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Comparing high pressure thermal processing and thermosonication with thermal processing for the inactivation of bacteria, moulds, and yeasts spores in foods

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ABSTRACT

Bacterial and fungal spores might germinate/grow after food processing, causing food-borne diseases or food spoilage. High pressure processing (HPP) and power ultrasound are two technologies that can be combined with heat (HPP-thermal or HPTP, TS) to increase the rate of spore inactivation. The objective of this study was to compare HPP, HPTP and TS with exclusively thermal processing to inactivate spores of two pathogenic bacteria (*Clostridium perfringens*, *Bacillus cereus*) as well as fruit spoilage microorganisms (*Alicyclobacillus acidoterrestris* bacteria, *Byssochlamys nivea* and *Neosartorya fischeri* moulds, and *Saccharomyces cerevisisae* yeast). For a 20 min process at 75 °C, 600 MPa HPTP was the best technology for the inactivation of spores of *C. perfringens* in beef slurry (2.2 vs. 0.9 log for 0.33 W/g TS and no inactivation for thermal treatment). Likewise, at 70 °C 600 MPa HPTP was better to inactivate *B. cereus* spores in milk than single thermal treatment (4.0 vs. 0.3 log after 20 min process). At 75 °C 600 MPa HPTP was also the best technology for the inactivation of mould ascospores of *N. fischeri* in apple juice (4.3 log) and *B. nivea* in strawberry puree (2.0 log) vs. no effect for thermal and even increase in the spores with TS after a 20 min process. TS was a better technology than thermal treatment to reduce *A. acidoterrestris* spores in orange juice (0.6 vs. 0.2 log after 20 min at 78 °C). Regarding *S. cerevisiae* ascospores inactivation in beer, non-thermal HPP was much better than TS and thermal processing: 3 log reductions required only 30 s at 400 MPa vs. 5 min for 16.2 W/mL-TS-55 °C and 84 min for 55 °C thermal. Overall, the heat assisted HPP technology was better than TS and thermal treatment alone for the inactivation of bacteria, mould, and yeast spores, requiring lower processing times thus allowing a better food quality.

1. Introduction

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, foodborne illnesses and outbreaks. Among the main bacterial and fungal spore-formers of concern for the food industry are *Clostridium perfringens*, *Bacillus cereus*, *Alicyclobacillus acidoterrestris*, *Byssochlamys nivea*, *Neosartorya fischeri*, and *Saccharomyces cerevisiae*. *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; García Armesto and Sutherland, 1997). *A. acidoterrestris* bacteria, *B. nivea* and *N. fischeri* moulds, and *S. cerevisiae* yeasts, are typical microbes associated with spoilage of high-acid and acidified foods (pH < 4.6). Thus, incidents or spoilage with these species have been

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registered in 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 and pasteurization method used for industrial food production. Microbial spores can exhibit very high resistance to heat and typically the inactivation is log linear with the time, according a first order kinetics. For example, decimal reduction values at 100 °C ($D_{100°C}$) of 7.1 min in beef slurry for *C. perfringens* NZRM 898 spores was obtained (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 was found for *A. acidoterrestris* spores, compared with major spoilage microbes of high-acid shelf-stable foods (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 and 1.8 min for *N. fischeri* JCM 1740 in apple juice and *B. nivea* JCM 12806 in strawberry pulp were obtained, respectively (Evelyn et al., 2016; Evelyn and Silva, 2015c). Much lower heat resistance of *S. cerevisiae* spores was reported, with $D_{60°C}$-values ranging between 4.6 and 11.2 min (Milani et al., 2015).

A number of different non-thermal technologies and their combination with heat have been investigated for microbial spore inactivation in foods, and they have emerged as an alternative to minimize changes of the food sensory properties induced by heating, as processing times with combined technologies can be shortened. These include heat assisted high pressure processing (HPP-thermal or HPTP) and simultaneous power ultrasound and heat (thermosonication, TS). TS induces spore inactivation in which the spores lose their inherent resistance after sonication and are also inactivated by subsequent treatment condition (heat) due to acquired sensitivity. HPTP 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 and Silva, 2017; Silva et al., 2012; Sulaiman et al., 2015a, 2015b; Uchida and Silva, 2017). This in turn will increase the process productivity and product quality.

It is very important to compare the efficacy of different processes such as thermal assisted HPP and ultrasound processes with the thermal process alone. In previous studies, we generated data for modeling the spore inactivation kinetics of novel technologies combined with heat at different temperatures. In this study, we are compiling and reanalyzing the same data with the focus of comparing two emerging technologies with the exclusively thermal process at the same temperature. The main objectives were to compare: (i) 600 MPa HPP-thermal, TS, and thermal inactivation of *C. perfringens* spores in beef slurry at 75 °C; (ii) 600 MPa HPP-thermal and thermal inactivation of *B. cereus* spores in milk at 70 °C; (iii) TS and thermal inactivation of *A. acidoterrestris* spores in orange juice at 78 °C; (iv) 600 MPa HPP-thermal, TS, and thermal inactivation of 4 week old of *B. nivea* ascospores in strawberry puree and *N. fischeri* ascospores in apple juice at 75 °C; and (v) 400 MPa non-thermal HPP, 55 °C TS, and 55 °C thermal inactivation of *S. cerevisiae* ascospores in beer.

2. Materials 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) psychrotrophic *Bacillus cereus* ICMP 12442 (= ATCC 9139, ATCC 21) was obtained from the Landcare Research New Zealand; (iii) *Alicyclobacillus acidoterrestris* NZRM 4447 (= ATCC 49025, NCIMB 13137) type strain was obtained from the NZRM; (iv) *Byssochlamys nivea* JCM 12806 (= CBS 696.95), isolated from pasteurized strawberry; and (v) *Neosartorya fischeri* JCM 1740 (= ATCC 1020, DSM 3700), isolated from canned apples, were obtained from the Japan Collection of Microorganisms (JCM); and (vi) *Saccharomyces cerevisiae* DSMZ 1848 (= DSM 1848, CECT 1990, brewing strain) was obtained from the German collection of microorganisms. All cultures were revived according to the suppliers' instructions. For spore production and enumeration please refer to methodology in Evelyn et al. (2016), Evelyn and Silva (2015a, 2015b, 2015c, 2017), Milani and Silva (2016), and Silva et al. (2012).

2.2. Foods

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

2.3. Units used for HPP, HPP-thermal, TS and thermal processes

The non-thermal HPP and HPTP 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; Milani and Silva, 2016). Regarding TS, an UP200S ultrasonic processor at 24 kHz by Hielscher (Hielscher-Ultrasonic Gmbh, Germany) was used for the induction of ultrasonic waves. The processor was operated at 100% amplitude and continuous energy supply. Sonotrodes with a tip-diameter of 3 mm (460 W/cm^2) and 14 mm (105 W/cm^2) were inserted in direct contact with the food and used to generate different acoustic sound intensities (Hielscher, 2007). The specific acoustic power (W/mL) was calculated from multiplication of the probe's surface area and sound intensity (W/cm^2 at 100% amplitude supplied in the manufacturer manual), and then divided by the volume of food sample. The maximum temperature supported by the sonotrode was 78 °C. For thermal processing, a thermostatic water bath was used.

2.4. Processing food containing spores

For HPP, the pouches containing the yeast inoculated food samples were submitted to 400 MPa high pressure at room temperature (no additional heat, non-thermal, T ≤ 36 °C) for up to 10 min. The other packed foods inoculated with bacteria and mould spores were submitted to 600 MPa high pressure combined with moderate temperature (70 or 75 °C) for times between 1 and 40 min. The average temperature during the HPTP constant pressure phase was considered as the HPTP temperature. The process hold-times did not include the pressure come-up (≤45 s for 400 MPa and ≤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.

The procedure for *C. perfringens* and moulds TS experiments (0.33 W/mL and 75 °C) was described previously (Evelyn and Silva, 2015a, 2015c, 2017; Evelyn et al., 2016). The temperature during TS processing was monitored by inserting a thin temperature probe in