Innovative Food Science and Emerging Technologies 27 (2015) 35–40

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Apparatus for the simultaneous processing of mesophilic spores by heat-only and by high pressure and heat in a high pressure vessel to investigate synergistic spore inactivation

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ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 7 November 2014
Accepted 15 December 2014
Available online 20 December 2014

Keywords:
High pressure thermal processing
Spore inactivation
Synergy
Clostridium sporogenes
Bacillus amyloliquefaciens

ABSTRACT

We sought to develop a paired sample container system that permits simultaneous processing of two samples in the same high pressure vessel, one pressurized and one protected by a pressure-resistant shell (heat-only), and subsequently compared the effect of pressure (600 MPa) on the inactivation of spores of *Bacillus amyloliquefaciens* and *Clostridium sporogenes* at high temperature. Reducing the compression rate to 200 MPa/min saw near-identical thermal profiles (R^2 0.990) delivered to paired samples, thereby facilitating a means to directly attribute increases in inactivation in pressurised samples to the synergistic effect of pressure and heat. At 115 °C (3.5 min hold time), inactivation of *B. amyloliquefaciens* spores was around 7 $\log_{10}$ cfu/mL greater with pressure while the high pressure thermal (HPT) inactivation of *C. sporogenes* spores was approximately 2 $\log_{10}$ cfu/mL more than by heat alone after 1–2 min at 100 °C. Using this system we have demonstrated unequivocally that high pressure acts synergistically with heat to inactivate spores of *B. amyloliquefaciens* and *C. sporogenes*.
Industrial relevance: To facilitate commercialization of HPT processing for low-acid foods, a significant improvement in spore inactivation by HPT compared to traditional thermal processing, or HPT synergy, needs to be demonstrated. The development of a system that permits unequivocal assessment of the synergistic effect of pressure and heat on spore inactivation is an important tool to assist validating such processes.

1. Introduction

High pressure thermal (HPT) processing has been reported to be more effective than conventional thermal processing for bacterial spore inactivation and has the potential to deliver novel chilled or shelf-stable products with improved sensory and nutritional qualities through reduction in overall thermal exposure during processing (Black et al., 2007; Juliano et al., 2006; Leadley, 2005; Wilson, Dabrowski, Stringer, Moezelaar, & Broklehurst, 2008). Given HPT processing is considered an alternative to thermal processing, many studies aim to determine if inactivation of relevant safety and spoilage microorganisms is comparably better under HPT conditions, ultimately assessing if pressure acts synergistically, or otherwise, with heat to inactivate spores.

We have previously discussed the difficulty in making such comparisons; specifically, establishing a basis on which to compare inactivation that takes into account the relevant process parameters of both heat and HPT processes (Bull, Olivier, van Diepenbeek, Kormelink, & Chapman, 2009; Olivier et al., 2011). The approach used in many early studies compared inactivation as a function of hold time at the desired processing temperature, ignoring lethality that may be delivered during the preheating, pressure come-up and depressurization phases of the overall HPT process (Ahn, Balasubramaniam, & Yousef, 2007; Patazca, Koutchma, & Ramaswamy, 2006; Rajan, Ahn, Balasubramaniam, & Yousef, 2006; Rajan, Pandrangi, Balasubramaniam, & Yousef, 2006). Our approach has been to compare spore inactivation based on the accumulated thermal lethality of the processes (F^Z_T value). As in thermal processing, calculating F^Z_T for HPT processes both acknowledges the significant thermal component of the entire HPT process and accounts for its non-isothermal nature (Bull et al., 2009; Olivier et al., 2011; Olivier, Bull & Chapman, 2012). However, this approach is dependent on knowledge of kinetic inactivation parameters, particularly z_T value (the temperature difference required to result in a 10-fold change in heat resistance, or D_T value), for which there is no current standardised approach for determining under HPT conditions. In our previous studies (Bull et al., 2009; Olivier et al., 2011; Olivier, Bull & Chapman, 2012) we elected to assume that calculated heat-only z_T values did not change under pressure (for practical reasons), recognising that this assumption potentially affected observations of synergistic inactivation by HPT processing (Olivier et al., 2011). Bull et al. (2009) also discussed the effect differences in the shape of the heat-only and HPT thermal profile, including critical temperatures, might have on calculated thermal lethality and subsequent comparison of inactivation.

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http://dx.doi.org/10.1016/j.ifset.2014.12.003
1466-8564/

Ideally, delivery of near identical thermal processes, with and without applied pressure, would facilitate direct comparison of heat-only and HPT spore inactivation without requiring a means to 'normalize' a basis on which to compare, i.e. F^{z}_{T} value. Margosch et al. (2006) sought to match a HPT thermal profile under heat-only conditions using glass capillaries. This approach was suitable given the high-pressure microsystem utilized (150 μL sample volume) was unique in its ability to undergo very rapid compression and heating (<20 s), and maintain isothermal and isobaric conditions during hold-time. Replicating the thermal profile delivered during a HPT process where compression rate is not near-instantaneous, nor which is isothermal or strictly isobaric during hold-time, as will be the case in a commercial setting, requires a different approach. Following on from previous development of a purpose-designed pressure-resistant shell engineered to protect a miniature temperature logger during HPT processing (Knoerzer et al., 2010), we sought to develop paired sample containers that would permit simultaneous processing of two samples in the same HPT vessel, one with pressure (HPT) and one without (heat-only), but both 'experiencing' the same thermal profile. Being inherently thermally equivalent both in lethality (F^{z}_{T}) and with respect to profile shape, this system would provide a means of direct comparison of inactivation as affected by pressure.

In this study we report the development and performance evaluation of high-pressure-resistant and high-pressure transmitting sample containers and subsequently compare inactivation achieved in paired spore samples processed simultaneously within these sample containers, in the same high-pressure vessel. *Bacillus amyloliquefaciens* and *Clostridium sporogenes* were selected for this investigation given our previous efforts to characterize the effect of HPT on their inactivation (Bull et al., 2009; Olivier et al., 2011) and their general importance with respect to the sterilization of low acid, shelf stable foods.

2. Materials and methods

2.1. Sample container development

Two sample container systems were designed and manufactured from highly stress resistant aircraft aluminium (7075-T6, with a tensile strength of 600 MPa and a high thermal conductivity of 130 W/(m · K)). One container ('open') transmits pressure to the sample through a channel sealed off with a moveable piston, while the second ('closed') shields the sample from pressure, i.e., maintains the sample close to atmospheric pressure (Fig. 1). The open container sample therefore undergoes compression heating while the closed container sample will only undergo temperature changes via heat conduction through the container walls. By controlling the pressure rate such that the compression heat can dissipate into the aluminium shell (with high thermal conductivity), near-identical temperature profiles can be achieved in each sample.

The dimensions of the aluminium shell and the feasibility of achieving near-identical temperature profiles was initially evaluated by numerical simulations in COMSOL Multiphysics™ (COMSOL AB, Stockholm, Sweden; data not shown). The models were based on earlier validated models describing HPT processes (Knoerzer & Chapman, 2011; Knoerzer, Juliano, Gladman, Versteeg, & Fryer, 2007). This approach fast-tracked development and avoided multiple iterations in trial and error experiments. Fig. 1a shows the CAD drawing of the open container; the closed container was identical in design and dimensions albeit with a sealed, solid bottom.

Regarding the volume of the internal cavity inside the sample containers, approximately 3 mL of liquid sample is added to the cavity initially. In order to seal the sample container, excluding as much air as possible, the cap is screwed down while a bleed-hole cap on the side of container is left loose to release any air. As both the top and then bleed-hole caps are sealed tight, excess liquid drains from the bleed-hole resulting in an internal volume of less than 3 mL. Upon opening the sample containers, a useable volume of 1.5–2 mL generally remains for sampling purposes.

2.2. Sample container performance evaluation

In order to determine the closeness of temperatures reached in samples within the open and closed containers during HPT processing, temperature profiles were recorded and compared as affected by the compression rate. All samples were processed at 600 MPa and held for 5 min in a Stansted ISO-LAB FPG11501 High-Pressure 3.6 L unit (Stansted Fluid Power Ltd., Stansted, Essex, UK) filled with a water-propylene glycol mixture (~35% glycol). Pressure rates from 100 to 600 MPa/min were investigated for samples pre-equilibrated to 80 to 85 °C to reach maximum temperatures (T_{max}) under pressure in the order of 100 to 110 °C. Some experiments were conducted where the decompression rate was also varied to evaluate the impact on the closeness of temperature profiles. It was found that this had only a marginal influence (data not shown); therefore, the fastest decompression rate (600–900 MPa/min) was selected for all subsequent trials.

The containers were filled with water to allow for good heat transfer from the container's cavity into the shell and to simulate the expected temperature response in spore suspensions eventually investigated

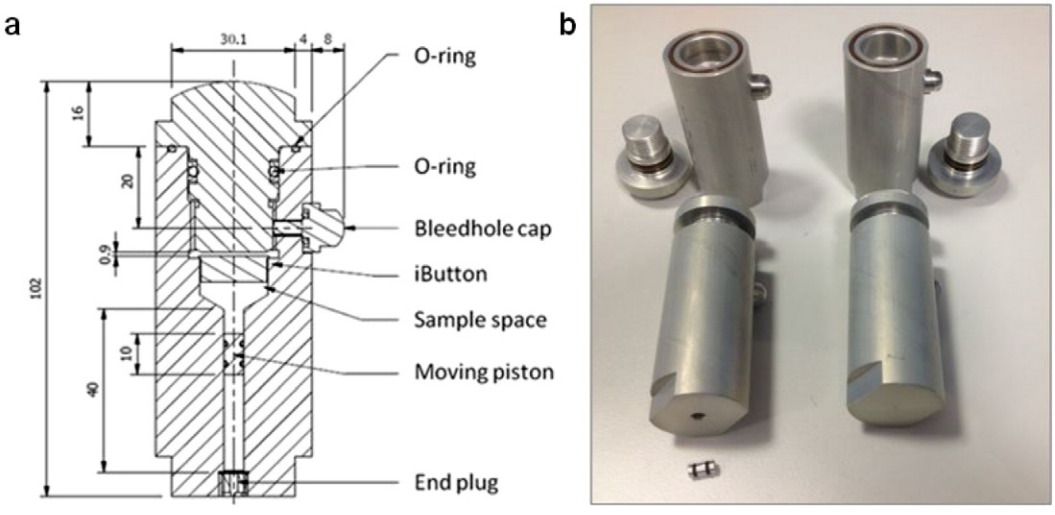

Fig. 1. (a) CAD drawing of the 'open' sample container. (b) Pair of sample containers: 'open' system for HPT spore inactivation (left) and 'closed' system for thermal-only inactivation (right).