ABSTRACT – the aim of this work was to evaluate the survival of a probiotic culture in an acai juice matrix, an Amazonian fruit already known by its nutritional and functional value worldwide. The acai berries were harvested in a rural property near Manaus, Amazon state, Brazil. The berries were washed, sanitized and bleached to extract the pulp. The juice was sweetened with xylitol in a concentration of 5%, pasteurized at 75 ºC for 20 min and the probiotic culture (L. acidophilus-HOWARU® Dophilus) was added at a 0.05%.

The probiotic ready to drink acai juice was stored in a refrigerator at 5ºC. The pH determination and viable cells counts were monitored for 70 days. The results obtained in this study showed that the probiotic culture L. acidophilus used was able to survive in the acai juice matrix stored at 5ºC for 70 days.

KEYWORDS: amazonian fruit beverage, functional food, Lactobacillus

1. INTRODUCTION

Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts (FAO/WHO 2001). Various reports have described their health benefits on gastrointestinal infections, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimicrobial activity, anti-mutagenic and anti-carcinogenic properties, anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of Helicobacter pylori infection by addition of selected strains to food products.

The most common probiotics functional foods marketed worldwide are dairy products containing microorganisms belonging to the genera Lactobacillus and Bifidobacterium. In recent years non-dairy probiotic products have been studied as potential carriers for these microorganisms to provide probiotic food free of cholesterol and lactose present in dairy products. Many published studies reported that fruit and vegetable beverages may be the next category of food matrices to serve as carriers of probiotic bacteria (Prado et al 2008). A chilled fruit-based beverage with probiotic already exists in the market, e.g. the Proviva™ juice concept (Makinen et al 2012).

The development of a probiotic fruit beverage should take into account the factors known to interfere in the survival of probiotic microorganisms in food products during production, processing and storage (Tripathi and Giri 2014). These factors include food parameters (pH, titratable acidity, molecular oxygen, water activity, presence of salt, sugar and chemical composition); processing parameters (heat treatment, packaging material and storage method) and microbiological parameters (strains of probiotics, rate and proportion of inoculation) (Mattila-Sandholm et al. 2002).
Acai, a palm fruit native to South America, is traditionally consumed in Brazil and has gained popularity abroad due to its nutritional value and functional properties, being classified as one of the new “super fruits” (Portinho et al., 2012). With increasingly competitive markets, the beverages manufacturers have targeted functionality as an extremely important marketing tool to create competitive advantages in the marketplace (Sorenson and Bogue, 2005). Therefore, besides the nutritional value and functional properties of acai, the development of an acai beverage containing probiotic has a promising future.

It is well known that food composition influence the survival, viability and functionality of probiotics, which determine their effectiveness. From the published studies, the stability of microorganisms in fruit juice depends on the species and strains as well as of the fruit used (Vergara et al. 2010; Nualkaekul and Charalampopoulos 2011; Mousavi et al 2011; Pereira et al 2011; Sheela and Suganya 2012; Ellendersen et al 2012). The aim of this work was to evaluate the survival of a probiotic culture in an acai juice matrix, an Amazonian fruit already known by its nutritional and functional value worldwide.

2. MATERIALS AND METHODS

2.1. Preparation of ready to drink acai juice
The acai berries were harvested in a rural property near Manaus, Amazon state, Brazil. The berries were washed with water, sanitized with sodium hypochlorite for 20 min and washed with water again to remove all the residues of hypochlorite. The acai berries were bleached at 50ºC for 20 min to facilitate pulp extraction by mechanical equipment. The pulp was obtained using 10 kg of acai berries and 6 L of water. The pulp obtained was filtered twice in an inox sieve, sweetened with xylitol in a concentration of 5%, pasteurized at 75 ºC for 20 min and immediately refrigerated at 5ºC.

2.2 Probiotic culture and culture addition in the juice
The culture used was the Lactobacillus acidophilus (HOWARU® Dophilus 200B, Danisco) provided by MasterSense Ig. Alim. Ltda. The probiotic culture was kept frozen until the use and it was added to the ready to drink acai juice at a concentration of 0,05% in aseptic conditions and homogenized. The acai juice containing the probiotic culture was dispensed into 250 mL sterile screw cap glass recipients.

2.3. Storage of the probiotic ready to drink acai juice and cell viability
The probiotic acai juice was stored in a refrigerator at 5ºC. Viable counts were performed after 1 h of the culture addition into the juice and after 10, 17, 24, 31 and 70 days. It was used the MRS agar (Merck) and the plates were incubated in an anaerobic jar with anaerobic (Probac) for 72 h at 37ºC.

2.4. pH analysis
The pH was determined by direct measure in a pHmeter (Hanna HI-2212).

2.5. Enumeration of coliforms, total Enterobacteriaceae, Bacillus cereus, Clostridium perfringens, Staphylococcus aureus and detection of Salmonella sp.
The enumeration of coliforms was performed by the Most Probable Number (MPN). For the total coliforms MPN it was used the brilliant green lactose broth and incubation for 48 h at 35°C and for the fecal coliforms MPN it was used the EC broth for 24 h at 44,5°C. The total Enterobacteriaceae was performed by the colony count method using MacConkey glucose agar and incubation for 24 h at 35°C. The enumeration of pathogenic bacteria Bacillus cereus, Clostridium perfringens and Staphylococcus aureus were performed according to Downes and Ito (2001). For the detection of Salmonella sp. it was used the immunoassay system miniVIDAS (Biomerieux).

3. RESULTS AND DISCUSSION
The selection of the food matrix is an important factor that should be considered in developing probiotic products. The chemical composition and physic-chemical properties of food carriers used for probiotic delivery are very important factors that influence survival of the probiotics. The components acting synergistically with properties such as pH from the food seems to be one of the best ways of improving probiotic efficacy. Fat content, concentration and type of proteins, sugars and pH of the product are some factors that could affect probiotic growth and survival in food (Ranadheera et al. 2010).

Acai is one of the most popular functional foods in Amazon. The main constituents found in dry matter are lipids (50%), fibers (25%) and proteins (10%). The amount of carbohydrates (glucose, fructose and sucrose) is relatively low, between 2.96% and 3.55% of total dry matter. Acai is also a good source of inorganic compounds, such as phosphorus, sodium, zinc, iron, manganese, copper, boron, chromium, calcium, magnesium, potassium and nickel (Yamagushi et al. 2015). Acai pulp has many essential properties for human nutrition and has received much attention in recent years due to the presence of bioactive substances such as phenolic compounds. Due to the health benefits, the acai has a high potential for the development of a beverage to reach the higher market demand nationally and all over the world.

The acai constituents could make it as a substrate for the delivery of probiotics and the already amount of fibers in it gives rise to the development of a symbiotic beverage. As shown in Figure 1 the cells of Lactobacillus acidophilus survived well in the ready to drink acai juice during 70 days at 5°C. Figure 1. Cell viability enumeration $(\log_{10}$ UFC/mL) of Lactobacillus acidophilus in ready to drink acai juice during 70 days storage at 5°C.

As can be seen in the Figure 1, the initial cell number was 8,3 Log UFC/mL and after 70 days the cell number was 7,7 Log UFC/mL , showing the ability of the L. acidophilus probiotic culture to survive in the acai juice matrix when stored at 5°C.

The pH of the acai juice just prepared was 5,23 and the pH of the ready to drink acai juice after pasteurization was 4,97. As shown in Figure 2, during storage there was a decrease in the pH and this profile was more pronounced after 17 days. Although it has been occurred a profile of pH decreasing, the pH registered in the end of the storage period was 4,54, pH which would be expected to still provide a good viability of the cells.

Figure 2. Evolution of pH in the ready to drink acai juice during storage for 70 days at 5°C.
Regarding the pH decreased during storage, it is presumably due to the fact that the L. acidophilus cells metabolized the available energy sources such as the sugar present naturally or xylitol added in the acai juice. During the total refrigerated storage period, the pH change corresponded to a decrease of 0.43 units. Badet et al. (2004) studied the possible adaptation of oral strains of lactobacilli to xylitol and demonstrated that some lactobacilli strains were able to grow at the expense of xylitol and to produce acids. Twenty-one strains were able to produce acids from xylitol, ten strains after a 15-days culture and eleven strains after a 40-days culture. No strains were able to produce acids after a 5 days culture in the adaptation medium. According to these authors, L. acidophilus ATCC 4356 produced acid after 20 days exposure to xylitol. Daneshi et al. (2013) reported a similar profile for Lactobacillus acidophilus in milk/carrot juice mix drink. Over the total storage period of 20 days at 4ºC, pH values of the probiotic drink was almost constant, although a very small decrease in pH was observed after 15 days storage.

Taking into account the results from the compositional analysis of the juices and the model, it was deduced that in certain juices, other compounds seemed to protect the cells during storage; these were likely to be proteins and dietary fibre. In contrast, in certain juices, such as pomegranate, cell survival was much lower than expected; this could be due to the presence of antimicrobial compounds, such as phenolic compounds (Nualkaekul and Charalampopoulos 2011).

Various authors reported the high contamination of acai pulp and juice by total and fecal coliforms, salmonella, moulds and yeasts (Sousa et al. 2006, Oliveira et al. 2011, Cohen et al. 2011, Faria et al. 2012, Ribeiro et al. 2015). In this work, the microbial analysis performed in the acai juice demonstrated contamination levels greater than those established by the current Brazilian legislation. The result obtained for the MPN of both total and fecal coliforms were > 1100/ mL. To achieve a safe product and extend the product shelf-life it is necessary to use a conservation process.

Pasteurization of juices is known to inactivate pathogenic and spoilage bacteria to achieve a safe product and extend the product shelf-life. For acai pulp, industries use tubular type heat exchanger and employ temperatures of 80ºC to 85ºC for 30 to 60 s, and after pasteurization, it is immediately frozen. Sousa et al. (2006), considering the high contamination of acai juice commercialized by local small vendors, reported that heat treatments using pasteurization at 90ºC for 5 min or boiling for 1 min were able to maintain the sensorial characteristics and to reach a safe product when stored frozen (-18ºC) for 120 days.

The acai juice just prepared was pasteurized at 75 ºC for 20 min and immediately refrigerated at 5ºC. As can be seen in the Table 1, the microbial analysis performed after 1 hour and 31 days storage demonstrated that heat treatment used was able to guaranty the microbial safety of the acai juice.

Table 1. Enumeration of microorganisms in the acai juice during the refrigerated storage period.
The pasteurization processes designed in this study was able to inactivate the pathogenic and spoilage microorganisms present in acai juice. However, the thermal processing of acai juice is not a popular option in the north region of Brazil due to the perceived negative effects of pasteurization on natural color of the juice. In fact, the pasteurization process used in this study caused a visual change on color of the acai juice and the color modification increased during the storage period. Despite potential sensory challenges, the acai juice can be an ideal carrier for probiotic delivery as a fruit based functional beverage because it inherently contain a high content of fiber, protein and lipids, components known to act synergistically to improve probiotic efficacy, and a number of other beneficial and health nutrients. Future research will be conducted to address non-thermal alternative processing technology for the control of pathogenic and spoilage microorganisms in acai juice and to maintain the color characteristic of the fruit.

4. CONCLUSIONS

Considering the experimental conditions employed and the results presented in this study, it can be stated as a general conclusion that the probiotic culture *L. acidophilus* used was able to survive in the acai juice matrix stored at 5°C for 70 days. However, for the development of a probiotic acai juice for the market a non-thermal alternative processing technology must be used to reach a safe product and to maintain the sensorial properties of the fruit.

5. ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by the Extension Program of the Institute Federal of Amazon and the MasterSense Ingredientes Alimentícios Ltda by provide the probiotic culture HOWARU® Dophilus.

6. REFERENCES


