

Article

Development of a Prototype of a Fermented Peanut Beverage Using Plant-Derived Lactobacilli

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Abstract

The growing demand for plant-based functional foods has encouraged the development of non-dairy fermented beverages able to deliver viable microorganisms and improve the technological and nutritional value of plant matrices. This study aimed to develop a prototype fermented peanut beverage using *Lactiplantibacillus plantarum* strains and to evaluate their technological performance, stability, and functional potential. Two plant derived strains, *L. plantarum* F1B and LpAv, and the commercial probiotic *L. plantarum* 299v were assessed in animal-free culture media and used to ferment peanut extract supplemented with sucrose and yeast extract. Fermented beverages were characterized through microbiological, physicochemical, rheological, peptide profile, refrigerated storage, and simulated gastrointestinal digestion. The selected formulation allowed all strains to reach pH ≤ 4.5 after 6.5 h, with LAB counts of 8.6–9.0 log CFU/mL. Lactic acid was the main fermentation product, and oxalic acid levels were significantly reduced. Fermentation increased peptide signals and improved rheological behavior, generating pseudoplastic beverages with higher viscosity than the control. LAB counts remained ≥ 8 log CFU/mL after 28 days at 4 °C, although gastrointestinal resistance after storage was strain-dependent. Taken together, *L. plantarum* strains showed promising technological and functional properties for developing fermented peanut-based beverages. The nutritional properties of peanut, together with the fact that it supports the growth and survival of LAB in a fermented beverage, positions peanuts as a candidate which deserves further studies in the space of fermented functional foods.



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1. Introduction

Peanut (*Arachis hypogaea* L.) is among the most widely consumed oilseed crops worldwide. From a nutritional perspective, peanuts are valued for their favorable lipid composition and for the presence of several bioactive compounds, including phytosterols, phenolics,

stilbenes, lignans, and isoflavonoids [1]. These components have been associated with protective effects against chronic diseases such as cardiovascular disorders, type 2 diabetes mellitus, and certain types of cancer. Peanut intake is included in healthy dietary patterns, such as the Dietary Approaches to Stop Hypertension diet, in which peanut consumption is recommended several times per week, and the traditional Mediterranean diet, in which its regular intake is encouraged. Therefore, current evidence supports the relevance of peanuts and peanut-based products as part of dietary strategies aimed at improving health outcomes [2].

Although peanuts are commonly considered oilseeds because of their high lipid content, they are botanically legumes, which makes them a potential substrate for promoting a healthy gut microbiota [3]. The gut microbiota is the community of microorganisms, mainly bacteria, that inhabits the gastrointestinal tract and performs essential functions for health, including digestive and immunological functions, prevention against infections, and communication with the central nervous system [4]. Diet is one of the main factors shaping the gut microbiota, and dietary fiber represents a key substrate for maintaining a diverse and abundant microbial community. In this context, legumes are considered an important source of fermentable substrates for the gut microbiota [5]. Additionally, according to the CEPEA (Center for Studies on Food Policy and Economics), the Argentine population shows a deficit of more than 90% in legume consumption compared with nutritional recommendations [6]. This gap between recommended and actual legume intake may reduce the availability of dietary substrates (fiber) for the microbiota and contribute to the development of chronic diseases with inflammatory or immunological bases, in association with a progressively depleted gut microbiota [7].

Fermented foods are defined as foods or beverages produced through microbial growth and the conversion of food components by microbial enzymatic activity [8]. In recent years, fermented foods have gained popularity mainly because of their potential health benefits [9]. Lactic acid bacteria (LAB), the microbial group most widely used in the development of fermented dairy foods, are also capable of fermenting legumes for the development of new plant-based functional foods [10,11]. Although traditionally the most popular industrial fermented foods are dairy-based, mainly yogurt and cheese, interest in plant-based beverages as alternatives to dairy products has increased in recent years. This trend is driven by factors such as lactose intolerance, the rise of vegan diets, environmental sustainability, dietary diversification, and the demand for functional foods [12]. Fermentation has emerged as a key technological strategy to improve the sensory, nutritional, microbiological, and functional properties of these plant matrices [13]. However, the development of plant-based fermented beverages still presents technological challenges. These include weak texture and phase separation due to plant proteins, the need for carefully selected starter cultures that acidify quickly, the maintenance of LAB viability during storage, the reduction of anti-nutritional compounds, and the improvement of sensory acceptance because bitterness, astringency, or beany notes may appear. Strategies such as exopolysaccharide (EPS)-producing LAB, heat treatments, homogenization, stabilizers, and flavoring can help improve stability, texture, safety, and acceptability [14]. Based on this background, the present work aims to explore the less-promoted leguminous aspect of the peanut for the development of a new plant-based fermented beverage. This approach seeks to combine the nutritional potential of peanuts as a legume-derived matrix with the delivery of viable microorganisms, with the ultimate goal of supporting the diversity, abundance, and functionality of the gut microbiota and, consequently, digestive health.

2. Materials and Methods

2.1. Microorganisms and Culture Media

Two *Lactiplantibacillus plantarum* strains isolated from plant materials were used in this study: *L. plantarum* F1B-GW (LpF1B; ITA culture collection) was isolated from local strawberries and *L. plantarum* LpAv (LpAv; INLAIN culture collection) was isolated from oat [15]. Commercial probiotic strain *L. plantarum* 299v (Lp299v; DMS 9843) was used as a reference strain. All strains were kept frozen at -70°C in de Man Rogosa Sharpe (MRS) broth (Biokar, Beauvais, France) supplemented with 17% (v/v) glycerol (Cicarelli, Buenos Aires, Argentina). Before use, lactobacilli were cultured twice (2% (v/v)) in MRS during 18 h at 37°C .

2.2. Production of ‘Vegan-Friendly’ LAB Cultures for Use in Plant Based Products

The culture broths used in this study were prepared in-house from individual ingredients (obtained from Cicarelli and Microquim, Santa Fe, Argentina). Standard MRS was prepared according to the composition of commercial MRS (Biokar), whereas plant-based MRS (MRSv) was obtained by replacing meat peptone with the same amount of soy peptone, as previously reported by Fontana et al. [16]. Additionally, simplified MRSv (without MgSO_4 , MnSO_4 , triammonium citrate and sodium acetate) supplemented with either glucose (MRSv SG) or fructose (MRSv SF) was formulated. Culture media were sterilized in autoclave (121°C , 15 min). Growth kinetics were carried out as described by Puntillo et al. [15] with slight modifications. Briefly, overnight cultures of the tested strains under study (ca. 10^9 CFU/mL) were washed twice and resuspended in sterile Phosphate-Buffered Saline Solution (PBS, pH 7.4). Then, each suspension was inoculated 1% (v/v) into the corresponding culture medium, yielding an initial concentration of ca. 10^7 CFU/mL and distributed in 96-well microplates (300 μL /well). The plates were incubated at 37°C in aerobiosis and optical density at OD570nm was recorded every 30 min in a microplate photometer (Thermo Scientific Multiskan FC, Thermo Fisher Scientific, Waltham, MA, USA). Experiments were performed in independent triplicates for each strain, and counts (MRS agar, 37°C , 48 h) were performed at time 0 and 18 h. The resulting growth curves were fitted to a model proposed by Baranyi and Roberts [17] and the kinetic parameters μ_{max} (maximum growth rate as variation in OD with time, in h^{-1}) and λ (lag phase, in h) were obtained using the DMFit add-in version 3.5 (Institute of Food Research, Norwich, UK). Subsequently, to mimic the production of ‘vegan-friendly’ cultures for industrial use, concentrated frozen (F) and freeze-dried (L) cultures were produced. Each strain was cultivated in MRSv (37°C , 18 h, aerobiosis); the biomass was recovered by centrifugation ($6000 \times g$, 10 min, 5°C), washed with sterile PBS and resuspended in 15% (w/v) maltodextrin to obtain a three-fold concentration. For F stocks, cell suspensions were distributed in Eppendorf tubes (2 mL/tube) and kept at -70°C . For L cultures, the same volume was distributed in sterile glass cryovials, frozen at -70°C for 24 h, and freeze-dried at -55°C and 0.0010 mBar for 22 h, using an Alpha 1–4 LD plus freeze-dryer (Christ, Osterode, Germany). L cultures were sealed under vacuum and stored at 4 and 22°C . LAB viability on F and L cultures was assessed periodically over 12 months.

2.3. Production of a Prototype Fermented Peanut Beverage (PB)

2.3.1. Preparation of the Peanut Extract and Preliminary Fermentation Tests

Unsalted skinless roasted peanuts, obtained from a local store, were soaked overnight in 0.5% w/v NaHCO_3 in a 1:3 peanut:solution ratio, rinsed, treated with flowing steam (3 min), and finally processed in a domestic blender in a 1:6 ratio with water. The suspension was filtered (cloth filter) and the liquid fraction (peanut extract, PE) was recovered and autoclaved (121°C , 15 min) to obtain a sterile PE. Preliminary fermentation tests (37°C)

were performed with the *L. plantarum* strains. Overnight cultures (MRSv, ca. 10^9 CFU/mL) were washed and resuspended in sterile PBS, and inoculated (1% *v/v*) in unsupplemented PE or PE supplemented with sucrose (S; 0.5 and 1.0% *w/v*) and/or yeast extract (YE; 0.25 and 0.5% *w/v*) to determine the PB formulation. Fermentation was monitored through pH measurements and stopped at $\text{pH} \leq 4.5$ when LAB counts were performed (MRS agar, 37 °C, 48 h).

2.3.2. Growth Kinetics in the Prototype PB

After selecting the final PB formulation, PE was prepared as described before, and supplemented with sterile sucrose and yeast extract solutions (to final concentrations of 0.5 and 0.25% *w/v*, respectively). The supplemented PE was then distributed into Falcon tubes in 50 mL aliquots. For each strain, overnight cultures (ca. 10^9 CFU/mL) grown in MRSv broth (37 °C, aerobiosis, 18 h) were washed and resuspended in sterile PBS, and inoculated (1% *v/v*) in the PB. Fermentations were conducted at 37 °C and the pH and LAB counts (MRS agar, 37 °C, 48 h, aerobiosis) were determined at the beginning and after 2, 4, 5 and 6.5 h of fermentation. Each fermentation was performed in independent duplicates. The kinetic parameters μ_{max} and λ were obtained using the DMFit add-in version 3.5 as described before.

2.4. Characterization of the Prototype Fermented PB

2.4.1. Microbiological Analysis

LAB counts (MRS agar, 37 °C, 48 h, aerobiosis) were determined at the beginning and after reaching $\text{pH} \leq 4.5$ (around 6.5 h of fermentation). LAB growth during fermentation was defined as $\Delta \log$ and was calculated as the difference between LAB counts after and before fermentation expressed as log CFU/mL. Microbiological indicators such as molds and yeasts (YGC agar, 28 °C, 7 d) and total aerobic psychrotrophic microorganisms (plate count agar, 7 °C, 10 d) were also determined.

2.4.2. Physicochemical and Composition Analysis of the PB

The pH value was determined using a pH meter (LAQUAtwin pH-11, Horiba, Kyoto, Japan) and total soluble solids (SS) were determined with a digital brix refractometer (MA871, Milwaukee Instruments, Rocky Mount, NC, USA). Total titratable acidity (TTA) was determined homogenizing at least 3 g of each sample with distilled water, and TTA was determined as the volume of 0.11 N NaOH used to obtain a pH value of 8.3 [18]. Crude protein was determined using the Kjeldahl method-AOAC-954.01 applying the conversion factor 5.46 for peanut ($\text{N} \times 5.46$) [19]. For short-chain fatty acids (SCFAs) and content of sugars, samples were frozen at -70 °C for 24 h and freeze-dried (Christ). Before analysis, freeze-dried samples were rehydrated (1 g in 10 mL of distilled water). To quantify the production of SCFAs, calibration curves of lactic, acetic, propionic, formic, oxalic, succinic, butyric and isobutyric acid standards in concentrations ranging from 0.15 g/L to 5.0 g/L for lactic, acetic, propionic, formic, oxalic, succinic and from 0.05 g/L to 5 g/L for propionic, butyric and isobutyric were used. In this case, freeze-dried samples were rehydrated (1 g in 10 mL of distilled water) and filtered through a 0.45 μm nylon membrane filter (Sartorius, Göttingen, Germany) and an aliquot of 10 μL was loaded into the equipment. An HPLC (Prominence, Shimadzu, Kyoto, Japan) coupled to DAD SPD-20A was used to quantify the SCFAs. The stationary phase used was a column Rezex ROA-Organic Acid H+ (8%) (7.8 $\times$ 300 mm) (Phenomenex, Torrance, CA, USA) and the mobile phase used was 0.009 N of H_2SO_4 in water. The SCFA peaks were read at 210 nm and integration of area under the curve of each peak was carried out for quantification. Glucose, fructose, and sucrose contents were determined with a K-SUFRG kit (Megazyme, Bray, Ireland) according to the

manufacturers' instructions. At least two independent measurements were performed for each sample and the results were expressed as mean value $\pm$ standard deviation.

2.4.3. MALDI-TOF Mass Spectral Profiling of pH 4.6-Soluble Fractions from Fermented PB

Two independent experimental replicates of each fermented PB were analyzed by MALDI-TOF following Peralta et al. [20], with some modifications. The pH 4.5-soluble extracts were prepared by adjusting the samples to pH 4.5 using HCl or NaOH, as appropriate, followed by incubation at room temperature for 30 min and subsequent readjustment to pH 4.5. The mixtures were then centrifuged at $16,000\times g$ for 30 min, and the resulting supernatants were filtered through filter paper. The filtrates were transferred into 10 mL vials, frozen at $-80\text{ }^{\circ}\text{C}$ for 24 h, and freeze-dried (Christ). The freeze-dried samples (10 mg) were reconstituted in 1 mL of water. The extracts were centrifuged at $10,000\times g$ for 10 min and filtered through a $0.22\text{ }\mu\text{m}$ membrane. Aliquots ($0.5\text{ }\mu\text{L}$) were spotted onto a stainless-steel target plate, air-dried at room temperature, and overlaid with the matrix (α -cyano-4-hydroxycinnamic acid) prior to analysis. The droplet was allowed to dry completely before the target plate was introduced into the mass spectrometer (AXIMA-iD Plus, Shimadzu). Analyses were conducted in positive-ion reflector mode over an m/z range of 500–7000. The acquired spectra were subsequently processed using mMass (version 5.5.0; Martin Strohm, Czech Republic).

2.4.4. Rheological Characterization of the Prototype Fermented PB

The rheological properties of PB fermented by strains Lp299v, LpF1B and LpAv, were evaluated using rotational rheometry. Flow behavior was characterized by recording shear stress (τ , Pa) as a function of shear rate ($\dot{\gamma}$, s^{-1}), together with dynamic viscosity (μ , Pa·s). Measurements were carried out in a HAAKE MARS 40 rheometer (Thermo Scientific, Waltham, MA, USA), equipped with a parallel plate geometry, using a 1 mm gap, and at controlled temperature ($5.0 \pm 0.5\text{ }^{\circ}\text{C}$). Sweeps were performed in the range of $\dot{\gamma}$: $0\text{--}200\text{ s}^{-1}$ [21] with modifications.

2.5. Survival of LAB and Characterization of the Fermented PB During Shelf Life

Cell counts of LAB, microbiological indicators, pH, SS and TTA measurements were performed on the prototype fermented PB immediately after fermentation and at days 7, 14, 21, and 28 of storage at $4\text{ }^{\circ}\text{C}$.

Survival of LAB to Simulated Gastrointestinal Digestion (GID)

The INFOGEST protocol with slight modifications was used immediately after fermentation and at the end of the storage period (28 d). The simulated salivary fluids (SSF), simulated gastric fluids (SGF) and simulated intestinal fluids (SIF) were prepared as previously described [22]. Prior to the actual digestion of the samples, a set of control digestions were performed to determine the amounts of HCl and NaOH needed for reaching the set pH of the gastric chyme. For the oral phase, the fermented PB (1.5 mL) were mixed with SSF, 0.3 M CaCl_2 (without amylase supplementation) and distilled water and pH was adjusted at pH 7.0, as described by Brodkorb et al. [22]. After incubation at $37\text{ }^{\circ}\text{C}$ for 2 min, the oral bolus was diluted 1:1 (volume ratio) with SGF, 0.3 M CaCl_2 , pepsin with activity of 2000 U/mL in final digesta (from porcine gastric mucosa ≥ 250 units/mg solid, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), 3.0 M HCl to reach pH 3.0 and distilled water. The samples were incubated and mixed at $37\text{ }^{\circ}\text{C}$. After 120 min of gastric digestion (GD), the samples intended for intestinal digestion (ID) were diluted 1:1 (v/v) with SIF, 0.3 M CaCl_2 , 10 mM (in final digesta) bile salts (bile extract from porcine, Sigma-Aldrich), pancreatin with trypsin activity in 100 U/mL in final digesta (from porcine pancreas, Sigma-Aldrich) and distilled water. The pH was adjusted at pH 7.0 if necessary

and immediately the samples were incubated at 37 °C for 120 min as described above. Cell counts were performed before and after the gastric and intestinal stages in MRS agar.

2.6. Validation of the ‘Vegan-Friendly’ LAB Cultures for the Development of a Prototype Fermented PB

Frozen and freeze-dried cultures were inoculated on the formulated PB and pH; SS (°Bx) and TTA (%) were measured as described before, at the end point of fermentation (pH ≤ 4.5). Values were compared to those obtained for fresh LAB cultures produced in MRSv.

2.7. Statistical Analysis

Microbiological and physicochemical determinations were performed at least in triplicate and the results were presented as mean ± standard deviation. One-way ANOVA procedure of GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, CA, USA) was used for data analyses. Tukey’s multiple range test was used to detect significant differences between means of the control and/or among fermented PB. Differences were considered statistically significantly different when $p < 0.05$.

3. Results and Discussion

3.1. Production of ‘Vegan-Friendly’ LAB Cultures for Use in Plant Based Products

When aiming at developing plant-based products, studying potential consumer profiles is essential. One of the target demographics interested in plant-based fermented beverages are vegans and vegetarians. In this context, all the ingredients involved in the production need to be animal-free, starting with the composition of the starter cultures. In our study, a culture broth without animal components (MRSv) was prepared in-house by replacing meat peptone in MRS with soy peptone. The ability of the LAB strains to grow on it was evaluated and compared to regular MRS. Also, two simplified versions of MRSv with either glucose or fructose as a carbon source were tested. The kinetic growth parameters ($\mu_{\max}$ and λ) for *L. plantarum* 299v, F1B and LpAv were determined in the four culture media (Table 1). Similar $\mu_{\max}$ values in MRS and MRSv were observed for all strains. However, significantly lower $\mu_{\max}$ values ($p < 0.05$) for simplified MRSv versions were detected. As for the extension of lag phases, only differences for LpAv were observed between MRS and MRSv, with higher times for the latter. Final counts (at 18 h) showed comparable ($p > 0.05$) and satisfactory yield growth of the LAB strains under study on MRS and MRSv (9.13 ± 0.21 and 9.22 ± 0.11 log CFU/mL, respectively) whereas lower growth was observed for simplified MRSv, irrespective of the carbon source (8.61 ± 0.10 and 8.68 ± 0.17 log CFU/mL for MRSv SG and MRSv SF, respectively).

Following biomass production, the subsequent phase in manufacturing ‘vegan-friendly’ LAB cultures involves generating stable, concentrated formulations. Starter and probiotic cultures are traditionally commercialized in frozen or lyophilized (freeze-dried) formats. Maltodextrin is widely used as a cryoprotectant due to its effectiveness during drying, minimal crystallization during storage, low cost, and mild flavor [16]. The counts of the strains immediately after freeze-drying were 10.8 ± 0.1 for Lp299v and LpF1B and 10.7 ± 0.0 log CFU/g for LpAv (survival rate $88 \pm 13\%$ for the three LAB strains). After 12 months, freeze-dried cultures stored at 4 °C remained stable, yielding dehydrated cultures with levels of 10.8 ± 0.1 log CFU/g and an average water activity of 0.057 ± 0.011 for the three strains. Regarding storage at controlled room temperature (22 °C), the cultures had an average water activity of 0.068 ± 0.025 , while viable cell counts were 9.0, 9.0, and 10.1 log CFU/g (with cumulative viability losses of 1.8, 1.7 and 0.6 log CFU/g) for Lp299v, LpF1B, and LpAv, respectively. This shows a significant higher resistance of the dehy-

drated LpAv culture compared to Lp299v and LpF1B ($p < 0.05$). The differences in survival along storage observed in this work highlight that even belonging to the same species, the resistance to technological processes is strain-specific. To improve survival for Lp299v and LpF1B to freeze-drying, applying sub-lethal stresses during biomass production or evaluating different cryoprotectants, could be useful strategies. As for the frozen cultures, they did not exhibit a significant change in viability ($p > 0.05$) after 12 months at $-70\text{ }^{\circ}\text{C}$.

Table 1. Kinetic parameters ($\mu_{\max}$ and λ) of the growth of *L. plantarum* 299v, *L. plantarum* F1B and *L. plantarum* LpAv on MRS, MRSv, MRSv SG and MRSv SF media.

Medium	Strain	$\mu_{\max}$ (h^{-1})	λ (h)	R^2	SE
MRS	Lp 299v	0.31 ± 0.05^a	2.98 ± 0.06^a	0.995	0.044
	Lp F1B	0.32 ± 0.06^a	3.20 ± 0.02^a	0.994	0.051
	LpAv	0.31 ± 0.07^a	3.04 ± 0.16^b	0.996	0.037
MRSv	Lp 299v	0.26 ± 0.03^a	2.89 ± 0.08^a	0.995	0.028
	Lp F1B	0.26 ± 0.02^a	3.24 ± 0.16^a	0.999	0.021
	LpAv	0.33 ± 0.06^a	3.50 ± 0.03^a	0.999	0.025
MRSv SG	Lp 299v	0.15 ± 0.01^b	2.20 ± 0.05^c	0.996	0.025
	Lp F1B	0.12 ± 0.01^b	1.80 ± 0.00^b	0.992	0.034
	LpAv	0.15 ± 0.01^b	2.09 ± 0.01^d	0.994	0.034
MRSv SF	Lp 299v	0.13 ± 0.01^b	2.50 ± 0.13^b	0.996	0.025
	Lp F1B	0.11 ± 0.00^b	1.94 ± 0.03^b	0.994	0.028
	LpAv	0.13 ± 0.00^b	2.63 ± 0.04^c	0.993	0.035

^{a,b,c,d} Values with different superscripts differ significantly ($p < 0.05$) for each strain between culture media. Values are the means ($\pm$ SD) of at least three replicates.

3.2. Production of a Prototype of a Fermented PB

A PE ($\text{pH } 6.9 \pm 0.2$; $\text{SS } 4.9 \pm 0.1\text{ }^{\circ}\text{Bx}$) with $2.44 \pm 0.02\%$ w/v of total protein content and $0.21 \pm 0.03\%$ w/v of total sugars, mainly sucrose, was obtained. When PE was used unsupplemented, the growth of LAB under study on PE was unsatisfactory, and only Lp299v was able to reach $\text{pH} \leq 4.5$ after 18 h of fermentation ($8.42 \pm 0.07 \log \text{CFU/mL}$). With the individual addition of either sucrose or yeast extract a similar situation was observed (fermentation times higher than 16 h, Table S1) whereas with the combination of both growth factors (S 1% + YE 0.5% or S 0.5% + YE 0.25% w/v), fermentation was possible in shorter times (from 6 to 8 h) and with satisfactory counts (ranging from 8.5 to 9.0 $\log \text{CFU/mL}$), without significant differences among conditions ($p > 0.05$). The combination of PE supplemented with sucrose 0.5% w/v and yeast extract 0.25% w/v was selected as final PB formulation and the fermentation kinetics were studied for the three *L. plantarum* strains (Figure 1). All three strains were able to ferment the PB, reaching the pH end point after 6.5 h, with similar lag phases ($1.46 \pm 0.46 \text{ h}$) and $\mu_{\max}$ slightly lower for LpF1B (0.323 h^{-1}) compared to LpAv (0.462 h^{-1} ; $p < 0.05$), with Lp299v having an intermediate behavior (0.416 h^{-1}).

Several authors have studied the production of peanut extract and its fermentation. Yamamoto et al. [23] studied the fermentation of unsupplemented PE with the strain *L. plantarum* Kinko SU4 isolated from the flower of *Hydrangea macrophylla*. In their work, even if LAB counts increased 2.4 $\log \text{CFU/mL}$ after 10 h of fermentation and pH decreased from 6.8 to 4.5, the final product (FPM) was fermented a total of 48 h, which could be considered not an efficient process from an economic point of view. Bansal et al. [24] aimed to develop a yogurt-like product from peanut ‘milk’ utilizing single probiotic cultures

and without any supplements. A PE with 3.93% and 6.6% of protein and fat content, respectively, was obtained (information about initial pH, total solids or carbohydrates content was not reported). After a fermentation screening with seven different probiotic strains, *Streptococcus faecalis* T110 was selected, and a minimum time of 12 h of fermentation was necessary to reach cell counts of the probiotic between 6.2 and 7.7 log CFU/mL [24]. Previous attempts have also explored PE supplementation with growth factors or other ingredients in order to improve the fermentation or the rheological characteristics of the fermented product. Schaffner et al. [25] studied the kinetics of fermentation of PE, with or without the addition of 2% (*w/v*) glucose, by a commercial strain of *L. bulgaricus*. After 24 h of fermentation, the pH of unsupplemented PE dropped only from 7.0 to 6.2, with LAB counts of ca. 8 log CFU/mL, whereas the supplemented PE reached a pH ≤ 4.5 at about 8 h of fermentation and counts close to 9 log CFU/mL. Yadav et al. [26] studied in an experimental design the impact of the addition of skim milk powder and carboxy methyl cellulose to produce an acceptable peanut milk-based curd, and even with the addition of these ingredients, fermentation times were 16–20 h. Bulca and Büyükgümüş [27] explored the addition of peanut pulp as a protein source to PE, and the use of two different commercial starter cultures (with *S. thermophilus* and *L. bulgaricus*). In this case, fermentation was stopped after 4 h of fermentation. The composition of the initial PE had an initial pH 6.2; 0.5% carbohydrate, 1.43% protein and 2.12% fat. After 4 h of fermentation, beverages with pH between 4.25 and 4.81, TTA 0.1–0.29% and counts of *L. bulgaricus* and *S. thermophilus* ranged 7.04–8.34 and 5.04–8.46 log CFU/mL, respectively, were obtained. These results showed that the composition of the PE varies greatly, depending on the production process or peanut variety. Also, the fermentation process is affected by the selection of the starter cultures, oftentimes oriented to dairy products, and the formulation of the final beverage. Our study focused on the selection of plant-derived strains of *L. plantarum*, a known species for the development of plant-based functional products, and aimed to develop a simple PB formulation that led to short fermentation times and satisfactory characteristics.

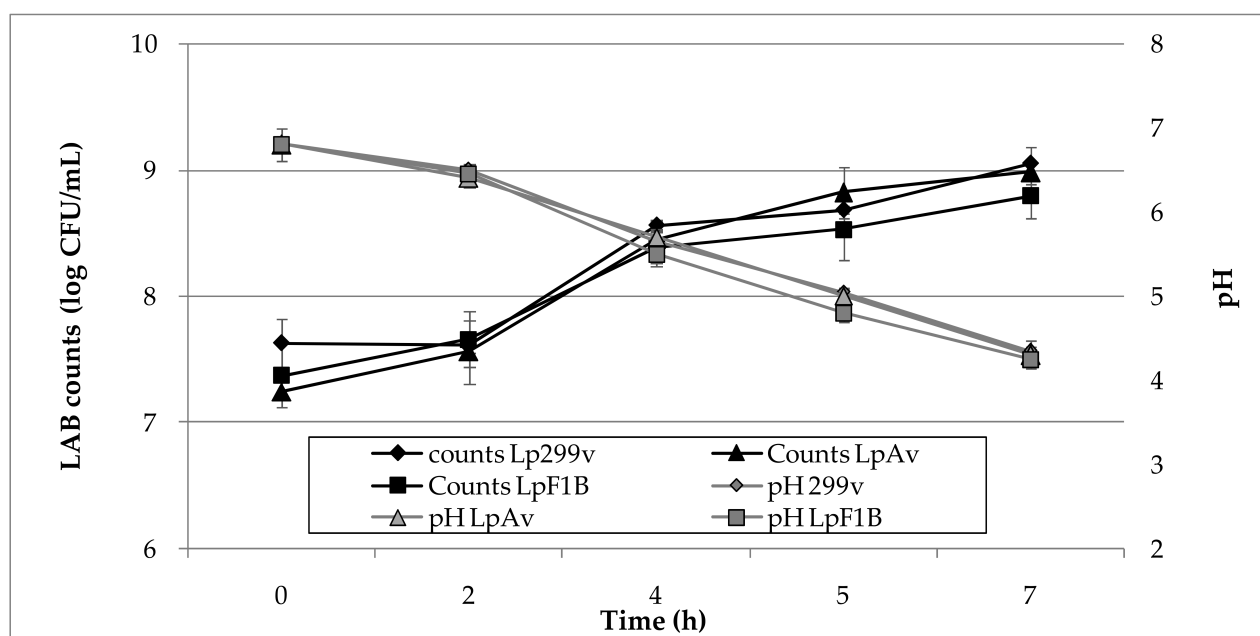


Figure 1. Total LAB counts (log CFU/mL) and pH on the prototype peanut beverage (PB; peanut extract + 0.5% sucrose + 0.25% yeast extract) fermented by *L. plantarum* F1B, *L. plantarum* LpAv and *L. plantarum* 299v, at the beginning and after 2, 4, 5 and 6.5 h of fermentation.

3.3. Characterization of the Prototype Fermented PB

Figure 2 shows the final appearance of the fermented PB. The physicochemical and microbiological characteristics of the prototype fermented PB are shown on Table 2. Beverages with pH of 4.3 ± 0.1 and SS of 1.5 ± 0.4 °Bx were obtained, with no differences among the LAB under study. Cell growths after 6.5 h of fermentation were 1.4, 1.3 and 1.6 log CFU/mL for Lp299v-PB, LpF1B-PB, and LpAv-PB, respectively. All microbiological indicators were under the limit of detection (<10 CFU/mL).

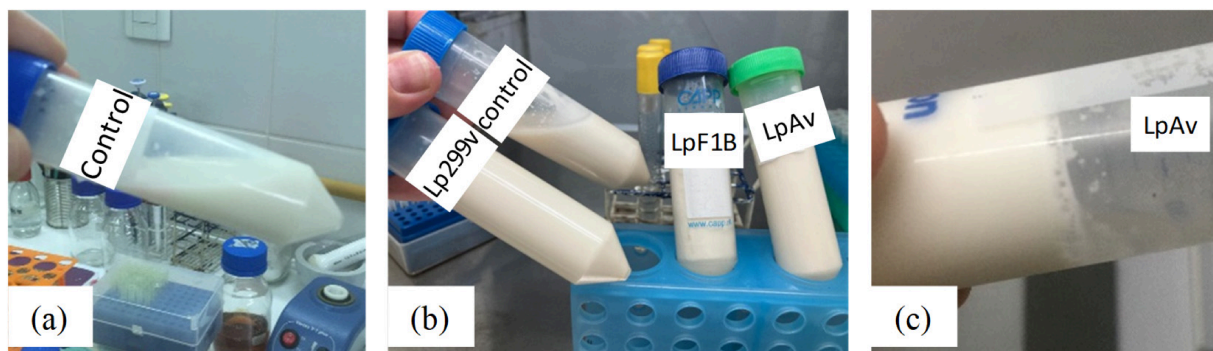


Figure 2. Peanut beverage (a) non-fermented (control) and (b) after 6.5 h of fermentation by *L. plantarum* 299v (Lp299v), *L. plantarum* F1B (LpF1B) and *L. plantarum* LpAv (LpAv); (c) close-up of LpAv fermented beverage to appreciate its consistency.

Table 2. Physicochemical and microbiological analysis of peanut beverages (PBs) non-fermented or fermented by Lp299v, LpF1B and LpAv.

	pH	SS (°Bx)	TTA (% AL)	LAB Counts (log CFU/mL)	LAB Growth (Δlog CFU/mL)	Molds and Yeast (log CFU/mL)	Total Psychrotrophic (log CFU/mL)
Lp299v-PB	4.3 ± 0.1^a	1.5 ± 0.3^a	0.44 ± 0.03^a	9.0 ± 0.1^a	1.4 ± 0.2^a	<1	<1
LpF1B-PB	4.3 ± 0.1^a	1.4 ± 0.5^a	0.43 ± 0.06^a	8.6 ± 0.3^b	1.3 ± 0.2^a	<1	<1
LpAv-PB	4.3 ± 0.1^a	1.5 ± 0.4^a	0.40 ± 0.07^a	$8.8 \pm 0.1^{a,b}$	1.6 ± 0.2^a	<1	<1
PB	6.9 ± 0.2^b	4.9 ± 0.2^b	0.08 ± 0.02^b	<2	-	<1	<1

^{a,b} Values with different superscripts on the same column differ significantly (ANOVA + Tukey's post hoc test; $p < 0.05$). Values are the means ($\pm$ SD) of at least three replicates.

TTA on fermented PB ranging from 0.40 to 0.44% were detected. Usually, plant-based yogurt-like products have less lactic acid than the dairy control (TTA 0.8–1.2%). Bulca & Büyükgümü [27] reported yogurt analogs from peanut extract with TTA of 0.12–0.28%. Our results showed intermediate values of TTA, which could be explained by the sucrose supplementation on the PB formulation. As expected, a significant reduction of sucrose on the fermented PB was evidenced (Table 3). Neither glucose nor fructose was detected on the PB, either fermented or un-fermented. Regarding the production of SCFAs and organic acids of interest, it is possible to observe that lactic acid was the main product of fermentation, with values ranging from 3.81 to 4.75 g/L. These levels of lactic acid measured by HPLC had corresponded with TTA values, reinforcing its utility as a quick test to monitor lactic fermentations. Propionic, acetic and isobutyric acids were also detected in significantly lower concentrations and without differences compared to the control samples (un-fermented PB) (Table 3). Interestingly, a significant decrease in oxalic acid levels in the fermented beverages was observed compared to the control PB (Table 3). One of the limitations to a broader use of legumes in food formulations is often the presence of anti-nutritional factors (ANF) and other compounds that may negatively affect

digestibility, technological performance, and sensory acceptability [28]. Peanuts are known to have high oxalate contents (up to 187.0 mg/100 g roasted peanuts) and a few studies have even described peanut-induced oxalate nephropathy [29]. In recent years, different technological and biotechnological strategies have been explored to enhance the nutritional and functional properties of legume-derived products, including fermentation. Several studies have provided evidence regarding the capacity of some lactic acid bacteria to degrade oxalates [30]. Two key genes present in LAB involved in this activity code for a formyl coenzyme A transferase (frc) and an oxalyl coenzyme A decarboxylase (oxc) [31]. Interestingly, Youssef [32] screened 15 LAB isolates for oxalate degrading activity and one of the strains with higher degrading ability (and presence of both genes) was *L. plantarum* 37. Our results could indicate the usefulness of the fermentation process to reduce an ANF such as oxalate [33]. Finally, the PB showed values of $2.34 \pm 0.02\%$ *w/v* total protein content before fermentation, 2.16 ± 0.06 , 2.38 ± 0.09 and $2.33 \pm 0.01\%$ *w/v* for Lp299v-PB, LpF1B-PB and LpAv-PB, respectively. Given the Kjeldahl's method inability to distinguish between different nitrogen sources, the exploration of the peptidic profiles of the fermented PB results are of interest to get further knowledge on the characteristics of the prototype.

Table 3. Determination of short-chain fatty acids, sugars and protein in peanut beverages (PBs) non-fermented or fermented by Lp299v, LpF1B and LpAv.

	Formic Acid (g/L)	Acetic Acid (g/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Isobutyric Acid (mg/L)	Oxalic Acid (g/L)	Lactic Acid (g/L)	Sucrose (% <i>w/v</i>)	Protein (N $\times$ 5.46) (% <i>w/v</i>)
Lp299v-PB	0.03 ± 0.00^a	0.11 ± 0.01^a	1.14 ± 0.03^a	n.d.	0.14 ± 0.00^a	0.02 ± 0.00^a	3.81 ± 1.46^a	0.32 ± 0.00^a	2.16 ± 0.06^a
LpF1B-PB	0.03 ± 0.00^a	0.25 ± 0.04^a	1.16 ± 0.03^a	n.d.	0.13 ± 0.01^a	0.03 ± 0.00^a	4.75 ± 0.20^a	0.30 ± 0.03^a	2.38 ± 0.09^a
LpAv-PB	0.03 ± 0.00^a	0.23 ± 0.14^a	1.25 ± 0.16^a	n.d.	0.11 ± 0.03^a	0.06 ± 0.04^a	4.30 ± 0.11^a	0.32 ± 0.05^a	2.33 ± 0.01^a
PB	0.02 ± 0.01^a	0.35 ± 0.17^a	1.39 ± 0.09^a	n.d.	0.08 ± 0.02^a	0.19 ± 0.02^b	0.23 ± 0.02^b	1.18 ± 0.05^b	2.21 ± 0.02^a

^{a,b} Values with different superscripts on the same column differ significantly (ANOVA + Tukey's post hoc test; $p < 0.05$). Values are the means ($\pm$ SD) of at least two replicates. n.d. non-detected.

Peanuts are rich in lipids and bioactive compounds that confer health benefits. Whereas lactic acid bacteria, in particular *L. plantarum* strains, are considered non-lipolytic or only very weakly lipolytic [34,35], their activity may impact the bioactivity of other components such as phytosterols, phenolic compounds, stilbenes or lignans. For example, isoflavonoids, one of the main phenolic compounds present in plant materials, have low biological activity but can be transformed to their aglycone (more active) forms by certain LAB, including strains of *L. plantarum* [36]. These activities were not addressed in this work and remain as a limitation of the present study.

3.3.1. Peptidic Profile of PB

MALDI-TOF mass spectral profiling of soluble fractions has been widely used to characterize peptide profiles in fermented dairy products such as cheese and yogurt [37–39]. On the other hand, information on peptide profiling in fermented plant-based milks remains limited, particularly for peanut-based products, highlighting a gap in the literature addressed in the present study. The mass spectra of the soluble fractions obtained from fermented peanut-based milks are shown in Figure 3 (500–3500 *m/z*) and Figure 4 (3500–7000 *m/z*). Overall, the fermented samples exhibited a higher number of peptide signals compared to the control, indicating enhanced proteolytic and peptidolytic activity associated with the incorporation of *L. plantarum* strains.

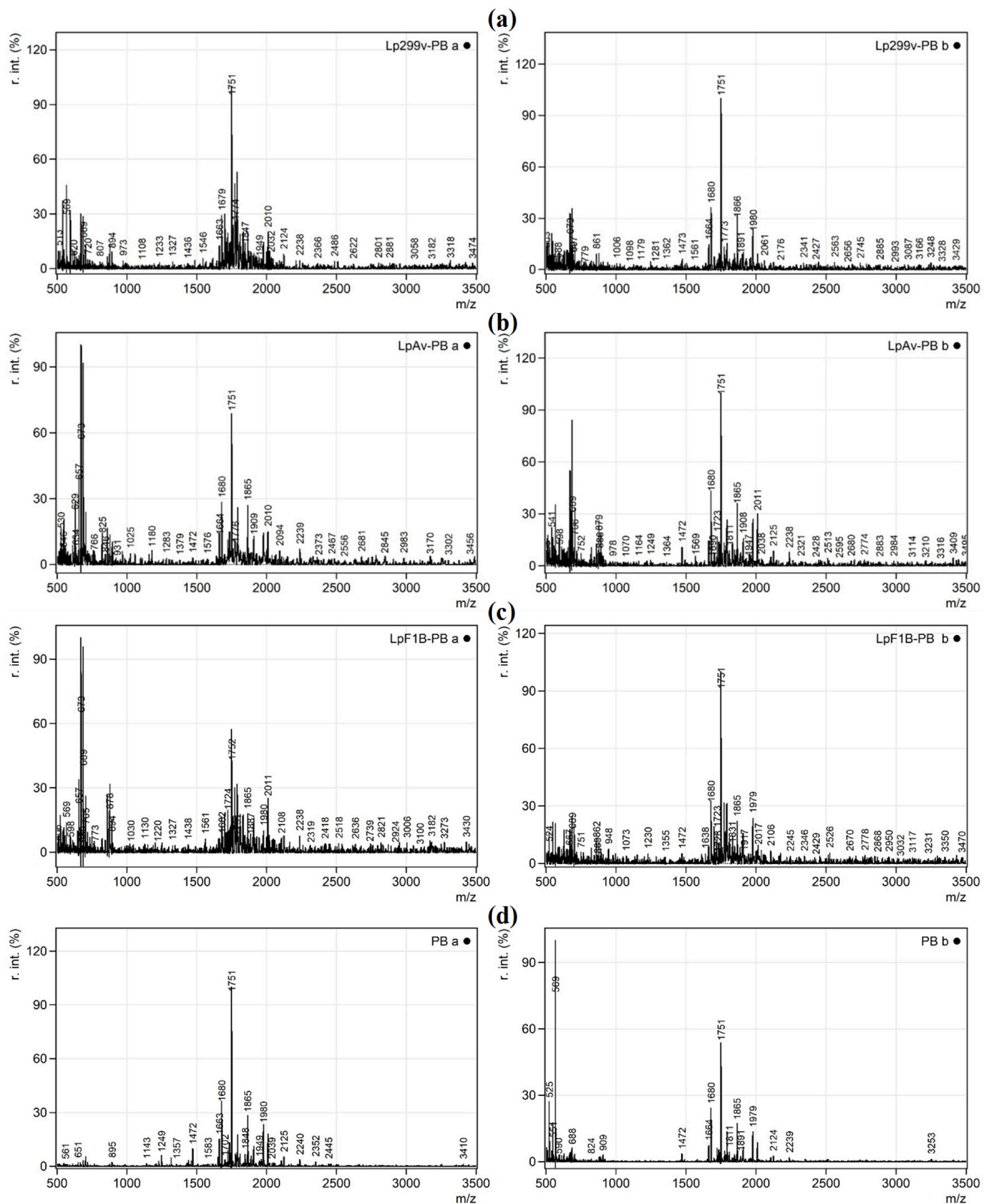


Figure 3. MALDI-TOF mass spectra (500–3500 m/z) of the pH 4.5-soluble extracts obtained from fermented peanut beverages (PBs): (a) Lp299v-PB; (b) LpAv-PB; (c) LpF1B-PB and (d) the non-fermented control PB. Left and right panels correspond to two independent experimental replicates (Trial 1 and Trial 2), respectively.

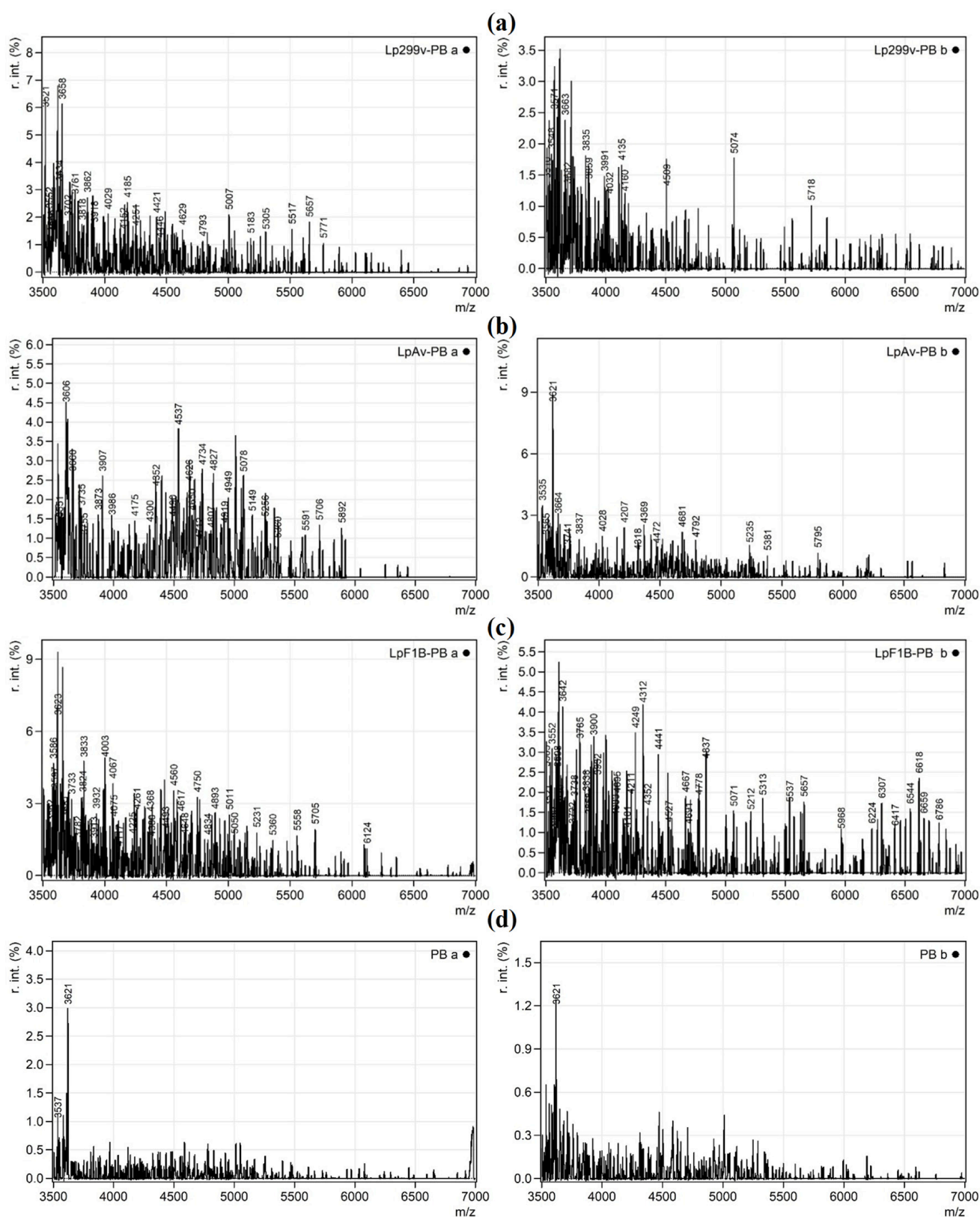


Figure 4. MALDI-TOF mass spectra (3500–7000 m/z) of the pH 4.5-soluble extracts obtained from fermented peanut beverages (PBs): (a) Lp299v-PB; (b) LpAv-PB; (c) LpF1B-PB) and (d) the non-fermented control PB. Left and right panels correspond to two independent experimental replicates (Trial 1 and Trial 2), respectively.

Fermentation led to an increase in the intensity of several peaks in the low molecular weight range (500–1000 m/z), reflecting the generation of smaller peptides during the process. In the intermediate mass range (1000–2000 m/z), several peaks—such as m/z 1751, one of the most intense signals across all samples—were also detected in the control, suggesting that they originate from the peanut extract matrix rather than being exclusively generated during fermentation. A better definition of the peaks on the range from 500 to 2500 m/z can be observed in Supplementary Figures S1–S4. In the high mass range (3500–7000 m/z), signal intensity was lower overall, although differences between the fermented samples and the control remained evident. The slight differences observed among the fermented samples likely reflect strain-dependent variability in proteolytic and peptidolytic activities, which has been widely reported in the literature [40,41].

It is widely reported that some species of lactic acid bacteria contribute to proteolysis in fermented foods through their well-developed proteolytic systems, which include cell-envelope proteinases and a broad range of intracellular peptidases [42]. In general, proteolysis is associated with improvements in texture and flavor in fermented food. In cheese, proteolytic and peptidolytic activities contribute to flavor development by degrading bitter peptides and increasing the concentration of free amino acids, which serve as key precursors of aroma compounds [43,44]. In fact, it has been estimated that up to 50% of volatile compounds in many cheese varieties originate from amino acid catabolism.

The proteolytic system of lactic acid bacteria has also been associated with potential health benefits, as numerous studies report the release of bioactive peptides during fermentation. In this context, Skrzypczak et al. [45] suggested that *L. plantarum* 299v promotes the generation of peptides with predicted biological activity during the fermentation of chickpea-derived beverages. While peptide release was observed in the present study, no conclusions can be drawn regarding their potential bioactivity, as this aspect was beyond the scope of the applied methodology. Similarly, peptidolytic activity has been suggested to influence sensory properties in fermented quinoa-based beverages produced with *L. plantarum* LpAv [16].

Overall, the peptide profiles obtained by MALDI-TOF support the role of these strains in enhancing proteolysis and suggest potential beneficial effects in the fermented PBs. However, further studies are required to confirm these effects and to assess the possible bioactivity of the peptides generated. It should be noted that, although the detected signals are mainly attributed to peptides based on the extraction procedure and the analyzed mass range, MALDI-TOF analysis does not provide unequivocal identification, as peptide assignment typically requires tandem mass spectrometry and database matching [46]. Therefore, the contribution of other low-molecular-weight compounds cannot be completely excluded.

3.3.2. Rheological Analysis of Fermented PB

The rheological behavior of the PB was studied in both fermented (Lp 299v-PB, Lp F1B-PB and LpAv-PB) and non-fermented forms (control). Overall, the fermented samples presented higher viscosity and shear stress values than the control across most of the evaluated range. As shown in Figure 5a, viscosity decreased while the shear rate increased, a characteristic of pseudoplastic fluids [47]. In contrast, the control exhibited significantly lower and practically constant viscosity values throughout the evaluated range, indicating a behavior close to Newtonian. In terms of shear stress, the fermented samples showed a nonlinear response to increasing shear rate, while the control showed an approximately proportional relationship. Particularly, the shear stress of Lp F1B-PB showed an intermediate behavior between the other fermented products and the non-fermented control throughout the shear rate range. This differential behavior in the shear stress (Figure 5b) with higher values for PB fermented by Lp299v and LpAv than for PB fermented by LpF1B,

could indicate a more unstable structure for the later. Taken together, these results indicate that fermentation promotes the formation of internal structures that increase viscosity and generate a dependence on the applied stress, which progressively weaken under shear conditions. These findings are consistent with the rheological behavior reported by Silva et al. [48] for plant-based beverages formulated from Brazil nut combined with Baru or Macadamia, which also exhibited non-Newtonian and pseudoplastic characteristics. According to these authors, the decrease in viscosity with increasing shear rate is a desirable attribute in plant-based beverages because it facilitates flow during pumping, mixing, and heat transfer operations while maintaining higher viscosity under low-shear conditions. Such behavior contributes to product stability and improves processing performance without compromising consumer perception. Silva et al. [48] further highlighted that pseudoplastic fluids represent the typical rheological profile of many food systems and can be readily processed using conventional industrial equipment. Therefore, the pseudoplastic behavior observed in the fermented PB suggests that fermentation not only modified the beverage structure but also generated rheological properties that may be advantageous for both industrial processing and physical stability of the final product.

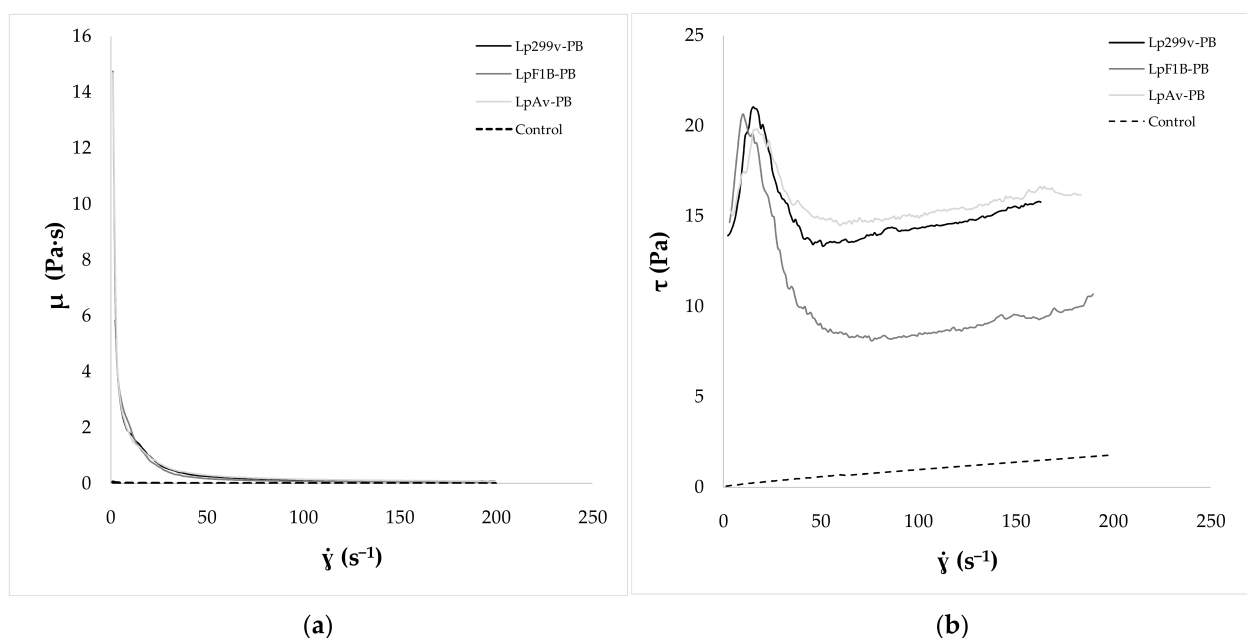


Figure 5. Apparent viscosity (μ) versus shear rate ($\dot{\gamma}$) (a), and shear stress (τ) versus shear rate ($\dot{\gamma}$) (b) for the PB, including the non-fermented control and samples fermented by Lp299v, LpF1B and LpAv.

3.4. Survival of LAB and Characterization of the Fermented PB During Shelf Life

The counts of *L. plantarum* 299v, *L. plantarum* F1B and *L. plantarum* LpAv in fermented PB were evaluated during refrigerated storage (4 °C) for 4 weeks (Figure 6). The bacterial counts at the beginning of the storage period were higher than 8.5 log CFU/mL in all fermented samples, with lower levels for LpF1B-PB compared to Lp299v-PB ($p < 0.05$). Interestingly, the levels of both plant-derived lactobacilli, LpF1B and LpAv remained stable throughout the storage period, with viable counts of 7.9 ± 0.6 log CFU/mL and 8.7 ± 0.2 log CFU/mL on the LpF1B-PB and LpAv-PB, respectively, after 28 days. Differently, the prototype PB fermented by the commercial probiotic Lp299v showed significant losses starting on the third week of refrigerated storage. However, despite reductions in viability, the final concentration of all LAB remained ≥ 8 log CFU/mL.

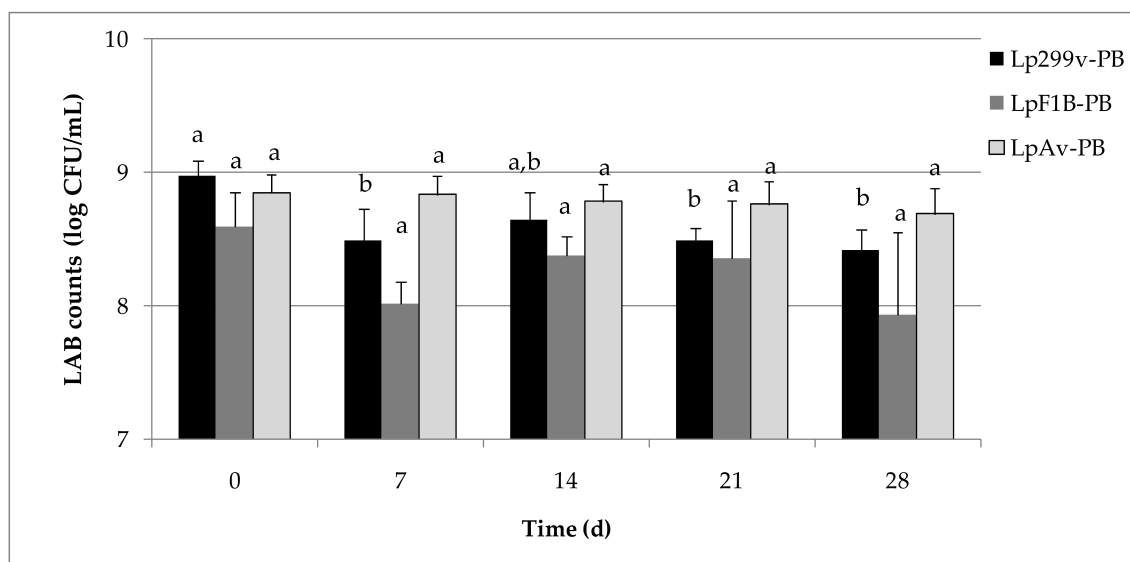


Figure 6. Total LAB counts on the peanut beverages fermented by Lp299v, LpF1B and LpAv at the beginning and after 7, 14, 21 and 28 days of storage at 4 °C. Values are means ($\pm$ SD) of five repetitions. ^{a,b} Values with different letters differ significantly ($p < 0.05$; ANOVA + Tukey's post hoc test) for each strain between times of storage.

The lactic acid concentration values measured as total titratable acidity of the fermented samples (Figure 7) displayed in general a significant increase ($p < 0.05$) during storage, but with no significant differences at each time point among the different LAB strains. Particularly, in LpF1B-PB total acidity remained stable after 2 weeks of storage whereas in the other fermented beverages acidity only stabilized in the last week. The increase in TTA was accompanied with a drop of pH for all fermented PB during the first week of storage, remaining stable until the end of the storage period (pH after 28 d: 3.8 ± 0.1 ; 3.7 ± 0.2 and 3.7 ± 0.2 for Lp299v-PB, LpF1B-PB and LpAv-PB, respectively). Finally, SS did not change along the refrigerated shelf life of the products.

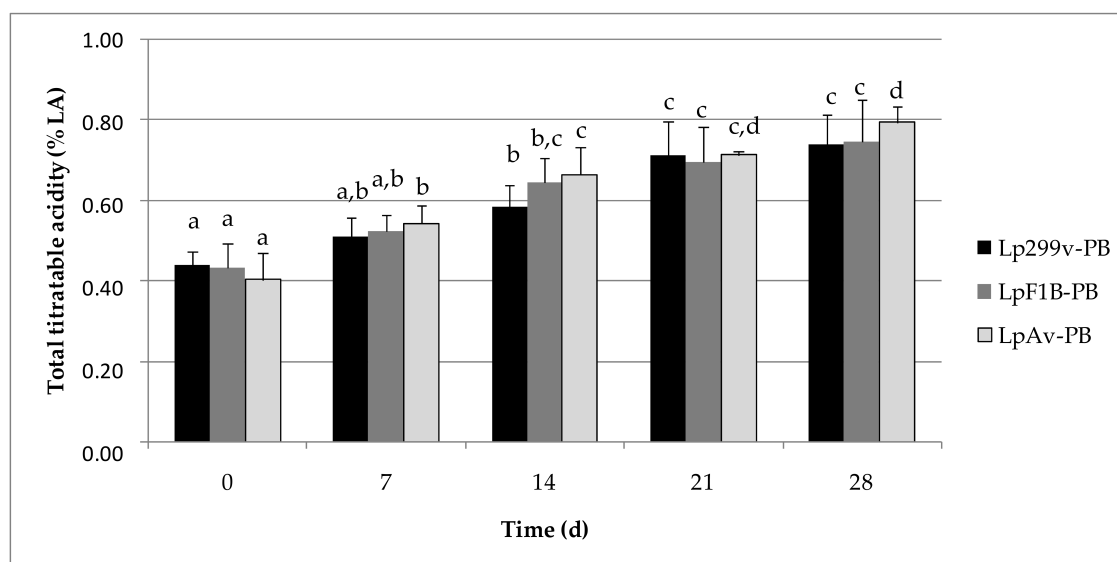


Figure 7. Total titratable acidity on PBs fermented by Lp299v, LpF1B and LpAv at the beginning and after 7, 14, 21 and 28 days of storage at 4 °C. ^{a,b,c,d} Values with different letters differ significantly ($p < 0.05$; ANOVA + Tukey's post hoc test) for each strain between times of storage.

All the attributes measured during storage show that the fermented prototypes are stable for at least a month at refrigerated temperature. It is also important to note that the rheological studies (performed at the midpoint of storage) showed that the fermented PB had a pseudoplastic behavior, a desirable characteristic for a plant-based fermented product [48]. The development of plant based yogurt analogs usually has to overcome major challenges related to appearance and texture since these products generally have low viscosity and phase separation [49]. In our case, the fermented PB had a satisfactory texture without the necessity of adding stabilizers or gelling agents. Until the end of the storage period the gel-like structure of the beverages remained stable, with scarce syneresis. Several lactic acid bacteria, including strains of *L. plantarum*, have the ability to produce exopolysaccharides. EPS produced by lactobacilli have been reported to be involved in various biological functions, such as adhesion to human cells or to other bacteria, and immunomodulation [50]. When utilized as starter cultures or probiotics, this capacity of some LABs is reported to have a significant impact on the rheology and texture of fermented foods [51]. Zepeda-Hernández et al. [52] have reported the usefulness of producing skyr-type yogurts with the probiotic strain *L. plantarum* 299v. Besides its functional characteristics, the production of EPS by Lp299v showed positive effects on attributes of the product such as creaminess, shininess, and reduced syneresis. Moreover, Bulca & Büyükgümü [27] produced yogurt analogs with peanut extract and explored the addition of microbial transglutaminase and/or the utilization of EPS-producing cultures to overcome the challenges related to texture. This later strategy resulted in an improved microstructure of peanut yogurt gels. Our PB was formulated with sucrose, a key carbon source for the EPS production by *L. plantarum* strains [51,53]. Considering this, EPS synthesis could explain some of the characteristics of our fermented PB and should be further studied in the future.

Survival of LAB to Simulated Gastrointestinal Digestion

At t0 (right after fermentation), all three *L. plantarum* strains withstood the GID simulation without significant viability losses (Figure 8).

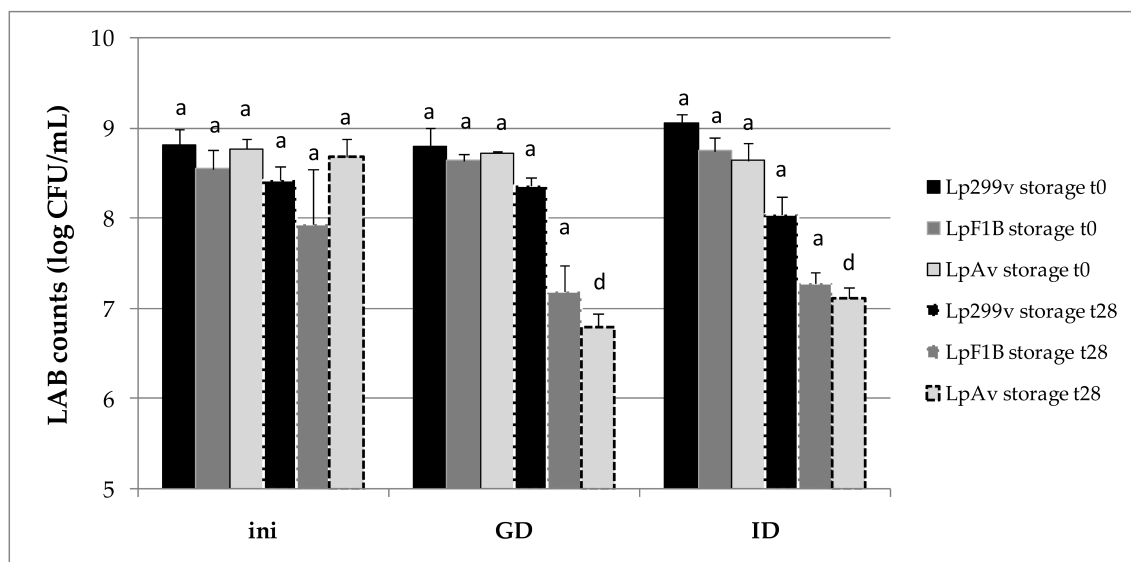


Figure 8. Total LAB counts on the PBs fermented by Lp299v, LpF1B and LpAv at the beginning (t0) and after 28 days (t28) of storage at 4 °C (ini), and after simulated gastric (GD) and intestinal digestion (ID) according to INFOGEST protocol. ^{a,d} Values with different letters differ significantly ($p < 0.05$; ANOVA + Tukey's post hoc test) for each strain at t0 or t28.

After 28 d at 4 °C, the microorganisms exhibited different behaviors. As mentioned in Section 3.4, LpF1B levels showed a not statistically significant decrease of approximately 0.7 log CFU/mL at the end of the storage period compared to initial values ($p > 0.05$). The high tolerance to both low pH in the stomach and high pH as a result of the presence of bile salts in the duodenum, is one of the many features that makes Lp299v a highly esteemed probiotic [54]. During the GID simulation after refrigerated storage, Lp299v remained stable after each stage. Similarly, LpF1B maintained its viability and no significant losses were observed after the GD and ID steps ($p > 0.05$). In contrast, while LpAv values only decreased by 0.1 log CFU/mL during storage, after 28 d the strain was highly sensitive to the GD stage, with a significant decrease in values from 8.7 ± 0.2 to 6.8 ± 0.2 log CFU/mL ($p < 0.05$). The ID stage did not further affect this decrease. These differences in the susceptibility to simulated GID suggest that the survival depends on the intrinsic characteristics of each particular strain. The differences in the accumulation of lactic acid during the storage period, with TTA being stable for LpF1B-PB from the second week onwards whereas for LpAv-PB it went up gradually until the last week, could explain the lack of adaption of the oat-derived strain to the acidic environment and consequently a higher sensitivity to simulated GD.

The use of probiotics in foods requires that the microorganisms survive processing and storage, along with gastrointestinal transit [55]. In order to efficiently exert a benefit, probiotics must reach their site of action in sufficient numbers where the active cells can produce active metabolites such as lactic acid and interact with the host and gut microbiota [56]. It is not possible to define a general dose for probiotics since it should be based on human studies showing a health benefit [57]. However, a concentration of 10^9 CFU per serving is the consensus in some countries as the minimum dose for probiotics [58]. The high survival during the in vitro gastric phase observed in this study suggests that particularly LpF1B may have the capacity to reach its site of action in levels comparable to a probiotic commercial strain such as *L. plantarum* 299v. It is convenient to note that the survival in the gastrointestinal tract is a desirable trait but it is not a requisite to qualify as a probiotic, the condition to be regarded as a probiotic is the capacity to confer a health benefit when administered alive to the host.

3.5. Validation of the ‘Vegan-Friendly’ LAB Cultures for the Development of a Prototype Fermented PB

Frozen (F) and freeze-dried (L) cultures were inoculated on the formulated PB and pH, SS (°Bx) and TTA (%) were measured at the end point of fermentation. The fermentation times were the same as those observed for fresh cultures, about 6 h at 37 °C. The pH values and SS were comparable for the three formats, for each LAB strain. Regarding TTA, significantly lower concentrations of lactic acid were detected for F and L in Lp299v-PB and LpF1B-PB, whereas beverages fermented with LpAv showed similar titratable acidity on its three culture formats (Figure 9). When each format was individually compared among the three strains of *L. plantarum*, LpF1B produced significantly less lactic acid in the F format (vs. Lp299v and LpAv) and in the L format (vs. LpAv). It should be noted that, even if F and L cultures were diluted before inoculation in order to imitate the experiences with the fresh cultures on the PB, slightly higher initial LAB counts were detected for L inoculated beverages compared to fresh ones ($p < 0.05$). These results showed that even if LAB cultures are stable in the frozen or freeze-dried format, performance tests should always be conducted to validate that their functionality has not been affected by the technological processing.

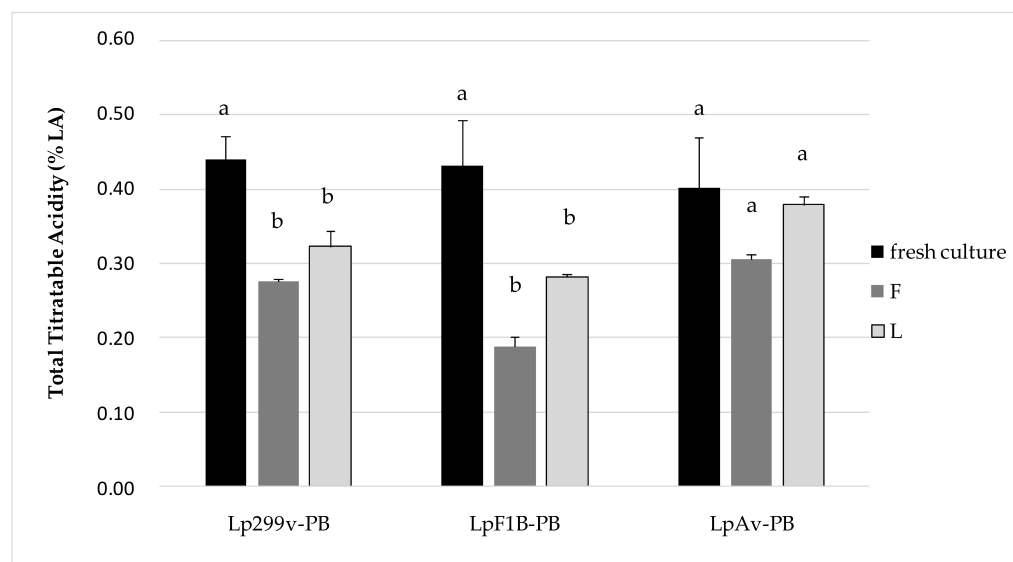


Figure 9. Total titratable acidity (expressed as % lactic acid) on the PB fermented by Lp299v, LpF1B and LpAv as fresh, frozen (F) or freeze-dried (L) cultures. ^{a,b} Values with different letters differ significantly ($p < 0.05$; ANOVA + Tukey's post hoc test) for each strain between formats of the inoculated culture.

The “live dietary microbes” concept refers to the idea that foods naturally containing living microorganisms, especially fermented foods, may support human health by interacting with the gut microbiome and the gut-associated immune system. Rather than focusing only on isolated probiotic microorganisms, this concept emphasizes that the regular consumption of microbe-rich foods as part of a normal diet is able to improve digestion, immune function, metabolism, and overall microbial diversity in the gut. The concept was developed after a pioneering publication made by Marco et al., where a classification system was proposed [59]. Since then, more than 100 publications can be found in PubMed, using the searching words “Live Dietary Microbes”, associating the intake of food and beverages rich in live microbes with a diversity of positive health outcomes.

4. Conclusions

Considering all results together both LAB isolated from plant materials showed technological and functional promising characteristics to be used on the development of a peanut fermented beverage. In particular, LpF1B showed to be a robust strain during gastrointestinal simulation, whereas LpAv showed great stability to be produced as a ‘vegan-friendly’ culture, maintaining its capacities as F or L culture, resistant to low pH along storage and conferring positive characteristics to the beverage rheology and functional characteristics. The nutritional properties of peanuts, together with the fact that it supports the growth and survival of lactic acid bacteria in a fermented beverage, positions peanuts, a relatively underexplored substrate, as a candidate which deserves further studies in the space of fermented functional foods. The fermented peanut beverage also contributes to the emerging concept of “Live Dietary Microbes” [60].

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fermentation12070335/s1>, Figures S1: MALDI-TOF mass spectra of pH 4.5-soluble extracts from peanut beverage fermented with Lp299v (Trial 1): (a) 500–1000 m/z ; (b) 1000–1500 m/z ; (c) 1500–2000 m/z ; and (d) 2000–2500 m/z ; Figures S2: MALDI-TOF mass spectra of pH 4.5-soluble extracts from peanut beverage fermented with LpAv (Trial 1): (a) 500–1000 m/z ; (b) 1000–1500 m/z ; (c) 1500–2000 m/z ; and (d) 2000–2500 m/z ; Figures S3: MALDI-TOF mass spectra of pH 4.5-soluble extracts from peanut beverage fermented with LpF1B (Trial 1): (a) 500–1000 m/z ;

(b) 1000–1500 m/z ; (c) 1500–2000 m/z ; and (d) 2000–2500 m/z ; Figures S4: MALDI-TOF mass spectra of pH 4.5-soluble extracts from non-fermented control (Trial 1): (a) 500–1000 m/z ; (b) 1000–1500 m/z ; (c) 1500–2000 m/z ; and (d) 2000–2500 m/z ; Table S1: Fermentation time (h), pH and LAB counts of the preliminary tests to produce a fermented PB.

Author Contributions: Conceptualization, M.F.Z., M.P. and G.V.; methodology, M.F.Z., J.M.O. and G.H.P.; validation, M.P., G.H.P., J.d.R. and M.F.Z.; formal analysis, J.d.R., D.L.H.T., S.B., G.H.P. and M.F.Z.; investigation, M.P., G.H.P., J.d.R., D.L.H.T., S.B. and M.F.Z.; resources, M.F.Z., J.M.O. and G.V.; data curation, M.F.Z.; writing—original draft preparation, M.F.Z., G.H.P. and M.P.; writing—review and editing, G.V.; visualization, M.F.Z.; supervision, M.F.Z. and G.V.; project administration, M.F.Z.; funding acquisition, M.F.Z. and G.V. All authors have read and agreed to the published version of the manuscript.

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