Food Control xxx (2016) 1–7

Contents lists available at ScienceDirect

Food Control

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

Alicyclobacillus acidoterrestris spore inactivation by high pressure combined with mild heat: Modeling the effects of temperature and soluble solids

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ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form
21 July 2016
Accepted 21 August 2016
Available online xxx

Keywords:
Acidophilic
High pressure thermal processing
Alicyclobacillus acidoterrestris
Fruit juices
Fruit concentrates
Brix

ABSTRACT

High pressure processing (HPP) comprises the application of pressures between 100 and 1000 MPa to foods for microbial inactivation and food preservation. HPP has been commercially applied to pasteurize fruit juices with the advantage of retaining its bioactive constituents and original organoleptic properties. *Alicyclobacillus acidoterrestris* has been suggested as a reference in the design of pasteurization for high-acid fruit products, due to spore resistance and spoilage incidents in fruit juices. In this study, *A. acidoterrestris* spore inactivation by 600 MPa combined with mild heat (35–65 °C) in malt extract broth adjusted to 10, 20 and 30 °Brix was carried out and the inactivation was modeled.

The soluble solids increased the resistance of the spores to 600 MPa-thermal process, while the temperature decreased its resistance. Although the nonlinear Weibull model gave better fittings, the first-order kinetic parameters were also determined. For example for 600 MPa at 55 °C $D_{10^{\circ}Brix} = 4.2$ min, $D_{20^{\circ}Brix} = 7.6$ min, $D_{30^{\circ}Brix} = 13.7$ min, and z_T-values were 20–21 °C. The z-values for the effect of soluble solids on D_T-values were 39–40 °Brix for 45 and 55 °C 600 MPa HPP. The results obtained with broth were validated with fruit juices and concentrates. The combination of HPP with heat was an effective alternative to conventional thermal processing for the inactivation of *A. acidoterrestris* spores in juices up to 30 °Brix, allowing the use of less 30–40 °C of temperature for the same microbial inactivation, which potentially results in more nutritious, fresher and tastier juices/concentrates.

1. Introduction

Although the first scientific publication demonstrating the extension of milk shelf life by high pressure processing (HPP) dates back to 1899 (Hite, 1899), its commercial scale was initiated in 1990 in Japan to produce three different jams (Hayashi, 1995). There are several successful high pressure processed products sold globally (Rastogi, 2013): fruit juices, jams, jellies, rice cakes and raw squids in Japan; salsa, guacamole meal kits, oysters in shells, ready-to-eat meat in the US; fruit juices, especially apple and orange juice in France, Portugal, Italy, UK, Mexico and the USA; and apple sauce in Canada. High hydrostatic pressure (HPP) is another term used for the same technology, since water is used to surround and transmit the pressure to the food uniformly and rapidly (Hayashi, 1989). Although HPP is mostly used for the inactivation of microorganisms and enzymes (Sulaiman, Soo, Yoon, Farid, & Silva, 2015), the technology can also be applied for protein gelation, cold denaturation of proteins, freezing and thawing of foods, or meat tenderization (San Martín, Barbosa-Cánovas, & Swanson, 2002). Molecules such as amino acids, vitamins, responsible for flavor, and low molecular weight are hardly affected by the process. As a consequence, the organoleptic and nutritional properties are retained with the process, and HPP treated food presents higher quality (Rendueles et al., 2011).

Alicyclobacillus acidoterrestris is an acidophilic, thermophilic, non-pathogenic, Gram-positive, strictly aerobic, spore-forming bacterium which has been related to several spoilage incidents in a variety of products such as apple juice (Cerny, Hennlich, & Poralla, 1984), shelf stable iced tea containing berry juice (Duong & Jensen, 2000) and carbonated fruit drink (Pettipher & Osmundson, 2000). Therefore it has been suggested as reference microorganism to design pasteurization processes in acidic fruit products (Silva & Gibbs, 2001, 2004, 2009; Silva, Gibbs, Nunez, Almonacid, & Simpson, 2014; Silva, Gibbs, Vieira, & Silva, 1999).

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http://dx.doi.org/10.1016/j.foodcont.2016.08.034

The microorganism cell is rod-shaped measuring 2.9–4.3 μm long and 0.9–1.0 μm wide and the oval-shaped spores are 1.5–1.8 μm long and 0.9–1.0 μm wide (Walker & Phillips, 2008), both able to grow between 25 and 60 °C (optimum: 42–53 °C) and low pH (Pontius, Rushing, & Foegeding, 1998; Yamazaki, Teduka, & Shinano, 1996). One of the main challenges related to the specific spoilage by *A. acidoterrestris* is the difficulty to be identified, since it is not linked with acid or gas production. The most evident signs of spoilage are the off-flavor and off-odor, described as "medicinal, phenolic and antiseptic" due to the production of 2-methoxyphenol, the guaiacol (Yamazaki et al., 1996) and other halophenols. One of the most distinctive characteristics of *Alicyclobacillus* spp. is the presence of ω-alicyclic fatty acids as the principal membrane component. Research demonstrated these closely packed fatty acids contribute to the heat resistance of the microorganism when a protective layer with strong hydrophobic bonds is formed. In extreme acidic and high temperature environment, these bonds stabilize and reduce the membrane permeability (Jensen, 1999; Kannenberg, Blume, & Poralla, 1984). The thermal processing D-value at 95 °C of endospores of different *A. acidoterrestris* strains in fruit juices vary between 1.5 and 8.7 min (Eiroa, Junqueira, & Schimdt, 1999; Evelyn & Silva, 2016a; Silva & Gibbs, 2001).

Bacterial spores are also extremely resistant to HPP. It has been accepted the impossibility to achieve high levels of *Bacillus*, *Clostridium* and mold spore inactivation by applying pressure alone in the commercial range of 200–600 MPa (Black et al., 2007; Evelyn, Kim & Silva, 2016; Evelyn & Silva, 2015a, 2015b, 2016b; 2016c). Therefore, HPP combined with mild heat is recommended: HPP-thermal or HPTP (high pressure thermal processing). The mechanism of spore inactivation by HPP is not completely known. It has been postulated that spores germinate first under certain temperature/pressure conditions, losing their resistance and readily inactivated by the HPP treatment. Under very high pressure (400–800 MPa), there is an induced spore germination accompanied by the release of dipicolinic acid with calcium – Ca-DPA (Rendueles et al., 2011; Wuytack, Boven, & Michiels, 1998). The release of Ca-DPA leads to cortex lysis, possibly due to the effects on DPA channels in the inner membrane or on the spore's membrane itself (Black et al., 2007). After germination, spores are much more sensitive to agents such as heat, pH and pressure compared to the dormant state (Hongkang & Mittal, 2008).

In the fruit juice/concentrates industry, soluble solids (expressed in °Brix) is one of the most important parameters, given its influence on the microbial resistance to pressure and heat (Basak, Ramaswamy, & Piette, 2002; Palou et al., 1998; Silva et al., 1999). The inactivation of *A. acidoterrestris* spores by HPP and HPTP in fruit juices and concentrates has barely been explored, with only a few studies found in the literature (Lee, Chung, & Kang, 2006; Lee, Dougherty, & Kang, 2002; Silva, Tan, & Farid, 2012; Sokołowska et al., 2012, 2013). There is no literature report on modeling the effect of soluble solids on the inactivation of *A. acidoterrestris* spores using combined HPP and thermal processing (HPP-thermal or HPTP) in fruit juice concentrates. Therefore the objectives of this research were: (1) to model the HPP-thermal inactivation of *A. acidoterrestris* spores in 10, 20 and 30 °Brix broths, (2) to investigate the effect of HPP temperature and soluble solids on the spore inactivation, (3) to validate the model with commercial fruit juice concentrates.

2. Material and methods

2.1. *Alicyclobacillus acidoterrestris* microbiology

2.1.1. Strain and growth medium

A freeze dried strain of *Alicyclobacillus acidoterrestris* NZRM 4447 (New Zealand Reference Culture Collection, Medical Section) was acquired from the Institute of Environmental Science and Research (ESR) in New Zealand. This is the type strain, same as ATCC 49025^T, NCIMB 13137^T, DSM 3922^T, GD3B^T and CIP 106132^T. The culture was grown for 3 days at 45 °C on potato dextrose agar (PDA) (BD Difco, North Ryde, Australia) adjusted to pH 4.0 after sterilization with 0.1 g/mL of tartatic acid.

2.1.2. Spore production

Fresh culture was spread onto PDA adjusted to a pH of 5.6 and incubated at 45 °C for 21 days or until at least 80% of the cells were sporulated. Sporulation was monitored by microscope examination after staining with 0.005 g/mL safranin and 0.05 g/mL malachite green solutions. When the desired sporulation was obtained, the spores were harvested by adding 1–2 mL of sterile water onto PDA plates and gently removing the surface growth with a sterile inoculator. The suspensions obtained from 20 plates were centrifuged at 4000*g* for 20 min at 4 °C (Centrifuge Sigma 4K15, UK) and the pellet was resuspended in sterile water and centrifuged again at 4000*g* for 10 min at 4 °C. This last step was repeated 3 more times to wash the spores. The final pellet was resuspended in sterile sodium phosphate buffer (pH 7.2) and stored at 4 °C until use.

2.1.3. Spore enumeration

In order to determine the spore concentration (N), a serial dilution technique was used, where 0.1 mL of spore suspension was diluted with 0.9 mL of sterile water in test tubes down to a dilution of 10^{-6}. The test tubes were vortex mixed before taking a portion for further dilution to ensure uniform concentration of spores. After the dilution, test tubes were heated at 80 °C for 10 min in a water bath to destroy any remaining vegetative cells. For each tube dilution, 2 × 0.1 mL was spread plated in two PDA plates (pH 4.0) and incubated at 45 °C for 3 days. After incubation, the colonies formed (cfu) were counted when ranging between 30 and 300 cfu and the average counts were calculated for each dilution and spore concentration was expressed in cfu/mL.

2.2. Preparation of broth, juices and concentrates for HPP-thermal processing

The malt extract broth (MEB) (BD Difco, North Ryde, Australia) was adjusted to 10, 20 and 30 °Brix by adding sucrose crystal and using a refractometer (Atago, Abbe Refractometer DR-A1, Japan). The pH was adjusted to 3.8 using a pH meter (Mettler Toledo, S20 – SevenEasy, USA) with a 10% w/v (0.1 g/mL) solution of tartatic acid (Univar, Ajax Finechem, Australia). The MEB solution was then sterilized using the autoclave. A small volume (0.1 mL) of spore suspension was added to the sweetened MEB to reach a concentration of ca. 10^6 CFU/mL. Several 6 cm × 10 cm transparent retort pouches (Cas-Pak, New Zealand) were filled with 2 mL of the inoculated MEB and thermosealed under vacuum (Multivac C200, Germany). The plastic film was composed by polyester coated with silicon oxide, laminated to nylon and laminated to cast polypropylene (PETSIOX(12)/ON(15)/RCPP(70)). These bags were 1.0 mm thick, presented a low oxygen transmission rate (<2 cc/m^2/24 h) and could withstand temperatures up to 130 °C, appropriate for high pressure thermal processing. The same packaging film was used for fruit juice and concentrate samples.

2.3. Experimental design

First, packed MEB samples with different soluble solids (10, 20 and 30 °Brix) were submitted to HPP combined with mild temperature (T) of 35, 45, 55, and 65 °C with processing times (t) up to 45 min. A 600 MPa HPP pressure was selected, the maximum

Please cite this article in press as: Uchida, R., & Silva, F. V. M., *Alicyclobacillus acidoterrestris* spore inactivation by high pressure combined with mild heat: Modeling the effects of temperature and soluble solids, *Food Control* (2016), http://dx.doi.org/10.1016/j.foodcont.2016.08.034