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REVIEW



Recent advances in dual effect of power ultrasound to microorganisms in dairy industry: activation or inactivation

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ABSTRACT

There is a growing interest on ultrasonic processing of dairy products, especially fermented dairy products which are a basis to functional foods. The studies have shown that power ultrasound can enhance the fermentation process of lactic acid bacteria by modifying their metabolic activity while reducing fermentation time and improving the quality characteristics of fermented milk products. Fermentation is one of the important stages in the processing of dairy products, but it is also one of the most time and resource consuming stages during production. Thus, the benefits of ultrasound to the fermentation process due to microbial activation become increasingly important. In fact, ultrasound applications have the dual effect on microorganisms. Besides being used for microbial activation in dairy industry, it can also be used for inactivation of microorganisms depending on ultrasound power and frequency, sonication time, microorganism type, pH, and temperature. This review article summarizes the effect of power ultrasound on microbial inactivation and microbial growth based on fermentation profile of dairy products, with a theoretical background on ultrasound, including research findings. Also, the details on the activation and inactivation mechanisms of power ultrasound to microorganisms are presented.

KEYWORDS

Acoustic cavitation; dairy; microbial activation and inactivation; power ultrasound

Introduction

Ultrasound is an emerging and fast-growing technology due to its wide range of applications in food science and technology. It has been applied to minimize food related processing parameters such as duration, energy consumption, etc., and to improve food quality and safety (Firouz, Farahmandi, and Hosseinpour 2019).

Ultrasound is a form of energy generated by sound waves with frequency which exceeds the hearing limit of the human ear (>20 kHz) (Higuera-Barraza et al. 2016; Shanmugam, Chandrapala, and Ashokkumar 2012). It is considered a developing innovative technology for dairy processing, due to its promising effects in food processing and preservation with minimum changes in products and less energy requirement (Cheng et al. 2015; Guimarães et al. 2018). Ultrasound is called green technology as it enables the creation of environmentally friendly processes through sound waves that are considered safe, nontoxic, simple and fast. It is also a relatively inexpensive technology. These properties give ultrasound a major advantage over both conventional methods and other innovative technologies (Arzeni et al. 2012; Frydenberg et al. 2016). Ultrasound applications in the food industry are classified into two main categories, low power ultrasound and high power (power) ultrasound (Awad et al. 2012; Sango et al. 2014). Low power ultrasound refers to low intensity-high frequency systems using smaller power levels (<1 W/cm²) and

frequencies higher than 100 kHz and it is mostly utilized for quality assurance, process control, non-contact inspection and monitoring as a nondestructive measuring technique (Mohammadi et al. 2014; Mohammadi, Ghasemi-Varnamkhasti, and Gonzalez 2017). It is applied to determine the physicochemical properties of food products without affecting the food structure (Ojha et al. 2017). Although it has a weak physical phenomenon, it shows a great effect on the formation of free radicals (Noci 2017). Power ultrasound refers to high intensity-low frequency systems using power levels between 10 and 1000 W/cm² and frequencies between 20 and 100 kHz which leads to a strong acoustic cavitation. It is mostly employed for process intensification (Chandrapala and Leong 2015; Gao, Hemar, Ashokkumar, et al. 2014; Loveday, Sarkar, and Singh 2013; Ojha et al. 2017).

Ultrasound applications have a strong potential for utilization specifically in the dairy industry because of its flexibility and capability on both processing and analyzing of the products. Ultrasound technology can be used in various dairy processing fields such as emulsification (O'Sullivan et al. 2015; Zwieten et al. 2017), homogenization (Akdeniz and Akalın 2020; Ertugay, Sengül, and Sengül 2004; Sfakianakis, Topakas, and Tzia 2015), creaming (Juliano et al. 2013), crystallization of ice and lactose (Akdeniz and Akalın 2019; Zamanipoor and Mancera 2014; Zhang et al. 2015), freezing (Kiani et al. 2013; Zheng and Sun 2006), cutting (Arnold et al. 2009); extraction of functional foods

(Ashokkumar et al. 2010), enhancement of whey ultrafiltration (Chandrapala et al. 2012a; Chemat, Huma, and Khan 2011), monitoring and quality control (Jiménez et al. 2017; Mohammadi et al. 2017; Richard et al. 2012), intensification of milk fermentation and enhancing fermentation rates (Nguyen, Lee, and Zhou 2009; Wu, Hulbert, and Mount 2000), increasing of bioactive compounds (Monteiro et al. 2018), and microbial inactivation (Bermudez-Aguirre and Barbosa-Canovas 2008; Cameron, McMaster, and Britz 2008; Huang et al. 2017). It improves food properties including emulsification, gelation, antioxidation, foamability, water holding capacity, viscosity, syneresis and gel strength (Akdeniz and Akalin 2017; Ashokkumar 2015; Erkaya et al. 2015; Pingret, Fabiano-Tixier, and Chemat 2013; Riener et al. 2010).

In ultrasound application, when sound waves pass through a liquid, longitudinal waves are created. Thus, high and low pressure regions are created due to consecutive compression and expansion cycle. This pressure change induces cavitation and gas bubble formation from gas nuclei existing in the liquid (Chouliara et al. 2010; Islam, Zhang, and Adhikari 2014). These bubbles distributed throughout the liquid and have a larger surface area during the expansion cycle. They reach a critical size after a few cycles and collapse of bubbles occur (Herceg et al. 2013; Soria and Villamiel 2010). Depending on the ultrasound frequency used, transient or stable cavitation generates in the liquid medium. When the low frequency ultrasound within 20–100 kHz is used, the transient cavitation occurs. The bubbles could not retain the vapor phase and collapse violently creating shock waves, shear forces, micro-jets and turbulence (Herceg et al. 2013; Soria and Villamiel 2010). At the high frequencies over 200 kHz, stable cavitation is observed resulting in the regular oscillation between high and low acoustic pressure for thousands of cycles. If the megahertz frequency ultrasound is used, no cavitation occurs (Ashokkumar et al. 2010; Galván-D'Alessandro and Carciochi 2018).

The violent collapse of bubbles generates excessive temperatures and pressures reaching up to 5000 K and up to 50 MPa, respectively due to energy accumulations in hot spots (Akdeniz and Akalin 2019; Ashokkumar 2011; Chouliara et al. 2010). This is known as acoustic cavitation. The main mechanical effects of acoustic cavitation include shock wave induced damage and microjet impacts (Abesinghe et al. 2019). In addition, agitation, turbulence, vibration, pressure, shear forces, and acoustic streaming are other mechanical phenomena of ultrasound. Besides these mechanical effects of cavitation, there are also chemical effects. One of the most important is the production of free radicals (Akdeniz and Akalin 2019; Galván-D'Alessandro and Carciochi 2018). In low power ultrasound, the chemical effects are stronger, and the physical effects are relatively weaker. Conversely, in power ultrasound, the physical effects of acoustic cavitation are stronger, and the radical production/reaction is insignificant (Ashokkumar et al. 2010).

Although ultrasound was started to be evaluated as a microbial inactivation method in the 1960s after discovering

that sound waves used in submarine warfare killed fish (Herceg et al. 2013; Piyasena, Mohareb, and McKellar 2003), the use of ultrasound to promote or control the activity of microorganism is much more recent (Ojha et al. 2017). In fact, ultrasound is a two-sided technique for microorganisms in terms of both microbial inactivation and growth of starter cultures in dairy technology (Bevilacqua, Campaniello, et al. 2019).

Most research investigating the effects of ultrasound on microorganisms was about using a power ultrasound at a lethal level (Herceg et al. 2013; Ojha et al. 2017). But recently it is considered that power ultrasound improves microbial growth and fermentation process by increasing cell permeability and mass transfer of components across the cell wall and membrane, leading to improved process efficiency and production rates (Ojha et al. 2017). Except from ultrasound power and frequency, the type of microorganisms, sonication time, pH and temperature (Chemat, Huma, and Khan 2011; Ojha et al. 2017) influence the effects of ultrasound applications on microorganisms. Depending on these factors, its effects may be either negative, such as inhibition and inactivation, or positive, such as stimulation of growth and modulation of some functional properties (Bevilacqua, Campaniello, et al. 2019). It is considered that ultrasound has this dual effect on microorganisms due to the level of sonoporation, which is the formation of transient cavities or pores on cell membrane resulting from sonication. Higher degrees of irreversible sonoporation could lead to microbial death due to leakage of cellular content and disruption or alternation of the cell membrane, while lower degrees of sonoporation could improve microbial growth due to facilitated mass transfer of oxygen and nutrients necessary for cell growth from the cell membrane and removal of waste products away from the cell (Lentacker et al. 2014; Pitt and Ross 2003; Wu and Nyborg 2008).

Although there are limited reviews on the effect of ultrasound application to dairy microorganisms, there is not any comprehensive review on relation between ultrasound and dairy microorganisms. This review aims to provide an overview of current information and potential applications regarding the effect and action mechanism of ultrasound on both microbial inactivation and also microbial growth as well as fermentation processes enhancement.

Effects of power ultrasound on microbial activation

Microbial activation is especially important for fermented dairy products, which are one of the most important sources of bioactive peptides, essential amino acids, fatty acids, vitamins, minerals and other biologically active compounds including lactic acid bacteria (LAB) and/or probiotics for human nutrition and health. Nowadays, functional foods are in greater demand by health-conscious consumers and fermented milk is widely used as basis for functional foods.

There are several ways to manufacture functional dairy products based on the modification of processing methods or fortification with different substances to increase the

functionality of the product (Dinkçi, Akdeniz, and Akalın 2019). In this context, the intensification of milk fermentation through ultrasonication is a recent area of interest in the dairy industry.

Fermentation that affects the product quality significantly is the most time- and resource-consuming stage during the processing of fermented dairy products (Abesinghe et al. 2019). For this reason, intensification of fermentation process without negative effect on a final product is important for dairy industry (Shershenkov and Suchkova 2015). Several studies have revealed that ultrasound can improve the quality characteristics of fermented milk gels such as texture profile, syneresis and water holding capacity (Akdeniz and Akalın 2019; Riener et al. 2010; Sfakianakis, Topakas, and Tzia, 2015). Therefore, the application of ultrasound on biological effects, such as the acceleration effect on proliferation of microbial cells is an exciting new area of research in the field of fermentation (Huang et al. 2017).

Mechanism of microbial activation

In fact, ultrasound can display a dual effect on microorganisms; a lethal effect or the stimulation of growth, depending on the intensity and the frequency of ultrasound application (Bevilacqua, Campaniello, et al. 2019). Ultrasound in higher frequencies and low powers does not affect on mechanical or chemical properties of material, but only causes vibrations in the molecules of the material. However, power ultrasound waves with lower frequencies carry more acoustic energy which cause physical, mechanical and chemical changes in the material depending on cavitation (Firouz, Farahmandi, and Hosseinpour 2019). It has been reported that appropriate ultrasound can promote the growth of microbial cells. Ultrasound waves with frequencies above 100 kHz did not cause any negative effects on cells due to the low power that produces stable cavitation providing repairable damages to cells. It changes the metabolic activity of microbial cells leading to acceleration of their proliferation and more products of metabolism increasing enzyme production and membrane permeability (Dahroud et al. 2016; Huang et al. 2017). The positive effect of low intensity ultrasound was reported on biofilm formation by means of increasing the transport of oxygen and nutrients to deeper layers of the biofilm and increasing the hydrophobicity and membrane permeability of the microorganism forming biofilm (Erriu et al. 2014; Bevilacqua, Racioppo, et al. 2019).

In the field of ultrasonic effects on living cells, Sinisterra (1992) initially reported that low intensity ultrasound could modify cellular metabolism or improve the mass transfer of products through the boundary layer or through the cellular wall and membrane. Afterwards, Chisti (2003) suggested that suitably controlled ultrasonication has shown beneficial effects on the metabolic performance of live systems though power ultrasound is detrimental to cell viability. The cell growth in low intensity insonation is due to its' ability to increase the transport of small molecules in solution by increasing the convection. It is often desirable to increase the transport of oxygen and nutrients to the cells and to

increase the transport of cellular waste products away from the cells in order to increase the growth rate of cells (Ojha et al. 2017).

Low intensity effects that increase convection in liquid are arisen from the acoustic streaming and microstreaming (Pitt and Ross 2003). Acoustic streaming that is dominant at frequencies above 1 MHz with less physical and chemical effects is a consequence of acoustic cavitation and relies upon the dissipation of the cavitation energy by means of shear energy and turbulence. It is the physical force of the sound capable of displacing ions and small molecules due to a pressure gradient. Microstreaming is produced by stable cavitating gas bubbles in the liquid. When the ultrasound intensity is sufficiently low the bubbles do not collapse completely during their contraction cycle and stable cavitation occur that greatly increases convective transport. In that case, little or no cavitation damage will occur and the beneficial effects are obtained such as improvement in microbial reactions, activation of enzymes and increased transfer of materials across the cell membrane (Ashokkumar et al. 2010; Bevilacqua, Campaniello, et al. 2019; Pitt and Ross 2003).

On the other hand, the permeability of cell membranes improve through the formation of pores, or temporary holes, in the cell membrane in a process known as sonoporation. Although these pores lead to sublethal injury to the microbial cell, they supply a channel for transport of essential nutrients and removal of toxic substances across these membranes. Therefore ultrasound treated cells with less physical damage can recover from injury and subsequently increase in number during fermentation, depending on the microorganism type and ultrasound process conditions (Galván-D'Alessandro and Carciochi 2018). The transient, temporary pores on cell membrane allow the channeling of non-permeable extracellular substances such as proteins and macromolecules to enter the cells and releasing of intracellular enzymes such as β -galactosidase out from the cell. Therefore these pores, microbubbles, provide important biological effects on the growth and metabolic processes of ultrasound treated cells enhancing membrane permeabilization (Ewe et al. 2012a; Huang et al. 2019).

According to Ojha et al. (2017), it is possible to modify the acoustic energy entering a cell suspension to reduce the effects of cavitation by altering the exposure time, the acoustic power or the frequency of the ultrasound treatment. As high power and long exposure time with low frequency causes cell damage, the parameters of ultrasonication should be arranged for expected cell activity. In another words, ultrasound may reveal positive or negative effects on bacterial cell performance due to the level of sonoporation. Various interactions of microbubbles with cell membranes has been reported such as push and pull effect of cavitating microbubble, rupturing of cellular membrane due to jet formation and penetration of microbubbles into a cell. A low level of sonoporation has been reported to improve microbial growth through mass transfer of substrates or reagents across cell membrane and removal of byproducts of cellular metabolism.

The effect of ultrasound on microbial cells is also influenced by microbial ecology (e.g. type of microorganism, food or medium composition), pH and temperature as well as ultrasound parameters (e.g. power and frequency, and sonication time). In general, gram (+) bacteria are more resistant to ultrasound depending on their cell wall characteristics. The effect of sonication is also culture specific because of distinctive resistance of microorganism toward ultrasound due to variations in cell characteristics. In addition, any temperature increase, induced by ultrasound exposure, could change the physicochemical state of the cell membranes (Lentacker et al., 2014; Ojha et al. 2017).

Although power ultrasound, particularly of 20–40 kHz, damages to biological molecules due to the generation of cavitation bubbles, which produce harmful physical effects and highly oxidizing hydroxyl radicals, the use of sub-lethal doses of ultrasonic irradiation has been shown to be beneficial for many bioprocesses. It is believed that controlled cavitation is not always detrimental and the use of enzyme/ultrasound combination in bioprocessing enhances the transport of enzyme macromolecules to the surface of substrate by means of the mechanical impact of cavitation (Kwiatkowska et al. 2011). Most of the ultrasonic beneficial effects for dairy fermentation appear over short times and at low frequencies. So, using mid-power short-time ultrasonic treatment allows minimize some of the ultrasound negative effects and energy costs (Ashokkumar et al. 2010). Because power ultrasound has shown versatility and effectiveness to enhance the productivity and process effectiveness in the food industry, the applications of power ultrasound in food fermentation, especially mid power ultrasound treatment at sublethal levels have already aroused researchers' concern due to its' positive effect on living cells (Galván-D'Alessandro and Carciochi 2018).

Power ultrasound effects on microbial growth

The interactions between ultrasound and microorganisms, particularly at sub-lethal levels to stimulate activity, are complex depending on the external and internal control parameters which can be altered for a range of fermentation applications (Ojha et al. 2017). Several research studies have revealed that power ultrasound can improve the fermentation profile and proliferation of LAB and probiotics by modifying their metabolism depending on the ultrasonic conditions (Abesinghe et al. 2019; Guimarães et al. 2019). Therefore, the intensity and duration of sonication should be carefully selected for application in fermented and also probiotic dairy products to improve the permeability of cell membranes and to accelerate the supply of oxygen and nutrients for microorganisms as intense sonication causes unrepairable cellular injuries such as beaking and shearing of the cell (Abesinghe et al. 2019; Guimarães et al. 2019; Racioppo et al. 2017). Depending on the ultrasound conditions, the nature of waves and type of sonication has been reported to affect microbial viability. Less cell damage, occur by the standing wave fields compared to propagating wave fields due to lower acoustic pressure and mechanical stress

in the standing wave fields. Intermittent or continuous sonication also has an impact on the process efficiency as some studies showed that the intermittent sonication improved the growth of microorganism (Chisti 2003). The viable cell count is a critical parameter in determining the shelf life of probiotic dairy products. The growth and viability of probiotics and LAB under various ultrasonic conditions obtained by different researchers are given in Table 1. Dahroudi et al. (2016) reported that the major ultrasonic event influencing the probiotic cells is microstreaming, not causing physical damage, but facilitating the mass transfer of gases and nutrients to stimulate the cells. The studies on the ultrasound effect to cell viability revealed that the frequency and/or power of ultrasonication that exerts a lethal effect toward microbial cells is dependent on the microorganism type; different strains have a different response to ultrasound (Huang et al. 2017; Ojha et al. 2017). Nguyen, Lee, and Zhou (2009) investigated the effect of ultrasonication (100 W, 20 kHz, 7, 15 and 30 min) on the viability of four different strains of *Bifidobacterium* (i.e., *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium animalis* ssp. *lactis* and *Bifidobacterium longum*) in fermented milk. The viable counts in sonicated samples were lower at the beginning of fermentation in comparison with the control sample while the final counts were not found significantly different between control and ultrasound-treated samples even being slightly higher for the strains *B. breve* and *B. infantis*. Effect of ultrasonic application at 40 kHz 116 W for 5, 10, 15 and 20 min on the growth of five different strains of probiotic bacteria (*Lactobacillus acidophilus* (LA-5), *Lactocaseibacillus casei* (LC), *Limosilactobacillus reuteri* (LR-MM53), *Bifidobacterium bifidum* Bb-12, *Bifidobacterium longum* (Bb-536)) in fermented skim milk was studied (Niamah 2019). Ultrasound treatment of 10 min increased the viable cells for *Lactobacillus acidophilus* (LA-5), *Lactocaseibacillus casei* (LC), *Limosilactobacillus reuteri* (LR-MM53), but decreased the viable cells for *Bifidobacterium bifidum* Bb-12, *Bifidobacterium longum* (Bb-536) (Niamah 2019).

Therefore, ultrasound may affect the viability of different LAB in different ways and extents. Although, ultrasound efficiency regarding cell viability can be easily assessed by enumeration of microorganisms before and after treatment, differences in ultrasound conditions and parameters (temperature, amplitude, pressure and duration of sonication) used in previous studies make comparison of results difficult. In addition, the physical and biological properties of the microorganism (growth phase, size and shape, capsular thickness and structures such as cell wall composition) influence the effect of ultrasound on growth and viability of cells (Gao et al. 2014a). For example, yeasts are more susceptible than bacteria due to their larger cells, aerobes are more resistant than anaerobes, and cocci are more resistant than bacilli to power ultrasound treatment (Chandrapala et al. 2012b). Similarly, the volume and the properties (composition, viscosity and size of particles as well as pH, temperature) of food being processed may influence both stimulation and inactivation effects of ultrasound on probiotics and LAB (Piyasena, Mohareb, and McKellar 2003).

Table 1. Effects of power ultrasound on the growth and viability, membrane permeability, enzyme activity and fermentation profile of LAB.

Application	Ultrasound parameters	Types of LAB and growth medium	Main effects obtained	References
Effect of sonication on lactose hydrolysis	5 mL of milk was sonicated at 20 kHz for 20 min during fermentation	<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> or <i>Lb. helveticus</i> in milk	Higher glucose level 71%–74% hydrolyzation of the initial lactose with sonication in compared with 39%–51% for unsonication	Toba et al. (1990)
β -galactosidase activity of sonicated <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> culture with different ions, pH and temperature	50 ml of inoculated milk was sonicated at 75 W for 4 min with a 19 mm probe in an ice water bath; energy density 360 J mL ⁻¹	<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> LB 11842 in skim milk	Stability and activity of enzyme in sonicated cultures was higher in K ⁺ presence Enzyme was relatively stable at all pH levels at 25 °C, while it was stable at pH 6 and 7 at higher temperatures	Kreft and Jelen (2000)
Effect of ultrasound on yoghurt fermentation	150 mL of inoculated milk sonicated before fermentation at 20 kHz, 180, 270 and 450 W for 8 min using a 13 mm diameter probe; energy density 576–1440 J mL ⁻¹	<i>Str. thermophilus</i> , <i>Lb. bulgaricus</i> , <i>Bifidobacterium</i> and <i>Lb. acidophilus</i> in cows' milk	Acid development accelerated Fermentation time decreased	Wu, Hulbert, and Mount (2000)
Effect of different ultrasonic frequencies on fermentation promotion 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	<i>Str. lactis</i> , <i>Str. cremoris</i> , <i>Streptococcus diacetylactis</i> , <i>Leu. cremoris</i> , <i>Lb. plantarum</i> and <i>Lb. casei</i> in cows' milk	Fermentation time shortened exponentially within frequency range studied	Shimada, Ohdaira, and Masuzawa (2004)
Effect of ultrasound on cell wall permeability	Sonication at 20 kHz for 5, 10, 15, 20, 25 and 30 minutes	<i>Lactobacillus acidophilus</i> (strain, LAI), <i>Lactocaseibacillus casei</i> (strain AB) <i>Lactococcus lactis</i> .spp <i>cremoris</i> and <i>Lactococcus lactis</i> .spp <i>lactis</i> in milk	The cell wall permeability of the cells increased according to TEM picture	Tabatabaie and Mortazavi (2008)
Stimulating fermentative effect of ultrasound on 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	<i>B. breve</i> ATCC 15700, <i>B. infantis</i> , <i>B. longum</i> (BB-46) and <i>B. animalis</i> ssp. <i>lactis</i> (BB-12) in reconstituted skim milk	Promoted growth of <i>B. breve</i> , and <i>B. infantis</i> Reduced fermentation time for <i>B. breve</i> , <i>B. infantis</i> and BB-12 Reduced cell counts with the processing time Increased the activity of β -galactosidase Lower the lactose concentration and higher the amount of oligosaccharides	Nguyen et al. (2009)
Effects of ultrasound on the growth and isoflavones bioconversion ability of lactobacilli in biotin-supplemented soymilk	10 mL cell suspension sonicated at 30 kHz, 20, 60 and 100 W for 60, 120 and 180 s before inoculation with a 3 mm diameter sonotrode; energy density 120–1800 J mL ⁻¹	<i>Lb. acidophilus</i> (BT 1088), <i>Lb. fermentum</i> (BT 8219), <i>Lb. acidophilus</i> (FTDC 8633) and <i>Lb. gasseri</i> (FTDC 8131) in soy milk	Increased membrane fluidity and permeability Increased growth Increased viable counts by >9 log cfu mL ⁻¹ with higher amplitudes and longer durations Enhanced β -glucosidase activity of lactobacilli leading to increased bioconversion of isoflavones	Ewe et al. (2012a)
Effects of ultrasound on the growth, isoflavones bioconversion and probiotic properties of <i>Limosilactobacillus fermentum</i> in biotin-supplemented soymilk	10 mL cell suspension sonicated at 30 kHz at amplitude of %60 (60 W) for 2 min with a 3 mm diameter sonotrode; energy density 720 J mL ⁻¹	<i>Limosilactobacillus fermentum</i> BT 8633 in biotin-soy milk	Increased growth of parent cells (3.23%–9.14%) by ultrasonication Enhanced intracellular and extracellular β -glucosidase activity Demoted probiotic properties of parent cells, but improving by subsequent passages	Ewe et al. (2012b)
Effect of mild sonication intensities on <i>Lb.</i>	500 mL of cultures were sonicated before	<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> LB-12 in skimmed milk	The best improvement at 14.68 W cm ⁻² was	Moncada, Aryana, and Boeneke (2012)

(continued)

Table 1. Continued.

Application	Ultrasound parameters	Types of LAB and growth medium	Main effects obtained	References
<i>delbrueckii</i> ssp. <i>bulgaricus</i> attributes at different temperatures	inoculation at 20 kHz and 8.07, 14.68, 19.83 and 23.55 W cm ⁻² at 4, 22 and 40 °C		obtained at 4 °C for the bile tolerance and growth, at 40 °C for the protease activity 23.55 W cm ⁻² had the best for the protease activity at 22 °C	
Carbohydrate metabolism of bifidobacteria in milk fermentation by ultrasonication	100 mL of inoculated milk was sonicated before fermentation at app. 100 W, 20 kHz for 7 min, 15 min and 30 min at 30–40 °C; energy density 420, 900 and 1800 J mL ⁻¹	<i>B. breve</i> ATCC 15700, <i>B. infantis</i> , <i>B. longum</i> (BB-46) and <i>B. animalis</i> ssp. <i>lactis</i> (BB-12) in reconstituted skim milk	Accelerated lactose hydrolysis and transgalactosylation reaction Stimulated major organic acids Decreased acetic acid:lactic acid	Nguyen et al. (2012)
Thermosonication effect on sweet whey fermentation with sonicated dairy cultures	Sonication of cultures (37 °C for La-5 and 43 °C for YC-380) 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; energy density 63–153 J mL ⁻¹	Commercial yoghurt culture YC-380 (<i>Str. thermophilus</i> , <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>) and <i>Lb. acidophilus</i> (La-5) in whey	Shorter time of fermentation for sonicated <i>Lb. acidophilus</i> culture Increased viable cell count at the end of fermentation (1–2 log cycles) with sonication of cultures at 84 W 150 s in thermosonicated (480 W, 55 °C for 8) whey	Barukcic et al. (2015)
Comparison of ultrasonic homogenization and conventional homogenization for yoghurt 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 density 180–900 J mL ⁻¹	<i>Str. salivarius</i> subsp. <i>thermophilus</i> and <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> in skimmed bovine milk	Lower pH reduction rate Lower duration of pH lag phase Formation of protein molecule aggregates	Sfakianakis et al. (2015)
Intensification of milk fermentation process of cows' milk by ultrasonication	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	Mix culture containing <i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i> and <i>Lc. lactis</i> subsp. <i>cremoris</i> (biovar <i>diacetylactis</i>) and yoghurt culture <i>Str. thermophilus</i> , <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> in reconstituted skim milk	Accelerated fermentation process by 10% Increased titratable acidity Reduced lactose content	Shershenkov and Suchkova (2015)
Effect of ultrasound on enhancement of β -glucosidase activity in soymilk	100 ml MRS medium was sonicated before inoculation at 20 kHz for 40, 50 and 60 W and different times; energy density 48–72 J mL ⁻¹	<i>L. acidophilus</i> BCRC 10695, <i>Lb. bulgaricus</i> BCRC 10696, <i>Lb. casei</i> BCRC 14080, <i>Str. thermophilus</i> BCRC13680, <i>Str. thermophilus</i> ATCC BAA-250 in MRS broth	<i>L. acidophilus</i> BCRC 10695 showed the best ability to release β -glucosidase At stationary phase of <i>L. acidophilus</i> growth, irradiation 40 W for 2 min provided 1.82 times higher β -glucosidase activity	Liu et al. (2018)
Effect of ultrasound on microflora and biologically active compounds	1 L reconstituted skim milk was sonicated before fermentation at 22 \pm 1.25 kHz, 60, 90 and 120 W for 1, 3, 5 min with a 25 mm probe; energy density 3.6–36 J mL ⁻¹	Kefir culture LAT LC K, yogurt culture LYOBAC YOYO, kefir fungi in reconstituted skim milk	Treatment of 120 W, 3 min for kefir grain and 90 W, 3 min for kefir and yogurt culture affected positively microbial composition and nutritional value	Potoroko et al. (2018)
Effect of ultrasound on probiotic bacteria growth and fermentation profile	1 L of skim milk sonicated at 40 kHz at amplitude of 30 % (116 W) for 5, 10, 15 and 20 min; energy density 34.8–139.2 J mL ⁻¹	<i>Lactobacillus acidophilus</i> (LA-5), <i>Lactocaseibacillus casei</i> (LC), <i>Limosilactobacillus reuteri</i> (LR-MM53), <i>Bifidobacterium bifidum</i> Bb-12, <i>Bifidobacterium longum</i> (Bb-536) in skim milk	Increased viable cells and total acidity, and decreased fermentation time for LA-5, LC and LR-MM53 with 10 min Increased extracellular release of β -galactosidase Higher enzymatic activity with increased exposure time	Niamah (2019)
Fermentation stimulation for the peptides by <i>Lactocaseibacillus paracasei</i>	Seed culture of <i>Lb. paracasei</i> was sonicated at 28 kHz 100 W/L for 1 h; energy density 360 J mL ⁻¹ . Fermented milk was sonicated at 28 kHz 100 W/L for 30 min; energy density 180 J mL ⁻¹	<i>Lb. paracasei</i> CICC 20241 in MRS medium <i>Lb. paracasei</i> CICC 20241 in fermented skim milk	The viable cells and the peptide content increased by 43.5% and 49.5%, resp., in the sonicated fermented skim milk medium with its seed culture without sonication	Huang et al. (2019)

Barukcic et al. (2015) reported that ultrasound treatment with nominal power of 84 W over 150 s for activation of starter cultures resulted in a significant increase in the viable count of yogurt culture bacteria and *Lb. acidophilus* LA-5 in thermosonicated (480 W, 55 °C for 8 min) fermented whey. In the study of Huang et al. (2019) *Lacticaseibacillus paracasei*-fermented skim milk medium was treated with ultrasonic pulsed model of on-time 100 s and off-time 10 s, 100 W/L for the treatment time of 30 min. The viable cells increased by 43.5% in the sonicated *Lacticaseibacillus paracasei*-fermented skim milk medium compared with those in the untreated samples.

The chemical composition of the culture media had also showed an important effect on probiotic survival. High intensity ultrasound did not affect the viability of probiotic bacteria in distilled water at 45 °C or pH 9, however, when the cultures were submitted to detrimental pH values (pH 4), a decrease in the probiotic viability was determined (Racioppo et al. 2017). The treatment of power ultrasound in culture media with LAB uses much lower energy densities than in dairy products, as the microorganisms do not have the milk components to protect them from acoustic cavitation (Gera and Doores 2011). The cell viability lactobacilli (*L. acidophilus* BT 1088, *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, *L. bulgaricus* FTDC 1311 and *L. casei* BT 1268) sonicated at 30 kHz and intensities of 20, 60 or 100 W levels for 1, 2 or 3 min at 25 °C were studied by Lye et al. (2012). Ultrasound treatment significantly increased the viability ($P < 0.05$) with increasing treatment intensities up to 60 W, while a decrease in cellular viability was observed ($P < 0.05$) upon treatment at 100 W for 3 min. The decrease in viability was attributed to the disruption of membrane lipid bilayer, cell lysis and membrane lipid peroxidation upon ultrasound treatment at higher intensity and duration. Nevertheless, the effect of ultrasound on membrane properties was reported as reversible by researchers, depending on the viability of ultrasound-treated lactobacilli was increased ($P < 0.05$) after fermentation at 37 °C for 20 h. Another important factor is the difference of inoculation rates for starter cultures that may cause different results during sonicated fermentation (Abesinghe et al. 2019).

Power ultrasound effects on cell membrane permeability

Ultrasound application can improve the microbial growth at low level sonoporation due to the interaction between microbubbles and cell membrane leading to improve the permeability of cell membrane. Related studies are given in Table 1. A high level of sonoporation can lead to a leakage of cellular content due to the physical disruption and modification of the membrane lipid bilayer, resulting in cell death. Therefore, ultrasound process parameters must be truly quantified and controlled to achieve the desired level of cell permeability (Abesinghe et al. 2019; Guimarães et al. 2019).

Lentacker et al. (2014) reported that the relatively small amplitude at lower ultrasound intensities exhibited higher

impact on the cell membrane, compared with non-adhered microbubbles. Besides direct microbubble contact, microstreaming around cavitating microbubbles provides a second possible origin of mechanical stress on the cellular membrane influencing permeability (Abesinghe et al. 2019). In addition, stable microbubble oscillations can induce the formation of free radicals and molecular products such as H_2O_2 (Gao et al. 2014a; Gao, Hemar, Ashokkumar, et al. 2014), which play a crucial role in lipid bilayer reposition and membrane disruption by lipid peroxidation. Furthermore, it was also reported that peroxidation of membrane lipids and location of proteins to the surface of the cell membrane increase membrane fluidity and membrane permeabilization upon ultrasound treatment (Ewe et al. 2012a; Lentacker et al. 2014).

Ultrasonication (30 kHz, 100 W) at different amplitudes (20%, 60% and 100%) for 60, 120 and 180 s was applied to strains of lactobacilli (*Lactobacillus acidophilus* BT 1088, *L. fermentum* BT 8219, *L. acidophilus* FTDC 8633, *L. gasseri* FTDC 8131) prior to inoculation and fermentation in biotin-soymilk (Ewe et al. 2012a). According to the results of that study, the treatment increased lipid peroxidation ($P < 0.05$) affecting the fatty acids chain of the cellular membrane lipid bilayer. This modification led to increased membrane fluidity and subsequently, membrane permeability of lactobacilli cells, with increased permeability in tandem with increasing treatment amplitudes. The permeabilized cellular membranes had facilitated nutrient utilization and subsequent growth enhancement of lactobacilli in biotin-supplemented soymilk promoting upon treatment at higher intensity and longer duration. ($P < 0.05$).

Tabatabaie and Mortazavi (2008) reported that ultrasound treatment at 20 kHz for 5, 10, 15, 20, 25 and 30 min have produced three types of microdamage, namely, microcracks, microvoids and ruptures in the cell membranes of LAB (*Lactobacillus acidophilus* (strain, LAI), *Lacticaseibacillus casei* (strain AB) *Lactococcus lactis* spp. *cremoris* and *Lactococcus lactis* spp. *lactis*) according to the Transmission Electron Microscope (TEM) pictures. The authors also indicated that ultrasound application provided an increase in the cell wall permeability of the cells.

Power ultrasound effects on enzyme activity and fermentation profile

Ultrasound treatment can increase the activity of certain enzymes depending on the treatment intensity and processing time (Table 1). Under suitable conditions, ultrasound changes the conformation of enzyme to accelerate the contact between enzyme and substrate generating cavitation, magnetostriptive and mechanical oscillation effects. Thus the bioactive molecules takes place and biological activity of enzymes is promoted (Galván-D'Alessandro and Carciochi 2018; Huang et al. 2017). Moreover, ultrasound can alter the characteristics of substrates and thereby reactions between enzyme and substrates. It can also contribute to provide an optimal environment for the reactions (Huang et al. 2017).

Several authors determined that ultrasound accelerated the activity of β -galactosidase, the major intracellular enzyme possessed by LAB to catalyze the hydrolysis of lactose (Abesinghe et al. 2019). The increase in enzyme activity may be due to the combined effects of ultrasound (i) improved membrane permeabilization of probiotic or LAB cultures causing the release of intracellular enzymes to the substrate medium (Ewe et al. 2012a; Wang and Sakakibara 1997), (ii) reduction of activation energy of the enzymes (Delgado-Povedano and de Castro 2015), (iii) increase in the affinity of enzymes to substrates by alteration of their characteristics (Dahroud et al. 2016; Huang et al. 2017).

In the study of Liu, Yang, and Fang (2018) the strategic ultrasonic treatment on *L. acidophilus* BCRC 10695 to induce stress response proved enhancement of β -galactosidase activity in soymilk. Similar findings were obtained by Ewe et al. (2012a, 2012b) that indicated an increase in extracellular and intracellular β -galactosidase activity of lactobacilli irradiated before inoculation to soymilk. Niamah (2019) reported that ultrasonic application to milk (at 40 kHz 116 W for 10 min) containing *Lactobacillus acidophilus* (LA-5), *Lactocaseibacillus casei* (LC), *Limosilactobacillus reuteri* (LR-MM53) caused an increase in the extracellular release of β -galactosidase activity in fermented milks. Moreover, total acidity increased and fermentation time decreased in the sonicated fermented milks compared with the control sample.

Ultrasound application decreased the fermentation time in bifidobacteria-fermented milk, due to the releasing intracellular β -galactosidase enzyme and promoting lactose hydrolysis and trans-galactosylation compared with non-sonicated samples (Nguyen, Lee, and Zhou 2009). Similarly lactose hydrolysis enhanced up to 2-4 times and also major organic acid levels increased with sonoporation in bifidobacteria-fermented milk (Nguyen, Lee, and Zhou 2012).

Depending on the results regarding the treatment of power ultrasound in the fermented milk processing; it could be stated that this treatment provided an increase in the activity of the probiotic and LAB cultures resulting in acceleration of lactose hydrolysis and reduction of fermentation time. This effect was associated to the alteration in the culture bacteria cells provided by power ultrasound, releasing β -galactosidase and accelerating carbohydrate metabolism during fermentation. Therefore, power ultrasound may shorten milk fermentation time depending on its promoting effect on the lactose hydrolysis and forming major organic acids during fermentation. Industrially, increase in lactose hydrolysis in fermented dairy products is also important for lactose intolerant individuals. In addition, availability of hydrolyzation products may enhance the growth of LAB. Undoubtedly, some other process parameters such as pH, temperature, food composition, bacterial strain type can affect the activity of enzyme and rate of lactose hydrolysis (Abesinghe et al. 2019; Galván-D'Alessandro and Carciochi 2018; Guimarães et al. 2019).

On the other hand, the potential of power ultrasound to generate bioactive peptides for the development of functional dairy products and to modify or improve the

functional properties of milk protein concentrates (MPCs) such as solubility and foaming has focused in recent years (Uluko et al. 2016). Milk is the primary source of multiple bioactive peptides that not only help to provide consumers' nutritional requirements but also play an important role in preventing several health disorders. Dairy peptides possess diverse biological activities for human health such as antihypertensive (ACE inhibitory), antioxidant, antimicrobial, immunomodulating and opioid activities (Punia et al., 2020).

Huang et al. (2019) reported that the peptide content of ultrasound treated *Lactocaseibacillus paracasei*-fermented skim milk media significantly increased compared with those in the untreated samples. The authors proposed the underlying mechanism of the peptide increment arising from the increase in extracellular enzyme activity due to ultrasound treatment.

ACE inhibitory and antioxidant activity of MPC hydrolyzates increased in the hydrolyzates that were exposed to high intensity ultrasound prior to enzymatic hydrolysis (Uluko et al. 2015; Uluko, Li, et al. 2013; Uluko, Liu, et al. 2014; Uluko, Zhang, et al. 2014). In this field, the effectiveness of power ultrasound on enzyme activity is highly dependent on protein source, enzyme type, equipment design and ultrasound treatment conditions (Ozuna et al. 2015). Application of high intensity ultrasound in pretreatment and during the hydrolysis process of proteins can modify protein conformation by affecting hydrogen bonds and hydrophobic interactions, while the effect of cavitation play a role in disrupting the quaternary and/or tertiary structure of proteins. Therefore, these structural modifications may expose more hydrolysis sites to the enzyme, providing an increase in degree of hydrolysis and bioactivity of proteins (Ozuna et al. 2015). Industrially, novel protein hydrolysates and bioactive peptides, which are generated as a result of enhancement of enzymatic hydrolysis of proteins by power ultrasound, could be used as ingredients in the production of functional foods (Ozuna et al. 2015).

Chandrapala et al. (2011) reported that power ultrasound can change the structural and/or functional properties of dairy proteins by altering their molecular characteristics. Similarly, ultrasound-induced structural changes of whey proteins were reported by Arzeni et al. (2012) and Silva, Zisu, and Chandrapala (2018). Power ultrasound provided a significant decrease in particle size and molecular weight of whey proteins solutions (Jambrak et al. 2014; Zisu et al. 2011), particle size of milk protein concentrate (Yan Jun et al. 2014), and sodium caseinate solutions (O'Sullivan et al. 2014), and denaturated casein-whey protein mixtures (Leong et al. 2018). Liu et al. (2014) obtained higher decrease in particle size of casein micellar solutions at alkaline conditions. Munir et al. (2019) reported that homogenization effects of moderate ultrasound treatment and protein unfolding can improve mass transfer and enzyme-substrate interactions leading to higher enzymatic activity. A significant increase in trypsin and alkaline protease activities of ultrasound pretreated milk protein concentrate was determined as a result of protein aggregate dissociation that

increased enzyme-protein interaction (Uluko, Zhang, et al. 2013). Ma, Wang, and Guo (2018) also reported improvements in the trypsin and pepsin activities in sonicated β -lactoglobulin solutions due to protein unfolding and the exposure of more cleavage sites.

In brief, power ultrasound can improve the fermentation profile and functional properties of fermented dairy products through increasing the viability, membrane permeability and enzyme activity of microbial (cultural) cells. Therefore, the technology of power ultrasound in dairy streams can be applied to intensify fermented milk product processing provided that optimum ultrasonication parameters are carefully determined before applying sonication.

Effects of ultrasound on microbial inactivation

Milk is one of the most complete food for human which provide a complex mixture of all macronutrients including proteins, carbohydrates, fat, and micronutrients such as minerals, vitamins, besides being a worldwide product with high commercial demand (Gao, Hemar, Lewis, et al. 2014; Herceg et al. 2012). But its composition makes it a highly perishable food and its the natural flora contains human pathogens (Vijayakumar et al. 2015). Traditional heat treatments such as pasteurization and ultra-high temperature treatment (UHT) are the most common and dominate methods in dairy technology to reduce the microbial spoilage (Gao et al. 2014a; Manas and Pagan 2005). On the other hand, protein denaturation, vitamin destruction, enzyme inactivation, Maillard reaction and production of lysinoalanine as a result of the high temperature used in traditional heat treatments cause deterioration of functional and sensory properties of dairy products and decrease in nutritional value. As a result of reactions catalyzed by temperature, product quality decreases due to structure and texture deformations, modification of macromolecules and creation of new compounds (Bermudez-Aguirre, Mawson, et al. 2009; Paniwnyk 2017). The effectiveness of heat treatment is dependent on the treatment temperature and time. Unfortunately, the amount of nutrient loss and development of undesirable flavors are increasing in proportional to the magnitude of treatment, time and process temperature (Awad et al. 2012; Marchesini et al. 2012). Thus, the increasing consumer demand for fresh-like foods and the undesirable side effects of high heat treatment have led producers to focus on alternative food preservation methods (Manas and Pagan 2005). Ultrasound is one of these emerging non-thermal technologies that promising in the dairy industry (Arvanitoyannis, Kotsanopoulos, and Savva 2017; Huang et al. 2017; Piyasena, Mohareb, and McKellar 2003). It is possible to inactivate microorganism with minimal changes in the natural properties of milk by ultrasound (Chouliara et al. 2010; Shamila-Syuhada et al. 2016). It is also more cost-efficient than conventional heat treatment and environmentally friendly, thus considered as green technology (Akdeniz and Akalin 2019; Awad et al. 2012).

Microbial inactivation mainly results from acoustic cavitation generated by power ultrasound due to strong shear

and mechanical forces (Gao et al. 2014b; Huang et al. 2017). Gao, Hemar, Lewis, et al. (2014) have investigated the effect of low frequency (20 kHz) and high frequency (850 kHz) ultrasound on microbial inactivation in skim milk and found that low frequency acoustic cavitation caused lethal damage to *Enterobacter aerogenes*, while high frequency ultrasound was not able to inactivate *E. aerogenes* in milk. It is considered that this is mainly due to the radical scavenging properties of milk. Similarly, Joyce et al. (2003) have found that low power ultrasound with high frequency has no significant reduction in bacterial cell numbers, but power ultrasound with low frequency showed a significant increase in killing for *Bacillus* species. In accordance with these studies, contrary to low power ultrasound, power ultrasound is effective in microbial inactivation.

Mechanism of microbial inactivation

Initially, ultrasound was accepted as a microbial inactivation method in the 1960s after discovering that sound waves used in submarine warfare killed fish (Herceg et al. 2013; Piyasena, Mohareb, and McKellar 2003). Many studies have been done to understand the mechanism of ultrasound that plays a role in the disruption of microorganisms (Bermudez-Aguirre, Corradini, et al. 2009; Gao et al. 2014a, 2014b).

These studies have shown that ultrasound is a food preservation technology in which the enzyme and microorganisms are inactivated due to the mechanical effects of cavitation (Sulaiman, Farid, and Silva 2017). It is considered that the forces generated on collapse of the cavitation bubbles and extremely high temperatures and pressures due to these collapses are the main reason of the reduction in the number of live microorganism cells (Gao et al. 2014b; Villamiel and de Jong 2000). Microbial inactivation in liquid products such as milk by ultrasound is occurred due to the thinning of cell membranes, breaking and shearing of cell walls, formation of hotspots, production of free radicals and DNA damage (Chandrapala et al. 2016; Chemat, Huma, and Khan 2011). Ultrasound gives lethal damage to the microorganisms because of some mechanism of cavitation microbubble. These mechanisms are pulling, pushing, jetting, microstreaming, shock waves and translation (Figure 1). Cavitation microbubble pulls the cell membrane during compression phase and pushes it during expansion phase leading to the disruption. Asymmetric collapse of a microbubble near a membrane surface generates a microjet toward the cell membrane resulting in pore formation on the membrane. The oscillation of microbubbles in the fluid forms microstreamings creating a pore on the cell membrane due to the mechanical stress. Shock waves created by the collapse of microbubbles cause membrane disruption because of high stress on the cell membrane. High intensity ultrasonic radiation forces diffuse across the boundary, compressing the microbubbles into the cell membrane, thereby disrupting (Bevilacqua, Campaniello, et al. 2019; Lentacker et al. 2014; Ojha et al. 2017). These mechanisms also weaken the cell wall and membrane by changing the membrane permeability and selectivity (Kwiatkowska et al. 2011). These effects of

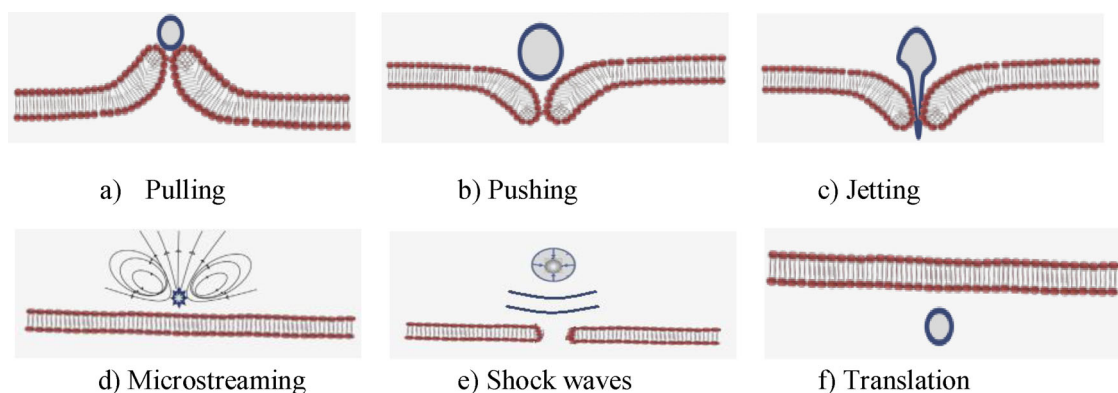


Figure 1. Microbial inactivation mechanisms of cavitation microbubble (Lentacker et al. 2014; Ojha et al. 2017).

acoustic cavitation cause cell death by damaging the structural and functional components of the bacterial cell (Chandrapala et al. 2016; Chemat, Huma, and Khan 2011).

In addition to these mechanical effects, the conditions caused by the collapse of microbubbles produce free radicals by decomposing water molecules. The free radicals containing hydroxyl radicals attack the chemical structure of the cell wall leading to DNA damage, liposomes and bacterial membranes damage, destruction of enzymatic activity (Gao, Hemar, Lewis, et al. 2014). Besides free radicals, the mechanical forces created by micro-streaming and shock waves and/or the enormous temperatures achieved during cavitation also cause enzyme inactivation (Nikitenko and Chemat 2015).

Power ultrasound effects on microbial inactivation

Microorganisms do not react in the same way to ultrasound treatment. In the destruction of microorganisms; sonication time, wave amplitude and ultrasound power are important factors (Gao, Hemar, Lewis, et al. 2014; Noci 2017). There are many studies about the effects of power ultrasound on microbial inactivation in milk and dairy products. As seen in Table 2, the results of these studies indicate that power ultrasound is effective to inactivate pathogens in milk and dairy products. Crudo et al. (2014) has detected 95% micro-organism reduction in fresh milk within 10 min by 370 W ultrasound. *Escherichia coli*, *Pseudomonas fluorescens*, *Listeria monocytogenes* and *Saccharomyces cerevisiae* were reduced in milk by power ultrasound at 20 kHz without any reduction in milk composition such as protein, casein and lactose amounts (Cameron, McMaster, and Britz 2008, 2009). Lethal damage of *E. aerogenes* in skim milk was observed by power ultrasound (Gao, Hemar, Lewis, et al. 2014). The studies show that prolonged sonication times increase the effectiveness in reduction of bacterial load (Beatty and Walsh 2016; Cameron, McMaster, and Britz 2008, 2009; Gao, Hemar, Lewis, et al. 2014). Some studies show that ultrasound could be considered as a processing alternative to pasteurization. Pasteurization, traditional heat treatment in the dairy industry, causes protein denaturation, vitamin destruction, enzyme inactivation, Maillard reaction and lysinoalanine production. On the other hand, ultrasound provides at least the same microbial reduction with

pasteurization and does not cause negative effects of pasteurization on the nutritional value, functional and sensory properties of dairy products (Barukcic et al. 2015; Crudo et al., 2014).

Ultrasound can be used alone as well as with the support of additional factors such as temperature (thermosonation) and pressure (manosonation) or a combination of both (manothermosonation) to increase the effectiveness of inactivation and improve chemical and sensory properties of milk and dairy products (Chandrapala and Zisu 2016; Vercet et al. 2002; Vijayakumar et al. 2015). Bermudez-Aguirre, Mawson, et al. (2009) has found that thermosonicated milk has better color and similar physicochemical characteristics with shorter processing time compared to conventional pasteurized milk besides positive effects of power ultrasound on bacterial inactivation. Other studies have shown that thermosonation provided complete destruction in all coliforms and at least 99% reduction in total bacterial count in skim milk and cream (Vijayakumar et al. 2015), inactivation of *Listeria innocua*, mesophilic bacteria and total aerobic bacteria in raw milk (Bermudez-Aguirre, Corradini, et al. 2009; D'Amico et al. 2006), inactivation of *Listeria monocytogenes* in UHT milk (D'Amico et al. 2006), better microbiological quality and sensory properties in sweet whey (Barukcic et al. 2015) and prevention the growth of yeast and mold in ayran (Erkaya et al. 2015).

Noci et al. (2009) found that the application of ultrasound in combination with temperature and pulsed electric field have provided inactivation of the *Listeria innocua* in milk to a degree comparable with conventional pasteurization by reducing the severity of time/temperature exposure.

In addition, liquid medium and food composition affect the effectiveness of ultrasound in microbial inactivation. Higher solids concentration contributes to more bactericidal effect due to the transfer of higher amounts of energy or acoustic power into the medium (Beatty and Walsh, 2016; Evelyn and Silva 2015). Ultrasound propagation rate in solid medium is higher than that in liquid medium depending on reduced compressibility in the solid medium (Awad et al. 2012). Evelyn and Silva (2015) have found that ultrasonic effects were higher in the high solid content foods such as beef slurry (24% wt/wt) compared with milk (9% wt/wt). On the other hand, Gao, Hemar, Lewis, et al. (2014) have found that bacteria (*E. aerogenes*) are more sensitive to ultrasound

Table 2. Effects of power ultrasound on microbial inactivation in dairy technology.

Application	Ultrasound parameters	Main effects obtained	References
Effect of ultrasound on inactivation of <i>Listeria monocytogenes</i> in UHT milk and total aerobic bacteria in raw milk	45 mL sample with a rate of 50 mL/min continuous flow was sonicated at 24 kHz, 150 W within 18 min at 57 °C; energy density 3.60 kJ mL ⁻¹	5 log reduction of <i>L. monocytogenes</i> in UHT milk and 5 log reduction in total aerobic bacteria in raw milk were obtained	D'Amico et al. (2006)
Effect of ultrasound on inactivation of <i>Escherichia coli</i> and <i>Saccharomyces cerevisiae</i> in milk	40 mL milk was sonicated at 20 kHz, 750 W up to 10 min; energy density 2.81–11.25 kJ mL ⁻¹	<i>Escherichia coli</i> and <i>Saccharomyces cerevisiae</i> were reduced by >99% after ultrasonication	Cameron, McMaster, and Britz (2008)
Effect of ultrasound on inactivation of <i>Listeria innocua</i> and mesophilic bacteria in raw whole milk	500 mL milk sample was sonicated at 24 kHz, up to 400 W for 10, 20 and 30 min at 63 °C; energy density 0.14–1.44 kJ mL ⁻¹	5 log reduction was obtained after 10 min when ultrasound is used in combination with heating process	Bermudez-Aguirre, Corradini, et al. (2009)
Effect of ultrasound on inactivation of <i>Pseudomonas fluorescens</i> , <i>Escherichia coli</i> , <i>Listeria monocytogenes</i> in milk	40 mL milk was sonicated at 20 kHz, 750 W for 2.5 to 10 min at 24–26 °C; energy density 2.81–11.25 kJ mL ⁻¹	Viable cell counts of <i>E. coli</i> and <i>P. fluorescens</i> were destroyed entirely and viable cell counts of <i>L. monocytogenes</i> were reduced (99%) within 10 min	Cameron, McMaster, and Britz (2009)
Effect of ultrasound alone or in combination with temperature and/or pulsed electric field on inactivation of <i>Listeria innocua</i> in low fat UHT milk	21 mL sample was sonicated at 24 kHz, up to 400 W and up to 160 s, energy density up to 3.05 kJ mL ⁻¹	Results showed that termosonication (400 W, 160 s) alone reduced <i>L. innocua</i> by 1.2 log cfu mL ⁻¹ , while combined with temperature (preheating to 55 °C over 60 s) and pulsed electric field (30 and 40 kV cm ⁻¹) reduced by 4.5 to 6.9 log cfu mL ⁻¹	Noci et al. (2009)
Effect of ultrasound on raw, thermized and pasteurized milk	200 mL sample was sonicated at 24 kHz, 200 W and up to 16 min, energy density up to 0.96 kJ mL ⁻¹	Results showed a 1–2.1 log cfu mL ⁻¹ reduction in total viable counts and psychrotrophs for raw, thermized and pasteurized milk up to 6 days of storage	Chouliara et al. (2010)
Effect of ultrasound on inactivation of <i>Staphylococcus aureus</i> and <i>E. coli</i> in milk containing 4% milk fat	200 mL milk sample was sonicated at 20 kHz, up to 600 W for 6, 9 and 12 min at 20, 40 and 60 °C; energy density 1.08–2.16 kJ mL ⁻¹	It was found that ultrasound improved the inactivation of both <i>S. aureus</i> and <i>E. coli</i> in all studied parameters. The results indicated that gram (–) bacteria (<i>E. coli</i>) were more susceptible to ultrasound than gram (+) bacteria (<i>S. aureus</i>)	Herceg et al. (2012)
Effect of ultrasound on microbial inactivation and sensory properties of rennet cheese whey in comparison with conventional pasteurization	100 mL sample was sonicated at 24 kHz, 240–400 W for 5, 6.5, 8 min at 35, 45 and 55 °C, energy density 0.72–1.92 kJ mL ⁻¹	Thermosonication at 400 W, 55 °C for 8 min resulted in a greater reduction of the total viable cells count (2.46 log cycles) in comparison with conventional pasteurization	Jelcic et al. (2012)
Effect of ultrasound on pasteurization of fresh cow milk	70 mL milk sample was sonicated at 35 kHz, up to 370 W for up to 10 min; energy density up to 3.17 kJ mL ⁻¹	95% microorganism reduction was ensured within 10 min at 370 W	Crudo et al. (2014)
Effect of ultrasound on inactivation of <i>E. aerogenes</i> in skim milk	15 mL skim milk was sonicated at 20 kHz, up to 9 W and up to 60 min; energy density up to 2.16 kJ mL ⁻¹	Lethal damage of <i>E. aerogenes</i> was obtained	Gao, Hemar, Lewis, et al. (2014)
Effect of ultrasound on microbial reduction of sweet whey	100 mL whey was sonicated at 20 kHz, 480 W and 600 W for 6.5, 8 and 10 min at 45 °C and 55 °C; energy density 1.87–3.60 kJ mL ⁻¹	Power ultrasound combined at 480 W and 55 °C resulted in best synergistic effect regarding lethal effects required for bacterial inactivation	Barukcic et al. (2015)
Effect of ultrasound on inactivation of yeast and mold in ayran	300 mL ayran was sonicated at 35 kHz, for 1, 3 and 5 min at 60, 70 and 80 °C	Ultrasound combined with 60 °C temperature for 1 min was enough to protect the lactic acid bacteria and to prevent the growth of yeast and mold in ayran production	Erkaya et al. (2015)
Effect of thermosonication on the psychrotrophic <i>Bacillus cereus</i> spores inactivation in skim milk.	100 mL sample was sonicated at 24 kHz, 200 W and up to 20 min at 50, 60, 70 °C, energy density up to 2.40 kJ mL ⁻¹	Results showed that thermosonication is much more effective in reducing psychrotrophic <i>B. cereus</i> spores in skim milk than thermal treatment alone at the same temperature	Evelyn and Silva (2015)
Effect of ultrasound combined with heat on skim milk and cream	100 mL sample was sonicated at 20 kHz, 104 W and 115 W for 1 and 3 min at 72 °C; energy density 0.06–0.21 kJ mL ⁻¹	Complete destruction in all coliforms and a > 99% reduction in total bacterial count in both skim milk and cream were obtained	Vijayakumar et al. (2015)

(continued)

Table 2. Continued.

Application	Ultrasound parameters	Main effects obtained	References
Effect of ultrasound on inactivation of <i>Geobacillus stearothermophilus</i> vegetative cells and spores in skim milk powder which was reconstituted to between 8 and 55% total solids	6 mL sample was sonicated at 20 kHz, for 5–30 s between 45 and 75 °C	Thermosonication proved to be more effective than heat treatment alone in reducing the microbial population of <i>G. stearothermophilus</i> . Optimization of cell reduction (4.8 log) was found to be at 19.75% total solids, 45 °C and 30 s, while optimization of spore reduction (0.45 log) was found to be at 31.5% total solid, 67.5 °C and 17.5 s	Beatty and Walsh (2016)
Effect of ultrasound on microbiological quality of semi-skimmed sheep milk	40 mL sample was sonicated at 20 kHz, 78 W for 6 and 8 min and 104 W for 4 and 6 min, energy density 0.62–0.94 kJ mL ⁻¹	Inactivation of total aerobic mesophilic bacteria, total coliform bacteria and <i>Staphylococcus</i> spp. in semi-skimmed sheep milk were provided, while acceptable amount of lactic acid bacteria (<i>Streptococci</i> and <i>Lactobacilli</i>) were maintained	Balthazar et al. (2019)
Effect of ultrasound on inactivation of pathogenic bacteria <i>Escherichia coli</i> O157: H7 and <i>Salmonella typhimurium</i> in camel milk	100 mL sample was sonicated at 20 kHz, 900 W for 15 min; energy density 8.10 kJ mL ⁻¹	Ultrasound processing of camel milk resulted in a 2 log cfu mL ⁻¹ reduction in total aerobic bacteria compared to the control and the total elimination of <i>E. coli</i> O157: H7 and a 4.4 log reduction in <i>S. typhimurium</i>	Dhahir et al. (2020)

in water than in milk. This result is attributed to the protective effect of fat in milk. It is considered that Evelyn and Silva (2015) have reported lower reductions in the cheese slurry than in beef slurry and rice porridge for similar reasons. The effectiveness of ultrasound also varies depend on the type of bacteria (gram (+) vs. gram (-)) (Herceg et al. 2012) and the state of microorganism (vegetative vs. spore) (Beatty and Walsh 2016). The studies have shown that gram (+) bacteria were more resistant to ultrasound treatment than gram (-) bacteria (D'Amico et al. 2006; Herceg et al., 2012, 2013; Villamiel and de Jong 2000). It is thought that this may be due to the fact that gram (+) cells have thicker cell walls, as well as the peptidoglycan layer that adheres more tightly to gram (+) cells (Chemat, Huma, and Khan 2011; D'Amico et al. 2006). Besides the type of bacteria, also their shapes are among the factors affecting the microbial inactivation of ultrasound. Cocci are more resistant than bacilli due to the relationship of cell surface and volume (Chemat, Huma, and Khan 2011). Since spores are the most resistant forms of microbial cells, their inactivation is one of the most striking problems in microbial inactivation (Impe et al. 2018). The studies showing the effect of ultrasound on spore inactivation are limited. Beatty and Walsh (2016) have informed that the log reductions observed for spores treated with power ultrasound were less compared with vegetative cells of *Geobacillus stearothermophilus* in reconstituted skim milk powder. Evelyn and Silva (2015) have found that thermosonication at 70 °C was more effective than sole thermal treatment in reducing psychrotrophic *Bacillus cereus* spores inactivation in skim milk and cheese slurry.

In brief, power ultrasound can inactivate microorganisms in milk and dairy products depending on many factors mentioned above. Thus, power ultrasound can be applied in dairy industry to provide bacterial destruction and to improve chemical and sensory properties of milk and dairy products.

Conclusion

The effect of power ultrasound on dairy microorganisms can result in two ways, both activation and inactivation, depending on factors such as ultrasonic conditions (temperature, amplitude, intensity, pressure and duration of sonication), microorganism type and properties (growth phase, size and shape, wall composition and thickness), medium composition. Studies have revealed that power ultrasound especially mid-power short-time ultrasound at sublethal levels can improve the fermentation profile and proliferation of LAB and probiotics by increasing the permeability of cell membranes and accelerating the supply of oxygen and nutrients for microorganisms and minimizing some of the negative effects of the ultrasound on living cells. Moreover, ultrasound application has been reported to shorten the fermentation time, to accelerate the enzyme activity of cultural cells especially intracellular β -galactosidase enzyme, and to promote lactose hydrolysis and trans-galactosylation. On the contrary, intense sonication causes irreparable cellular injuries such as breaking and shearing of the cell. The studies have shown that power ultrasound has advantages over conventional heat treatment and can be used as a processing technique for microbial inactivation due to lower processing temperatures, less energy requirement, minimization of valuable nutrients and flavor loss in dairy industry providing a better quality compared to traditional heat treatment.

In this context, as power ultrasound is promising in dairy industry, there is a strong need to focus on studies revealing sonication effects on dairy microorganisms for the sustainability of the industry.

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