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Differences in the resistance of microbial spores to thermosonication, high pressure thermal processing and thermal treatment alone

Evelyn [a, b, **], Filipa V.M. Silva [a, *]

[a] Department of Chemical and Materials Engineering, University of Auckland Private Bag 92019, Auckland 1142, New Zealand
[b] Department of Chemical Engineering, University of Riau, Pekanbaru 28293, Indonesia

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ABSTRACT

Bacterial and fungal spores can survive pasteurization treatments, and germinate/grow after food processing, causing food spoilage and/or outbreaks. High pressure processing (HPP) and power ultrasound technologies can be combined with heat (HPP-thermal or HPTP, TS) to increase the rate of spore inactivation. In this study, the differences in the resistance of bacterial and mould spores of several microbial species to thermal, 600 MPa HPP-thermal, and TS (0.33 W/mL or g) treatments were investigated. Thermal processes in the range of 70–78 °C almost did not affect the five microbial species' spores tested (<1 log after 40 min). On the contrary, 600 MPa-75 °C HPP treatment reduced all microbial spores (2.8 to >5.8 log after 40 min): *C. perfringens* presented the highest resistance, followed by *B. nivea* and *N. fischeri* moulds, and lastly *B. cereus*. TS treatments (70–75 °C) up to 60 min had almost no effect on *A. acidoterrestris* and *C. perfringens*, while *B. cereus* in beef slurry was readily inactivated (6 log after 2 min). Activation shoulders were registered for both moulds. As opposed to HPP-thermal and thermal treatments, the type of food had a significant effect in the spore inactivation by TS.

1. Introduction

Only some microbial species can produce spores. Spores are resistant structures able to survive various environmental stresses such as nutrients deprivation. The outgrowth of contaminating spores in foods can cause food spoilage. If the microbe is pathogen, foodborne illnesses and outbreaks can also occur. *Clostridium perfringens*, *Bacillus cereus*, *Alicyclobacillus acidoterrestris*, *Byssochlamys nivea*, and *Neosartorya fischeri* are among bacterial and fungal spore-formers of concern for the food industry. *C. perfringens* has been identified as the most common cause of food outbreaks in ready-to-eat and partially cooked meat and poultry products as result of improper handling, and preparation of large quantities of foods (Evelyn and Silva, 2015a, 2016a; Silva and Gibbs, 2009). *B. cereus* is another pathogenic bacterium commonly found in meat, rice, cereals and spices, resulting in food poisoning similar in many respects to *C. perfringens*. Psychrotrophic strains of *B. cereus* are frequently found in low acid chilled foods (Carlin et al., 2000a, 2000b; Dufrenne et al., 1995; Silva and Gibbs, 2010; Silva et al., 2014) due to their ability to grow at low temperatures (T < 8 °C) (Choma et al., 2000; Dufrenne et al., 1995). *A. acidoterrestris* bacteria, and *B. nivea* and *N. fischeri* moulds are typically associated with spoilage of high-acid and acidified foods (pH < 4.6) such as juices, purees, jellies, jams, and canned fruits (Beuchat, 1998; Cerny et al., 1984; Jay, 2000; Pitt and Hocking, 1997; Silva and Gibbs, 2004, 2009; Silva et al., 2014).

Thermal processing is a common sterilization/pasteurization method used for the industrial production of stable/durable foods. The heat causes damages in bacterial/mould/yeast spore cells, which become unviable. The heated spores' deformations and damages, and the release of intracellular components are visible through scanned electron microscopy (Rozali et al., 2017). Microbial spores can exhibit very high resistance to heat and typically the inactivation is log linear with time, according a first order kinetics. For example, a decimal reduction time of 7.1 min at 100 °C ($D_{100°C}$) was obtained in beef slurry for *C. perfringens* NZRM 898 spores (Evelyn and Silva, 2015a). The $D_{90°C}$-value of 2.0 min was registered for psychrotrophic *B. cereus* ICMP 12442 spores in skim milk (Evelyn and Silva, 2015b). A higher heat resistance for *A. acidoterrestris* spores, compared with major spoilage

* Corresponding author.
** Corresponding author. Department of Chemical Engineering, University of Riau, Indonesia.
E-mail addresses: evelyn@unri.ac.id (Evelyn), filipavinagresilva@gmail.com (F.V.M. Silva).

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microbes of high-acid shelf-stable foods was reported (1.0 min < $D_{95°C}$ < 5.3 min and 6.0 min < $D_{90°C}$ < 23.0 min) (Evelyn and Silva, 2016b; Silva and Gibbs, 2001; Silva et al., 2014). With respect to mould spores, $D_{90°C}$-values of 4.9 min for *N. fischeri* JCM 1740 in apple juice and 1.8 min for *B. nivea* JCM 12806 in strawberry pulp were reported (Evelyn et al., 2016; Evelyn and Silva, 2015c). In addition, previous studies demonstrated an increase in the resistance of *B. nivea* and *N. fischeri* mould ascospores with their age (Chapman et al., 2007; Evelyn and Silva, 2017; Wyatt et al., 2015).

A number of different emerging non-thermal technologies and their combination with heat have been investigated for microbial spore inactivation in foods, as an alternative to minimize the heat induced changes in the food sensory properties. The combined technologies allow the processing times to be shortened, thus less heat exposure. Heat assisted high pressure processing (HPP-thermal or HPTP) and simultaneous power ultrasound and heat treatment (thermosonication, TS) were investigated in this study. TS induces loss of spore inherent resistance, with subsequent heat inactivation due to acquired susceptibility. HPP-thermal and TS methods reduced the treatment temperatures and/or processing times for bacterial/mould/yeast spores and enzyme inactivation (Evelyn and Silva, 2015a, 2015b, 2015c, 2015d, 2016a, 2016b, 2016c; Evelyn et al., 2016; Garcia et al., 1989; Milani et al., 2016; Milani and Silva, 2016, 2017; Silva et al., 2012; Sulaiman et al., 2015a, 2015b; Uchida and Silva, 2017). Therefore the combination of mild heat with non-thermal technologies will increase the process productivity and product quality. Evelyn et al. (2017) showed that HPP-thermal and TS treatments are better than thermal processing alone for the inactivation of spores of bacteria, moulds, and yeasts in foods.

The spores of *Clostridium perfringens*, psychrotrophic *Bacillus cereus*, *Alicyclobacillus acidoterrestris, Byssochlamys nivea*, and *Neosartorya fischeri* present high resistance to several food pasteurization methods. Thus, the differences in the resistance of these specific microbial spores to the following physical treatments were investigated in this study: (i) thermal processing at 70–78 °C; (ii) 600 MPa HPP-thermal at 70–75 °C; (iii) 0.33 W/mL (or W/g) TS at 70–75 °C. The last objective was (iv) to study the effect of type of food after 70 °C thermal, 600 MPa HPP-70 °C and 0.33 W/mL (or W/g) 70 °C TS inactivation on psychrotrophic *Bacillus cereus* spores.

2. Material and methods

2.1. Microorganisms

Six different microorganisms were used: (i) *Clostridium perfringens* NZRM 898 (= ATCC 14809 and NCTC 10239) type A strain isolated from salted beef, was obtained from the Medical Section-New Zealand Reference Culture Collection (NZRM); (ii) *Alicyclobacillus acidoterrestris* NZRM 4447 (= ATCC 49025 and NCIMB 13137) isolated from apple juice concentrate, was obtained from the NZRM; (iii) psychrotrophic *Bacillus cereus* NZRM 984 (= ATCC 11778, NCTC 10320) was also obtained from the NZRM; (iv) psychrotrophic *Bacillus cereus* ICMP 12442 (= ATCC 9139, ATCC 21) was obtained from the Landcare Research New Zealand; (v) *Byssochlamys nivea* JCM 12806 (= CBS 696.95), isolated from pasteurized strawberry; and (vi) *Neosartorya fischeri* JCM 1740 (= ATCC 1020, DSM 3700), isolated from canned apples, were obtained from the Japan Collection of Microorganisms (JCM). All cultures were revived according to the suppliers' instructions. For spore production and enumeration please refer to methodology from Evelyn et al. (2016), Evelyn and Silva (2015a, 2015b, 2015c, 2016b, 2016c, 2017), and Silva et al. (2012). Four week old *B. nivea* and *N. fischeri* moulds' ascospores were used for this study, as age increased their resistance to thermal, HPP-thermal and TS processes (Evelyn and Silva, 2017). As opposed to moulds' spores, preliminary experiments and previous findings from other studies showed that bacterial spore resistance is not much affected by age, even after a long period of refrigerated storage such as 16 months (Fernández-Coll and Silva-Negrón, 1991; Rodriguez-Palacios and LeJeune, 2011).

2.2. Foods

The following foods were chosen since they are prone to contamination by the microorganisms studied: beef slurry (pH 6.5) for *C. perfringens*; orange juice (pH 3.8, 9.5°Brix) for *A. acidoterrestris*; skim milk and beef slurry (both at pH 6.5) for *B. cereus*; strawberry puree (pH 3.4, 8.1°Brix) for *B. nivea*; and apple juice (pH 3.7, 10.6°Brix) for *N. fischeri*. Each microbial spore preparation was inoculated in the specific food to yield a final concentration of approximately ~ 10^6–10^7 cfu/mL or cfu/g prior to processing. For HPTP and thermal processes, the inoculated samples were packed in 8 × 8 cm Cas-Pak (New Zealand) plastic retort pouches and vacuum sealed.

2.3. Thermal processing of food containing spores

Transparent packed food samples containing the inoculated spores were processed for up to 60 min in a thermostatic water bath with the temperature set to 70 °C for *B. cereus*, 75 °C for *C. perfringens* and moulds, and 78 °C for *A. acidoterrestris*. 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. Thermal treatments were carried out up to 60 min.

2.4. HPP-thermal processing of food containing spores

The HPP-thermal treatments were carried out using a QFP 2L-700 HPP processor from Avure Technologies-USA (Evelyn and Silva, 2015b, 2015c, 2016a, 2016c, 2017; Evelyn et al., 2016). The pouches containing the food samples inoculated with bacteria or mould spores, were submitted to 600 MPa HPP combined with moderate temperature (70 or 75 °C) for times between 1 and 40 min, as this was the maximum temperature supported by the equipment. The average temperature during the HPP-thermal constant pressure phase was considered as the HPTP treatment temperature. The process times did not include the pressure come-up (≤1.5 min for 600 MPa) and the depressurization times (<30 s), and change in the spore population during the come-up time was not accounted.

2.5. Thermosonication of food containing spores

An UP200S ultrasonic processor at 24 kHz by Hielscher (Hielscher-Ultrasonic Gmbh, Germany) was used for the induction of the ultrasonic waves. A sonotrode with a tip-diameter of 3 mm was inserted in direct contact with the food. The specific acoustic power of 0.33 (W/mL or W/g) was calculated from the multiplication of the 3 mm probe's surface area (0.0707 cm^2) with the sound intensity (460 W/cm^2) at 100% amplitude (information listed in the manufacturer manual), and then divided by the volume (100 mL) or mass (100 g) of the food sample. The procedure for TS experiments at 70–75 °C was described previously (Evelyn and Silva, 2015a, 2015c, 2015d, 2016b, 2017; Evelyn et al., 2016). The temperature during TS treatments was monitored by inserting a thin probe in the food sample contained in a round bottom flask, and a thermostatic water bath was used to keep it at the desired value during the treatment. The foods containing the spores of each microorganism were processed for up to 60 min.