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Treatment with high hydrostatic pressure and supercritical carbon dioxide to control *Alicyclobacillus acidoterrestris* spores in apple juice☆

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ABSTRACT

Alicyclobacillus acidoterrestris is a thermoacidophilic and spore-forming bacterium that can develop in pasteurized juices. However, there are innovative technologies for food processing and preservation such as high hydrostatic pressure (HHP) and supercritical carbon dioxide (SCCD) that can be used to inactivate these bacteria.

The objective of this study was to investigate the effect of SCCD and HHP on inactivation and germination of spores of two *A. acidoterrestris* strains.

Spores were suspended in apple juice (70.7, 35.5, 23.7, 11.2 °Bx) and treated with SCCD: 60 MPa/50–75 °C/20–40 min and HHP: 300 MPa/50–75 °C/5–15 min. The number of surviving population was determined by the plate method on BAT-agar with or without heat treatment (80 °C, 10 min).

After 40 min of SCCD treatment at 60 MPa, 75 °C, germination of *A. acidoterrestris* TO-169/06 spores in apple juice (11.2 °Bx) was 3.9 log of which 3.4 log were inactivated. Under the same process conditions but in the 70.7 °Bx juice much lower degree of germination (0.9 log) and inactivation (0.5 log) was obtained.

In the case of *A. acidoterrestris* TO-169/06 strain HHP processing at 300 MPa, 50 °C, 15 min in apple juice (11.2 °Bx) 4.4 log of spores germinated of which 3.7 log were inactivated.

In the contrary, under the same conditions, in apple juice concentrate germination and inactivation of the spores was 1.5 log and 0.9 log resp. The spores of the *A. acidoterrestris* TO-117/02 strain were significantly more resistant to SCCD and HHP on the used parameters.

These results demonstrate that SCCD and HHP combined with moderately elevated temperature may be a useful technique for inactivation of *A. acidoterrestris* spores in single strength juices.

1. Introduction

Alicyclobacillus acidoterrestris is a gram-positive, spore-forming and thermoacidophilic bacterium, which survives typical pasteurization, causing spoilage of juices by producing compounds associated with a disinfectant-like odour (guaiacol, 2,6–dibromophenol, 2,6–dichlorophenol) (Gocmen, Elston, Williams, Parish, & Housett, 2005). These compounds are very potent odorants. The odour detection threshold of guaiacol in apple juice was estimated as 0.57- mg/L (Siegmund & Pöellinger-Zierle, 2006) or 0.91 mg/L (Eisl & Semon, 2005), and recognition threshold as 2 mg/L (Siegmund & Pöellinger-Zierle, 2006) or 2.23 mg/L (Orr, Shewfelt, Huang, Tefera, & Beuchat, 2000). Sensory detectable amounts of aromatic compounds were produced by *A. acidoterrestris* when the count of bacteria reached the level of 10^6 to 10^7 cfu/mL (Pettipher, Osmundson, & Murphy, 1997).

Bacterial spores are extremely resistant to environmental stresses such as elevated temperature, and can survive conventional pasteurization. The use of higher temperatures during heat treatment, which might inactivate spores, is one of the possibilities of avoiding problems with spoilage of juices caused by *A. acidoterrestris*, but is usually connected with a deterioration in the nutritious and sensory quality of the product. Therefore it is necessary to look for innovative, preferably non-thermal technologies for juice preservation, enabling the elimination of these bacteria, such as high hydrostatic pressure (HHP) (Huertas, Esteban, Antolinos, & Palop, 2014; Oey, Lille, Van Loey & Hendrickx, 2008;

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Wang, Faeder, Setlow, & Li, 2015) and supercritical carbon dioxide (SCCD) (Garcia-Gonzalez et al., 2007).

As a non-thermal processing technique, HHP and SCCD applies to inactivate vegetative microorganisms, some enzymes and to preserve product quality attributes. Those methods are a novel food preservations that are already applied for example to sliced or ready –to–eat: e.g.: meat products and fruits and vegetables juices to reduce microbiological risk while preserving their quality characteristic. Alternative technologies have the potential to be a tool for preserving bioactive compounds in crucifereus vegetables, especially glucosinolates and polyphenols, by providing a mild treatment for controlled enzyme inactivation (Moris, Brody & Wicker, 2007). Prior to industrial application there is need to understand and map the effect of HHP and SCCD on bioactive compounds and the degrading enzymes. The adaption and commercialization of HHP is continuing to emerge in many food processing categories. These method has many commercial applications now on the market place around the world. Food processors are using nonthermal methods as practical solutions to produce microbiologically safe foods but also products with better quality and more often with enhanced health benefits (Moris et al., 2007; Raso & Barbosa-Canovas, 2003).

No thermal processing of foods has emergered as a viable alternative to those conventional processing techniques by offering safe products of excellent quality and very reasonable cost. These emerging technologies utilize nonthermal microbial stress factors as the main inactivation mechanism. These methods could be used in combination or with other preservation approaches seeking effects in order to have shorter processes and very good quality food products (Ortega-Rivas & Salmeron Ochoa, 2014).

Many studies have demonstrated that the application of HHP, combined with an elevated temperature, can efficiently inactivate bacterial spores, e.g. approximately 2 log reduction of *A. acidoterrestris* spores after processing with 200 MPa at 65 °C for 10 min, was obtained by Silva, Tan, and Farid (2012), and more than 5.5 log reduction was achieved at 70 and 90 °C using pressure ranging from 207 to 621 MPa (Lee, Dougherty, & Kang, 2002).

The efficacy of carbon dioxide, in both a supercritical and gaseous state, under high pressure, has been examined to inactivate microorganisms, both pathogenic and food-borne, including yeasts, moulds, as well as lactic acid bacteria, in fruit and vegetable products. Treatment at 20 MPa and 45 °C for 15 min reduced levels of *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes, Staphylococcus aureus*, by > 7.0 log (Garcia-Gonzalez et al., 2007; Jung, Choi, & Rhee, 2009; Rawson et al., 2012). In the case of *Bacillus* spores 2 log reduction was observed during treatment with pressure 30 MPa at 35 °C (Watanabe et al., 2003). Applying CO_2 at 15 MPa, 60 °C for 30 min reduced the number of *A. acidoterrestris* spores by 3 log in apple cream (Casas, Valverde, Marin-Iniesta, & Calvo, 2012) and total inactivation was achieved at above 65 °C, 10 MPa for 40 min and 70 °C at 8 MPa for 30 min in apple juice (Bae, Lee, Kim, & Rhee, 2009).

To enhance the effectiveness of the process, inducing spore germination and transforming them into a vegetative form is recommended as this greatly increases their susceptibility to inactivation using physical or chemical agents, whereas their metabolic activity remains unchanged (Setlow, 2003).

The inactivation of *Alicyclobacillus* spores trough the HHP occurs in two steps: within the first phase the high pressure induces the spores to germinate, and in the second step causes the inactivation of germinated spores (Luu et al., 2015; Setlow, 2003). Spores of *Bacillus* species could also be germinated at HHP by the activation of the GRs (germinant receptors). However, spores lacking GRs also germinate with HHP, which indicates that HHP likely open the spores Ca-DPA channels directly (Paidhungat et al., 2002; Wuytack, Soons, Poschet, & Michiels, 2000).

An alternative approach to efficient spore inactivation by HHP could be the combination of pressure with some compounds or the use of pressure oscillations, to induce spore germination at low pressure followed by higher pressures to destroy cells (Parades-Sabja Setlow & Sarker, 2011; Wei et al., 2010).

Spore germination in *Bacillus subtilis* is triggered by HHP which acts through the GerA, GerB and GerK receptors. The germination signal from the GRs is transduced and amplified in manner by the GerD protein, and this leads to release of monovalent cations and then dipicolinic acid and changes in cortex strain, degradation of cortex, allowing and initiation of spore outgrowth, and finally completion spore germination (Doona et al., 2014; Parades-Sabja et al., 2011; Reineke, Mathys, Heinz, & Knorr, 2013; Setlow, 2014a).

Spores are killed by damaging a number of different components, including DNA, the spore's inner membrane, proteins in the spore core. Spores also utilize a variety of strategies to generate their extreme resistance, including the maintenance of special outer layers to help protect sensitive spore components such as peptidoglycan from enzymatic attack and DNA from chemical attack (Setlow, 2014b).

Inactivation of spores by highly pressurized CO_2 follows different pathway and occurs in two steps: a first step would indicate penetration of CO_2 into the cells, while the second step corresponds to destruction and inactivation of germinated cells (Setlow, Korza, Blatt, Fey, & Setlow, 2016).

Although the effect of HHP and SCCD on spore inactivation has been broadly studied, there are only a few papers on the germination of *A. acidoterrestris* spores induced by HHP (Porębska, Rutkowska, & Sokołowska, 2015a; Sokołowska et al., 2015, 2013; Vercammen, Vivijs, Lurquin, & Michiels, 2012) and SCCD (Porębska, Sokołowska, & Łaniewska- TrokenheimŁ, 2016). These papers were focused on single strength juices and buffers.

The aim of this study was to investigate the effect of two innovative non-thermal preservation methods, SCCD and HHP, on the inactivation and germination of spores of two *A. acidoterrestris* strains in apple juice with various soluble solids content.

2. Materials and methods

2.1. Tested organism

The *A. acidoterrestris* strains TO-169/06 and TO-117/02 used in this research were isolated from Polish concentrated apple juice, using the International Federation of Fruit Juice Producers' method (2004/2007). These strains were chosen from among eight wild strains tested in a previous study (Skąpska et al., 2012) because of their different response to external factors. The TO-117/02 strain was highly resistant to HHP and the TO-169/06 was the most sensitive.

2.2. Spore production and preparation of samples

Spores were produced based on the method described by Sokołowska et al. 2012. Just before the experiments, spores were suspended in apple juice of various soluble solids content. The final microbial concentration of inoculated juices was 6 log cfu/mL. Two-, three- and six-fold dilutions were made from apple juice concentrate (70.7 °Bx; pH 3.06; a_w 0.774) with sterile deionized water. The soluble solid content and water activity ranged from 35.5 °Bx (a_w 0.950) in the two-fold diluted concentrate, trough 23.7 °Bx (a_w 0.982) in the three - fold diluted concentrate to 11.2 °Bx (a_w 0.996) in the six-fold diluted concentrate. The pH of the juices ranged from 3.20 to 3.30. Water activity was measured in temperature 25 °C with using AQUALAB,