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Effects of ultrasonic on microorganisms and enzymes

Bahram Hosseinzadeh Samani[1], Zahra Lorigooini[2]

[1]Department of mechanics of biosystem, Shahrekord University, Shahrekord, Iran.
[2]Medical Plants Research Center, Shahrekord University of Medical Sciences, Sharekord, Iran.

*Corresponding author's E-mail: b.hosseinzadehsamani@gmail.com

Abstract
Removing on pathogens is the key concern of non-thermal technology in food products. Recently, inclusion of ultrasound into the food industry has been a subject of research and development. The application of ultrasound in food industry is undoubtedly the notion of consideration and development and, the same as other cases in other areas, the sound ranges can would fall into the following categories of high frequency, low energy, diagnostic ultrasound in the MHz range and low frequency, high energy, power ultrasound in kHz range. The advantageous application of power ultrasound is reflected in its chemical, mechanical, or physical effects on the process or product. Microorganisms Inactivation mechanism, based on physical and chemical factors that ultrasonic was caused on liquid food. When it comes to physical and chemical effects, one would distinguish between them as thinning cell membrane, localized heat and pressure changes (5500°C, 50Mpa); hydroxyl radical and sonochemical reactions, respectively. Ultrasound-assisted inactivation of microorganisms and enzymes brings about the extension of shelf life of raw materials or prepared foods. The study focuses on the related literature having to do with the effect of ultrasonic on microorganism and enzymes.
Keywords: Ultrasonic, Innovation Technology, Pasteurization, Microorganism

Introduction
Ultrasonic is defined as the frequency above 20 kHz. It ascends to MHz range and finally, at around 1 GHz, progresses into what is conventionally called the hypersonic regime, where an average sound velocity is about 5000 m /s. In water, the most widely used liquid, the sound velocity is about 1500 m /s, having wavelengths of 3 mm to 30µm for the above frequency range.
The application of ultrasound in food industry is undoubtedly the notion of consideration and development and, the same as other cases in other areas, the sound

ranges can would fall into the following categories of high frequency, low energy, diagnostic ultrasound in the MHz range and low frequency, high energy, power ultrasound in kHz range. During the expansion cycle, high intensity ultrasonic waves make small bubbles grow in liquid. When such waves achieve a level where there is no energy absorption, violent implode occurs to be called cavitation. What happens during implosion is that very high temperatures (approximately 5000 K) and pressures (estimated at 50000 kPa) are included in these bubbles (Raso et al., 1998).

A number of changes are made by high-intensity acoustic radiation among which is the propagation occurring through a medium. Several mechanism are at work to demonstrate such changes, however, it is not to say that al mechanisms are known.

These changes can be explained by several mechanisms, but not all mechanisms involved are known or well understood. Following is the summary of the reported effects and proposed mechanisms by different scholars:

Heating (Lehmann and Krusen, 1954, Suslick et al., 1985, Fayyazi et al, 2012). Cavitation: (Suslick, 1988, Fayyazi et al, 2012, HosseinzadehSamani, 2014). Structural effects: (Ensminger, 1986).Compression and rarefaction: (Ensminger, 1986, Hosseinzadeh Samani, 2014).Turbulence: (Ensminger, 1988).

The use of ultrasound in food processing is embedded into two basic branches:

1. Monitoring a process or product
2. Affecting a process or product

Power ultrasound

The inclusion of ultrasound in line with improving processes and products is not welcomed by food manufacturing, however, it is considered in other industries. Based on the application, High-intensity sound is commonly used for the applications with frequency either in the sonic (<18 kHz) or ultrasonic (≥ 18 kHz) range. The advantageous application of sound is reflected in its chemical, mechanical, or physical effects on the process or product. Sonochemistry, which is a new term to be developed in chemistry, takes the advantage relevant to ultrasonic chemical effects (Suslick, 1986).

Common uses encompass acceleration of conventional and decomposition reactions, degradation of polymers, and polymerization reactions (HosseinzadehSamani, 2014). When sonication is run on particles of material in a liquid, different physical and mechanical effects are brought about.

Large particles are subject to surface erosion (via cavitation collapse in the surrounding liquid) or particle size reduction (due to fission through interparticle collision or the collapse of cavitation bubbles formed on the surface). The results of conducted researches in the field of the effect of sonication on suspended powders have manifested that the particles can be forced into violent collision that, in the case of metals, fusion can occur in some cases, the colliding powders are affected by chemical reaction. Thus, when copper and sulphur are sonicated together in hexane for 1 hour, 65% Cu2S is generated (Goh et al., 1994).

Effect of ultrasonic on microbial and enzyme

Removing on pathogens is the core concern of non-thermal technology in food products. Relying on the related literature one would claim that a number of non-thermal methods can reduce thermal effect and sometimes help destroying microorganisms cell membrane through remobing thermal effect (Piyasena et al., 2003). The use of ultrasonic in food products has been discussed in some review papers (Knorr et al., 2004, Pingret et al., 2013).

Microorganisms Inactivation mechanism, based on physical and chemical factors that ultrasonic was caused on liquid food. When it comes to physical and chemical effects, one would distinguish between them as thinning cell membrane, localized heat and pressure changes (5500°C, 50Mpa); hydroxyl radical and sonochemical reactions, respectively(Butz and Tauscher, 2002, Piyasena et al., 2003, Valdramidis et al., 2010).The effectiveness and efficiency of ultrasonic in microorganism inactivation rely heavily on physical and chemical properties, the volume of food product, microorganism type, the processing temperature, ultrasound intensity and time exposed (Piyasena et al., 2003, Lee et al., 2009a).Ultrasonic which is known as a potential technology in lione with reducing the amount ofg microorganisms to 5 log cycles enjoying FDA standard has been developed in juices. When compared with conventional thermal method, ultrasonic is not able to remove microorganisms in food products. Attaining considerable inactivation effect necessitates that the process be integrated with other methods such as heat and pressure, called hybrid technology (Guerrero et al., 2005, Lee et al., 2009b).

Ultrasound-assisted inactivation of microorganisms and enzymes brings about the extension of shelf life of raw materials or prepared foods. One would demonstrate that the contribution of ultrasound to diverse microbial samples depends highly on the shape and size of the microorganisms (bigger cells being more sensitive than smaller ones, and coccal forms are more resistant than rod-shaped bacteria), type of cells (gram positive being more resistant than gram negative, aerobic being more resistant than anaerobic), and physiological state (younger cells being more sensitive than older ones, spores being much more resistant than vegetative cells) (Piyasena et al. 2003).

The findings of the studies conducted on the effect of ultrasonic on mircoorganisms and enzyme in differentmedium have been provided.

Ordonez et al. (1987) showed a decline in the effectiveness of ultrasound on bacteria inactivation at elevated temperatures (50°C–60°C). Garcia et al. (1989) concluded that this efficiency loss could be derived from the rise of vapor pressure in the sonicated medium that impaired or reduced the intensity of cavitational collapse, which was avoided by increasing the applied pressure of the sonicated medium. Monothermosonication (MTS) is a method in which ultraonication is combined with thermal and pressure inducing. Lopez et al. (1994) found that the monothermosonication treatment was much more efficient when compared with heat treatment alone aiming to inactivate enzymes such as *lipoxygenase*, POD, and PPO in buffer systems. The combination of ultrasound with chlorine treatment seemed to decline in 4-log of *Salmonella* on poultry surfaces (Lillard 1994). Proteases and lipases are found to be the affective factors for the shelf life of ultraheat-treated milk. These enzymes were inactivated up to 10 times faster employing monothermosonication treatment (Vercet et al. 1997). The capability of ultrasound to inactivatingobserved pathogenic microorganisms such as *L. monocytogenes*, a number of strains of *Salmonella* spp., *E. coli*, or *S. aureus*, which are increasingly found in outbreaks of food poisoning, has been noticed (Sala et al., 1995, Pagan et al. 1999). *Pectin methylesterase* from orange to be regarded as a thermostable enzymewas inactivated almost 500 times faster by monothermosonication treatment when compared with heat treatment at the same temperature (Vercet et al. 1999). The success of ultrasound application has been reported at ambient pressure in line with inactivatin food-quality-based enzymes. POD was inactivated by integrating heat with ultrasound at neutral (Gennaro et al. 1999) or low pH (Ku et al. 2000) and lipoxygenase was inactivated at low sonication intensities (Thakur and Nelson 1997).the combination of flow ultrasound treatment and mild heat (57°C) for 18 min yielded 5-log reduction of *L. monocytogenes*in milk, a 5-log reduction in total aerobic bacteria in raw milk and a 6-log reduction in *E. coli* O157:H7 in pasteurized apple cider Villamiel. Jong (2000) found no contribution of ultrasoundto endogenous milk enzymes such as alkaline phosphatase, g-glutamyltranspeptidase and lactoperoxidase at room temperature,however, synergistic inactivation was found to exist at higher temperatures (60°C–75°C).In another experiment, Seymour et al. (2002) reported microbial decontamination on the surface of minimally processed fruits and vegetables (lettuce, cucumber, carrots, parsley, and others) by ultrasound. In this research, the microorganisms were subsequently killed by inclusion of chemical sanitizers such as chlorine. Pectin methylesterase and polygalacturonase in tomato juice were also inactivated by monothermosonication treatment with higher efficiency (Vercet et al. 2002). Other researches such as Scouten and Beuchat(2002) indicated the decontamination of alfalfa seeds inoculated with *Salmonella* or *E. coli* O157 by combined treatments of ultrasound and Ca (OH) 2, which could be taken into account as an alternative to chlorine treatments so as to ignore contamination.

The activity of *pectinesterase* (PE) at themoderate pressure (100-300 kPa) of temperature below 100°C can be affected by Monothermosonication (Kuldiloke *et al.*, 2002).

A continuous ultrasound system accompanied by steam injection has emitted to fourfold higher inactivation rates of *E. coli* and *Lactobacillus acidophilus* in several liquid foods such as milk and fruit juices (Zenker et al. 2003). Guerrero et al. (2005) highlighted that the inactivation of *S. cerevisiae*could be enhanced by incubating with low molecular weight chitosan prior to including ultrasound.Ugarte et al. (2006) investigated the application of acoustic energy to secure apple juice safety. Sonication was found to give rise to*E. coli* K12 cell destruction by 5.3-log, 5.0-log, and 0.1-log cycles at 40°C, 50°C, and 60°C, respectively. D'Amico et al. (2006) asserted that ultrasound treatments distinguished by inclusion or not including mild heating were effective in reducing *L. monocytogenes* in raw milk and *E. coli* O157:H7 in apple cider. Ferrante et al. (2007) reported that that the control of *L. monocytogenes*in orange juice could be developed by integrating high intensity ultrasound with mild heat treatment and natural antimicrobials.Salleh-Mack and Roberts, (2007) in their research studied the effects of temperature, sugar concentration (8, 12, and 16 g/100 ml), organic acids (citric and malic acids) and pH (2.5 and 4.0) on ultrasound pasteurization. In this research, *E.coli* was used as model organism. Based on the obtained results one would claim that ultrasound increased the sensitivity of *E. coli* to thermal inactivation. The presence of soluble solids had a protective effect where the sonication time requirement increased. Similar to heat sensitivity, the lower pH environment resulted in *E. coli* having less resistance to sonication (Salleh-Mack and Roberts, 2007).In the study conducted by Valero et al (2007) the influence of ultrasound and conventional heating under different processing conditions on the inactivation and potential subsequent growth of micro-organisms in orange juice was highlighted. This research gave its attention to ultrasonic parameter adjusting as frequency 500 kHz, power 240 W and exposed time 15 min. the findings revealed that the presence of pulp in the juice increased the resistance of micro-organisms to ultrasound. The combination of ultrasound and pressure and/or heat shows considerable promise for the inactivation of microorganisms and enzymes. Hence, techniques such as thermosonication, monosonication, and monothermosonication may be of more relevance in the future as an energy-efficient processing alternative for the food industry. Another research made use of thermosonication (TS) method categorized by frequency 24kH, power 400W, at amplitudes of 25, 50 and 75μm at 60, 65 and 70°C or heat only treatments on inactivation *pectinmethylesterase* in tomato juice. the obtained results indicated that the TS treatment at 60 °C, 65 °C and 70 °C for 41.8, 11.7 and 4.3 min exposure, respectively reduced *pectinmethylesterase* (PME) activity by 90% (Wu *et al.*, 2008).

Another experiment relied on investigating the impact of thermosonication (TS) and pulsed electric field (PEF), individually and combined, on the survival of *Listeria innocua* 11288 (NCTC) in milk. TS (400 W, 160 s) without pre-heating declined*L. innocua* by 1.2 log10 cfu mL^{-1}, while shorter treatment times produced negligible inactivation, showing that TS was a hurdle rather than an effective standalone treatment (Noci et al, 2009).

Tiwari et al (2009) drew his attention to investigating the effect of sonication on *pectinmethylesterase* (PME) activity and cloud stability of orange juice .Ultrasonic acoustic energy density (AED) levels of 0.42, 0.47, 0.61, 0.79 and 1.05 W/mL and treatment times of 0(Control), 2, 4, 6, 8 and 10 min were investigated. The highest PME inactivation level noticed was 62% for sonication at the highest AED level and treatment time. These findings highlighted that the cloud stability of sonicated orange juice depends both on PME inactivation and particle size reduction. Industrial relevance: Power ultrasound is taken into account as a non-thermal pasteurization method that has been found to enjoy the US FDA requirement for a 5 log reduction in E. coli relevant to fruit juices. another research focused on the effect of Ultrasonic treatment 20 kHz, monosonication, thermosonication, monothermosonication at temperature of 40, 37, 54 and 61°C and pressure of 100, 300, 400 and 500 kPa on exposed time of 4 min and conventional thermal method on *E.coli* inactivation. Derived conclusions from the study revealed that the mixed method could decrease inactivation time significantly (Lee *et al.*, 2009a). Recently, the research conducted by Pulsed light (PL) and Thermosonication (TS) has been reported to be applied alone or in combination making use of a continuous system to investigate their effect on Escherichia coli inactivation in apple juice (Muñoz, et al, 2012).another in-field experiment relied itself on surveying possible effects of ultrasound probe diameter, reactor diameter, and juice level in the reactor upon effectiveness of ultrasound waves on decontamination of sour cherry juice. The obtained results demonstrated that the contribution of probe diameter, reactor diameter and reactor height to ultrasonic effect were significant t (P<0.01) (Hosseinzadeh Samani et al, 2013). The impacts of temperature (20–52 °C), acoustic intensity (60–120 W/cm^2) and treatment time (40–240 s) at a constant pressure (225 kPa) was the focus of another study through employing monothermosonication processing on microbial inactivation. *Escherichia coli*and *Pseudomonas fluorescens* showed to have increased by *c*. 1.6 log CFU/ml. *Staphylococcus aureus*was reported to have lower inactivation values (1.05 log CFU/ml). One would obtain such values on the condition of 36 °C, 90 W/cm2 and 240 s (Cregenzán-Alberti et al, 2014). Other relevant researches include Blume and Nies (2003) (*Facal coliforms* and *streptococci* in municipal wastewater), Duckhouse et al (2004) (*E.coli* in Saline), Furuta et al (2004) (*E.coli* in water), Tsukamoto et al (2004) (*Cryptosporidium parvaum* in Water), Lo¨rincz (2004) and Tsukamoto et al (2004) (*Saccharomyces cerevisiae* in Water), Lo´pez-Malo et al (2005) (*Aspergillusflavus* and *Penicilliumdigitatum* in Sabouraud growth medium),Raviyanet al (2005) (*pectinmethylesterase* in tomato), Zhang et al (2006) (*Microcystisaeruginosa*in BG11 growth medium), Dadjour et al (2006) (*Legionella pneumophila*in diluted medium), Alvarez et al (2006) (*Salmonella Senftenberg 775W* in McIlvaine citrate-phosphate buffer or nutrient broth), Borthwick et al (2005), Lee at al (2009) (*E.coli*in phosphate buffer) Hosseinzadeh Samani (2014) (*E.coli* and *Saccharomyces cerevisiae* in Sour Cherry).

Conclusion and recommendations

Based on the obtained results one would assert that it is feasible to make reduction in thermal impact through employing ultrasonic method where its addition to thermal effect would bring about destroying in microorganisms cell membrane intensity. The required time to do the operations is limited in this method, however, it necessitates to be mixed with other existing methods so as to reduce the time of processing and to give rise to ultrasonic effect. Having provided the related review of literature, we have made a number of suggestions regarding how to use the ultrasonic method. When it comes to manipulate the pasteurization systems, it is necessary to mix ultrasonic with heat and pressure. In addition, making use of optimized ultrasonic device e.g. probe and reactor dimensions is further suggested to achieve optimum ultrasonic effect.

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