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Prebiotic Potential of Pulp and By-products from Native Fruits of the Brazilian Savannah

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Abstract

Native fruits from the Brazilian Savannah, such as jatobá-do-Cerrado (*Hymenaea stigonocarpa*) and jenipapo (*Genipa americana* L.), have interesting sensory and nutritional qualities. Jatobá-do-Cerrado consumption is limited due to its aroma and texture. Jenipapo is widely used in food and cosmetics, though its by-products (peel and seeds) are often discarded. The present study evaluates the nutritional aspects (proximate composition, antioxidant capacity, phenolic compounds, and prebiotic potential) as well as the technological properties (water and oil holding capacity) of the jatobá-do-Cerrado pulp (JAP) and the jenipapo by-product (JEBP). JAP and JEBP exhibited high dietary fiber content, mainly insoluble fiber, as well as a high concentration of phenolic compounds and antioxidant activity superior to other Cerrado fruits. JAP and JEBP showed WHC of 3.16 and 4.06 g/g and OHC of 3.09 and 1.14 g/g, respectively, indicating their potential for food processing and product development. Fermentation tests showed that JAP and JEBP supported probiotic (*Lactobacillus* and *Bifidobacterium*) growth similarly to fructooligosaccharides (FOS), lowering the medium's pH. Fermentation also stimulated the synthesis of bioactive amines such as spermidine and phenylethylamine. Thus, they are promising prebiotic ingredients for functional foods and supplements.

Keywords: dietary fiber, jatobá-do-cerrado, jenipapo, probiotics, polyamines, spermidine

1. Introduction

Native fruits from the Brazilian Savannah, Cerrado, are valued for their appealing sensory qualities, such as vibrant color, aroma, and intense flavor (Bailão et al., 2015). The jatobá-do-Cerrado (*Hymenaea stigonocarpa*), a native fruit, has a farinaceous pulp with a creamy/yellowish color (Carvalho, 2007). The pulp is sweet, very flavorful, and has a characteristic odor. It has a high dietary fiber content and significant amounts of calcium, magnesium, sugar, carotenoids, polyphenols, and vitamin C (Batista et al., 2011). Despite its proven nutritional properties, the consumption of the fruit in natura by the local population is limited, and the development of products derived from its pulp has not been widely explored, which justifies the need for further studies to encourage the inclusion of jatobá-do-Cerrado in the human diet. The limited consumption of jatobá-do-Cerrado can be attributed to its aroma and farinaceous texture, which may be considered unpleasant (Carvalho, 2007).

Other native fruit of the Cerrado, the jenipapo (*Genipa americana* L.) has a yellow, rough, and wrinkled peel, and its pulp is light brown with numerous elongated seeds (Silva et al., 2014). It has an attractive flavor and odor, and exotic attributes, making it a versatile ingredient in the diet (Hamacek et al., 2013). It is consumed in various forms: fresh, in preparations (jams, sweets, and ice creams), and beverages (especially liqueurs). The pulp can also be used as a substitute for commercial pectin to aid in the gelation of low-pectin fruit juices (Hansen et al., 2008).

Unlike the jatobá-do-Cerrado, the jenipapo pulp is already common in human consumption, particularly in the production of sweet products (Hamacek et al., 2013). Additionally, the jenipapo has coloring properties attributed to the presence of a compound called genipin and its glycosylated forms (geniposide and genipinic acid) (Bentes & Mercadante, 2014). However, it is notable that the production of food products and pigments use only the pulp of the fruit, leaving its seeds and peel as waste (Madrona et al., 2019). It is estimated that 48% of the fruit consists of pulp, resulting in a significant amount of waste (peels and seeds) that remains unused. Therefore, a portion greater than the edible part of the fruit is discarded (Hamacek et al., 2013). Given that the use of this fruit's pulp is highlighted not only in food but also in the pharmaceutical and cosmetic industries (Sabino et al., 2021), it is necessary to consider the waste generated from its processing, which impacts the environment (Melo et al., 2023). Therefore, investigating the functional potential of its by-products (peel and seeds) becomes an important area of study to find alternative uses for these by-products and to promote sustainability.

Some studies have reported that native fruits pulp (Foltz et al., 2021; Guergoletto et al., 2016) and by-products (Andrade et al., 2020; Barbosa et al., 2022) can present functional characteristics, including the ability of stimulate the growth of probiotic bacteria, proving a prebiotic potential of this compounds, which can lead to health benefits (Gibson et al., 2017). These substrates can stimulate the colonic bacteria metabolism, producing important metabolites, such as short-chain fatty acids (SCFAs) and polyamines, that can regulate gut homeostasis (Fusco et al., 2023) and enhance human longevity (Kibe et al., 2014).

Considering the nutritional and functional potential of fruit pulps, the social, economic, and environmental relevance of using by-products for human consumption, this study aimed to evaluate the nutritional aspects (proximate composition, antioxidant capacity, presence of phenolic compounds, and prebiotic potential) as well as the technological properties (water and oil holding capacity) of the jatobá-do-Cerrado pulp and the jenipapo by-product.

2. Method

2.1 Samples Preparation

Jenipapo and jatobá-do-Cerrado fruits were manually harvested and selected based on their ripeness. Jenipapo was obtained from the Cerrado Fruit Collection at the School of Agronomy, Federal University of Goiás, in December 2021. Jatobá-do-Cerrado fruits were harvested in September 2021 in the municipality of Bela Vista de Goiás, Goiás. Both fruits were washed in running water, and only the jenipapo fruits were immersed in a sodium hypochlorite solution (2.5%) for 15 min.

To obtain the freeze-dried jenipapo byproduct (JEBP), an industrial pulping machine with a capacity of 0.25 DF (Bonina$^{®}$, Itabuna, Brazil) and 2.5 mm sieves was used to separate the pulp from the byproduct (peel and seeds). The byproduct samples were homogenized, freeze-dried (Liotop$^{®}$, L108, Brazil) (temperature: −50 ± 5°C, pressure: < 100 μHg) for 24 h, and ground in a blender until a fine powder was obtained. The pulp of jatobá-do-Cerrado (JAP) was manually collected and passed through 2.5 mm sieves to separate the pulp from the residue (peel and seeds). After processing, the fresh JAP and the freeze-dried JEBP samples were separately vacuum-packed (Selovac$^{®}$, Microvac, Brazil) in transparent packaging and stored under refrigeration, protected from light, until the time of analysis.

2.2 Chemical Composition

Proximate composition was determined by moisture, ash, total nitrogen, and conversion to crude protein. Total energy value was estimated using Atwater conversion factors of 4, 4, and 9 kcal/g to protein, carbohydrate, and lipid, respectively. The contents of total, insoluble and soluble dietary fibers were determined with an enzymatic-gravimetric method (Official methods of analysis, 2010).

2.3 Phenolic Compounds and Antioxidant Activity

Methanol extracts of JAP and JEBP were prepared to determine the total phenolic compounds. 2 g of the samples were added to 5 mL of 70% methanol and homogenized using a magnetic stirrer (IKA, Staufen, Germany) for 60 min. The samples were then centrifuged at 3000 rpm for 5 min, and the supernatant was manually separated from the solid part. An aliquot of the samples was placed in test tubes (10 mL) wrapped in aluminum foil. A volume of 0.25 mL of the extracts was mixed with 2.5 mL of water and 0.25 mL of Folin-Ciocalteu reagent. The mixtures were vortexed for 10 s. After 5 min at room temperature, 250 μL of 10% sodium carbonate solution was added, and the mixture was kept at room temperature in the dark for 60 min. The absorbance at 725 nm was determined using a UV/Vis spectrophotometer V-630 (Jasco, Tokyo, Japan), and the total phenolic content was calculated using a gallic acid standard curve (16 to 100 mg/L). The results were expressed as milligrams of gallic acid equivalent per 100 grams of fresh weight (mg GAE/100g) (Singleton & Rossi, 1965).

The free-radical scavenging capacity of DPPH was determined according to Brand-Williams et al. (1995). The antioxidant values were expressed as the number of moles of antioxidant per mole of DPPH. In 15 mL test tubes wrapped in aluminum foil, 3.9 mL of 60 µmol/L DPPH reagent solution (previously analyzed for absorbance, which was between 0.57-0.63) was added in triplicate. Subsequently, 0.1 mL of the sample extract was added, and the tubes were homogenized by vortexing for 10 s and left to stand for one hour and 30 min at room temperature, protected from light. The reduction in absorbance was determined using a spectrophotometer at 515 nm, calculated using the Trolox standard curve, and the results were expressed in micromoles of Trolox equivalent per gram of sample (µmol TE/g).

2.4 Water and Oil Holding Capacities

The water holding capacity (WHC) and oil holding capacity (OHC) of JAP and JEBP were measured according to the method described by Salih et al. (2017). A total of 30 mL of ultrapure water (Merck Milipore® Direct Q3, Brazil) for WHC or 30 mL of commercial soybean oil for OHC were added to exactly 1 g of the samples, agitated using a vortex mixer for 1 min, and stored at room temperature for 24 h. The tubes were then centrifuged at 3000 rpm for 20 min. The supernatant was decanted, and the residue was weighed and compared to the initial weight. WHC and OHC were calculated as g of water or oil absorbed per gram of dry sample, respectively.

2.5 Prebiotic Potential

2.5.1 Microorganism Preparation

Probiotic microorganisms were used in the *in vitro* prebiotic potential assays: *Lactobacillus acidophilus* LA-05, *Lacticaseibacillus casei* L-26 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (obtained from College of Biotechnology, Portuguese Catholic University - Porto, Portugal). Each probiotic was cultivated in De Mann, Rogosa and Sharpe (MRS) (Kasvi, Espanha) broth at 37 °C for 24 h under anaerobic conditions. For *B. animalis*, 0.5 g/L of l-cysteine-HCl was added to the broth (Albuquerque et al., 2020). The cell suspensions were standardized an optical density of 0.8 at 655 nm, and viable cell counts of approximately 6 log colony forming units (CFU) per mL (log CFU/mL).

Pathogenic strains, *Escherichia coli* (ATCC 25922 and ATCC 8739) were used as an enteric mixture inoculum (ratio 1:1). This mixture simulates the diverse microbial interactions within the gut, which is crucial for assessing prebiotic activity (Huebner et al., 2007; Zhang et al., 2018). The strains were activated in Brain Heart Infusion (BHI) broth (Kasvi, Spain) at a temperature of 37 °C for 20 h under aerobic conditions. For the enteric bacteria, cell suspensions were standardized an optical density of 0.1 at 655 nm. This suspension provided viable counts of approximately 6 log CFU/mL (Albuquerque et al., 2020).

2.5.2 Cultivation Media, Probiotic Viable Cell Counts and Metabolic Activity

Three different culture media were prepared with different carbon sources to assess the growth of probiotic bacteria. The medium containing glucose served as the non-prebiotic control, fructooligosaccharides (FOS) acted as the positive prebiotic control, and a medium without an added carbon source functioned as the negative control. Each carbon source was used at a concentration of 20 g/L. Probiotic microorganisms were then separately inoculated with 200 µL into the medium, mixed and incubated at 37 °C under anaerobic conditions (Albuquerque et al., 2020). These media were composed by tryptone (10 g/L), meat extract (8 g/L), yeast extract (4 g/L), dipotassium hydrogen phosphate (2 g/L), tween 80 (1 g/L), sodium acetate (5 g/L), tribasic ammonium citrate (2 g/L), magnesium sulfate (0.2 g/L), manganese sulfate (0.04 g/L), and the respectively sample as carbon sources (JAP or JEBP).

The viable cell count was performed using MRS agar. Tubes containing culture medium and inoculum were vortexed and serially diluted up to 10^{-8} in sterile saline solution. Aliquots of the dilutions were plated on MRS agar and incubated for different times. After 48 h of incubation at 37°C, under anaerobioses, viable cells were counted in CFU/mL.

To assess bacterial metabolic activity, pH values in the culture media were observed at incubation times (0, 18, 24, and 48 h) using a digital pH meter (Adwa®, AD1000, Hungary). Also, the concentrations of sugars (glucose, fructose and sucrose) of the media were evaluated during the fermentation time 0 and 18 h. 0.5 g of the samples was diluted in 10 mL of ultrapure water at 50°C in a digital sample homogenizer for 5 min, then the mixture was centrifuged (3000 × g, 5 min, 24°C) and the supernatant was filtered (0.45 µm). The extracts were analyzed in high-performance liquid chromatography (HPLC) and the sample peaks were measured by comparing their retention times with the previously mentioned standards of sugars (Purgatto et al., 2002).

The bioactive amines production (spermine, spermidine, putrescine and phenylethylamine) was evaluated in fermentation times 18 and 48 h. Extracts of samples JAP and JEBP were prepared and submitted in HPLC, composed of an LC-10 AD system connected to an RF-551 spectrofluorimetric detector at 340 and 445 nm of excitation and emission, respectively, and a CBM-10 controller AD (Shimadzu Corp. Kyoto, Japan), as performed according to Dala-Paula et al. (2021).

2.5.3 Prebiotic Activity Score

The prebiotic activity score was performed according to Huebner et al. (2007). Initially, M9 broth (Sigma-Aldrich, St. Louis, MO, USA) was added with 20 g/L of glucose, FOS, JAP and JEBP. 100 µL of the enteric mixture were serially diluted in sterile saline solution, and 20 µL aliquots of each dilution were plated on Eosin Methylene Blue (EMB) (Kasvi, Spain) agar by the drop plate technique at 0 h and 48 h of fermentation. The plates were incubated at 37°C for 48 h and the viable cells were counted (log CFU/mL). The probiotic viable cells counted in media with glucose added to *L. acidophilus* LA-05, *L. casei* L-26 and *B. animalis* BB-12, at 0 and after 48 h were counted. The results of viable cell counts were used on the following equation: Prebiotic activity score = [(probiotic log CFU/ml on the prebiotic at 48 h - probiotic log CFU/ml on the prebiotic at 0 h)/(probiotic log CFU/ml on glucose at 48 h - probiotic log CFU/ml on glucose at 0 h)] - [(enteric log CFU/ml on the prebiotic at 48 h - enteric log CFU/ml on prebiotic at 0 h)/(enteric log CFU/ml on glucose at 48 h - enteric log CFU/ml on glucose at 0 h)].

2.6 Statistical Analysis

Physical-chemical data were assessed in triplicate for JAP and JEBP, with results expressed as mean ± standard deviation. *In vitro* assays were conducted in triplicate across three separate experiments. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's test, with differences considered significant when $p \leq 0.05$ (RStudio version 2.15).

3. Results and Discussion

3.1 Chemical Composition, Total Phenolic Compounds and Antioxidant Capacity of JAP and JEBP

JAP and JEBP had a high content of total dietary fiber, mainly insoluble fiber, and low content of moisture, ash, lipid, and total energy value (Table 1). JAP total carbohydrates were higher than the pulps from São Paulo and Minas Gerais (Cardoso et al., 2013; Dias et al., 2013). High concentrations of total dietary fibers were also observed in a study with JAP from Minas Gerais (Cardoso et al., 2013). This content was higher than that observed in other fruits considered excellent sources of dietary fiber, such as guava (5.78 g/100 g) (FoRC, 2023).

Table 1. Chemical composition, total phenolic compounds and antioxidant capacity of jatobá-do-Cerrado pulp, freeze-dried jatobá-do-Cerrado by-product and freeze-dried jenipapo by-product

Parameter	JAP	JEBP
Proximate composition (g/100 g)		
Moisture	10.87 ± 0.03	19.23 ± 0.04
Ash	2.95 ± 0.01	2.59 ± 0.01
Protein	12.49 ± 0.10	6.26 ± 0.06
Lipid	0.66 ± 0.04	0.56 ± 0.04
Total carbohydrate	73.01 ± 0.99	79.58 ± 2.25
Total sugar	9.97 ± 0.18	8.20 ± 0.28
Total dietary fiber	48.54 ± 0.02	36.82 ± 0.95
insoluble dietary fiber	43.85 ± 1.01	30.58 ± 1.07
soluble dietary fiber	4.17 ± 0.13	5.63 ± 0.06
Energy value (kcal/100 g)	261.01 ± 0.26	241.90 ± 2.24
Total phenolic compounds (mg GAE/100g)	1235.46 ± 36.57	161.50 ± 4.20
Antioxidant capacity (µmol TE/g)	6.12 ± 1.63	2.40 ± 1.55

Values are expressed as mean ± standard deviation (n= 3). JAP: jatobá-do-Cerrado pulp; JEBP: Jenipapo by-product

The dietary fiber content in 100 g of JEBP exceeds the recommended daily intake for a healthy adult, that are 25 g/day for women and 38 g/day for men (DRI, 2005). JEBP presents a high pectin content, justifying the high content of soluble dietary fiber. Dietary fibers can affect the composition and metabolic activity of intestinal bacterial communities, including the production of SCFAs (Gibson et al., 2017).

JAP presented high content of total phenolic compounds. This fruit pulp presents high content of bioactive compounds when compared to other Cerrado fruits (Almeida et al., 2019). The consumption of high levels of phenolic compounds diets may offer protection against diseases, such as cancer and inflammatory disorders (Rudrapal et al., 2022). Additionally, certain dietary polyphenols have been shown to promote the proliferation of microorganisms (Cheng et al., 2019; Serreli & Deiana, 2019). These compounds also enhance the production of beneficial metabolites for health, such as SCFAs (Mithul Aravind et al., 2021).

Also, an important antioxidant activity was observed for JAP and JABP, higher than those found in passion fruit (*Passiflora* sp.) (Genovese et al., 2008). The JABP is composed by peel and seeds, which are alternative and natural sources of antioxidants, similar to peel and seeds of siriguela (*Spondia purpurea* L.) and umbu (*Spondia tuberosa*), that presents antioxidant activity and total phenol content higher than the edible portion of their respective fruits (Omena et al., 2012). The identification of functional compounds with antioxidant activity can contribute to adding value to the species, given the global trend of seeking foods with high nutritional value. This identification may also stimulate the inclusion of JAP in the human diet (Menezes Filho et al., 2019).

JAP contains a significant amount of protein, making it a valuable ingredient for food product development. Proteins are essential in food technology, improving the texture, structure, and sensory properties of products. They serve functional roles such as emulsifiers and gelling agents (Pam Ismail et al., 2020). Natural protein sources like JAP offer sustainable options for creating nutritionally enhanced foods, meeting the needs of health-conscious consumers.

3.2 Water and Oil Holding Capacities

In the search for functional foods and alternatives for the food industry, the water-holding capacity (WHC) and oil-holding capacity (OHC) were also evaluated as alternatives for products that can perform functions such as forming emulsions and adding nutritional value in processing and product development (Zahid et al., 2021). The WHC (g/g freeze-dried sample) of JAP and JEBP were 3.16 ± 0.02 and 4.06 ± 0.01, and the OHC were 3.09 ± 0.03 and 1.14 ± 0.01, respectively (Table 2).

Table 2. Water and oil holding capacity of jatobá-do-Cerrado pulp, freeze-dried jatobá-do-Cerrado by-product and freeze-dried Jenipapo by-product

Parameter (g/g freeze-dried sample)	JAP	JEBP
Water holding capacity	3.16 ± 0.02	4.06 ± 0.01
Oil holding capacity	3.09 ± 0.03	1.14 ± 0.01

Values are expressed as mean ± standard deviation (n= 3). JAP: jatobá-do-Cerrado pulp; JEBP: Jenipapo by-product

The WHC and OHC of JEBP were higher than those found by Salih et al. (2017) in an analysis of dry ripe banana peel flour (0.7 g water/g sample and 0.8 g oil/g sample). The fiber concentrate from mango, pineapple, guava, and passion fruit by-products showed higher water retention values (6.4 g water/g sample, 14.6 g water/g sample, 10.2 g water/g sample, and 13.5 g water/g sample, respectively) compared to JEBP (Zahid et al., 2021).

WHC may be related to the physical state of starch, dietary fiber, and protein in the sample. According to Rodríguez-Ambriz et al. (2008), amylase can effectively bind to water molecules, producing greater water retention capacity. The increase in this retention can occur with protein denaturation, solution properties of dietary fiber such as hemicelluloses and pectin polysaccharides. Water retention capacity refers to the hydrophilic nature of the by-products. This analysis is important for food processing and the development of new products, as this property can modify the texture and viscosity of a formulated product, reduce calories, and prevent degradation phenomena (Zahid et al., 2021). Also, the WHC and OHC capacity analysis can be useful for predict the textural properties of the products during frozen storage (Zuo et al., 2025).

3.3 Effects of JAP and JEBP on Probiotic Strains

So far, this is the first study that investigated the prebiotic potential properties of jatobá-do-Cerrado pulp and jenipapo by-products. The viable cell counts of *L. acidophilus* LA-05 in JAP and JEBP media were similar to those in glucose and FOS media ($p > 0.05$). The growth of *L. casei* L-26 cells was also similar in the JAP medium ($p > 0.05$) over 48 hours of fermentation. In 18 h of fermentation, *B. animalis* subsp. *lactis* BB-12 growth achieved 9.82 ± 0.52 log CFU/mL in media added with JAP, and 9.26 ± 0.85 log CFU/mL with FOS ($p > 0.05$) (Figure 1).

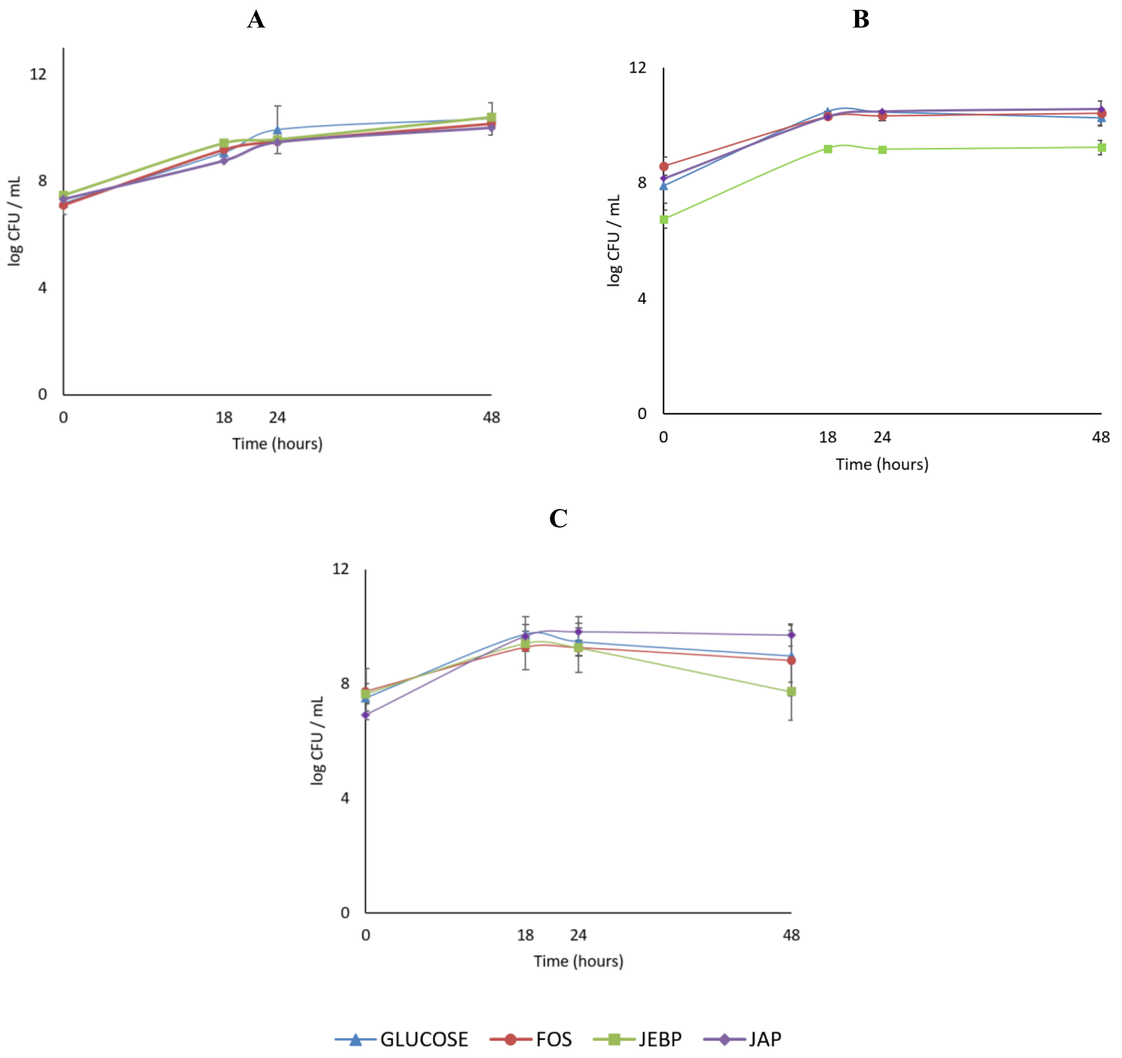

Figure 1. Viable cell counts of *L. acidophilus* LA-05 (A), *L. casei* L-26 (B), and *B. animalis* subsp. *lactis* BB-12 (C) in media with GLU, FOS, freeze-dried JAP and freeze-dried JEBP during 48 h of incubation. GLU: glucose; FOS: fructooligosaccharides; JAP: freeze-dried jatobá-do-Cerrado pulp; JEBP: jenipapo by-product

These results demonstrate that both samples were used as carbon sources by all tested microorganisms, in a similar way than FOS, an inulin-type fructans recognized as prebiotic (Hughes et al., 2022). This fact may be explained by the fermentation of the available pulp and by-products compounds, such as dietary fibers (Table 1). The difference in the growth of strains with the same substrate can be attributed to the specific genomic and carbohydrate metabolism diversity found between *Lactobacillus* genus and *Bifidobacterium* (Scott et al., 2020).

JAP and JABP were able to maintain the growth of *Lactobacillus* (LA-05 and L-26) until the end of the assay, as no reduction in the viable cell counts was observed in the respective medium at 48 h of fermentation. Carbon sources with prebiotic potential can stimulate the bacterial adhesion of *L. acidophilus*. An increased adhesion capacity of probiotics to the gastrointestinal tract can prolong bacterial residence, producing positive health effects (Celebioglu et al., 2017).

The use of JAP, JEBP, glucose, and FOS as carbon sources by the tested probiotics decreased the pH of the cultivation media at 48 h compared to the initial pH (Table 3). In 48 h of fermentation, media added with JAP and JEBP in the presence of *B. animalis* subsp. *lactis* BB-12 reached pH values (4.33 and 4.17) compatible with FOS (4.51) ($p > 0.05$). These results indicate intense fermentative metabolic activities of these microorganisms on the analyzed carbon sources, related to organic acids production (Gibson et al., 2017). The production of SCFA by probiotics, such as butyric acid, propionic acid and lactic acid, stimulated by prebiotic compounds, maintain a low pH value and inhibiting the growth of pathogenic microorganisms (Fusco et al., 2023). Additionally, the increased

SCFAs appears to be one of the main therapeutic avenues in several approaches that aim to modulate the intestinal microbiome, and a mediational role in the microbiota-gut-brain axis (Dalile et al., 2019).

Table 3. pH values of *L. acidophilus* LA-05, *L. casei* L-26, and *B. animalis* subsp. *lactis* BB-12 in media with GLU, FOS, freeze-dried JAP and freeze-dried JEBP during 48 h of incubation. GLU: glucose; FOS: fructooligosaccharides; WCS: Without carbon source; JAP: freeze-dried jatobá-do-Cerrado pulp; JEBP: jenipapo by-product

Time (hours)	Carbon Source	*L. acidophilus* LA-05	*L. casei* L-26	*B. animalis* subsp. *lactis* BB-12
0	FOS	6.11 ± 0.02^{ab}	6.10 ± 0.01^{ab}	5.40 ± 0.04^{a}
	GLU	6.00 ± 0.03^{ab}	5.99 ± 0.02^{bc}	5.27 ± 0.03^{b}
	WCS	6.22 ± 0.04^{ab}	6.20 ± 0.01^{a}	5.41 ± 0.03^{a}
	JAP	6.59 ± 0.65^{a}	5.86 ± 0.11^{c}	5.32 ± 0.03^{ab}
	JEBP	5.59 ± 0.02^{b}	5.60 ± 0.00^{d}	5.03 ± 0.04^{c}
18	FOS	3.89 ± 0.02^{c}	3.87 ± 0.00^{d}	4.81 ± 0.03^{b}
	GLU	3.94 ± 0.00^{c}	3.93 ± 0.00^{d}	3.57 ± 0.05^{e}
	WCS	5.57 ± 0.05^{ab}	5.55 ± 0.02^{a}	5.04 ± 0.04^{a}
	JAP	5.74 ± 0.90^{a}	4.88 ± 0.08^{b}	4.27 ± 0.04^{c}
	JEBP	4.50 ± 0.05^{bc}	4.47 ± 0.01^{c}	4.14 ± 0.04^{d}
24	FOS	3.85 ± 0.00^{b}	3.74 ± 0.00^{e}	4.84 ± 0.08^{b}
	GLU	3.82 ± 0.02^{b}	3.80 ± 0.00^{d}	3.54 ± 0.05^{d}
	WCS	5.67 ± 0.01^{a}	5.58 ± 0.02^{a}	5.08 ± 0.02^{a}
	JAP	5.72 ± 1.02^{a}	4.64 ± 0.03^{b}	4.29 ± 0.05^{c}
	JEBP	4.52 ± 0.05^{ab}	4.49 ± 0.01^{c}	4.15 ± 0.04^{c}
48	FOS	3.74 ± 0.02^{b}	3.45 ± 0.01^{c}	4.51 ± 0.16^{b}
	GLU	3.58 ± 0.11^{b}	3.52 ± 0.00^{c}	3.42 ± 0.04^{c}
	WCS	5.68 ± 0.06^{a}	5.62 ± 0.11^{a}	5.14 ± 0.14^{a}
	JAP	5.71 ± 1.11^{a}	4.45 ± 0.01^{b}	4.33 ± 0.07^{b}
	JEBP	4.52 ± 0.01^{ab}	4.32 ± 0.00^{b}	4.17 ± 0.06^{b}

$^{a-e}$: different superscript small letters in the same column at each time denote differences between carbon sources based on Tukey's test ($p \leq 0.05$). GLU: glucose; FOS: fructooligosaccharides; WCS: Without carbon source; JAP: freeze-dried jatobá-do-Cerrado pulp; JEBP: jenipapo by-product

The concentration of sugars (glucose, fructose and sucrose) in most of the cultivation media decreased at 18 h of fermentation, compared to the initial contents (Table 4). Sucrose was found in notable contents in JEBP medium, and after fermentation time considerable decrease of this sugar was found, when compared to the initial time ($p < 0.05$). In some cases, genus *Lactobacillus* survives in significantly greater numbers in the digestive tract of mice fed a diet rich in sucrose compared to a diet rich in plant polysaccharides (Kleerebezem et al., 2003). It was also notable the use of the fructose during the fermentation of JAP and JEBP by *L. acidophilus* LA-05 and *B. animalis*. Carbohydrate metabolic abilities may vary considerably between strains, however, most of *Bifidobacterium* species can utilize fructose to produce organic acids (Pokusaeva et al., 2011).

Table 4. Sugar content (g/L) in media with GLU, FOS, freeze-dried JAP and freeze-dried JEBP inoculated with *L. acidophilus*, *L. casei*, and *B. animalis* during 18 h of incubation. GLU: glucose; FOS: fructooligosaccharides; WCS: Without carbon source; JAP: freeze-dried jatobá-do-Cerrado pulp; JEBP: jenipapo by-product

Sugars	Carbon source	Strains					
		LA-05		L-26		BB-12	
		0 h	18 h	0 h	18 h	0 h	18 h
Glucose	GLU	2.57 ± 6.48^{a}	1.18 ± 9.77^{b}	2.48 ± 3.59^{a}	1.17 ± 10.15^{b}	2.29 ± 2.36^{a}	0.78 ± 6.21^{b}
	FOS	0.04 ± 0.95^{a}	0.04 ± 0.50^{a}	0.07 ± 4.92^{a}	0.05 ± 2.17^{ab}	0.05 ± 1.38^{a}	ND
	JAP	0.03 ± 8.07^{a}	0.02 ± 0.50^{ab}	0.08 ± 0.55^{a}	0.12 ± 0.94^{ab}	0.00 ± 0.02^{a}	0.00 ± 0.05^{a}
	JEBP	0.33 ± 3.39^{a}	0.01 ± 0.36^{b}	0.30 ± 6.92^{a}	0.03 ± 3.77^{b}	0.29 ± 3.65^{a}	0.01 ± 0.19^{b}
Fructose	GLU	0.03 ± 1.28^{a}	ND	0.03 ± 0.48^{a}	ND	0.08 ± 0.74^{a}	ND
	FOS	0.09 ± 2.82^{a}	0.06 ± 1.42^{b}	0.03 ± 0.63^{a}	0.05 ± 0.76^{a}	0.09 ± 1.88^{a}	0.00 ± 0.04^{b}
	JAP	0.12 ± 0.01^{a}	0.06 ± 1.26^{b}	0.05 ± 0.23^{a}	0.05 ± 0.32^{a}	0.06 ± 0.76^{a}	0.06 ± 0.07^{a}
	JEBP	0.18 ± 1.45^{a}	0.00 ± 0.30^{b}	0.17 ± 2.90^{a}	0.01 ± 0.74^{b}	0.15 ± 2.05^{a}	0.00 ± 0.03^{b}
Sucrose	GLU	0.08 ± 2.32^{a}	0.04 ± 4.22^{b}	0.13 ± 3.84^{a}	0.13 ± 0.52^{a}	0.13 ± 1.38^{a}	0.12 ± 2.30^{a}
	FOS	0.04 ± 6.16^{a}	0.02 ± 4.77^{b}	0.10 ± 4.61^{a}	0.15 ± 1.71^{ab}	0.13 ± 2.62^{a}	0.15 ± 2.83^{ab}
	JAP	0.07 ± 4.85^{a}	0.01 ± 0.99^{b}	0.34 ± 4.85^{a}	0.05 ± 0.55^{b}	0.05 ± 1.46^{a}	0.04 ± 0.66^{ab}
	JEBP	0.33 ± 1.66^{a}	0.29 ± 4.55^{b}	0.30 ± 4.47^{a}	0.28 ± 0.68^{ab}	0.28 ± 2.50^{a}	0.15 ± 1.77^{b}

$^{a\text{-}c}$: different superscript small letters in the same row and different measured sugars for each isolate among media with GLU, FOS, PBP, and GBP denote differences based on Tukey's test ($p \leq 0.05$). ND: not detected; GLU: glucose; FOS: fructooligosaccharides; JAP: freeze-dried jatobá-do-Cerrado pulp; JEBP: jenipapo by-product

Intense metabolic activity of the probiotic strains was also confirmed by the production of bioactive amines during 48 h of fermentation (Figure 2). After 48 h of fermentation by BB-12, there was a greater production of spermidine in the medium containing JAP (17.32 mg/kg) and JEBP (18.83 mg/kg) ($p < 0,05$), when compared to the media added with FOS. Spermidine is an important polyamine for multiple cellular processes, including the maintenance of cellular homeostasis (Madeo et al., 2018). Polyamines produced by intestinal bacteria in the presence of potentially prebiotic components can promote benefits to the intestinal microbiota (Dong et al., 2021).

The endogenous spermidine (from gut) can present anti-inflammatory and antioxidant activity, and prebiotics that induce gut probiotic bacteria to synthesize polyamines have strong potential for easing aging effects and age-related metabolic disorders (Yu et al., 2023). Corresponding concentration of bioactive amine phenylethylamine, was also detected in all media fermented by *B. animalis* subsp. *lactis* BB-12, in higher concentration on JAP (18.70 mg/kg) and JEBP (18.83 mg/kg), when compared to FOS media (11,52 mg/kg) ($p < 0.05$). Some probiotic bacteria biosynthesize phenylethylamine, that controls satiety and mood, and in specific bacteria species, these amines can be informational messengers for microbial cross-talk and for stress control (Konings, 2006; Pessione, 2012). Therefore, the presence of JAP and JEBP in media with the probiotic bacteria stimulated the production of these bioactive amines that may bring health benefits.

However, it is important to emphasize that bioactive amines production can also lead to adverse effects on the nervous, respiratory, and cardiovascular systems. Additionally, high levels of these compounds may trigger allergic reactions (Dala-Paula et al., 2023). Therefore, while bioactive amines, like polyamines, contribute positively to various biological processes, careful consideration of their concentrations is necessary to prevent potential health risks.

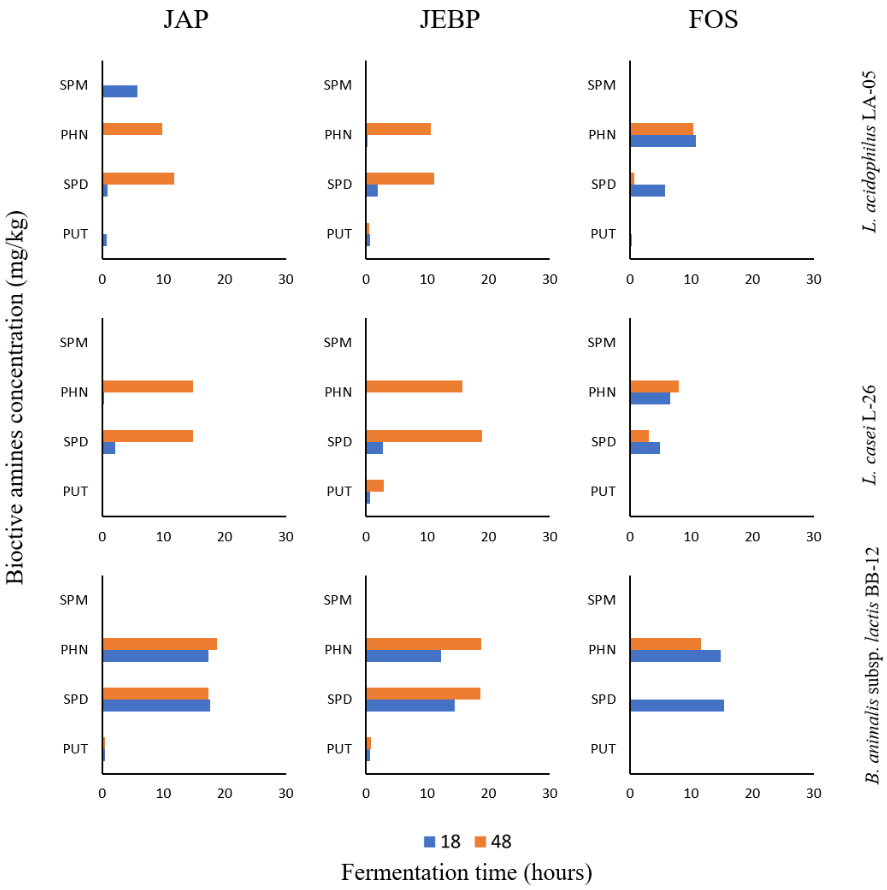

Figure 2. Bioactive amines concentration (mg/kg) production in media with fructooligosaccharides (FOS), freeze-dried jatobá-do-Cerrado pulp (JAP) and jenipapo by-product (JEBP) during 18 h and 48 h of fermentation. PUT: putrescine; SPD: spermidine; PHN: phenylethylamine; SPM: spermine

3.4 Prebiotic Activity Scores

Positive prebiotic activity scores were observed for all samples, indicating that JAP and JEBP were selectively metabolized by the probiotic bacteria but not by the pathogenic strains used in the enteric mixture (Figure 3). In assays with *B. animalis* subsp. *lactis* BB-12, no significant differences were found between JAP and JEBP scores with FOS ($p > 0.05$). In fermentation by *L. casei* L-26, the medium added with the JEBP showed a higher prebiotic activity score (3.65 ± 1.40) than the medium added with FOS (1.16 ± 1.05) ($p < 0.05$).

The JEBP prebiotic activity score in *L. casei* L-26 was higher than other scores for by-products fermented by the same probiotic bacteria. Barbosa et al. (2022) found 0.39 and 0.53 prebiotic activity scores for puçá and gabiroba by-products, from Cerrado. Extract chitosan from mushroom waste presented prebiotic activity score of approximately 0.80 (Yadav et al., 2024). This high prebiotic activity in *L. casei* L-26 in agreement with the intense metabolic activity found by these bacteria in the media added with JEBP, since the consumption of glucose, fructose and sucrose was considerable (Table 4). Carbohydrate metabolism of *L. casei* stands out for its ability to use sucrose through its acid hydrolysis (Mousavi & Mousavi, 2019).

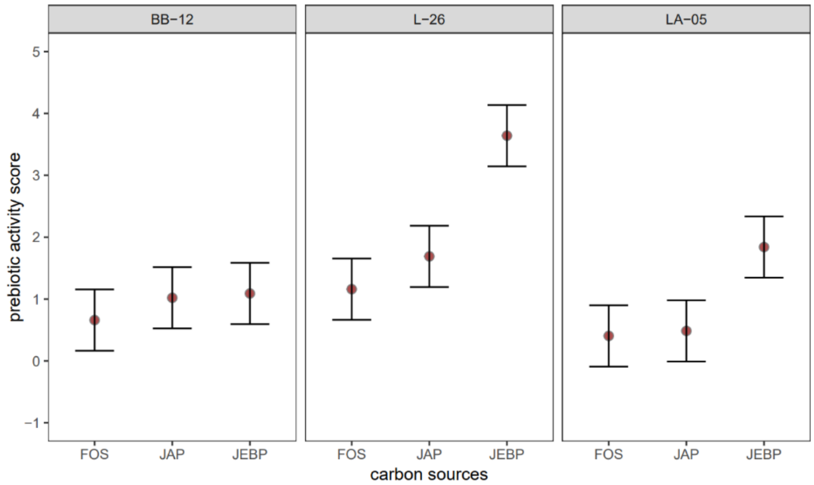

Figure 3. Prebiotic activity scores of freeze-dried jatobá-do-Cerrado pulp (JAP), Jenipapo by-product (JEBP) and fructooligosaccharides (FOS) under different probiotic strains fermentation

Finally, jatobá pulp and jenipapo by-products presented prebiotic properties, associated with their nutritional compositions, including total phenolics and dietary fibers that selectively stimulated probiotic bacteria of the genus *Lactobacillus* and *Bifidobacterium*. JAP and JEBP present relevant concentrations of carbohydrates, antioxidant activity and insoluble fibers. Also, interesting technological propriety has been observed, associated with good water and oil holding capacity. Probiotic tested strains produced polyamines, including spermidine, during fermentation of JAP and JEBP. This is the first study that evaluated prebiotic property of these native fruits, revealing that Jatobá pulp and jenipapo by-products should be considered potential prebiotic ingredients for human health, adding in functional foods and dietary supplements.

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Authors contributions

Deborah Dias Oliveira, Luciana Conrado Duarte de Souza and Jéssica Pereira Barbosa were responsible for study design and revising. Deborah Dias Oliveira and Luciana Conrado Duarte de Souza were responsible for data collection and analysis. Bibiana da Silva, Ana Carolina de Oliveira Costa, Eduardo Purgatto and José Eduardo Gonçalves were responsible for specific analysis. Deborah Dias Oliveira, Luciana Conrado Duarte de Souza and Jéssica Pereira Barbosa drafted the manuscript and Prof. Patrícia Amaral Souza revised it. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal’s policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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