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Banana Pseudostem By-Product: A Sustainable Source of Prebiotics and Protection for Probiotic Lactic Acid Bacteria Under Gastrointestinal Conditions

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Abstract

Agricultural by-products, such as banana pseudostems (BPS), present a sustainable solution for waste reduction and the recovery of valuable metabolites with biotechnological applications. This study investigated the potential of BPS as a substrate for bio-fermentation, specifically for the cultivation of lactic acid bacteria (LAB). *Maçã* cultivar BPSs (MBPS) and *Nanica* cultivar BPSs (NBPS) flour samples showed differences in carbohydrate composition, especially in resistant starch (16.7 and 2.7%), cellulose (27.0 and 52.4%), and hemicellulose (25.4 and 33.8%), respectively. Phenolic compound content in NBPS was higher than in MBPS (193.9 and 153.5 GAE/100 g, respectively). The BPS starches and flour were well assimilated by the probiotic LAB cultures. *Limosilactobacillus fermentum* SJRP30 and SJRP43 showed significant growth in media with gelatinized *Maçã* flour (GMF) and non-gelatinized *Nanica* flour (NGNF) BPS by-products (Log 9.18 and 9.75 CFU/mL, respectively), while *Lacticaseibacillus rhamnosus* GG exhibited the highest growth (Log 11.31 CFU/mL) in the medium with NGNF BPS by-products. The probiotic *Lbs. casei* SJRP146 and *Lmb. fermentum* SJRP30 and SJRP43 presented high enzymatic activity and the ability to assimilate D-xylose. Only *Lactobacillus delbrueckii* subsp. *bulgaricus* SJRP57 and SJRP49 were able to assimilate starch. Their prebiotic potential under in vitro gastrointestinal digestion was evidenced by promoting the selected probiotic bacteria's protection and maintaining their viable cells after challenging conditions, likely associated with the BPS composition. *Lab. delbrueckii* subsp. *bulgaricus* SJRP57, *Lacticaseibacillus casei* SJRP145, and *Lmb. fermentum* SJRP43 performed similarly to the commercial strain *Lbs. rhamnosus* GG. These results demonstrate the feasibility of using cost-effective and abundant agricultural waste as a promising sustainable ingredient with potential prebiotic activity, via eco-friendly production methods that do not require chemical or enzymatic extraction.

Keywords: agricultural waste; lactobacilli; biochemistry characterization; fermentation

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1. Introduction

Agricultural production generates a massive quantity of by-products, presenting both an environmental challenge and an opportunity. A pivotal strategy to address this is the valorization of these residues through fermentation. Using readily available and inexpensive materials like sugarcane bagasse and banana crop waste as substrates for microbial fermentation, particularly with lactic acid bacteria (LAB), transform waste into valuable biochemical products. This process not only reduces environmental pollution but also drives the circular bioeconomy, as recent studies confirm its potential for cost-effective and eco-friendly metabolite production [1–3]. Additionally, it also entails the innovation of novel food fermentation strategies. However, these methods address challenges such as the presence of pentose sugars in lignocellulosic biomass, which are not readily metabolized by conventional LAB strains [2]. Overcoming challenges related to substrate optimization and the fine-tuning of fermentation parameters remain essential for the successful implementation of such processes.

Banana production holds significant importance in global agriculture, with Brazil ranking as the fourth-largest producer worldwide. In 2022, according to the Food and Agriculture Organization (FAO) [4], banana (*Musa* spp.) ranks among the most economically significant crops globally. Global banana production is expected to increase at an annual growth rate of approximately 1.5%, reaching an estimated 135 million metric tons by the year 2028. However, banana cultivation generates substantial amounts of agricultural waste. Post-harvest, approximately 60% of the banana plant's biomass, including banana pseudostem (BPS), leaves, and peels, remain as residue. It is estimated that every 1 ton of banana produced generates about 3 tons of pseudostem [5]. On the other hand, BPS constitutes a promising matrix for the cultivation of LAB, owing to its complex composition rich in fermentable carbohydrates (such as starch), structural polysaccharides (including cellulose, hemicellulose, and pectin), minerals (e.g., potassium, sodium, calcium, magnesium, and phosphorus), and various bioactive compounds. This substrate holds potential for the generation of value-added products, particularly as nutritional inputs for both human consumption and animal feed [6].

Since the middle of the 20th century, BPS starch has been investigated as a promising starch alternative source due to the presence of considerable quantities of starch. To develop healthier and more sustainable food products, Ho et al. [6] incorporated BPS flour into bread to assess its starch digestive behavior. Their findings reveal that the resulting composite breads are rich in minerals, total sugars, and resistant starch. The functional properties were significantly improved, with the breads exhibiting a slower starch hydrolysis rate, which led to a lower hydrolysis and estimated glycemic index. This study not only addresses agricultural waste but also aligns with the One Health Global initiative by creating novel, healthier ingredients and food products.

Ghutke et al. [7] conducted a study to assess an efficient method for decomposing BPS waste and evaluating the nutrient composition in the decomposed material. The shorter duration for compost preparation (83.27 days) resulted in higher total iron (7.73 mg/kg), manganese (5.72 mg/kg) content, total nitrogen, phosphorus, and potassium content in BPS compost (2.57, 0.49, and 3.28%, respectively). These findings underscore the BPS's dual role in both fermentation processes and as a source of health-promoting compounds.

Recent studies have highlighted the BPS potential in biotechnological applications, particularly in the production of lactic acid and prebiotic compounds. For defining a product or ingredient as prebiotic, the product needs to meet certain criteria: prebiotic compounds are required to withstand gastrointestinal digestion, undergo fermentation by the gut microbiota, and selectively promote the growth and/or metabolic activity of beneficial intestinal microorganisms linked to host health and well-being [8].

The key criterion is that a prebiotic compound must not be extensively metabolized or absorbed in the small intestine of healthy individuals but rather undergo microbial fermentation that favors the proliferation of health-promoting microorganisms within the native gut microbiota [9]. Prebiotics are non-digestible food ingredients, including fructans, galacto-oligosaccharides, xylo-oligosaccharides (XOS), chito-oligosaccharides, lactulose, resistant starch, and polyphenols. Resistant starch (RS) refers to all starch and starch derivatives that are not absorbed in the small intestine and travel to the colon, where it is fermented by the gut microbiota. While many gut microbiota express α-amylases that can break down soluble starch, only a small number of gut microbiota can break down resistant starch, which is insoluble and highly resistant to digestion [9]. Moreover, the metabolic utilization of prebiotic substrates depends on the presence of specific bacterial taxa within the host's gut microbiota that can ferment these compounds. Strains belonging to LAB, such as lactobacilli and enterococci, as well as *Bifidobacterium* spp., *Saccharomyces boulardii*, and certain non-pathogenic strains of *Escherichia coli*, have a well-established history of safe use and are among the most employed microorganisms with probiotic functionality. Recently, strains belonging to the new genera from the family Lactobacillaceae, such as *Lactiplantibacillus*, *Levilactobacillus*, *Ligilactobacillus*, and *Limosilactobacillus*, were recognized as probiotic cultures [10]. "Probiotic is live microorganisms which when administered in adequate amounts confer a health benefit on the host" [11]. The probiotic effects are essentially direct since they modify the composition of the gut microbiota and, consequently, they influence both intestinal and body functions [12]. These microorganisms promote or support a beneficial balance of the autochthonous microbial population in the gastrointestinal tract (GIT) [13].

In the colon, prebiotic and other food ingredients are fermented by probiotic saccharolytic bacteria using a range of enzymes to produce beneficial metabolites, including vitamins and short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. Organic acids, such as lactic acid, are byproducts of carbohydrate fermentation and play a key role in maintaining the host's functional balance, as well as in protecting against foodborne and spoilage pathogens.

These SCFAs have been associated with essential beneficial properties in the colon and intestinal immune responses. They elicit systemic effects, including the reduction in blood lipid levels, attenuation of postprandial glycemic response, modulation of neutrophil chemotaxis and phagocytic activity, anti-inflammatory effects, and regulation of apoptosis and immune responses. They are essential for maintaining the integrity of the intestinal barrier, providing energy for colonocytes, and contributing to intestinal eubiosis, which translates into improvements in gastrointestinal function and overall metabolic health [14,15]. The gut–brain axis, intrinsically linked to the intestinal microbiota, is crucial for maintaining physiological balance [16]. Recent evidence suggests that prebiotic and probiotic interventions can mitigate symptoms of anxiety and depression, a promising area for unconventional preventive and therapeutic strategies. The key mechanisms involved are the attenuation of inflammation and increased serotonin availability [17].

Considering that prebiotics can be metabolized and then serve as a source of SCFAs in the body, the demand for prebiotic sources has been increasing considerably, and research in this field has been intensified [18–20]. Recently, the BPS has been explored and well documented to produce XOS, which serves as prebiotics to promote the growth of beneficial gut microbiota. The study evaluated different treatments of BPS for XOS production and assessed their efficacy in supporting probiotic bacterial growth, indicating the BPS's versatility as a functional food ingredient [1].

This study aimed to produce and characterize the BPS by-product and evaluate its potential prebiotic effect on probiotic microorganisms. We also assessed the probiotic strains' ability to produce enzymes and assimilate carbohydrates. Strains demonstrating the highest bacterial growth (biomass and CFU/mL) with specific carbon sources were selected for further analysis of their resistance to gastrointestinal digestion.

2. Materials and Methods

2.1. Obtaining the BPS By-Product

BPSs (*Musa* spp.) were obtained from two local farms in the north-west of São Paulo State (SP), Brazil. *Nanica* cultivar BPSs (*Musa acuminata* Cavendish Subgroup, NBPS) were harvested immediately post-harvest of banana at a rural property in Santa Fé do Sul, SP, Brazil, at the geographic coordinates 20°15′ south latitude and 50°57′ west longitude. *Maçã* cultivar BPSs (*Musa acuminata* × *M. balbisiana*, AAB Group, 'Silk', MBPS) were also harvested immediately post-harvest of banana at rural property in Santa Rita D'Oeste, SP, Brazil, latitude 20°10′ S and longitude 50°46′ W. Before transportation, the BPS of both cultivars had 30 cm of edges (bottom and top) discarded, and the middle portion was used for flour preparation and starch extraction.

2.2. Preparation of BPS Flour

The harvested BPS samples were initially washed with potable water and subsequently rinsed with deionized water. Thereafter, the outer two epidermal layers of the BPS were carefully removed, and the underlying tissues were collected for further processing. These layers were further cut into small pieces and dried in a ventilated oven (Marconi, Piracicaba, SP, Brazil) at 50 °C for 40 h. The samples were then ground into flour and sieved by passing the material through a 0.210 mm mesh sieve (Granutest, São Paulo, SP, Brazil).

2.3. Characterization of the BPS Flour

2.3.1. Determination of Total Starch and Resistant Starch in the BPS Flour

The total starch content of the BPS flour was measured using a Total Starch Assay Kit (AA/AMG) (K-TSTA-100A) (Megazyme International Ltd., Wicklow, Ireland). The resistant starch of the BPS flour was measured using the 'Resistant starch AOAC Method 2002.02/AACC Method 32-40.01′ kit from Megazyme International Ltd. (K-RSTAR) (Wicklow, Ireland) following the protocol recommended by the manufacturer for samples of D-glucose and/or maltodextrins.

2.3.2. Analyses of Lignin and Carbohydrates in the BPS Flour

For lignin determination, the dried BPS flour samples (0.3 g) were shaken in 3.0 mL of sulphuric acid 72% (150 rpm; 30 °C; 30 min)and, subsequently, 84 mL of distilled water was added to the flask, the sample was then autoclaved for 1 h (121 °C) and filtrated on paper (Whatman, Little Chalfont, UK). Soluble lignin from this filtrate was evaluated directly at $\lambda = 240$ nm, using gallic acid as the standard. Hydrolyzed cellulose and hemicellulose were quantified by the amount of sugar content released (ionic chromatograph). The material retained on the filter paper was dried at 105 °C for dry weight determination and burned (500 °C; 4 h) for insoluble lignin quantification. These data were used to calculate the amount of insoluble lignin [21]. Cellulose and hemicellulose were quantified using an ICS 5000 Dionex HPAEC-PAD ionic chromatograph (Thermo Fisher Scientific, Waltham, MA, USA), according to Moretti et al. [22].

2.3.3. Characterization of Bioactive Compounds and Antioxidant Activity of BPS Flour

The extraction of bioactive compounds was performed using an extraction solvent composed of 70% acetone and 30% water (v/v, 7:3). The mixture was vortexed for 1 min and subsequently subjected to ultrasonic treatment (135 W, 40 kHz) for 15 min. Following sonication, the samples were centrifuged (Beckman Coulter, Brea, CA, USA) at 1935× g for 20 min, and the supernatant was collected into a 50 mL flask. This extraction procedure was repeated until the sample was fully exhausted, with all steps conducted under light-protected conditions.

Following extraction, the total phenolic content was quantified using the Folin–Ciocalteu reagent according to the protocol described by Singleton et al. [23], and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample (mg GAE/100 g). The FRAP assay was conducted based on a modified method by Benzie & Strain [24], with outcomes expressed as micromoles of ferrous sulfate equivalents per gram of sample (µmol ferrous sulfate/g). The ABTS+ radical scavenging activity was assessed using the DPPH assay following Rufino et al. [25] and the FRAP method as described by Pulido et al. [26], with all measurements performed in triplicate. Both antioxidant capacities were reported as micromoles of Trolox equivalents (µM TE) per gram of sample.

2.4. Starch Extraction from BPS By-Product

Starch extraction from BPS was conducted following the methodology of Utrilla-Coello et al. [27], with slight modifications. The BPS was cut into cubes and immediately immersed in water at a 1:2 (*w*/*v*) ratio, then macerated at low speed using a blender (Bermar BM31NR, São José do Rio Preto, SP, Brazil) for 2 min. The resulting homogenate was filtered through sieves with pore sizes of 0.59 mm and 0.44 mm until the wash water became clear; this grinding and sieving process was repeated twice. The filtrate was allowed to settle overnight at 5 °C for sedimentation, after which the supernatant was discarded. The sediment was then resuspended in distilled water, passed through a 0.210 mm mesh sieve, and centrifuged at 10,000× *g* for 10 min at 25 °C. The supernatant was again discarded, and the precipitate resuspended in distilled water. The extracted starch was then vacuum filtered and dried at 45 °C for 16 h. The dried starch was sieved through a 0.210 mm mesh and stored at 4 °C. The total starch and resistant starch were obtained as described in Section 2.3.1.

2.5. Microbiological Analysis

2.5.1. Strains

The probiotic strain *Lacticaseibacillus rhamnosus* GG was used as positive control in this research. In the research strategy, eight (8) microbial cultures: *Lactobacillus* (*Lab.*) *delbrueckii* subsp. *bulgaricus* SJRP49, SJRP57, and SJRP149, *Lacticaseibacillus* (*Lbs.*) *casei* SJRP145, SJRP146, and SJRP169, and *Limosilactobacillus* (*Lmb.*) *fermentum* SJRP30 and SJRP43, isolated from mozzarella cheese, previously characterized as potential probiotics [28,29], were evaluated and selected based on their potential for application. These strains belong to the São Paulo State University (UNESP) Culture Collection (CCLAB-UNESP, WDCM 1182), Brazil.

2.5.2. Pre-Inoculum Preparation

All the cultures were reactivated by transferring 0.1 mL of stock culture (kept at −80 °C in the presence of 20% glycerol, as a cryoprotector) to 10 mL of sterilized MRS broth (Difco Laboratories, Detroit, MI, USA) (autoclaved at 121 °C for 15 min). The culture was then incubated at 37 °C for 12 h in anaerobic jars, generating an anoxic atmosphere (Probac, São Paulo, SP, Brazil). Afterwards, the pre-inoculum was prepared by adding 0.1 mL of the reactivated culture to 10 mL of MRS broth (Difco) and culturing it at 37 °C for 12 h in anaerobic conditions, as described previously.

2.5.3. Fermentation with Different Carbon Sources

A carbohydrate-free culture medium (CFCM) was prepared to investigate the effects of the BPS starch and BPS flour on in vitro bacterial growth. CFCM consisted of trypticase peptone (10.0 g/L, Difco), yeast extract (5.0 g/L, Difco), $C_6H_{14}N_2O_7$ (2.0 g/L, Sigma-Aldrich, Saint Louis, MO, USA), $C_2H_3NaO_2$ (5.0 g/L, Labsynth, Diadema, SP, Brazil),

K_2HPO_4 (2.0 g/L, Labsynth), $MgSO_4$ (0.05 g/L, Labsynth), $MnSO_4$ (0.025 g/L, Labsynth), and Tween-80 (1 mL/L, Labsynth) to prepare 1 L medium.

Evaluated sources of carbohydrates were glucose (GLU, Synth, São Paulo, SP, Brazil), gelatinized (G) and non-gelatinized (NG) starches, and BPS flours extracted from the cultivars MBPS and NBPS. Gelatinized samples were prepared using starch and suspended flour (10 g/L each individual carbohydrate in fresh CFCM). The mixture was autoclaved at 121 °C for 20 min and then cooled to room temperature (24 ± 3 °C). On the other hand, the non-gelatinized samples were prepared using starch and flour and autoclaved separately in a hermetically sealed vial to prevent sample gelatinization. After heating at 121 °C for 20 min., the samples were cooled to room temperature and added (10 g/L of each individual carbohydrate) to the sterilized CFCM. CFCM supplemented with GLU was used as a control carbohydrate source for bacterial growth studies. The carbohydrate sources that provided the greatest growth of the strains (bacterial counts in CFU/mL) were selected and used in subsequent tests.

2.5.4. Growth of Probiotic Microorganisms in CFCM

The pre-inoculum of each bacterial culture was grown in MRS broth (Difco) for 12 h at 37 °C. Cells were harvested by centrifugation at 5000× *g* for 5 min at 5 °C, washed twice with sterile saline solution (0.85% NaCl, *w*/*v*), and finally resuspended at a ratio of 1:2 (*v*/*v*) in the same culture medium. Active bacteria were transferred to CFCM with GLU, gelatinized (G) and non-gelatinized (NG) starches, and *Maçã* and *Nanica* cultivar BPS flours. The post-inoculated samples were incubated at 37 °C. LAB cultures were evaluated for carbohydrate-dependent growth using plate count analysis. For sampling purposes, aliquots of 0.1 mL were taken manually over a 24 h period at times 0, 6, 12, and 24 h post-inoculation and suspended in 0.9 mL of saline solution. Serial dilutions were prepared and inoculated into plates containing MRS (Difco) supplemented with agar (15.0 g/L). Probiotic microorganisms were cultured at 37 °C in anaerobic jars, generating an anoxic atmosphere (Probac). Bacterial counts of each treatment were carried out in duplicate.

2.6. LAB Carbohydrate Fermentation and Enzyme Activities Profiles

The ability to ferment a variety of carbon sources and enzyme activities were determined by the API 50 CHL commercial kit (BioMerieux, Marcy I'Etoile, France) and API ZYM biochemical kits (BioMerieux), according to the manufacturer's guidelines; the assays were performed at 37 °C for 48 h and for 2 h, respectively. The LAB cultures of *Lactobacillus* spp., *Lacticaseibacillus* spp., and *Limosilactobacillus* spp. were cultivated in standard MRS broth (Difco) under anaerobic conditions at 37 °C for 12 h prior to inoculation.

Results were scored from yellow to black for API 50 CHL and white to dark red for API ZYM, according to the reaction color shown on a scale chart. Yellow or white is considered a negative reaction (−), whereas black or dark red describes the highest intensity for a positive reaction (+, ++, +++, and ++++) for API 50 CHL and API ZYM, respectively, according to the manufacturer's manual. The results obtained from the experiments of proliferation of the LAB strains in different carbon sources and characterization of assimilation of carbohydrates and enzyme activities profiles were used as quality characteristics for the selection of the LAB culture for the next steps.

2.7. INFOGEST Static Model for Simulation of Gastrointestinal Digestion

In vitro digestion simulations of both BPS flours were performed according to the INFOGEST static protocol [30,31]. The experiments were performed using carbon sources that provided higher growth for each of the strains. Gelatinized MBPS flour was used as a carbon source in the cultivation of *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 and *Lbs. casei* SJRP145 strains, while non-gelatinized NBPS flour was used as a substrate for *Lmb. fermentum* SJRP43 and *Lbs. rhamnosus* GG. The samples were submitted to the digestion protocols of all digests (oral, gastric, and intestinal), which were also analyzed by bacterial counts.

BPS flour digestion was simulated in vitro according to the harmonized protocol proposed by Minekus et al. [30] and Brodkorb et al. [31] using all reagents from Sigma-Aldrich and a magnetic stirrer (C-MAG HS 7, IKA®, Staufen, Germany) connected to a contact thermometer (ETS-D5, IKA®, Staufen, Germany), which allows precise temperature control for incubation. Five milliliters of CFCM supplemented with BPS flour (according to each strain, either gelatinized *Maçã* BPS flour [GMF], or non-gelatinized *Nanica* BPS flour [NGNF]) containing the probiotic bacteria cultured overnight were homogenized with 4 mL of simulated salivary stock solution (15.1 mmol/L KCl, 3.7 mmol/L KH_2PO_4, 13.6 mmol/L $NaHCO_3$, 0.15 mmol/L $MgCl_2$, 0.06 mmol/L $(NH_4)_2CO_3$, 1.1 mmol/L HCl), 0.22 mL of 1 mol/L NaOH, 0.025 mL of 0.3 mol/L $CaCl_2$, and 0.755 mL of ultrapure water) totaling 10 mL. The mixtures were incubated for 2 min at 37 °C. Then, 7.5 mL of the simulated gastric stock solution (6.9 mmol/L KCl, 0.9 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 47.2 mmol/L NaCl, 0.12 mmol/L $MgCl_2$, 0.5 mmol/L $(NH_4)_2CO_3$, 15.6 mmol/L HCl), 1.6 mL of pepsin solution (P7000, 250 units/mg solid), 0.005 mL of 0.3 mol/L $CaCl_2$, 0.240 mL of 1 mol/L HCl, and 0.655 mL of ultrapure water) was added, totaling 20 mL. The pH was adjusted to 3.0, and the mixture was incubated at 37 °C for 2 h to simulate gastric digestion. Finally, 11 mL of the simulated intestinal stock solution (6.8 mmol/L KCl, 0.8 mmol/L KH_2PO_4, 85 mmol/L $NaHCO_3$, 38.4 mmol/L NaCl, 0.33 mmol/L $MgCl_2$, 8.4 mmol/L HCl), 5 mL of pancreatin solution (P1750, 1 g/L), 2.5 mL of bile solution (10 g/L), 0.04 mL of 0.3 mol/L $CaCl_2$, 0.55 mL of 1 mol/L NaOH, and 0.91 mL of ultrapure water) was added to the gastric chyme, totaling 40 mL. The pH was adjusted to 7.0, and the mixture was incubated at 37 °C for 2 h to simulate intestinal digestion. The experiment was conducted in duplicate. Before and after the incubation time of each phase (oral, gastric, and intestinal), one aliquot was immediately collected, and the serial dilutions were prepared and inoculated into plates containing MRS medium added with agar for bacterial counts.

2.8. Statistical Analysis

The results were submitted to a one-way analysis of variance followed by the Tukey test at $p < 0.05$ to compare means. All the analyses were considered significant at the 0.05 level, and they were carried out using Minitab Statistical Software, version 17 (Minitab LLC, State College, PA, USA).

3. Results and Discussion

3.1. Characterization of BPS By-Products

In both MBPS and NBPS starch samples, the resistant starch content was approximately 50% of the identified starch content (Table 1). The starch content present in the BPS flour samples showed significant differences between the MBPS and NBPS flour samples (39.7% and 8.1% [on a dry basis -db], respectively). Moreover, the MBPS and NBPS flour samples presented cellulose (27.0 and 52.4%) and hemicellulose (25.4 and 33.8%), respectively. However, no sample presented lignin content.

Phenolic compounds were quantified, and the values obtained from NBPS (193.6 GAE/100 g) flour samples were higher compared to MBPS flour (153.5 GAE/100 g) (Table 1). The antioxidant activity of BPS flour samples was determined using FRAP, DPPH, and ABTS assays. Higher values for ABTS (145.0 µM Trolox/g) and DPPH (102.8 µM Trolox/g) were observed for the NBPS and MBPS flour samples, respectively. The FRAP results were quite similar among the samples (Table 1).

Table 1. Carbohydrate composition, bioactive compounds, and antioxidant activity of the BPS by-products.

Analysis	**Flour Samples**		**Starch Samples**	
Carbohydrate composition	MBPS	NBPS	MBPS	NBPS
Starch (db-%)	39.7 ± 1.1 ^{a}	8.1 ± 1.4 ^{b}	98.0 ± 0.2 ^{a}	92.1 ± 0.6 ^{b}
Resistant starch (db-%)	16.7 ± 0.1 ^{a}	2.7 ± 0.0 ^{b}	49.3 ± 0.7 ^{a}	40.6 ± 3.0 ^{b}
Cellulose (db-%)	27.0 ± 1.2 ^{b}	52.4 ± 0.2 ^{a}	nd	nd
Hemicellulose * (db-%)	25.4 ± 0.4 ^{b}	33.8 ± 0.5 ^{a}	nd	nd
Insoluble lignin (%)	nd	nd	nd	nd
Soluble lignin (%)	nd	nd	nd	nd
Phenolic content (GAE/100 g)	153.5 ± 0.1 ^{b}	193.6 ± 0.1 ^{a}	nd	nd
Antioxidant activity				
ABTS (µmol Trolox/g)	145.0 ± 0.2 ^{a}	50.7 ± 0.1 ^{b}	nd	nd
DPPH (µmol Trolox/g)	72.5 ± 0.1 ^{b}	102.8 ± 0.4 ^{a}	nd	nd
FRAP (µmol sulfato ferroso/g)	14.9 ± 0.0 ^{a}	12.1 ± 0.1 ^{b}	nd	nd

MBPS, *Maçã* cultivar banana pseudostem; NBPS, *Nanica* cultivar banana pseudostem. * data obtained from the sum of xylose and arabinose contents. nd = not detected. Statistical analysis was performed for flour and starch samples separately. Different lowercase letters in the columns denote differences ($p < 0.05$) between flour and starch samples.

3.2. Effects of Prebiotics on LAB Count

At the onset of fermentation (0 h), all inoculated samples exhibited LAB populations ranging from 6 log to 8 log CFU/mL (Figure 1). Following 12 and 24 h of cultivation, the probiotic strains demonstrated robust proliferation, achieving bacterial densities exceeding 8 log CFU/mL in fermentation media supplemented with BPS by-products and glucose. Counts of the *Lbs. rhamnosus* GG showed an increase of approximately 4.7 log CFU/mL in medium with NGNF BPS by-product after 24 h of cultivation compared to the counts found at time zero. Likewise, *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 and *Lmb. fermentum* SJRP43 showed an increase of approximately 2.7 log CFU/mL after 24 h of cultivation in media with GMF and NGNF BPS by-product, respectively. *Lbs. casei* SJRP145 showed an increase of 1.8 log CFU/mL in the medium with the GMF BPS by-product after 12 h of cultivation. The reason for this increase in both strains is likely due to their metabolic capacities (enzyme production and carbohydrate utilization), as presented in the next sections.

3.3. Enzyme Production Assay Using API ZYM Kit

Lbs. rhamnosus GG (the reference strain) and the tested strains, *Lab. delbrueckii* subsp. *bulgaricus* SJRP149, *Lbs. casei* SJRP145, SJRP146, and SJRP169, and *Lmb. fermentum* SJRP30 and SJRP43, presented enzymatic activity for almost all enzymes (Figure 2). Esterases (EC 3.1.1.x) constitute a heterogeneous class of hydrolases, distinguished by their capacity to catalyze both the hydrolysis and synthesis of ester linkages [32]. Carboxylesterases, for example, can release ferulic acid from plant cell wall polysaccharides, including but not limited to pectin or xylan [32].

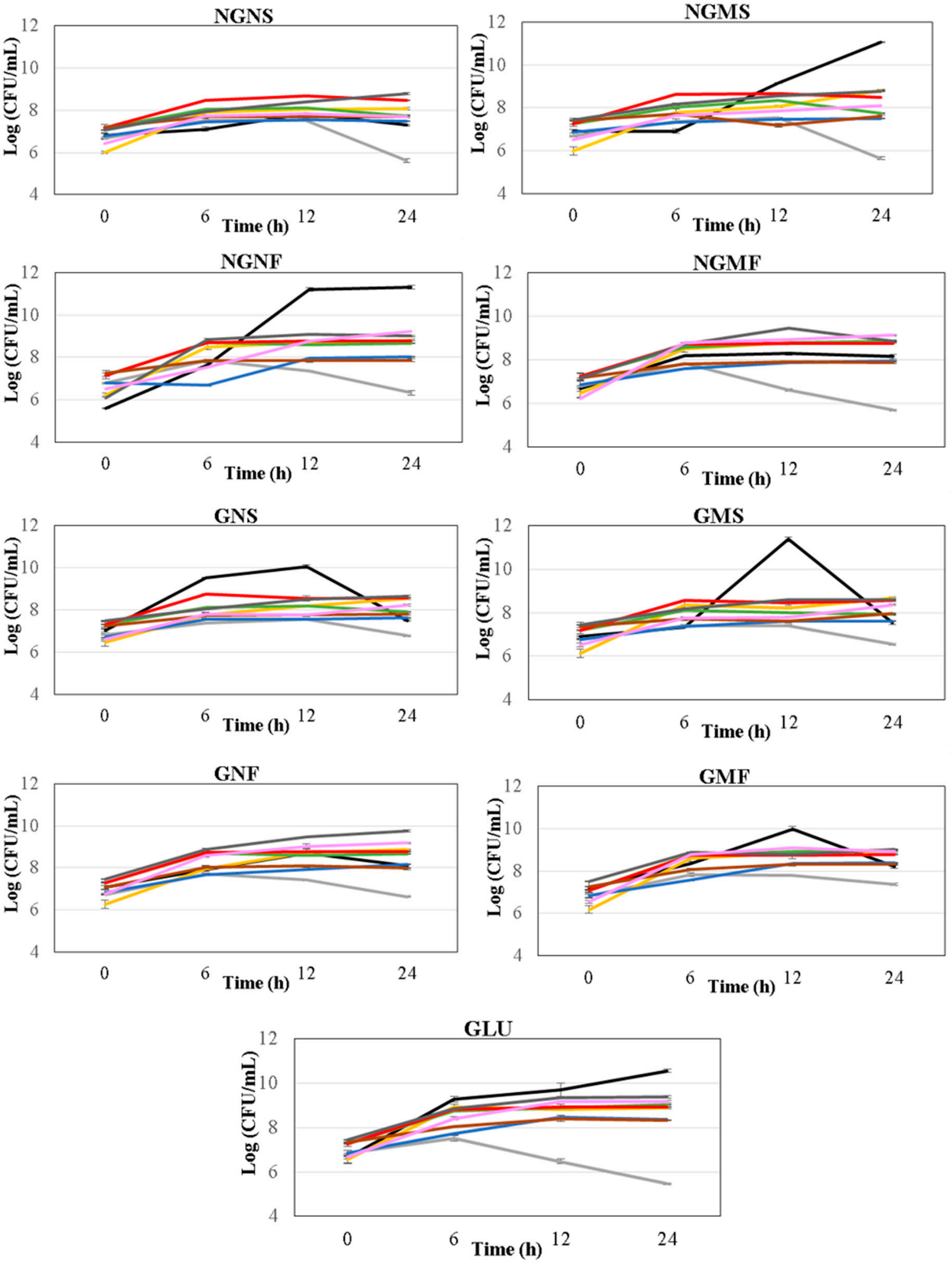
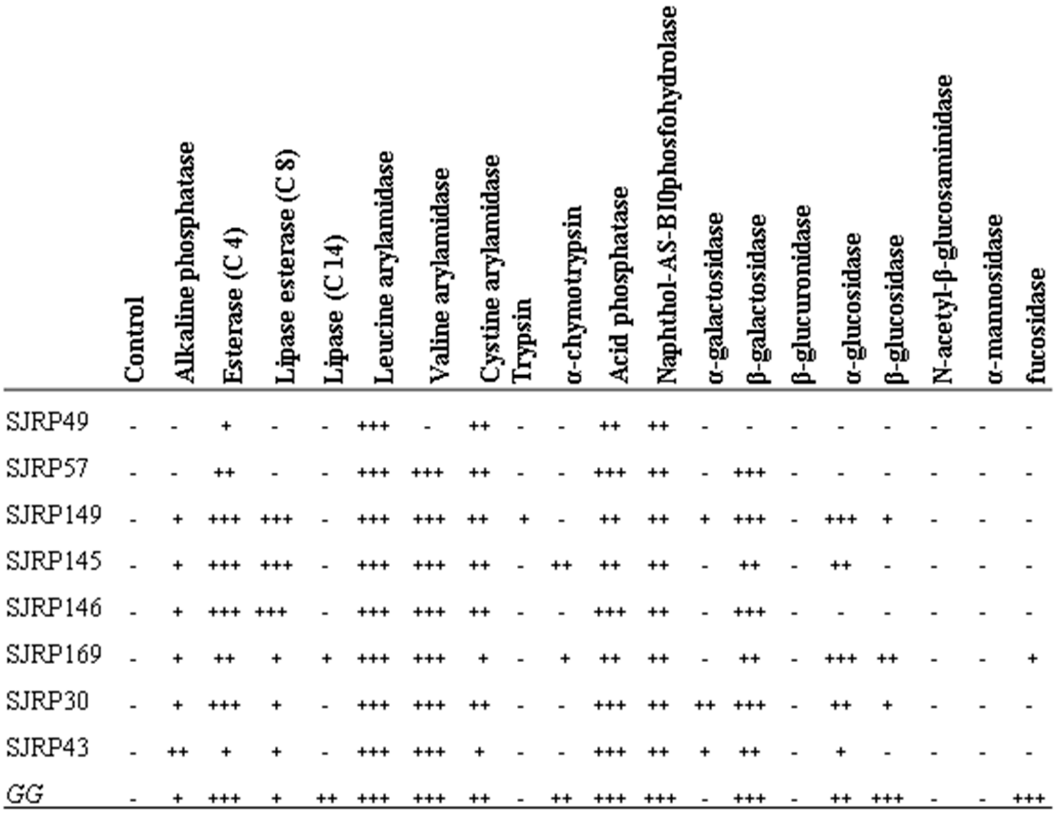


Figure 1. Effect of the supplementation with 1% of BPS by-product on the in vitro bacterial growth. *Lab. delbrueckii* subsp. *bulgaricus* SJRP49, SJRP57, and SJRP149, *Lbs. casei* SJRP145, SJRP146, and SJRP169, *Lmb. fermentum* SJRP30 and SJRP43, and *Lbs. rhamnosus* GG. Carbohydrate sources extracted from the two cultivars of BPS: Non-Gelatinized (NG) and Gelatinized (G), *Nanica* (N) and *Maçã* (M) cultivars; glucose (GLU). ▬ = GG; ▬ = SJRP49; ▬ = SJRP57; ▬ = SJRP149; ▬ = SJRP145; = ▬ SJRP146; ▬ = SJRP169; ▬ = SJRP30; ▬ = SJPR43.



	Control	Alkaline phosphatase	Esterase (C 4)	Lipase esterase (C 8)	Lipase (C 14)	Leucine arylamidase	Valine arylamidase	Cystine arylamidase	Trypsin	α-chymotrypsin	Acid phosphatase	Naphthol-AS-BI0phosfohydrolase	α-galactosidase	β-galactosidase	β-glucuronidase	α-glucosidase	β-glucosidase	N-acetyl-β-glucosaminidase	α-mannosidase	fucosidase
SJRP49	-	-	+	-	-	+++	-	++	-	-	++	++	-	-	-	-	-	-	-	-
SJRP57	-	-	++	-	-	+++	+++	++	-	-	+++	++	-	+++	-	-	-	-	-	-
SJRP149	-	+	+++	+++	-	+++	+++	++	+	-	++	++	+	+++	-	+++	+	-	-	-
SJRP145	-	+	+++	+++	-	+++	+++	++	-	++	++	++	-	++	-	++	-	-	-	-
SJRP146	-	+	+++	+++	-	+++	+++	++	-	-	+++	++	-	+++	-	-	-	-	-	-
SJRP169	-	+	++	+	+	+++	+++	+	-	+	++	++	-	++	-	+++	++	-	-	+
SJRP30	-	+	+++	+	-	+++	+++	++	-	-	+++	++	++	+++	-	++	+	-	-	-
SJRP43	-	++	+	+	-	+++	+++	+	-	-	+++	++	+	++	-	+	-	-	-	-
GG	-	+	+++	+	++	+++	+++	++	-	++	+++	+++	-	+++	-	++	+++	-	-	+++

Figure 2. Analysis of enzyme activities by LAB cultures using API ZYM method. *Lab. delbrueckii* subsp. *bulgaricus* SJRP49, SJRP57, and SJRP149, *Lbs. casei* SJRP145, SJRP146, and SJRP169, *Lmb. fermentum* SJRP30 and SJRP43, and *Lbs. rhamnosus* GG. (−) indicates a negative reaction, (+) indicates a low positive reaction, (++) indicates an intermediate reaction, and (+++) indicates a high reaction in the API ZYM panel, according to the manufacturer's manual.

As shown in Table 1, BPS fiber contains more than 25% hemicellulose, that is, a complex polysaccharide comprising a backbone of xylose residues, which may be covalently linked to ferulic acid through an ester linkage [33]. In the human colon, the polysaccharide in BPS fiber can be degraded into ferulic acid by microorganisms, such as LAB and other microorganisms. The ferulic acid can be further degraded into propionic acid derivatives that can interact with intestinal epithelium and gut microbiota, exhibiting a protective effect on the gut, providing beneficial activities in human health. These effects include enhancing antioxidant and anti-cancer activities, cardiovascular disease, and diabetes prevention activity [34–36]. A predominant proportion of LAB cultures exhibited both leucine and valine arylamidase activities. These enzymatic functionalities are considered advantageous attributes for probiotic strains, as they play a critical role in the adaptive physiological responses of the bacterium to its environmental milieu. These enzymes may participate in the intracellular protein and peptide catabolism (i.e., protein turnover) as well as the hydrolysis of endogenous proteins [37]. When applied in the dairy industries as a probiotic, the proteolytic system of LAB provides amino acids for the development of flavor and aroma, and/or production of bioactive compounds in dairy products [38].

These findings hold potential utility for industries involved in fermented food product development. Beyond proteolytic capabilities, the selection of probiotic microorganisms necessitates the fulfillment of additional criteria, including antimicrobial efficacy, antibiotic susceptibility profiles, auto-aggregation capacity, cell surface hydrophobicity, and in vitro antioxidant activity. Furthermore, candidate strains must demonstrate robust resistance to acidic environments (low pH), as well as simulated gastric juice and bile salts [39]. Previous studies conducted with LAB cultures (*Lbs. casei* SJRP145, SJRP146, and SJRP169, *Lmb. fermentum* SJRP30 [29], *Lmb. fermentum* SJRP43 [40], and *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 [28]) presented promising properties and/or superior probiotic characteristics (such as the aforementioned) compared to the reference strain *Lbs. rhamnosus* GG.

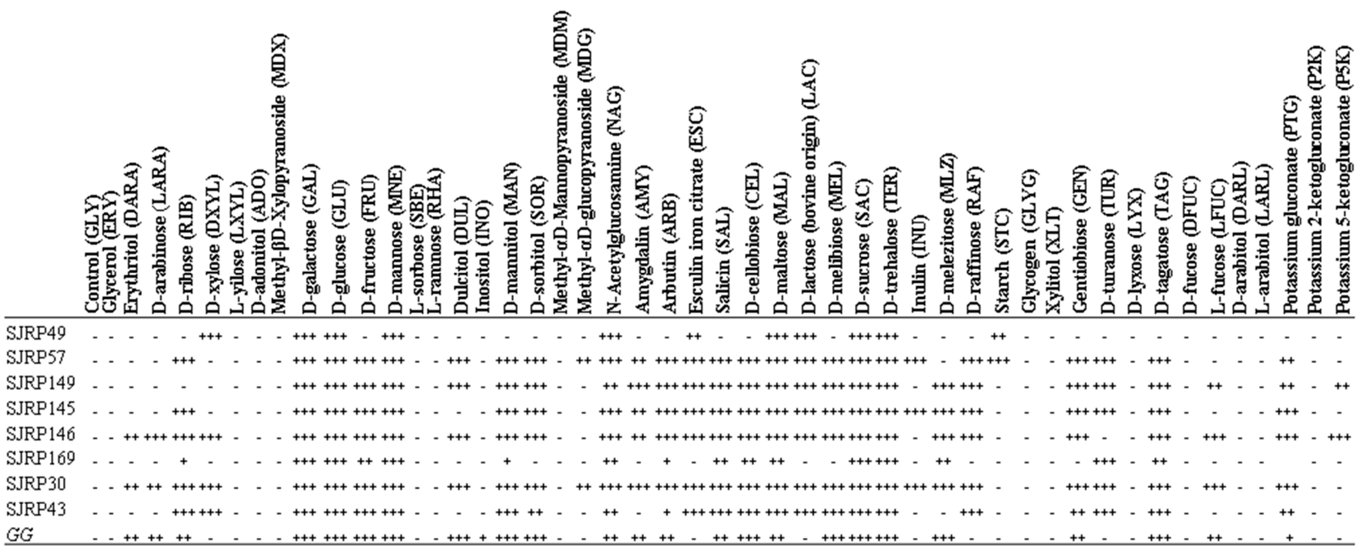
All LAB cultures presented acid phosphatase activity, as well as the control strain, *Lbs. rhamnosus* GG (Figure 2). This enzyme has been reported as significant in improving calcium and phosphorus absorption [41]. According to Pillai et al. [42] and Kumari et al. [43], calcium and phosphorus are among the most abundant minerals present in BPS. Also, all cultures showed an expressive increase in CFU during the fermentation. Considering the enzyme β-galactosidase, four cultures (*Lab. delbrueckii* subsp. *bulgaricus* SJRP57 and SJRP149, *Lbs. casei* SJRP146, and *Lmb. fermentum* SJRP30) exhibited high activities. β-galactosidase-producing bacteria help in lactose digestion and may potentially reduce lactose intolerance, thus aiding lactose-intolerant people in digesting dairy products [40].

In contrast to the reference strain, *Lbs. rhamnosus* GG, *Lab. delbrueckii* subsp. *bulgaricus* SJRP149, and *Lbs. casei* SJRP169 presented high activity of α-glucosidase. This enzyme holds significant importance due to its potential to enhance the fermentation of resistant starch, thereby promoting butyrate synthesis. This, in turn, may lead to improvements in bowel regularity and an increase in fecal bulk [44]. Other desirable enzymes for probiotic bacteria [45], such as Naphthol-AS-BI-phosphohydrolase, β-glucosidase, and fucosidase, were investigated, and the cultures had moderate to low activity for these enzymes, except for *Lbs. rhamnosus* GG, which presented high activity. Conversely, the detection of deleterious enzymes, such as β-glucuronidase, constitutes significant consideration during the in vivo safety assessment of probiotic strains. This enzyme possesses the enzymatic capacity to hydrolyze glucuronide conjugates, thereby releasing potentially harmful carcinogenic metabolites that can subsequently be reabsorbed in the colon and liver [46]. None of the cultures produced β-glucuronidase; hence, they are potentially safe to human health (Figure 2).

3.4. Carbohydrate Utilization Assay Using API 50 CHL Kit

All tested LAB cultures presented positive features for fermentation of D-galactose, D-glucose, D-manose, D-sucrose, and D-trealose. Moreover, approximately 70 to 80% of the tested cultures could ferment D-fructose, D-mannitol, D-sorbitol, esculin iron citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melitrose, D-turanose, and D-tagatose (Figure 3). Among the evaluated LAB cultures, *Lab. delbrueckii* subsp. *bulgaricus* SJRP49, *Lbs. casei* SJRP146, *Lmb. fermentum* SJRP30, and *Lmb. fermentum* SJRP43 were positive for the fermentation of D-xylose. It is important to underline that BPS flour presents significant hemicellulose content (Table 1). Hemicellulose is a heterogeneous polymer composed of different monosaccharides, such as xylose, arabinose, mannose, and galactose, which are pentose and hexose carbohydrates. The hydrolysis of the backbone of hemicellulose produces pentose (xylose and arabinose) and hexose (galactose, mannose, and glucose) sugars [47].

In the present study, only two LAB cultures were able to assimilate starch: *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 with a higher intensity, followed by *Lab. delbrueckii* subsp. *bulgaricus* SJRP49. However, *Lab. delbrueckii* subsp. *bulgaricus* SJRP149 could not do so, which reinforces the idea that probiotics and other beneficial properties are clearly strain-specific. The increase in the CFU among all by-products tested in the cultivation of *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 was not significantly different, likely because all substrates used as prebiotics present starch content (Figure 1). The LAB cultures that presented the largest capacity of carbohydrate utilization were *Lmb. fermentum* SJRP30 (28 carbohydrates), followed by *Lbs. casei* SJRP146 (27 carbohydrates).



	Control (GLY)	Glycerol (ERY)	Erythritol (DARA)	D-arabinose (LARA)	D-ribose (RIB)	D-xylose (DXYL)	L-yilose (LXYL)	D-adonitol (ADO)	Methyl-βD-Xylopyranoside (MDX)	D-galactose (GAL)	D-glucose (GLU)	D-fructose (FRU)	D-mannose (MNE)	L-sorbose (SBE)	L-ramnose (RHA)	Dulcitol (DUL)	Inositol (INO)	D-mannitol (MAN)	D-sorbitol (SOR)	Methyl-αD-Mannopyranoside (MDM)	Methyl-αD-glucopyranoside (MDG)	N-Acetylglucosamine (NAG)	Amygdalin (AMY)	Arbutin (ARB)	Esculin iron citrate (ESC)	Salicin (SAL)	D-cellobiose (CEL)	D-maltose (MAL)	D-lactose (bovine origin) (LAC)	D-melibiose (MEL)	D-sucrose (SAC)	D-trehalose (TER)	Inulin (INU)	D-melezitose (MLZ)	D-raffinose (RAF)	Starch (STC)	Glycogen (GLYG)	Xylitol (XLT)	Gentiobiose (GEN)	D-turanose (TUR)	D-lyxose (LYX)	D-tagatose (TAG)	D-fucose (DFUC)	L-fucose (LFUC)	D-arabitol (DARL)	L-arabitol (LARL)	Potassium gluconate (PTG)	Potassium 2-ketogluconate (P2K)	Potassium 5-ketogluconate (P5K)
SJRP49	-	-	-	-	-	+++	-	-	-	+++	+++	-	+++	-	-	-	-	-	-	-	-	+++	-	-	++	-	-	+++	+++	-	+++	+++	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-
SJRP57	-	-	-	-	+++	-	-	-	-	+++	+++	+++	+++	-	-	+++	-	+++	+++	-	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	+++	-	-	+++	+++	-	+++	-	-	-	-	++	-	-
SJRP149	-	-	-	-	-	-	-	-	-	+++	+++	+++	+++	-	-	+++	-	+++	+++	-	-	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	+++	-	-	-	+++	+++	-	+++	-	++	-	-	++	-	++
SJRP145	-	-	-	-	+++	-	-	-	-	+++	+++	+++	+++	-	-	-	-	+++	+++	-	-	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	+++	+++	-	+++	-	-	-	-	+++	-	-
SJRP146	-	-	++	+++	+++	+++	-	-	-	+++	+++	+++	+++	-	-	+++	-	+++	+++	-	-	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	+++	-	-	-	+++	-	-	+++	-	+++	-	-	+++	-	+++
SJRP169	-	-	-	-	+	-	-	-	-	+++	+++	++	+++	-	-	-	-	+	-	-	-	++	-	+	-	++	++	++	-	-	+++	+++	-	++	-	-	-	-	-	+++	-	++	-	-	-	-		-	-
SJRP30	-	-	++	++	+++	+++	-	-	-	+++	+++	+++	+++	-	-	+++	-	+++	+++	-	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	+++	+++	-	+++	-	+++	-	-	+++	-	-
SJRP43	-	-	-	-	+++	+++	-	-	-	+++	+++	+++	+++	-	-	-	-	+++	++	-	-	++	-	+	+++	+++	+++	+++	+++	+++	+++	+++	-	-	+++	-	-	-	++	+++	-	+++	-	-	-	-	++	-	-
GG	-	-	++	++	++	-	-	-	-	+++	+++	+++	+++	-	-	+++	+	+++	+++	-	-	++	++	++	-	++	+++	++	-	+++	+++	+++	-	+++	-	-	-	-	++	-	-	+++	-	++	-	-	+	-	-

Figure 3. Analysis of carbohydrate utilization by LAB cultures using API 50 CHL method. *Lab. delbrueckii* subsp. *bulgaricus* SJRP49, SJRP57 and SJRP149, *Lbs. casei* SJRP145, SJRP146, and SJRP169, *Lmb. fermentum* SJRP30 and SJRP43, and *Lbs. rhamnosus* GG. (−) indicates a negative reaction, (+) indicates a low positive reaction, (++) indicates an intermediate reaction, and (+++) indicates a high reaction in the API 50 CHL.

3.5. In Vitro Simulation of Gastrointestinal Digestion

During the oral phase, there was a significant difference in viability (CFU/mL) among the strains compared to the initial phase (Figure 4). There was a decrease of 1.7% in the viability of *Lmb. fermentum* SJRP43 after oral digest, while the viability of *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 and *Lbs. casei* SJRP145 was not affected after the oral phase. On the other hand, the viability of *Lbs. rhamnosus* GG increased 1.3% after the oral phase. This stage of digestion entails mechanical fragmentation, a process accomplished through mastication [30]. However, the prevailing conditions within the oral cavity, such as pH, are notably mild.

There was no significant difference in the CFU counts before and after the gastric and intestinal phase of digestion among the tested LAB cultures (Figure 4). The gastric phase of digestion is defined by the mechanical and enzymatic breakdown of the ingested bolus. During this digestive stage, a significant reduction in pH is observed, alongside the action of pepsin, which remains the sole proteolytic enzyme active within the stomach [30]. The intestinal digestion is marked by the pH adjustment after the addition of the pancreatic juice, consisting of simulated intestinal stock solution, bile, and pancreatin [48].

In general, when a probiotic culture is exposed to the challenging conditions of the gastrointestinal tract and the diverse resident microbiota, probiotic cultures face continuous selective pressures. Consequently, probiotic cells can exist in various physiological states: viable (active and cultivable), dormant (inactive but cultivable), viable but non-cultivable (active but not cultivable), or non-viable (inactive and non-cultivable) [49]. In this study, BPS flour promoted probiotic bacteria protection, maintaining their viable cells after challenging conditions, which is considered an important feature for the development of symbiotic products. Additionally, *Lab. delbrueckii* subsp. *bulgaricus* SJRP57, *Lbs. casei* SJRP145, and *Lmb. fermentum* SJRP43 presented the same performance as the commercial strain *Lbs. rhamnosus* GG.

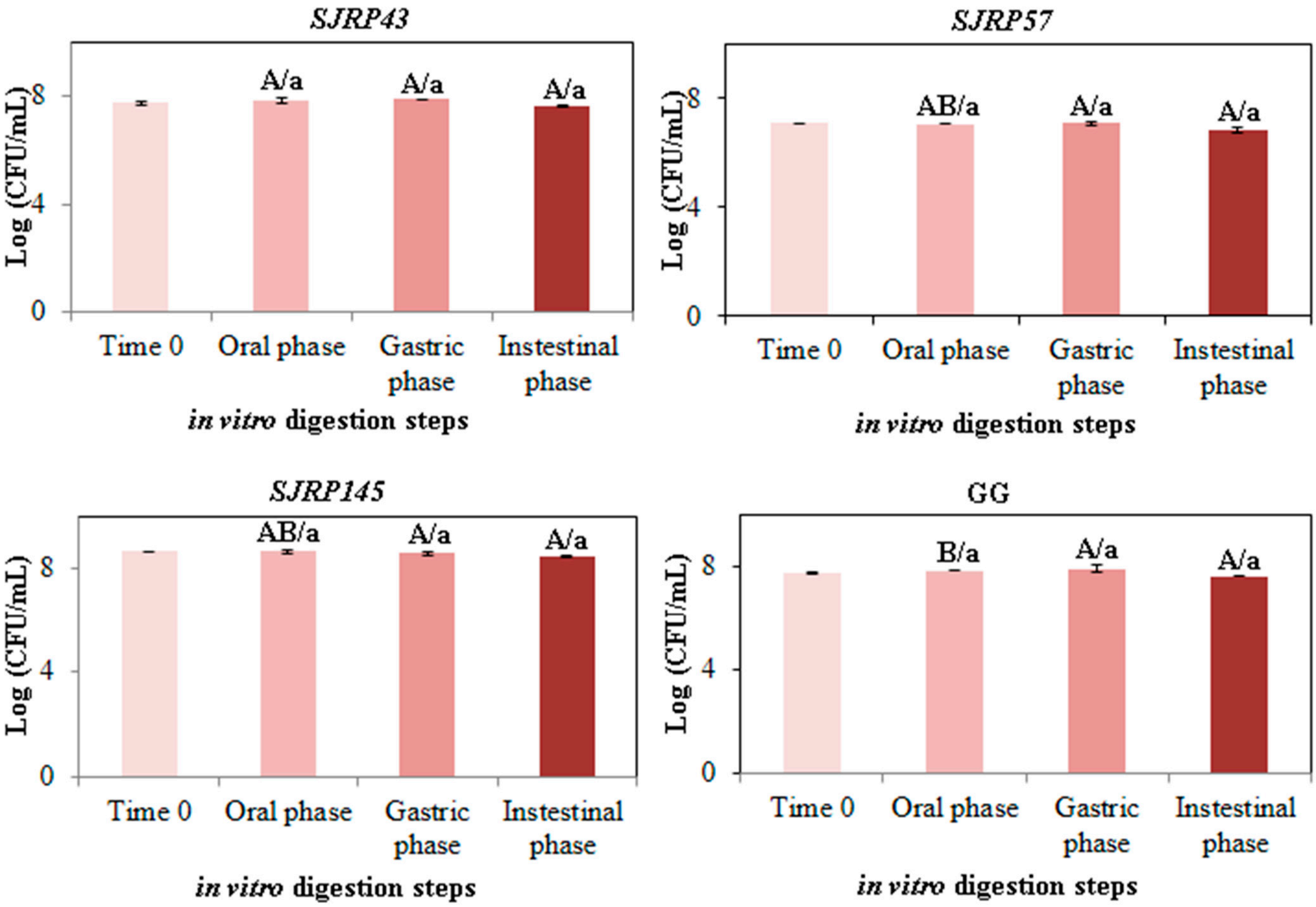


Figure 4. Survival of probiotic LAB cultures (log CFU/mL): SJRP43—*Lmb. fermentum* SJRP43, SJRP57—*Lab. delbrueckii* subsp. *bulgaricus*SJRP57, SJRP145—*Lbs. casei* SJRP145 and GG—*Lbs. rhamnosus* GG in fermented BPS by-product, at: ■ = time zero (0 h), ■ = after exposure to simulated oral (2 min, pH 7.0), ■ = gastric (2 h, pH 3.0), and = ■ intestinal conditions (2 h, pH 7.0), SD are indicated. Different capital letters denote differences ($p < 0.05$) of the same phase for different strains. Different lowercase letters denote differences ($p < 0.05$) among phases for the same strain.

The efficacy of prebiotic substrate is contingent upon its initial fermentative utilization by targeted microorganisms within the gastrointestinal tract and its subsequent selective modulation of the proliferation of potentially beneficial microbial genera, such as *Bifidobacterium* and probiotic LAB [50]. Therefore, the prebiotic potential of a specific substrate can be evaluated by using the growth of these microorganisms as one of the parameters. *Lbs. rhamnosus* GG and *Lmb. fermentum* SJRP43 showed increased growth in the NGNF BPS by-product. Likewise, *Lab. delbrueckii* subsp. *bulgaricus* SJRP57 and *Lbs. casei* SJRP145 showed increased growth in the GMF BPS by-product. The disparities in the growth kinetics among these LAB cultures can be ascribed to inherent variations in their carbohydrate metabolic pathways and enzymatic repertoires, reflecting their significant genomic and metabolic diversity [51,52]. In this study, microbial consumption of BPS starch was not evident for LAB cultures; they showed an apparent selectivity for the different BPS components. Starch is synthesized by glucose and its hydrolysis, depending on several enzymes, including α-glucosidase, which breaks starch and disaccharides into glucose. However, only two LAB cultures showed high enzyme activity for breaking down this carbohydrate and using it as a carbon source (Figure 2). On the other hand, in our study, approximately 70 to 80% of the tested cultures were able to degrade D-cellobiose, and four cultures were able to degrade D-xylose. It is well established in the literature that probiotic LAB can metabolize a range of non-digestible carbohydrates from the host diet, including oligo- and polysaccharide fractions derived from plant cell walls. Consequently, these compounds emerge as potential prebiotics [53,54], in which the role of beneficial microorganisms is essential for driving metabolic transformations. Beyond its role as a carbon source for microbial proliferation, the insoluble fiber fraction also contributes to the regulation of digestive functions by enhancing intestinal motility [55]. Furthermore, it offers protective effects for probiotic microorganisms against the adverse conditions in the digestive tract, a phenomenon corroborated by an in vitro digestion simulation assay (Figure 4).

Further supporting these positive findings, a prior investigation by Casarotti et al. [29] demonstrated the safety profile of *Lmb. fermentum* SJRP30 and *Lbs. casei* SJRP145 and SJRP146. This assessment considered key parameters such as the hemolytic activity, mucin degradation, presence of genes encoding virulence factors, antibiotic resistance and biogenic amines, and antibiotic susceptibility. Additionally, *Lbs. casei* SJRP146 and SJRP145 and *Lmb. fermentum* strains exhibited robust growth on MRS agar supplemented with 0.5% (w/v) TDCA acid sodium salts, whereas the growth of *Lab. delbrueckii* subsp. *bulgaricus* was completely inhibited. Furthermore, these strains possess similar or superior probiotic characteristics compared to the reference strain *Lbs. rhamnosus* GG (ATCC 53103), enhancing their potential to be applied for biotechnological degradation of agricultural by-products.

4. Conclusions

The present study highlights the promising potential of BPS by-products as a sustainable and high-value substrate for microbial fermentation. BPS flour, characterized by a high content of insoluble fiber, lignocellulosic compounds, resistant starch, and phenolic compounds with antioxidant activity, provided a favorable matrix for the growth of lactic acid bacteria (LAB), supporting viable populations in the range of 6–8 log CFU/mL. Furthermore, in vitro gastrointestinal digestion assays revealed a significant prebiotic potential of BPS flour, as it contributed to the protection and survival of probiotic bacteria under simulated harsh digestive conditions. These findings suggest that BPS by-products can be valorized through fermentation processes to develop functional food ingredients or bioactive compounds, promoting both environmental sustainability and human health.

Author Contributions: M.M.d.S.M. and T.F.B. performed the experiments. M.M.d.S.M. designed the initial draft and reviewed the manuscript. S.D.T. and A.L.B.P. performed the conceptualization, reviewing, and editing. A.L.B.P. was responsible for funding acquisition, supervision, and project administration. All authors have read and agreed to the published version of the manuscript.

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