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Thermosonication versus thermal processing of skim milk and beef slurry: Modeling the inactivation kinetics of psychrotrophic *Bacillus cereus* spores

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ABSTRACT

Non-thermal processed foods are generally cold stored and distributed. The use of ultrasound for food preservation has attracted the interest of many research groups. In the current study, the thermosonication (TS, simultaneous ultrasound and thermal process) inactivation of psychrotrophic *Bacillus cereus* spores was investigated (24 kHz, 210 μm, 0.33 W/mL or W/g). First, the effectiveness of a 1.5 min TS process at 70 °C in skim milk, beef slurry, cheese slurry, and rice porridge was investigated. The TS was more effective than sole thermal treatment in reducing *B. cereus* spores in rice porridge, beef slurry and cheese slurry by 7, 6, and 4 fold, respectively. Then, the first-order *D*- and *z*-values for TS and thermal processing in skim milk and beef slurry, and the best model to fit TS inactivation of *B. cereus* spores in beef slurry were determined. The $D_{70\ °C}$-values in skim milk were 2.9 min for TS and 8.6 min for the thermal treatment. And in beef slurry, values of 0.4 min for TS and 2.3 min for thermal were estimated. It was found that the Log-logistic model better described the TS spore inactivation in beef slurry. The ultrasound technology required 20–30 °C lower temperatures for the same spore inactivation, which resulted in better food quality and energy saving gains.

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1. Introduction

Psychrotrophic microorganisms can grow at temperatures of 7 °C or less, regardless of the optimum growth temperature (Collins, 1981). Psychrotrophic bacteria are of great concern in pasteurized, low-acid, cold distributed foods (pH > 4.6, e.g. milk and dairy products) and also chilled prepared foods such as *sous vide* and cook-chill foods (Carlin, Girardin, et al., 2000; Carlin, Guinebretiere, et al., 2000; Silva & Gibbs, 2010; Silva, Gibbs, Nunez, Almonacid, & Simpson, 2014). The population of bacteria can increase when foods are prepared under poor hygienic conditions and during distribution (Barbano, Ma, & Santos, 2006). Spore-forming psychrotrophic pathogens such as non-proteolytic *Clostridium botulinum* and certain strains of *Bacillus cereus* have been linked to the microbiological safety of this type of foods because they can survive pasteurization, grow under refrigerated conditions and cause food poisoning (Silva & Gibbs, 2010). Yet control of psychrotolerant *B. cereus* spores in this class of food products is one of the most important concerns (Choma et al., 2000; Dierick et al., 2005; Evreux, Delaporte, Leret, Buffet-Janvresse, & Morel, 2007; Ghelardi et al., 2002; Luby, Jones, Dowda, Kramer, & Horan, 1993; Silva & Gibbs, 2010; Slaten, Oropeza, & Werner, 1992).

B. cereus is a Gram-positive, rod-shaped, spore-forming facultative anaerobic bacterium which is able to grow over a wide range of temperatures (4–55 °C), pH (4.9–9.3), and water activities values (0.92–1.0) (EFSA, 2005). This organism is able to regenerate to large numbers at refrigerated temperatures (Choma et al., 2000; Christiansson, Naidu, Nilsson, Wadström, & Pettersson, 1989; Valero, Hernandez-Herrero, & Giner, 2007), and produce toxins in foods (Samapundo et al., 2011). When the level exceeds 10^5 cfu/g, food intoxication by *B. cereus* can cause diarrhea or emesis depending on the type of toxin produced (Dierick et al., 2005; Luby et al., 1993; Schoeni, 2005; Slaten et al., 1992). Fatal meningitis has also been reported (Evreux et al., 2007). Contamination is difficult to detect since the organoleptic properties of the foods do not change (Christiansson et al., 1989). In addition, mild, short duration and self-limiting symptoms, and infrequent routine laboratory analysis, result in a low number of reported incidents (FSANZ, 2013). Nevertheless, the following contaminated foods have been reported in outbreaks of *B. cereus* around the world: starchy foods (for instance rice, potato, pasta) and cheese products (USFDA, 2012a), raw and pasteurized milk (Ahmed, 1983; Røssland et al., 2005), meat (Luby et al., 1993; Slaten et al., 1992), vegetable puree as well as other chilled-foods containing vegetables (Carlin, Girardin, et al., 2000; Carlin, Guinebretiere, et al., 2000; Jenson, Moir, & Hocking,

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2003) and cake and other desserts (Ghelardi et al., 2002; Granum & Lund, 1997). The decimal reduction times of psychrotrophic strains of *B. cereus* spores at 90 °C ($D_{90\ °C}$-value) ranged from 2.2 to 9.2 min in buffer (Dufrenne, Bijwaard, Te Giffel, Beumer, & Notermans, 1995) and from 4.4 to 6.6 min in reconstituted skim milk (Shehata & Collins, 1973), while others have reported values of 4 and 10 min in water and pork roll, respectively (Byrne, Dunne, & Bolton, 2006; Fernández, Ocio, Fernández, & Martínez, 2001). According to Silva and Gibbs (2010), psychrotrophic *B. cereus* appears to have a higher heat resistance than psychrotrophic non-proteolytic *C. botulinum* strains, exhibiting *D*-values in the magnitude of seconds at >95 °C for *B. cereus* and at lower temperatures (85 °C to >95 °C) for *C. botulinum*.

The use of non-thermal technologies has emerged as an alternative to minimize changes of the food sensory properties induced by heating. The inactivation of bacteria using ultrasound was first initiated in the 1920s (Harvey & Loomis, 1929). This technology relies on the application of pressure waves (frequency ranging from 20 to 100 kHz) to the food/beverage, causing microbial cell death. This phenomenon, called cavitation (Chen, 2012; Feng et al., 2009; Feng & Yang, 2011; Piyasena, Mohareb, & McKellar, 2004), creates microgas bubbles due to regions of pressure change. Some authors may also use the term cavitation to describe the bubble growth and subsequent collapse with considerable energy release, inducing localized extreme conditions, which leads to bacterial cell death (Feng & Yang, 2011; Gogate, 2011; Wu, Guo, Teh, & Hay, 2013). Microbial killing involves the thinning of the cell membranes, localized heating, and the production of free radicals (Butz & Tauscher, 2002; Fellows, 2000; Piyasena et al., 2004), which induces adverse chemical changes in the DNA or protein denaturation (Riesz & Kondo, 1992).

The effect of ultrasound alone has been considered ineffective for the inactivation of bacterial spores (Butz & Tauscher, 2002). Hence, the combination with other treatments such as temperature, pressure, or both heat and pressure to increase the lethal effect has been investigated. The combination of ultrasound with chemicals and ionizing radiation has also been studied. Spore inactivation was significantly enhanced and the treatment times were also significantly reduced compared to ultrasound, chemical or radiation alone (Ahmed & Russell, 1975; Sagong et al., 2012; Sierra & Boucher, 1971). Regarding ultrasound assisted (before or after) thermal processing for spore inactivation, heat sensitization of *B. cereus*, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus stearothermophilus* spores is generally observed after ultrasonic treatments (Burgos, Ordonez, & Sala, 1972; Ordonez & Burgos, 1976; Sanz, Palacios, Lopez, & Ordogez, 1985). In addition, the release of low molecular weight substances from the spore protoplast after ultrasonication was registered (Palacios, Burgos, Hoz, Sanz, & Ordóñez, 1991).

With respect to the simultaneous use of ultrasound and heat, often referred to as thermosonication (TS), a process of 20 kHz, 5 W/mL and 70 °C was much more effective for the spore inactivation of two strains of *B. subtilis* in milk than thermal processing alone (Garcia, Burgos, Sanz, & Ordonez, 1989). López-Malo, Jimenez-Fernandez, and Palou (2001); López-Malo, Palou, Jiménez-Fernández, Alzamora, and Guerrero (2005) also observed the synergistic effect of ultrasound and heat on fungal spore inactivation which resulted in lower TS decimal (*D*-) reduction values (20 kHz, 40–60 °C) than the corresponding thermal *D*-values. To date, there have been only two publications on TS microbial spore inactivation, and there is almost no documentation regarding the kinetic modeling of bacterial spore inactivation by TS. Due to the importance of psychrotrophic *B. cereus* spores and the lack of reports on TS inactivation modeling, as well as the benefits over thermal inactivation alone, in this research, *B. cereus* spore inactivation by TS in skim milk, beef slurry, cheese slurry, and rice porridge was investigated as follows: (i) to study the effectiveness of 1.5 min of thermosonication (TS) vs. thermal processing at 70 °C to reduce the spores in different foods; (ii) to model the thermosonication and thermal inactivation kinetics in skim milk and beef slurry; (iii) to compare the first-order kinetic parameters (*D*- and *z*-values) for TS and thermal inactivation of spores in skim milk and beef slurry.

2. Materials and methods

2.1. Food sample preparation

Skim milk, beef, cheese, and rice were chosen for this research since they are prone to contamination by *B. cereus* (USFDA, 2012a), and the major composition was determined by an accredited laboratory in New Zealand (Table 1). All foods under study were commercial products to avoid variability of food composition between each treatment. The foods were also prepared in the same way for all the treatments.

2.1.1. Skim milk

Reconstituted skim milk was prepared by diluting New Zealand skim milk powder with 100 mL of sterile distilled water (SDW).

2.1.2. Beef slurry

Sirloin beef mince was stored overnight at 4 °C before use. After autoclaving, the beef was mixed with SDW in a sterile laboratory scale blender (100 mL of SDW was added to every 100 g of minced meat).

2.1.3. Cheese slurry

Cheese slurries were prepared by mixing and blending 100 g of New Zealand grated cheddar cheese with 100 mL SDW.

2.1.4. Rice porridge

Raw jasmine rice was cooked with water (ratio of 1:7) to make porridge. The cooked rice porridge was immediately blended.

2.2. B. cereus microbiology

2.2.1. Strain

B. cereus strain NZRM 984 (NCTC 10320, ATCC 11778, DSMZ 345), obtained from the New Zealand Reference Culture Collection, was selected for this study since preliminary tests with NZRM 984 and other psychrotrophic ATCC 9139 demonstrated higher heat resistance for NZRM 984. The psychrotrophic behavior (growth at 4 °C) of NZRM 984 was previously demonstrated (Wimalaratne, 2009). The original freeze-dried culture was initially suspended into test tubes containing 5 mL of Brain–Heart Infusion (BHI) broth (Oxoid, Hampshire, UK) for 20 min. The culture was then inoculated into a larger volume (50 mL) of BHI broth and grown overnight at 37 °C with continuous shaking at 200 rpm in an orbital shaker.

2.2.2. Sporulation

The cells from the overnight culture in BHI were used as a starting culture for sporulation. Aliquots of 0.1 mL were spread plated onto tryptic soy agar (TSA; Difco, Becton Dickinson, USA) supplemented with 0.05 g/L $MnSO_4 \cdot 4H_2O$ to induce the sporulation. The plates were incubated aerobically for 14 days at 37 °C for sporulation. The level of

Table 1
The composition and pH of foods processed in this study.*

	Composition (g/100 g)			
	Skim milk	Beef slurry	Cheese slurry	Rice porridge
Moisture	90.5	76.0	68.2	75.9
Fat	<0.1	7.0	16.1	<0.1
Protein	2.7	14.0	10.6	1.9
Carbohydrate	4.9	2.6	3.3	22.1
Sugars	*4.7*	*0.3*	*0.2*	*<0.1*
Ash	1.8	0.4	1.8	0.1
pH	6.5	6.5	5.8	6.7

* The analyses were carried out by an accredited laboratory and the average values are presented.

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