CLARIFIED NON-ALCOHOLIC FERMENTED AÇAÍ BEVERAGE (*Euterpe oleracea*) WITH *Lactiplantibacillus plantarum*: INFLUENCE OF SUCROSE AND FRUCTOOLIGOSACCHARIDES ON CELL VIABILITY

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ABSTRACT

The study focuses on developing a non-alcoholic fermented açaí beverage using *Lactobacillus plantarum* B135 and assessing its viability over 42 days. Açaí (*E. oleracea*), known for its antioxidant properties, is chosen due to its nutritional benefits and rising popularity. Five formulations with clarified açaí, supplemented with sucrose and/or fructooligosaccharides, are fermented at controlled pH. Microbial viability starts at 6.5 log CFU/mL and reaches 13 log CFU/mL during fermentation, with pH below 4.5 after 8 hours. Lactic acid concentration varies during fermentation. Testing reveals the beverage's shelf life, with the sucrose-supplemented variant maintaining viable cells above 6 log CFU/mL for 35 days at pH 3.7 when stored at 4°C. This finding suggests the formulation's potential as a probiotic fermented açaí beverage. The research addresses the growing demand for non-dairy fermented products, catering to individuals with dietary restrictions such as lactose intolerance and milk allergies, while also providing a novel way to enjoy the nutritional benefits of açaí. Further exploration into optimizing formulations and understanding microbial dynamics during fermentation could enhance the beverage's commercial viability and health benefits.

Keywords: Açai. Fermented beverage. *Lactiplantibacillus*. Bioeconomy.

1 INTRODUCTION

The market for fermented fruit-based beverages is expanding and evolving daily. This growth is attributed to dietary restrictions among a significant portion of the global population, such as milk protein allergies, lactose intolerance, and elevated cholesterol levels, which deter these individuals from consuming dairy-based beverages.[1] Açaí (*Euterpe oleracea*) stands as the most significant fruit of the socio-biodiversity within the Amazon region. Nutritionally, açaí boasts lipids (32.5 to 50.5% of total solids - TS), total dietary fiber (20.9 to 21.8% TS), and proteins (8.1 to 12% TS) as its main constituents. However, it is renowned as a superfruit due to its high content of antioxidants such as anthocyanins (1,365.2 mg/kg fruit) and α-tocopherol (45 mg/100g TS).[2]

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have established that probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. However, they do not specify a minimum concentration of probiotics in food, as they emphasize that the quantity may vary depending on the strains used, intended health claims, type of food product, among other factors. It is important to emphasize that the effectiveness and benefits of probiotics are not solely linked to cell concentration but also to the viability and survival capacity of the microorganisms in the final product (shelf life) and in the human gastrointestinal tract.[3,4]

The aim of this study was to produce, for the first time, a fermented açaí beverage using the *Lactiplantibacillus plantarum* B135 strain, isolated by the research group.[5] This involved developing formulations supplemented with sucrose and/or fructooligosaccharides (FOS) to evaluate quality attributes and cell viability during fermentation and throughout a storage period at 4°C for 42 days.

2 MATERIAL & METHODS

Açaí fruits (10 kg) were sourced from local farmers in Abaetetuba, Pará, Brazil, transported under refrigeration (4°C) to the Centre for Valorization of Amazonian Bioactive Compounds (CVACBA), and processed to obtain the product known as clarified açaí, initially described in the patent (PI1003060-3)[6] and regulated by MAPA.[7] Clarified açaí is characterized as a beverage with reduced total solids content (≤ 2%), sugars (≤ 1%), no lipids, total polyphenols (≥ 150 mg/100g), anthocyanins (≥ 40 mg/100g), capable of preserving the characteristic color, aroma, and flavor of açaí. Five beverage formulations were prepared: 50 g/L of sucrose (50S); 50 g/L of fructooligosaccharides (FOS) (50F); 40 g/L of sucrose + 10 g/L of FOS (40S10F); 40 g/L of FOS + 10 g/L of sucrose (40F10S); and without supplementation (Control - C). The beverages had a final volume of 200 mL, pH 6, and were pasteurized (82.5°C for 1 min.).

The *L. plantarum* B135 strain was reactivated in MRS broth (37°C for 24 h) under anaerobic conditions, subsequently centrifuged (4000 rpm at 4°C for 10 min), and the pellet adjusted to 9 log CFU/mL in a 0.1% peptone water solution. The beverages were inoculated with 2% (v/v), reaching a concentration of 6.5 log CFU/mL, and then incubated on a shaker for 24 h

at 37°C and 100 rpm. Following the determination of the appropriate fermentation time (cells in logarithmic growth phase) for each formulation, new açaí beverages were produced under the same previously described parameters. However, the fermentation process was reduced to 10 h (C) and 16 h (50S, 50F, 40S10F, 40F10S), after which the beverages were stored at 4°C for 42 days.

Cell quantification was conducted using a method widely described for *Lactobacillus*.[8] pH was monitored through direct readings using a pH meter. Lactic acid determination was carried out through potentiometric titration, applicable to fruit-derived products with intense colorations.[9] Finally, sugar content was assessed using HPLC with RI detector.[10]

3 RESULTS & DISCUSSION

Cell viability monitoring in the açaí beverages was assessed over 24 h (Fig. 1a) and 42 days (Fig. 1b). All beverages exhibited an average cell viability growth of 11.6 ± 1.3 log CFU/mL over the 24-hour period, representing a five-order logarithmic increase. This indicates adaptation of *L. plantarum* B135 to the medium, even under nutrient-limiting conditions (C). In formulations supplemented with sucrose and FOS, the onset of the stationary phase was identified after 16 h (10.7 and 13.2 log CFU/mL for formulations 40F10S and 40S10F, respectively), while in the C, it occurred at 10 h of fermentation.

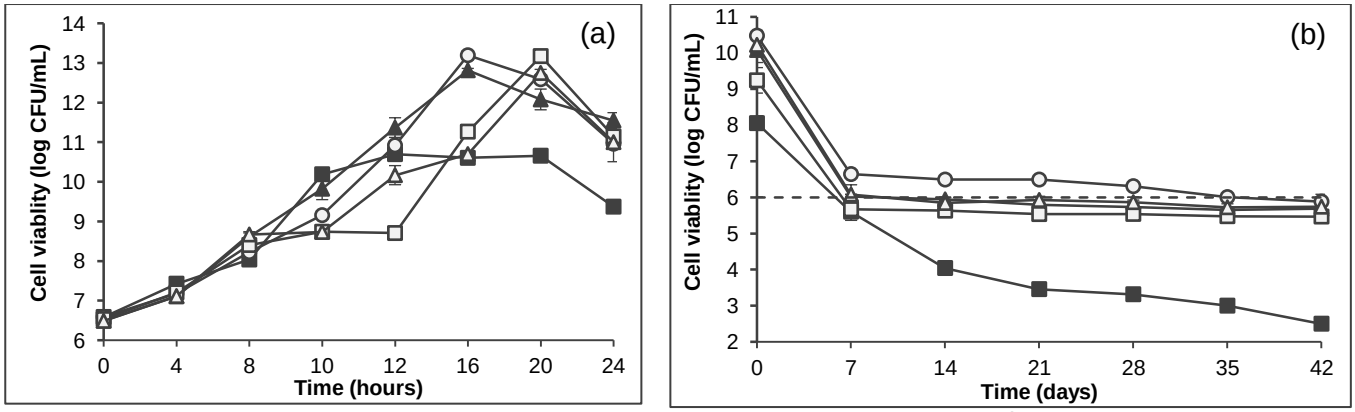

Figure 1: Viability (log CFU/mL) of *Lactiplantibacillus plantarum* B135 during fermentation of clarified açaí drink for 24 hours at 37°C (a). Cell viability (log CFU/mL) of *L. plantarum* B135 in the storage of clarified açaí fermented beverage for 42 days at 4°C (b). (■) Drink without supplementation (Control-C), (○) Drink with 50 g/L of sucrose (50S), (▲) Drink with 50 g/L of FOS (50F), (□) Drink with 40 g/L of sucrose + 10 g/L of FOS (40S10F), (△) Drink with 40 g/L of FOS + 10 g/L of sucrose (40F10S) and (--) Minimum limit of BAL concentration.

The use of Amazonian fruits as a base for fermented beverages is relatively recent. For instance, a study utilizing açaí pulp (8% TS) fermented with *L. casei* supplemented with sucrose and FOS at the same concentrations as this study resulted in a cell viability increase from 7.8 to 8.9 log CFU/mL (one logarithmic order) after 24 h (28°C at pH 6.1). Cocoa pulp (*Theobroma cacao*) diluted in water (9.6% TS) was fermented with *L. casei* (7.0 log CFU/mL) and showed a cell viability increase of 9.9 log CFU/mL (three logarithmic orders) after 24 h at 33°C (pH 6.2).[11] Another study utilizing cupuaçu pulp (*Theobroma grandiflorum*) at a concentration of 17% TS for fermentation with *L. casei* achieved a cell viability increase from 7 to 9.2 log CFU/mL (two logarithmic orders) after 24 h at 30°C (pH 5.8).[12] In summary, the use of clarified açaí as the base for fermented beverage was effective, as it demonstrated a five-logarithmic order cell growth within 24 h of cultivation.

Cell viability, pH monitoring (Fig. 2), lactic acid content, and sugar levels in the fermented açaí beverages were assessed over 42 days of cold storage (4°C). The highest cell decay was observed within the first 7 days of storage, with an average decrease of 3.6 log CFU/mL. However, from the seventh day of storage until the 42nd day, the rate of cell decay decreased to an average < 1 log CFU/mL, with values of 6 log CFU/mL (50S), 5.7 log CFU/mL (40F10S), and 5.6 log CFU/mL (50F and 40S10F). The unsupplemented beverage (C) exhibited the lowest cell viability value at 2.5 log CFU/mL (Fig. 1b). In food products such as fermented beverages, the viable microorganism count typically ranges from 6 to 7 log CFU/mL, as their intake ranges between 100 to 250 mL two or more times a day. Under these conditions, a dose of 9 log CFU (required quantity for fermented products due to dilution in the human digestive system) is achieved.[3,4]

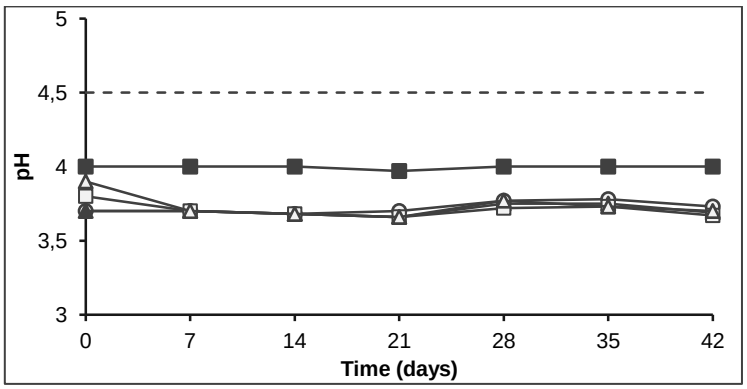

Figure 2. Monitoring the pH in the storage of clarified açaí fermented drink at 4°C for 42 days. (■) Drink without supplementation (Control-C), (○) Drink with 50 g/L of sucrose (50S), (▲) Drink with 50 g/L of FOS (50F), (□) Drink with 40 g/L of sucrose + 10 g/L of FOS (40S10F), (△) Drink with 40 g/L of FOS + 10 g/L of sucrose (40F10S) and (--) Maximum limit of pH value.

Throughout the monitoring period, none of the fermented beverages had a pH higher than 4.5, thus remaining protected from the development of pathogenic and spoilage microorganisms, while also staying stable within the appropriate range for

fermented products (pH 3.6 - 4). The sucrose concentration decreased by approximately 10 % during storage at 4°C, except for formulations with higher FOS content, where no significant reduction in sucrose was observed. One possible explanation is the hydrolysis of FOS and subsequent increase in the amount of sugars available to microorganisms. Lastly, the lactic acid content in all fermented beverages remained between 199 to 293 mg/100 mL, indicating that any negative sensory effects resulting from the formation of this acid would be negligible upon consumption of the beverages.

4 CONCLUSION

This study demonstrated, for the first time, the use of clarified açaí as a raw material for the production of a fermented beverage. The açaí beverage was fermented by *L. plantarum* B135 and exhibited a cell viability of 6 log CFU/mL for up to 42 days of storage at 4°C, particularly for the formulation containing 50g/L of sucrose (50S). pH and acidity values remained within the standards for fermented beverages. Understanding how the *L. plantarum* B135 strain survives in the human digestive system will further contribute to the technological development of the product. Moreover, the results provide prospects for advancements in the development of functional beverages with Amazonian fruits.

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