Food Microbiology 74 (2018) 125–131

Contents lists available at ScienceDirect

Food Microbiology

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

Enhanced control of *Bacillus subtilis* endospores development by hyperbaric storage at variable/uncontrolled room temperature compared to refrigeration

Carlos A. Pinto, Mauro D. Santos, Liliana G. Fidalgo, Ivonne Delgadillo, Jorge A. Saraiva*

QOPNA, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 21 July 2017
Received in revised form
18 February 2018
Accepted 17 March 2018
Available online 20 March 2018

Keywords:
Hyperbaric storage
Refrigeration
Endospores
Bacillus subtilis

ABSTRACT

The effect of hyperbaric storage on *Bacillus subtilis* endospores, as a new food preservation methodology with potential to replace the conventional refrigeration processes, was assessed and compared to refrigeration. To do so, three different matrices (McIlvaine buffer, carrot juice and brain-heart infusion broth, BHI-broth) were inoculated with *B. subtilis* endospores and stored at 25, 50 and 100 MPa at variable/uncontrolled room temperature (18–23 °C), under refrigeration (4 °C), and room temperature at atmospheric pressure (0.1 MPa), up to 60 days. Two different quantification procedures were performed to assay both vegetative and endospores (unheated samples) and endospores (heated samples), to assess germination under pressure.

The results showed that hyperbaric storage yielded pronounced endospore loads reductions in carrot juice and BHI-broth at 50 and 100 MPa, while in McIlvaine buffer, lower endospore loads reductions were observed. At 25 MPa, the endospores germinated and outgrew in carrot juice. Under refrigeration conditions, both carrot juice and BHI-broth underwent endospore germination and outgrowth after 60 and 9 days of storage, respectively, while in McIlvaine buffer there were no endospore outgrowth.

These results suggest that hyperbaric storage at room temperature might not only be a feasible preservation procedure regarding endospores, but also that the food product (matrix characteristics) seems to influence the microbial inactivation that occurs during hyperbaric storage.

1. Introduction

Environmental concerns towards global warming are raising issues concerning the need of environmentally friendlier domestic/industrial practises. Regarding food industry, it is responsible for considerable emissions of carbon dioxide (CO_2), along with other greenhouse-effect gases. For instance, James and James (2010) reported that 35 to 50% of the energetic consumptions in super and hypermarkets are due to the refrigeration (RF) and freezing facilities, being responsible for approximately 1% of the CO_2 emissions worldwide. RF is also the third major source of CO_2 of all food industry (with 490 megatons of CO_2 released to the atmosphere in 2008) (Gilbert, 2012). Thus, the adoption of alternative and more efficient food preservation procedures than RF are required, without compromising food quality and safety.

When it comes to food safety, pasteurized low acidic and high water activity (a_W) food products are to be permanently kept at RF temperatures in order to slowdown/inhibit the germination and outgrowth of bacterial spores. Pasteurization only destroys vegetative microorganisms, being many endospores resistant structures (Soni et al., 2016), which limit the product shelf-life. So, a preservation methodology that is not only environmentally-friendlier but that could perform equally or even better than RF to slowdown/inhibit endospore germination and outgrowth is of upmost interest.

Lately, a new preservation methodology is being increasingly studied with potential to be a feasible alternative to RF. Under the name of hyperbaric storage (HS), it states that instead of controlling the storage temperature, it is more advantageous to control the storage pressure. In fact, energy is only required for the short compression and decompression phases of the pressure vessel, and not to keep it along storage, together with the needless temperature control (performed at naturally variable/uncontrolled room temperature, RT) (Fernandes et al., 2014). This allows substantial

* Corresponding author.
E-mail address: jorgesaraiva@ua.pt (J.A. Saraiva).

https://doi.org/10.1016/j.fm.2018.03.010
0740-0020/

energetic savings and, consequently, economic gains and reduced CO_2 emissions. In fact, Bermejo-Prada et al. (2017) demonstrated that keeping 800 Kg of strawberry juice under HS at RT (HS/RT) conditions for 15 days had an energetic cost of 0.002$, against 0.034$ of RF. Still, equipment costs for HS were estimated by the same authors as being currently higher compared to RF. In an industrial point of view, the aforecited author also stated that, if a liquid food product is to be stored under HS/RT conditions, it could be used as the pressurization fluid itself.

HS performance at room-like temperatures is being increasingly investigated regarding the preservation of highly perishable food products (low acidity and high a_W), namely watermelon juice (Fidalgo et al., 2014; Lemos et al., 2017; Pinto et al., 2017, 2016; Santos et al., 2015), carrot soup (Moreira et al., 2015), *requeijão* (Portuguese whey cheese) (Duarte et al., 2015), cooked ham (Fernandes et al., 2015), raw bovine meat (Freitas et al., 2016) and tilapia fillets (Ko and Hsu, 2002). Moreover, the effect of HS/RT has been also extensively evaluated for strawberry juice (acidic food product) when it comes to its microbiological, physicochemical and enzymatic parameters (Bermejo-Prada et al., 2016, 2015; Bermejo-Prada and Otero, 2016; Segovia-Bravo et al., 2012). Moreover, it was recently proved that HS/RT performed similarly (50 MPa) to better (75 and 100 MPa) than the conventional RF regarding the development of pathogenic surrogated microorganisms (*Escherichia coli* and *Listeria innocua*) (Pinto et al., 2017).

All these studies concluded that HS/RT performed similarly or even better than RF concerning the preservation of the quality attributes (colour, volatile profiles (strawberry juice), phenolic compounds, among others) and microbial development control, resulting in potential shelf-life extensions when compared to RF (Freitas et al., 2016; Lemos et al., 2017; Pinto et al., 2017, 2016).

When it comes to enzymatic activity, Bermejo-Prada et al. (2015) reported a significant increase of polyphenol oxidase (PPO) activity on strawberry juice stored under different HS/RT conditions (50 and 200 MPa/15 days) compared to RF storage. Contrarily, significant peroxidase (POD) inactivation on longer HS/RT periods (200 MPa/15 days) was found, while pectin methylesterase (PME) catalytic activity was not affected by HS/RT compared to samples stored under RF (Bermejo-Prada et al., 2016). These results are generally in agreement with those reported by Pinto et al. (2017), who evaluated the impact of HS/RT (50, 75 and 100 MPa/10 days) on the enzymatic parameters of PPO, POD and PME of watermelon juice.

The aforementioned studies only reported the effect of HS on vegetative microorganisms, and even though information regarding the effect of low pressures on some *Bacillus* spp. and *Clostridium* spp. endospores is available, the cases reported studied only short periods of time (few minutes/hours). For example, Aoyama et al. (2005) reported a germination rate of about 4 and 1.5 log-cycles at 100 MPa for 1 h, at 40 and 60 °C, respectively for *Bacillus subtilis* endospores suspended in glucose broth, as well the reduction of about 1 log cycle on endospore counts at 80 MPa for 1 h at 60 °C in phosphate buffer. However, literature concerning the HS effect (25–220 MPa over days of storage) on endospores is unavailable.

In fact, only three related papers are available, as the authors are aware, concerning this subject, suggesting that a combination of mild pressures (40–100 MPa) and moderate temperatures (30–80 °C) for periods up to 4 days enhances the germination and inactivation of *Bacillus* spp. and *Clostridium* spp. (Aoyama et al., 2005, 2004; Shigeta et al., 2007). The authors of the aforecited studies meant to trigger endospore germination by combining low hydrostatic pressures with moderate/higher temperatures (than those of the HS range), and with a different final objective, consisting only in endospore germination induction for subsequent inactivation by further processing.

The spore-former *B. subtilis* is a gram-positive, facultative aerobic, non-pathogenic and rod-shaped bacteria whose endospores are widely used for food processing design. In fact, they are used as surrogated endospores of the pathogenic *B. cereus* (that are quite heat-resistant and its vegetative form produces cereulide, a heat-resistant emetic toxin) resulting in food poisoning illness (such as vomits and nausea) (Agata et al., 2002; Checinska et al., 2015). *B. cereus*, along with *B. subtilis* endospores, are prevalent in low acidic food products such as meat (Soni et al., 2016), raw and pasteurized milk (Christiansson et al., 1999; Eneroth et al., 2001) and carrot juice (Aneja et al., 2014), among others. These products need to be preserved at RF conditions to inhibit endospore germination and outgrowth, since both pH and a_W do not hurdle the microbial development on the aforementioned products.

Given the importance of these biological structures on food safety, HS/RT of three different matrices was performed, consisting of McIlvaine buffer (pH 6.00), carrot juice and brain-heart infusion broth (BHI-broth, a general, non-selective culture media) (both at pH 6.00). Each matrix was inoculated with *B. subtilis* endospores and stored at 25, 50 and 100 MPa for up to 60 days at naturally variable/uncontrolled RT (18–23 °C) and compared with atmospheric pressure (AP) storage at both RT and RF (4 °C). These three different matrices were used since the easiness of *B. subtilis* endospores germination increases in the order McIlvaine buffer (a nutrient-free matrix), carrot juice (an intermediate nutrient matrix) and BHI-broth (optimal growth matrix), allowing to evaluate the endospore behaviour at HS/RT under very different conditions, as well the matrix composition influence on the endospore behaviour under pressure.

2. Materials and methods

2.1. Reagents and solutions

Physiological solution (0.9% NaCl) and citric acid were purchased from Applichem Panreac (Darmstadt, Germany), BHI-broth and BHI-agar were obtained from Oxoid (Cheshire, United Kingdom), and sodium phosphate dibasic was purchased from Riedel-de Haën (Seelze, Germany).

2.2. Matrices preparation

The McIlvaine citrate-phosphate buffer (0.2 M of Na_2HPO_4 and 0.1 M of citric acid) at pH 6.00 and BHI-broth were prepared according to McIlvaine (1921) and the instructions provided by the supplier, respectively.

Fresh carrots (*Daucus carota* subsp. *Sativus*) were purchased at a local supermarket. Then, the carrots were washed with distilled water to remove dust and other adhered particles and cut in small pieces that were crushed with a blender, (for each 150 g of carrots, 300 mL of distilled water were added). The juice was then filtered with a cotton filter to remove coarse particles.

The inoculation matrices were sterilized at 121.1 °C for 15 min and were used on the same day of its preparation. Moreover, as the main purpose of this study concerns the HS evaluation on endospores, and as both a_W (Sevenich et al., 2015) and pH (Black et al., 2007; Reineke et al., 2013a) are known to influence endospore behaviour under hydrostatic pressure, the pH of both carrot juice and BHI-broth were adjusted to 6.00 with sterile citric acid (0.1 M), while the a_W was just measured using a hygrometer (Lab Swift – a_W, Novasina AG, Switzerland), being verified a similar value for the three matrices.