Accepted Manuscript

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

Rafael Uchida, Filipa V.M. Silva

PII: S0956-7135(16)30461-3

DOI: 10.1016/j.foodcont.2016.08.034

Reference: JFCO 5204

To appear in: *Food Control*

Received Date: 26 February 2016

Revised Date: 21 July 2016

Accepted Date: 21 August 2016

Please cite this article 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), doi: 10.1016/j.foodcont.2016.08.034.

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

Rafael Uchida, Filipa V.M. Silva*

Department of Chemical and Materials Engineering, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.

* Corresponding author Filipa Silva. Tel: +64 9 3737999, Fax: +64 9 3737463, Email: filipavinagresilva@gmail.com.

Abstract

High pressure processing (HPP) comprises the application of pressures between 100-1000 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 $D_{10°Brix}$ = 4.2 min, $D_{20°Brix}$ = 7.6 min, $D_{30°Brix}$ = 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.

Keywords: acidophilic, high pressure thermal processing, *Alicyclobacillus acidoterrestris*, fruit juices, fruit concentrates, Brix.

1. Introduction

Although the first scientific publication demonstrating the extension of milk shelf life by high pressure processing (HPP) dates back to1899 (Hite, 1989), 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, Vieira, & Silva, 1999; Silva & Gibbs, 2001; 2004; 2009; Silva, Gibbs, Nunez, Almonacid, & Simpson, 2014). 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 – 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 characteristic 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 to 600 MPa (Black, et al., 2007; Evelyn & Silva, 2015a, 2015b, 2016b; 2016c; Evelyn, Kim, & Silva, 2016). 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 to 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, Dougherty, & Kang, 2002; Lee, Chung, & Kang, 2006; 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 to 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 x 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 – 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 x 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 pressure (P) allowed by the equipment, since HPP by itself is not sufficient for *A. acidoterrestris* spore inactivation (Lee, et al., 2002; Silva, et al., 2012). Two samples were processed for each P-T-t and soluble solids content conditions and the average ± standard deviation of log survivors was plotted.

To validate the spore inactivation results, commercial apple juice and fruit juices concentrates were submitted to 600 MPa HPP combined with 45°C. The soluble solids were adjusted in the fruit juice concentrates by dilution with sterilized water. Apple juice (10.6°Brix, pH=3.38), adjusted lime juice concentrate (20.2°Brix, pH=2.50) and adjusted blackcurrant concentrate (30.3°Brix, pH=3.05) were processed and *A. acidoterrestris* spore inactivation was compared with 10, 20 and 30°Brix MEB, respectively. HPP-thermal processing and spore inoculation/enumeration were carried out as described previously.

2.4. High Pressure Processing (HPP)

The HPP unit Avure 2 L Laboratory Food Processing System (Avure Technologies, Columbus, Ohio, USA) was used. The machine can operate at a maximum temperature and pressure of 90°C and 600 MPa, respectively. The equipment comprises a 2 L cylindrical shaped pressure treatment chamber pumping system, water circulation for heating and is controlled by a software provided by the manufacturer through a desktop computer. During the process, the chamber containing the packed MEB, fruit juice or concentrate samples, was

filled with distilled water. The chamber was equipped with two thermocouples which registered the water temperature during the HPP treatment inside the chamber. A pre-heating of the pressurized fluid (distilled water) inside the chamber was required. The treatment time considered for the experiment was the holding pressure time and did not include the pressurization and depressurization phases. The pressure come up time to reach 600 MPa was approximately 1.5 min and adiabatic heating was observed during this period, resulting in approximately 5°C higher than the average/target temperature during the constant pressure phase. During the pressure holding phase (isobaric), a decrease in the chamber temperature was registered. Care was taken to adjust the initial temperature of the water inside the pressure vessel in order to obtain an average temperature during the treatment as close as possible to the desired treatment temperature. The depressurization time was approximately 30 sec. After treatment the broth/juice samples were immediately cooled in ice water. The spore counting was carried out as described in section 2.1.3.

2.5. Modeling the kinetics of *A. acidoterrestris*

The survival curves of log of spores numbers versus time were plotted for modeling the data. The microbial survivors for thermal inactivation usually follow the first-order kinetic, with log linear reduction of the microorganism population. However, several studies reported deviations from linearity (e.g., tail, biphasic curves, concave and convex curves) in survival curves of different microorganisms, especially after HPP. Below are the two models used in this study.

2.5.1. First-order kinetic

HPP-thermal inactivation of microorganisms' population is dependent on the treatment time at a constant pressure and temperature, and expressed in number of viable spores. For each

pressure-temperature condition, the inactivation can be modeled by the following equation:

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D_T}t \quad (1)$$

where N is the final number of viable spores (cfu/mL) at time t (min), N_0 is the initial population of spores (cfu/mL) at time zero, and log (N/N_0) is the number of log reductions in spores during the processing time t. For first order kinetics the log of the spore ratio (N/N_0) is linear with time, and the slope of the survival curve is $-1/D_T$. The decimal reduction time D_T, is defined as the time in minutes to achieve one log cycle reduction in the spore concentration. D_T is temperature (T)/soluble solids content (SS) dependent according to the following equations:

$$\log\left(\frac{D_T}{D_{ref}}\right) = \frac{T_{ref}-T}{z_T} \quad (2)$$

$$\log\left(\frac{D_{SS}}{D_{ref}}\right) = \frac{SS_{ref}-SS}{z_{SS}} \quad (3)$$

where the z_T- and z_{SS}-values are the increase of temperature and soluble solids that reduces D_T by a factor 10, respectively.

2.5.2.Weibull model

The Weibull equation has been successfully used to model the inactivation of several microorganisms by different processes (Evelyn & Silva, 2015a, 2015b, 2015c; Evelyn, et al., 2016; Weibull, 1951). This model considers that spores have different resistances (as opposed to the first-order kinetic) and a survival curve represents the cumulative form of a distribution of lethal agents. The Weibull model distribution is represented by the following equation:

$$\log\left(\frac{N}{N_0}\right) = -bt^n \quad (4)$$

where b and n are the scale and shape parameters respectively (Peleg & Cole, 1998). Normally for HPTP $n < 1$, and the survival shape is concave upward. When $n = 1$ is linear (equal the first-order kinetic, Equation 1) and if $n > 1$ a concave downward is registered.

224

225 *2.5.3. Data analysis and model performance*

226 The *A. acidoterrestris* spore logarithmic reduction (Log N/N_0) was calculated and the average
227 was plotted for each HPP-thermal processing time. The linear and several nonlinear models
228 (Weibull, modified Gompertz and Log-Logistic) were attempted using the software
229 *TableCurve 2D* (Systat, USA). The goodness of fit of the models was assessed by the
230 coefficient of determination (R^2) and the mean square error (MSE). Weibull was the best
231 model, and *b* and *n* parameters ± standard error were estimated (Eq. 4). In addition the first-
232 order kinetic parameter D_T and *z*-values were also estimated to compare with previous results.

233

234

235 **3. Results and discussion**

236 3.1 HPP-thermal inactivation of *A. acidoterrestris* in broth

237 The *A. acidoterrestris* spores survival lines in broth after HPP at 600 MPa combined with
238 mild temperature (35, 45, 55 and 65°C) for different soluble solids content (10, 20 and
239 30°Brix) are presented in Figures 1 and 2. The higher the temperature/soluble solids content,
240 the higher the spore inactivation. A 2.3 decimal reduction was obtained for 10°Brix after
241 HPP-55°C-10 min, and a value of 2.8 log reductions was obtained for 20°Brix MEB after
242 HPP-65°C-7 min and for 30°Brix after HPP-65°C-10 min. Thermal processing by itself
243 without HPP cannot achieve any inactivation of *A. acidoterrestris* spores at these
244 combinations of °Brix/temperatures. For example Evelyn and Silva (2016a) worked with the
245 same strain in 9.5°Brix orange juice, and obtained less than 0.1 log reductions after 10 min at
246 78°C, the lowest temperature tested. Silva et al (1999) determined 0.15 log reduction of *A.*
247 *acidoterrestris* type strain spores in 32.5°Brix broth after 10 min thermal treatment at 85°C,
248 the lowest temperature tested.

249

250 *3.1.1. Effect of HPP temperature*

251 The HPP temperature has a significant role in the inactivation of *A. acidoterrestris* spores.
252 When the temperature increased, the rate of microbial destruction also increased (Fig. 1). For
253 instance, 5 min treatment at 600 MPa at 55°C resulted in an approximately 1.2 log reduction
254 of *A. acidoterrestris* spores in MEB 10°Brix, while at 35°C more than 45 min was needed to
255 obtain the same degree of inactivation. Previous NZRM 4098 *A. acidoterretsris* spore
256 inactivation suspended in orange juice (9.2°Brix) processed by 600 MPa-45°C-10 min
257 (Silva, et al., 2012) was similar to our experiment with 10°Brix MEB with type strain (around
258 1 log reduction). At 600 MPa-55°C, while after 5 min the log inactivation results in orange
259 juice and broth were similar (~1.2), for 10 min processing time the log reduction in broth
260 almost double the value to 2.2. Studies with a cocktail of NFPA1013 and NFPA1101 strains
261 17.5°Brix apple juice subjected to 621 MPa-45°C resulted in approximately 2.2 and 2.5 log
262 reductions after 5 and 10 min, respectively (Lee et al., 2006). Our inactivation with NZRM
263 4447 in 20°Brix MEB processed for 10 min at 600 MPa-45°C was much lower, with a value
264 of 0.6 log reduction.

265

266 *3.1.2 Effect of soluble solids*

267 Figure 2 shows the effect of soluble solids on the 600 MPa-55°C *A. acidoterrestris* spore
268 inactivation. After 10 minutes the results for 10, 20 and 30°Brix broth were respectively 2.2,
269 1.1 and 0.5 log reductions. Therefore, when applying HPP-thermal process in to fruit
270 concentrates, and similar to thermal resistance alone, the higher the soluble solids content, the
271 lower the spore inactivation, demonstrating a protective effect. Concentrated juice with high
272 soluble solids presents a low water activity. Clearly, water availability has an effect on the
273 reaction of microorganisms to high pressure thermal treatment (Lee, et al., 2006). The

baroresistance may be in part explained by the incomplete spore germination under low water availability conditions (Black, et al., 2007). Other authors observed the same baroresistance in apple juice/concentrate (Lee, et al., 2002; Sokołowska, et al., 2013), although no modeling was attempted.

3.2. Modeling the HPP-thermal inactivation of *A. acidoterrestris* spores in 10, 20 and 30 °Brix broths

An upward concavity was observed for 10 and 20°Brix broths, while downward concavities were registered for 30°Brix at 45 and 55°C. The possible explanation is that the microorganism populations are composed by several subpopulations, each one presenting distinct inactivation patterns, which causes the nonlinear curves (van Boekel, 2002). Furthermore, previous authors postulated the spore number and age, protoplast dehydration and sporulation temperature might also affect HPP-thermal resistance (Chang & Kang, 2004; Jay, Loessner, & Golden, 2005). The best fitting was obtained with the Weibull model, presenting in general higher R^2 and lower MSE than the first order kinetics (Tables 1 and 2).

3.2.1. Nonlinear Weibull model

The Weibull model fitting for the inactivation curves of *A. acidoterrestris* by combined high pressure and mild heat is represented in Fig. 1. These curves indicates that the Weibull model fits were very satisfactory, confirming the performance indices shown on Table 1. The shape factor (n) less than 1 (Table 1), confirmed the upward concavity of the *A. acidoterrestris* survival curves at 10 and 20°Brix, except 10°Brix - 35°C which was close to linear n=1. The n<1 means that the remaining cells have less chance of dying, being most resistant ones, or maybe the cells have the ability to adapt to the stress. Therefore, the concave upward might be understood as evidence of quick inactivation of the weak or sensitive cells of the

populations and the remaining survivors were the sturdy ones. Similar upward trend was observed with HPP-thermal of orange juice (Silva et al., 2012). On the contrary, a downward concavity (n >1) for 30° Brix broths was obtained at 45 and 55°C. In addition, no change or a slight decrease trend in n was observed with the HPP temperature. Buzrul et al. (2005) reported at certain pressures, shape parameter was dependent on temperature. Van Boekel (2002) reported in only 7 out of 55 studies n seemed to be dependent on temperature. Cunha et al. (1998) suggested the shape parameter n should indicate the kinetic pattern of the model that controls the process studied and thus, be independent from the external factor, temperature.

Regarding the scale parameter b from the Weibull equation, also known by the rate parameter, a linear increase of log b with T of the HPP-thermal process was noted (Fig. 3), analogous to $1/D_T$-value. Evelyn and Silva (2015a) observed the same log-linear trend for b parameter of *B. cereus* HPP-thermal inactivation in milk and temperature dependency was also registered in HPP-thermal inactivation of spores of *Byssochlamys nivea* and *Neosartorya fischeri* moulds in fruit products (Evelyn and Silva, 2015b; Evelyn et al., 2016).

3.2.2 Linear first order kinetics

The D_T-values were estimated for different HPP temperatures and broths soluble solids contents from the reciprocal of the slope of log-survivor versus treatment time (Table 2). As expected the D_T-*values* decreased with temperature. For 10° Brix, at 35, 45 and 55°C the D_T-*values* were 38.08, 10.32 and 4.17 min, respectively. Previous 600 MPa HPP-thermal inactivation studies carried out with a different *A. acidoterrestris* spore strain (NZRM 4098) in orange juice (9.2°Brix and pH=3.8) resulted in: $D_{45°C}$ -value =12.9 min and $D_{55°C}$= 7.0 min (Silva, et al., 2012). The z_T-values for 10, 20 and 30°Brix at 600 MPa were similar to the three soluble solids broths ranging from 20 to 21°C. The usual z-value for thermal process

alone is approximately half 7-13°C (Silva & Gibbs, 2009), this being an indication of lower susceptibility to temperature change in a HPP-thermal process than a thermal process.

The protective effect of soluble solids is evident reaching around threefold increase from 4.2 min at 10°Brix-55°C to 13.7 min at 30°Brix-55°C. The *z*-value can also be calculated for constant temperature and varying soluble solids content (Equation 3). In order to reduce one $\log_{10}$ in the D_T value, it is necessary to reduce approximately by 38-40°Brix (z_{SS}-value) the soluble solids content.

3.3 Model validation with commercial fruit juices/concentrates

Table 3 shows a comparison of HPP-thermal inactivation results in MEB and fruit juices/concentrates for 10, 20 and 30°Brix. The results were good and both models parameters were similar for broth and real fruit products. For example, D_T-value obtained with 10°Brix MEB/apple juice and 20°Brix MEB/lime juice from concentrate were 9-10 min and 20-22 min, respectively. Regarding 30°Brix blackcurrant juice concentrate, the D_T-value in MEB was lower than the corresponding fruit concentrate. However, very close *b* and *n* were obtained when using Weibull model.

4. Conclusion

Although in conventional thermal processes, a temperature of ≥ 95°C is required for *A. acidoterrestris* spore inactivation, much lower temperatures of 45 to 65°C were needed when using HPP-thermal technology. This can represent more retention of original fruit organoleptic properties, nutrients and bioactive components, which address the current consumers demand for fresher, healthier, convenient and tastier foods.

A. acidoterrestris spores are very resistant to HPP-thermal processes. Similar to thermal processing we have demonstrated the higher the temperature of the 600 MPa HPP-thermal process and the lower the soluble solids content of the medium processed, the higher was the inactivation of *A. acidoterrestris* spores. For example 1.2 log reductions was obtained in 10°Brix broth after 5 min-55°C, 10 min-45°C and 45 min-35°C, 2.0-2.3 log reductions was observed in 20°Brix broth, after 2.5 min-65°C, 15 min-55°C and 45 min-45°C, and regarding 30°Brix, 1.5 to 1.9 log reductions were observed after either 7.5 min-65°C, 20 min-55°C or 45 min-45°C.

The *A. acidoterrestris* inactivation under 600 MPa HPP combined with mild heat was well modelled with Weibull model, however first order kinetics was also reasonable. The $D_{55°C}$-value was 4.17, 7.59 and 13.71 min in 10, 20 and 30°Brix broths, respectively, showing the protective effect of the soluble solid content against HPP-thermal process. The z_T-value of 20-21°C was similar for the three different soluble solids investigated and the z_{SS}-value for the soluble solids was 38-40°Brix. The model predictions from experiments with broth were validated with commercial fruit juices/concentrates, which showed close HPP-thermal inactivation results between broths and commercial juices of analogous soluble solids content.

Acknowledgements

The authors gratefully acknowledge Mr. Peter Buchanan and Mr. Rick Coetzer with the operation and maintenance of the high pressure processing equipment and Mrs. Laura Liang for laboratory assistance.

References

Basak, S., Ramaswamy, H. S., & Piette, J. P. G. (2002). High pressure destruction kinetics of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and concentrated orange juice. *Innovative Food Science & Emerging Technologies, 3*(3), 223-231.

Black, E. P., Setlow, P., Hocking, A. D., Stewart, C. M., Kelly, A. L., & Hoover, D. G. (2007). Response of Spores to High-Pressure Processing. *Comprehensive Reviews in Food Science and Food Safety, 6*(4), 103-119.

Buzrul, S., Alpas, H., & Bozoglu, F. (2005). Use of Weibull frequency distribution model to describe the inactivation of *Alicyclobacillus acidoterrestris* by high pressure at different temperatures. *Food Research International, 38*(2), 151-157.

Cerny, G., Hennlich, W., & Poralla, K. (1984). Spoilage of fruit juice by *Bacilli*: Isolation and characterization of the spoiling microorganism. *Z Lebensm Unters Forch, 179*(3), 224-227.

Chang, S.-S., & Kang, D.-H. (2004). *Alicyclobacillus spp*. in the fruit juice industry: history, characteristics, and current isolation/ detection procedures. *Critical Reviews in Microbiology, 30*(2), 55-74.

Cunha, L. M., Oliveira, F. A. R., & Oliveira, J. C. (1998). Optimal experimental design for estimating the kinetic parameters of processes described by the Weibull probability distribution function. *Journal of Food Engineering, 37*(2), 175-191.

Duong, H. A., & Jensen, N. (2000). Spoilage of iced tea by *Alicyclobacillus*. *Food Australia, 52*(7), 292-292.

Eiroa, M.N.U., Junqueira, V.C.A., & Schimdt, F.L. (1999). *Alicyclobacillus* in orange juice: occurrence and heat resistance of spores. *Journal of Food Protection, 62(*8), 883-886.

Evelyn, & Silva, F. V. M. (2015a). High pressure processing of milk: Modeling the inactivation of psychrotrophic *Bacillus cereus* spores at 38–70 °C. *Journal of Food Engineering, 165*, 141-148.

Evelyn, & Silva, F. V. M. (2015b). Inactivation of *Byssochlamys nivea* ascospores in strawberry puree by high pressure, power ultrasound and thermal processing. *International Journal of Food Microbiology, 214*, 129-136.

Evelyn, & Silva, F. V. M. (2015c). Use of power ultrasound to enhance the thermal inactivation of *Clostridium perfringens* spores in beef slurry. *International Journal of Food Microbiology, 206*, 17-23.

Evelyn, & Silva, F. V. M. (2016a). High pressure processing pretreatment enhanced the thermosonication inactivation of *Alicyclobacillus acidoterrestris* spores in orange juice. *Food Control*, *62*, 365-372.

Evelyn, & Silva, F. V. M. (2016b). High pressure thermal processing for the inactivation of *Clostridium perfringens* spores in beef slurry. *Innovative Food Scence and Emerging Technologies, 33*, 26-31.

Evelyn, & Silva, F. V. M. (2016c). Modeling the inactivation of psychrotrophic *Bacillus cereus* spores in beef slurry by 600 MPa HPP combined with 38-70°C: comparing with thermal processing and estimating the energy requirements. *Food and Bioproducts Processing, 99*, 179-187.

Evelyn, Kim, H. J., & Silva, F. V. M. (2016). Modeling the inactivation of *Neosartorya fischeri* ascospores in apple juice by high pressure, power ultrasound and thermal processing. *Food Control*, *59*, 530-537.

Hayashi, R. (1989). Application of high pressure to food processing and preservation: Philosophy and development. In W. E. L. Spiess & H. Schubert (Eds.), *Engineering and Food Species* (Vol. 2, pp. 815-826). England: Elsevier Applied Science.

Hayashi, R. (1995). Chapter 9 - Advances in high pressure food processing technology in Japan. In A. G. Gaonkar (Ed.), *Food Processing* (pp. 185-195). Amsterdam: Elsevier Science B.V.

Hite, B. H. (1899). The effect of pressure in the preservation of milk. *West Virginia Agricultural Experimental Station Bulletin, 58*, 15-35.

Hongkang, Z., & Mittal, G. S. (2008). Effects of High-Pressure Processing (HPP) on Bacterial Spores: An Overview. *Food Reviews International, 24*(3), 330-351.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Food Protection with High Temperatures, and Characteristics of Thermophilic Microorganisms. In J. M. Jay, M. J. Loessner & D. A. Golden (Eds.), *Modern Food Microbiology* (pp. 415-441): Springer US.

Jensen, N. (1999). *Alicyclobacillus* - a new challenge for the food industry. *Food Australia, 51*(1-2), 33-36.

Kannenberg, E., Blume, A., & Poralla, K. (1984). Properties of ω-cyclohexane fatty acids in membranes. *FEBS Letters, 172*(2), 331-334.

Lee, S. Y., Dougherty, R. H., & Kang, D.-H. (2002). Inhibitory Effects of High Pressure and Heat on *Alicyclobacillus acidoterrestris* Spores in Apple Juice. *Applied and Environmental Microbiology, 68*(8), 4158-4161.

Lee, S. Y., Chung, H.-J., & Kang, D.-H. (2006). Combined Treatment of High Pressure and Heat on Killing Spores of *Alicyclobacillus acidoterrestris* in Apple Juice Concentrate. *Journal of Food Protection, 69*(5), 1056-1060.

Palou, E., López-Malo, A., Barbosa-Cánovas, G. V., Welti-Chanes, J., Davidson, P. M., & Swanson, B. G. (1998). Effect of oscillatory high hydrostatic pressure treatments on *Byssochlamys nivea* ascospores suspended in fruit juice concentrates. *Letters in Applied Microbiology, 27*(6), 375-378.

Peleg, M., & Cole, M. B. (1998). Reinterpretation of Microbial Survival Curves. *Critical Reviews in Food Science and Nutrition, 38*(5), 353-380.

Pettipher, G. L., & Osmundson, M. E. (2000). Methods for the detection, enumeration and identification of *Alicyclobacillus acidoterrestris*. *Food Australia, 52*(7), 293.

Pontius, A. J., Rushing, J. E., & Foegeding, P. M. (1998). Heat resistance of *Alicyclobacillus acidoterrestris* spores as affected by various pH values and organic acids. *Journal of Food Protection, 61*(1), 41-46.

Rastogi, N. K. (2013). *Recent developments in high pressure processing of foods*. New York: New York: Springer

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Science and Technology, 44*(5), 1251-1260.

San Martín, M. F., Barbosa-Cánovas, G. V., & Swanson, B. G. (2002). Food Processing by High Hydrostatic Pressure. *Critical Reviews in Food Science and Nutrition, 42*(6), 627-645.

Silva, F. V. M., Gibbs, P., Vieira, M. C., & Silva, C. L. M. (1999). Thermal inactivation of *Alicyclobacillus acidoterrestris* spores under different temperature, soluble solids and pH conditions for the design of fruit processes. *International Journal of Food Microbiology, 51*(2–3), 95-103.

Silva, F. V. M., & Gibbs, P. (2001). *Alicyclobacillus acidoterrestris* spores in fruit products and design of pasteurization processes. *Trends in Food Science & Technology, 12*(2), 68-74.

Silva, F.V.M., & Gibbs, P. (2004). Target selection in designing pasteurization processes for shelf-stable high-acid fruit products. Critical Reviews in Food Science and Nutrition, 44, 353−360.

Silva, F. V. M., & Gibbs, P. (2009). Principles of Thermal Processing. In R. Simpson (Ed.), *Engineering Aspects of Thermal Food Processing*: CRC Press.

Silva, F. V. M., Tan, E. K., & Farid, M. (2012). Bacterial spore inactivation at 45–65 °C using high pressure processing: Study of *Alicyclobacillus acidoterrestris* in orange juice. *Food Microbiology, 32*(1), 206-211.

Silva, F.V.M., Gibbs, P.A., Nunez, H., Almonacid, S., & Simpson, R. (2014). Thermal processes: Pasteurization. In C.A. Batt, & M.L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (pp. 577−595). Amsterdam: Elsevier.

Sokołowska, B., Skąpska, S., Fonberg-Broczek, M., Niezgoda, J., Chotkiewicz, M., Dekowska, A., & Rzoska, S. (2012). The combined effect of high pressure and nisin or lysozyme on the inactivation of *Alicyclobacillus acidoterrestris* spores in apple juice. *High Pressure Research, 32*(1), 119-127.

Sokołowska, B., Skąpska, S., Fonberg-Broczek, M., Niezgoda, J., Chotkiewicz, M., Dekowska, A., & Rzoska, S. (2013). Factors influencing the inactivation of *Alicyclobacillus acidoterrestris*

spores exposed to high hydrostatic pressure in apple juice. *High Pressure Research, 33*(1), 73-82.

Sulaiman, A., Soo, M. J., Yoon, M. M. L., Farid, M., & Silva, F. V. M. (2015). Modeling the polyphenoloxidase inactivation kinetics in pear, apple and strawberry purees after High Pressure Processing. *Journal of Food Engineering, 147*, 89.

van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology, 74*(1–2), 139-159.

Walker, M., & Phillips, C. A. (2008). *Alicyclobacillus acidoterrestris*: an increasing threat to the fruit juice industry? *International Journal of Food Science and Technology, 43*(2), 250-260.

Weibull, W. (1951). A statistical distribution function of wide applicability. *Journal of Applied Mechanics, 18*, 292-297.

Wuytack, E. Y., Boven, S., & Michiels, C. W. (1998). Comparative Study of Pressure-Induced Germination of *Bacillus subtilis* Spores at Low and High Pressures. *Applied and Environmental Microbiology, 64*(9), 3220-3224.

Yamazaki, K., Teduka, H., & Shinano, H. (1996). Isolation and Identification of *Alicyclobacillus acidoterrestris* from Acidic Beverages. *Bioscience, Biotechnology, and Biochemistry, 60*(3), 543-545.

Table 1. Weibull *b* and *n* parameters and model performance for *A. acidoterrestris* spores inactivation by 600 MPa HPP combined with mild heat in malt extract broth adjusted 10, 20 and 30°Brix.*

Soluble solids (°Brix)	HPP temperature (°C)	Weibull *b* ± SE	Weibull *n* ± SE	R^2	MSE
10	35	0.02 ± 0.01	1.10 ± 0.11	0.960 - 0.982	0.00 - 0.04
	45	0.15 ± 0.04	0.86 ± 0.09		
	55	0.60 ± 0.06	0.55 ± 0.05		
20	45	0.09 ± 0.02	0.81± 0.07	0.920 - 0.972	0.01 - 0.10
	55	0.23 ± 0.08	0.80 ± 0.13		
	65	1.47 ± 0.18	0.31 ± 0.08		
30	45	0.01 ± 0.00	1.49 ± 0.25	0.801 - 0.916	0.04 - 0.13
	55	0.02 ± 0.03	1.48 ± 0.51		
	65	0.35 ± 0.10	0.86 ± 0.14		

*Strain NZRM 4447 (=ATCC 49025); *b* and *n* are Weibull scale and shape factors respectively; low mean square errors (MSE) and R^2 close to 1.00 are indication of good fit.

Table 2. First order kinetics parameters (D_T- and z_T-values) for *A. acidoterrestris* spores inactivation by 600 MPa HPP combined with mild heat in malt extract broth adjusted 10, 20 and 30°Brix.*

Soluble solids (°Brix)	HPP temperature (°C)	D_T-value ± SE (min)	z_T-value ± SE (°C)	R^2	MSE
10	35	38.08 ± 1.24	20.82 ± 2.17	0.829 - 0.980	0.00 - 0.10
	45	10.32 ± 0.40			
	55	4.17 ± 0.34			
20	45	21.63 ± 0.98	20.07 ± 1.01	0.508 - 0.948	0.02 - 0.53
	55	7.59 ± 0.37			
	65	2.18 ± 0.27			
30	45	32.26 ± 2.07	21.43 ± 2.52	0.703 - 0.909	0.03 -0.17
	55	13.71 ± 2.02			
	65	3.76 ± 0.21			

*Strain NZRM 4447 (=ATCC 49025); low mean square error (MSE) and R^2 close to 1.00 are indication of good fit

Table 3. High pressure processing (HPP) at 600 MPa combined with 45°C: *A. acidoterrestris* spores resistance parameters in malt extract broth and the analog commercial juice/concentrate.*

Soluble solids (°Brix)	Media	First-order	Weibull	
		D_T (min) ± SE	b ± SE	n ± SE
10	Malt extract broth	10.32 ± 0.40	0.15 ± 0.04	0.86 ± 0.09
	Apple Juice	8.63 ± 0.44	0.27 ± 0.07	0.73 ± 0.08
20	Malt extract broth	21.63 ± 0.98	0.09 ± 0.02	0.81± 0.07
	Lime juice concentrate	19.88 ± 1.15	0.10 ± 0.05	0.81 ± 0.14
30	Malt extract broth	32.26 ± 2.07	0.01 ± 0.00	1.49 ± 0.25
	Blackcurrant juice concentrate	46.11 ± 4.64	0.00 ± 0.01	1.44 ± 0.35

*Strain NZRM 4447 (=ATCC 49025)

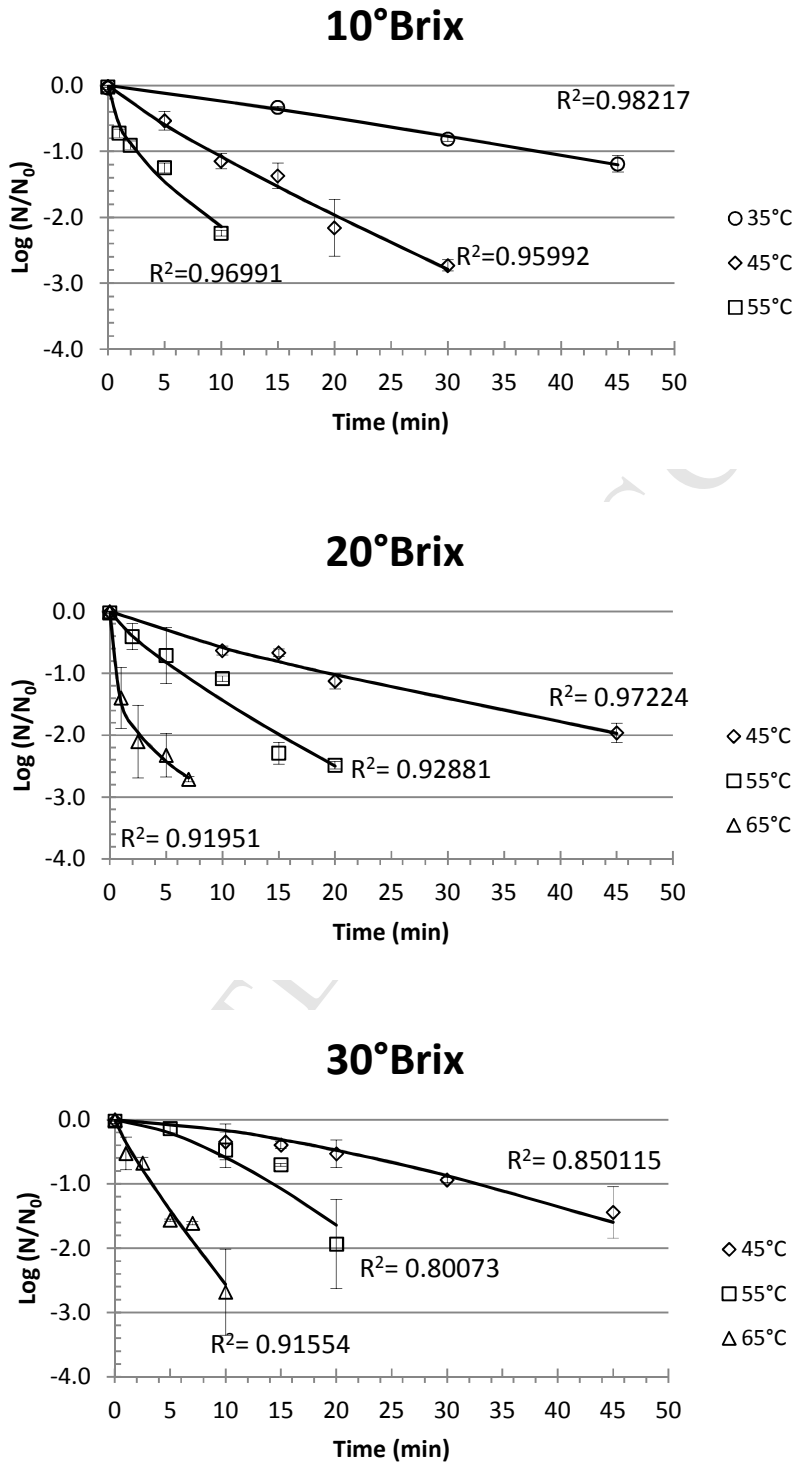

Figure 1. 600 MPa HPP combined with mild heat for the inactivation of *A. acidoterrestris* spores in malt extract broth adjusted 10, 20 and 30°Brix: effect of temperature and Weibull model fitting (values are log inactivation average ± standard deviation).

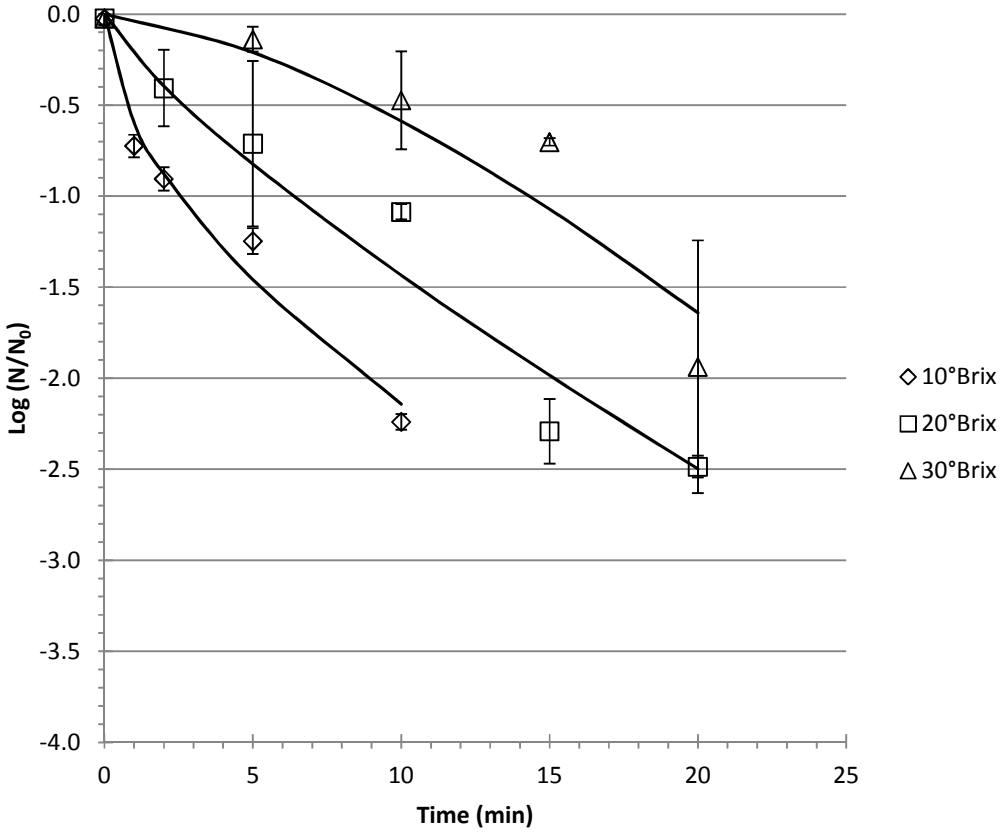

Figure 2. 600 MPa HPP combined with 55°C for the inactivation of *A. acidoterrestris* spores in malt extract broth adjusted 10, 20 and 30°Brix (values are average ± standard deviation).

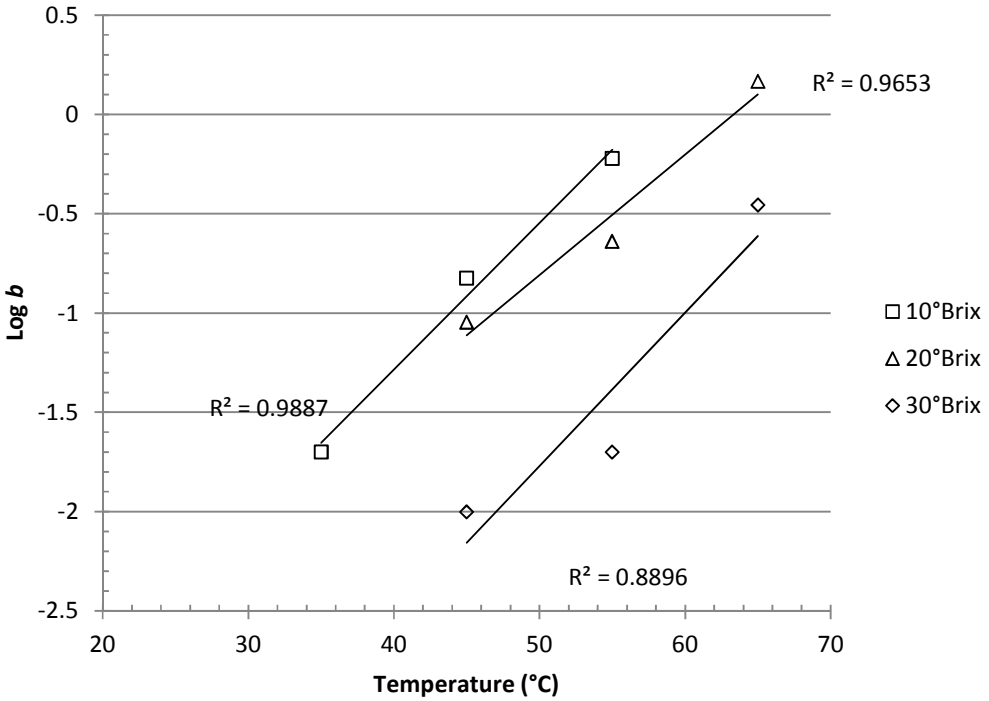

Figure 3. The effect of HPP temperature on the Weibull log *b* values for *A. acidoterrestris* spore inactivation at 600 MPa.

Highlights:

- HPP-thermal processing allows the use of lower pasteurization temperatures
- Higher soluble solids increased the resistance of *A. acidoterrestris* spores to HPP-thermal processes
- Weibull model described the nonlinear inactivation of *A.acidoterrestris* spores