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High pressure processing pretreatment enhanced the thermosonication inactivation of *Alicyclobacillus acidoterrestris* spores in orange juice

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ABSTRACT

The spoilage of high acid fruit juices and nectars by *Alicyclobacillus acidoterrestris* is a major concern to juice manufacturers around the world since it is difficult to detect. In this study, thermosonication (ultrasound and heat, TS) and thermal inactivation of *A. acidoterrestris* spores in pretreated orange juice were carried out and resistance parameters were estimated. First, the effect of TS acoustic energy density (AED, 0.3–20.2 W/mL) on the inactivation at 75 °C was investigated. Then, the influence of TS temperature (70–78 °C) on the spore inactivation (AED = 20.2 W/mL) was studied. Next, we explored the effect of high pressure processing (HPP) pretreatment of juice on the 20.2 W/mL TS inactivation at the best temperature (78 °C). Lastly, the thermal inactivation of spores in juice heat shocked + 1 min sonicated vs. untreated juice was also investigated.

Results of TS showed higher spore inactivation for higher AED ($D_{75°C}$-value of 49 min for 20.2 W/mL vs. 217 min for 0.33 W/mL). Lower *D*-values were obtained at higher temperatures ($D_{78°C}$-value of 28 min vs. $D_{70°C}$-value of 139 min at 20.2 W/mL). The TS $D_{78°C}$-value (at 20.2 W/mL) decreased further from 28 min to 14 min when the orange juice was previously submitted to 600 MPa for 15 min. Thermal treatment alone at 78 °C resulted in almost no spore inactivation, whereas the heat shock + ultrasound pretreatment of juice enhanced the thermal inactivation of spores ($D_{85°C}$-value decreased from 69 to 29 min). To conclude, HPP-assisted TS provided the best method for spore inactivation, indicating the benefit of high pressure and power ultrasound technology in addition to heat. TS required at least 8 °C lower temperatures than thermal treatments to achieve the same spore inactivation, which could enhance juice quality and energy savings.

1. Introduction

Alicyclobacillus acidoterrestris (AAT) is an aerobic, rod-shaped, gram-positive, endospore-forming bacterium which is able to grow at a pH range of 2.0–7.0 and a temperature range of 25–60 °C. Optimal growth occurs at a pH of around 4.0–4.5, and a temperature around 40–45 °C (Bevilacqua, Sinigaglia, & Corbo, 2008a). The spores of AAT survive the thermal pasteurization (generally between 80 and 100 °C) employed by the fruit beverage industry, and exhibit very high heat resistance 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) (Silva & Gibbs, 2001; Silva, Gibbs, Nunez, Almonacid, & Simpson, 2014). AAT spore germination and growth up to a level of 10^5–10^6 cfu/mL can occur after pasteurization (cycle of up to 5 days) in high-acid fruit juices when the storage and distribution temperatures are around 40 °C (Splittstoesser, Churey, & Lee, 1994). Product spoilage is difficult to detect visually since AAT does not produce gas during growth. However, juice/beverage spoilage is evident by the off flavour, caused by guaiacol and other halophenols (ppb) (Gocmen, Elston, Williams, Parish, & Rouseff, 2005). Therefore, AAT was suggested as reference microorganism for pasteurization processes in high-acid fruit products (Silva & Gibbs, 2001; 2004).

Large-scale AAT spore germination and spoilage was first reported in 1982 in aseptically packaged apple juice (Cerny, Hennlich, & Poralla, 1984). Since then, other incidents have been reported in USA, Europe and Japan (Jensen, 2000) and in different types of fruit products such as lemonade carbonated fruit juice

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drinks, shelf-stable ice tea containing berry juice, fruit pulps, and canned diced tomatoes (Duong & Jensen, 2000; Pettipher & Osmundson, 2000; Walls & Chuyate, 1998). Today, food and beverage spoilage by AAT spores has become an industrial issue.

The effectiveness of heat alone or combined with antimicrobials for inactivating AAT has been investigated: nisin was added to fruit juices (Komitopoulou, Boziaris, Davies, Delves-Broughton, & Adams, 1999), chlorine dioxide was added to the surface of apples (Lee, Gray, Dougherty, & Kang, 2004), grape polyphenols were added to grape juice (Oita & Kohyama, 2002), enterocin AS-48 was added to fruit juices (Grande et al., 2005), ascorbic acid was added to apple juice (Bahçeci & Acar, 2007), and eugenol and cinnamaldehyde were added to acidified malt extract broth (Bevilacqua, Corbo, & Sinigaglia, 2008b).

A number of different non-thermal technologies and their combination with heat have also been investigated for microbial spore inactivation in juices, fruit products and other foods (Evelyn, Kim, & Silva, 2016). These include high hydrostatic pressure combined with heat or HPP-thermal (Evelyn & Silva, 2015a; S. Lee, Chung, & Kang, 2002; Shearer, Hoover, Dunne, & Sikes, 2000; Silva, Tan, & Farid, 2012; Sokołowska et al., 2012), high pressure carbon dioxide (Bae, Lee, Kim, & Rhee, 2009; Casas, Valverde, Marín-Iniesta, & Calvo, 2012), and radiation (Nakauma, Saito, Katayama, Tada, & Todoriki, 2004). Power ultrasound is another non-thermal method that has been studied for microbial spore (Evelyn & Silva, 2015b, 2015c) and enzyme inactivation (Sulaiman, Soo, Farid, & Silva, 2015). Ultrasonic waves at sufficient intensity can cause microbial cell death by a phenomenon called cavitation (Chen, 2012). The microgas bubbles are formed during the rarefaction cycle of the acoustic wave within a liquid, collapse violently during the compression cycle (Leong, Ashokkumar, & Kentish, 2011), and create micro-mechanical shocks leading to disruption of cellular components and hence cell lysis (Guerrero, López-Malo, & Alzamora, 2001). Lower decimal reduction values (*D*-values) of bacterial and mould spores were registered after simultaneous use of ultrasound and heat (thermosonication [TS]) and ultrasound-assisted (before or after) thermal processing (Burgos, Ordonez, & Sala, 1972; Evelyn & Silva, 2015b, 2015d; Garcia, Burgos, Sanz, & Ordonez, 1989; López-Malo, Palou, Jiménez-Fernández, Alzamora, & Guerrero, 2005; Ordonez & Burgos, 1976).

To date, limited information is available on the inactivation of AAT by power ultrasound, especially on spores (Ferrario, Alzamora, & Guerrero, 2015; Wang, Hu, & Wang, 2010; Yuan, Hu, Yue, Chen, & Lo, 2009). Therefore, in this research, orange juice inoculated with AAT spores was processed by TS. The effects of varying energy density, temperature, and juice pretreatments were investigated, and the spore first-order TS resistance parameters (*D*- and z-values) were determined and compared with thermal inactivation processes. The specific objectives were: (i) to determine the best acoustic energy density (AED) for TS inactivation at 75 °C; (ii) to determine the effect of TS temperature on the *D*-values, (iii) to study the effect of high pressure pretreatment on the TS spore inactivation and compared with thermal inactivation alone; (iv) to compare the thermal resistance of spores in orange juice pretreated with ultrasound vs. no pretreatment; and (v) to recommend optimal TS conditions for the pasteurization of orange juice.

2. Material and methods

2.1. A. acidoterrestris microbiology

2.1.1. Strain

Alicyclobacillus acidoterrestris type strain NZRM 4447 (same as ATCC 49025 and NCIMB 13137) was obtained from the New Zealand Reference Culture Collection. This strain was isolated from apple juice concentrate. It was precultured on potato dextrose agar (PDA, Difco North Ryde, Australia) adjusted to a pH 4.0 with 10% w/v (0.1 g/mL) tartaric acid. The PDA plates were incubated at 45 °C for 3 days and used as source of inoculum for sporulation.

2.1.2. Sporulation

The sporulation procedure described by Silva et al. (2012) was used. Briefly, the fresh cells from the initial culture were inoculated on PDA (pH 5.6) and incubated for 21 days at 45 °C to obtain spores. The spores were confirmed by phase contrast microscopy (Motic microscope BA410 Series, Canada). Then the spores were harvested by flooding the plates with 1–2 mL of sterile distilled water and dislodging the spores from the agar surface with a sterile bent glass rod. After harvesting, the spores were washed three times by centrifugation with sterile distilled water (Centrifuge Sigma 4K15, UK) at 4,000 g and 4 °C for 10 min, resuspended in 50 mL sterile phosphate buffer (pH 7.2), and stored at 2 °C until use.

2.1.3. Orange juice inoculation

The orange juice used in this study (pH 3.8, 9.5 ± 0.1°Brix) was purchased from a local supermarket and used as the treatment medium for the AAT spore inactivation. The juice contained added pulp, flavour, food acid (citric acid), and preservatives (potassium sorbate). For 0.33 W/mL TS experiments, a small portion (ca. 1–2 mL) of spore solution was inoculated into 99 mL of orange juice, whereas for thermal and other TS experiments, 1 mL of the spore solution was inoculated into 49 mL of orange juice. A final spore concentration of approximately 10^6 or 10^7 cfu/mL was obtained in orange juice.

2.1.4. Spore enumeration

The *A. acidoterrestris* spore concentration in the juice before and after processing was determined by spread plating into acidified (pH 4) PDA plates. The spore concentration before processing was determined after a heat shock treatment (80 °C, 10 min) of 5 mL juice in a thermostatic water bath to eliminate any vegetative cells. Orange juice samples were decimal diluted ten times with 0.1% (w/v) sterile buffered peptone water (Difco, Becton Dickinson, USA). Each tube dilution was mixed repeatedly using a high-speed vortex mixer to yield a uniform spore suspension, and plated twice. The PDA plates were then inverted inside a sealed plastic bag, to avoid drying of the medium and keep the moisture away from the agar surface, and incubated at 45 °C for 3–5 days. Plates showing 30 to 300 colonies were used for enumeration, and spore concentration was expressed in colony forming units per milliliter (cfu/mL) of juice sample after calculations for corresponding dilution.

2.2. Experimental design and data analysis

2.2.1. Experimental design

The first experiment examined the effect of TS acoustic energy density (AED) at 75 °C on AAT spore inactivation in orange juice. The AED of 0.33, 4.10 and 20.20 W/mL were used and evaluated, with 20.20 W/mL being the maximum energy of the equipment for the tip and juice volume used in the TS process. Because AED 20.20 W/mL was also the best performing AED, the following TS experiments were carried out at 20.20 W/mL. In the next experiment, TS inactivation of AAT spores was carried out at three temperatures (70, 75, and 78 °C) for up to 60 min; 78 °C is the maximum temperature recommended for this ultrasound unit by the manufacturer. Thirdly, we investigated the effect of TS on AAT spore inactivation with and without juice HPP pretreatments, and compared with TS and thermal inactivation alone at 78 °C, the best temperature. Two 15 min HPP pretreatments at 200 and 600 MP