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EVALUATION OF TOTAL ANTHOCYANIN CONTENT IN NON-ALCOHOLIC FERMENTED AÇAÍ BEVERAGE (*Euterpe oleracea*) BY *Lactiplantibacillus plantarum*

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ABSTRACT

The development of fermented fruit-based beverages is a global trend. The Amazon region harbors a high richness of fruits that are still biotechnologically underexplored, such as açaí (*E. oleracea*). This study evaluated, for the first time, the monitoring of anthocyanin content in a non-alcoholic fermented açaí beverage. Four formulations containing clarified açaí supplemented with sucrose and/or fructooligosaccharides (FOS) were fermented with *Lactiplantibacillus plantarum* B135 for 16 hours at 37°C. Subsequently, the beverages were stored under refrigeration (4°C) for 42 days. The concentration of total anthocyanins decreased by an average of 14.4 ± 3.6% during the fermentation and cold storage process for the beverages. The study presents promising prospects in the development of fermented açaí-based beverages, however, future studies should be conducted to assess the probiotic potential of the product.

Keywords: Bioeconomy, Fermented Beverage, *L. plantarum*, Açaí

1 INTRODUCTION

The increase in life expectancy should be accompanied by healthy lifestyle habits, including good nutrition. There is scientific evidence that diets rich in fruits and vegetables from an early age substantially contribute to reducing the risk of chronic diseases in adulthood.[1] The consumption of fermented fruit-derived products, especially those derived from Amazonian fruits like açaí (*Euterpe oleracea*), can promote benefits related to the absorption of vitamins, phenolic compounds, and antioxidants. Açaí stands as the most significant fruit of the socio-biodiversity within the Amazon region. Additionally, it addresses dietary restrictions for part of the global population, which includes milk protein allergy, lactose intolerance, and high cholesterol levels.[2]

Regulated by the MAPA (BR) in 2018, clarified açaí beverage can be an alternative for the production of a fermented beverage, as it has a low total solids content (≤ 2%), sugars (≤ 1%), no lipids, total polyphenols (≥ 150 mg/100g), and anthocyanins (≥ 40 mg/100g), capable of preserving the characteristic color, aroma, and flavor of açaí.[3] This product has been gaining prominence in the international market.[4]

In this study, the fermentation of clarified açaí beverage with the endophytic strain *Lactiplantibacillus plantarum* B135 (isolated by the research group[5]) was evaluated for the first time to assess the anthocyanin profile during storage 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] 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 7 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.

The quantification of anthocyanin compounds in açaí beverage was performed using an adapted method employing UHPLC.[7] The sample was diluted (10x) in methanol/water solution (50:50 v/v) acidified with 1% formic acid. 5 µL of the sample was injected into a Kinetex EVO column (100 × 4.6 mm, 2.6 µm, Phenomenex, USA) at a flow rate of 0.3 mL/min, at 25°C. The mobile phases used were ultrapure water (A) and methanol (B), both acidified with 2.5% acetic acid, with a gradient of 10% B

(0-12.9 min), 40% B (13-14.9 min), 70% B (15-15.4 min), 90% B (15.5-17.9 minutes), and 10% B (18-22 minutes). Chromatograms were recorded at 515 nm. In each assay, the compound was identified by its retention time and spectral data compared to commercial standards of cyanidin 3-glucoside and cyanidin 3-rutinoside (Sigma-Aldrich) and quantified through a calibration curve.

3 RESULTS & DISCUSSION

Figure 1 depicts the concentration of total anthocyanins in clarified açaí beverages fermented with *L. plantarum* B135 during cold storage (4°C) for 42 days. The total anthocyanin content in the beverages showed an average value of 56.1 ± 3.6 mg/100mL before the fermentation process. The impact of the fermentation process and cold storage of clarified açaí beverages did not greatly influence the reduction in total anthocyanin content, which remained above 40 mg/100mL as recommended by Brazilian legislation.

A study on the fermentation of açaí pulp reported that the anthocyanin content, specifically Cy3Rut, reached a maximum of 0.313 ± 0.005 mg/100 mL and increased by approximately 15.93 ± 3.70% after 22 hours of fermentation. [8] This significant difference underscores that the clarified açaí beverage retains a much higher concentration of total anthocyanins compared to the açaí pulp. All samples exhibited a reduction in total anthocyanin content over the storage time with average values of 11.3% (11 days, C), 9.7 ± 3.2% (28 days, C, 50S, 50F, 40S10F, and 40F10S), and 4.0 ± 1.7% (42 days, 50S, 50F, 40S10F, and 40F10S). The decrease in total anthocyanin concentration was already expected due to the natural oxidative process occurring during fermentation.[9]

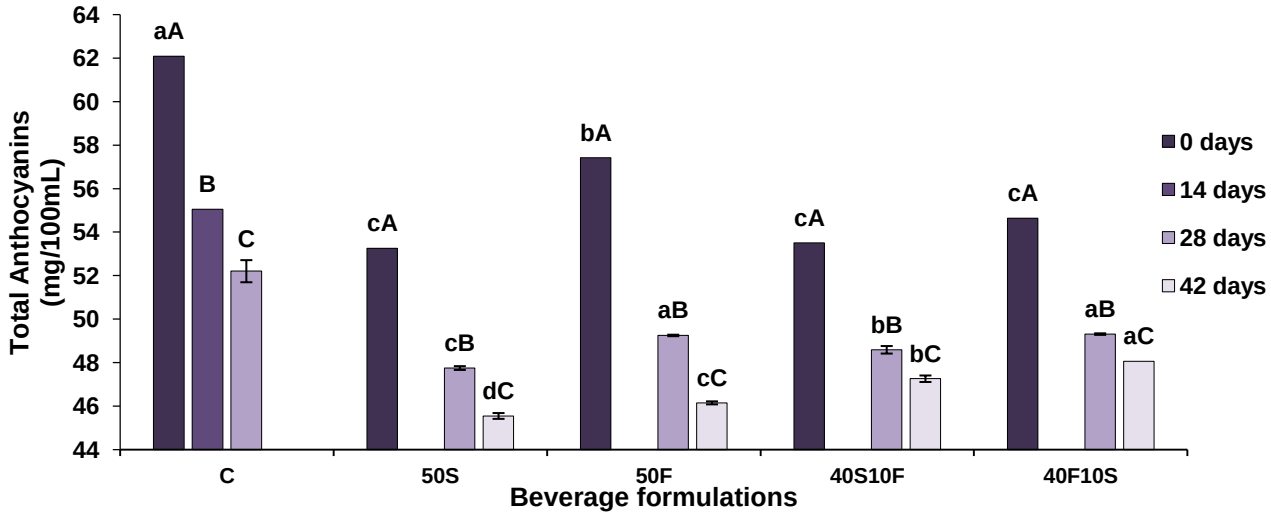

Figure 1: Concentration of total anthocyanins (mg/100mL) in clarified açaí beverages fermented by *L. plantarum* B135 during storage at 4°C for 42 days. 50 g/L of sucrose (50S); 50 g/L of fructooligosaccharides (FOS) (50F); 40 g/L of sucrose + 10 g/L of FOS (40S10F); 40 g/L of FOS + 10 g/L of sucrose (40F10S); and without supplementation (Control - C). Different lowercase (time) and uppercase (samples) letters represent significant differences ($p < 0.05$).

Evaluating the type of formulation of clarified açaí fermented beverage on the total anthocyanin content, there is no significant difference after 28 days (48.7 ± 0.7 mg/100mL) and 42 days (46.7 ± 1.1 mg/100mL) of storage, respectively. This indicates that even when varying the type of substrate in the fermentation process, the total anthocyanin content is not affected (Fig. 1). Therefore, other factors such as viable cells, pH, acidity, among others, should guide the choice of the best formulation.

CONCLUSION

The present study highlights the production potential of five clarified açaí fermented beverages by *L. plantarum* B135 in maintaining the total anthocyanin content over 42 days of cold storage. Initially, the total anthocyanin content showed an average value of 56.1 ± 3.6 mg/100mL before the fermentation process, reaching 46.7 ± 1.1 mg/100mL after 42 days of storage. The anthocyanin concentration result complies with legal standards for the use of clarified açaí. Finally, the pioneering results of the study bring prospects for advancements in the development of functional beverages with Amazonian fruits, underscoring the biotechnological potential of this rich and biodiverse region.

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