Modelling *Alicyclobacillus acidoterrestris* inactivation in apple juice using thermosonication treatments

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Abstract

A spore-forming bacterium, *Alicyclobacillus acidoterrestris*, is often responsible for fruit juices quality degradation. The objective was to study the influence of ultrasounds and combinations with temperature (thermosonication) on *A. acidoterrestris* spores inactivation in apple juices. Commercially available juice was artificially inoculated with the bacterium (~10^5 CFU/mL). Sonication was carried out in an ultrasonic bath (35 kHz; 120-480 W) at room temperature and at 70, 80, 85, 90 and 95 ºC for different times. Thermal treatments at the same temperatures were performed as a control. Sonication had no significant effect on *A. acidoterrestris* spores inactivation. However, when applied at 70 and 80 ºC, it allowed 1 log-cycle more of inactivation when compared to thermal treatments and at the end of the processes. Ultrasounds at higher temperatures required approximately half of the treatment time to attain the same inactivation that occurred when the thermal processes were applied alone. In thermosonicated juices, spores decreased by 4.8, 4.7 and 5.5 log-cycles (at 85, 90 and 95 ºC, respectively) after 90, 60 and 20 minutes.

The Weibull model satisfactorily fitted inactivation data of thermosonicated juices.

Thermosonication is efficient for *A. acidoterrestris* spores inactivation, with a drastic impact on spores' loads when high temperatures are used.

Keywords: Ultrasounds; pasteurization; spores inactivation; Weibull model

1. Introduction

Fruit juices are healthy beverages as a consequence of their beneficial micronutrients, such as minerals, vitamins and phytochemicals. It is of the utmost importance the production of safe and stable juices, which are also highly nutritious and with characteristics associated with natural products. Novel technologies that avoid the negative impact of high temperatures on the quality characteristics of pasteurized juices are emerging.

In apple juice production, *Alicyclobacillus* spp. and its spores are concerns because are resistant to acid and high temperature environments, therefore, alternatives to conventional pasteurization are a challenge (Clotteau, 2014; Murakami, Tedzuka, & Yamasaki, 1998; Walker & Philips, 2008). In this context, non-thermal based processes have been studied: high hydrostatic pressure (Buzrul, Alpas, & Bozoglu, 2005), ultraviolet radiation (Keyser, Muller, Cilliers, Nel, & Gouws, 2008), natural antimicrobials (Bevilacqua, Campaniello, Speranza, Sinigaglia, & Corbo, 2013), pulsed electric fields (Moody, Marx, Swanson, & Bermúdez-Aguirre, 2014), ultrasounds (Abid et al., 2013; Gao, Lewis, Ashokkumar, & Hemar, 2014; Mohideen et al., 2015; Wang, Hu, & Wang, 2010), pulsed light (Palgan et al., 2011), or combination of these processes with mild heat treatments (Bermúdez-Aguirre & Barbosa-Cánovas, 2012; Bevilacqua et al., 2013; Lee, Dougherty, & Kang, 2002; Muñoz et al., 2012).

Among these methods, ultrasounds, which are also called as sonication, have the advantages of enhancing product quality, reducing energy consumption, while being environmentally friendly (Abid et al., 2013; Chemat, Huma, & Khan, 2011; Mohideen et al., 2015). Ultrasounds are a method that uses sound waves above the threshold of human hearing (>14-16 kHz) (Bermúdez-Aguirre & Barbosa-Cánovas, 2012). According to the frequency, it can be classified as high frequency ultrasounds (5-10 MHz) and low frequency or power ultrasounds (20-100 kHz) (Bermúdez-Aguirre & Barbosa-Cánovas, 2012; Piyasena, Mohareb, & McKellar, 2003). Power ultrasounds are capable of inducing cavitation to inactivate microorganisms in foods (Mohideen et al., 2015; Piyasena et al., 2003). Cavitation is the process whereby micro bubbles are grown and collapsed within a liquid medium (Gabriel, 2012; Gao et al., 2014). This action results in hot spots and microbial cell disruptions because of increased temperature and pressure, respectively (Bermúdez-Aguirre & Barbosa-Cánovas, 2012; Koda, Miyamoto, Toma, Matsuoka, & Maebayashi, 2009; Mohideen et al., 2015). However, as a preservation method, application of ultrasounds alone is not efficient enough to kill all microorganisms. Moreover, high power level of ultrasounds may adversely affect foods nutritional and sensorial properties (Ferrario, Alzamora, & Guerrero, 2015).

Therefore, combination of ultrasounds with other processes, such high temperature (thermosonication), high pressure (manosonication), or both (manothermosonication), may induce synergistic effects in terms of quality retention and efficiency in microbial inactivation (Chemat et al., 2011; Cruz, Vieira & Silva, 2006; Cruz, Vieira, & Silva, 2008; Lee, Zhou, Liang, Feng, & Martin, 2009). Among these combinations, thermosonication has been reported as more efficient than ultrasounds applied alone (Coronel, Jimene, López-Malo, & Palou, 2011; López-Malo, Palou, Jimenez-Fernandez, Alzamora, & Guerrero, 2005). Application of this technology for the processing of fruit juices has a great potential, since milder temperatures may be used to attain microbial decontamination, while original characteristics of fruit juices may be retained (Bhat, Kamaruddin, Min-Tze, & Karim, 2011). Important impacts in the process can also be achieved by reducing processing time, higher throughput and lower energy consumption, which is interesting for industrial application.

The objective of this study was to evaluate the influence of power level ultrasounds and thermosonication at different temperatures, on *A. acidoterrestris* spores' survival in apple juices. The Weibull model was used to describe the inactivation kinetics when thermosonication processes were applied.

2. Materials and Methods

2.1 Spores suspension

A. acidoterrestris CCT 4384 spores were obtained according to methodology described by Tremarin, Brandão, & Silva (2017). Six-month-old spores were used in the experiments.

2.2 Apple juice samples

Experiments were carried out using a clear and pasteurized apple juice commercially available, with no *A. acidoterrestris* contamination (assessed experimentally).

A refractometer (Palette PR-32, Atago, Tokyo, Japan) and a pH meter (GLP 22, Crison Instruments, Barcelona, Spain) were used to evaluate samples soluble solids content (°Brix) and pH value. The measurements were performed in triplicate.

A volume of 0.05 mL of the spores' suspension (2×10^7 CFU/mL) was inoculated in 25 mL of apple juice, and then samples were submitted to inactivation treatments.

2.3 Inactivation processes

An ultrasonic bath (RK 102 H, BANDELIN, Berlin, Germany), with 120-480 W power levels and 35 kHz frequency, was used to carry out sonication treatments (US). Artificially contaminated apple juice (25 mL) was placed in stirred (magnetic stirring) Erlenmeyer flask and inserted in the bath. After different exposure times, up to a maximum of 60 minutes, 1 mL of sample was collected from the flask. Thermosonication processes were also performed in the ultrasonic bath, as previously described. The following temperatures were imposed: 70, 80, 85, 90 and 95 ºC. Samples of 1 mL were collected after different exposure times, depending on the temperature used, up to a maximum of 90 minutes.

In a thermostatic bath with stirring capability (FP40, Julabo, Seelbach, Germany), thermal treatments at 70, 80, 85, 90 and 95 °C were carried out, and after used as a control of the applied thermosonication processes. Depending on the temperature used, samples of 1 mL were taken at different exposure times, till a maximum of 90 minutes. Microbiological analyses of the juices were carried out after each treatment.

In all experiments, Erlenmeyer flasks were previously autoclaved and kept covered with aluminum foil to avoid contamination.

A minimum of three replicates of each treatment was performed.

2.4 Enumeration of *A. acidoterrestris*

To quantify survival spores in untreated and treated juice samples, the diluted samples were spread plated onto *Bacillus acidoterrestris* agar (BAT agar, Merck, Darmstadt, Germany; pH=4), and plates were incubated at 45 °C for 2 days. Counting colonies were performed in triplicate and expressed as CFU/mL.

2.5 Modeling of inactivation behaviour

Log-survival data of *A. acidoterrestris* spores obtained in thermosonication treatments were described by the Weibull model (Mafart, Couvert, Gaillard, & Leguerinel, 2002):

$$log\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\alpha}\right)^{\beta} \qquad (1)$$

where N is the microbial load (CFU/mL) at a given treatment time t (min), N_0 the initial microbial load of the juice (CFU/mL), α is a scale parameter (min), and β is the shape parameter (dimensionless), showing upward (β <1) and downward concavity (β >1).

Also, the classical log-linear model was used (Evelyn, Kim, & Silva, 2016):

$$log\left(\frac{N}{N_0}\right) = -\left(\frac{t}{D}\right) \quad (2)$$

where D is the decimal reduction time, *i.e.*, the time expressed in minutes for the survivor curve to traverse one log cycle.

Both equations 1 and 2 were fitted to log-survival data of *A. acidoterrestris* spores' observed in thermosonication treatments. The temperature coefficient z-value was also estimated as the temperature increase that results in a 10-fold decrease in D value (Evelyn & Silva, 2016).

Regression analysis was done using IBM SPSS Statistics 24 for Windows® (SPSS Inc., Chicago, USA). The quality of the regressions was assessed by checking randomness and normality of the residuals (Shapiro-Wilk test), and by the coefficient of determination (R^2). Precision of the model parameter estimates was evaluated by calculating the margin of confidence interval at 95% (*i.e.*, half of the confidence interval at 95%).

3. Results and Discussion

In the characterization of apple juice, values of soluble solids content obtained were 11.0 ± 0.3 °Brix and pH averaged 3.29 ± 0.03.

Results of *A. acidoterrestris* spores' inactivation in apple juices are included in Figure 1. To prevent the influence of the initial spores' loads, data were presented in terms of log (N/N_0). The magnitude of N_0 used in all the experiments was around 10^5 CFU/mL.

Ultrasound treatments applied at 35 kHz frequency and 120-480 W power levels had a small impact on spores' inactivation. A maximum reduction of 0.8 log-cycles was observed after 60 minutes of treatment, which was equivalent of using a thermal treatment at 70 °C. Djas, Bober, & Henczka (2011) observed less than 0.12 log-cycles reduction of *A. acidoterrestris* spores after the application of US (10 min, 330 W) in concentrate apple juice. Ferrario et al. (2015) did not observe reductions on *A. acidoterrestris* ATCC 49025 spores in commercial and natural squeezed apple juices artificially inoculated and treated with US at 20 kHz/600 W for 30 minutes.

When ultrasounds were coupled with high temperature (70, 80, 85, 90 and 95 ºC), results showed that spores' inactivation was higher than the one observed when the thermal treatment was applied alone (Fig. 1). This occurred for all tested temperatures. For lower temperatures (70 and 80 ºC) and at the end of the process, inactivation obtained by thermosonication differs in 1 log-cycle, when compared to a simple thermal treatment at the same temperatures. For higher temperatures (85, 90 and 95 ºC) the difference between thermal treatments and thermosonication was higher. At these temperatures, *A. acidoterrestris* spores in thermosonicated juices decreased 4.8, 4.7 and 5.5 log-cycles (at 85, 90 and 95 ºC, respectively) after 90, 60 and 20 minutes of treatment.

Figure 1 here, please

Table 1 here, please

Concerning the regression analyses performed to data obtained for thermosonication treatments, it can be concluded that the Weibull model was adequate in data fits. Randomness, normality and homoscedasticity of the residuals were verified ($p > 0.05$) and the coefficient of determination was above 0.97 in all cases (Table 1). Model parameters and corresponding margin of confidence intervals at 95% are also in Table 1. The scale parameter α can be interpreted as the reciprocal of the inactivation rate, and the higher the value the slowest the process. The α estimates for thermosonication at the lowest temperature of 70 ºC (38.53 ± 0.34 min) was 8 times higher than the one obtained for the highest temperature of 95 ºC (4.67 ± 0.46 min). This means that increasing the temperature of the US treatment greatly affects the rate of inactivation.

In terms of the shape parameter β, for US+90 ºC, US+80 ºC and US+70 ºC the values were lower than 1, indicating that the log-survival curve presented an upward concavity. For US+95 ºC and US+85 ºC treatments β was higher than 1 indicating a downward concavity. Upward concave curves are associated with the adaptation of the remaining cells to the applied stress. Downward concave indicates the increased damage of the remaining cells in applied stress (van Boekel, 2002). A linear model was also used aiming at estimation of D-values of *A. acidoterrestris* CCT 4384 spores related to thermosonication processes applied to the apple juice (Table 1). When compared to Weibull model fits, linear model lacked adequacy, also proven by the lower values of R^2 obtained. For the lowest temperature tested, D-value related to the US+70 ºC treatment was 44.3 ± 1.6 min; for the highest temperature imposed (US+95 ºC), D-value obtained was 3.9 ± 0.5 min. Based on the results, the estimated z-value was 23.6 ºC.

Evelyn & Silva (2016) used thermosonication (20.2 W/mL) to inactivate *A. acidoterrestris* NZRM 4447 spores in orange juice, estimating D-values at 70, 75 and 78 °C (139, 49 and 28 min, respectively). The z-value was 11.5 °C. When compared to results obtained in our study, differences of thermosonication resistance and temperature sensitivity may be due to *A. acidoterrestris* strains, type of fruit (more acidic in the case of orange), or even to the severity of the process applied.

For apple juice, Evelyn et al. (2016) applied power ultrasounds (24 kHz, 0.33 W/mL) at 75 ºC to inactivate *Neosartorya fischeri* JCM 1740 ascospores. In these conditions, inactivation did not occur, revealing mould resistance to the process.

4. Conclusions

Ultrasounds applied individually were not effective in *A. acidoterrestris* spores’ inactivation in apple juices. However, when ultrasounds were combined with high temperature, a synergetic effect occurred, being the processes more efficient in spores’ inactivation than a thermal process at the same temperature. The shorter process times required in thermosonication processes may allow milder impacts on juices overall quality.

The Weibull model was adequate in fitting survival data of *A. acidoterrestris* spores' in thermosonicated juices and the model parameters allowed comparing shape of the curves and influence of temperature on the rate of inactivation. Generated kinetic models can be used to design thermosonication processing conditions for apple juices preservation.

Thermosonication applied to apple juices can be an alternative to conventional thermal pasteurization, with potential to be industrially applied. However, studies of the impact of thermosonication on overall quality of the juices are required for a convenient optimization of the process conditions.

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REFERENCES

Abid, M., Jabbar, S., Wu, T., Hashim, M.M., Hu, B., Lei, S., …, & Zeng, X. (2013). Effect of ultrasound on different quality parameters of apple juice. *Ultrasonic Chemistry, 20*, 1182-1187.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G.V. (2012). Inactivation of *Saccharomyces cerevisiae* in pineapple, grape and cranberry juices under pulsed and continuous thermo-sonication treatments. *Journal of Food Engineering, 108,* 383-392.

Bevilacqua, A., Campaniello, D., Speranza, B., Sinigaglia, M., & Corbo, M.R. (2013). Control of *Alicyclobacillus acidoterrestris* in apple juice by citrus extracts and a mild heat-treatment. *Food Control, 31*, 553-559.

Bhat, R., Kamaruddin, N.S.B.C., Min-Tze, L., & Karim, A.A. (2011). Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrasonics Sonochemistry, 18,* 1295–1300.

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, 151–157.

Chemat, F., Huma, Z., & Khan, M.K. (2011). Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics Sonochemistry, 18,* 813–835.

Clotteau, M.S. (2014). *Alicyclobacillus* spp. control in the fruit juice industry. Food and Beverage https://www.pall.com/pdfs/Food-and-Beverage/FBTBTABFJEN.pdf

Coronel, C., Jimene, M., López-Malo, A., & Palou, E. (2011). Modelling thermosonication inactivation of *Aspergillus flavus* combining natural antimicrobial at different pH. *Procedia Food Science, 1,* 1007-1014.

Cruz, R.M.S., Vieira, M.C., & Silva, C.L.M. (2006). Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). *Journal of Food Engineering, 72*, 8−15.

Cruz, R.M.S., Vieira, M.C., & Silva, C.L.M. (2008). Effect of heat and thermosonication treatments on watercress (*Nasturtium officinale*) vitamin C degradation kinetics. *Innovative Food Science & Emerging Technologies, 9,* 483-488.

Djas, M., Bober, M., & Henczka, M. (2011). New methods for inactivation of *Alicyclobacillus acidoterrestris* spores in apple juice concentrate. *Challenges of Modern Technology,* 2, 46-49.

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

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.

Ferrario, M., Alzamora, S.M., & Guerrero S. (2015). Study of the inactivation of spoilage microorganisms in apple juice by pulsed light and ultrasound. *Food Microbiology, 46*, 635-642.

Gabriel, A.A. (2012). Microbial inactivation in cloudy apple juice by multi-frequency *Dynashock* power ultrasound. *Ultrasonics Sonochemistry*, *19,* 346-351.

Gao, S., Lewis, G. D., Ashokkumar M., & Hemar Y. (2014). Inactivation of microorganisms by low-frequency high-power ultrasound: A simple model for the inactivation mechanism. *Ultrasonics Sonochemistry, 21*, 454–460.

Keyser, M., Muller, I.A., Cilliers, F.P., Nel, W., & Gouws, P.A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science & Emerging Technologies, 9*, 348–354.

Koda, S., Miyamoto, M., Toma, M., Matsuoka, T., & Maebayashi, M. (2009). Inactivation of *Escherichia coli* and *Streptococcus mutans* by ultrasound at 500 kHz. *Ultrasonics Sonochemistry 16*, 655–659.

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*, 4158-4161.

Lee, H., Zhou, B., Liang, W., Feng, H., & Martin, S. E. (2009). Inactivation of *Escherichia coli* with sonication, manosonication, thermosonication and manothermosonication: microbial responses and kinetics modeling. *Journal of Food Engineering, 93,* 354-364.

López-Malo, A., Palou, E., Jimenez-Fernandez, M., Alzamora, S. M., & Guerrero, S. (2005). Multifactorial fungal inactivation combining thermosonication and antimicrobials. *Journal of Food Engineering, 67*, 87-93.

Mafart, P., Couvert, O., Gaillard, S., & Leguerinel, I. (2002). On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology, 72,* 107-113.

Mohideen, F.W., Solval, K.M., Li, J., Zhang, J., Chouljenko, A., Chotiko, A., …, & Sathivel, S. (2015). Effect of continuous ultra-sonication on microbial counts and physico-chemical properties of blueberry (*Vaccinium corymbosum*) juice. *LWT - Food Science and Technology, 60,* 563–570.

Moody, A., Marx, G., Swanson, B.G., & Bermúdez-Aguirre, D.A. (2014). Comprehensive study on the inactivation of *Escherichia coli* under nonthermal technologies: High hydrostatic pressure, pulsed electric fields and ultrasound. *Food Control, 37,* 305-314.

Muñoz, A., Caminiti, I. M., Palgan, I., Pataro, G., Noci, F., Morgan, D.J., …, & Lyng, J.G. (2012). Effects on *Escherichia coli* inactivation and quality attributes in apple juice treated by combinations of pulsed light and thermosonication. *Food Research International, 45,* 299–305.

Murakami, M., Tedzuka, H., & Yamasaki, K. (1998). Thermal resistence of *Alicyclobacillus acidoterrestris* spores in different buffers and pH. *Food Microbiology, 15,* 577-582.

Palgan, I., Caminiti, I. M., Muñoz, A., Noci, F., Whyte, P., Morgan, D.J., …, & Lyng, J.G. (2011). Effectiveness of high intensity light pulses (PL) treatments for the control of *Escherichia coli* and *Listeria innocua* in apple juice, orange juice and milk. *Food Microbiology, 28,* 14–26.

Piyasena, P., Mohareb, E., & McKellar, R.C. (2003). Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology, 87,* 207–216.

Tremarin, A., Brandão, T.R.S., & Silva, C.L.M. (2017). Inactivation kinetics of *Alicyclobacillus acidoterrestris* in apple juice submitted to ultraviolet radiation. *Food Control, 73,* 18-23.

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,* 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*, 250–260.

Wang, J., Hu, X., & Wang, Z. (2010). Kinetics models for the inactivation of *Alicyclobacillus acidiphilus* DSM14558T and *Alicyclobacillus acidoterrestris* DSM 3922T in apple juice by ultrasound. *International Journal of Food Microbiology, 139*, 177–181.

Figure captions

Figure 1. Thermosonication and thermal inactivation of *A. acidoterrestris* spores' in apple juice at: (-) US, (○) 70 ºC, (●) US+70 ºC; (◊) 80 ºC, (♦) US+80 ºC; (Δ) 85 ºC, (▲) US+85 ºC; (□) 90 ºC, (■)US+90 ºC, (+) 95 ºC, (x) US+95 ºC. Solid lines represent data fits of the Weibull model.

Table captions

Table 1. Weibull model parameters (α and β) and D-values of *A. acidoterrestris* in apple juice estimated for thermosonication treatments (margin of confidence intervals at 95% included). Coefficient of determination (R^2) of the regression analysis of Weibull and linear models.