



Thermosonication Technology in the Dairy Industry: A Review

Sachin P. Ramteke¹, R. J. Desale², D. H. Kankhare³ and Y. G. Fulpagare⁴

¹Ph.D. Research Scholar, Discipline of Dairy Science, Department of Animal Husbandry and Dairy Science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.H.), India-413722.

E-mail: sachinramtekej87@gmail.com

²Associate Professor, Department of Animal Husbandry and Dairy Science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.H.), India-413722.

E-mail: desaleraahul@yahoo.co.in

³Associate Professor, Division of Animal Husbandry and Dairy Science, College of Agriculture Dhule, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.H.), India-413722.

E-mail: dkankhare2001@yahoo.com

⁴Head of Department, Department of Animal Husbandry and Dairy Science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.H.), India-413722.

E-mail: hodasds@gmail.com

Abstract

Milk processors seek methods to improve the shelf life of fluid milk. Conventional pasteurization destroys all pathogens and many spoilage microorganisms, increases milk shelf life upto 14 to 21 days when bottled and stored under refrigerated conditions but extreme heating of milk can lead to Maillard or caramelization reactions, off-flavors and loss of nutrients. Thus, it is important that any proposed treatment does not compromise the sensory quality and nutritional properties of milk. Thermosonication is a novel and good alternative technique to replace the conventional heat treatment process. It increases the shelf life of the product, making it safe for human consumption without altering its nutritional composition and organoleptic attributes.

Keywords: Thermosonication, milk, dairy processing, ultrasound

Introduction

Milk is a wholesome food which provides a great source of nutrition for the body but it is a highly perishable in the nature. Milk processors and dairy scientists seek methods to improve the shelf life of fluid milk. The shelf life of milk depends on various factors, such as the quality of incoming raw milk, processing time and temperature, survival of spoilage microorganisms, storage temperature, exposure to

light, and post-processing contamination. Conventional pasteurization [i.e., HTST continuous pasteurization and low-temperature, long-time (LTLT) batch pasteurization] destroys all pathogens and many spoilage microorganisms, rendering milk a shelf life of approximately 14 to 21 days when bottled and stored under refrigerated conditions. Ultra High temperature processing, where the milk is heated to 138°C and

held there for at least 2 sec, can extend milk's shelf life to at least 60 days at room temperature. However, extreme heating of milk can lead to Maillard or caramelization reactions (Clare *et al.*, 2005), off-flavors and loss of nutrients. According to Silva and Gibbs (2010), psychrotrophic *B. cereus* appears to have a higher heat resistance than psychrotrophic non-proteolytic *C. botulinum*. Thus it is important that any proposed treatment does not compromise the sensory quality and nutritional properties of milk. The use of non-thermal technologies has emerged as an alternative to minimize changes of the food sensory properties induced by heating. The inactivation of bacteria using ultrasound was first initiated in 1920 (Harvey & Loomis, 1929). This technology relies on the application of ultrasonic waves (frequency ranging from 20 to 100 kHz) to the liquid food, causing microbial cell death.

The effect of ultrasound alone has been considered ineffective for the inactivation of bacterial spores (Butz & Tauscher, 2002). Hence, the combination with other treatments such as temperature, pressure, or both heat and pressure to increase the lethal effect has been investigated.

What is Thermosonication ?

Thermosonication is one of the methods of ultrasound technology, which combines moderate heat of 37 to 75 °C with low frequency ultrasound waves (20 kHz) treatment to enhance inactivation of enzymes and pathogenic microorganisms (Lee *et al.*, 2013). When

heat and ultrasound is used together, the process temperature and time is considerably reduced (16 to 55%) compared to the conventional heating process.

Principles of sonication process

The Sonication process described by Altaf *et al.*, (2018) that, longitudinal waves with a frequency higher than 20 kilohertz are created. When a sonic wave passed through a liquid food material (medium), regions of alternating compression and expansion of the medium particles are created. These regions of pressure change cause cavitation in the medium. Cavitation is the process whereby micro bubbles or cavities filled with vapors are formed. Sonication causes tiny bubbles to expand and contract thousands of times every second. A point is reached where the ultrasonic energy provided is not sufficient to retain the vapour phase in the bubble; therefore, rapid condensation occurs. The condensed molecules collide violently, creating shock waves. These shock waves create regions of very high temperature and pressure, reaching 5,000 degrees Kelvin and 2,000 bar pressure and finally bubble collapses. Cavitation can result in the occurrence of micro-streaming which is able to enhance heat and mass transfer in the milk, because of streaming, the bulk milk is treated results in a localized pasteurization effect. Destruction of microorganisms or inactivation of enzymes can be induced by one or more of these consequences of sonication. Microbial killing involves the thinning of the cell membranes and localized heating. Cavitation depends on ultrasound characteristics (e.g. frequency, intensity), product properties (e.g. viscosity, surface tension) and ambient conditions (e.g. temperature, pressure).

Fig 1. Sonication process

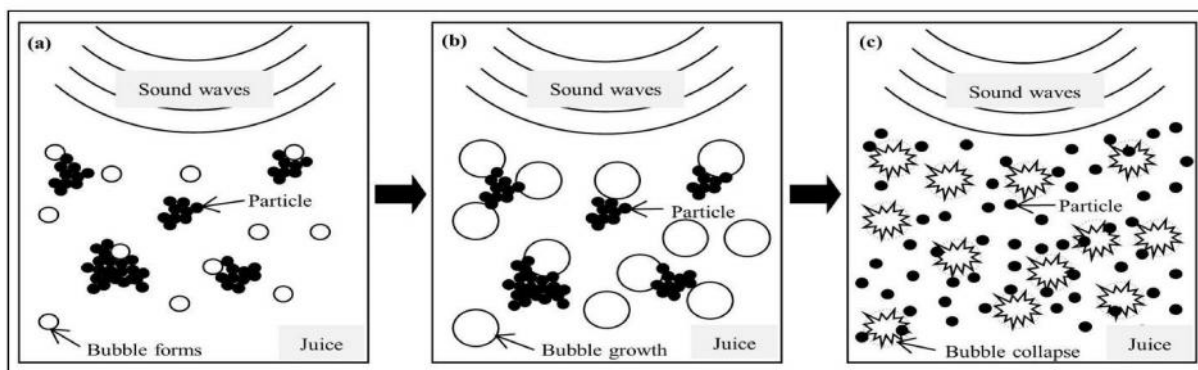


Fig. Cavitation phenomenon. (a) bubbles formation in juice (medium) by sound waves; (b) bubbles growth to the maximum size and (c) bubbles collapse, and particle dispersion and cell disruption (Abdulla and Nyuk Ling Chin, 2014)

Applications of Thermo-sonication in Dairy Industry

- 1) Microbial inactivation
- 2) Enzyme inactivation
- 3) Homogenization
- 4) Development of Yoghurt
- 5) Preparation of cream cheese
- 6) Emulsification

Microbial inactivation

Pasteurization as a thermal processing technology has long been used for microbial inactivation to ensure safety and extended shelf life of a variety of liquid foods. Traditional thermal pasteurization methods, however, adversely affect the sensorial quality and nutritional values of the processed foods. For this reason, in the last decades the study of nonthermal processing has gained growing interest within the food technologists' community, looking for alternative treatments able to guarantee food's microbiological safety along with its nutritional and sensory quality. Thermo-sonication process increases the shelf life of the product, making it safe for human consumption without altering its nutritional composition and organoleptic attributes.

Thermophilic spore-forming microbes are organisms capable of growing from 40-65°C and are often present during the manufacture of milk powder (Scott *et al.* 2007; Hill and Smythe 2012). thermophilic spore-forming microbes is their capability of producing acids, lipases, and proteases, causing spoilage and decreased quality in dairy products (Hill and Smythe 2012). *Listeria monocytogenes* has been reported as one of the most common pathogenic

microorganisms related to foodborne outbreaks in the dairy industry. This microorganism is a short rod that can grow under aerobic and microaerophilic conditions. The optimum temperature for growth is 35–37 °C, but it can also grow under refrigerated conditions, over a wide range of pH (4–9), and with salt concentration upto 10%. The use of sound waves in liquid media to disrupt cells, either alone (sonication), or in combination with heat (thermo-sonication), is an alternative approach to the inactivation of such bacteria.

The lethal effect of thermo-sonication is not the same for all microorganisms because the efficiency of microbial inactivation is also influenced by their morphological differences such as type, shape or diameter of the microorganisms. Generally, cavitation of sonication is more effective on gram-positive bacteria, spores, spherical-shaped and small round cells. Gram-positive bacteria are more resistant to ultrasound than gram-negative because of their thicker cells wall that give them a better protection against ultrasound effects. Differences in cell sensitivity could be caused by the more tightly adherent layer of peptidoglycans in gram-positive cells. Bacterial spores and fungi are more resistant to ultrasound than vegetative bacteria. Spores are more difficult to be destroyed than vegetative cells which are in phase of growth. The cocci or spherical-shaped cell is more resistant to ultrasound than bacilli or rod-shaped cell because of the relationship of cell surface and volume. Cells with larger surface area are more sensitive to ultrasound than the small and round cells. In summary, the effectiveness of ultrasound in treating the microorganisms is different depending on the species and its cell wall structures.

Table 1. Microorganism's morphology and its lethal effect of thermo-sonication

Microorganism	Shape	Cell wall Structure	Frequency (kHz)	Time(min)	Temperature (°C)	Microbial reduction	Reference
<i>Salmonella spp.</i>	Bacill	Gram negative	24	2-10	52-58	5 log	Chemat <i>et al.</i> , (2011)
<i>S.boydii</i>	Bacill	Gram negative	22.3	10	37	5 log	Ugarte-Romero <i>et al.</i> , (2007)
<i>L.monocytogenes</i>	Bacill	Gram positive	22.3	2.5	65	5 log	-do-
<i>S.aureus</i>	Cocci	Gram positive	30	20	55	3.3 log	Walkline-Ribeiro <i>et al.</i> , (2009)
<i>E. coli</i>	Bacill	Gram negative	20	3.8	59	5 log	Lee <i>et al.</i> , (2013)

Bermúdez-Aguirre (2009) studied the inactivation of *Listeria innocua* and mesophilic bacteria in raw whole milk. Five systems were evaluated in an ultrasonic processor (24 kHz, 120 μ m, 400 W). Tested amplitudes of ultrasonic waves were 0, 40, 72, 108 and 120 μ m, with a constant temperature of 63 °C and treatment time of 30 min. After 10 min of treatment, thermal pasteurization achieved a 0.69 log and a 5.3 log-reduction after 30 min. However, after using ultrasound at 60, 90 or 100% in combination with temperature, a 5 log-reduction was obtained after 10 min. Inactivation of mesophilic bacteria was similar to those for *Listeria*. As intensity of thermo-sonication increased, pH was slightly lower (6.64), acidity was increased (0.141%), and color of samples was whiter (92.37). Thermo-sonication is an emerging technology that has shown positive effect in bacterial inactivation, reducing treatment times considerably. Thermosonicated milk has better colour and similar physicochemical characteristics compared to conventional pasteurized milk, but the processing time is shorter. He concluded that Industrially, thermo-sonication seems to be a viable option for pasteurization of milk; however, there are advantages to using conventional pasteurization in combination with ultrasound, as the results in this manuscript show. The possibility of using HTST processing in addition to ultrasound is also feasible, which could reduce treatment times even more. Vijaykumar (2012) Studied the effects of thermosonication on proteases and characteristics of milk and cream. He observed that thermosonication at 133 and 152 μ m (p.p) for 1 and 3 min completely destroyed coliforms and destroyed over 99% of the total aerobic bacteria. The total aerobic bacteria count of thermosonicated skim milk and cream samples were less than 20,000 cfu/mL on day 30. Gabriela and Raúl Avila-Sosa (2012) *Listeria innocua* inactivation by thermo-sonication along with vanillin was investigated. High-intensity/low-frequency ultrasound (20 kHz/400 W) at selected wave amplitudes (60, 75, or 90 μ m), temperature (40, 50, or 60 °C) and vanillin (200, 350, or 500 mg/kg). The combination of ultrasound, vanillin, and temperature increased the inactivation rate and decreased inactivation time of *L. innocua*. In general, a multi-target inactivation effect was observed at a temperature range from 45 to 55 °C, achieving four log-cycle reductions of *L. innocua*. He concluded that further studies are needed in order to scale up thermo-sonic pasteurization to an industrial level. Beatty (2016) Studied the effects of thermosonication (high intensity ultrasound coupled with thermal treatment), on the reduction of thermophilic spore-

forming microorganisms (*Geobacillus stearothermophilus*) and its effects on the solubility index in reconstituted skim milk powder (RSMP) were evaluated. Thermosonication yielded a significantly higher level of microbial destruction for both vegetative cells and spores than heat treatment alone. The solubility of RSMP treated with HIU did not significantly differ from the solubility of RSMP not treated with HIU. All research findings and observations suggest HIU, or thermosonication, to be a successful method for reducing microbial populations during milk powder processing without sacrificing skim milk powder solubility.

Enzyme inactivation

Enzyme may be defined as proteinaceous substance that catalyses a biochemical reactions. There are desirable and undesirable enzyme found in milk.

Desirable Enzyme in milk

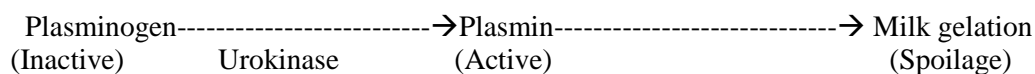
Lactoperoxidase, sulphhydryl oxidase, superoxide dismutase help to protect the quality.

Undesirable Enzyme in milk

Lipoprotein lipase, protease, acid phosphatase and xanthine oxidase cause milk quality deterioration, while alkaline phosphatase, which is inactivated at conventional pasteurization temperature, serves as an indicator for proper pasteurization. In addition of indigenous enzymes in milk, enzymes are also produced by the microorganisms like *Pseudomonas* spp., *Bacillus* spp. (Vijaykumar, 2012). These microorganisms produce enzymes such as proteases, lipases etc. which are heat resistant and cause spoilage of milk.

Shelf-life Limiting Proteases

Proteases are one of the major types of enzymes that limit the shelf-life of fluid milk, apart from lipoprotein lipase, acid phosphatase and xanthine oxido-reductase (Fox and Kelly, 2006). Proteases hydrolyze milk proteins into various peptides and one result of these cleavage reactions is age gelation. The peptides are also responsible for bitterness, which often accompanies flocculation of aged milk. Proteases can be indigenous (the plasmin system) or produced by microorganisms that survive milk pasteurization or are postpasteurization contaminants.



Enzyme inactivation

Plasmin (A fibrinolysin that is secreted into the milk from blood plasma through the mammary cells) is heat stable and survives pasteurization; UHT processing can only reduce plasmin activity but cannot completely inactivate the enzyme (Newstead *et al.*, 2006). A minimum of 30% of the activity of plasmin exists in milk after UHT processing (Alichanidis *et al.*, 1986). Heating to 120°C for 15 min can completely inactivate plasmin in milk (Chen *et al.* 2003) and heating to 85°C for 5 min can completely inactivate plasmin in phosphate buffer of pH 6.6 (Borda *et al.*, 2004). This implies that plasmin inactivation depends on the media the enzyme is present in. Similar to plasmin, proteases produced by the psychrotrophic and thermophilic bacteria that survive pasteurization are heat stable. Proteases produced by *Pseudomonas* sp. are stable to pasteurization and UHT processing. Heat stable native and microbial proteases greatly limit the shelf-life of pasteurized milk and ways to inactivate these enzymes are being extensively investigated by scientists. Emerging technologies such as thermosonication is being explored to investigate their potential to inactivate the shelf-life limiting enzymes in milk, particularly proteases.

Vijaykumar S. (2012) evaluated the effects of combined heat and ultrasound on the activity of *Staphylococcus aureus* protease and total plasmin, as well as the impact on sensory properties of milk and cream. The samples were heated at 60 °C and

Sonication at 133 µm (p.p) for 2.5 min was applied, results showed that ultrasound, when combined with heat, is capable of decreasing the activity of *Staphylococcus aureus* protease in skim (by approx. 72%), reduced-fat (by approx. 92%) and whole milk (by approx. 92%) and total plasmin activity in fresh skim milk (by approx. 81 to 94%) and cream (by approx. 96%). He concluded that Ultra-high temperature processing inactivates proteases but detrimentally affects milk's sensory quality, thermosonication did not induce off-aromas or viscosity changes, but inactivated microorganisms and protease enzymes, thermosonication may be an appropriate alternative to pasteurization.

Homogenization

Homogenization is refers to the process of forcing the milk through the homogenizer with the object of subdividing the fat globules for the better product development. The size of fat globules and the composition of the membrane are of tremendous importance in the technical and sensory properties of dairy products. Stabilization of fat, creamy texture and resistance of milk to oxidized flavour are some of the advantages of the homogenization process, which consists of pumping the milk at high pressures through small eddies at pasteurization temperatures. Fat globules in milk vary in diameter ranged from 0.1 to 22 microns (De 2017). Thermosonication is responsible for the breakage of fat globules in milk.

Table 2. Milk fat globules size of different animal species

Animal Species	Average size of fat globules(µm)	Reference
Buffalo	8.7	El-Zeini (2006)
Cow	3.78	El-Zeini (2006)
Goat	2.99	El-Zeini (2006)
Sheep	3.76	El-Zeini (2006)

The main mechanism of action in ultrasonication technology is called cavitation, a phenomenon that can be either stable or transient. Stable cavitation is associated with the small bubbles dissolved in a liquid, while transient cavitation occurs when the bubble size changes quickly and collapses and as a result produce very high pressure (100 MPa) and high temperature (5000 K). Ultrasonication at higher temperatures (between 70° and 75°C) resulted in a more homogeneous particle distribution than heat or

sonication alone (Villamiel and Jong, 2000). High-power ultrasound (450 W) is capable of homogenizing fluid milk better than commercial homogenization (Wu *et al.*, 2001). Raw whole milk was thermosonicated (400 W, 24 kHz, 120 µm amplitude), using a 22-mm probe at 63 C for 30min. Heat treatment involved heating the milk at 63 C for 30 min. The results showed that the surface of the fat globule was completely roughened after thermosonication.

The overall structure of milk after sonication showed smaller fat globules (smaller than 1 μm) and granular surface (Bermúdez-aguirre *et al.*, 2008). This was due to the interaction between the disrupted MFGM and some casein micelles. Minor changes in the aspect of the globules between thermal and raw milk were detected. The advantages of thermosonicated milk are that it can be pasteurized and homogenized in just 1 step, it can be produced with important cost savings, and it has better characteristics, making thermosonication a potential processing method for milk and most other dairy products. Vijaykumar S. (2012) observed in his studies that thermosonication decreased the fat globule size in both skim milk and cream and the homogenizing effect increased with increase in treatment time.

Thermosonication for Yoghurt development

A key aspect of quality in yoghurt is associated with the physical properties of the yoghurt gel which should possess a smooth textural character in the mouth during consumption, as well as a low tendency to serum separation during storage. Such features are promoted by pre-culture homogenisation of fat globules and by a relatively severe (e.g. 90–95 $^{\circ}\text{C}$ for 10 min) pre-inoculation heat treatment of milk, which results in fairly extensive denaturation of whey proteins. Technology, which could be of benefit in the manufacture of cultured milk products, such as yoghurt, is ultrasonication, since it offers the possibility of combining homogenisation and pasteurisation of milk in a single operation prior to inoculation with starter culture. Yoghurt produced from thermosonicated milk (skim, reduced or full fat) had superior physical properties, illustrated, for example, by higher viscosities and greater water-holding capacities (Riener *et al.*, 2010). Study was undertaken to compare additional quality aspects (rheological, sensory and nutritional) of TS-derived and conventionally produced yoghurt. Thermosonication (TS) of preheated (45 $^{\circ}\text{C}$) milk (0.1%, 1.5% and 3.5% fat) for 10 min at an ultrasound frequency of 24 kHz allowed the preparation of

yoghurts with rheological properties superior to those of control yoghurts produced from conventionally heated milk (90 $^{\circ}\text{C}$ for 10 min). Texture profile analysis and flow curves showed that yoghurts from the TS milks had stronger gel structures which displayed higher water-holding capacities (WHC) and lower syneresis. Based on averaged data from a sensory panel (n = 30), TS yoghurts showed superior texture and colour properties and samples with a fat content of 0.1% scored best in terms of overall acceptability. Retentions of water-soluble (thiamine and riboflavin) and fat-soluble (retinol and tocopherol) vitamins were similar in TS and conventionally prepared yoghurts.

Preparation of cream cheese

Thermosonication (TS) of the milk reduced the size of milk-fat globules and increased the fat content and yield of cream cheese. Cream cheeses were made with milk sonicated at varying combinations of ultrasound power (0–100 W), temperatures (4–63 $^{\circ}\text{C}$) and times (0–30 min). The TS of the milk improved the thermostability of the cream cheese significantly. The best textural and rheological properties of cream cheeses were observed in those cheeses made with milk TS at 50W for long times (630 min) and applying temperatures between 35 and 50 $^{\circ}\text{C}$ (Almanza-Rubio, 2016).

Emulsification

Emulsification is the process of mixing two immiscible phases (e.g., oil and water) with the aid of a surface active agent (emulsifier) into homogeneous dispersion or emulsion. The process requires an energy input by means of mechanical agitation or ultrasonication to facilitate the formation of small droplets. With ultrasonication, cavitation releases high energy micro-jets near interfaces and facilitate emulsification. Compared to mechanical agitation, the use of ultrasound requires less amounts of surfactants and produces smaller and more stable droplets (Altaf, 2018).

Difference between thermosonication and conventional pasteurization

Thermosonication	Conventional pasteurization
• Retained nutritive value	• Loses nutritive value
• More energy efficient	• Energy consuming
• Effective against all microbes	• Not effective against all microbes
• It can pasteurized and homogenized milk in just one step	• Only pasteurized milk
• Shelf life of milk increases upto 42 days	• Shelf life of milk increases upto 21 days

Conclusion

The combination of low frequency ultrasound with mild heat will help in reducing processing temperature and time by 16 and 55%. Thermosonication can prove to be a cost efficient technology as the process temperature is reduced due to the use of ultrasonication when compared to the conventional heating techniques. Thermophilic bacteria and spores could be easily inactivated with the help of thermosonication. Yoghurt produced from thermosonicated milk results in superior physical properties, higher viscosity greater water-holding capacities, superior texture, colour properties and best in terms of overall acceptability in comparison to conventional heat treatment. Thermosonication is a promising pretreatment method for shelf-life extension as an adjunct to pasteurization. However, more studies are needed to understand the commercial feasibility of the process. If thermosonication is seen to bring about the desired effects in milk, then it can be used as a commercial method to treat and homogenize milk in the future.

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