O., & González-García, J. (2011). Acoustic cavitation. In H. Feng, G. V. Barbosa-Cánovas & J. Weiss (Eds.), *Ultrasound technologies for food and bioprocessing* (1st edn., pp. 13–64). New York, NY, USA: Springer-Verlag.

Lucey, J. A. (2004). Cultured dairy products: an overview of their gelation and texture properties. *International Journal of Dairy Technology*, *57*, 77–84.

Lucey, J. A., & Singh, H. (1997). Formation and physical properties of acid milk gels: a review. *Food Research International, 30*, 529–542.

Maciulevičius, M., Tamošiūnas, M., Jakštys, B., Jurkonis, R., Venslauskas, M. S., & Šatkauskas, S. (2016). Investigation of microbubble cavitation-induced calcein release from cells in vitro. *Ultrasound in Medicine & Biology, 42*, 2990–3000.

Ma, H., Huang, L., Jia, J., He, R., Luo, L., & Zhu, W. (2011). Effect of energy-gathered ultrasound on Alcalase. *Ultrasonics Sonochemistry, 18*, 419–424.

Marcela, B. S., Silvana, S., Fabiana, W. R., Renata, S., & Lisiane, D. M. T. (2018). Effects of pretreatment ultrasound bath and ultrasonic probe, in osmotic dehydration, in the kinetics of oven drying and the physicochemical properties of beet snacks. *Journal of Food Processing and Preservation, 42,* Article 13393.

Marchesini, G., Balzan, S., Montemurro, F., Fasolato, L., Andrighetto, I., Segato, S., et al. (2012). Effect of ultrasound alone or ultrasound coupled with CO_2 on the chemical composition, cheese-making properties and sensory traits of raw milk. *Innovative Food Science & Emerging Technologies, 16*, 391–397.

Marchesini, G., Fasolato, L., Novelli, E., Balzan, S., Contiero, B., Montemurro, F., et al. (2015). Ultrasonic inactivation of microorganisms: A compromise between lethal capacity and sensory quality of milk. *Innovative Food Science & Emerging Technologies, 29*, 215–221.

Martini, S. (2013a). Common uses of power ultrasound in the food industry. In *Sonocrystallization of fats* (pp. 27–33). New York, NY, USA: Springer.

Martini, S. (2013b). An overview of ultrasound. In *Sonocrystallization of fats* (pp. 7–16). New York, NY, USA: Springer.

Moncada, M., Aryana, K. J., & Boeneke, C. (2012). Effect of mild sonication conditions on the attributes of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12. *Advances in Microbiology, 2*, 104–111.

Nguyen, N. H. A., & Anema, S. G. (2010). Effect of ultrasonication on the properties of skim milk used in the formation of acid gels. *Innovative Food Science & Emerging Technologies, 11*, 616–622.

Nguyen, N. H. A., & Anema, S. G. (2017). Ultrasonication of reconstituted whole milk and its effect on acid gelation. *Food Chemistry, 217*, 593–601.

Nguyen, T. M. P., Lee, Y. K., & Zhou, W. (2009). Stimulating fermentative activities of bifidobacteria in milk by high intensity ultrasound. *International Dairy Journal, 19*, 410–416.

Nguyen, T. M. P., Lee, Y. K., & Zhou, W. (2012). Effect of high intensity ultrasound on carbohydrate metabolism of bifidobacteria in milk fermentation. *Food Chemistry, 130*, 866–874.

Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2015). Homogenisation improves the microstructure, syneresis and rheological properties of buffalo yoghurt. *International Dairy Journal, 46*, 78–87.

Nöbel, S., Protte, K., Körzendörfer, A., Hitzmann, B., & Hinrichs, J. (2016a). Sonication induced particle formation in yogurt: Influence of the dry matter content on the physical properties. *Journal of Food Engineering, 191*, 77–87.

Nöbel, S., Ross, N.-L., Protte, K., Körzendörfer, A., Hitzmann, B., & Hinrichs, J. (2016b). Microgel particle formation in yogurt as influenced by sonication during fermentation. *Journal of Food Engineering, 180*, 29–38.

Novoa-Díaz, D., Rodríguez-Nogales, J., Fernández-Fernández, E., Vila-Crespo, J., García-Álvarez, J., Amer, M., et al. (2014). Ultrasonic monitoring of malolactic fermentation in red wines. *Ultrasonics, 54,* 1575–1580.

O'Leary, V. S., & Woychik, J. H. (1976). Utilization of lactose, glucose, and galactose by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk treated with lactase enzyme. *Applied and Environmental Microbiology, 32,* 89–94.

Ojha, K. S., Mason, T. J., O'Donnell, C. P., Kerry, J. P., & Tiwari, B. K. (2017). Ultrasound technology for food fermentation applications. *Ultrasonics Sonochemistry, 34,* 410–417.

Paniwnyk, L. (2017). Applications of ultrasound in processing of liquid foods: A review. *Ultrasonics Sonochemistry, 38,* 794–806.

Patist, A., & Bates, D. (2008). Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science & Emerging Technologies, 9,* 147–154.

Peng, Y. (2010). *Impact of altering the gelation conditions on the physical properties of yogurt*. PhD thesis. Madison, WI, USA: University of Wisconsin-Madison.

Peshkovsky, A. S. (2017). From research to production: overcoming scale-up limitations of ultrasound. In D. Bermudez-Aguirre (Ed.), *Ultrasound: Advances for food processing and preservation* (pp. 409–424). New York, NY, USA: Academic Press.

Pitt, W. G., & Ross, S. A. (2003). Ultrasound increases the rate of bacterial cell growth. *Biotechnology Progress, 19,* 1038–1044.

Piyasena, P., Mohareb, E., & McKellar, R. (2003). Inactivation of microbes using ultrasound: a review. *International Journal of Food Microbiology, 87,* 207–216.

Puvanenthiran, A., Williams, R., & Augustin, M. (2002). Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios. *International Dairy Journal, 12,* 383–391.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2009). Characterisation of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal, 19*, 269–272.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2010). A comparison of selected quality characteristics of yoghurts prepared from thermosonicated and conventionally heated milks. *Food Chemistry, 119*, 1108–1113.

Salazar, J., Chávez, J. A., Turó, A., & García-Hernández, M. J. (2009). Effect of ultrasound on food processing. In J. Ahmed, H. S. Ramaswamy & S. Kasapis (Eds.), *Novel food processing* (pp. 65–84). Boca Raton, FL, USA: CRC Press.

Sancheti, S. V., & Gogate, P. R. (2017). A review of engineering aspects of intensification of chemical synthesis using ultrasound. *Ultrasonics Sonochemistry, 36,* 527–543.

Sfakianakis, P., Topakas, E., & Tzia, C. (2015). Comparative study on high-intensity ultrasound and pressure milk homogenization: Effect on the kinetics of yogurt fermentation process. *Food and Bioprocess Technology, 8,* 548–557.

Shanmugam, A., Chandrapala, J., & Ashokkumar, M. (2012). The effect of ultrasound on the physical and functional properties of skim milk. *Innovative Food Science & Emerging Technologies, 16,* 251–258.

Shershenkov, B., & Suchkova, E. (2015). Upgrading the technology of functional dairy products by means of fermentation process ultrasonic intensification. *Agronomy Research, 13,* 1074–1085.

Shimada, T., Ohdaira, E., & Masuzawa, N. (2004). Effect of ultrasonic frequency on lactic acid fermentation promotion by ultrasonic irradiation. *Japanese Journal of Applied Physics, 43*, Article 2831.

Tabatabaie, F., & Mortazavi, A. (2008). Studying the effects of ultrasound shock on cell wall permeability and survival of some LAB in milk. *World Applied Sciences Journal*, *3*, 119–121.

Tabatabaie, F., Mortazavi, A., & Ebadi, A. (2009). Effect of power ultrasound and microstructure change of casein micelle in yoghurt. *Asian Journal of Chemistry, 21*, 1589–1594.

Toba, T., Hayasaka, I., Taguchi, S., & Adachi, S. (1990). A new method for manufacture of lactose-hydrolysed fermented milk. *Journal of the Science of Food and Agriculture, 52*, 403–407.

Torley, P., & Bhandari, B. R. (2007). Ultrasound in food processing and preservation. In M. S. Rahman (Ed.), *Handbook of food preservation* (2nd edn., pp. 713–740). Boca Raton, FL, USA: CRC Press, Taylor and Francis Group.

Vercet, A., Oria, R., Marquina, P., Crelier, S., & Lopez-Buesa, P. (2002). Rheological properties of yoghurt made with milk submitted to manothermosonication. *Journal of Agricultural and Food Chemistry, 50*, 6165–6171.

Wang, D., & Sakakibara, M. (1997). Lactose hydrolysis and β-galactosidase activity in sonicated fermentation with *Lactobacillus* strains. *Ultrasonics Sonochemistry, 4*, 255–261.

Wang, D., Sakakibara, M., Kondoh, N., & Suzuki, K. (1996). Ultrasound-enhanced lactose hydrolysis in milk fermentation with *Lactobacillus bulgaricus*. *Journal of Chemical Technology and Biotechnology*, *65*, 86–92.

Wang, B., Shi, L., Zhou, J., Yu, Y., & Yang, Y. (2003). The influence of Ca^{2+} on the proliferation of *S. cerevisiae* and low ultrasonic on the concentration of Ca^{2+} in the *S. cerevisiae* cells. *Colloids and Surfaces B: Biointerfaces, 32,* 35–42.

Wang, Z., Lin, X., Li, P., Zhang, J., Wang, S., & Ma, H. (2012). Effects of low intensity ultrasound on cellulase pretreatment. *Bioresource Technology, 117,* 222–227.

Welti-Chanes, J., Morales-de la Peña, M., Jacobo-Velázquez, D. A., & Martín-Belloso, O. (2017). Opportunities and challenges of Ultrasound for food processing: an industry point of view. In D. Bermudez-Aguirre (Ed.), *Ultrasound: Advances for food processing and preservation* (pp. 457–497). New York, NY, USA: Academic Press.

Wu, H., Hulbert, G. J., & Mount, J. R. (2001). Effects of ultrasound on milk homogenization and fermentation with yogurt starter. *Innovative Food Science & Emerging Technologies, 1,* 211–218.

Zhao, L., Zhang, S., Uluko, H., Liu, L., Lu, J., Xue, H., et al. (2014). Effect of ultrasound pretreatment on rennet-induced coagulation properties of goat's milk. *Food Chemistry, 165,* 167–174.

Figure legends

Fig. 1. Main components of laboratary-scale ultrasound devices: (a) ultrasound probe; (b) ultrasound bath.

Fig. 2. Transmission electron micrographs of *Lactobacillus acidophilus* untreated (a) and ultrasonicated (b–d); bar = 1000 nm. Adapted from Cameron, McMaster, and Britz (2008).

Fig. 3. Growth curve of *Lactobacillus casei* subsp. *casei* ATTC 39392 in MRS broth treated with ultrasound (◆; amplitude 60%, 15 s, 10 g L^{-1} peptone) and control sample without ultrasound (■): (a) OD_{600} nm; (b) bacterial counts. Adapted from Dahroud et al. (2016)

Fig. 4. Transmission images of stirred yoghurt samples sonicated at different pH values during fermentation. Average sample mass: 13 g; average layer thickness: 1.2 mm. Adapted from Nöbel et al. (2016a).

Table 1

Application of high-intensity US to lactic fermentation of milk.

Applications	Ultrasonic conditions	Type of bacteria and growth medium	Main effects observed	References
Accelerate lactic acid production	50 mL sample was sonicated at amplitudes of 20%, 40% and 60% for 15, 30 and 45 s every 2 h during fermentation using an ice bath	*Lb. casei* subsp. *casei* ATTC 39392 in permeate powder medium (Pegah Co.,Tabriz, Iran)	Increased production of lactic acid, cell reproduction and substrate consumption Increased growth indexes (specific growth rate and logarithmic phase duration) Increased the membrane permeability (3%)	Dahroud et al. (2016)
Stimulate milk fermentation of bifidobacteria	100 mL of inoculated milk was sonicated before fermentation at 100 W, 20 kHz for 7 min., 15 min. and 30 min. using an ice bath, energy density 420, 900 and 1800 J mL^{-1}	*B. breve* ATCC 15700, *B. infantis*, *B. longum* (BB-46) and *B. animalis* ssp. *lactis* (BB-12) in skim milk	Reduced fermentation time for *B. breve*, *B. infantis* and BB-12 Promoted growth of bifidobacteria Lower the lactose concentration and higher the amount of oligosaccharides Increased the activity of β-galactosidase	Nguyen, Lee, and Zhou (2009)
Enhance cell production of lactic and propionic acid bacteria for industrial purposes	Sonication during fermentation using a fermenter with a flow rate of 10 mL s^{-1} at 880 kHz and 0.1–0.7 W cm^{-3} for 100–120 s	*Lc. lactis* (VPKM B-2092), *Lb. plantarum* (VPKM B-4173), and *Prop. acidipropionici* (VPKM B-2092) under submerged cultivation	Increased the biomass of cells producing lactic and propionic acid	Durnikin, Silantyeva, and Ereshchenko (2016)
Whey fermentation with selected dairy cultures	Sonication of cultures before inoculation at 84 W and 102 W for 75 s and 150 s with a 12 mm diameter probe and frequency of 20 kHz. Sonication temperatures: 37 °C for La-5 and 43 °C for YC-380	*Str. thermophilus, Lb. delbrueckii* subsp. *bulgaricus* and *Lb. acidophilus* (La-5) in thermosonicated whey (480 W, 8 min, 55 °C)	Shorter time of fermentations Increased viable cell count Improved sensory properties	Barukčić, Jakopović, Herceg, Karlović, and Božanić (2015)
Kinetics of sugar and organic acid production during milk fermentation	100 mL of inoculated milk sonicated before fermentation with 20 kHz and an amplitude of ≈100 W for 7 min, 15 min and 30 min at 30–40 °C; energy density 420, 900 and 1800 J mL^{-1}	*B. breve* ATCC 15700, *B. infantis*, *B. longum* (BB-46) and *B. animalis* ssp. *lactis* (BB-12) in skimmed milk	Accelerated lactose hydrolysis and accelerate transgalactosylation Decreased acetic acid: lactic acid Decreased total acetic and propionic acids: lactic acid	Nguyen, Lee, and Zhou (2012)
Isoflavones bioconversion ability of lactobacilli in biotin-supplemented soymilk	10 mL sample sonicated at 30 kHz, 20 W, 60 W and 100 W for 60, 120 and 180 s before inoculation with a 3 mm diameter sonotrode; energy density 120–1800 J mL^{-1}	*Lb. acidophilus* (BT 1088), *Lb. fermentum* (BT 8219), *Lb. acidophilus* (FTDC 8633) and *Lb. gasseri* (FTDC 8131) in soy milk	Induced lipid peroxidation Increased membrane fluidity and permeability Increased growth Enhanced β-glucosidase activity of lactobacilli Promoted bioconversion of glucosides to aglycones in soymilk	Ewe, Abdullah, Bhat, Karim, and Liong (2012)

Yoghurt fermentation	150 mL of inoculated milk sonicated before fermentation at 20 kHz and 450 W, 225 W and 90 W for 1, 6 and 10 min. using a 13 mm diameter probe; energy density 36–1800 JmL^{-1}	*Str. thermophilus, Lb. bulgaricus, Bifidobacterium* and *Lb. acidophilus* in cows' milk	Faster acid development Increased water holding capacity Decreased syneresis Decreased fermentation time	Wu, Hulbert, and Mount (2001)
Lactose hydrolysis and the cell viability of lactic acid bacteria in sonicated fermentation	Sonication during fermentation using a 400 cm^3 fermenter at 200 kHz, 135 W and 17.2 kW m^{-2} for 30 min, 37 °C	*Lb. delbrueckii* subsp. *bulgaricus* B-5b, *Lb. helveticus* LH-17, *Lb. delbrueckii* subsp. *lactis* SBT-2080 and *Lb. acidophilus* SBT-2068 in reconstituted non-fat dry milk	Lower viable cell counts Higher total β-galactosidase activity High degree of lactose hydrolysis	Wang and Sakakibara (1997)
Enhancement of lactose hydrolysis by sonication to produce hydrolysed lactose fermented milk	Sonication during fermentation using a 500 cm^3 fermenter at 200 kHz, 135 W and 17.2 kWm^{-2} for 30 min, 37 °C	*Lb. delbrueckii* subsp. *bulgaricus* B-5b in 10% (w/v) non-fat dry milk	Released intracellular β-galactosidase Higher lactose hydrolysis activity Decreased cell viability	Wang, Sakakibara, Kondoh, and Suzuki (1996)
Compare ultrasonic homogenisation and conventional homogenisation on fermentation kinetics	500 mL milk sample sonicated before inoculation at 20 kHz and output power of 150, 262, 375, 562, and 750 W for 10 min without temperature control using a 13 mm probe; energy density180–900 J mL^{-1}	*Str. salivarius subsp. thermophilus and Lb. delbrueckii* subsp. *bulgaricus* in skimmed bovine milk	Low pH reduction rate Low duration of pH lag phase Higher coagulum viscosity Formation of protein molecule aggregates	Sfakianakis et al. (2015)
Investigate the correlation between exopolysaccharide synthesis ability of starter cultures and the effect of sonication during fermentation of yoghurt	100 mL milk sample sonicated during fermentation using an ultrasonic bath (35 kHz, 300 W) for 5 min.	*Lb. delbrueckii ssp. bulgaricus* and *Str. thermophilus* in skimmed cows' milk	Induced syneresis in set-gels Increased particle numbers under low exopolysaccharide production	Körzendörfer, Nöbel, and Hinrichs (2017)
Effect of different ultrasonic frequencies on fermentation kinetics of Kefir	500 mL milk sample was sonicated during fermentation using an ultrasonic bath at four 28, 40, 100 and 200 kHz and 14 kPa sound pressure at 30 °C	*Str. lactis, Str. cremoris, Streptococcus diacetylactis, Leu. cremoris, Lb. plantarum* and *Lb. casei* in cows' milk	Fermentation time shortened exponentially with frequency	Shimada, Ohdaira, and Masuzawa (2004)
Effect of mild sonication intensities at different temperatures	500 mL of cultures were sonicated before inoculation at 20 kHz and 8.07, 14.68, 19.83 and 23.55 W cm^{-2} at 4, 22 and 40 °C	*Lb. delbrueckii* ssp. *bulgaricus* LB-12 in skimmed milk	14.68 W cm^{-2} improved the bile tolerance, growth and protease activity	Moncada, Aryana, and Boeneke (2012)
Effect of the presence of Na$^+$ and K$^+$ ions on the stability and enzyme activity of sonicated cultures under various temperature and pH levels	50 mL of inoculated milk sample was sonicated at 75 W for 4 min. using a 19-mm probe in an ice water bath; energy density 360 J mL^{-1}	*Lb. delbrueckii* ssp. *bulgaricus* LB 11842 in skimmed milk	Stability of the β-galactosidase activity in sonicated cultures was higher in K+ Enzyme was relatively stable at all pH levels at 25 °C Stability of the enzyme higher at pH 6 and 7 under 51 and 56 °C	Kreft and Jelen (2000)
Impact of sonication on lactose hydrolysis	5 mL of milk was sonicated during fermentation at 20 KHz for 20 min, 0	*Lb. delbrueckii* subsp. *bulgaricus B-6, Lb. delbrueckii* subsp. *bulgaricus B-5b or Lb.*	Higher glucose level 71–74% of the initial lactose was hydrolysed	Toba, Hayasaka, Taguchi, and Adachi (1990)

	°C	*helveticus* LH-17 in milk	Increased syneresis	
Influence of sonication before fermentation on the properties of acid milk gels of skimmed milk	18 g of milk was sonicated before inoculation at 22.5 kHz and 50 W up to 30 min. with (20–70 °C) and without temperature control; energy density 5000 J g^{-1}	*Str. thermophilus* *Lb. delbrueckii* subsp. *bulgaricus* in skimmed milk	Increased in firmness (final G′) Whey proteins denaturation Reduced casein micelle size κ-Casein dissociated from the micelles	Nguyen and Anema (2010)
Comparison of traditional heat treated and thermosonicated milk in terms of their gelation properties	Milk was sonicated before inoculation at 24 kHz and 400 W for 10 min. with a 22 mm diameter tip at 45 °C	Yogotherm yoghurt culture 77570 in skimmed milk	Higher gelation pH Firmer structure Honeycomb-like microstructure Low storage modulus (G`)	Riener et al. (2010)
Intensify the fermentation process of cows' milk	25 mL of milk sonicated at the beginning and after 2 h fermentation using a 2.5 mm probe for 1–3 min.; 30 kHz and from 2 W to 8 W; energy density 4.8–57.6 J mL^{-1}	*Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*	Accelerated fermentation process by 10% Increased shelf-life Reduced syneresis Increased viscosity Enhanced thixotropic properties and structure characteristics	Shershenkov and Suchkova (2015)

Table 2

Growth and viability of LAB upon US treatment.

Treatment conditions	Types of LAB/microorganisms	Observed effects on VCC and growth	References
40 mL milk sample sonicated with a 13 mm probe at 20 kHz, 750 W for 10 min after inoculation; 24–26 °C; energy density 11.25 kJ mL^{-1}	*Lb. acidophilus*	Reduced by log$_{10}$ 0.82	Cameron et al. (2008)
100 mL of whey was thermosonicated with 12 mm probe; 20 kHz, 480 W and 85 Wcm^{-2} for 8 min, 55 °C; energy density 2.3 kJ mL^{-1}	Total plate count	Reduced by log$_{10}$ 2	Barukčić et al. (2015)
100 mL pasteurised whey with 0.08% (w/v) culture was treated with 12 mm probe sonicator at 20 kHz and 84 W for 150 S before inoculation under 43 °C; energy density 0.126 kJ mL^{-1}	*Streptococcus thermophilus* *Lb. delbrueckii* subsp. *bulgaricus*	Increased by log$_{10}$ 2	Barukčić et al. (2015)
Continuously sonication of the cell suspension at 880 kHz and 0.3-0.5 W cm^{-3} for 100-120 s	*Lc. lactis, Lb. plantarum, Prop. acidipropionici*	Increased viability by 28.6, 9, and 16.7 times respectively	Durnikin et al. (2016)
50 mL sample sonicated at an amplitude of 60% for 15 s every 2 h during fermentation using an ice bath	*Lb. casei* subsp. *casei*	Increased biomass production and substrate consumption by ≈25%	Dahroud et al. (2016)
10 mL cell suspension sonicated with 3 mm probe at 30 kHz, 20 W, 60 W and 100 W for 60, 120 and 180 s before fermentation; energy density 0.12–1.8 kJ mL^{-1}	*Lb. acidophilus, Lb. fermentum, Lb. gasseri*	Increased viable counts by >9 $_{\log}$ cfu mL^{-1} with higher amplitudes and longer durations whereas the low amplitude of short duration decreased in viability	Ewe et al. (2012)
100 mL inoculated milk treated at 20 kHz and 50 W for 7–30 min and 40 °C before fermentation; energy density 0.21–0.9 kJ mL^{-1}	*B. breve, B. infantis, B. longum, B. animalis* ssp. *lactis*	Cell counts reduced with the processing time	Nguyen et al., 2009
Sonication while fermentation using a 400 cm^3 fermenter at 200 kHz, 135 W and 17.2 kW m^{-2} for 30 min, 37 °C	*Lb. delbrueckii subsp. bulgaricus Lb. helveticus, Lb. delbrueckii* subsp. *lactis, Lb. acidophilus*	Cell viability decrease in the later period of sonicated fermentation sonication.	Wang and Sakakibara (1997)
Sonication while fermentation using a 400 cm^3 fermenter at 200 kHz, 135 W, 17.2 kW m^{-2}, 37–39 °C for 30 min followed by the incubation in static state (without sonication, agitation and pH control)	*Lb. delbrueckii* subsp. *bulgaricus Lb. helveticus, Lb. delbrueckii* subsp. *lactis, Lb. acidophilus*	Cell viability increased during the static incubation	Wang and Sakakibara (1997)

Table 3

Impact of US on sensory attributes of fermented dairy products.

Product	Type of starter culture	Sonication equipment	Sonication condition	Properties after sonication	Reference
Set yoghurt and stirred- yoghurt	*Lb. delbrueckii* ssp. *bulgaricus*, *Str. thermophilus*	Ultrasonic water bath (RK 1028/ H; Bandelin electronic GmbH& Co. KG, Berlin, Germany)	35 kHz and 300 W for 5 min at 42 °C during fermentation	Set yoghurt: Increased syneresis Reduced firmness Stirred yoghurts: Increased large particles (d > 0.9 mm) Higher viscosity	Körzendörfer et al. (2017)
Stirred yoghurt	*Yo-Mix 215* *YC-471* *(Danisco Deutschland GmbH, Niebull, Germany)*	Ultrasonic water bath (USC1200TH, VWR International GmbH, Darmstadt, Germany)	45 kHz, 200 W and 17 kW m^{-3} for 5 min at 42 °C during fermentation	Increased large particles	Nöbel et al. (2016b)
Set yoghurt	*Str. thermophilus, Lb. bulgaricus*	Piezoelectric source, Hielscher, Germany	20 KHz, 30 min before fermentation	Improved the gel texture Improved viscosity Decrease in milk turbidity and lightness	Tabatabaie, Mortazavi, and Ebadi (2009)
Set yoghurt	*Str. thermophilus, Lb. bulgaricus, Bifidobacterium, Lb. acidophilus*	Model CP502, Cole-Parmer Instrument Company, USA	150 mL inoculated milk sonicated before fermentation at 20 kHz and 450 W for 8 min using a 13 mm diameter probe; energy density 1.44 $kJmL^{-1}$	Reduce syneresis Improve viscosity	Wu et al. (2001)
Ayran (fermented milk drink)	*Str. thermophilus* *Lb. bulgaricus*	Ultrasonic bath; Model No. RK103H, Bandelin, Berlin, Germany	300 mL sample treated at 35 kHz and 60–80 °C for 1, 3 and 5 min	Increased the viscosity Decreased serum separation Whiter in colour	Erkaya, Başlar, Şengül, and Ertugay (2015)
Set yoghurt	*YBCN 143*	Branson 450 sonicator	Manothermosonication of 6 mL milk circulated and treated at 32 $mLmin^{-1}$, 20 kHz and 12 s under 2 kg cm^{-2} pressure, 40 °C	Firmer structure Improved texture Higher gumminess and chewiness Less structure loss upon compression	Vercet et al. (2002)
Stirred yoghurt	*Yo-Mix 215 (Danisco Deutschland GmbH, Niebull, Germany)*	Ultrasonic bath (RK1028H; Bandelin electronic GmbH & Co. KG, Berlin, Germany	100 mL milk sample sonicated at 35 kHz, 300 W, 15 Wm^{-3} at 42 ^oC for 5 min during fermentation; energy density 0.9 $kJmL^{-1}$	Induced the formation of large particles, no significant effect of the sonication to the yoghurts above 14.2% dry matter	Nöbel, Protte, Körzendörfer, Hitzmann, and Hinrichs (2016a)

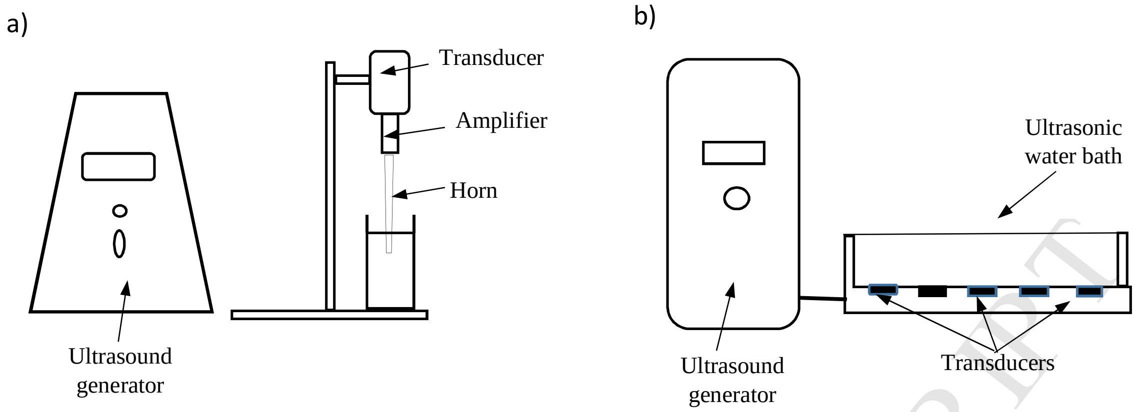
a)
Transducer
Amplifier
Horn
Ultrasound generator
b)
Ultrasonic water bath
Ultrasound generator
Transducers

Figure 1

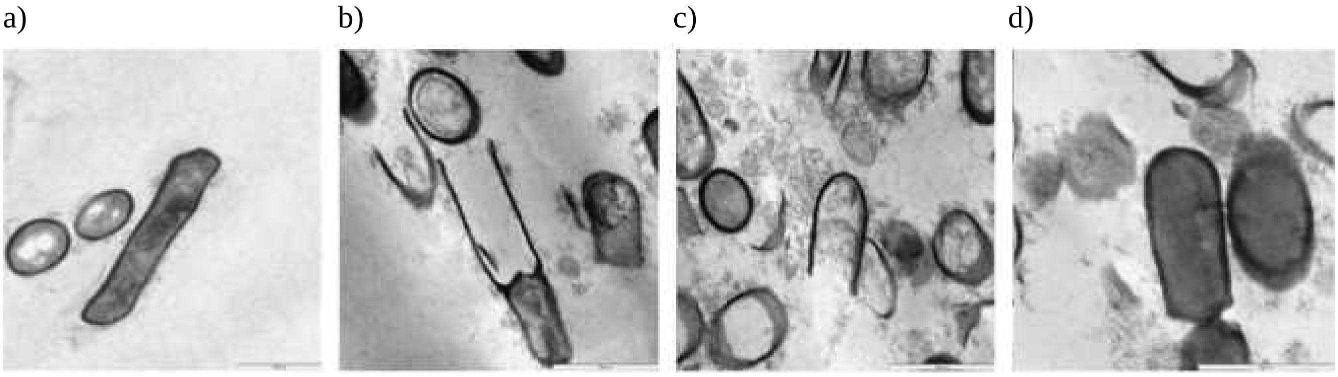

Figure 2.

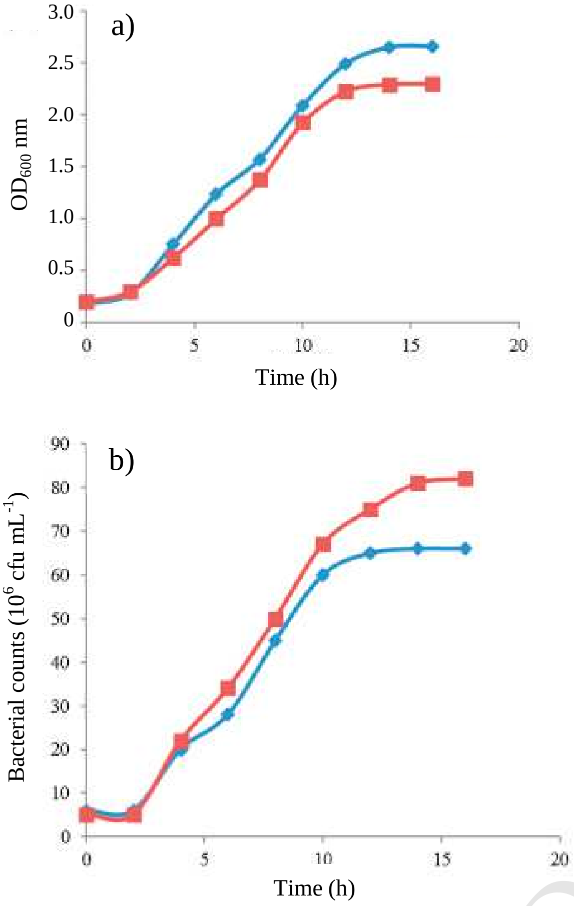
a)
3.0
2.5
2.0
1.5
1.0
0.5
0
OD$_{600}$ nm
0
5
10
15
20
Time (h)
b)
90
80
70
60
50
40
30
20
10
0
Bacterial counts (10^{6} cfu mL^{-1})
0
5
10
15
20
Time (h)

Figure 3.

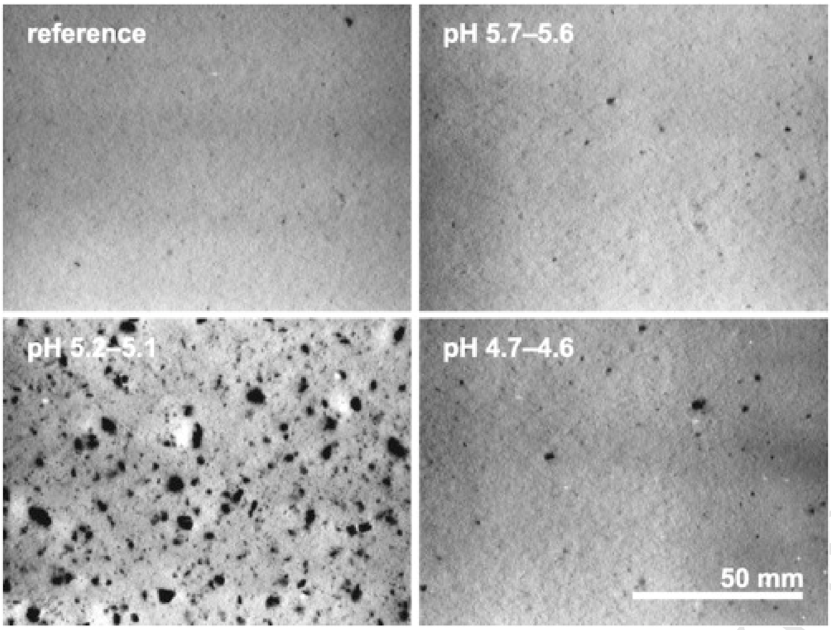

Figure 4.