INACTIVATION OF ALICYCLOBACILLUS ACIDOTERRESTRIS SPORES AND ESCHERICHIA COLI IN AÇAÍ (EUTERPE OLERACEA MARTIUS) BY HIGH ISOSTATIC PRESSURE

A.L.T. Jesus¹, M.Cristianini²

¹- Emerging Technology Laboratory - Department of Food Technology – University of Campinas, Faculty of Food Engineer – CEP: 13083-862 – Campinas – SP – Brazil, Phone: 55 (19) 3521-0226 – e-mail: (analaurati@hotmail.com)

²- Emerging Technology Laboratory - Department of Food Technology – University of Campinas, Faculty of Food Engineer – CEP: 13083-862 – Campinas – SP – Brazil, Phone: 55 (19) 3521-0226 – e-mail: (olecram@unicamp.br)

ABSTRACT – Food processing using an emerging non-thermal technology (high pressure) could be an alternative to conventional pasteurization processes for thermosensitive products. This study evaluated the effect of high isostatic pressure (HIP) technology on the inactivation of Escherichia coli and Alicyclobacillus acidoterrestris spores. The samples were submitted to the processes of 400/500/600MPa for 5-15 min at 25°C-65°C. The açaí pulp was inoculated with 10⁶ CFU/mL of A. acidoterrestris spores and 10⁷ CFU/mL of E. coli. Results indicated 7-log reduction of E. coli for all the treatments. For A. acidoterrestris, when HIP treatment was conducted at 65°C, reductions of 3.3-log and 5.0-log after process at 600MPa/5min/65°C and 600MPa/20min/65°C, were achieved, respectively, while at 400MPa/5min/25°C it was obtained 2.1-log reduction cycles. Results showed that different microorganisms react distinctly to the HIP process and that combination of high pressure and mild temperature can be used to inactivate vegetative cells and spores on açaí pulp.

KEYWORDS: High hydrostatic pressure; spores; bacterial inactivation.

1. INTRODUCTION
Açaí (Euterpe oleracea Martius) is a fruit that has an important economic contribution to Brazil, mainly in the north and northeast regions. The consumption of açaí pulp had a recent increase in both the South and Southeast regions as in the international market due their important nutritional value and the large amount of bioactive compounds, including anthocyanins. In addition to açaí beverages are exported all over the world as an energetic drink (Roge, 2000; Yamaguchi et al., 2015).

The açaí berry is highly perishable fruit, and its maximum shelf life, even under refrigeration, is 12 hours. The factor responsible for this high perishability is the high microbial load and enzymatic degradation (Souza et al, 1999). Being a highly perishable fruit, açaí needs treatments that prolong its shelf life, inactivating enzymes naturally present and spoilage and pathogenic microorganisms. However, during the heat pasteurization, the functionality of these bioactive compounds might be drastically reduced as well as the modification of the characteristic aroma and color, making difficult the commercialization and the market growth (Butz and Tauscher, 2002). Food processing using an emerging non-thermal technology (high pressure) could be an alternative to conventional pasteurization processes for thermosensitive products, due the advantages it offers, like minimizing...
organoleptic and nutritional losses, additionally making available microbiologically safe food (Cardello et al., 2007; Patterson, 2005).

Alicyclobacillus acidoterrestris (AAT) and Escherichia coli (E.coli) are microorganism important for the fruit industry. AAT is an aerobic, thermoacidophilic, gram-positive, endospore-forming bacterium and capable of producing guaiacol (the main compound responsible for the deterioration of juices) (Álvarez-Rodríguez et al., 2003; Chang and Kang, 2004). The AAT spores survive the thermal pasteurization employed by the fruit beverage industry being used as reference microorganism to design pasteurization processes in shelf-stable fruit juices and beverages (Silva and Gibbs, 2001).

E.coli is a fecal coliform bacteria commonly found in the intestines of animals and humans. This bacteria is considered the most specific indicator of fecal contamination and the possible presence of pathogens in food (Mohammad, 2005).

The aim of this study was evaluated the effect of high isostatic pressure (HIP) technology on the inactivation of a pathogenic microorganism (Escherichia coli) and a heat-resistant spoilage (Alicyclobacillus acidoterrestris), allowing to establish adequate process parameters to ensure the microbiological safety of the product and preserve the functional and sensory properties of fruit.

2. MATERIAL AND METHODS

2.1. Preparation of the Sample for Microbial Inactivation

The açaí samples of type B (12% total solids, pH 4.9, 4 - 6 ° Brix) were purchased from an establishment of the North (Abaetetuba - Pará - Brazil). For microbial inactivation tests, the açaí pulp (27g pulp in natura) was vacuum-packed in flexible bags (low density polyethylene coextruded with ethylene vinyl alcohol and activated carbon, and an inner layer of black pigmented from Dixie Toga-Ltda, São Paulo/Brazil) and sterilized (121°C/15 minutes) in an autoclave to inactivate the natural microbiota of the pulp.

2.2. Bacterial Strain and Preparation of Cell Suspension

A. acidoterrestris strain and growth medium: A. acidoterrestris CCT 7547 was obtained from Tropical Cultures Collection André Tosello (Campinas, Sao Paulo, Brazil). Strains were grown at 45°C for 3 days in potato dextrose agar (PDA) media adjusted to pH 4.0 with filter sterilized 10% w/v (0.1 g/mL) tartaric acid.

A. acidoterrestris sporulation: AAT was incubated on PDA at 45°C for 3 days to obtain a stock culture. The cells from stock culture were spread onto PDA agar in Petri dishes and incubated at 45°C for 18 days. After reaching more than 80% of sporulation, confirmed by microscopy following staining with malachite green, spores were collected with a sterile swab and resuspended in sterile distilled water. The pool of spores collected from different plates was centrifuged at 4000×g for 20 min at 4 °C, washed two times with sterile distilled water by repeated centrifugation, and finally resuspended in phosphate buffer (pH 7.2) and stored at 4°C until use.

A. acidoterrestris spore enumeration: According to the methodology proposed by Silva et al., 2012, for AAT inactivation, 3 ml of a spore suspension (10⁷ CFU/ml) was inoculated into 27 mL of açaí pulp thus obtaining an initial count 10⁶ CFU/ml. To determine the spore concentration (N) in the non-processed and processed açaí pulp, 0.1 mL of spore suspension/açaí pulp was serial diluted with 0.1% (w/v) peptone water solution in test tubes. Each dilution was vortex mixed to ensure uniform concentration of spores in the tube dilutions. The dilutions were heated at 80°C for 10 min to eliminate any vegetative cells remaining, and 0.1 mL of each dilution was spread plated twice in two acidified PDA plates, and incubated at 45°C for 3 days. After incubation, the colonies formed (cfu) were
counted for each dilution and average counts were calculated. Spore concentration in the açaí pulp, N, was expressed as cfu/mL açaí pulp.

*E.coli* strain, growth and enumeration: *E. coli* CCT 0923 was obtained from Tropical Cultures Collection André Tosello (Campinas, Sao Paulo, Brazil). For *E. coli* inactivation, 0.3 ml of a cell suspension (10^8 CFU/ml) was inoculated into 30 ml of açaí pulp thus obtaining an initial count 10⁷ CFU/ml. For enumeration of processed samples, 1 mL was added to 9 mL of sterile 0.1% (w/v) peptone water solution. Counting was carried out by plating on the surface and in duplicate using culture media violet red bile glucose agar (VRBG). The VRBG plates were incubated at 35 ± 2° C for 72 h (Ariefdjohan et al., 2004).

2.3. High Pressure Processing

The HPP unit used in this study was QFP 2 L-700 Avure Technologies, Ohio, USA. The temperature of the chamber was measured by two type K thermocouples inserted into the chamber, one located in the top and other in the middle. The process conditions selected for inactivation of *Alicyclobacillus acidoterrestris* spores were based on the use mild temperature (65°C) and high pressures. The process parameters were chosen at: 600MPa at 65°C for 2.5, 5, 10, 15, 20, 25 and 30 minutes, and a condition with lower pressure and lower temperature for comparison of effects (400MPa/5min/25°C). The process conditions selected for inactivation of *E. coli* were the lowest and highest pressure of this study: 400MPa/5min/25°C, 400MPa/5min/65°C, 600MPa/5min/25°C and 600MPa/5min/65°C.

3. RESULTS AND DISCUSSION

Among the microorganisms studied, *E.coli* was the lesser resistant to the HIP in açaí pulp. The results showed that for all conditions there was a reduction of 7.1 log cycles, thus demonstrating that this microorganism would not be an açaí contamination problem after processing for all the conditions of this study. Some studies report that gram negative bacteria are less resistant to the effects of the HIP than gram positive (have cell wall more resistant) (Wuytack et al., 2002). Similar results were found by Torres et al, 2015 in a study with *E. coli* O157: H7 in orange juice, the authors were able to reduce 6.5 log cycles when processed juice to 400MPa/3minutes/25°C with reduction of 2.43 log cycles at 100 MPa and complete inactivation (7.31 log cicles) at 200 MPa.

Figure 1 –Inactivation of *Alicyclobacillus acidoterrestris* CCT07547 after HIP in açaí pulp.

The results to AAT inactivation (Figure 1) showed that for the lower condition (400MPa/5min/25°C) there was a reduction of 2.14 log cycles. The increase of pressure from 400 MPa
to 600 MPa and increase of temperature 25°C to 65°C, caused further reduction of AAT spores at all the times tested. At 600MPa/25min/65°C, a complete inactivation of spores (6.3 log cycles reduction) it was achieved.

The Figure 2 shows the kinetics of AAT inactivation in açaí pulp processed by HIP. It was observed that AAT inactivation did not follow a linear profile. The Weibull model was the mathematical model's able to describe the inactivation profile of AAT by HIP ($R^2=0.9303$; RMSE=0.6586; $\alpha=1.64$ min; $\beta=0.59$; Log$_{10}$($N_0$) = 5.16) and the 4D reduction is reached at ±17.25 min. This model was generated from GlnaFIT 1.6 software in excel. The model of Weibull, among many, assumes that the population of cells or spores have different heat-resistant and survival curve is a cumulative lethal distribution. In terms of the survival curve, Equation 1 can describe Weibull distribution:

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

Where:
- $N_0$ = initial concentration of spore/ml;
- $N$ = concentration of survivors/ml (after treatment for $t$ minutes)
- $t$ = time (minute or second)
- $\alpha$ = scale parameter (minute or second)
- $\beta$ = shape parameter used as performance index

The reduction results found in this study with açaí, show that a process with a reduction of 4 log cycles would be advantageous, once $10^9$CFU/ml spore concentrations can be found in juices, but these do not show deterioration characteristics. AAT produce guaiacol from concentrations of $10^5$CFU/ml of spores (Gocmen et al., 2005; Perripher et al., 1997).

Figure 2- *Alicyclobacillus acidoterrestris* spore survivor curves in açaí pulp at 65°C in combination with high pressure

4. CONCLUSIONS
Results showed that different microorganisms react distinctly to the high pressure processing and the use of the HIP combined with mild temperature (65°C) is effective to inactivate A. acidoterrestris spores and E. coli in açaí pulp. The results from this study revealed that resulted in non-detectable levels of E. coli in açaí pulp. In addition, these results complied with the 7.1 log reduction of E. coli, more than the recommended by the FDA guidelines for fruit juices (5-log reduction). Under the evaluated conditions, HIP can be considered an alternative technology for açaí pulp preservation.

5. ACKNOWLEDGMENTS
The authors are grateful to the São Paulo Research Foundation (FAPESP) for awarding a scholarship (2015/01570-0).

6. REFERENCES
