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The Use of Ultrasonic Waves in Food Technology

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Abstract: Heat treatment is the most utilized method in order to eliminate harmful microorganisms and inactivate enzymes in foods. On the other hand, there is a growing interest in searching for methods able to reduce the intensity of the heat treatments needed for food preservation. Ultrasonication is an important non-thermal method which can be combined with heat treatment or can be used as an alternative method to heat. Some of the other industrial applications of ultrasound include texture, viscosity and concentration measurements, composition determination of eggs, meats, fruits and vegetables, dairy products; thickness, flow level and temperature measurements for monitoring and controlling several processes and non-destructive inspection of egg shells and food packages.

Key words: Manothermosonication, non-thermal methods, ultrasonication, food technology, ultrasonic waves

INTRODUCTION

In the food industry, eliminating harmful microorganisms and inactivating enzymes are important for food quality and also for public health. That is the reason why heat treatment is the most utilized method for stabilizing foods because of its capacity to destroy microorganisms and also to inactivate enzymes. However, since heat can alter the organoleptic properties of foods and diminish the contents or bioavailability of some nutrients, there is a growing interest in searching for methods that are able to reduce the intensity of the heat treatments needed for food preservation (Lopez *et al*., 1994).

In order to reduce the detrimental effects of heat treatment, heat is combined with other physical and chemical agents to increase the lethal action; in addition, non-thermal alternatives are being tried. Some of the common non-thermal alternatives to conventional thermal processing of foods include; pulse-electric field inactivation, microfiltration, pulse-light inactivation, high pressure and ultrasonication (Crosby, 1982; Lopez *et al*., 1994; McClements, 1995). Ultrasonication combined with heat can accelerate the rate of sterilization of foods, thus lessening both duration and intensity of thermal treatment and the resulting damage. The advantages of ultrasound over heat treatment include: Minimization of flavor loss, especially in sweet juices; greater homogeneity and significant energy savings (Earnshaw *et al*., 1995). There are many other methods that can be combined with ultrasound other than heat.

SHORT HISTORY OF ULTRASONIC TREATMENT IN THE FOOD INDUSTRY

Ultrasonic irradiation has the potential to be used for the inactivation of bacterial populations. 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).

According to another source; the inactivation of microorganisms by Ultrasonic Waves (UW) was reported in the early 1930s but its scant lethal effect prevented its use as a sterilization method. However, improvements in UW generation technology over the last few decades have stimulated the interest of investigators in microbial inactivation by UW (Pagan *et al*., 1999).

Sanz *et al.* (1985) studied the lethal effects of ultrasound on spore forming bacteria. In 1987, combination of heat and high power UW (20 kHz) was first explored by Ordondez *et al*. (1987) and the term of thermoultrasonication was used. According to this study of Ordondez *et al*. (1987) the inactivation effect of thermoultrasonication was greater than UW at room temperature. More recently, the term Manothermosonication (MTS), which is the combination of heat, pressure and ultrasound, has been coined for the combined treatments (Ordondez *et al*., 1987).

In 1994, a resistometer was designed and built to apply high-power UW under pressure at nonlethal (manosonication) and lethal (manothermosonication)

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temperatures. The results indicated that the rate of vegetative cell inactivation by Manosonication (MS) increased when the static pressure was raised. It was also observed that the inactivation rate by MS increased exponentially with the amplitude of UW (Oagan, 1977; Palacios *et al.*, 1991).

THE APPLICATIONS OF ULTRASONICATION IN FOOD TECHNOLOGY

The use of ultrasonication is still being studied with because of the advent of many applications. Altough the possibility of deactivating enzymes or destroying microorganisms by ultrasound waves, alone or in combination with other physical treatments, has been widely used in for laboratory, the same is not true for industry. One of the reasons for that is the lack of information needed for design and scale-up procedure (Mason *et al.*, 1992).

MTS (Manothermosonication) has been shown to inactivate several enzymes and microorganisms much more rapidly than heat treatment at identical temperatures. The industrial use of MTS could therefore reduce substantially treatment times of several foods. But before MTS can be industrially used, its effects on several quality parameters of these individual foods need to be studied (Vercet *et al.*, 2002).

Ultrasonic methods other than microbial inactivation: The industrial applications of ultrasound include texture, viscosity and concentration measurements of many solid or fluid foods; composition determination of eggs, meats, fruits and vegetables, dairy and other products; thickness, flow level and temperature measurements for monitoring several processes; and non-destructive inspection of egg shells and food packages. Also, the direct process improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, disruption of cells, degassing of liquids, acceleration of heat transfer and extraction process and enhancement of any process dependent upon diffusion are ways that UW can be used in food technology.

Homogenization is an important and necessary pretreatment in milk processing. Ultrasound application in milk homogenization is noteworthy in recent studies. Improvements over conventional homogenization can lead to yogurt of superior quality (Wu *et al.*, 2001).

In order to evaluate the effect of ultrasound on the fermentation, three treatments were investigated. One of these was the control group in which the yogurts were manufactured by the conventional method. The other group was treated by ultrasound before the milk was inoculated with yogurt starter cultures. For the last treatment group, ultrasonication was applied after inoculation with starter but before the fermentation stage. Treatment with UW before inoculation resulted in an increase in water holding capacity and a decrease in syneresis. Treatment after inoculation resulted in a decreased fermentation time by 0.5 h and increased water holding capacity but arguably no beneficial effect on syneresis (Wu *et al.*, 2001).

In the experimental study of Willamiel and De Jong (2000) a reduction of 73 % in the size of the fat globule was observed after the treatment of milk by continuous-flow ultrasonic treatment at temperatures close to 62°C. They concluded that continuous-flow ultrasonic treatment of milk could be a promising milk preservation technique, especially when used in combination with sub-pasteurization temperatures such as thermisation. The microbiological quality of milk could be enhanced comparable to conventionally thermised milk and, at the same time, the reduction in the size of the fat globules makes this technique practical. However, more research is needed (Willamiel and Jong, 2000).

LIU (Low Intensity Ultrasound) can be applied to several stages of the cheese making process (Benedito *et al.*, 2002). This technique was tried for determining the optimum cut time in the coagulation step. The quality control of some manufactured cheese includes the detection of internal cracks due to abnormal fermentations, classification according to the ripening time as well as the non-destructive assessment of the composition of cheese blocks. The optimum rennet cut time, cheese composition and textural properties can be non-destructively determined using LIU. The existence of internal cracks resulting from freezing can also be detected. These technologies should be considered for different types of cheese for a wide range of applications including mould and pocket distribution assessment. As long as these measurements are non-destructive, the equipment should be designed to consider the assessment of a company's entire production (Benedito *et al.*, 2002).

A specially designed ultrasound device has been applied to cheese manufacturing. The results showed that this process reduced waste, lowered energy consumption and could be used to produce softer cheeses (Gaffney, 1996).

Toba (1990) reported that ultrasound improved the action of *Lactobacilli* in the rate of nearly 50% and also induced a sweetening effect in yogurt without increasing the caloric content.

Ultrasonic applications can be applied to all types of products in the food industry. Ultrasonic techniques also can be used to asses the ripenning degree of some fruits. In these applications, the change of physicochemical characteristics, such as textural properties or sugar content, has been related to ultrasonic parameters such as velocity and attenuation. These ultrasonic techniques have been mainly used to asses the concentration, structure, location and physical state of different components in food products (Benedito *et al*., 2002).

The use of ultrasonic velocity to estimate the concentration of a given componenet in a multiphase system has been quite successful. This technique will need to be commercially developed further to be used on a large scale in the food industry (Javanaud, 1998).

As we mentioned above, ultrasonication is commonly used for inactivation of enzymes. Although ultrasound waves deactivate enzymes or destroy microorganisms alone, combinations with other physical treatments are being tried (Mason *et al*., 1992). The action of ultrasound in combination with conventional heat treatment is quite effective in deactivating peroxidase suggesting that this technique has interesting possibilities in food technology. It has been demonstrated that the efficiency of the combined treatment can be related to the ultrasound power density, i.e. the ultrasound power per unit area of tip of the probe and unit volume of liquid treated. In addition, the experimental evidence suggests that there exists a frequency-power density combination which corresponds to the maximum efficiency of the treatment and that the deactivation dynamics is the same whether the treatment is performed in batch or continuous mode (Gennaro *et al*., 1999).

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Lopez *et al*. (1994)studied the combined effects of heat and ultrasonic waves operating at absolute pressures between 1.5 and 7.2 kg^{-2} cm on peroxidase, polyphenol oxidase and lipoxygenase inactivation. According to this study, a synergistic effect which can substantially reduce enzyme resistance and heat treatment required for inactivation was observed in all cases. The enzyme destruction efficiency of the combined process greatly increases with ultrasonic wave amplitude; decimal reduction times at constant temperature decreased logarithmically with increasing amplitudes. As a conclusion of this study it's reported that this combined treatment could help to solve the problems caused by thermostable enzymes in milk, juices and other drinks.

Vercet *et al*. (1998) investigated free radical formation by manothermosonication under different conditions. According to the results of their study, increasing temperature resulted in a decrease in the rate of hydroxyl radical production. Temperature effects were studied between 30 and 140°C at 117 μm ultrasound amplitude. The ultrasound amplitude was varied between 20 and 145 μm at two different temperatures and pressures (70°C/200kPa and 130°C/500kPa). In both cases, free radical production rate increased linearly with increasing ultrasound amplitude. The pressure effects on free radical formation were studied under two different conditions at 117 μm: 70 and 130°C. At 70°C an increase of hydrostatic pressure resulted in an increase in free radical production rate; whereas increasing hydrostatic pressure at 130°C had a negligible effect on free radical production (Vercet *et al*., 1998).

A new prototype of a multi-sample ultrasonic dehydration system based on the application of high-amplitude ultrasonic vibrations in direct contact with food samples at low temperatures and together with vacuum, forced-air and static pressure, at pre-industrial stage has been designed, constructed and tested by Fuente *et al*. (2006). First experimental trials were carried out to study the influence of ultrasonic power (0, 25, 50, 75 and 100 W) in the kinetics of the dehydration process. In all trials the temperature and relative h umidity were kept between 24-26°C and 30-46%, respectively. The applied static pressure was fixed at 0.06 kg^{-2}cm, the suction at 60 mbar and the air flow velocity and temperature at 2 m^{-1}s and 30°C, respectively. Moisture content of samples was measured by weighing them at fixed intervals of 15 min. The results clearly show the strong influence of the acoustic intensity in the process. The curves obtained up to a maximum power applied of 100 W reveal a direct increase of the drying effect with the acoustic intensity and no saturation was reached. The use of the present prototype of ultrasonic drying system has so confirmed the role of the main ultrasonic parameter when the other thermo-mechanical parameters (temperature, flow rate, suction, etc.) are kept constants. It is provided with electromechanical and pneumatic elements together with the software and hardware necessary for the automatic control and monitoring of all the variables of the process. (Fuente *et al*., 2006).

Ultrasonic methods used for microbial inactivation: Ultrasound technology can be very useful for minimal processing because transfer of acoustic energy to the food product is instantaneous and is distributed throughout the whole product volume. This means a reduction of the total processing time, higher throughput and lower energy consumption. As Crosby (1982) reported, the advantages of ultrasound include: the minimizing of flavor loss, especially in sweet juices; greater homogeneity and significant energy savings.

Ultrasound in its most basic definition refers to pressure waves with frequency of 20 kHz or more (Butz and Tauscher, 2002). Higher power ultrasound at lower frequencies (20-100 kHz), which is referred to as "power ultrasound" has the ability to cause cavitation, which has uses in food processing to inactivate microbes (Corsby, 1982). The ultrasonic frequency must be under 2.5 MHz, because cavitation does not occur above that level (Alliger, 1975). The effects of cavitation on microbial suspensions are; dispersion of clumps of microorganisms, modification of cellular activity, puncturing of the cell wall and an increased sensitivity to heat. The mechanism of ultrasound for biological cell destruction may be explained by the collapse of cavitation bubbles. Usually a 20 kHz frequency is used. Ultrasonic waves generate cavitational fields which damage the cell wall and maybe also the cytoplasmic membrane. Furthermore they lead to modifications in the cellular structure of yeasts (Ciccolini *et al*., 1997).

The mechanism of microbial inactivation can be attributed to intracellular cavitation. Micro-mechanical shock waves are created by making and breaking microscopic bubbles induced by fluctuating pressures under the ultrsonication process. These shock waves disrupt cellular structural and functional components and lead to cell lysis (USDA, 2000).

The effect of ultrasound on killing of Gram-negative and Gram-positive bacteria is unclear. It has been reported that Gram-negatives are less resistant to ultrasound waves than Gram-positives (USDA, 2000). As a result of another study, no significant difference was found in killing rate of two kinds of microorganisms by ultrasonication (Scherba *et al*., 1991; Ahmed and Russell, 1975). Alliger (USDA, 2000) reported that Gram-negative and rod-shaped bacteria seem to be more vulnerable to sonication than Gram-positive and coccus-shaped cells (Alliger, 1975). Other factors that affect the inactivation of microbes by ultrasound are known to be the amplitude of the ultrasonic waves, exposure/contact time, volume of food being processed, the composition of the food and the treatment temperature (USDA, 2000).

The limited current literature indicates that pathogens are resistant to ultrasound treatment, especially when it is used alone. If ultrasound is combined with other methods a greater antimicrobial potency can be obtained. In many cases, combinations of conventional method with ultrasound treatment gave the best result (Garcia *et al*., 1991). The combination of heat and ultrasound was reported to be more efficient with respect to treatment time and energy consumption compared to either treatment individually (Manas *et al*., 2000; Ordondez *et al*., 1984, 1987). Because of the benefits of these combinatorial treatments, many studies have been performed and the sonication term was derived to manosonication (combination with pressure treatment), thermosonication (combination with heat treatment) and manothermosonication (combination with heat and pressure). McClements (1995) also suggested that inactivation of microbes using ultrasound is effective when used in combination with other decontamination methods such as decreasing pH or chlorination.

Reducing a_w also acts synergistically to enhance the effectiveness of ultrasonic treatment. Such a synergy was demonstrated for the inactivation of *Salmonella enterica* serovar Enteridis by ultrasound (Alvarz *et al*., 2003).

According to Raso *et al*. (1998) pressure alone (600 kPa) did not influence the heat resistance of *Yersinia enterocolitica*. At a temperature of 58°C, the lethality of UW under pressure was greater than that of heat treatment alone at the same temperature. At higher temperatures, this difference disappeared. Heat and UW under pressure seemed to act independently. The lethality of MTS treatments appeared to result from the added effects of UW under pressure and the lethal effect of heat. The individual contributions of heat and UW under pressure to the lethal effect of MTS depended on temperature (Raso *et al*., 1998).

In a study evaluating the germicidal efficacy of ultrasonic energy, aqueous suspensions of specific bacteria, fungi and viruses were exposed to an ultrasonic frequency of 26 kHz (Scherba *et al*., 1991). This frequency was chosen to maximize the potential for ultrasonically induced cavitation and also to be above the frequency level of human hearing. The selected microorganisms were the bacteria *Escherichia coli, Staphylococcus aureus, Bacillus subtilis;,Trichophyton mentagrophtes,* a fungus and feline herpes virus type 1 and feline calivirus. As a result of this study, a significant effect of intensity was observed for all the bacteria, except *E. coli.* A significant reduction was detected in fungal growth compared to the control group as well as the reduction in populations of feline herpes virus, but there was no apparent effect of ultrasound on feline calicivirus (Scherba *et al*., 1991).

There is a paucity of literature on the application of ultrasonics to solid foods such as poultry. Sams and Feria (1991) exposed pre- and post-chill broiler drumsticks submerged in deionized water to 47 kHz in an ultrasonic cleaning tank. Sonication was applied for 15 or 30 min at 25 or 40°C and for shorter intervals (0.5, 2 and 3.5 min) in the presence of lactic acid, with pH adjusted to 2 or 4. There was no significant difference in total aerobic bacteria for controls or sonicated thighs when stored for 0, 7 or 14 days at 4°C (Sams and Feria, 1991).

Sonication of *Salmonella* inoculated on broiler skin with and without chlorination was studied by Lillard. Sonication of *S.* Typhimurium cell suspensions (10^8 cells mL^{-1} of peptone) at 20 kHz confirmed the time-dependent reduction shown by Wrigley and Llorca (1992) the log_{10} count decreased to nondedectable levels after sonication for 55 min Lillard showed that salmonellae which were attached to broiler skin were reduced by 1-1.5 log_{10} by sonication in peptone at 20 kHz for 30 min; by < 1 log_{10} by chlorine alone; but by 2.5-4 log_{10} by sonicating skin in a chlorine solution with 0.5 ppm free residual chlorine.

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 in BHI broth for 30 min, 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 treatment at 50°C. It was proposed that this was a result of egg constituents protecting the microorganism from the inhibitory effects of cavitation (Wrigley and Llorca, 1992).

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. 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. *S.* Seftenberg which is the most resistant of the *Salmonella* serovars to heat treatment could only be reduced by 0.5 log cycle; however, a 3-log cycle reduction was obtained when manothermosonication was applied (Manas *et al.*, 2000).

Munkacsi and Elhami (1976) found that the treatment of milk with ultrasound caused an elimination of 93% of coliforms.

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.*

Ultrasonic treatment (20 kHz and amplitude of 117 µm) at ambient temperature was found to be ineffective on *Listeria monocytogenes* giving a decimal reduction time of 4.3 min (Pagan *et al.*, 1999). By combining sonication with a pressure of 200 kPa, 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. On the other hand, *L. monocytogenes* cultures that were incubated 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 (Pagan *et al.*, 1999).

Comparisons between *Bacillus subtilis* spores treated with manosonication and manothermosonication showed that the heat treatment provided by manothermosonication makes the inactivation process more effective (Raso *et al.*, 1998). The simultaneous use of heat and ultrasonic waves could reduce the heat resistance of two strains of *B. subtilis* substantially at atmospheric pressure and temperatures in the range 70-90°C (Garcia *et al.*, 1991).

Heat resistance of the spores of *Geobacillus stearothermophilus* was reduced when subjected to ultrasonic treatment (Palacios *et al.*, 1991). It is 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 (Palacios *et al.*, 1991).

Ananta *et al.* (2005) examined cellular injury in cells of *E. coli* and *Lactobacillus rhamnosus* in response to a high-intensity ultrasound treatment. According to their results, the Gram-positive bacterium, *L. rhamnosus*, was more resistant to the lethal effect of ultrasound in comparison with the gram-negative *E. coli* (Ananta *et al.*, 2005).

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