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Improving the efficiency of ultra-high pressure homogenization treatments to inactivate spores of *Alicyclobacillus* spp. in orange juice controlling the inlet temperature

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ABSTRACT

Samples of orange juice inoculated with strain CECT 7094 of *Alicyclobacillus acidoterrestris* or strain CECT 5324 of *Alicyclobacillus hesperidum* were pre-heated to five different temperatures (20, 50, 60, 70 and 80 °C) before applying a 300 MPa Ultra High Pressure Homogenization (UHPH) treatment. Treated and control samples were kept during 30 days at 22 °C, 30 °C and 43 °C to evaluate the ability of the surviving spores to germinate and grow. UHPH treatments hardly affected the spore counts at the lowest inlet temperature (20 °C), but significant reductions were observed when inlet temperature raised to 60 °C, achieving lethality values above 5 Log CFU/ml when juice samples were pre-heated at 70 for *A. hesperidum* and 80 °C for *A. acidoterrestris*. During the later storage of the juice samples *Alicyclobacillus* counts increased significantly during the first 15 days in samples pre-heated at 20 °C and 50 °C when they were stored at both 30 °C and 43 °C, achieving similar counts than control samples, but not at 22 °C. Nevertheless, in samples pre-heated at 70 and 80 °C spore counts remained below the detection limit indicating that these treatments were the most efficient inactivating *Alicyclobacillus* spp. spores.

1. Introduction

Ultra high pressure homogenization (UHPH) is an emerging technology that allows to process in continuous fluid foodstuffs with proved efficiency inactivating pathogenic and most of the spoiling microorganisms in different kind of foods. The application of UHPH treatments at pressures above 200 MPa with inlet temperatures of 4 °C and 20 C has been shown effective in reducing the endogenous microbiota of apple juice (Suarez-Jacobo, Gervilla, Guamis, Roig-Sagues, & Saldo, 2010) and orange juice (Velazquez-Estrada, Hernandez-Herrero, Guamis-Lopez, & Roig-Sagues, 2012; Velazquez-Estrada et al., 2011) at a level comparable to that achieved by the industrially used thermal pasteurisation. From the point of view of safety, UHPH treatments of 300 MPa can reduce by at least 5 ULog counts of major pathogenic bacteria (*Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Listeria monocytogenes* or *Staphylococcus aureus*) (Briñez, Roig-Sagues, Hernandez-Herrero & Guamis-Lopez, 2006; Briñez, Roig-Sagues, Hernandez-Herrero, & Guamis-Lopez, 2007; Velazquez-Estrada, Hernandez-Herrero, Lopez-Pedemonte, Guamis-Lopez, & Roig-Sagues, 2008), as required by the U.S. Food and Drug Administration in fruits, vegetables and their juices (Choi & Nielsen, 2005). One of the main advantages of this technology is that it is less harmful over the nutritive and functional properties of fruit juices, such as the antioxidant capacity, polyphenol composition, vitamin C and provitamin A content, than the commonly used thermal treatments, as have recently been described by Suarez-Jacobo et al. (2011) and Velazquez-Estrada, Hernandez-Herrero, Ruefer, Guamis-Lopez, and Roig-Sagues (2013) in apple and orange juices, respectively.

Different species of the genus *Alicyclobacillus* have been described as common spoilage bacteria in different types of fruit juices when stored above room temperatures (Silva, Gibbs, Vieira, & Silva, 1999). These sporulated Gram positive rods are acidophilic, with optimum growth at pH values between 2.5 and 5.5, as well as thermophilic, growing at temperatures between 25 and 60 °C (Murakami, Tedzuka, & Yamazaki, 1998). The spores of *Alicyclobacillus* are also very heat resistant. Silva and Gibbs (2004) proposed that *Alicyclobacillus acidoterrestris* should be the target used for the

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fruit products industry in the design of pasteurization processes. Bevilacqua, Cibelli, Corbo, and Sinigaglia (2007) reported that UHPH treatments from 50 to 170 MPa hardly reduced by 1–2 log CFU/ml the counts of vegetative cells of *A. acidoterrestris* and less than one log CFU/ml the counts of its spores in culture broth. Similar results were observed when the microorganism was inoculated in apple juice (Bevilacqua, Corbo, & Sinigaglia, 2012). However, during the later storage of the juice a reduced survival rate of the microorganisms was appreciated indicating that some sublethal structural damages could have been caused in the spores by UHPH making them more vulnerable to the acidic conditions. This effect of UHPH treatments increasing sensitization to unfavourable environmental conditions was previously described for different pathogenic microorganisms present in fruit juices (Briñez et al. 2006, 2007; Velazquez-Estrada et al., 2008), Milk (Roig-Sagues et al., 2009) and whole egg (Velazquez -Estrada et al., 2011).

The combination of UHPH treatments with some chemical agents, such as benzoate, dimethyl-dicarbonate or eugenol, has been evaluated but it did not increased significantly the lethality of *A. acidoterrestris* spores in culture media (Chen, Harte, Davidson, & De Oro, 2013) or apple juice (Bevilacqua et al., 2012). Concerning the combination of UHPH with other physical treatments, Chaves-Lòpez et al. (2009) evaluated the efficiency of applying UHPH treatments at 150 MPa alone or in combination with a non-isothermal heat treatment (from 20 °C to 85 °C) on spores of *Bacillus cereus* and *B. Subtilis* suspended in water, reporting that the combination of a single cycle of UHPH with the heat treatment reduced in less than two Log CFU/ml the spore counts.

Although UHPH is usually described as a non-thermal technology, temperature usually increases significantly when the sample passes through the valve as a consequence of the adiabatic heating generated in the machine in addition to the high turbulence, shear and cavitation forces suffered (Hayes & Kelly, 2003; Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003). It has been suggested that the increment of the inlet temperature improves the rate of microorganism inactivation in a synergic effect with the pressure applied, enhancing the effectiveness of the treatment (Diels, Wuytack, & Michels, 2003; Dumay et al., 2013; Vachon, Kheadr, Giasson, Paquin, & Fliss, 2002). In UHPH treatments below 200 MPa the inactivation obtained is mainly due to mechanical effects than the thermal mechanisms in the homogenization valve, but when the fluid reaches temperatures about 80 °C in the valve, the observed microbial inactivation could mainly be explained by the shear-induced temperature effects (Pathanibul, Taylor, Davidson, & Harte, 2008). Velazquez-Estrada et al. (2013) reported that orange juice temperatures increased to above 90 °C when was submitted to an UHPH treatment at 300 MPa at an inlet temperatures of 20 °C, while the maximum temperature achieved was around 70 °C at 200 MPa and around 45 °C at 100 MPa. Recently, Amador- Espejo, Hernandez-Herrero, Juan, and Trujillo (2014) described that milk samples submitted to UHPH treatments increased 20.1 °C for each100 MPa when passing through the valve when the inlet temperatures was 55 °C while this increase was reduced to 17.8 °C/100 MPa when the inlet temperatures increased to 85 °C, registering in the valve a maximum temperature of 139 °C. As a consequence, a complete inactivation of spores of different species of the genus *Bacillus* was reported. This temperature is into the range of temperatures achieved during ultra-high temperature treatments used to sterilize commercial milks, but with the difference that during the UHPH treatments the sample remains at this maximum temperature for less than a second which a priori would be better for the nutritive and sensorial quality.

The aim of this study was to investigate the effect of increasing the inlet temperatures on the efficiency of UHPH treatments to inactivate spores of *Allicyclobacillus* spp. in orange juice determining the role of the maximum temperature achieved on it. The capability of the surviving spores to germinate and grow during the shelf life of the juice stored at room and abuse temperatures was also investigated in order to evaluate whether UHPH is a suitable technology for obtaining commercially sterile fruit juices or not.

2. Materials and methods

2.1. Alicyclobacillus spp. spore preparation

Alicyclobacillus acidoterrestris (CECT 7094) and *Alicyclobacillus hesperidum* (CECT 5324) strains were obtained from the Spanish Type Culture Collection (CECT). K broth (Chang & Kang, 2004) was used to recover the spores of the *Alicyclobacillus* spp. strains. K broth was incubated at 43 °C for 48 h. One millilitre of the culture broth was transferred to a 250 ml Roux flask containing 50 ml of Potato Dextrose Agar (APD, Oxoid, Basingstoke, UK) medium. Roux bottles were kept at 43 °C between 7 and 14 days until obtaining a sporulation rate above 80%. After this period, spores were collected by adding at least four aliquots of 5.0 ml of sterile distilled water into the Roux bottles and scratching gently the surface with a sterile loop. All the fractions were collected in a sterile centrifuge tube and centrifuged at 10.000 g for 20 min at 4 °C using a Sigma 4K15 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The pellet was suspended with 15 ml of sterile distilled water and centrifuged again during 10 min under the same conditions. This washing procedure was repeated four times. The resulting pellet was finally suspended with 15 ml of sterile distilled water and submitted to a heat treatment at 80 °C for 10 min placing afterwards the suspension on an ice bath for additional 10 min.

2.2. UHPH treatments

Spores were inoculated into sterilized commercial orange juice in order to achieve a final concentration between 10^5-10^6 spores/ml. Samples were UHPH treated at 300 MPa using a benchtop UHPH homogenizer (model/DRGFPG7400H:350, Stansted Fluid Power Ltd., Essex UK). Inlet temperature (InT) of samples was previously adjusted to 20 °C, 50 °C, 60 °C, 70 °C or 80 °C using a heat exchange coil connected to the admission of the UHPH equipment.

2.3. Microbiological analysis

SK Agar prepared according to Chang and Kang (2004) was used to count the initial load of spores (N_0) as well as the surviving spores after the UHPH treatments (N_f). SK agar plates were incubated at 43 °C for 72 h. The lethal effect of the treatment was assessed comparing the N_f with the N_0 counts expressed as Log CFU/ml of juice. To evaluate the ability of the surviving spores to germinate and grow after the treatments samples were divided in three batches which were stored at three different temperatures: 22 ± 1 °C (room temperature), 30 ± 1 °C and 43 ± 1 °C, performing the aforementioned microbiological analysis after 15 and 30 days of storage.

2.4. pH measurements

The pH of samples was evaluated after de treatments and during the storage period using a GLP 22 Crison pH metre (Crison Instruments, Alella, Barcelona).