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Review article

Inactivation of microbes using ultrasound: a review☆

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Abstract

Alternative methods for pasteurization and sterilization are gaining importance, due to increased consumer demand for new methods of food processing that have a reduced impact on nutritional content and overall food quality. Ultrasound processing or sonication is one of the alternative technologies that has shown promise in the food industry. Sonication alone is not very effective in killing bacteria in food; however, the use of ultrasound coupled with pressure and/or heat is promising. Thermosonic (heat plus sonication), manosonic (pressure plus sonication), and manothermosonic (heat and pressure plus sonication) treatments are likely the best methods to inactivate microbes, as they are more energy-efficient and effective in killing microorganisms. Ultrasonic processing is still in its infancy and requires a great deal of future research in order to develop the technology on an industrial scale, and to more fully elucidate the effect of ultrasound on the properties of foods.

Keywords: Ultrasound; Thermosonication; Manosonication; Manothermosonication; Bacteria; Microorganisms; Food; Inactivation

1. Introduction

Conventional thermal pasteurization and sterilization are the most common techniques currently used to inactivate microorganisms in food products; however, the demand for new methods that have a reduced impact on the nutritional content and overall food quality is increasing. New preservation techniques have been developed that could eliminate microbial activity while significantly reducing or completely eliminating the amount of heat required. These processes are, for the most part, less energy-intensive and therefore more cost-efficient and environmentally friendly than conventional thermal processing. Thermal processing does kill vegetative microorganisms and some spores; however, its effectiveness is dependent on the treatment temperature and time. Unfortunately, the magnitude of treatment, time and process temperature are also proportional to the amount of nutrient loss, development of undesirable flavours and deterioration of functional properties of food products. Some of the common nonthermal alternatives to conventional thermal processing of foods include pulse-electric field inactivation, microfiltration, pulse-light inactivation, high pressure and ultrasonication.

In recent years, the food industry has discovered that ultrasonic waves have a wide variety of applications in the processing and evaluation of products. From grading beef to sterilization, ultrasound has a number of applications in an increasing number of

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areas in the food industry. In combination with heat, it can accelerate the rate of sterilization of foods, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound over heat pasteurization include: the minimizing of flavor loss, especially in sweet juices; greater homogeneity; and significant energy savings (Crosby, 1982). This paper provides a comprehensive summary of literature recently published on food processing and microbial food safety using ultrasound.

1.1. Ultrasound waves

Ultrasound, in its most basic definition, refers to pressure waves with a frequency of 20 kHz or more (Brondum et al., 1998; Butz and Tauscher, 2002). Generally, ultrasound equipment uses frequencies from 20 kHz to 10 MHz. Higher-power ultrasound at lower frequencies (20 to 100 kHz), which is referred to as ''power ultrasound'', has the ability to cause cavitation, which has uses in food processing to inactivate microbes.

Types of transducers that can accomplish the generation of ultrasonic waves, equipment and their functions are given in details by Povey and Mason (1998). This review will focus on those aspects applicable to microbial food safety.

1.2. Microbial inactivation

The elimination of microorganisms is an area of increasing concern to consumers. Effective microbial destruction is imperative in food processing; a single report of contamination could put the reputation and future success of a manufacturer at risk. In order to minimize the bacterial load of a product, the manufacturer must reduce initial contamination, inactivate any microorganisms present in the food, and implement procedures to prevent or retard the subsequent growth of microbial populations which have not been inactivated (Sala et al., 1995). Ultrasonic irradiation has the potential to be used for the inactivation of bacterial populations.

Conventional methods of bacterial inactivation usually involve thermal treatment (i.e., pasteurization, ultra high temperature). These treatments often result in the formation of undesirable flavors and loss of nutrients. However, in the ultrasound process, cavitation caused by the changes in pressure created by the ultrasonic waves is responsible for the killing of bacteria.

1.3. Mechanism of microbial inactivation

Investigation of ultrasound as a potential microbial inactivation method began in the 1960s, after it was discovered that the sound waves used in anti-submarine warfare killed fish (Earnshaw et al., 1995). The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and production of free radicals (Butz and Tauscher, 2002; Fellows, 2000). During the sonication process, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (Sala et al., 1995). These regions of pressure change cause cavitation to occur, and gas bubbles are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas, causing the bubble to expand. 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 up to 5500 °C and 50,000 kPa. The pressure changes resulting from these implosions are the main bactericidal effect in ultrasound. The hot zones can kill some bacteria, but they are very localized and do not affect a large enough area.

The cavitation threshold of a medium (that is, the minimum oscillation of pressure that is required to produce cavitation) is determined by a number of factors (Rahman, 1999). Among these are dissolved gas, hydrostatic pressure, specific heat of the liquid and the gas in the bubble, and the tensile strength of the liquid. Another extremely important variable is temperature, which is inversely proportional to cavitation threshold. The ultrasonic frequency used must be under 2.5 MHz, as cavitation will not occur above that level (Alliger, 1975).

The effects and efficiency of ultrasound treatment have been studied by a number of different groups. Even though patents exist on ultrasonic bacterial

inactivation systems, Sala et al. (1995) stated that the use of ultrasound by itself in food processing might be a very challenging task. The effectiveness of an ultrasound treatment is dependent on the type of bacteria being treated. Microorganisms (especially spores) are relatively resistant to the effects, thus extended periods of ultrasonication would be required to render a product safe. If ultrasound were to be used in any practical application, it would most likely have to be used in conjunction with pressure treatment (manosonication), heat treatment (thermosonication) or both (manothermosonication). The enhanced mechanical disruption of cells is the reason for the enhanced killing when ultrasound is combined with heat or pressure. Pioneering work in this area was done by Ordonez et al. (1984) using ultrasound of 20 kHz and 160 W combined with temperatures ranging from 5 to 62 °C. The combination of heat and ultrasound was much more efficient with respect to treatment time and energy consumption compared to either treatment individually (Ordonez et al., 1984). McClements (1995) also suggested that inactivation of microbes using ultrasound is effective when used in combination with other decontamination techniques, such as heating, extremes of pH or chlorination.

Sonication, manosonication, thermosonication and manothermosonication processes have been studied by several researchers (Manas et al., 2000a; Miles et al., 1995; Raso et al., 1998a; Ordonez et al., 1984). Raso et al. (1998a) explained these processes in detail. Temperature control during ultrasonication experiments was achieved by dissipating excess heat evolved during ultrasonication, using cold water circulated through the cooling coil that was placed inside the treatment chamber. Pressure was supplied by a nitrogen cylinder and was monitored by a manometer that was placed in the treatment chamber. The temperature and pressure were regulated independently inside the treatment chamber.

Other factors that are known to affect the effectiveness of microbial inactivation are amplitude of the ultrasonic waves, exposure/contact time, volume of food being processed, the composition of the food and the treatment temperature (USDA, 2000).

As mentioned previously, success with ultrasound in the inactivation of bacteria is dependent on the organism being treated. Research has been done on *Listeria monocytogenes*, a number of strains of *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and some other microorganisms.

1.3.1. L. monocytogenes

Several reports have indicated that *L. monocytogenes* can be inactivated by ultrasound combined with other treatments. Ultrasonic treatment (20 kHz and amplitude of 117 μm) at ambient temperature was not very effective on *L. monocytogenes* (Pagan et al., 1999a), giving a decimal reduction time (the time to reduce bacterial activity by 90%, referred to as the *D*-value) of 4.3 min. By combining sonication with an increased pressure of 200 kPa (i.e., using manosonication), the *D*-value of the ultrasonic treatment was reduced to 1.5 min. A further increase in pressure to 400 kPa reduced the *D*-value to 1.0 min. An amplitude increase of 100 μm decreased the resistance of *L. monocytogenes* to manosonication by a factor of 6. Temperatures up to 50 °C did not have any significant effect on inactivation, but once temperatures exceeded this, a considerable enhancing effect was noted. Over the range that the experiments were conducted, the following relationships were developed with respect to amplitude on manosonication inactivation rate (Pagan et al., 1999b):

$$\log D_{ms} = \log D_o - 0.0091 \times (A - 62) \quad (1)$$

where D_{ms} is the decimal reduction time (min) for each manosonication treatment, D_o is the control decimal reduction time (min) for a manosonication treatment at an amplitude of 62 μm, and A is the ultrasonic wave amplitude (μm). With respect to pressure on manosonication, the inactivation rate was related to pressure as:

$$\log D_{ms} = \log D_o - 0.0026 \times p + 2.2 \times 10^{-6} \times p^2 \quad (2)$$

where D_o (min) is defined at an amplitude of 117 μm, 40 °C and ambient pressure, and p is the static relative pressure (kPa).

Pagan et al. (1999a) stated that the temperature at which the cultures were grown affected the heat resistance of *L. monocytogenes*. Cultures that were grown at 37 °C were found to be twice as heat-resistant as those grown at 4 °C; however, the cell growth temperature did not change the effect of

manosonication treatment. Lower pH values resulted in greater inactivation rates, while greater sucrose concentrations increased *D*-values. Pagan et al. (1999b) also found that the *D*-value for *L. monocytogenes* treated by an ultrasonic wave with an amplitude of 117 μm and a frequency of 20 kHz at 64 °C and 200 kPa was 0.34 min. The combined effect of manosonication and heat treatment was additive, rather than synergistic, for *L. monocytogenes*.

Heat shocks, which often aid microorganisms in developing greater resistance to heat treatments, did not affect the response of cells to manosonication treatment (Pagan et al., 1999c). Non-heat-shocked *L. monocytogenes* cells displayed an additive effect of inactivation when exposed to manothermosonication, while a synergistic response to the combined treatment was noted with heat-shocked cells.

It was possible to predict the effect of manosonication treatments on *L. monocytogenes* by the amount of power entering the medium from the ultrasonic transducer (Manas et al., 2000b). This was accomplished by comparing the power consumed by the ultrasound generator (P_E), input power to the transducer (P_T) and the ultrasonic power entering the treatment medium (P_C) with the effect that they have on *L. monocytogenes* treatment with ultrasonic waves. The D_{ms} value for *L. monocytogenes* was found to have a linear relationship with P_C calculated using calorimetric methods. This relationship (at 40 °C, amplitudes of 62, 90 and 117 μm and pressures of 0, 100, 200 and 300 kPa) was represented by:

$$D_{ms} = 8.0 - (1.37 \times P_C) \quad (3)$$

1.4. Salmonella spp.

Salmonella spp. are widely known as food-borne pathogens, due to numerous outbreaks which have occurred over the last few decades, usually involving eggs, poultry, fruits and vegetables. The effect of ultrasound treatment on *Salmonella* spp. has been studied quite extensively.

In an experiment using broiler drumstick skin as the medium, little effect of sonication was noted in *Salmonella* spp. (Sams and Feria, 1991). Pre-chill treatment of skins with sonic waves of 47,000 Hz and power of 200 W showed no reduction of aerobic plate counts (APC) after 0, 7 or 14 days of storage. It was suggested that the lack of effect might be due to irregular skin surface, which may have provided physical shielding to the microbes. It was observed, however, that the APC was greater after a period of 7 days in ultrasonicated samples, but returned to the same levels as the non-ultrasonicated samples after 14 days. Sams and Feria (1991) concluded that this was due to increased nutrient availability that may have been provided by the sonication, which is known to increase the extractability of nutrients.

Chocolate is another potential source of *Salmonella* spp. This product is of concern, since the high sugar content of chocolate might increase the heat resistance of the microorganism. A 4-log reduction in viable cell count was observed when *Salmonella* spp. were subjected to ultrasound of 160 kHz at a power of 100 W for 10 min in a peptone water (Lee et al., 1989). A slight difference was observed between the *D*-values of *Salmonella* Eastbourne (3.0 min) and *Salmonella* Anatum (2.1 min) treated with ultrasound at 100 W and 160 kHz at 5 °C. In the ultrasonic treatment of chocolate inoculated with *S.* Eastbourne under the same conditions, a 26% reduction after 10 min and a 74% reduction after 30 min were observed; however, heat was also generated around the ultrasonic probe (73 °C), which could be responsible for the some of the cell inactivation. The 74% killing effect shows that ultrasound might be useful for the conching process in chocolate manufacture.

The use of chlorine and sonication in combination proved more effective in reducing bacterial populations on broiler breast skin than either treatment alone (Lillard, 1993; Lillard, 1994). Samples that were sonicated in a 0.5-ppm chlorine solution had reduced bacterial counts compared to non-sonicated controls. This implied that sonication aids the sterilizing effect of the chlorine solution. The use of ultrasound alone in reducing the bacterial count in leafy lettuce was also not very successful; ultrasound treatment gave a maximum reduction of 90% compared to 99% by chlorinated (100–200 mg/l) water, and 99.95% by ozonated (0.1–1.5 mg/l) water (Kim et al., 1999). Other studies showed that power ultrasound (32–40 kHz) increased the antimicrobial activity of chlorine against *Salmonella* Typhimurium on lettuce by removing cells attached to the produce (Seymour et al., 2002).

Wrigley and Llorca (1992) examined the use of ultrasonication to destroy *S.* Typhimurium in brain heart infusion broth, skim milk and liquid whole egg. When *S.* Typhimurium was treated for 30 min in brain heart infusion broth, cell numbers decreased by more than 3 log at 40 °C, and by 1 log at 20 °C. In skim milk, a 30-min treatment at 50 and 40 °C resulted in 3.0- and 2.5-log reductions, respectively. The microorganisms were more resistant in liquid whole egg; a maximum of <1-log reduction was found with 30 min of treatment at 50 °C. Wrigley and Llorca (1992) proposed that this was a result of the egg protecting the microorganism from the inhibitory effects of cavitation. This might be due to high viscosity of the liquid whole egg, as cavitation is reduced with increased viscosity (Hülsen, 1999). This study also found that the age of the culture did not play a role in the success of the treatment. Cultures grown for either 3 or 18 h gave similar results in both brain heart infusion broth and skim milk.

Munkacsi and Elhami (1976) found that the treatment of milk with ultrasound resulted in the elimination of 93% of coliforms (from an initial of 2.38 10^4 cfu/ml). However, when milk was subjected to 20 s of UV irradiation after sonication at 800 kHz for 1 min with a power intensity of 8.4 W/cm^2, the death of coliforms was increased to 99%. A possible explanation for this observation is that the fat globule was broken up by the ultrasound, allowing deeper penetration of the UV, thus resulting in a more efficient process.

Salmonella Enteritidis, *S.* Typhimurium and *Salmonella* Senftenberg were investigated for their resistance to heat treatment, manosonication and manothermosonication in liquid whole eggs and citrate phosphate buffer solution (Manas et al., 2000a). With manosonication (117 μm, 200 kPa, 40 °C), *S.* Enteritidis, *S.* Typhimurium and *S.* Senftenberg had decimal reduction times of 0.76, 0.84 and 1.4 min in whole egg and 0.73, 0.78 and 0.84 min in citrate phosphate buffer, respectively. In comparison, the *D*-values at 60 °C were 0.068, 0.12 and 1.0 min for the buffer and 0.12, 0.20 and 5.5 min for the whole egg, respectively. A linear increase in ultrasonic wave amplitude resulted in an exponential increase in the inactivation rate of the manosonic treatment. When manothermosonication (117 μm, 200 kPa, and 60 °C) was attempted, an additive effect of the two other treatments (heat and manosonication) resulted. The most resilient of the *Salmonella* species to heat treatment was *S.* Senftenberg, which could only be reduced by 1/2-log cycle; however, when it was subjected to manothermosonication, a 3-log cycle reduction was attained.

1.5. E. coli

E. coli has been greatly publicized in recent years due to a number of outbreaks that have resulted in loss of life. The effects of ultrasound on *E. coli* in an aqueous medium, using a frequency of 24 kHz with varying intensities, has been examined by Scherba et al. (1991). Significant reduction of the bacterial population was achieved which increased with treatment time. Intensity did not affect the killing rate, as it remained relatively similar for all intensities used.

The separation of *E. coli* from suspensions by concentrating them in a single location using ultrasonication was explored (Miles et al., 1995). *E. coli* (6×10^7 cfu/ml) suspended in a cuvette of 1% milk was treated with a frequency of 2.05 MHz at 360 kPa for 3 min. Separation of the bacteria was identified by the formation of grayish bands in the cuvette. The mechanism of separation is not well established as there are discrepancies between the different theories and published observation (Miles et al., 1995). Coakley et al. (1989) regarded the net force on a particle (bacteria) in a stationary acoustic field to be composed of three components: a radiation force, a viscous force derived from acoustic streaming and a vertical gravitational force. According to these authors, when particles are levitated into bands, these three forces must be balanced, and the net force is zero. By manipulating the frequency and intensity of applied ultrasound, these forces could be balanced so that particles or bacteria clump together and settle in a band near half wavelength of the ultrasound used.

Limaye and Coakley (1998) also concentrated *E. coli* and *Saccharomyces cerevisiae* using 1- or 3-MHz ultrasound waves and obtained 95.5% removal at 4.5 and 11.5 min, respectively. Utsunomiya and Kosaka (1979) noticed that the initial temperature, medium and pH influenced the survival of *E. coli* treated at 700 kHz. When *E. coli* was suspended in saline at an initial temperature of 32 °C, survival of 0.83% and 0.2% were obtained after 10 and 30 min of treatment, respectively; however, at an initial temperature of

17 °C, these values increased to 37.86% and 8.1%, respectively, at the above treatment times. No inactivation occurred in milk. When 10% orange juice was added to milk, only 0.3% survival was found at the pH of 2.6, with 100% survival at the pH of 5.6.

The use of ultrasound to inactivate *E. coli* in biofilms could be beneficial to the food (Johnson et al., 1998; Rediske et al., 1999) and water (Ince and Belen, 2001) industries. Johnson et al. (1998) reported that the combination of 70 kHz with an antibiotic (gentamicin sulfate) reduced the *E. coli* count in a biofilm by up to 97% in 2 h. Rediske et al. (1999) also noticed enhanced killing when the antibiotic erythromycin was added to *Pseudomonas aeruginosa* and treated at a nonlethal frequency of ultrasound (70 kHz). The killing was enhanced by 2 log over the use of the antibiotic alone. The reason for the enhanced killing was mainly due to increased diffusion of antibiotic through the cell membrane as the lipopolysaccharide layer of the outer cell membrane was destabilized by ultrasound. This method has potentially greater application for the removal of microbial biofilms in medical devices than in food processing equipment.

Ince and Belen (2001) observed that the concentration of *E. coli* in deionized water decreased with treatment time at 20 kHz of sonication, and that added solids (ceramic granules, metallic zinc particles, and activated carbon) improved the inactivation of *E. coli*. Activated carbon was the most effective material due to its activated surface, which favored adsorption of the bacteria. It is also important to note that the particles increased cavitation during sonication (Jimenez-Munguia et al., 2001). Ince and Belen (2001) also developed a model to predict the inactivation of *E. coli* during this process,

$$\ln N = N_0 + \frac{k}{m+1} t^{m-1} \tag{4}$$

where, N_0 is initial count, N is the count at time t, k is the rate coefficient, and m is an empirical constant. While this is potentially useful, its application in food industry might be limited, as most liquid foods are more complex than deionized water, and there will be competition from the macromolecules that are found in foods for the activated surfaces. However, if successful, this combined process could be used for clarification and microbial removal where activated charcoal is traditionally used in the clarification processes of liquid foods.

1.6. B. subtilis

An effective antimicrobial action has been observed in a number of studies that involved *B. subtilis*. The effect of heat and ultrasonic waves on *B. subtilis* in three different media (distilled water, milk and glycerol) was examined by Garcia et al. (1989). Treating the spores with ultrasound (20 kHz and 150 W) and then exposing them to heat (at 100 °C) had no greater effect than just simply heating them; however, thermosonication treatment at the above temperature resulted in significant decreases in decimal reduction times for both of the strains of *B. subtilis* (niger-40 and ATCC 6051) in milk and glycerol: 63% (niger-40) and 74% (ATCC 6051) in glycerol and 79% (niger-40) and 40% (ATCC 6051) in milk. The effect of thermosonication with water as the medium resulted in a reduction of the heat resistance of the spores of between 70% and 99.9% over the range of 70 to 95 °C, but diminished significantly as the temperature approached 100 °C (due to boiling). When using manothermosonication between the range of 110 and 112 °C, the heat resistance of *B. subtilis* spores was reduced by 1/10 of that observed under regular heat treatment (Sala et al, 1995).

Comparisons between *B. subtilis* spores treated with manosonication and manothermosonication show that the heat treatment provided by manothermosonication makes the inactivation process more effective (Raso et al., 1998a). Manosonication treatments at 20 kHz and 150 μm at 500 kPa for 12 min were seen to have the most dramatic killing effect. Increases in amplitude resulted in an increase in the lethality of the treatment. Increasing pressure to 500 kPa resulted in increasing microbial inactivation; however, any further increase did not result in greater spore inactivation. In fact, increasing pressure over 500 kPa actually inactivated fewer spores. Raso et al. (1998a) noted that the maximum inactivation pressure of 500 kPa was different than that discovered by Neppiras and Hughes (1964). It was hypothesized that the discrepancy was due to differences in ultrasonic fields, microorganism sensitivity or medium

characteristics such as pH or amount of total solids present in the liquid.

1.7. Other microbial inactivation experiments using ultrasound

While the majority of experiments have focused on those bacteria listed above, other microorganisms have been examined. Palacios et al. (1991) found that the heat resistance of spores of *Bacillus stearothermophilus* was reduced when subjected to ultrasonic treatment. *B. stearothermophilus* released calcium, nitrogen and dipicolinic acid into the medium during ultrasonication. Glycopeptides [Ser (16.8%), Gly (15.51%), Ala (14.45%), Glu (9.615%), Asp (8.84%), Cys (6.74%), Orn (5.39%), Leu (4.62%), Val (4.64%) and others (13.4%)] were also released, as well as some fatty acids, acyl glycerols and glycolipids, but no phospholipids. Palacios et al. (1991) suggested that the high pressure due to sonication affected the permeability of the spore protoplast membrane, resulting in the release of dipicolinic acid, calcium and other low molecular weight substances. It may also have allowed the entrance of water from the external environment, which would have reduced the heat resistance. It was concluded that the reduction in heat resistance was the result of a modification of the hydration state of the spore.

A study on the influence of temperature, amplitude and pressure on *Yersinia entercolitica* by Raso et al. (1998b) showed that the increase in amplitude (at 30 °C and 200 kPa) from 21 to 150 μm reduced the *D*-value exponentially (from 4 to 0.37 min). Increasing pressure at 30 °C and 150 μm from 0 to 600 kPa resulted in a *D*-value decrease from 1.52 to 0.2 min. The magnitude of decrease in *D*-value lessened as pressure increased. The *D*-value was greater as the amplitude of the ultrasonic waves increased. Pressure, when used exclusively, had no effect on heat resistance of the bacteria. Also, above the temperature of 58 °C, the heat and ultrasonic effects appeared to be unrelated, since the *D*-values for heat and manothermosonic treatments were equal. It was also shown that inactivation using ultrasound was not a result of titanium particles eroded from the sonication horn. These experiments showed that inactivation by manosonication was due to only one mechanism: the mechanical disruption of cells resulted in inactivation of the bacteria. Raso et al. (1998b) suggested an equation for predicting *D*-values when using manothermosonication. Assuming that heat and ultrasonic waves affect the medium independent of one another, the *D*-value of manothermosonication can be predicted using the following equation:

$$D_{MTS} = (D_T \times D_{MS})/(D_T + D_{MS}) \quad (5)$$

where D_{MTS} is the decimal reduction time of manothermosonication (min), D_T is the decimal reduction of thermal treatment (min) and D_{MS} is the decimal reduction of manosonic treatment (min).

The inactivation kinetics of *S. cerevisiae* were examined at various temperatures combined with ultrasonication at 20 kHz (Lopez-Malo et al., 1999). *D*-values for heat treatments with ultrasound were significantly lower than those with heat alone, with significant results even at low temperatures. The *D*-value for *S. cerevisiae* at 45 °C with heat treatment alone was 739 min; however, when ultrasound was combined with heat treatment, the *D*-value was reduced to 22.3 min.

Ultrasonic treatment was used in a continuous flow process to inactivate total bacteria in milk and *P. fluorescens* and *Streptococcus thermophilus* in tryptic soy broth (Villamiel and de Jong, 2000). It was found that gram-negative bacteria (*P. fluorescens*) were more susceptible to the ultrasonic treatment than gram-positive bacteria (*S. thermophilus*). This observation was in accordance with those of Hülsen (1999) and Alliger (1975) who reported that gram-negative, rod-shape bacteria were more sensitive than gram-positive, coccus-shaped bacteria. Hülsen (1999) also reported that the best result was observed at a pH of between 6.8 and 7.1 for milk samples; however, effectiveness of the inactivation decreased with increasing viscosity, and increased with increasing pressure up to 3000 kPa. In contrast, Scherba et al. (1991) did not find any differences in resistance to ultrasound between gram-negative (*P. aeruginosa* and *E. coli*) and gram-positive (*S. aureus* and *B. subtilis*) bacteria. Gram-positive bacteria (*S. aureus* and *B. subtilis*) usually have a thicker and more tightly adherent layer of peptidoglycan than gram-negative (*P. aeruginosa* and *E. coli*) bacteria; however, Scherba et al. (1991) argued that this morphological feature did not seem to be a differentiating factor in ranking the

organisms by percent killed by ultrasonic treatment. They proposed instead that the target of ultrasonic damage might be the inner (cytoplasmic) membrane, which consists of a lipoprotein bilayer.

Villamiel and de Jong (2000) reported that continuous-flow ultrasonic treatment could be a promising technique for milk processing. A continuous ultrasonic process has the additional benefits of homogenization as well as lower costs due to low energy consumption when compared to a batch ultrasonic system. Homogenization is traditionally done using a homogenizer as a separate unit operation; however, during ultrasonication, size reduction of fat globules was observed by Villamiel and de Jong (2000).

Scherba et al. (1991) examined the effects of ultrasound at 24 kHz on *S. aureus*, *B. subtilis*, *P. aeruginosa*, the fungus *Trichophyton mentagrophytes* and the viruses, feline herpesvirus type 1 and feline calicivirus. All of the bacteria were affected by the ultrasound with the bactericidal effect increasing with time and intensity. The reduction of bacterial population increased from 68% to 72% for *P. aeruginosa*, from 52% to 76% for *B. subtilis* and from 42% to 43% for *S. aureus* when the treatment time was increased from 2 to 30 min at the a ultrasound power intensity of 3 W/cm^2. The reduction of bacterial population increased from 31% to 78% for *P. aeruginosa*, from 11% to 100% for *B. subtilis* and from 22% to 39% for *S. aureus* when the treatment intensity was increased from 1 to 3 W/cm^2 after 15 min of treatment. Fungal growth was inhibited with ultrasonic treatment, and the virus feline herpesvirus type 1 was significantly affected as well, with reductions of 4.0×10^5 in 60 min related to intensity. The virus feline calicivirus was not affected by ultrasonic treatment.

Lopez-Malo et al. (2001) demonstrated that thermosonication could effectively inactivate *Penicillium digitatum.* Thermal and thermosonication treatments were evaluated to determine optimal pH, water activity (a_w), temperature and ultrasonic amplitudes. At an a_w of 0.99, increasing ultrasonic amplitude and decreasing pH resulted in a decrease in *D*-values. When pH was kept constant, a higher a_w resulted in a lower *D*-value. It was found that when thermosonication was used, lower *D*-values were obtained when compared to heat treatment without ultrasound. The authors did not provide any numerical data for the *D*-values or the composition of the laboratory broth they used.

Inactivation of *P. digitatum* as well as *Aspergillus niger* spores using ultrasonic treatment was studied by Jimenez-Munguia et al. (2001). Boiling chips and air bubbles were added to the treatment medium (Sabourand broth) to determine their effect on the inactivation. In general, *A. niger* (sonicated at 45 °C) showed lower *D*-values than *P. digitatum* (sonicated at 40 °C). Boiling chips and bubbles enhanced the effect of the cavitation from the sonication, reducing *D*-values further. This enhanced cavitation effect could be applied to sonication treatment processes to increase efficiency.

Oulahal-Lagsir et al. (2000) showed that ultrasound (40 kHz for 10 s) could be used to remove the biofilms of the spore-forming bacterium, *B. stearothermophilus*, from stainless steel equipment in a beef processing plant. It was reported that removal of the biofilm by ultrasound was fourfold greater as compared to the swabbing method. Mott et al. (1998) showed that up to 87.5% of *Proteus mirabilis* biofilms formed on water-filled glass tubes could be removed using 20-kHz ultrasound axially propagated along the tube. According to these workers, the axial propagation of ultrasound along the lengths of water-filled tubing offers the prospect of on-line cleaning of systems such as heat-exchanger pipes.

2. Conclusion

Ultrasonic technologies could have a strong presence in the future of the food industry. The use of ultrasound on its own in the food industry for bacterial destruction is currently unfeasible; however, the combination of ultrasound and pressure and/or heat shows considerable promise. The future of ultrasound in the food industry for bactericidal purposes lies in thermosonication, manosonication and manothermosonication, as they are more energy-efficient and result in the reduction of *D*-values when compared to conventional heat treatment.

Ultrasound has been successfully used by the food industry for: the measurement of thickness of pipes, chocolate layers, fat, lean tissues in meat, canned liquids and shell eggs; detection of contaminants such as pieces of metal, glass or wood in foods; measurement of flow rates through pipes; determination of food composition; and measurement of particle size

distribution in dispersed systems. However, further research is required before ultrasound becomes an alternative method of food preservation. Hoover (2000) identified the following research needs for further advancement of this technology: a determination of the effect of ultrasound on microbial inactivation efficiency when used with other processing technologies (high pressure, heat or others); the identification of the mechanisms of microbial inactivation when used in combination with other technologies; the critical process factors when ultrasound is used in hurdle technology; and evaluation of the influence of food properties (such as viscosity and size of particulates) on microbial inactivation. Use of antibacterial agents (natural and artificial), bubble-inducing agents and activated adsorption surfaces may also be explored. Though much work has been conducted on ultrasound technologies in food applications, it is still in its infancy and leaves the opportunity for a great deal of future research in order to produce equipment on an industrial scale.

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