Ultrasound Processing of Milk and Dairy Products

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Contents

Abstract

The application of ultrasound to conventional dairy processes has the potential to provide significant benefits to dairy industry such as possible cost savings and improved product properties. Moreover, the appeal of ultrasound as a processing technique has been regarded safe compared to other emerging technologies. During the past decade, the technology has rapidly emerged as a mild nonthermal

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processing tool capable of replacing or assisting many conventional dairy processing applications such as inactivation of microbes and enzymes, homogenization and emulsification, creaming, crystallization, and functionality modifications within dairy systems. These aspects are highlighted in this chapter.

Keywords
Milk and dairy products • Dairy powders • Emulsification • Gel formation • Microbial inactivation • Sonocrystallisation • Ultrasound assisted filtration • Viscosity modifications

Introduction

Low-frequency, high-power ultrasound induces strong cavitation effects that influence the physical, mechanical, or chemical/biochemical properties of food systems [1]. In contrast, high-frequency low-power ultrasound generates physical effects that are comparatively gentle and can be utilized for noninvasive analysis, monitoring food materials, and nondestructive separations of multicomponent mixtures [2]. The application of high-power ultrasound in dairy processing may lead to the generation of physical forces that include cavitation, acoustic streaming, acoustic radiation, shear, micro-jetting, and shockwaves and chemical reactions that include the generation of very small amount of highly reactive radicals [3]. Both the physical forces and chemical effects are utilized in some applications while most dairy applications concentrated in solely utilizing the physical forces.

Microbial Inactivation

The dairy industry most commonly uses heat treatment to inactivate microorganisms as a means of preservation and ensuring food safety. However, thermal treatment causes significant changes to the composition and surface properties of the colloidal particles present and alters the physical properties of milk. Some changes such as those used to improve the texture of products like yogurt are desirable, while gel formation during the manufacture of ultrahigh temperature milk is highly undesirable [4]. Hence, novel preservation technologies that maintain the quality of milk have attracted some interest within the dairy industry. This trend toward the development of ultrasound as an alternative nonthermal technique is further driven by other advantages including reduced energy consumption, ability to target specific organisms, and no requirement for the introduction of preservatives [5].

Intense power and long contact times are required to inactivate microorganisms at ambient conditions in real food systems when ultrasound is applied alone [6, 7]. Hence, recent improvements recognize the combination of more than one established technique in combination with ultrasound such as heat (thermosonication, TS), pressure (manosonication, MS), or heat and pressure (manothermosonication, MTS) and provide enhanced benefits for microbial inactivation. The effectiveness of microbial inactivation by these methods is dependent on the amplitude of the ultrasonic waves, exposure/contact time, volume of food being processed, the composition of the food including the types and initial number of the microbes present, and their aggregate state, viscosity of the medium, and the treatment conditions.

D'Amico et al. [8] showed that ultrasound treatment combined with mild heat (57 °C) for 18 min resulted in a 5-log reduction of *L. monocytogenes* in milk and a 5-log reduction in the total aerobic bacteria in raw milk. Juraga et al. [9] investigated the inactivation of *Enterobacteriaceae* in raw milk with the use of high-intensity ultrasound. Temperature (20 °C, 40 °C, and 60 °C), amplitude (120, 90, and 60 μm), and time (6, 9, and 12 min) were varied in the study. The results indicated significant inactivation of microorganisms under longer period of treatment with ultrasonic probe particularly in combination with higher temperature and amplitude. Use of ultrasound at 124 μm amplitude was effective without heat against spoilage microorganisms and potential pathogens including *E. coli*, *Pseudomonas fluorescens*, and *L. monocytogenes* even when inoculum loads of five times higher than permitted were present in raw and pasteurized milk [6]. In this study 100 % *E. coli* within 10 min, 100 % *Pseudomonas fluorescens* within 6 min, and 99 % *L. monocytogenes* within 10 min were reduced (Fig. 1). *Listeria monocytogenes* and *E. coli* were also thermosonicated in milk by others [10–12]. Gera and Doores [12] not only showed that pulsed sonication at 24 kHz caused mechanical damage to the bacterial cell wall and cell membrane when treated at temperatures between 30 °C and 35 °C but milk had a microbial protective effect with lactose exerting the most positive effect on bacterial survival. In a separate study, the synergistic effect of heat (63 °C) combined with sonication (24 kHz) was used to inactivate *Listeria innocua* and reduce the *mesophilic* bacteria count in raw whole milk by 0.69 log after 10 min and 5.3 log after 30 min [13] resulting in an extended shelf life [14]. Treating UHT milk with the same sonication parameters (63 °C and 24 kHz) prevented *mesophilic* growth higher than 2 log during ambient and refrigerated storage for 16 days [15]. Many documented outbreaks associated with infant formula are linked to *Cronobacter sakazakii*. Thermosonication at 20 kHz and temperatures up to 50 °C were used to inactivate *Cronobacter sakazakii* and reduce the microbial count in reconstituted infant formula by up to 7.04 $\log_{10}$ reduction after 2.5 min of treatment [16]. Thermosonication was also used against *Bacillus subtilis* [17], *Staphylococcus aureus* [18], *Salmonella typhimurium* [19], coliforms, and total plate counts [20] in milk.

Noci et al. [21] investigated the impact of thermosonication (TS) and pulsed electric field (PEF), individually and combined, on the survival of *Listeria innocua* 11288 (NCTC) in milk. TS (400 W, 160 s) without preheating declined *L. innocua* by 1.2 log10 cfu mL^{-1}, while shorter treatment times produced negligible inactivation. This highlighted the fact that TS was a hurdle rather than an effective standalone treatment [21]. Gabriel et al. [22] established the inactivation behavior

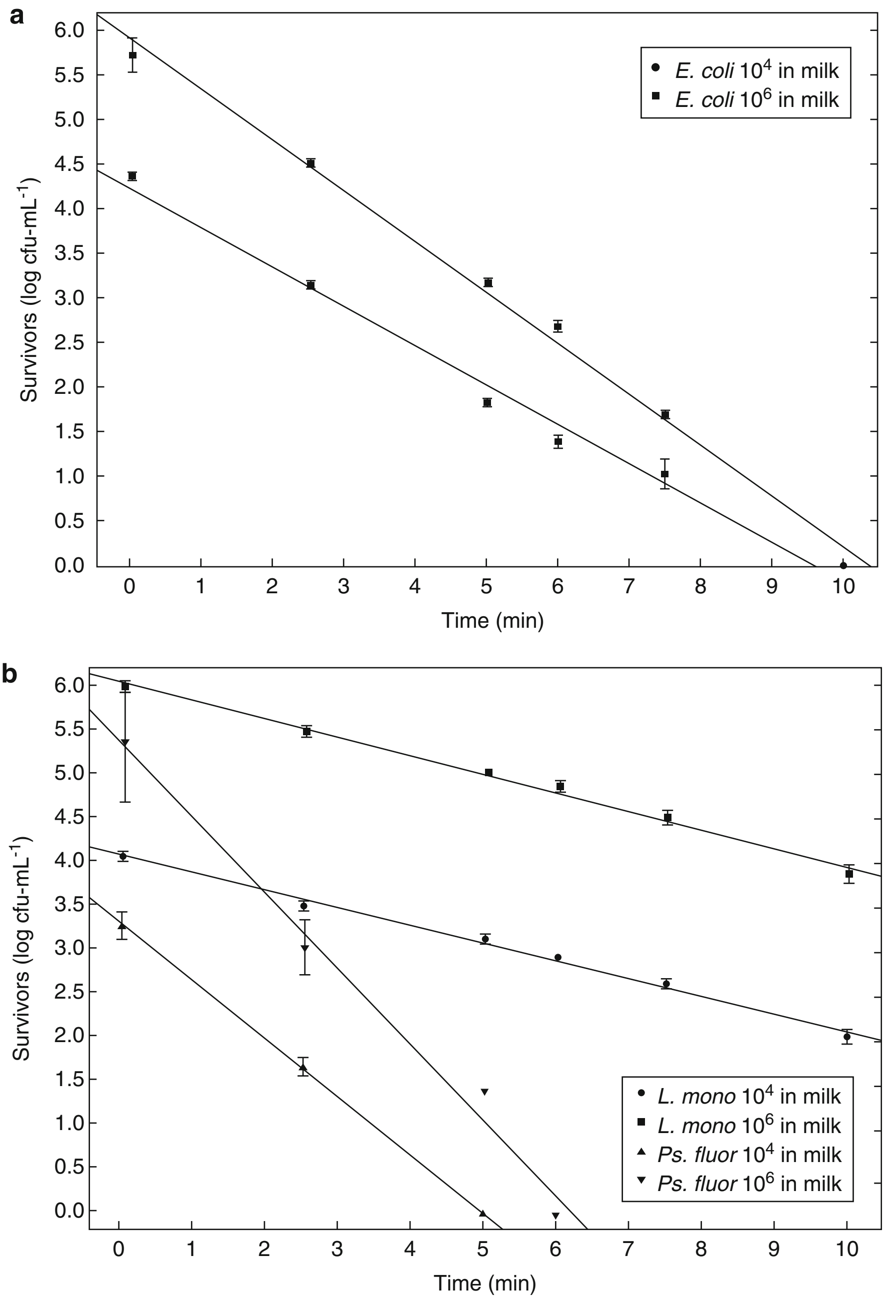


Fig. 1 The impact of ultrasonication at 20 kHz on (**a**) *Escherichia coli*, (**b**) *Listeria monocytogenes*, and *Pseudomonas fluorescens* at different starting concentrations in UHT milk [6]

of *Listeria monocytogenes* in nonfat, low-fat, and full-cream milk treated with multifrequency *Dynashock* ultrasound. Inactivation in all samples was biphasic, with an inactivation lag phase where injury accumulated, prior to log-linear inactivation phase. *L. monocytogenes* exhibited shortest lag phase of 20.57 min in full-cream milk. *L. monocytogenes* exhibited slowest log-linear inactivation rate of −0.24 log colony-forming unit cfu/min in full-cream milk and fastest inactivation rate of −0.37 log cfu/min in low-fat milk. Inactivation rate was slowest in full-cream milk at −0.24 log cfu/min and fastest in low-fat milk at −0.37 log cfu/min. Corrected decimal reduction time was shortest in full-cream milk at 24.81 min, followed by those in nonfat and low-fat milk at 29.17 and 30.64 min, respectively.

The main mechanism responsible for the ultrasonic microbial deactivation is the physical forces generated by acoustic cavitation. The asymmetric collapse of a cavitation bubble leads to a liquid jet rushing through the center of the collapsing bubble. Microorganisms that have hydrophobic surfaces will promote the collapse of cavitation bubbles on the surface and lead to severe damage of the cell wall (Fig. 2). Similarly, microstreaming effects can lead to the erosion of cell walls, again resulting in inactivation of the microorganisms. The effects of localized heating, free radical production causing DNA damage which in turn causes thinning of cell membranes are also crucial in the inactivation [23, 24]. Furthermore, the presence of a thick bacterial capsule (biopolymer layer) prevents cavitation bubbles from collapsing near the plasma membrane thus preventing the breakup of the bacterial cell [25]. On the other hand, the presence of a highly hydrated capsule may help absorbing the mechanical forces exerted on the bacterial cell. Gao et al. [25] proposed to consider the thickness and softness of the bacteria capsules as one of the most important parameters when using high-power ultrasound for the deactivation microbes.

Some microorganisms, in particular bacterial spores, are more resistant under certain conditions than others, and achieving inactivation can be relatively difficult. *Bacillus* and *Clostridium* spores were found to be more resistant to heat and similarly resistant to ultrasound [26]. Raso et al. [27] investigated the inactivation of *Bacillus subtilis* spores by ultrasonic treatments under pressure and combined pressure and heat treatment conditions. They showed that manosonication (MS) treatment at 500 kPa and 117 μm of amplitude for 12 min inactivated ~ 99 % of the *B. subtilis* spore population. MS treatment (20 kHz, 300 kPa, 70 °C, 12 min) at 90 μm amplitude inactivated 75 % of the *B. subtilis* spore population; the same treatment at 150 μm amplitude inactivated 99.9 % of this population. The MS treatments at temperatures higher than 70 °C, which is manothermosonication (MTS), also led to more spore inactivation. In the range 70–90 °C, the combination of heat with a MS treatment (20 kHz, 300 kPa, 117 mm, 6 min) had a synergistic effect on spore inactivation. Table 1 highlights some other literature that has investigated the influence of different parameters on microbial inactivation of different dairy systems with the use of ultrasound.

The demand for food safety projected to rise annually in the world. The major trends associated with consumers in future are toward the use of dairy products that

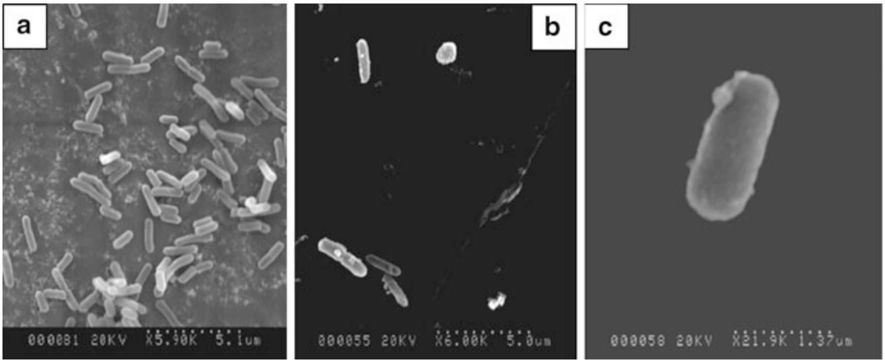


Fig. 2 SEM images of *L. Innocua* cells inoculated in raw whole milk (**a**) without treatment; (**b**) after 10 min of thermosonication treatment (63 °C and 120 μm) (20 kV, magnification 5.9 K); (**c**) closer view of a cell after treatment (20 kV, magnification 21.9 K) [28]

are less "heavily" preserved, high quality, more convenient, more "natural", free from additives, nutritionally healthier, and still with high assurance of microbiological safety. Ultrasound is a nondestructive-targeted technique without introducing preservatives. However, current research to enhance microbial inactivation in dairy systems by ultrasound is focused mostly in combination of another technique or combining ultrasound with other preservation factors [29] and moreover conducts in small-scale operations. Hence, the industry transition is yet to accelerate within the companies.

Enzyme Inactivation

Thermo-resistant enzymes in milk such as lipases and proteases that withstand UHT treatment can reduce the quality and shelf life of heat-treated milk and other dairy products. The potential of ultrasound to inactivate food enzymes has mostly been studied in model systems. General trends observed from all these studies suggested that thermo-labile enzymes were more sensitive to ultrasonication than heat-resistant enzymes. Such enzymes have been efficiently inactivated at tenfold the rate of thermal treatment alone, by MTS (20 kHz, 145 μm amplitude, and 650 kPa for protease, 117 μm amplitude and 450 kPa for lipase, 109–140 °C) treatment [32]. Molecular size and structure are thought to play a role in the sensitivity of enzymes to MTS, with large and less globular enzymes displaying more sensitivity [33].

The effectiveness of ultrasound for control of enzymatic activity is strongly influenced by many factors such as enzyme concentration, temperature, pH, and composition of the medium including treatment volume and gas concentration and processing variables such as sonotrode type and geometry, frequency, and acoustic energy density [34]. Enzyme inactivation generally increases with increasing US

Table 1 Some literature data on microbial inactivation in milk systems with regard to different parameters and combination of techniques

Target parameters	Food matrix	Target microbes	Conditions	Comments	Reference
Presence of butter fat	Fat-free milk Whole milk	*L. innocua*	400 W, 24 kHz, 63 °C/30 min 200 μm amplitude	Inactivation occurs in fat-free milk Inactivation increased with increase in fat content Mechanism: adhering of microbes to the newly formed fat globules and protected either by rough surface of the fat globules or by concealments within the globules providing a protective fat layer	[13]
Thermosonication (TS)	Raw whole milk	*L. innocua* Mesophilic bacteria	400 W 24 kHz	Pasteurization reduced 0.69 log and 5.5 log after 10 and 30 min Combined with US 5 log reduction was achieved after 10 min	[14]
Thermosonication (TS)	Milk		400 W, 24 kHz, 63C 30 min Energy delivered: 129 mW/ml	Increased shelf life Viable option for pasteurization	[30]
Different media Presence of lactose, casein, and β-LG	Whole milk Skim milk SMUF Phosphate buffer	*E. coli* *L. monocytogenes*	85 W/cm^2 24 kHz, 100 μm amplitude, pulsing 80 %	D values of 2.43, 2.4, 2.19 and 9.3, 8.61, 7.63 min for *E. coli* and *L. monocytogenes* was obtained, respectively, with whole, skim, and phosphate buffer Presence of lactose showed significantly higher D values Mechanism: stabilization of the bacterial membrane and proteins by lactose or accumulation of compatible solutes in the presence of lactose	[12]
Temperature Amplitude Time	Milk	*S. aureus* *E. coli*	20 kHz	Optimum conditions: T = 59.99 °C, time = 12 min, amplitude = 117.27 μm to *S. aureus*, and 110.41 μm to *E. coli* Longer periods of time and with higher amplitude and temperature increased the inactivation of microbes	[31]

power, frequency, exposure time, amplitude, temperature, and pressure but decreases as volume of sample increases [35]. It has been reported that enzyme inactivation increases with an increase in solid content and decreases with increase in enzyme concentration [7]. No effect on milk enzymes was observed when ultrasound was applied without thermal treatment. However inactivation effects were reported when sonication was carried out above 61 °C. Similarly, TS (150 W, 20 kHz, 120 μm amplitude, 30–75.5 °C, 40.2–102.3 s) was reported to be more effective at inactivating milk enzymes (alkaline phosphatase, lactoperoxidase, and γ-glutamyl transpeptidase) than heat alone. However, the extent of inactivation was both enzyme and media specific [7]. Several studies have demonstrated that the effect of ultrasonic waves increases at higher total solid concentration [35]. In skim milk, the concentration of solids is lower than in whole milk resulting in a reduced ultrasonic effect. However, the concentration of enzymes in skim milk (alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGTP)) is also lower than in whole milk leading to a more pronounced effect, as these enzymes are linked to fat globules and can be liberated by the ultrasound effect to the serum phase. As an example, the enhanced decrease of enzyme activity in whole milk than in skim milk by the effect of ultrasound and heat (75.5 °C; 102.3 s) could be due to the higher concentration of solids in the former [7]. Ertugay et al. [36] reported greater inactivation of LPO and AP enzymes which have a significant function in dairy processing at 40 °C compared to 20 °C.

According to the literature, inactivation of monomeric enzymes generally involves either defragmentation of the enzyme or formation into aggregates [37], whereas polymeric enzymes tend to fragment into monomeric subunits during ultrasonication primarily attributed to cavitation. In addition, the extreme agitation created by microstreaming could disrupt van der Waals interactions and hydrogen bonds in the polypeptide, causing protein denaturation [38]. Prolonged exposure to high-intensity ultrasound has been shown to inhibit the catalytic activity of a number of food enzymes due to the intense pressures, temperatures, and shear forces generated by the ultrasonic waves which denature protein. Özbek and Ülgen [39] reported that ultrasonic inactivation mechanisms depend on amino acid composition and the conformational structure of the enzyme. For example, splitting of the heme group from peroxidase by MTS was reported to inactivate the enzyme [40], while free radical-mediated deactivation of lipoxygenase by MTS was reported [41]. On the other hand, the inactivation of trypsin has been partly attributed to the large interfacial area created by ultrasound, which disrupts hydrophobic interactions and hydrogen bonds [38].

The combination of sonication with heat can assist thermal processing by reducing the thermal resistance of various enzymes. However, in some cases, solutions containing enzymes have been found to have increased activity following short exposures to ultrasound [1]. This may be due to the ability of ultrasound to break down molecular aggregates, making the enzymes more readily accessible for reaction. Therefore the key enzymes of concern to each dairy system should be investigated to ascertain the critical control parameters which can be specific to the enzyme, the dairy system, or both.

Homogenization

High-pressure homogenization is the most used technique within the dairy industry. In recent years, the use of ultrasound has attracted much interest [42–44] compared to microfluidization and high shear mixing due to its lower processing times and its controllable nature toward a desired favorable output [45]. However, there remains some uncertainty as to which technique is more efficient for homogenization and the primary mechanism for its effectiveness. Koh et al. [46] found that shear forces generated in the absence of cavitation were mainly responsible for homogenization effects with the use of high-pressure homogenization. Similar effects were found by the authors with the use of high-intensity ultrasound highlighting the minor contribution from cavitational effects toward homogenization. However, the efficiency of sonication toward homogenization is driven by several important factors such as power, frequency, amplitude, diameter of the ultrasonic probe, and the composition of the medium being sonicated.

High-power, low-frequency milk homogenization reporting a reduction in the size of milk fat globules has been widely studied [42–44]. Bermúdez-Aguirre et al. [30] showed that ultrasonic homogenization (400 W, 24 kHz, using a 22 mm probe) of milk at 63 °C for 30 min reduced the diameter of the milk fat globules to < 1 μm compared to the native fat globule size of 4.3 μm. Villamiel and de Jong [7] reported a milk fat globule size reduction of up to 82 % during continuous flow, high-intensity (150 W, 20 kHz, using a Branson sonifier with 18.76 mL cavity) ultrasonication of milk. Bosiljkov et al. [43] showed that an increase of the amplitude (20, 60, and 100 %) and time (2–15 min) of ultrasound (30 kHz, using 7 and 10 mm probes) significantly influenced the degree of homogenization of milk. Sonication disrupts the milk fat globule membrane (MFGM) and the resulting fat globules become heavily coated by proteins [30, 47]. Michalski et al. [47] stated that these complexes are stabilized neither by calcium bridges nor by hydrogen bonds, but through association of casein hydrophobic regions with lipid particles and possibly whey proteins. They further presumed that its αs_{2-} and κ-caseins are more likely to be involved in the complex. Fox et al. [48] suggested that van der Waals forces can also exist within casein–fat interactions.

High-fat dairy systems responded differently to sonication. This different behavior is totally dependent on processing conditions such as temperature, energy delivered, and processing times. Vijaykumar [49] studied the effect of thermosonication (60 °C, 20 kHz, 107–152 μm amplitude for 1–3 min) on cream samples containing 45.5 % fat content. They found increased viscosities where they attributed it toward the swelling of proteins. Cavitation caused by sonication can denature the proteins where it losses the tertiary structure or unfolding of globular proteins which in turn swells up resulting in increased hydrodynamic radius and greater molecular associations and thereby leads to viscosity increases. Furthermore, they stated that the specific area of fat globules of cream samples increased from 1.8–10.8 $\mu m^2 g^{-1}$ with thermosonication at 152 μm for 1 min.

Use of ultrasound as a homogenization technique in dairy processing is one of the limited number of processes that are mature enough to be implemented by the dairy industry in large scale. With the development of large-scale equipment customized for specific dairy processing applications (Prosonix & Hilscher), it is anticipated that more work will be implemented by the dairy industry in coming years.

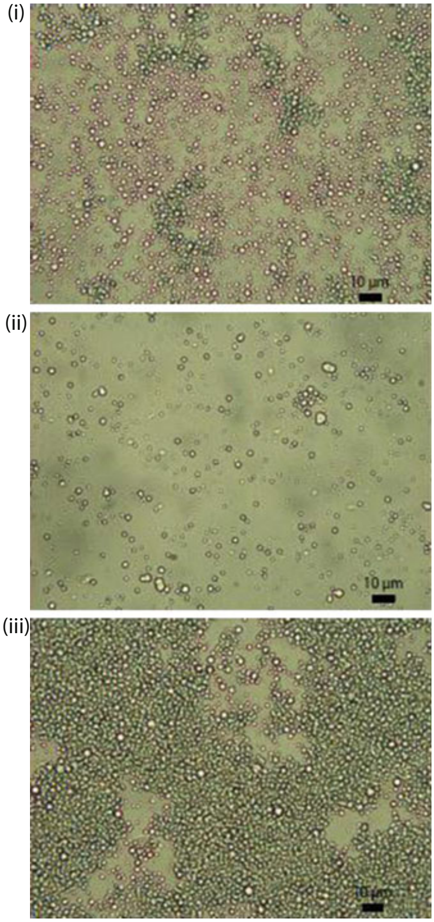
Creaming

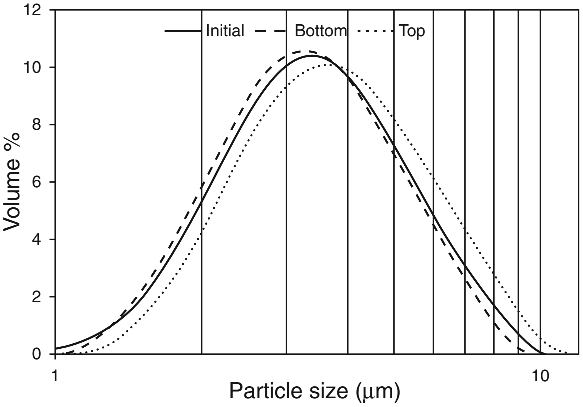
Milk fat separation is a key process in producing some dairy products such as butter, cheese, yogurt, and skim milk. This is typically performed in large scale by centrifugal separators operated at high temperatures to maximize the separation efficiency. Juliano et al. [50, 51] used high frequencies (>400 kHz) than those associated with conventional food processing to destabilize fat and assist creaming in batch systems ranging in scale from mL to L as an alternative separation technology that can enhance the rate of milk creaming by gravitational sedimentation. This may provide advantages such as lower maintenance, less cleaning costs, and lower energy usage as it reduces the residence time of milk inside the ultrasonic separator. Moreover, no disturbance on the structure of fat globules is considered as an important aspect [2].

Leong et al. [52] established suitable ultrasonic parameters that enable successful separation of natural whole milk in large scale, demonstrating the importance of energy density and effectiveness of high-frequency ultrasound (1 and 2 MHz) to separate the small fat globules distributed in milk (~4 μm diameter) (Fig. 3). Furthermore, it was demonstrated that operation in the temperature range of around 25–40 °C is more optimal to the fat separation process due to the influence of temperature on the physical properties of the fat globules such as density, viscosity, and liquid/solid ratio [53]. When an ultrasonic standing wave is set up in a container, the fat globules distributed in the milk experience acoustic radiation forces that cause them to migrate specifically to the pressure antinodes. Since the acoustic forces are proportional to $(\text{radius})^3$, the speed of response of the particles is a function of $(\text{radius})^2$. Therefore larger particles will be driven to the node faster than smaller particles. As higher proportion of larger globules been represented by fat globules, then it’s easy to separate the fat globules in milk (Fig. 3). The acoustic forces can be manipulated by adjusting the applied frequency and energy density.

One possible concern when using high-frequency ultrasound for the separation of milk fat is the potential for oxidation of fat to occur (i.e., lipolysis). Lipid oxidation and development of rancid off-flavors may greatly decrease the acceptability of lipid-containing dairy products. Consequently, ultrasound may have a detrimental effect on lipid functionality and integrity by promoting radical-driven oxidation processes which also consequently limit the application of power ultrasound to lipid-containing dairy systems. Juliano et al. [54] have shown that when sonicating milk within this high-frequency range used, oxidative volatiles derived from sonication were detectable above human sensory threshold limits only when very high specific energies were delivered to the milk. In contrast, Torkamani et al. [55] showed no significant oxidation of fat with sonication of cheddar cheese whey at similar frequencies and energy densities.

Fig. 3 Microscopy images obtained of (i) initial milk samples, (ii) bottom and (iii) top products after 20 min of sonication, with 1 MHz and sound source to reflector distance of 45 mm [52]





Another concern for using high-frequency ultrasound is the potential for alteration of fatty acids within the systems. The type of lipids that are present in foods is becoming of great importance to food manufacturers due to concern about “good” and “bad” lipids. Leong et al. [56] found that there was minimal production of monoglycerides and diglycerides upon sonication of triglyceride containing emulsions. Furthermore, Pandit and Joshi [57] found that high-energy inputs ($\sim$1000 J/mL) were required for hydrolysis of fatty oils by cavitation. However, lipids may also indirectly be modified by application of high-intensity ultrasound by influence the activity of lipases that may be used to alter lipids in milk systems.

Hence, these small-scale studies showed promising signs in using ultrasound as a fat separation technique within the dairy industry without any concerns. However, the cost involved within the initial stages of manufacturing ultrasonic equipments is of a hurdle for the dairy industry to embrace this technology in the near future.

Emulsification

Emulsification in dairy industry has been used through mechanical shaking, colloid mills, high- or ultrahigh-pressure homogenization, and microfluidization. However, ultrasonic emulsification offers several benefits over conventional emulsification methods such as improved energy efficiency, higher emulsion stability, lowered requirement of surfactants, and narrow-size distributions under controllable conditions [58, 59]. As an example, Jafari et al. [45] showed that increasing the microfluidization energy input beyond moderate pressures (40–60 kPa) led to overprocessing of emulsion droplets due to coalescence and found that decrease in emulsion droplet size <0.5 μm by microfluidization was not possible. In contrast, increased energy input helped to reduce emulsion droplet size with minimum re-coalescence of new droplets with US emulsification.

Ultrasound-assisted emulsification is influenced by many variables such as irradiation power, position of the ultrasonic source with liquid–liquid interface, tip diameter, vessel size, viscosity of the continuous phase, pre-emulsification, oil–water ratio, surfactant concentration, hydrostatic pressure, presence of dissolved gases, and exposure time [58–60]. Ultrasonic emulsification is primarily driven by cavitation, wherein collapse at or near the oil–water interface bubble causes disruption and mixing of the two phases, resulting in the formation of very fine emulsions [61]. Shear forces generated are very strong at low frequencies (e.g., 20 kHz) compared to 211 kHz, whereas an efficient emulsification is obtained at 20 kHz while a similar experiment at 211 kHz does not produce an emulsion. Only high-intensity, low-frequency ultrasound (16–100 kHz) is able to produce emulsions. Leong et al. [56] showed that pressures up to 400 kPa improved the efficiency of ultrasonic emulsification of food oils, whereas operating with ambient pressures over 450 kPa suppressed the cavitation activity and no emulsification could be achieved. Increasing the amount of gas in the system tends to increase the gas/vapor pressure ratio inside the bubbles which can cushion the bubble collapse and hence reduce the shock wave intensity and thereby be less efficiently emulsified. In contrast, Behreud and Schubert [60] showed that increased concentration of gas neither influence the magnitude of the cavitational effects per unit volume nor the intensity of the cavitation collapse, although the energy dissipation per unit volume was found to control the particle size distribution of a system.

Recently, Shanmugam and Ashokkumar [62] demonstrated the possibility of incorporating novel food oils into milk systems by ultrasonic emulsification. A 20 kHz ultrasound horn was used to emulsify flaxseed oil in skim milk for delivering as a ready to drink formulae. A minimum process time of 3 min at an applied acoustic power of 176 W was sufficient to produce emulsion droplets with an average diameter of 0.64 μm. Furthermore, they found that those emulsions were stable for 9 days where no addition of surfactants was required to stabilize the emulsion. The authors attributed the stability of the emulsion due to the denatured whey proteins in the sonicated emulsion systems. Furthermore, they attributed the adsorption of proteins toward the emulsion surface stabilized the emulsion droplets due to the electrostatic repulsions and also highlighted the importance of residence time and power density toward achieving stable emulsions.

Although several studies have not been concentrated on formation of emulsions within the dairy industry, the increased trends toward ready to eat and drink dairy products may embrace the ultrasound as a technology toward manufacturing secondary dairy products with extended benefits of such as increased stability and less production times.

Ultrasound-Assisted Filtration

Membrane technology is currently used in the dairy industry for a variety of applications such as separation of milk components, concentration of protein levels prior to spray drying and water purification, and treatment of liquid effluents. One of the critical issues during filtration is the decline in permeate flux as a result of both concentration polarization and membrane fouling. Heat treatment of milk increases viscosity that will result in excessive membrane fouling due to pore blockage and cake formation [63], which in turn has a detrimental influence on the permeation rate and limits the economic efficiency of the processing operation. There are a number of different chemical (use of acid, alkali, enzymes, and hypochlorite) and physical (forward flushing and back flushing) methods currently used for cleaning a fouled membrane. These methods are time consuming, damage the membranes, cause secondary pollution, reduce the lifetime of the membrane, and are unsafe and/or expensive.

The application of ultrasound has proven to be an effective approach to enhance the flux to improve the cleaning of fouled membranes. It can be expected that the increased permeability observed due to sonication may have been affected by the physical processes caused by acoustic cavitation which may occur on the surface of the fouled membrane, on the solid material, and in the vicinity of the pores where the dislodgement of particles that block the pores can be expected. Lamminen et al. [63] found increases in cleaned flux ratio as the power intensity of the system increased. This increase was attributed to an increase in the number of cavitation bubbles in the system and an increase in acoustic energy in the system by the cavitation bubbles. At the same time, although higher frequencies may have more cavitation bubbles, the bubbles are smaller in size and collapse less energetically; thus, they may not be capable of detaching particles from the cake layer as readily as lower frequencies [63–66]. Although, ultrasound has more positive attributes, cavitation may damage the membrane surfaces. At the highest powers (>12.2 W), some damage to the membrane was observed by Lamminen et al. [63], while at lower applied powers (<7.2 W), no damage to the membrane was found by Muthukumaran et al. [64, 65]. Hotrum et al. [67] hypothesized that non-inertial cavitation (cavitation bubble formation and bubble growth and oscillations) and acoustic streaming would be the main mechanisms of importance for the prevention of fouling of cheese milk whereas inertial cavitation which is the bubble collapse considered as an undesirable phenomenon due to the risk of erosion of equipment surfaces and or changes in the system. On the other hand, non-inertial cavitation induces microstreaming which can enhance the heat transfer, mass transfer, and membrane flux processes. However, they found that surface vibration may serve as a mechanism for prevention of a fouled layer.

Muthukumaran et al. [64] studied the ultrasonic cleaning of polysulfone ultrafiltration membranes fouled with dairy whey solutions. It was suggested that the ultrasonic effect is more significant in the absence of a surfactant but is less influenced by temperature and transmembrane pressure. Their experimental results in the whey ultrafiltration process revealed that ultrasound can significantly enhance the permeate flux, with an enhancement factor of between 1.2 and 1.7 across the full range. An increase of the mass transfer coefficient within the concentration polarization layer was also observed. In another study [65], they extended this aspect to consider the effect of ultrasonic frequency. Their results showed that the use of continuous low-frequency (50 kHz) ultrasound is most effective in both the fouling and cleaning cycles, whereas the application of intermittent high-frequency (1 MHz) ultrasound is less effective. Furthermore, their results showed that continuous low-frequency sonication generally reduces the components of the total flow resistance that are readily reversed during water flush [66]. This included the mass transfer resistance arising from both concentration polarization and labile protein deposits that are readily removed. Some other recent filtrations with the use of ultrasound in dairy processing are highlighted in Table 2.

Another recent study by Koh et al. [68] used a different approach in accompanying ultrasound to reduce the fouling of heat-treated dairy whey systems. They used ultrasound as a pretreatment process to break down big protein particles generated through thermal processing of whey protein solutions in order to improve the downstream ultrafiltration performance. The use of ultrasound followed after a heat treatment reduced membrane pore blockage and growth of the foulant cake layer greatly compared to heat-treated systems that has not been subjected to

Table 2 Some literature on use of ultrasound during filtration with regard to different parameters for dairy systems

Target	Food matrix	Conditions	Comments	Reference
Effects of different sonication modes with different frequencies on permeation flow and fouling during UF	Skim milk	Frequency = 37, 80 kHz, and tandem Modes = continuous, pulsed, sweeping, and degassing	Permeation flow increased with decreasing frequency Pulsed mode had the most effect on enhancement of flux and reduction of fouling percentage	[69]
Effects of ultrasound toward ultrafiltration of whey solution	2 % w/w whey protein solutions	Frequency = 30kHZ UF – cellulose membranes Power = 100 W	Ultrasound decreased the membrane fouling caused by concentration polarization Use of high-power US led to lowered retentions	[70]
Effects of ultrasound toward production and purification of peptides	Milk protein concentrates	Frequency = 20 kHz Power = 800 W Time = 4 min	A successful pilot scale membrane filtration was established as US-enhanced hydrolysis characteristics of proteins	[71]
Effects of ultrasonic waves on flux of MF	Fresh cow milk	Feed pressure = 0.5-0.8-1.4 bar Power = 20, 30, 50 W Distance between probe and surface = 2.6 and 4.4.cm	Higher flux was achieved with 0.5 bar, 40 W distance 2.6 CM Continuous irradiation increased flux by 33 % compared to pulsed irradiation	[72]

ultrasound. The extent of changes to pore blockage and cake growth was greater at higher solid concentration which leads to advantages of using ultrasound for processing of concentrated milk within the dairy industry.

These experiments have shown that the use of ultrasound in membrane processing within the dairy industry is generally positive (Table 2). However, there are conditions under which it can be less effective or even has a negative effect on filtration performance depending on circumstances. At present, the embracement of this technology by dairy industries has found to be slow mainly due to the need of processing line adjustments.

Sonocrystallization

Sonocrystallization is the use of power ultrasound to aid and control crystallization. The most effective sonocrystallization can be achieved when sound energy is delivered at the nucleation phase [73]. The use of ultrasound plays a key role in controlling the crystal structure and shape, decreasing the crystallization induction times, increasing yields, reducing size distribution, and increasing the rate of crystallization, which in combination increase the efficiency of some traditional processes, leading to cost effectiveness [74].

Lactose Crystallization

Lactose which is the most abundant component found in whey waste stream is removed in order to increase the further processing ability of these streams such as spray drying due to the fact that lactose makes the material sticky and thereby hard to process. A typical commercial process for lactose crystallization within the dairy industry can take up to 20 h to achieve a yield of ~80 % crystallized lactose. Hence, a rapid recovery of lactose by ultrasound-assisted crystallization has been reported [73, 75]. The lactose recovery was found to be much higher.

In a typical reaction, crystallization takes place on the surface of existing crystals and these crystals act as nucleation sites. Cavitation bubbles act as nucleation sites [73, 75]. Shockwaves cause further agitation and bubble disruption increasing the number of nuclei available for nucleation [76], and the greater number of nuclei reduces crystal size, improves uniformity, and increases crystallization rate [77]. The cavitation hot spots where bubbles collapse are thought to be privileged nucleation centers, where the critical energy for crystal formation is decreased. Furthermore, the rapid collapse of bubbles reduces the crystallization temperature and thereby increases super saturation [78]. Even small changes in super saturation were found to significantly reduce the nucleation rate. The application of ultrasound can reduce the metastable zone width (MZW) which provides information for developing a controlled crystallization process, and this can have many significant benefits such as improved control over crystal size and habit. In comparison to mechanical agitation, ultrasound can provide more uniform mixing that can avoid unwanted zones of excessive super saturation in the vessel.

Patel and Murthy [79, 80] used sonocrystallization for lactose recovery from whey waste streams. The crystallization was reportedly completed with yields in the range of 80–92 % within 4 min of sonication. A recent study by Zisu et al. [81] used sonocrystallization at 20 kHz frequency to concentrated whey solutions containing ~32 % lactose at ~22 °C in a noncontact approach delivering a low applied energy density that varied between 3 and 16 J/mL. The control solution was passed through the ultrasonic rig at the appropriate flow rate without sonication. Crystallization of lactose in commercially concentrated whey was significantly increased by the application of ultrasound at a low-energy density of 3 J/mL and a flow rate of 2 L/min as can be seen from Fig. 4. Regardless of the sonication intensity and flow rate,

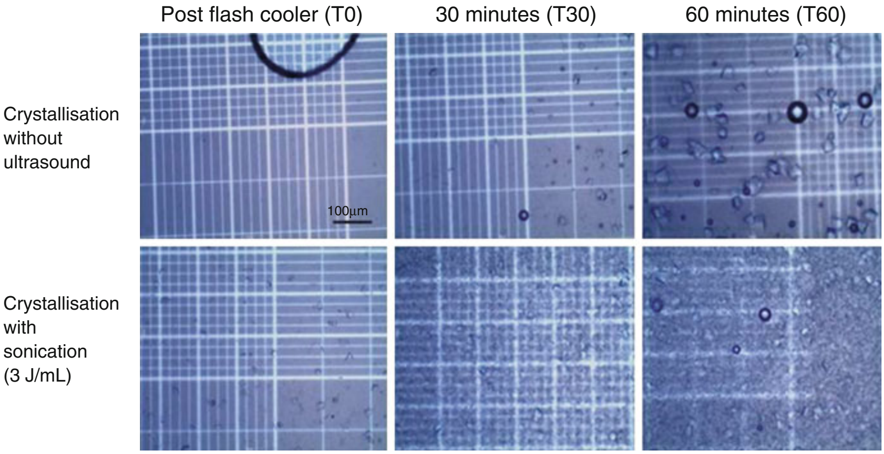


Fig. 4 Concentrated whey viewed under a light microscope at 10× magnification immediately after flash cooling at a flow rate of 2 L/min and after 60 min [81]

the least number of lactose crystals was observed in the control solutions. A greater number of lactose crystals were present in whey immediately after sonication at all energy densities (3–16 J/mL). Ultrasound generated a large number of nuclei resulting in the growth of many small crystals. The rate of sonocrystallization was greater than stirring for approximately 180 min but slowed down between 120 and 180 min as the metastable limit was reached. A second treatment with ultrasound at 120 min delivering an applied energy density of 4 J/mL stimulated further nuclei formation, and the rate of crystallization was maintained for >300 min. Yield on the other hand was limited by the solubility of lactose and could not be improved. The crystal size distribution was narrower than that with stirring and the overall crystal size was smaller. The study highlighted the importance of the conditions used for promoting crystallization.

Fat Crystallization

There has been considerable interest in the application of ultrasound with regard to crystallization of fats within the dairy industry [82, 83]. The crystal size and shape of fats within a product play a significant role to the texture and mouth feel. Martini et al. [82] studied the use of ultrasound as an additional processing condition to alter the crystallization behavior of anhydrous milk fat (AMF). It was shown that ultrasound decreases induction time of crystallization and generates smaller crystals and higher viscosities. However, the effectiveness of this technology depends entirely on the processing conditions. In contrast, Sizuki et al. [83] showed that ultrasound induced primary and secondary nucleations in the lipid, generating smaller crystals and as a consequence resulted in harder materials. Ultrasound affected hardness more efficiently when applied at higher crystallization temperatures (26 °C and 28 °C).

In addition to changes in hardness, AMF networks obtained after sonication were characterized by a steeper and sharper melting profile [83]. This research showed that ultrasound can be used as an additional processing tool to tailor the functional and physicochemical properties of lipids with the potential to be used in the processing of *trans*-free shortenings.

Ultrasound affects the rates of polymorph-dependant crystallization, crystal size, and morphology [82, 83]. A primary effect of sonocrystallization may be due to the high pressure generated when a sonication-induced cavity collapses. The different polymorphic forms have different stabilities that form specifically as a function of the supercooling temperature, mechanism, and lifetime of the collapsing bubbles. This points to the fact that ultrasound irradiation is an effective tool for controlling polymorphic crystallization of fats and reducing induction times. Furthermore, a range of crystal structures can be controlled. This means it is possible to tune the desired texture conditions for a particular dairy product. However, the ultrasound-induced cavitation that produces free radicals appears to have restricted the use of sonocrystallization for systems containing fats and oils, which are susceptible to oxidation by free radicals creating off-flavors [84]. On the contrary, Patrick et al. [85] reported that sonication at 66 kHz did not cause any off-flavor production due to oxidative changes to palm oil. Their results indicate that the optimum conditions for obtaining small crystals in the shortest time period are just below the cavitation intensity threshold.

Ice Crystallization

The ice crystal size directly influences the texture and taste of ice creams which are consumed in a frozen stage. The ice crystals are required to be as small as possible for a desired creamy mouth feel. When US is applied during the crystal growth phase, fragmentation of large crystals under acoustic stress will occur and lead to crystal size reduction. Ice cream contains up to 50 % by volume of entrapped air. Ultrasonic degassing can occur during the application of ultrasound and this process can result in undesirable modifications to the ice-cream texture. Acton and Morris [86] overcame this issue by increasing the initial gas content so that the proportion of air lost due to US can be compensated. But the question that remained unanswered was how much extra air needs to be added to obtain the desired texture.

Furthermore, high-intensity ultrasound may lead to fat oxidation which can lead to off-flavors and improper textures in ice creams. However, by keeping the cooling regime constant, it has been found that the structure of the crystallized product can be adjusted by varying the ultrasonic intensity [87]. Mortazavi and Tabatabai [88] found that application of 20 min-pulsed ultrasound resulted in the best sensory flavor, texture, and mouth feel evaluations of ice creams. Flavor and texture of samples prepared with 5 and 20 min pulse time also had better mouth feel than the control. Hence, the conditions need to be carefully monitored in using ultrasound on ice-cream applications.

The use of ultrasound for crystallization of lactose has been widely embraced by the dairy industry, although fat and ice crystallization aspects still remain in doubt due to its detrimental effects leading to consumer sensory concerns within dairy products. However, more work is needed to get the appropriate conditions optimized specific to a certain dairy product of interest.

Solubility of Dairy Powders

Rapid dissolution of dairy powders is desirable to avoid prolonged processing times, increased production costs, and reduced product quality. Micellar casein (MC) and milk protein concentrates (MPC) have generally poor solubility, which is known to decrease during storage, particularly at high ambient temperature and humidity [89]. The use of shear to accelerate the solubilization of these powders is therefore of interest. Some attempts have been made to produce high-protein dairy powders with increased solubility through the application of static high pressure [90], high shear [91], or ultrasound [91, 92] to concentrates, the addition of sodium caseinate or polydextrose [93], and the addition of mineral salts before drying [94]. A recent study by McCarthy et al. [95] showed that ultrasound (20 kHz/70.2 W) increased the solubilization of MPC powders with ultrasound (20 kHz/70.2 W). However, their study involved a stirring pretreatment step at 50 °C, which aids the dissolution of the powder particles to an extent. However, a thorough investigation of the effects of ultrasound on the dissolution of dairy powders was performed by Chandrapala et al. [96].

Chandrapala et al. [96] work investigates the effect of shear on powder solubilization by examining in detail the behavior of low-solubility MPC and MC powders during ultrasonication and was compared for low-shear overhead stirring, rotor-stator mixing, and high-pressure homogenization (Fig. 5). The initial solubility of the MPC and MC powders was between 60 % and 70 %. Ultrasonication achieved much more rapid solubilization of the powders, with 90–95 % solubility achieved in less than 10 min. The shear forces generated increase the mass transfer at the surface of these particles resulting in an increase in the rate of solubilization. To better contrast the solubilization behavior, the particle size distribution data can be compared for MPC and MC that had achieved approximately 80–100 % powder solubilization (Fig. 6). Ultrasonication appeared to be even more effective than rotor-stator mixing at breaking apart particle aggregates, presumably due to the high localized shear that can be created [97]. The average size of the largest particles was reduced for both MPC and MC powders to about 10–20 μm, with the emergence of a casein micelle peak at 200 nm now evident (Fig. 6). To observe if there were any structural changes to the proteins, native PAGE was performed on MPC solutions sonicated at different time intervals (30 s, 3 min, and 10 min) and compared to powder dissolved by low-shear overhead stirring. Bands representing casein, β-lactoglobulin, and α-lactalbumin and higher-molecular weight proteins such as immunoglobulins and BSA were all observed to remain unchanged after processing

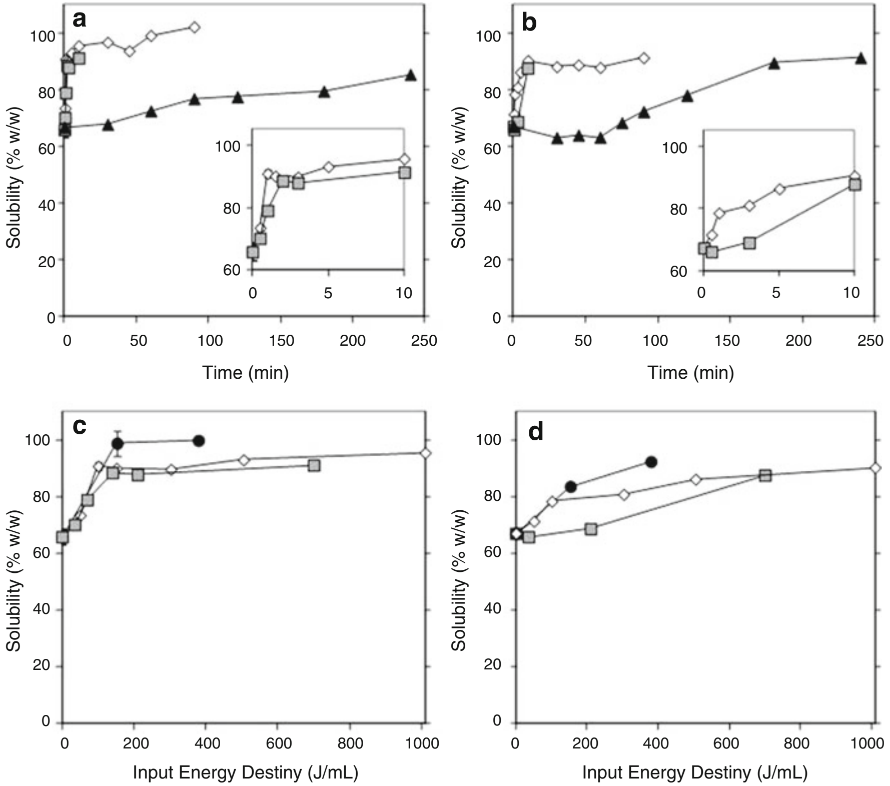


Fig. 5 Rate of solubilization of MPC (**a**) and MC (**b**) powders by sonication (◊), rotor-stator mixing (■), and low-shear overhead stirring (▲) and comparison of solubilization of MPC (**c**) and MC (**d**) powders by sonication (◊), rotor-stator mixing (■), and high-pressure homogenization (●) as a function of energy input [96]

by either sonication or homogenization [96]. This indicates that ultrasound did not have any noticeable effect on the individual protein components in the reconstituted milk or their interactions with each other. The current results suggest that these techniques only affect the large powder particles, increasing their rate of solubilization and disaggregating casein micelles without affecting the protein components liberated during dissolution.

Functionality Modification of Dairy Systems

Viscosity Modifications

Controlling the viscosity of food systems by ultrasound is one of the most promising processes that have been developed due to the facts that the process does not require any chemicals and additives, is simple and rapid, is cost effective, and will not

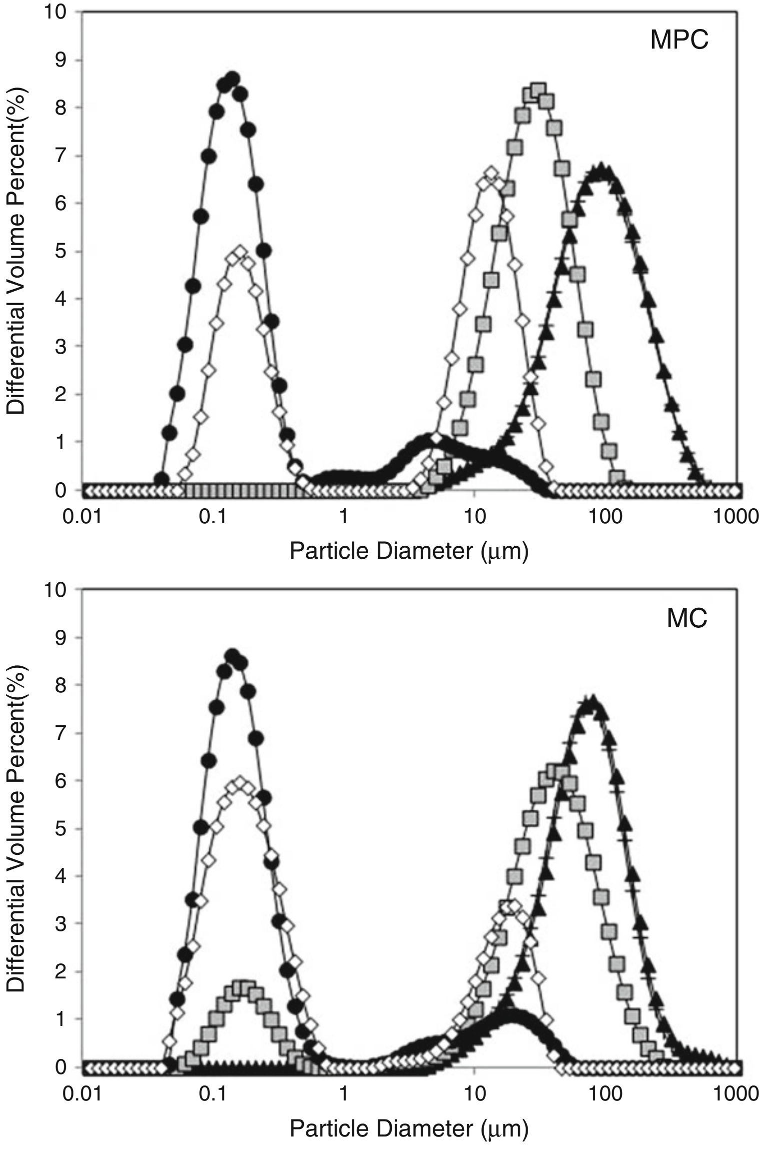


Fig. 6 Comparison of volume-weighted particle size distribution of MPC and MC powders solubilized to between 80 % and 100 % by different methods; ultrasonication (◇; sonicated for 3 min to 89 % and 81 % solubility of MPC and MC), rotor-stator mixing (■; mixed for 3 min and 10 min to 88 % solubility of MPC and MC), high-pressure homogenization (●; homogenized at 80 bar to 99 % and 84 % solubility of MPC and MC), and low-shear mixing time (▲; mixed for 240 min to 86 % and 92 % solubility of MPC and MC) [96]

induce large changes in the chemical structure of particles present in dairy systems which will be of a benefit.

Whey Proteins

During manufacture of whey protein powders, whey protein solutions consisting significantly high levels (4–15 % by weight) of protein are subjected to heat treatment. Issues with significantly increased viscosity of the protein solution ultimately limit the extent to which heat treatment is applied and the total solid concentration that can be used. The viscosity then increases further upon a second heat treatment during manufacture of secondary dairy products. Ashokkumar et al. [98, 99] and Zisu et al. [100] outlined a novel approach to overcome this problem. The application of ultrasound for a very short duration after such a heating step breaks down these aggregates and prevents their reformation on subsequent heating, thereby reducing the viscosity increase that is usually associated with this process (Fig. 7). This functionality (low viscosity) was preserved even after freeze or spray drying and then reconstitution into aqueous solution. Initially, it was argued that these observed viscosity changes might have been caused by the physical or chemical effects of acoustic cavitation. To investigate possible chemical effects due to radical generation, further experiments were carried out in which reconstituted whey protein concentrate (WPC) solutions were sonicated over a range of frequencies (20 kHz to 1 MHz) [98, 99]. It was found that whey solutions sonicated at 20 kHz showed the highest viscosity reductions [99, 100] even though 20 kHz ultrasound formed the least amounts of radicals [101]. The authors therefore attributed the observed viscosity reduction primarily to the physical forces generated during acoustic cavitation.

A thorough understanding of the mechanism of these ultrasound-assisted viscosity modifications was investigated by Chandrapala et al. [102]. The three types of main interactions within protein solutions were investigated: surface charge of the protein aggregates (indicative of electrostatic interactions), reactive thiol groups (indicative of thiol–disulfide interactions), and surface hydrophobicity (indicative of hydrophobic interactions). Interestingly, it was found that surface charge and reactive thiol groups remained unchanged with the sonication step applied in between the preheating and postheating steps (Fig. 8). However, the surface hydrophobicity of these aggregates was altered markedly (Fig. 8). Preheating denatures the whey proteins and thereby exposes the hydrophobic groups. This was indicated by an increase in the surface hydrophobicity of these preheated aggregates. This increase in surface hydrophobicity was greatly reduced with the introduction of a sonication step. It was speculated that sonication broke down the protein aggregate networks through physical shear caused by acoustic cavitation, leading to the formation of smaller aggregates with lower-surface hydrophobicity. These smaller aggregates are resistant toward further aggregation during postheating, thereby improving heat stability.

Similarly, Kresic et al. [103] also investigated the rheological and thermophysical properties of WPC and WPI solutions subjected to sonication. According to these results, the use of ultrasound changed the flow behavior and this was attributed to

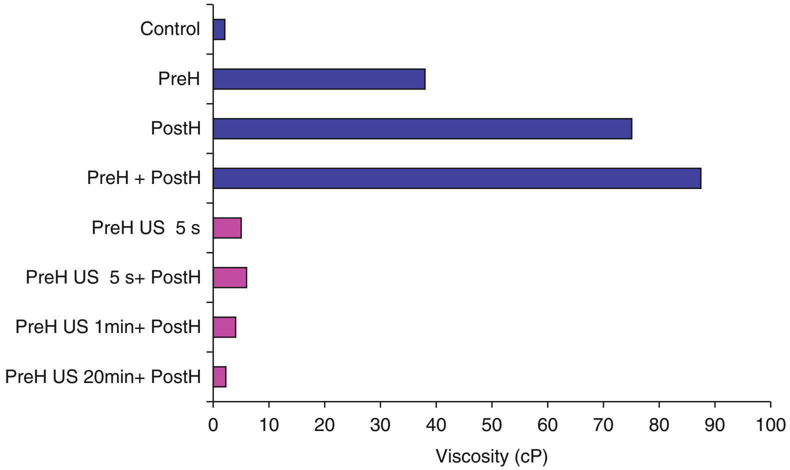


Fig. 7 The effects on solution viscosity for a 6.4 % protein (by weight) solution reconstituted from whey protein concentrate and sonicated with a 20-kHz horn at a calorimetric power of 31 W in a batch mode. Dark gray (*blue*) bars represent solutions without sonication; light gray (*pink*) bars indicate sonicated solutions. *PreH* preheating, *PostH* postheating, *US* sonication [99]

altered protein structure, namely, that the hydrophilic parts of amino acids are opened toward the surrounding aqueous phase, leading to an increased binding of water molecules. There has been some concern that the sonication of proteins in solution can lead to the formation of amyloid-type fragments [104]. This formation was observed when excessively high specific energy was delivered to a small volume. However, recent studies by Chandrapala et al. [105, 106] showed that no significant protein structural changes were observed up to 60 min of sonication, although these minor changes cannot be completely omitted from the observed functional property changes for both complex and model systems.

Casein + Whey Protein Mixtures

Although casein micelles are considered relatively stable particles, their composition and size respond to alterations in pH, temperature, and milk protein concentration [107]. It is possible that the localized high temperatures and shear forces created by sonication can physically alter the casein micelles or their interactions with other milk components. Madadlou et al. [108] found that the average size of reassembled casein micelles could be reduced by exposure to ultrasound (35 kHz frequency) for 6 h provided the pH was above 8. However it is unclear how this relates to native casein micelles since the reassembled casein particles were considerably larger (275 nm) and structurally and functionally different [109]. In another study involving true casein micelles in milk, a decrease in particle size resulting from sonication was observed. This particle size decrease was attributed to a reduction in casein micelle size by the authors, although this was not substantiated [110]. A recent study by Chandrapala et al. [111] showed that sonication did not appear to affect casein micelle size or composition or permanently affect the mineral balance in fresh skim

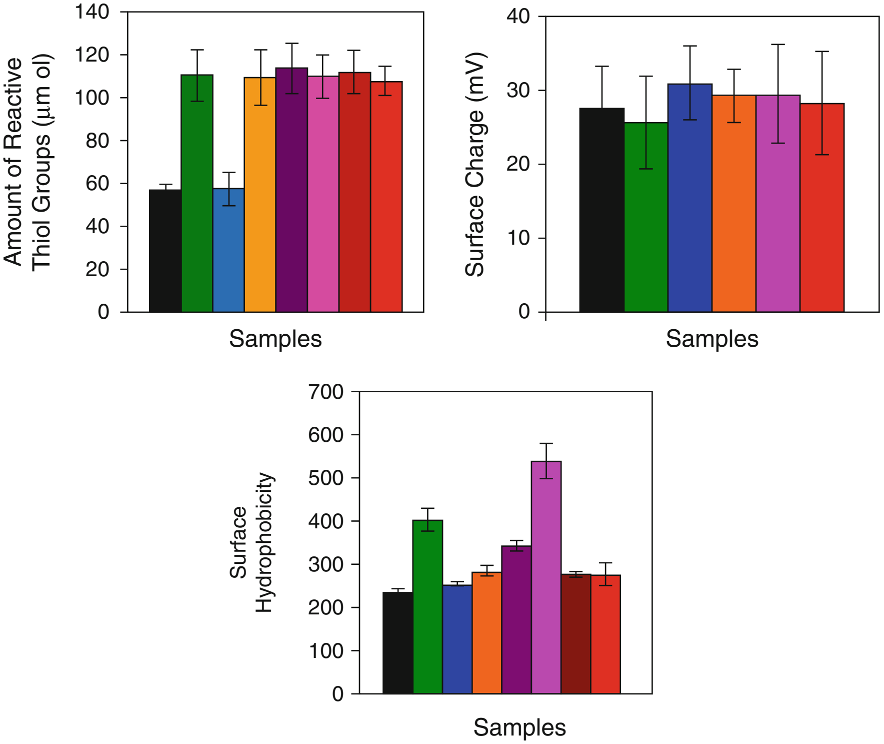


Fig. 8 Reactive thiols (*left*), surface charge (*middle*), and surface hydrophobicity (*right*) of proteins in heat-treated and sonicated 5 % (w/w) reconstituted WPC solutions. Colored bars from *left*: native (*black*), preheat (*green*), sonicated (*blue*), preheat + sonicated (*orange*), post-heat (excluding middle) (*purple*), preheat + post- heat (*pink*), sonicated + post-heat (dark *red*), preheat + sonicated + post-heat (light *red*) [102]

milk. Sonication did however reduce the size of the already small fat globules remaining in skim milk, appeared to break up whey protein aggregates and assisted in breaking apart casein micelle, non-micellar casein, and whey protein aggregates present in reconstituted casein powder systems. The results showed that controlled application of ultrasonic energy can help break up large casein and whey protein aggregates thereby influencing macroscopic properties such as viscosity, without inducing changes to the casein micelles or mineral balance. Similar results were obtained by Shanmugam et al. [112] when using up to 30 min of sonication, but prolonged sonication resulted in the partial disruption of some whey proteins from the whey–whey aggregates. In contrast, Liu et al. [113] found that casein micelles are disrupted at high pH values with sonication. In their study, reconstituted skim milk at 6.7–8 pH was sonicated at a specific energy input of 286 kJ/kg using 20 kHz. According to this study, ultrasound caused greater disruption of casein micelles causing release of proteins from the micellar to the serum phase at high pHs. Furthermore, they showed that the released proteins reassociated to form aggregates of smaller size but with surface charge similar to that of casein micelles in the original milk. However, in other studies the dissociation of κ-casein was found to be dominant with increase in pH, which can result in an increase to the total protein content in the supernatants [114]. They hypothesized that it is due to a pH-dependent conversion of the native colloidal calcium phosphate (CCP) to an alternative form of calcium phosphate, which is less capable of maintaining the micellar integrity, particularly at higher pH values where the charges of the proteins are greater. Hence, it is arguable as to whether the effects observed by Liu et al. [113] under high pH conditions are just an ultrasound effect or a pH effect and/or a combination of both.

Milk Concentrates

Milk is often concentrated commercially to high solids in preparation for spray drying (typically 40–55 %). However, increasing the solid content, the viscosity of concentrated milk increases with time in a process known as “age thickening” by structural buildup through weak interactions between casein micelles that can be disrupted by mechanical shear [115]. High-power low-frequency ultrasound (20 kHz) has potential industrial application to reduce the viscosity and to control the rate of age thickening of concentrated skim milk. A recent study by Zisu et al. [116] investigated the high-intensity low-frequency ultrasound on concentrated skim milk to lower viscosity through the process of acoustic cavitation. Batch sonication for 1 min at 40–80 W and continuous treatment delivering an applied energy density of 4–7 J/mL reduced the viscosity of medium-heat skim milk concentrates containing 50–60 % solids. Viscosity was reduced by approximately 10 %, but this has improved to 17 % in highly viscous age thickened material. Sonication also showed changes in the shear-thinning behavior at shear rates below 150 s^{-1}. Although ultrasound lowered the viscosity of skim milk concentrated to 50 % solids, the treatment could only delay the rate of thickening once the aging process was established. It was only when ultrasound was activated during concentration that sonication prevented the viscosity of skim milk concentrates from increasing rapidly.

Gel Formation

A key aspect of yogurt is associated with the physical properties of the gel, which should possess a smooth textural character in mouth during consumption along with low serum separation during storage. Vercet et al. [117] studied the use of manothermosonication (MTS), to obtain tailored functional properties of the products. The application of ultrasound allowed elaboration of yogurts with rheological properties such as flow curves, apparent viscosity, yield stress, and viscoelastic properties superior to those of control yogurts elaborated with non-sonicated milk. The authors further showed that MTS yogurts had stronger structures, which resulted in higher values of almost all of the many relevant rheological parameters. They suggested that ultrasound effects are mainly related to the cavitation phenomenon. As a result of the cavitation conditions, water molecules can be homolyzed, generating highly reactive free radicals that can react with and modify several molecules. Mechanical stress, generated either by shock waves derived from bubble implosion or from microstreaming derived from bubble's size oscillations, is also able to disrupt large macromolecules or particles. Reiner et al. [84] also found that compared to conventional yogurts, cultures from thermosonication (TS) milk had higher gelation pH values, greater viscosities, and higher water-holding capacities. The authors further stated that the structure was different; it showed a honeycomb-like network and exhibited a more porous nature. Similarly, Reiner et al. [118] found superior rheological properties of yogurts prepared from ultrasonicated milk than the yogurts prepared from conventionally heated milk. Further, Bermudez-Aguirre et al. [30] found only minor changes to the nutritional properties of milk after ultrasound, with the advantage of extending the shelf life of the product for more than 16 days at 4 °C without the use of intensive heat treatments. Wu et al. [119] reported that high-intensity ultrasound (90, 225, and 450 W, 20 kHz) significantly improved the viscosity and water-holding capacity and reduced syneresis of yogurt produced from sonicated milk. These effects are directly related to the yogurt structure, which is based on strings or clusters of casein micelles interacting physically with each other and with denatured serum proteins entrapping serum and fat globules. Furthermore, ultrasound could cause some qualitative changes in the fat globule membrane which would modify the ability of fat globules to interact with themselves and/or casein micelles, thereby improving the gelling properties.

In another study by Liu et al. [120], observed the renneting properties of reconstituted skim milk at 6.7–8 pH that were sonicated at a specific energy input of 286 kJ/kg using 20 kHz. It was shown that gelation attributes were significantly modified (i.e., faster gelation) in rennet gels made from milk sonicated at pH 8.0 and readjusted back to pH 6.7 compared to those made from milk sonicated at pH 6.7. The renneting properties were also modified (i.e., firmer gels) in milk sonicated at pH 6.7 compared to those of non-sonicated control milk. The modified renneting behavior was attributed to ultrasound-induced changes to the proteins in milk. Chandrapala et al. [121] looked at the phosphate-induced micellar casein gelation and the influence of sonication (20 kHz) to this process. Gels were formed by the addition of 7.6 mM tetrasodium pyrophosphate (TSPP) to 5 wt% micellar casein (MC) solutions. It was shown that sonication at 20 KHz and 31 W for up to 30 min changed the surface hydrophobicity of the proteins, whereas surface charge was unaltered. Sonication before the addition of TSPP formed a firm gel with a fine protein network and low syneresis. Conversely, sonication after TSPP addition led to an inconsistent weak gel-like structure with high syneresis (Fig. 9). Gel strength in both cases increased significantly after short sonication times, while the viscoelastic properties were less affected. Overall, the results showed that sonication can have a significant effect on gelation of micellar casein systems, but the state of the casein micelle prior sonication is a dominant factor and should be carefully controlled. Hence, the effects observed in the study by Liu et al. [113] can be confirmed as a

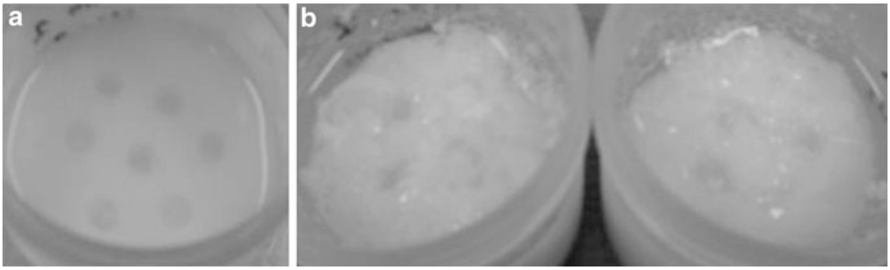


Fig. 9 Appearance of gels set by (**a**) sonication prior to TSPP addition and (**b**) TSPP addition after sonication [121]

combined effect of pH and ultrasound rather than just simply ultrasound being responsible.

The effect of sonication on milk gels has been reported. Acid gel firmness (*G'*) was found to be altered when skim milk was ultrasonically treated prior to acidification, although the effect was attributed largely to denaturation of whey protein caused simply by temperature increases (up to about 95 °C) resulting from sonication performed without temperature control [114]. However, direct effects of ultrasound on casein micelles or individual caseins cannot be ruled out completely, although negligible effects were found on the individual caseins/casein micelles [111]. This may be because big multimeric protein complexes are more sensitive to the shearing forces created by microstreaming and bubble implosion than single-dissolved monomeric proteins.

Zisu et al. [122] also investigated the changes in heat-induced gelation properties of WPC systems. It was found that heat-set gels formed from WPC solutions that had been sonicated at 20 kHz showed higher gel strengths, reduced syneresis, and differences in gel microstructure, compared with gels made from non-sonicated WPC. Conversely, whey protein isolate (WPI) solutions were relatively unaffected by sonication, possibly reflecting the absence of larger aggregates in the initial solution or differences in composition. Adjustment of pH prior to ultrasound treatment did not result in significant differences compared with samples that were sonicated at neutral pH. This suggests that the mechanism for gel promotion is different from effects induced by pH changes. According to their results, the use of ultrasound changed the flow behavior and this was attributed to altered protein structure; the hydrophilic parts of amino acids are opened toward the surrounding aqueous phase, leading to increased binding of water molecules.

Foaming Capacity

Ultrasound has been used for many years in the study of estimating changes in protein conformation. Jambrak et al. [123] showed that ultrasound with a high-intensity (20 kHz) probe has a major effect on whey protein's functional properties such as solubility and foam ability. Their results showed that ultrasound of 40 kHz frequency had less effect on whey protein than a 20 kHz probe. It was explained by the way of treatment. At probe treatment, the horn is inserted in solution which favors contact between tip and sample, whereas at baths flasks filled with solutions were immersed, so there was not direct contact with irradiating surface. A 15 min treatment using a 40 kHz bath showed the major impact: it decreased the conductivity of protein sample, increased solubility, and foaming ability of protein. The larger increases of foaming ability might be to the homogenization effect of ultrasound according to the authors. The homogenization effect of ultrasound usually disperses the protein and fat particles more evenly, which may improve the foaming property. During ultrasound treatment proteins probably became partially unfolded in structure which increases the foaming ability [123]. Ultrasound of 500 kHz did not impact the foaming ability of whey protein, but it affected solubility and conductivity.

Manipulating the functional properties of dairy ingredients is interesting from the point of protein functionality, although the actual mechanisms responsible for some observed effects are still under investigation. As mentioned earlier in this review, sonication of a liquid generates a number of different effects: mechanical vibration, agitation, shear forces, turbulence, acoustic cavitation, and free radicals. At this stage, it has been speculated that functional changes are due primarily to the physical effects of acoustic cavitation that slightly alter protein structure, since a higher-frequency ultrasound did not affect the functional properties of dairy ingredients. All these tailored desirable functional properties of end products with less nutritional loss and longer shelf lives are most welcoming for the wide use of ultrasound as an emerging technology in dairy streams.

Conclusion and Future Directions

Ultrasound is a promising technology suitable for a range of different applications in the dairy industry. In liquid media, the extreme physical forces generated by low-frequency, high-intensity ultrasound induces acoustic streaming, cavitation, shear, micro-jet, and shockwaves. These physical forces have been successfully used for the generation of dairy emulsions, functionality improvements of dairy systems, inactivation of microbes and enzymes, and crystallization of lactose, ice, and fat in dairy systems, among several other applications. High-frequency ultrasound on the other hand has been used to initiate rapid creaming of fat from milk. Ultrasound processing has advantages of achieving high product yields, minimizing flavor loss, increasing homogeneity, reducing energy requirements, reducing processing times, enhancing end-product quality, reducing chemical and physical hazards, and lowering the environmental impact, when compared with conventional dairy processes. Synergies with pressure and/or temperature have been reported but caution is advised to minimize nutritional losses and adverse flavor modifications if very high specific energies are to be delivered to the process.

Although the majority of these applications are only proven in the laboratory for a range of advantages, there is a high potential for large commercial scale-up operations. In the last couple years, significant improvements in product quality, process enhancement, and reduction of cost were successfully achieved on a commercial scale due to the availability of high-power units consisting large continuous flow chambers. Furthermore, production of improved energy-efficient ultrasonic equipments with efficient ultrasonic generators and transducers reduces the internal heating and subsequently prevents using expensive cooling systems which have often caused the whole system to fail in the past. Most up-to-date systems have an energy efficiency of greater than 80 % which simply indicates that most of the power sent to the transducer is transferred into the medium. In addition, small-sized new generators and absence of moving parts such as rotors lead to the easy installation of the ultrasonic equipments into an existing facility and have a low maintenance cost. Hence, commercial standard ultrasonic equipments are developing at a great pace, although no novel process for the application of ultrasound in food industry is possible without ultrasonic equipment manufacturers willing to build new designs according to the requirements. This implies that while the technology has great promises, it will have to be carefully developed and scaled up for every individual, unique application.

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