

Perspectives of high power ultrasound in food preservation

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Abstract. High Power ultrasound can be used to alter physicochemical properties and improve the quality of foods during processing due to a number of mechanical, chemical, and biochemical effects arising from acoustic cavitation. Cavitation creates pressure waves that inactivate microbes and de-agglomerate bacterial clusters or release ascospores from fungal asci. Bacterial and heat resistant fungal spores' inactivation is a great challenge in food preservation due to their ability to survive after conventional food processing, causing food-borne diseases or spoilage. In this work, a showcase of application of high power ultrasound combined with heat or thermosonication, to inactivate bacterial spores *i.e. Bacillus cereus* spores in beef slurry and fungal spores *i.e. Neosartorya fischeri* ascospores in apple juice was presented and compared with thermal processing. Faster inactivation was achieved at higher TS (24 KHz, 0.33 W/g or W/mL) temperatures. Around 2 log inactivation was obtained for *B. cereus* spores after 1 min (70 °C) and *N. fischeri* ascospores after 30 min (75 °C). Thermal treatments caused <1 log in *B. Cereus* after 2 min (70 °C) and no inactivation in *N. Fischeri* ascospores after 30 min (80 °C). In conclusion, temperature plays a significant role for TS spore inactivation and TS was more effective than thermal treatment alone. The mould spores were more resistant than the bacterial spores.

1. Introduction

Ultrasound technology utilizes the energy of sound waves that travel through a medium (solid, a liquid, or a gas) at a specific speed or velocity. This technology has a number of mechanical, chemical, and biochemical effects, which are used to modify the physicochemical properties and improve the quality of various food systems during processing [1]. A wide variety of application of ultrasound technology in industrial food processing has been known including drying, extraction, crystallization, freezing, emulsification, degassing, meat tenderization, defoaming, oxidation processes, and microbial or enzyme inactivation. High power ultrasound with a typical sound intensity between 10 and 1000 W/cm² and frequency between 20 and 100 kHz are commonly used for the inactivation of microorganisms. Microbial cell death is believed to arise from acoustic cavitation, which refers to the creation, growth, and collapse of microgas bubbles due to regions of pressure change [2]. Microbial killing involves thinning of the cell membrane, localized heating, and production of free radicals which induces adverse chemical changes in the DNA or protein denaturation [3-5].

Some microbial species can produce spores, which are resistant structures able to survive under environmental stresses such as nutrients deprivation. The outgrowth of these contaminating spores in



foods can cause food spoilage, and if the microbe is pathogen, food borne illnesses and outbreaks. Among the main bacterial and fungal spore-formers of concern for the food industry are *Bacillus cereus* bacteria and *Neosartorya fischeri* mould. *B. cereus* is pathogenic bacterium commonly found in meat, rice, cereals and spices, and food intoxication can cause diarrhea or emesis depending on the type of toxin produced [6]. Psychrotrophic strains of *B. cereus* are frequently found in low-acid chilled foods [7, 8] due to their ability to grow at low temperatures ($T < 8\text{ }^{\circ}\text{C}$) [9]. *N. fischeri* mould is typical microbes associated with spoilage of high-acid and acidified foods ($\text{pH} < 4.6$). Thus, incidents or spoilage with these species have been registered in pasteurised fruit products such as juices, purees, jellies, jams, and canned fruits [10, 11].

The objective of the present study was, therefore to: (i) study TS inactivation of *B. cereus* spores in beef slurry at different temperatures; (ii) study TS inactivation of *N. fischeri* ascospores in apple juice at different temperatures; and (iii) compare TS and thermal inactivation of *B. cereus* spores in beef slurry and *N. fischeri* ascospores in apple juice.

2. Materials and methods

2.1. Microorganisms and sporulation

Psychrotrophic *Bacillus cereus* NZRM984 (= ATCC 11778) from the New Zealand Reference Culture Collection and *Neosartorya fischeri* JCM 1740 (= ATCC 1020) from the Japan Collection of Microorganisms were selected for this study. For sporulation of *B. cereus*, fresh cells from initial cultures were inoculated on tryptic soy agar (TSA; Difco, USA) supplemented with 0.05 g/L $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ and incubated aerobically for 14 days at $37\text{ }^{\circ}\text{C}$. With respect to sporulation of *N. fischeri*, spores were obtained after growth for four weeks at $30\text{ }^{\circ}\text{C}$ on malt extract agar (MEA). The sporulation was confirmed using phase-contrast microscopy (Motic microscope BA410 Series).

2.2. Food inoculation and preparation

The following foods were chosen since they are prone to contamination by the microorganisms studied: beef slurry ($\text{pH}\ 6.5$) for *B. cereus*; and apple juice ($\text{pH}\ 3.7$, $10.6\text{ }^{\circ}\text{Brix}$) for *N. fischeri*. Each microbial spore was inoculated in each food in TS experiments to yield a final concentration of approximately $\sim 10^6$ – 10^7 cfu/g or cfu/mL prior to processing.

2.3. Spore enumeration

The *B. cereus* spore concentration in beef slurry before and after processing was determined by spread plating into nutrient agar (NA) supplemented with 0.1% soluble starch and incubation at $37\text{ }^{\circ}\text{C}$ for 24–48 h. Prior to plating, samples were placed in 110×230 mm sterilized stomacher bags (Inter science, France) and decimal diluted ten times with 0.1% (w/v) buffered peptone water. These dilutions were considered in the calculation of the final spore concentration. Samples were then homogenized in the stomacher (Masticator Stomacher, IUL Instruments, Germany) for 2 min prior to plating onto NA plates. With respect to *N. fischeri*, the mould ascospore concentration in apple juice before and after TS processing was determined by spread plating onto MEA and followed by incubation at $30\text{ }^{\circ}\text{C}$ for 3 to 5 days.

2.4. Thermosonication

A Hielscher UP200S ultrasonic processor (Hielscher-Ultrasonic GmbH, Germany) with a sonotrode tip diameter of 3 mm was used for all TS experiments. The processor has a frequency of 24 kHz and was operated at 100% amplitude (460 W/cm^2 , 0.33 W/g or W/mL). For each temperature (70 , and $50\text{ }^{\circ}\text{C}$ for *B. cereus*; 75 and $65\text{ }^{\circ}\text{C}$ for *N. fischeri*), a 200 mL round bottom-flask containing the food sample (100 g or 100 mL) was placed in a thermostatic water bath inside a biosafety cabinet (class II, type A2, Esco Micro Pte. Ltd., Singapore) to bring the sample temperature to the designated temperature. Inoculation of spores was carried out aseptically when the sample reached the designated temperature, and then the ultrasound was turned on. The temperature of the food sample during processing was monitored and a thermostatic water bath was used to keep it at the desired value ($\pm 1\text{ }^{\circ}\text{C}$). Ultrasonic treatments were

carried out for up to 70 min, depending on the microbes and TS temperature. Food samples were removed from the water bath at pre-specified intervals, and spore survivor counts were immediately performed.

2.5. Thermal processing

Regarding the thermal treatments, transparent packed food samples containing the inoculated spores were processed in a thermostatic water bath for up to 150 min with the temperature set to the processing temperature: 70 °C for *B. cereus*, and 80 °C for *N. fischeri*. The pouches were maintained at the desired temperature during the thermal treatments, taken out at different time intervals, and kept in an ice water bath until microbial enumeration. At least two independent survival experiments were carried out for each TS and thermal treatment temperature with duplicate samples processed for each time.

2.6. Statistical analysis

At least two survival experiments were run for each temperature, subjected to the same or different processing method, and average values $\pm$ standard deviation were calculated. Then, significant differences in the microbial log reductions from different temperatures under the same method and similar temperature under different methods were investigated by performing a one-way analysis of variance (ANOVA) followed by Tukey's tests, with a confidence level of 95% ($p < 0.05$).

3. Results and discussion

3.1. Effect of TS temperature on the inactivation of *B. cereus* spores in beef slurry

The effect of increasing temperature from 60 to 70 °C in a 0.33 W/g TS process on the log survivors of *B. cereus* spores in beef slurry is illustrated in Figure 1. A higher spore inactivation was obtained with a higher temperature. For example, for a 2 min process, >5.0 log was obtained at 70 °C compared to 1.7 log at 60 °C ($p < 0.05$). The higher spore inactivation at the higher temperature (i.e. around 70 °C) for the survival curves are in agreement with previous study [12] with *Bacillus subtilis* spores in milk. The results suggest benefit of TS method for the inactivation of this bacterial spores.

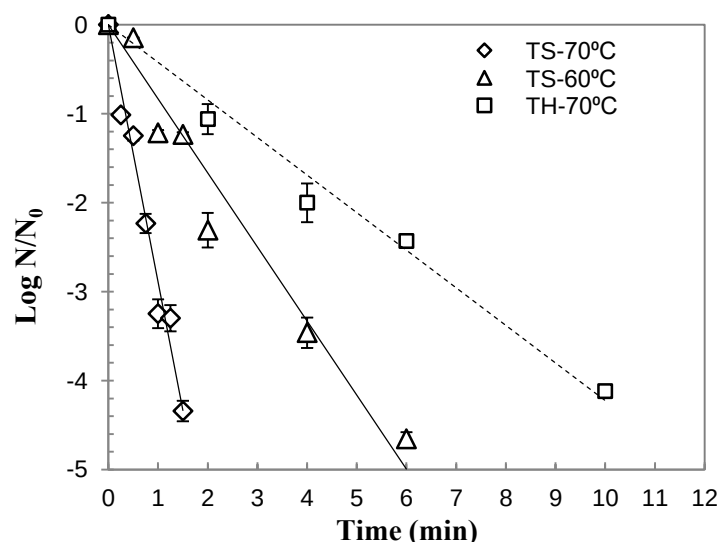


Figure 1. Effect of different temperatures on the thermosonication (TS, 24 kHz, 0.33 W/g) inactivation of *B. cereus* spores in beef slurry and its comparison with 70 °C thermal treatment (TH) (data from Evelyn and Silva, 2015 [13]).

3.2. Effect of TS temperature on the inactivation of *N. fischeri* ascospores in apple juice

The log survivors of *N. fischeri* ascospores in apple juice at 65 and 75 °C after 0.33 W/mL TS treatments are shown in Figure 2. Activation shoulders were observed for all TS temperatures tested, with a maximum at 10 min for 75 °C and 30 min for 65 °C, followed by approximately linear spore inactivation. Increase in the spore number at the beginning of the treatments could be due to the role of sonication in de-agglomerating fungal clusters and producing suspension of free ascospores [14-16]. Lower spore activation and faster inactivation were obtained as the TS temperature was increased from 65 to 75 °C, indicating significant role of temperature on the inactivation of these ascospores for up to 75 °C. The 75 °C was the maximum temperature supported by the ultrasound equipment. Overall while 30 min at 75 °C achieved ≈ 2 log reductions, > 80 min at 65 °C were required to obtain the same spore inactivation. Although more log reductions at 75 °C can be obtained at longer times (> 30 min), the long processing times and activation shoulders for this type of microorganism and food make TS should be re-evaluated for commercial application.

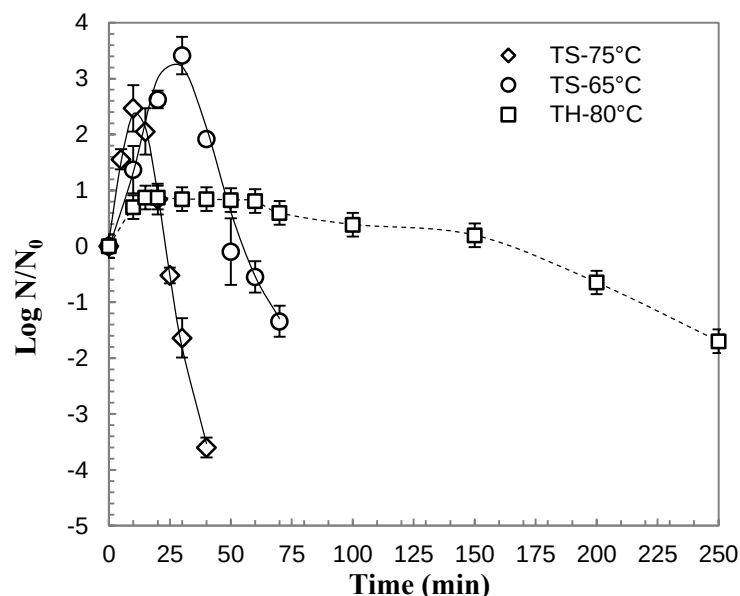


Figure 2. Effect of different temperatures on the thermosonication (TS, 24 kHz, 0.33 W/mL) inactivation of *N. fischeri* spores in apple juice and its comparison with 80 °C thermal treatment (TH) (TS data from Evelyn et al. 2016 [15]).

3.3. TS versus thermal inactivation of *B. cereus* and *N. fischeri* spores

Figure 1 and Figure 2 also show a comparison of the log survivors of *B. cereus* spores in beef slurry by 0.33 W/g TS-70 °C vs. 70 °C thermal process, and the log survivors of *N. fischeri* ascospores in apple juice by 0.33 W/mL TS-75 °C vs 80 °C thermal process, respectively. The TS inactivation for both microbes was much faster than thermal inactivation alone. Regarding *B. cereus* spores, > 5.0 log reduction for TS vs. 0.9 log reduction for thermal alone were obtained after a 2 min process at 70 °C (Figure 1, $p < 0.05$). In fact, similar log reduction (0.9 log, $p > 0.05$) were obtained after 2 min for the 70 °C thermal process and 50 °C TS process, thus demonstrating that TS treatment requires 20 °C lower temperature than a thermal process alone to obtain the same level of lethality. With respect to *N. fischeri* ascospores, in order to compare TS with thermal results, 80 °C was used for the thermal process since no inactivation of the ascospores by 75 °C thermal treatment was observed (not shown). Similar spore inactivation (≈ 1.7 log, $p > 0.05$) was obtained for the 75 °C TS process (after 30 min) and 80 °C thermal process (after 250 min), indicating the remarkable advantage of ultrasound. The benefit of TS vs. thermal

for spore inactivation suggesting less impact of heat in the food quality was also documented by other authors in past studies [12, 13, 17].

4. Conclusions

Increasing TS temperature for up to 70 or 75 °C caused an increase in the inactivation of *B. cereus* spores in beef slurry and *N. fischeri* ascospores in apple juice. 0.33 W/g TS–70 °C for a short time period (2 min) was a better method than 70 °C thermal for the inactivation of *B. cereus* spores in beef slurry, and 0.33 W/mL TS–75 °C was also a better process than 80 °C thermal treatment for *N. fischeri* ascospore inactivation in apple juice, confirming the benefit of ultrasound technology. A reduction of >5.0 log can be obtained for psychrotrophic *B. Cereus* spores after 2 min of TS–70 °C, however further research must be carried out for other bacterial spores. Activation shoulders and long treatment times for *N. fischeri* ascospore inactivation by TS–75 °C make TS not yet feasible for processing high acidic foods containing this heat resistant spores. The TS process might be applicable at longer treatment times (≥ 30 min) and higher temperatures (75 °C), thus further research must be conducted to reduce the activation shoulders and design an ultrasound probe that can withstand higher temperatures. The results from this study show that TS might be a better option over thermal treatment for the preservation of food products prone to contamination of these microbial spores, however caution must be taken for each microbial spores.

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