



Review Article

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Mano-Thermo-Sonication in Food Preservation

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ABSTRACT

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Food preservation is a process by which food materials are prevented from getting spoilt in order to retain in their best desirable condition for a long period of time. A major key mechanism involving food preservation is destruction or inactivation of spoilage microorganisms and/or enzymes. There are different types of emerging food preservation techniques and mano-thermo-sonication is one among them. Mano-thermo-sonication (MTS) is a food preservation technology that efficiently combines the effects of pressure, heat and ultrasonic waves at an optimal level to reach the desired levels of food stability and safety while ensuring minimum negative effects on quality of food material. It is a developing technique proved for its antimicrobial action and enzyme inactivation preventing food spoilage without altering organoleptic properties of foods subjected to it. The following review encompasses the research based findings and facts that support manothermosonication (MTS) as a potential food preservation technique which overcomes the deleterious effects of severe heat preservation processes on food.

Introduction

“Food preservation includes the processing and handling of food materials to stop or slow down spoilage and thus allow for longer storage”. It includes processing of food to prevent it from undergoing undesirable changes making it further shelf stable. Preservation usually involves preventing the growth of spoilage bacteria, yeasts, fungi and other micro-organisms as well as retarding the action of spoilage enzymes like lipases, proteases etc. Food preservation can also include processes which inhibit sensory deterioration that can occur during food preparation and/or storage. Traditional food

preservation techniques are primarily based on reducing the free moisture content in food thereby dampening the biological processes. Some of these include drying, refrigeration, curing, smoking, pickling, sugaring etc. Pasteurization or heat treatment is the most widely used method of food preservation technique. Recent advancements in non-thermal technologies have shown potential as alternative to conventional heat treatment, being able to inactivate pathogens, spoilage microorganisms and enzymes without the adverse effects on food quality associated with thermal pasteurization. The major drawback of heat is its non-specificity in processing. Heat treatments while inactivating microorganisms

can also modify the nutritional and sensory profile of foods in undesirable manner. Therefore, food industry is currently looking for alternative and more specific preservation techniques, which besides ensuring the stability and safety of foods, will not greatly modify their quality. During non-thermal processing, the temperature of foods is kept considerably less than the temperature employed in conventional thermal processing; therefore, least degradation of food quality is likely. However, non-thermal technologies must not only improve food stability but also augment safety levels, when compared with other procedures or techniques they replace. This approach has led to combining non-thermal methods of food preservation techniques with conventional or thermal processes which helps to reduce the severity of treatments needed to obtain a required level of safety. This combination can possibly augment the lethal potency of processing on microbes and/or prevent the proliferation of survivors following treatment. Manothermosonication is a combined preservation technique now gaining importance in food industry.

Mano-thermo-sonication

Manothermosonication (MTS) combines and synergises the ultrasound with moderate temperature and pressure in order to inactivate enzymes and/or micro-organisms. This technique has seen convincing developments in past three decades for food preservation owing to its ability to inactivate microorganisms and endogenous enzymes while retaining nutrients and flavour (Butzet *et al.*, 1995). Harvey and Loomis (1929) first documented the lethal effects of ultrasound on living organisms and since then, its use has been continuously recommended for disinfection and food preservation (Paci, 1953; Jacobs and Thornley, 1954; Boucher, 1980; Gaboriaud, 1984). Sound waves having

frequencies > 20 kHz are considered as ultrasounds and in context of food preservation, upper limit is usually taken to be 5 MHz in gases and 500 MHz in liquids and solids. The first studies on high hydrostatic pressure (HHP) preservation of foods were conducted in early 1890s(Hite, 1899).

It was demonstrated that microbial inactivation with ultrasound increases when treatment is applied under pressure (Manosonication, MS) (Raso *et al.*, 1998b). The lethality of ultrasound under higher static pressure was reported to be remarkably greater within a given pressure range (0 to 300 kPa). The application of MS treatment simultaneously with heat (Manothermosonication) surges the microbial inactivation manifolds. A major advantage of using MTS is a higher extent of specificity as acoustic energy is absorbed specifically at the interface of membranes causing targeted heating (Floros and Liang, 1994). This heating effect has also assumed to be responsible for increasing the permeability of the living membranes resulting in complete loss of their selectivity. For instance, significantly increased rate of diffusion of sodium ions through living frog skin under ultrasound was demonstrated by Lehmann and Krusen (1954).

Since, MTS is undertaken at comparatively lower temperatures than conventional thermal processes, a product with heat sensitive components can be treated. The local molecular temperature, however, is rising during the treatment. Therefore careful temperature control is required. Also, the treatment time is actually longer during the destruction and/or inactivation of micro-organisms and/or enzymes varying with product to product, which may cause high-energy requirement (Burgos, 1998). Thus, MTS is an emerging technology that efficiently exploits the effect of heat and ultrasonic waves synergised by pressure.

Mechanism

In MTS, major role of micro-organism and enzyme inactivation is played by ultrasound and temperature while pressure acts as a synergising energy that helps to optimize the overall intensity of the process. Here we can consider the individual effect of pressure, ultrasound and heat in order to understand their combined outcome. Ultrasound is defined as sound waves with frequencies above that of human hearing (typically higher than 18 kHz). These waves can be propagated in liquid media as alternating compression. If ultrasound has sufficient energy, cavitation takes place in the medium. This phenomenon involves the formation, growth, and sudden collapse of microscopic bubbles. These collapsing bubbles deliver very high temperatures (approximately 5000 K) and pressures (estimated at 50000 kPa) momentarily to the liquid media (Suslick 1988; Sala *et al.*, 1995). High pressure results in physico-chemical changes, often leading to longer shelf life. High pressure destroys the cell membrane function, leading to cell leakage.

Thus, summing up the mechanism of MTS, the ultrasound generates the cavitation or bubble implosion in the media. These implosions can cause inactivation and/or destruction of micro-organisms and enzymes. The simultaneous pressure treatment maximizes the intensity of the explosion, which results in greater levels of inactivation. The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and production of free radicals (Piyasena *et al.*, 2003). Very strong shaking of molecules takes place causing breakage of bonds. This result in liberation of dipicolinic acid and some low molecular weight polypeptides from the cortex of spores of certain bacterial species. Rehydration of the protoplast occurs resulting in loss of heat

resistance. The loss in heat resistance at acidic pH of the medium is also caused by the rehydration of protoplast as a result of cortex degradation (protonization) (Leistner and Gorris, 1997).

Another observed peculiarity of MTS treatment in microbial and enzyme inactivation is that it is a bi-phasic process i.e. a faster rate of inactivation in initial stages of treatment followed by decrease in inactivation for remaining course of treatment (Lopez *et al.*, 1994; Lee *et al.*, 2009). Although it is an established fact that efficacy of MTS treatment increases with increase in temperature but after a certain temperature increase (boiling point of the medium), the lethality of MTS has been observed to decrease (Lopez and Burgos, 1995; Vercet *et al.*, 1997; Kuldiloke, 2002). A possible reason for this weakened effect could be decreased intensity of bubble implosions because of elevation of the water vapour pressure inside the bubble with rise in temperature (Vercet *et al.*, 1997). It has already been proved that the effect of heat and ultrasonic waves in enzyme inactivation combines synergistically. This is actually derived from the result that the inactivation rate of combined treatment is larger than the sum of the rate of inactivation by ultrasound at room temperature and the rate of inactivation by simple heating. Microbial and enzyme inactivation by MTS follows first order kinetics and there are a number of models developed by researchers (Lopez *et al.*, 1994; Mañas *et al.*, 2000; Chen and Hoover, 2004; Gómez *et al.*, 2005 a, b; Álvarez *et al.*, 2007) to predict the process requirements that could interest the industry.

MTS in microbial inactivation

The decimal reduction time (D value) of *Yersinia enterocolitica* has been reported to decrease eight times after mano-sonication treatment (600 kPa, 150 µm). It has been

reported that MTS treatment reduced heat resistance of *Staphylococcus aureus* by 63 per cent (Ordoñez *et al.*, 1987) and that of *B. subtilis* by 43 per cent (Garcia *et al.*, 1989), as compared to their heat resistance at the same temperatures. Pagan *et al.*, (1999) investigated the effect of MTS (200 kPa, 117 μ m, 62 °C for 1.8 minutes) on heat-shocked and non-heat-shocked cells of *Listeria monocytogenes* and reported maximum levels of inactivation under MTS treatment as compared to thermally treated samples. Piyasena *et al.*, (2003) reviewed the possibility of ultrasound in microbial inactivation and suggested manothermsonication to be most effective among non-thermal preservation techniques.

Lee *et al.*, (2009) investigated the effect of MTS (20 kHz, 124 mm amplitude) at 40, 47, 54, and 61 °C and 100, 300, 400, and 500 kPa, on inactivation of *E. coli* in phosphate buffer (0.01 M, pH 7). They reported that the combination of lethal factors (heat, ultrasound and pressure) significantly shortened the exposure time necessary to attain a 5 log reduction. Investigations on other spore forming bacteria (*B. cereus*, *B. coagulans* and *B. stearothermophilus*), non-spore forming bacteria (*Aeromonas hydrophila*) and yeasts (*Saccharomyces cerevisiae*) show that lethality of MTS treatment was greater (5 to 30 times) than that of the corresponding heat treatment at the same temperature (Raso and Barbosa-Canovas, 2003).

The lethal effects of MTS treatment has been found to be additive in case of vegetative cells but on the other hand, a synergistic effect has been observed in case of spore of *Enterococcus faecium* and *B. subtilis* (Raso *et al.*, 1998c; Pagan *et al.*, 1999). Also, there is a direct correlation between the rate of microbial inactivation to the amplitude of ultra-sound and pressure for all microorganisms (Pagan *et al.*, 1999).

In order to optimize suitable MTS parameters with maximum 3 log reduction of *Listeria inocula* in milk based smoothie, Palgan *et al.*, (2012) conducted a study where, MTS (200 K Pa, 35°C) treatment was varied at different levels of amplitude (50, 75, 100 %) and residence time (2.1, 1, 0.7 min). It was reported that increase in residence time and amplitude significantly reduced the microbial count ($p < 0.001$) as compared to the untreated control samples. In another study Guzel *et al.*, (2014) evaluated the effect of MTS on inactivation of *Listeria monocytogenes* and *Escherichia coli* in acidic fruit juices like that of apple and orange. Variation in MTS (110 μ m amplitude, and 200 KPa pressure) was applied at different temperatures (50, 55, 60°C). It was concluded that MTS could be a credible alternative to existing pasteurization treatments for fruit juices as the combination of ultrasound and heat treatment synergistically inactivated *L. monocytogenes* STCC 5672 and *E. coli* O157:H7.

Kahraman *et al.*, (2017) reported the efficacy of MTS (40, 50, 60°C temperature and 100, 200, and 300 kPa, pressure) in reducing the *E. coli* O157:H7 population in apple-carrot juice to 5 log CFU/g in comparatively shorter time as compared to that of traditional HTST treatment. Along with microbial parameters, MTS was reported to be able to mend the chemical parameters such as antioxidant activity and total phenolic content. It was also noted that increase in temperature not only reduced the microbial population, but also the time required to achieve the same.

Sour cherry juice was mano-thermosonicated at varying levels of amplitude (50, 75, 100%) and temperatures (20, 30, 40°C) at different time intervals (2, 6, and 10 min) at a constant frequency of 20KHz by Turken and Erge (2017). The results revealed the positive influence of MST on reduction of *Escherichia coli* O157:H7 by temperature and treatment

time ($p < 0.05$) along with phenomenal increase in total monomeric anthocyanins and antioxidant capacity of juice. In another study conducted by Zhu *et al.*, (2017) on blueberry juice, it was reported that MTS (560 W, 5 min, 40 °C/350 MPa, 40 °C) for 5, 10, 15, 20 minutes was highly significant in inactivating *Escherichia coli* O157:H7 and reducing their population by 5.85 log.

MTS in enzyme inactivation

MTS has been demonstrated to be very effective in inactivation of enzymes associated with food spoilage which otherwise endure the conventional thermal treatment. This method can significantly decrease the activity of many enzymes like pectin esterase (PE) enzyme of various fruit juices at the moderate pressure (100-300 kPa) and temperature below 100°C. Kuldiloke (2002) achieved almost complete inactivation of PE (94 per cent inactivation at 70°C, 300 kPa, 2 min and 96 per cent inactivation at 80°C, 200kPa, 5 min) and also reported that the efficacy of the process depended upon pH, time of exposure, temperature, pressure and amplitude of the ultrasound

Effect of MTS treatment has been tested on food deterioration agents, enzymes and microorganisms mainly on model enzymes and microorganisms. For instance, enzyme inactivation efficacy of MTS treatment has been reported to be considerably greater than that of thermal processing at the same temperature. Some of such reports include greater inactivation levels of polyphenol oxidase (PPO), lipase and protease (Lopez *et al.*, 1994; Vercet *et al.*, 1995; Vercet *et al.*, 1999), soybean lipoxxygenase (Lopez and Burgos, 1995a), horseradish peroxidase (Lopez and Burgos, 1995b), tomato pectic enzymes (Lopez *et al.*, 1998), orange pectin methylestrase (PME) (Vercet *et al.*, 1999) and orange-carrot blend PME (Lyng *et al.*, 2012).

PME is a pectic enzyme present in citrus fruit juices and is responsible for their quality deterioration by objectionable precipitation of cloud particles, thus deactivation of this enzyme is critically required during juice processing. MTS has been proved to be an efficient tool to inactivate other enzymes native to milk such as lipoxxygenase, peroxidase and proteases and lipases from psychrotrophic bacteria (Lopez *et al.*, 1994; Sala *et al.*, 1995; Vercet *et al.*, 1997). Lee *et al.*, (2005) reported that application of heat (72°C) and ultrasound (20 kHz, 117 μ m) simultaneously under moderate pressure (200 kPa) surged the inactivation rate of PME in orange juice by 25 times in buffer, and over 400 times in orange juice. A 10-fold decrease in lysozyme activity was achieved by MTS treatment (117 μ m, 200 kPa, 70 °C) for 3.5 min (Condon *et al.*, 2006). Kuldiloke (2002) investigated the inactivation of PE by MTS in the pressure range 100 to 300 kPa at temperature varying between 40 and 80°C, and ultrasound 20 kHz. Treatment time was 5 minutes. Kuldiloke (2002) described the enzyme inactivation as a first order kinetic model.

Gamage *et al.*, (2007) reported better enzyme inactivation in tomato juice treated with MTS (20 kHz, 2 kg pressure, 117 μ m amplitude at 70 °C for 1 min) as compared to thermally treated (TT) samples. PME activity was found to fall by almost 38 per cent of the initial values in TT samples whereas in MTS-treated tomato juice it was undetected. Thermally treated tomato juice showed no decrease in polygalacturonase (PG) activity whereas MTS treated samples saw 62 per cent inactivation of total PG activity.

Maragoniet *al.*, (1989) investigated the effect of MTS on POD isozymes in tomato and reported that heat and ultrasound play a synergistic role in inactivation of the enzymes and increasing either of the parameters results

in synergistic increase in the process efficacy. Kuldiloke (2002) investigated the effect of MTS treatment on various food enzymes. He reported that the extent of inactivation by MTS increases with increase in temperature in the range of 40-80 °C. Based on lower *D*-values and higher *z*-values of MTS treatment, Kuldiloke (2002) also indicated that MTS could inactivate lemon, tomato and strawberry PE at temperature where thermal inactivation was insignificant. This fact implies that MTS treatment is more efficient at temperature lower than the corresponding thermal treatment. Reason behind this could be the impairment of protection provided to the enzymes by food molecules.

Effects on food material

The application of ultrasonic waves generating cavitation in suspensions, containing enzymes and micro-organisms, has a lethal result and deactivating action (Suslick, 1988). High power ultrasound waves when propagate through a liquid, it causes the pre-existing and newly formed micro bubbles to vibrate at an identical frequency. Increasing acoustic pressure causes the growth and powerful collapse of these bubbles, which is accompanied by a sudden increase of the temperature and the pressure in small local surrounding area. Food preservation by elevated temperature for short period of time is still the most common form of food preservation process (Davies, 1959; Kinsloe *et al.*, 1954; Pagan, 1997; Raso *et al.*, 1998e). In most cases the controls and process variables are derived by first-hand analysis of the effect of time and temperature of exposure on microbial survival kinetics with lesser emphasis on quality of food in relation to effects of heat treatment on food composition and structure. The damage to food quality occurs due to modification of macromolecules, deformation of plant and animal structures and production of new

substances from heat-catalyzed reactions. The non-covalent bonds in proteins, nucleic acids and carbohydrates undergo changes leading to different molecular structures.

Gamage *et al.*, (2007) compared the physical properties of MTS (20 kHz, 2 kg pressure, 117 µm amplitude and 70 °C for 1 min) and thermally treated tomato juice. They reported that the MTS treated samples were superior in terms of higher apparent viscosity (1.6 times) and yield stress values (2.2 times), better consistency (1.9 times higher) and lower flow index.

There have been a number of publications relating to the utility of ultra-sound in food industry. For example, ultra-sound treatment provides better emulsification properties (Mason *et al.*, 1996); aids in better extraction (Stasiak, 2005; Chendke and Fogler, 1975), crystallization (Mason *et al.*, 1996), dehydration (Ensminger, 1988) and freezing (Zheng and Sun, 2006). Dolatowski *et al.*, (2007) has also reported ultra-sonication to improve tenderness of meat which is a highly desired property among consumers.

MTS in dairy industry

From review of literature, it is established that MTS treatment by virtue of its mechanism is best suited for acidic pH products. A few researchers have examined the possible uses of this technology in dairy products. Dolatowski *et al.*, (2007) reported that the use of ultrasound as a processing aid can reduce the production time of yoghurt of up to 40 per cent. Lopez *et al.*, (2002) claimed MTS (20 kHz ultrasound amplitude, 2 kg pressure, and 40 °C for 12 s) as an effective tool for achieving better rheological and physical properties in yoghurt and also reported the treatment to attain a certain level of homogenization.

Halpinet *al.*, (2013) compared the microbial growth profile in raw milk treated with conventional thermal treatment (72 °C, 20 s) and MTS (frequency; 20 kHz, amplitude; 27.9 mm, pressure; 225 kPa) at two temperatures (37 and 55 °C) followed by pulsed electric field (electric field strength; 32 kV/cm, pulse width; 10 µs, frequency; 320 Hz). They reported significantly lower microbial counts in thermally treated samples than in MTS+PEF treated samples. A possible reason for this could be a higher pH conditions prevailing in raw milk as MTS is more effective in lower pH mediums. Condón *et al.*, (2011) claimed to achieve a 99.99 % inactivation of *C. sakazakii* cells (in milk) when treated with MTS (35 °C; 200 kPa; 117 µm for 4 min). They also reported that same level of inactivation could be reached within 1.8 minutes when the temperature is raised to 60 °C.

In conclusion, three of the energies viz. heat, pressure and ultrasound have been known and tested for their individual ability to aid in food preservation. But, there are respective demerits of each technique such as higher power requirement, greater exposure time, food tissue damage, loss of nutrients, rheological changes, incompetence in safety etc. Combined processing with these energies has been researched and gained interest as hurdle technology in past three decades and proved to be very much promising in overcoming or minimizing the detrimental effects on food material while achieving better food safety and stability levels. A higher specificity of MTS is not only confined to micro-organisms and enzymes only, but to the whole cellular structure. Thus, this targeted treatment can be modified in such a way to yield the desired levels of food preservation or processing. MTS has shown to be potential food preservation and processing technique while exhibiting least negative effects on food material which qualifies it to

advance from a lab scale technology to an industrial one. Nevertheless, the studies on food safety as well as the appropriate pre and post-treatment changes need to be investigated in detail.

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