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Investigation of the use of ultrasonication followed by heat for spore inactivation

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ABSTRACT

Conventional thermal sterilization is the most commonly used technique to inactivate microbial spores in low acidic liquid food products. In recent years, food researchers have investigated ultrasonication in combination with heat as an alternate technology for the reduction of microbial spores. However, the competitive advantage of this process over conventional thermal sterilization has not been properly assessed. In this study, energy delivered by ultrasound was used to provide the heating needed, which fulfils 55% of the thermal energy requirement in addition to the effect caused by cavitation. The effect of pre-treatment with ultrasonication (20 kHz, 750 W) on decimal reduction time (D values) of *Bacillus subtilis* (*B. subtilis*) spores ATCC 6633 was evaluated in three different suspending media (water, whole milk and rice porridge) and were compared with thermal treatment. Among these, pre-treatment with ultrasonication (114 μm, 1.1 W/ml, 5 min) of whole milk resulted in 35% reduction in D-value compared to thermal only treatment at 100 °C whereas under the same treatment conditions, water and rice porridge gave only 18% and 4% reduction respectively. These reduction in D values through the use of combined technology is minimal unless excessive ultrasonication is used, which is commercially not viable.

1. Introduction

In thermal sterilization, low acidic liquid food products are heated to very high temperatures to inactivate microbial spores and thereby preserve food. Prolonged exposure to high temperature (120 °C–140 °C) results in the deterioration of nutrition value, texture, colour and flavour of food. Over the last few years, numerous studies have been done to reduce the heat intensity during sterilization by using combination of thermal and non-thermal preservation methods (Raso and Barbosa-Canovas, 2003).

Ultrasound has been found to possess antimicrobial effects in addition to its positive role in extraction (Chavan and Singhal, 2013; Duba and Fiori, 2015; Guerrouj et al., 2016; Haque et al., 2016; Kadam et al., 2015; Piyasena et al., 2003; Vinatoru, 2001), homogenization (Wu et al., 2000), mixing, defoaming and emulsification (Delmas and Barthe, 2015; Feng and Yang, 2010). Antimicrobial role of ultrasound was first discovered in anti-submarine warfare that resulted in harmful effects on sea life. Later on research focuses on mechanism of ultrasonication for destruction of microorganisms (Earnshaw et al., 1995).

Cavitation is the phenomena that results in the production of microbubbles on exposure of a liquid to ultrasound waves. These microbubbles release high amount of energy and generates high pressure on collapse. The release of high amount of energy and pressure tends to provide microbial inactivation (Feng et al., 2011). Another theory which explains the lethal effect of ultrasound suggests that sonication of any liquid, results in the formation of free radicals that attack DNA within microbial cells (Earnshaw et al., 1995).

Microbial cells are sensitive to sonication treatment while sonication alone has only little effect on spores. However, some studies reported inactivation of spores when ultrasound was used in combination with heat (Evelyn and Silva, 2015b; Garcia et al., 1989), pressure (Raso et al., 1998) and chemicals (Sierra and Boucher, 1971). Table 1

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Table 1 – Overview of literature on ultrasonic inactivation of spores.

Equipment	Matrix	Spore strain	Conditions	Effect on spores	Source
Ultrasonication (at controlled temperature) pre-treatment followed by heat					
Not given	Ringer solution	*B. cereus* *B. licheniformis*	Ultrasonication (20 kHz, 10–12 °C for 12 min with 4 ml suspension) followed by heat treatment at 110 °C for *B. cereus* and 99 °C for *B. licheniformis*	87% reduction in D_{110C} 45.45% reduction in D_{99C}	Burgos et al. (1972)
Ultrasonic disintegrator MSE 60W	Ringer solution	*B. subtilis* 189	Ultrasonication (20 kHz, 0 °C for 10 min with 5 ml suspension) followed by heat treatment at 105 °C, 110 °C and 112 °C	Approx. 20% reduction in D_{105C}, 20% reduction in D_{110C}, 23% reduction in D_{112C} No effect on z-values	Ordo&ez and Burgos (1976)
Vibra cell ultrasonic processor VC 505 (Sonics & materials) 500W	Non-fat milk	*B. licheniformis* (ATCC) 6634 *B. coagulans* (ATCC) 12245 *G. stearothermophilus* ATCC (15952)	Ultrasonication (20 kHz, 3.679 W/ml, 91.2 μm, 0 °C–33 °C for 10 min with 20 ml suspension) followed by heat treatment 63 °C for 30 min	0.42 log reduction 0.56 log reduction 0.73 log reduction	Khanal et al. (2014)
Thermosonication (ultrasound + heat simultaneously)					
UP 200S Hielscher 200W	Beef slurry	*C. perfringens* (NZRM 2621 and NZRM 898)	24 kHz 0.33 W/g, 75 °C for 60 min with 100 ml suspension, 210 μm	Less than 1.5 log reduction in both strains	Evelyn and Silva (2015c)
UP 200S Hielscher 200W	Skim milk Beef slurry Cheese slurry Rice porridge	Psychotrophic *B. cereus* NZRM 984	24 kHz, 0.33 W/g or W/ml 70 °C for 1.5 min with100 ml suspension, 210 μm	0.3-0.4 log reduction (approx. 66% reduction in D_{70C}) Greater than 4 log reduction (approx. 84% reduction in D_{70C}) Greater than 3 log reduction (approx. 72% reduction in D_{70C}) Greater than 4 log reduction (Approx. 85 reduction in D_{70C})	Evelyn and Silva (2015b)
Heat system Ultrasonic wave generator mod (W-220 F) 150 W	Whole milk Glycerol	*B. subtilis* var. *niger*-40 *B. subtilis* ATCC 6051 *B. subtilis* var. *niger*-40 *B. subtilis* ATCC 6051	Thermosonication 20 kHz, 100 °C, 30 ml	79% reduction in D_{100C} 40% reduction in D_{100C} 63% reduction in D_{100C} 74% reduction in D_{100C}	Garcia et al. (1989)
Manothermosoni cation (ultrasound + heat + mild pressure)					
PG Branson sonifier ultrasound	Sterile distilled water	*B. subtilis* var. niger ATCC (9372)	20 kHz, 300 kPa, 70 °C for 12 min, 150 μm	3 log reduction	Raso et al. (1998)