

REVIEW ARTICLE

Improving the Characteristics of Dairy Products Using Ultrasound Technology: A Modern, Green and Sustainable Approach

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ABSTRACT

Development of novel dairy products is gaining importance due to an increased consumer demand for palatable, healthy, and minimally processed products. Ultrasonic processing or sonication is a promising alternative technology in the food industry as it has potential to improve the technological and functional properties of milk and dairy products. This review presents a detailed summary of the latest research on the impact of high-intensity ultrasound techniques in dairy processing. It explores the ways in which ultrasound has been employed to enhance milk properties and processes of interest to the dairy industry, such as homogenization, emulsification, yogurt and fermented beverages production, and food safety. Special emphasis has been given to ultrasonic effects on milk components; fermentation and spoilage by microorganisms; and the technological, functional, and sensory properties of dairy foods. Several

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current and potential applications of ultrasound as a processing technique in milk applications are also discussed in this review.

KEYWORDS

- Sonication • Ultrasound • Piezoelectric material • Ultrasonication
- Acoustic cavitation

INTRODUCTION

Ultrasound technology has been widely applied in medicine, the chemical industry, and engineering since the early twentieth century. It is classified as a promising methodology for aspects of dairy food processing, such as gelling, fermentation, freezing, emulsification, and sterilization. In recent years, the research of high intensity ultrasound applied on dairy or dairy by products has been aimed at reducing processing time or enhance the physicochemical quality of different food items. Power ultrasound has a low frequency between 20 and 100 kHz and energy intensities between 10 and 1000 W/cm². When power US travel through a liquid medium, it generates microbubbles through “acoustic cavitation”. The violent collapse of these microbubbles induces localized strong shear forces and turbulence which cause disruption of milk fat globule. Ultrasound generates alternating high- and low-pressures, causing compression and expansion (rarefaction) cycles in the medium. Rarefaction leads to the formation of cavitation bubbles, which are tiny vacuum bubbles that occur when the negative pressure exerts. The vacuum bubbles grow over several compression/rarefaction cycles until they cannot absorb more energy and the cavitation bubble undergoes an implosive collapse releasing energy. This process of bubble generation, growth and implosion is regarded as acoustic cavitation or implosion. Ultrasound has been used in food and dairy processing, in applications such as, the enhancement of whey ultrafiltration, extraction of functional foods, reduction of product viscosity, homogenization of milk fat globules, crystallization of ice and lactose and for cutting of cheese blocks.

PROCESSING EQUIPMENT

The equipment for ultrasound available in the market offers a wide spectrum of frequencies. The other is the probe system, which is more powerful and leads to vibrations directly into the sample. The systems available

for sonochemistry are bath type, probe system, and other systems. Different types of instrumentation available for bath type systems are ultrasonic cleaning bath, bath with plane transducer as base, submersible transducer, and tube reactor. The types for the probe are horn, cup-horn, and push-pull system. The other type of instrumentation is near field acoustic processor (NAP) and ultrasonic bar emitting radial ultrasound. The other type is a tube containing piezoelectric transducers on both ends. Various types of reactors are employed in ultrasonic processing, depending on the application. These include:

Batch reactors: Stirred tanks equipped with ultrasonic transducers for processing materials in batches.

Continuous mixed flow reactors: Similar to batch reactors but designed for continuous processing.

Continuous plug flow reactors: Equipped with ultrasonic transducers for continuous processing in a tube like setup.

For large-scale applications, two types of plants are used:

Batch type: it utilizes an ultrasound cleaning bath for processes like cleaning and decontamination.

Flow type: it employs a liquid whistle or tube reactors for emulsification and other processes. Tube reactors, suitable for food sector applications, consist of a tube with shaking boundaries and transducers placed at the walls to rectify sound energy. The tube design can vary in cross-section shape. An alternative setup uses a coaxial arrangement with a spiral emitting rod, requiring minimal energy. Sonication gives better results when applied in synergy with other parameters like temperature and pressure. When combined with thermal, then it is termed as thermo-sonication when combined with pressure it is called mano-sonication and when coupled with both heat and pressure, it is called mano-thermo-sonication.

GEL FORMATION PROPERTY OF US

Shokri *et al.* (2022) reviewed that gel-based protein processes require partial protein unfolding to form a 3D structure. Acoustic cavitation from ultrasounds can unfold the secondary and tertiary structures of the protein, favoring the gelling properties. Milk proteins have been widely used due to their remarkable gelling properties (Shen *et al.*, 2017). The gelling strongly affects the food texture (microstructure and rheological properties) including cheeses and yogurts (Nunes & Tavares, 2019). Enzymes, acidification, heat, or ultrasounds can induce gelling of milk proteins (Frydenberg *et al.*, 2016).

In addition, acoustic cavitation can dissociate water molecules and dissolved oxygen and create highly reactive free radicals (\bullet OH, \bullet OOH). These reactive species can oxidize SH (free sulfhydryl) groups into S-S (total disulfide) bonds, thereby increasing aggregate formation and forming a firmer gel. Shen *et al.* (2017) evaluated the effect of low-intensity ultrasound (20 kHz, for 5 to 40 min) on the gelling properties of whey proteins, mainly induced by acid coagulation and observed that the strength and firmness of the gel was increased. Liu *et al.* (2014) examined the effects of ultrasounds on skimmed milk: 10% w/w total solids at natural pH 6.7 or alkaline adjusted to pH 8.0. The results revealed that the gelled curds were firmer than the control's (no ultrasound treatment). The authors concluded that these superior properties of rennet were attributed to the reduction in particle size of casein in the milk and, very likely, to the exposure to hydrophobicity. Therefore, ultrasounds are promising alternative for the dairy industry, particularly in cheese making, as the improved gelling properties decrease the processing time and increase the curd firmness.

APPLICATION OF US WAVES IN FERMENTATION PROCESS

The food fermentation process involves a quite complex biochemical reaction, and modern fermentation has invested in new technologies to be highly competitive and innovative to improve the quality yield processes of its products (Ojha *et al.*, 2017). High-intensity ultrasound treatments have been used as a non-destructive analytical technique to monitor fermentation processes.

On the other hand, low-intensity ultrasound treatments have been used to improve fermentation rates and the performance of enzymes and microorganisms (Umego *et al.*, 2022). The traditional method of monitoring fermentation processes is based on taking samples at regular intervals to estimate the main fermentation parameters, such as microbial growth, pH, acidity, turbidity, and chemical composition. These classic analyses are time-consuming and do not allow real-time control. However, high-intensity ultrasounds can provide useful information to characterize the fermentation process in real-time (Ojha *et al.*, 2017). In this case, a probe is placed in a fermentation tank, and the ultrasonic velocity of a wave traveling through a tank can be used to infer the concentrations of alcohol and sugars during the fermentation process (Novoa-Díaz *et al.*, 2014). These authors reported that a change in ultrasonic velocity is correlated with the concentration (0 to 8 g/L) of lactic acid and malic acid during red wine fermentation. They observed that the concentrations of malic acid and lactic acid have different impacts on ultrasonic velocity, occurring with a decrease rate of 0.2 ms per g/L and an increase of 0.3 ms per g/L (for the interval of 0-8 g/L) in malic and lactic acids, respectively.

Herrera-Ponce *et al.* (2022) evaluated the effect of high-intensity ultrasound (40 kHz and 11 W/cm²) for 0, 3, and 10 min as a pre-treatment for developing fermented whey and oat beverages. Applying ultrasound for 3 min (40 kHz, 11 W/cm²) and the proportion of 50% whey to 50% oat was the best beverage, as it generated the highest growth levels of *Lactocaseibacilluscasei*. The authors concluded that ultrasounds are a tool that accelerates beverage fermentation. The improvement of fermentation is related to changes, such as the hydrolysis of carbohydrates, leaving them more accessible for fermentation by bacteria (Guimarães *et al.*, 2019). On the other hand, Alouache *et al.* (2015) monitored yogurt fermentation using high-intensity (5 MHz) ultrasounds. The results showed that the ultrasound methods correlated with traditional methods (measuring acidity and pH). The authors concluded that high-intensity ultrasounds are an alternative that can be used to monitor yogurt fermentation. Therefore, ultrasounds can be applied in the dairy industry, mainly to intensify the processing of fermented dairy products.

ENHANCED FREEZING PROCESS DUE TO US WAVES

Food freezing is an important operation in the food industry, especially for perishable products, as it minimizes biochemical and enzymatic reactions. The development of non-uniform crystals, can result in the loss of the sensory quality of the food (Norton *et al.*, 2009). Ultrasound treatments applied to frozen foods provided a smaller crystal size distribution, reduced cell damage, and reduced the time between the beginning of crystallization and complete ice formation (Chemat *et al.*, 2011). Türker and Dogan (2021) evaluated the effect of the application time (3, 5, and 7 min) of ultrasounds on ice cream. The authors observed that using ultrasounds reduced the size of ice crystals in ice cream. It is essential to obtain the smallest crystals in the freezing process to avoid a gritty structure, achieving the desired smoothness and softness of the ice cream. Similarly, Adhikari *et al.* (2020) showed that low-power sonication (220 kHz for 60 s) reduced melting properties and ice cream hardness. It may be associated with the size of the ice crystal in the product (Wu *et al.*, 2019). Mortazavi and Tabatabaie (2008) also found that applying low-intensity ultrasound (20 kHz) for 60 min reduces the freezing time of ice cream and forms smaller ice crystals. Crystallization can often occur uncontrolled simply due to a slight decrease in temperature or pressure and can cause severe problems during the food manufacturing process (Chemat *et al.*, 2011). Martini *et al.* (2008) investigated the effect of ultrasounds with a frequency of 20 kHz on the crystallization of anhydrous milk fat. The samples were subjected to rapid cooling (10 °C/min) at different crystallization temperatures ($T_c = 22, 24, 26, 28, \text{ and } 30 \text{ }^\circ\text{C}$). Shortly after the cooling process, the samples were heated to 80 °C to allow complete melting of the triacyl glycerides and placed in a double-walled thermostatic crystallization cell. Then, they were cooled in a water bath, and as soon as they reached T_c , high-power ultrasounds were applied for 10 s to each sample individually. The samples were kept at T_c for 90 min. The authors concluded that high-intensity ultrasounds reduced the fat crystallization time and the crystal size, generating smaller crystals, which were more statistically significant in the sample with $T_c = 28 \text{ }^\circ\text{C}$. In addition, they verified higher viscosities when samples are crystallized

after ultrasound applications. Therefore, the effects of ultrasounds in the freezing and crystallization processes are promising for the dairy industry, especially in the production of ice cream.

IMPROVED HOMOGENIZATION DUE TO US APPLICATION

The main objective of high-pressure homogenization in the dairy industry is to reduce the diameter of fat globules, which inhibits creaming while storing liquid dairy products. However, this process has been reported to affect whey protein denaturation and protein adsorption on the surface of fat globules and to modify the structural properties of casein micelles, altering the functional properties of proteins and their coagulation (Jia *et al.*, 2022). In addition, these conventional homogenizers have high initial and maintenance costs. Homogenizer valves wear out easily and must be replaced periodically, increasing maintenance costs. The ultrasound processes have become an alternative to traditional homogenization processes to avoid this bottleneck. The reduction of fat globules is related to the mechanism of cavitation through the collision of the bubbles in the medium close to the surface of the boundary layer of the phase of the two immiscible liquids. This shock wave efficiently mixes the two layers, forming emulsions with an extremely narrow and highly stable fat-globule-size distribution. In addition, the energy needed to produce an emulsion by acoustic waves is less than that required using conventional methods. Monteiro *et al.* (2018) evaluated the effect of ultrasounds at different ultrasonic densities (0.3 to 3.0 kJ/cm³) on the size of milk fat globules from an unfermented chocolate milky beverage. The authors reported that the distributions of fat globules were influenced by ultrasound processing regardless of the energy density. The samples presented fat globules with sizes between 1 and 10 µm. However, the higher energy density led to greater product homogenization, thus avoiding separating the continuous and dispersed phases. Aernouts *et al.* (2015) also evaluated the effect of ultrasonic homogenization (20 kHz 20, 60, 120, 300, 600, and 1200 s) on the size of fat globules in raw milk. The authors found that the size of fat globules decreased with increasing exposure time to acoustic waves, obtaining globules between

0.8 and 12 μm in size. Furthermore, they reported that the fat globules were too small after the 1200-s ultrasonic homogenization to be observed under an optical microscope. Another study revealed that the ultrasound at 66 W for 15 min reduced the mean diameter of the fat globule from buffalo milk by 92%. Thus, ultrasound treatment is an alternative to traditional dairy homogenization processes.

STERILIZATION PROCESS OF US WAVES

Pasteurization and sterilization are mandatory treatments in Brazil (government regulations) for some products (e.g., Serra da Estrela cheese) in the dairy industry. However, high temperatures can have undesirable nutritional and sensory effects on dairy products; for example, they promote increased ionic calcium in heat-treated milk. This increase in ionic calcium content negatively affects the thermal stability of milk, forcing the dairy industry to add calcium chelators, for example, sodium citrate, EDTA, oxalate, and phosphate salts (Acosta *et al.*, 2023). These chelators stabilize the milk, preventing protein coagulation during heat treatment. However, some studies have revealed that high concentrations of these calcium chelators can form scale in heat exchangers. Because of this, the dairy industry has shown great interest in using gentler processing technologies such as membrane technology (used in France to retain/separate bacteria from milk) and ultrasounds. It was shown that ultrasounds (78 W/8 min, 104 W/4 min, or 104 W/6 min) with 4-s pulses was efficient in inactivating coliforms and *Staphylococcus* spp. in semi-skimmed sheep milk (Balthazar *et al.*, 2018). It was also reported that ultrasound treatment (20 kHz for 10 min) was as effective as the high-temperature short time (HTST) and low-temperature long time (LTLT) pasteurization processes in inactivating food pathogens such as *Brucella melitensis* type 3, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* inoculated in goat milk (Tavsanlı *et al.*, 2021). Another study reported a 2.37 log CFU/mL reduction in total aerobic mesophilic bacteria counts on goat milk with ultrasonication at 20 kHz, 300 W 10 min. The mechanism of microbial death is mainly due to the thinning of cell membranes, localized heating, and production of free radicals. Ultrasound applications are an alternative

to heating processes in dairy products, as effective bacterial inactivation can be achieved in a relatively cost-effective and eco-friendly way. However, prolonged milk exposure to ultrasounds can develop a metallic, burnt taste and a rubbery appearance.

The negative effect of ultrasound application of ultrasound on the processing of foods has evidenced negative impact of heat on thermo-labile compounds such as, vitamins, and pigments. But the application of ultrasound in beverages has also showed healthy benefits such as increase of levels of antioxidants and bioactive compounds. HIU have shown to increase the zeta-potential in milk and the stability of the product.

CONCLUSION

The main finding of this review was the incorporation of ingredients such as prebiotics, probiotics, and postbiotics, which have been of great interest in the food industry in general due to their health benefits. Due to its lower cost, high protein value, and interesting sensory aspects, the addition of whey protein in formulations continues to be used. Ultrasound technologies can reduce ice crystals in ice cream and fat globules in milk using low-intensity and short time, and are efficient in the inactivation of coliforms and *Staphylococcus* spp. In addition, using ultrasounds for a few minutes as a pre-treatment for developing fermented whey and oat beverages resulted in the highest growth levels of *Lactobacillus casei*. Another issue is assessing the correct proportion of ingredients in a reformulated food product. These trends of using new ingredients and ultrasound technologies positively affect sensorial, physicochemical, and nutritional properties of dairy products. Ultrasonication is a long-established system for the preparation of emulsions in sub-micron level and several researchers applied US in to cow's milk. It was found that ultrasonication reduced the size of milk fat globule by 175 nm during the first 5 min of the US treatment at 22.5 kHz and 50 W in bovine milk. Sonoporation is defined as the formation of transient cavities or pores on cell membrane due to sonication. At low level of sonoporation, ultrasound methodology can improve the microbial growth due to: improvement in the permeability of cell membranes, resulting in improved mass transfer of substrates across the membranes

and efficient removal of by-products of cellular metabolism, and acceleration of the supply of oxygen and nutrients for microorganisms. Therefore, the major ultrasonic phenomenon influencing the probiotic cells is the microstreaming, not causing physical damage, but facilitating the mass transfer of gases and nutrients to stimulate the probiotics.

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