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Development of Probiotic Fermented Sausages and Viability Monitoring of Supplemented *Lactiplantibacillus plantarum* BFL Strain

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Abstract: The reformulation of meat products is a pending task for the scientific-technological sector. Fermented meat products can carry probiotics, and studying their effect during the product shelf life currently represents a large area of vacancy. The objective of this work was to study the viability of microencapsulated (E) and unencapsulated (P) *Lactiplantibacillus plantarum* BFL as well as their effects on the microbiological and physicochemical parameters of fermented sausages preserved at 20 °C and 5 °C during 60 days of storage. The inoculated sausages (P and E) had significantly reduced pH values and potential pathogenic microorganism counts. The viability of encapsulated *L. plantarum* BFL (E) did not decline during storage as it did in its unencapsulated state (P). In addition, *L. plantarum* BFL could present an antioxidant effect at 20 °C towards the end of storage. The probiotic *L. plantarum* BFL generally tolerated the meat matrix conditions; it could be used as a biocontroller since its high viability rates would allow it to be projected as an adjunct culture for the meat industry. However, spray-drying microencapsulation of the probiotic *L. plantarum* BFL is not recommended as a viability-enhancing strategy in the Salamines Criollos studied in this work.

Keywords: probiotics; lactic acid bacteria; human health

1. Introduction

Many modern industrial practices that have been key to eliminating foodborne illnesses have also reduced human exposure to beneficial microorganisms. In this context, the consumption of fermented foods with live cultures contributes to the exposure of humans to microorganisms. In this way, humans could incorporate variability and a quantity of microorganisms into the intestinal microbiota. Fermented foods could mimic some of the beneficial effects of probiotic foods. However, despite many fermented foods containing live microorganisms, many of these microorganisms may not meet the minimum criteria to be classified as probiotics [1]. In this context, reformulated meat products are a pending task for the scientific-technological sector [2,3]. Meat products profiled as functional foods could become an interesting strategy to cover this need. One way to confer functionality to a food is by adding specific probiotics to its formulation [4]. Probiotic sausages have a high

degree of safety due to their ability to inhibit pathogenic microflora and reduce protein transformation and lipid oxidation, all of which are hazardous to health. The doses and viabilities of probiotics in meat products become crucial factors to guarantee benefits to the consumer by modulating their gut microbiota. Shelf-life studies of probiotic meat products demonstrated that they have good sensory qualities for up to 6 months, and they are marked with a high level of safety [5,6]. Furthermore, multiple factors must be considered when a probiotic meat product is designed [7]. According to others, effective probiotics should contain at least 7 log CFU/g of eaten food [2,3]. When considering this aspect, strategies such as the microencapsulation of probiotics have shown improvements related to the viability of probiotic strains during the processing and storage stages. Microencapsulation has proven effective in protecting sensitive bacteria from harsh environments where they cannot normally survive. In meat products, a promising scenario has been provided by some studies for advances in this strategy based on the microencapsulation of probiotics. Muthukumarasamy and Holley [8] studied the microbiological and sensory qualities of dry fermented sausages containing *Lactobacillus reuteri* microencapsulated in alginate, with the authors concluding that the encapsulation process allowed the probiotics to survive in sufficient quantities to be considered functional. Years later, Oliveira Gomes et al. [9] found that *Bifidobacterium animalis* ssp. *lactis* (BB-12) encapsulated by spray drying did not affect the quality and sensory characteristics of Italian salami, remaining viable during 45 days of storage. Despite the aforementioned studies, there are still very few studies that are focused on the viability of microencapsulated probiotic strains in meat matrices.

Salamín Criollo is a traditional fermented sausage from the Litoral area in Argentina. It has a diameter of 2.5–3.5 cm, a length of 13–15 cm, and the fat (bacon) is characterized by being coarsely chopped. Using this popular meat product as a vehicle for probiotics represents an interesting strategy for beneficial bacteria to be reached by most of the population.

Industrial Salamines Criollos can maintain *L. plantarum* BFL viability during storage by keeping the required dose to benefit the consumer. This viability can be enhanced by the microencapsulation of *L. plantarum* BFL. Therefore, the objective of this work was to study the viability of microencapsulated and unencapsulated *L. plantarum* BFL as well as their effects on the microbiological and physicochemical parameters of fermented sausages (Salamines Criollos) preserved at 20 °C and 5 °C for 60 days of storage.

2. Materials and Methods

2.1. Bacterial Cultures

The strain used as a probiotic, *Lactiplantibacillus plantarum* BFL, was isolated (only for research purposes) from the Bioflora ™ product (BIOSIDUS S.A, Buenos Aires, Argentina), which is marketed in Argentina as a probiotic with corresponding health certifications. The technological properties of different commercial probiotics were evaluated in vitro to perform as a probiotic culture in a dry cured sausage matrix. In addition, the possible implicit factors (antagonisms, synergisms, etc.) of the probiotic microorganisms with the members of the starter inoculum and pathogens were determined. In all the tests mentioned above, *L. plantarum* BFL showed a good performance.

An overnight MRS broth of *L. plantarum* BFL was used to inoculate 80 mL of sterile MRS broth at 2% (*v*/*v*), which was incubated at 37 °C for 18 h in aerobiosis. This pure *L. plantarum* BFL culture was used to inoculate, at 2% (*v*/*v*), 4 L of sterile economic culture medium prepared in a bioreactor (BIOSTAT® A Sartorius Stedim Biotech, mod. BB-8822000,Guxhagen, Germany). The formulation of the economic medium was as follows: 60 g/L whey permeate (VARIOLAC 850, Arla Foods, Porteña, Argentina); 10 g/L casein peptone (Microkin S.R.L., Santa Fe, Argentina); 8 g/L yeast extract (Biokar Diagnostics, Beauvais, France); 1 mL/L tween 80; and 0.05 g/L magnesium sulfate monohydrate ($MnSO_4$) (Merck, Darmstadt, Germany). Fermentation in the bioreactor was carried out at 37 °C under continuous agitation (120 rpm) and a constant pH of 6.0 ± 0.2 by automatic titration with a sterile 6 N NaOH solution for 18 h. Once the biomass production was finished, the concentration of the culture was evaluated by counting on a plate of

MRS agar medium (Oxoid, Basingstoke, United Kingdom) from decimal dilutions (0.85% physiological solution). After 18 h, the entire culture from the bioreactor was removed and centrifuged (at 8 °C for 10 min at 6000× *g*, Thermo Scientific™ Sorvall™ RC 6 Plus Centrifuge). The supernatant was discarded, and the pellet was resuspended in phosphate-buffered saline (PBS) (138 mM NaCl, 3 mM KCl, 8.1 mM Na_2HPO_4 (Biopack, Buenos Aires, Argentina) and 1.5 mM KH_2PO_4 (Biopack, Buenos Aires, Argentina)) to wash the cells. This procedure was repeated twice consecutively. With the obtained pellets, two total inocula of 13.3 ± 0.1 log CFU were prepared. Inoculum P: The pellet was mixed with a 10% whey protein isolate and a maltodextrin (WPI-MTX) cryoprotective solution and then preserved in an ultrafreezer at −80 °C for one week. The WPI (BIPROTM) was generously provided by Davisco Foods International Inc. (Le Sueur, MN, USA). The composition was 0.4% (*w*/*w*) fat, less than 0.5% (*w*/*w*) lactose, 2.0% (*w*/*w*) ashes, and 4.8% (*w*/*w*) moisture. The WPI protein content determined by the Kjeldhal method was 97.9% (*w*/*w*) (dry basis). The brand of corn maltodextrin was El Bahiense (Biotechnology y co., Huanggang, Hubei, China). Inoculum E: The pellet was mixed with 563 g of WPI-MTX, which formed the wall material of the microcapsules. The final WPI-MTX-pellet solution was at 40%, and it was fed to a laboratory Spray Dryer (Mini Spray Dryer ADL311S, Yamato, Japan). In this way, 330 g of a probiotic powder to be added to meat batter was obtained. The best drying conditions were previously determined (inlet temperature: 160 °C, outlet temperature: 65 °C). Microbiological counts were carried out before centrifugation, on the wet pellet, on the WPI-MTX solutions, and in a powdered inoculum.

2.2. Salamines Criollos Preparation

An 100 kg amount of already-homogenized meat batter (lean, fat, condiment, and additives) was provided by a local company (town of Esperanza, Santa Fe, Argentina) where the Salamines Criollos were made. The product formulation was as follows: bacon 25.09%, pork shoulder 15.05%, pork trim 25.09%, chicken thigh leg 10.04%, heart 17.56%, BQS 0.50%, corn starch 1.51%, sugar 0.15%, spices 3.51%, soy flour 1.51%, and 150 ppm $NaNO_3$. The 100 kg of meat paste was divided into three equal parts (33.3 kg), and three treatments were prepared: encapsulated probiotic treatment (E)—Salamín Criollo with experimental starter RC20 and the addition of 330 g of probiotic powder; free probiotic treatment (P)—Salamín Criollo with experimental starter RC20, with the addition of 110 mL of P inoculum and 496 g of sterile WPI-MTX powder (298 g WPI and 198 g MTX); control—Salamín Criollo with experimental starter RC20 and the addition of 563 g of sterile WPI-MTX powder (346 g WPI and 217 g MTX). RC20 is an experimental starter that is composed of *Staphylococcus xylosus* and *Pediococcus pentosaceus*. The different amounts of WPI-MTX were added to treatments P and control to equalize the chemical sample compositions since the P inoculum had WPI-MTX in its formulation. The pastes were stuffed into natural bovine casings with diameters of 25 mm. Once the products were stuffed, they were kept in the dryer for 5 days. The drying conditions were the following: 20 ± 1 °C and 72 ± 1% relative humidity (RH). The probiotic counts in the ready-to-eat products were 8.8 ± 0.1 log CFU/g (P) and 8.3 ± 0.2 log CFU/g (E). On the fifth day, all samples were removed from the dryer and left at room temperature (23 ± 1 °C) until day 9, when samples were vacuum-packed then kept at room temperature (20 °C) or under refrigeration (5 °C) for 60 days. Day 9 of preparation corresponds to day 0 of this current trial.

2.3. Characterization of L. plantarum BFL Microcapsules

The spray-dried WPI-MTX particles were placed onto carbon tapes on aluminum sample stabs. The morphologies of the WPI-MTX particles were observed using a Phenom World ProX microscope (Phenom-World, Eindhoven, Brabant, The Netherlands) in detector mode for backscattered electrons with an operating voltage of 10 kV in ultrahigh vacuum.

2.4. Experimental Design

On days 0, 15, 30, 45, and 60 of storage, three experimental units were taken from each treatment (control, P, and E) and from each temperature (20 °C and 5 °C) to perform the following determinations: pH changes, microbiological analysis, lipid oxidation, color measurement, and texture analysis.

2.5. Determination of pH

The pH values were determined at room temperature (24 ± 1 °C) with the pH/mV/Temp Meter (Altronix TPX-III™, Taipei, Taiwan) equipped with a spear-type gel electrode (Oakton ao-35805–18, Vernon Hills, IL, USA). The measurements were made directly and taken three times by changing the electrode insertion place each time.

2.6. Lactiplantibacillus plantarum BFL Strain Survival

Lactiplantibacillus plantarum BFL counts were determined using *Lactobacillus plantarum* selective medium (LPSM) at 37 °C for 72 h in anaerobiosis [3,4,10].

2.7. Microbiological Analysis

Microbiological analyses were established as described by Sirini et al. [4]. A 5 g aliquot of each sausage sample was aseptically obtained and then homogenized with 45 mL of sterile saline (8.5 g NaCl/l deionized water) in a Stomacher® 80 Biomaster (Seward, London, UK) for 2 min. Aliquots were ten-fold serially diluted in sterile saline and plated. LAB were determined using DeMan, Rogosa, and Sharpe agar (MRS) (Biokar, Beauvais, France) after 72 h at 37 °C in anaerobiosis. Enterobacteria were determined using Violet Red Bile Glucose Agar at 37 °C for 24 h in aerobiosis (Oxoid, Basingstoke, UK). The *E. coli* counts were determined using Tryptone Bile X-glucuronide Agar at 44 °C for 24 h in aerobiosis (Biokar, Beauvais, France), and *Staphylococcus* mannitol salt positives were determined in mannitol salt agar at 37 °C for 48 h in aerobiosis. The Mannitol salt agar was prepared by components according to the following formulation (g/L): Meat extract 1.0 (Microquin, Buenos Aires, Argentina); Peptone protease 10.0 (Oxoid, Basingstoke, UK); NaCl 75.0 (Biopack, Buenos Aires, Argentina); D-mannitol 10.0 (Mallinckrodt, Buenos Aires, Argentina); Phenol red 0.0250 (Mallinckrodt, Buenos Aires, Argentina); Bacteriological agar 15.0 (Biokar, Beauvais, France). The final Mannitol salt agar pH was 7.4 ± 0.2. The enumeration results were recorded as log CFU/g sausage sample.

2.8. Evaluation of Lipid Oxidation

Lipid oxidation was evaluated as a function of changes in 2-thiobarbituric acid-reactive substances (TBARs) in storage, following the method by Rosmini et al. [11].

2.9. Color Measurement

The determined CIELAB coordinates were lightness (L*), the red/green co-ordinate (a*), and the yellow/blue co-ordinate (b*), from which the psychophysical magnitudes hue (h*) and chrome (C*) were calculated (UNE 72–031, 1983). A Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer with illuminant D65, 10° observer, SCI mode, 11 mm aperture of the instrument for illumination, and 8 mm aperture for measurement was used.

2.10. Texture Profile Analysis

The texture profile analysis (TPA) was performed with an INSTRON texture analyzer (Universal Testing Machine, 3342, EUA) in accordance with Herrero et al. [12]. The texture profile parameters hardness, cohesiveness, springiness, and chewiness were determined.

2.11. Statistical Analysis

All data collected during the storage process were evaluated by applying an ANOVA according to a factorial design. The applied factors were: treatment (control group, free

probiotic, and encapsulated probiotic), storage temperature (room temperature at 20 °C and refrigeration temperature at 5 °C), and storage time (0, 15, 30, 45, and 60 d). For these analyses, InfoStat software version 2020 (National University of Córdoba, Province of Córdoba, Argentina) for Windows was used. The level of $p < 0.05$ was used to define the significant differences. Three complete replicates of the study were performed ($n = 3$). All determinations were performed in triplicate. The trial plan was a completely randomized design.

3. Results and Discussion

3.1. Characterization of L. plantarum BFL Microcapsules

SEM images of the spray-dried *L. plantarum* BFL microcapsules are illustrated in Figure 1. The electron microscopy studies showed that *L. plantarum* BFL cells were successfully entrapped inside WPI-MTX solids, leaving no cells outside. Some of these particles showed pores and concavities, commonly observed in spray-dried powders, due to the rapid evaporation of water from droplets [13,14] (Figure 1B,C). However, the probiotic powders showed no visual fractures or cracks on their surfaces. This confirms the formation of a protective film around the probiotics and a high encapsulation efficiency after the drying process. The microcapsules showed a partially spherical shape 2–15 µm in diameter, which is consistent with other authors' reports [15] (Figure 1A).

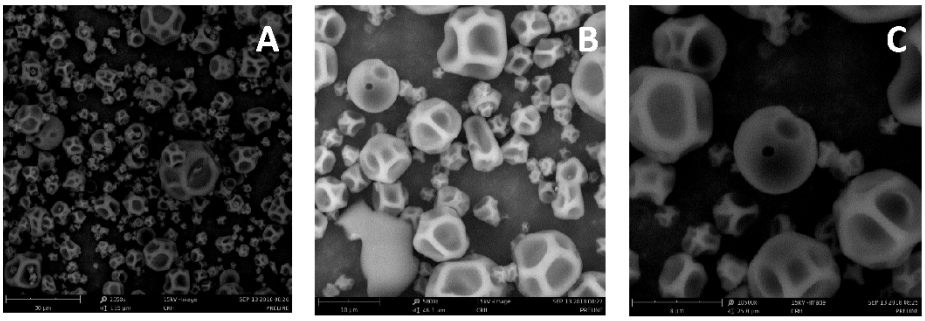

Figure 1. SEM photomicrographs of spray-dried encapsulates corresponding to the WPI/MDX systems: (**A**): magnification of 2350×; (**B**): magnification of 5800×; (**C**): magnification of 10,500×.

3.2. Changes in pH

Figure 2A,B show the results of the changes in pH during storage at 20 °C and 5 °C, respectively. The presence of encapsulated (E) and unencapsulated (P) *L. plantarum* BFL showed different pH values compared to the control throughout the 60 d at both studied temperatures ($p < 0.001$) [16]. This difference in the pH values was a consequence of a greater acidification caused by the probiotic (P and E) during the drying stage, which was maintained during the entire storage period. On the other hand, the storage temperature of Salamines Criollos did not affect the behavior of the pH of the control group or treatment P, but it did affect treatment E. Small differences in pH when encapsulating the probiotic and preserving the samples at 5 °C compared to 20 °C were observed ($p = 0.027$). This could be because possible cell damage caused by spray drying leads to decreased lactic acid production during regrowth. For this reason, the acidifying power of encapsulated *L. plantarum* BFL could be slowed by keeping Salamines Criollos at room temperature, where there were lower microbial counts. The similarity in acidification for the two treatments with probiotics (P and E) may be due to the fact that the capsules disintegrated from the moment they were incorporated into the meat matrix. This may be due to the water-soluble nature of the capsule and the high initial moisture content of the paste. Although one could initially assume that all capsules will lose integrity, the differences in pH between the probiotic treatments during storage at 20 °C suggests that at least a part of them could remain intact. However, more studies need to be conducted to confirm or rule out these claims. Oliveira Gomes et al. [9] studied the application of microencapsulated

Bifidobacterium animalis ssp. *lactis* BB-12 in Italian salami assuming that these encapsulated bacteria may not have contributed to the lowering and raising of the pH. Rather, the authors understand that the changes in pH must have been caused by lactic acid bacteria naturally present in the meat.

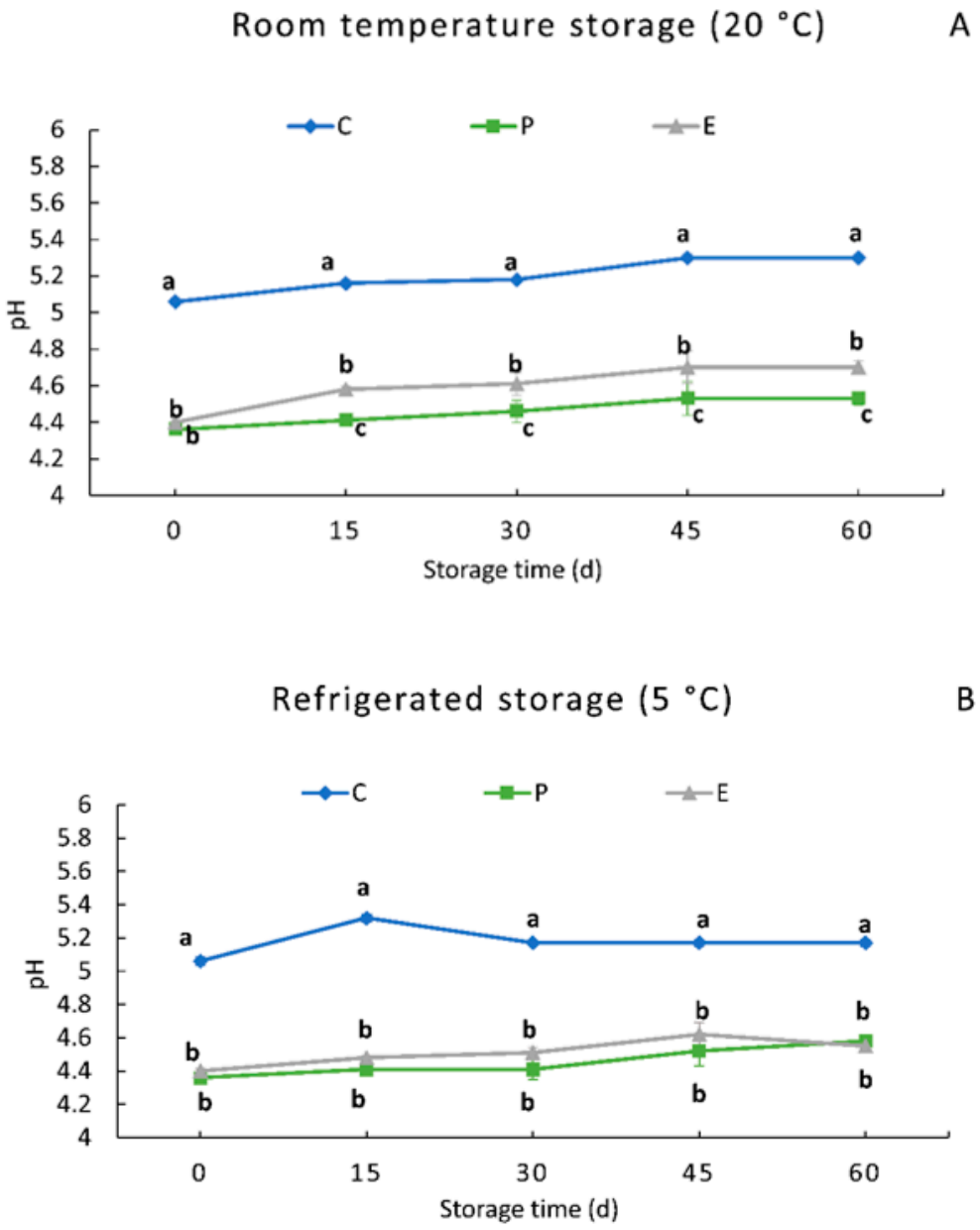

Figure 2. (**A**): Evolution of pH (means ± standard deviations) during room temperature storage (20 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). (**B**): Evolution of pH (means ± standard deviations) during refrigerated storage (5 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). The different letters indicate significant differences ($p < 0.05$) between treatments on the same day under the same storage temperature.

3.3. Monitoring L. plantarum BFL Strain Survival

The different treatments affected the viability of *L. plantarum* BFL during storage ($P < 0.001$). Up to day 30 and at both storage temperatures studied, higher counts of *L. plantarum* BFL were reached in the free probiotic treatment (P) compared to the encapsulated probiotic treatment (E) (Figure 3A,B). However, from day 45 the *L. plantarum* BFL counts

were equal for P and E both at room temperature (p = 0.875) and under refrigeration (p = 0.1013). The viability of encapsulated *L. plantarum* BFL (E) did not decline in the final stage of storage as it did in its unencapsulated state (P); therefore, there could be improvements in the viability of the probiotic due to encapsulation towards the end of shelf life. On the other hand, lactic acid bacteria are microorganisms commonly found in fermented sausages that are subject to a great variability in species depending on the region in which the products are manufactured. Regarding the Salamín Criollo studied in this article, *L. plantarum* sp. was found as a native strain in the control group. However, this did not represent any inconvenience when counting the inoculated probiotic strain since the native *L. plantarum* sp. was not a dominant strain, being detected in much lower counts compared to the P and E treatments (Figure 3A,B). The bibliography regarding the viability of food probiotics preserved at different temperatures is still scarce. However, Gardiner et al. [17] stated that the probiotic bacteria viability during storage is inversely related to the storage temperature, so probiotic food products should preferably be stored at a temperature of 4–5 °C. This statement is consistent with the findings in this work since the *L. plantarum* BFL counts were affected by the interaction between the storage time and the storage temperature. Higher counts of *L. plantarum* BFL were reported when Salamines Criollos were kept under refrigeration compared to storage at room temperature ($p < 0.001$). In addition, the survival of *L. plantarum* BFL above 7.7 log CFU/g for 60 days was observed by keeping Salamines Criollos refrigerated (5 °C) for both treatments (P and E). This result is promising since a population level > 6 log CFU/g is an essential probiotic dose for the development of a functional food [18]. However, similar probiotic counts were achieved at room temperature only after 15 days of storage. Salamín Criollo is considered an intermediate-moisture food (water activity < 0.90); therefore, it can be stored at room temperature. However, when the Salamín Criollo formulation is altered by adding *L. plantarum* BFL, its conservation under refrigeration should be recommended to ensure a high dose of the probiotic at the time of consumption. Ben Slima et al. [19] studied the viability of *L. plantarum* TN8 as a partial substitute of nitrite in a beef sausage formulation for 10 days at 4 °C, finding that probiotic counts increased by more than 1 log CFU/g. On the other hand, Blaiotta et al. [20] showed in their investigations that *L. plantarum* 299v, as an adjunct, maintained a concentration greater than 10^6 CFU/g in bovine salami after 60 days of storage at 4 °C. Pavli et al. [21] studied the inoculation of *L. plantarum* L125 with commercial starter cultures in Greek fermented sausages, finding that the strain maintained high population levels (>6 log CFU/g) during storage at 4 °C and 12 °C for 182 days. It is known that the genus and species *Lactiplantibacillus plantarum* is capable of adapting, surviving, and developing in meat matrices. The strain studied in this work, *L. plantarum* BFL does not escape this generality. The results during storage for treatment E at both storage temperatures (5 °C and 20 °C) revealed that *L. plantarum* BFL counts remained constant until day 30. However, for treatment P under refrigeration (5 °C), it was observed that *L. plantarum* BFL counts remained constant until day 45, while at room temperature (20 °C) the viability losses were staggered from 15 d to 60 d. Although probiotic viability improvements after day 45 could be attributed to microencapsulation, this is not a strong enough result to justify the spray-dry encapsulation of *L. plantarum* BFL as a viability enhancement. This is because spray-dry encapsulation entails high input costs and previous production steps that do not represent a significant improvement in the viability of the probiotic, which showed a high rate of viability as a free cell. In addition, a meta-analysis based on LAB viability in different refrigerated matrices, which was recently published, reported that the application of preservation methods such as immobilized, encapsulated, or freeze drying were not effective in protecting LAB [22]. This could be because in foods with fresh cultures the free microorganisms could grow during the storage period, while the application of preservation methods would generate stress on the cells by decreasing their counts. In this sense, preservation methods could be more effective in foods preserved for a time longer than 30 d, especially in foods with low aqueous activities, as shown in this article.

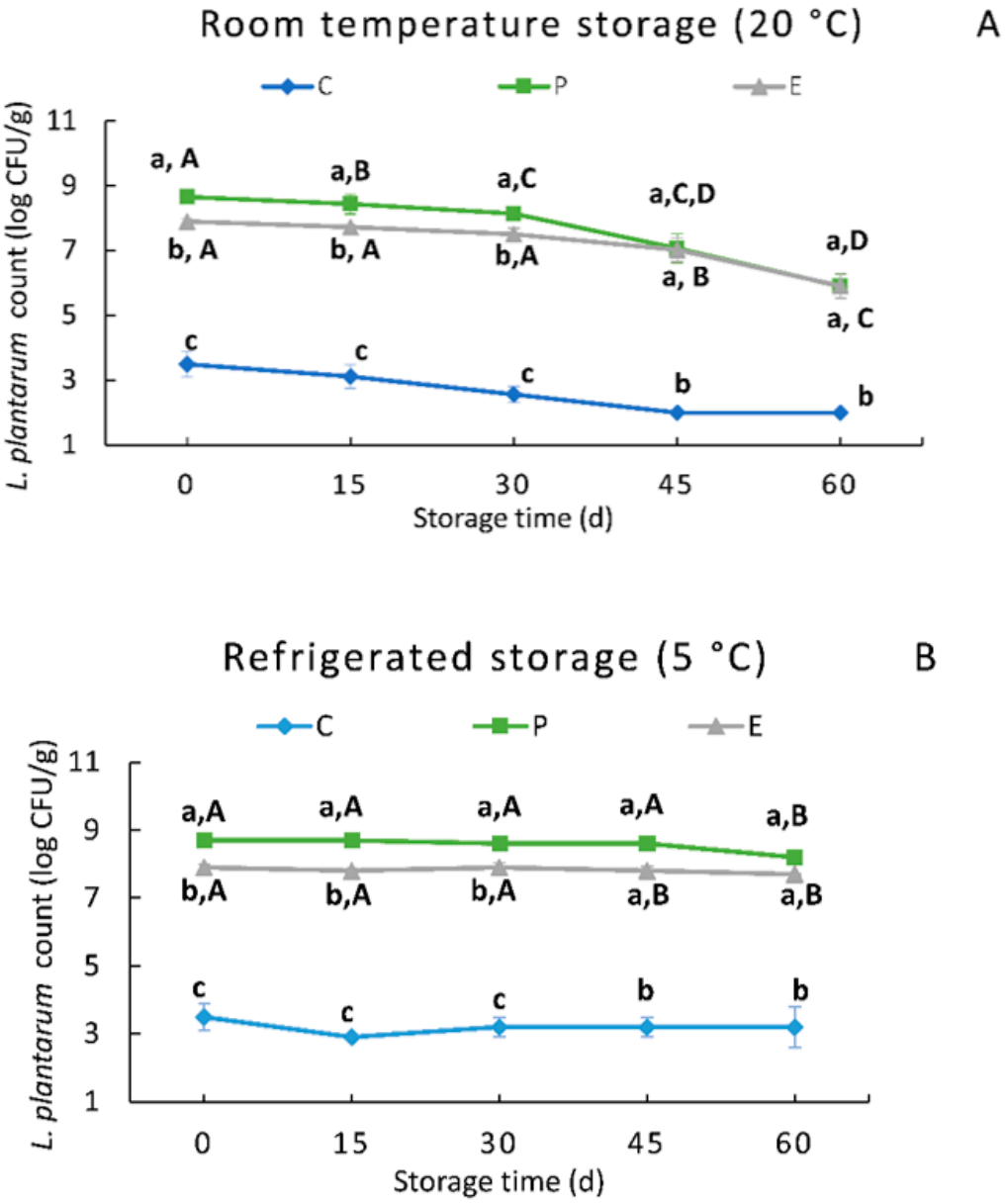

Figure 3. (**A**): Viability of microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P) (means ± standard deviations) during 60 d of room temperature storage (20 °C) of Salamines Criollos. (**B**): Viability of microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P) (means ± standard deviations) during 60 d of refrigerated storage (5 °C) of Salamines Criollos. Different capital letters express significant differences ($p < 0.05$) within the same treatment throughout the 60 days. Different lowercase letters express significant differences between treatments on the same day.

3.4. Microbiological Analysis

The LAB counts were affected by the interaction between the treatment and the storage time ($p = 0.005$). However, the LAB behaviors were similar in the three studied groups (control, P, and E) since a decrease in the LAB count was observed during the entire storage period. On the other hand, there was also an interaction between the storage time and the storage temperature ($p < 0.001$) (Figure 4A,B). There were no differences in LAB counts in Salamines Criollos preserved under refrigeration during the 60 days of storage. However, when the samples were kept at 20 °C, a difference in LAB counts between the treatments was observed. These results are consistent with the stability of the probiotic *L. plantarum* BFL under refrigeration. Figure 5A,B show the viability of mannitol-salt-positive *Staphylococcus*

bacteria (MSPS) during 60 d of storage at room temperature (20 °C) and under refrigeration (5 °C), respectively. The MSPS counts were affected by interactions between the treatment and storage time ($p < 0.001$), the treatment and storage temperature ($p < 0.001$); and the storage time and storage temperature ($p < 0.001$). For the control group and the P and E treatments at 5 °C and 20 °C, a general trend of MSPS count decrease was observed during the entire storage period. On the other hand, the P treatment reached lower MSPS counts compared to the control group throughout the 60 days of storage at 20 °C and 5 °C. This result could be beneficial in bromatological terms since the mannitol-salt-positive *Staphylococcus* bacteria could be directly related to the count of a pathogen commonly found in this type of meat product, such as *Staphylococcus aureus*. The E treatment showed a similar behavior to the P treatment at 5 °C, but at 20 °C an oscillating behavior in the MSPS count during storage by the E treatment was observed. This could be related to the less acidifying capacity of the encapsulated *L. plantarum* BFL at 20 °C, as shown in Figure 2A. Finally, differences in the MSPS counts at different temperatures in the control group and in treatment E were observed. However, for treatment P the MSPS counts were the same regardless of the storage temperature. With this last result, it could be inferred that free *L. plantarum* BFL has the same antagonistic effect against MSPS at both storage temperatures. Figures 6A,B and 7A,B show Enterobacteria and *E. coli* viabilities during 60 d of room temperature storage (20 °C) and under refrigeration (5 °C), respectively. The Enterobacteria counts were affected by interactions between the treatment and storage time ($p < 0.001$), the treatment and storage temperature ($p = 0.014$), and the storage time and storage temperature ($p < 0.001$). The presence of microencapsulated *L. plantarum* BFL (E) and as a free cell (P) caused lower counts of Enterobacteria compared to the control group up to day 30 in refrigerated samples (5 °C) and up to day 15 in samples stored at room temperature (20 °C). The storage temperature showed a marked effect on the Enterobacteria counts and *E. coli* counts ($p = 0.016$). Enterobacteria and *E. coli* viability was favored under refrigeration (5 °C) in the three studied groups (C, P, and E). These results are consistent with those of Nissen and Holck [23], who reported that storage at 20 °C is more effective in killing *E. coli* O157:H7 than storage at 4 °C. Furthermore, *E. coli* counts were affected by the different treatments ($p = 0.016$) and by the interaction between the storage time and storage temperature ($p < 0.001$). For both studied temperatures, the presence of *L. plantarum* BFL (P and E) led to lower *E. coli* counts compared to the control group up to 15 d of storage. At room temperature (20 °C), there were no significant differences in *E. coli* counts between the treatments. In addition, from 0 d to 15 d, the *E. coli* counts dropped to the detection limit of the plate count technique and remained constant until day 60 (Figure 6A). However, under refrigeration temperature (5 °C) the decrease in *E. coli* counts was gradual, with higher counts in the control group on days 15 and 30 ($p < 0.05$). As shown in Figures 6 and 7, despite the control group and P and E treatments reaching day 60 of storage with the same bacterial counts, the presence of *L. plantarum* BFL caused lower Enterobacteriaceae and *E. coli* counts at earlier points in storage.

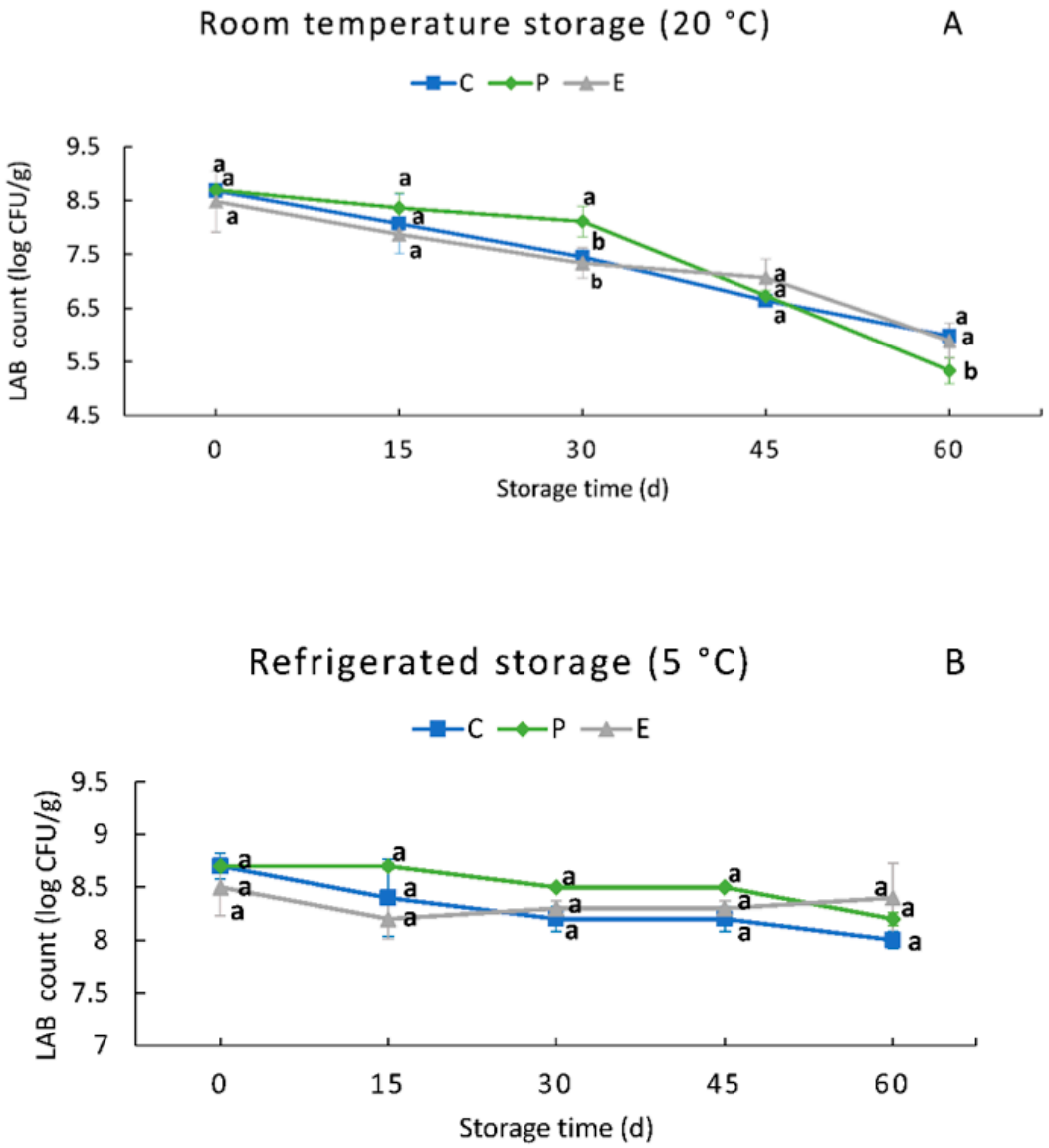

Figure 4. (**A**): LAB counts (means ± standard deviations) during 60 d of room temperature storage (20 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL. (**B**): LAB counts (means ± standard deviations) during 60 d of refrigerated storage (5 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). The different letters indicate significant differences between treatments on the same day under the same storage temperature.

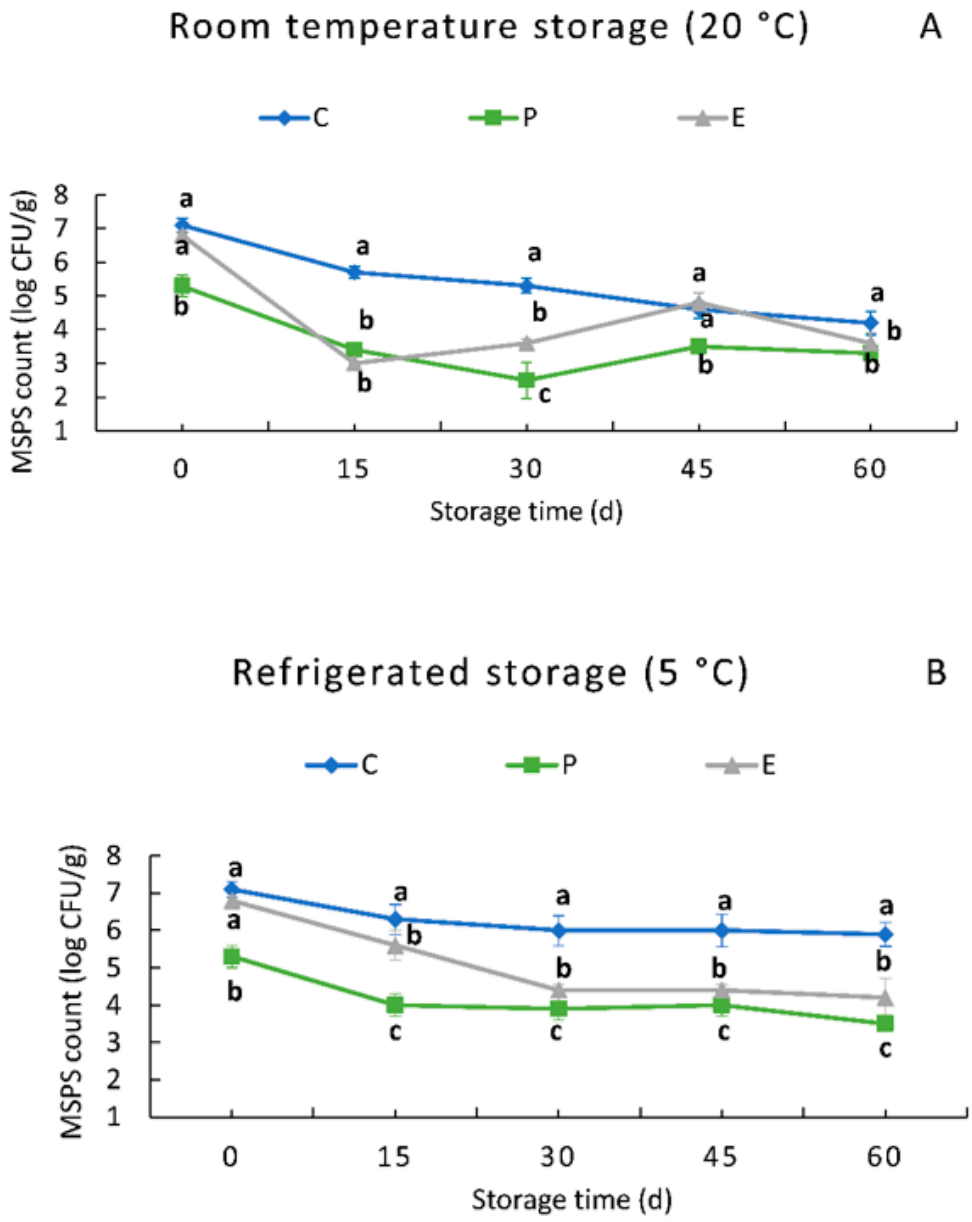

Figure 5. (**A**): MSPS counts (means ± standard deviations) during 60 d of room temperature storage (20 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). (**B**): MSPS counts (means ± standard deviations) during 60 d of refrigerated storage (5 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). The different letters indicate significant differences between treatments on the same day under the same storage temperature.

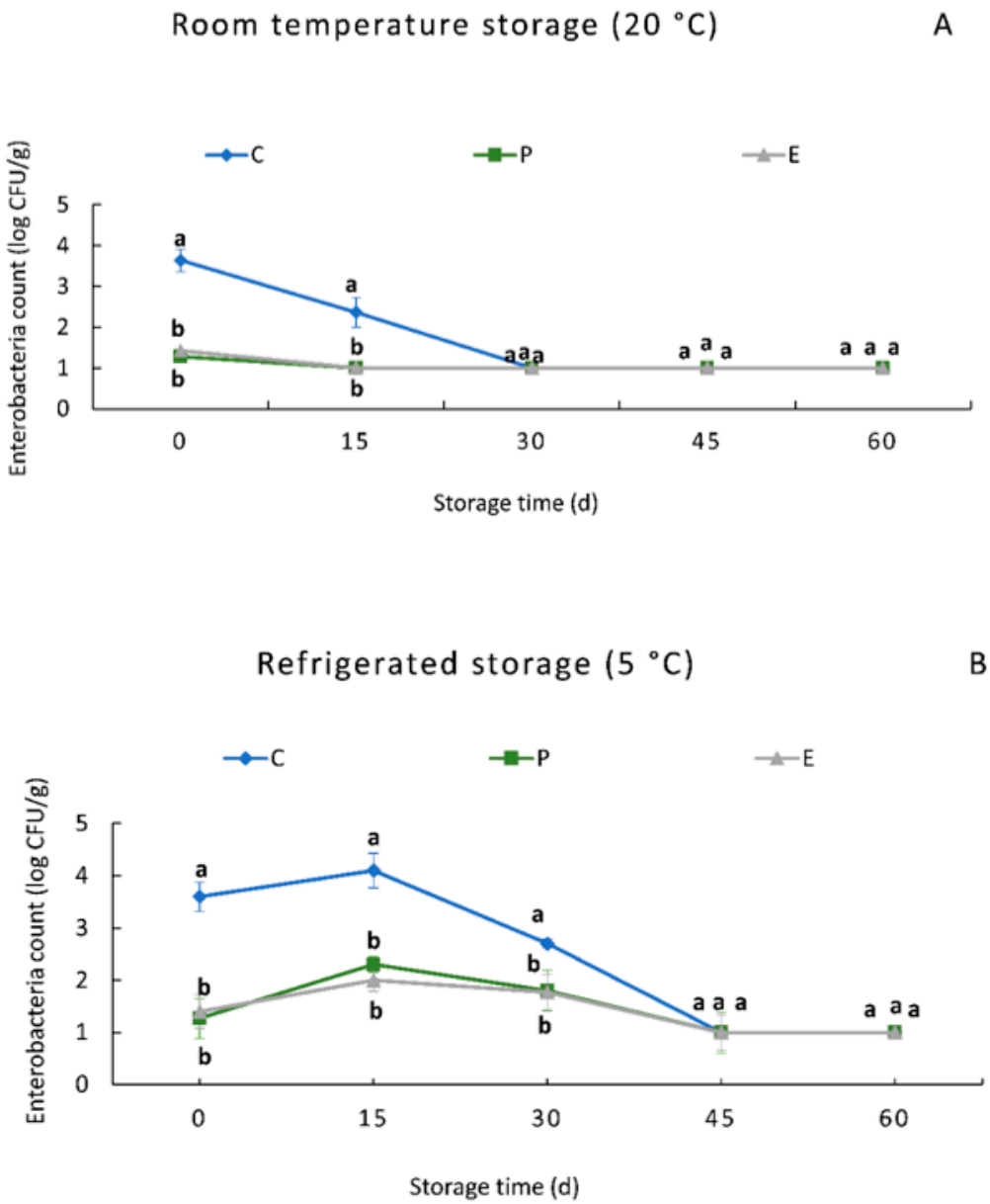

Figure 6. (**A**): Enterobacteria counts (means ± standard deviations) during 60 d of room temperature storage (20 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). (**B**): Enterobacteria counts (means ± standard deviations) during 60 d of refrigerated storage (5 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). The different letters indicate significant differences between treatments on the same day under the same storage temperature.

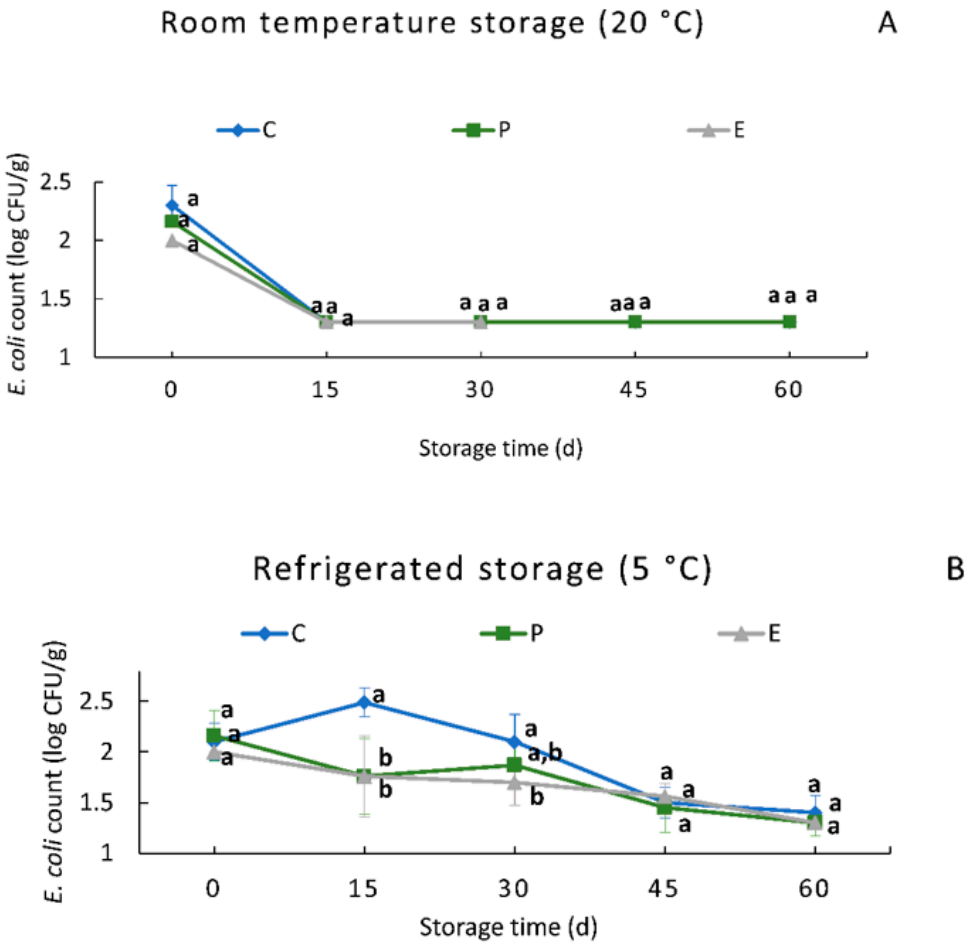

Figure 7. (**A**): *E. coli* counts (means ± standard deviations) during 60 d of room temperature storage (20 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). (**B**): *E. coli* counts (means ± standard deviations) during 60 d of refrigerated storage (5 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). The different letters indicate significant differences between treatments on the same day under the same storage temperature.

3.5. Lipid Oxidation

The lipid oxidation, measured as TBARs, was affected by interactions between the treatment and storage time ($p < 0.001$), the treatment and storage temperature ($p < 0.001$), and the storage time and storage temperature ($p < 0.001$). Figure 8A,B show Salamines Criollos lipid oxidation levels during 60 days of storage at 20 °C and 5 °C, respectively. All sausages stored at room temperature (control, P, and E) showed higher lipid oxidation levels than the refrigerated sausages stored for 60 d ($p < 0.001$). This result is expected since higher storage temperatures could accelerate oxidative rancidity and shorten the shelf life of the meat products. However, at 45 d and 60 d, lower TBARs values in the E treatments at both studied temperatures were detected. For the P treatments, only at 20 °C were the lower TBARs values observed ($p < 0.05$). These results agree with Pinto et al. [24], who found that *Enterococcus faecium* CRL 183 inoculation in salami decreased lipid oxidation. In addition, Ben Slima et al. [19] found that *Lactobacillus plantarum* TN8 inoculated into fermented sausages had the same antioxidant power as nitrites, which could be the result of the production of antioxidant compounds such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. Recently, Yu et al. [25] have investigated the influence of mixed starters (*Lactobacillus plantarum* CD101 and *Staphylococcus simulans* NJ201) with high protease activity on protein degradation and peptide formation with antioxidant activities during the fermentation and maturation of fermented sausages. The results suggest that

mixed starters could promote the synthesis of endogenous peptides with high antioxidant capacities while improvement to lipid stability. More studies should be carried out on the strain used in this work to confirm the mechanisms through which an antioxidant effect could be guaranteed by *L. plantarum* BFL. It should be clarified that Salamines Criollos preserved both at room temperature (20 °C) and under refrigeration (5 °C) reached day 60 of storage with lipid oxidation values lower than 2 mg MDA/kg, which is considered the limit of detection in the mouth.

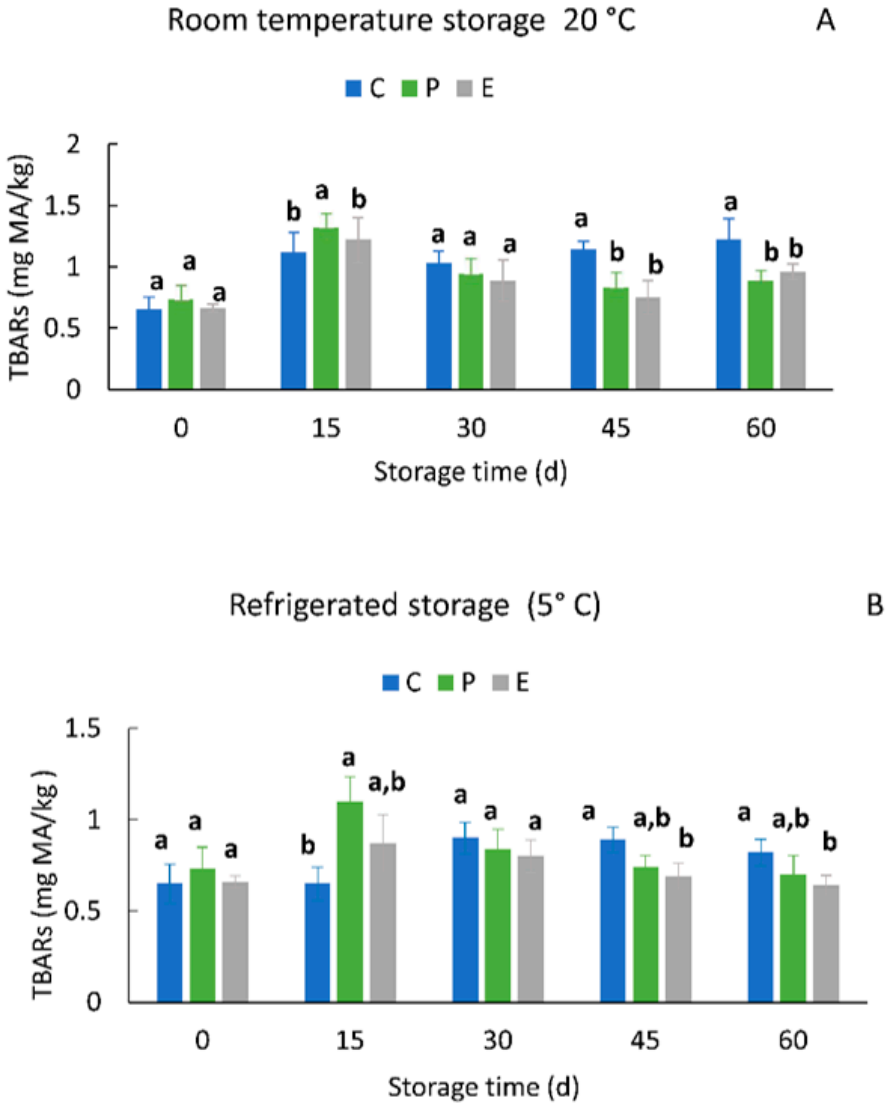

Figure 8. (**A**): TBARs values (means ± standard deviations) during 60 d of room temperature storage (20 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). (**B**): TBARs values (means ± standard deviations) during 60 d of refrigerated storage (5 °C) of Salamines Criollos formulated with microencapsulated *L. plantarum* BFL (E) and free *L. plantarum* BFL (P). The different letters indicate significant differences between treatments on the same day under the same storage temperature.

3.6. Color Measurement

Table 1 shows the color parameters of Salamines Criollos (C, P, and E) during 60 days of storage. The luminosity (L*) was in the range between 30–39 at room temperature and 29–38 under refrigeration, which is consistent with the values reported by other authors [26,27]. Although an interaction between the storage time and storage temperature ($p < 0.001$) was observed, the same pattern was followed by the L* variability at room

temperature (20 °C) and under refrigeration (5 °C). In addition, L* values were affected by the interaction between the treatment and storage time ($p < 0.001$). In the *L. plantarum* BFL treatments (P and E), higher L* values were achieved than the control during the entire storage period at both studied temperatures. This could be associated with a higher fermentative activity due to the presence of *L. plantarum* BFL and the consequent lactic acid formation (Figure 2A,B). The closer the food pH is to the isoelectric point of proteins, the lower its water-holding capacity. Although a single isoelectric point cannot be attributed to the meat batter, the pH differences between treatments could be the cause of the differences in the L* values. In addition, L* values are influenced by the movements of "free water" on the meat product surface, and therefore L* values will depend on the area or the way in which the measurement was made [28]. In this work, the instrumental measurement of color was carried out through cross-sections of the samples. The free water could have been exposed and released to the outside, thus provoking the detection of higher L* values. The redness (a*) and yellowness (b*) values were affected by the interaction between the treatment and storage time. At room temperature (20 °C), samples showed higher b* values than the refrigerated samples (5 °C) ($p < 0.001$). Since yellow is the color mainly related to the lipid oxidation parameter, this is a logical result that coincides with the recorded TBARs values (Figure 8A,B). The *L. plantarum* BFL treatments (P and E) achieved higher a* values than the control group ($p = 0.003$). This was observed after day 15 in Salamines at room temperature storage and after day 30 for salamines under refrigeration. The bibliographic data indicate that the higher the lactic acid content, the greater the myoglobin denaturation and the lower the a* values [28]. However, Fernández-López [29] studied a dry-cured sausage model system, finding that as the NaCl content increased (due to moisture loss), the a* values increased. The author also stated that the lactic acid concentration would increase along with the b* color coordinates. On the other hand, the redness increase could also be due to the ability of the inoculated *L. plantarum* BFL to reduce nitrites and thus continue to form red pigments. The h* values were typical of this type of meat product, and no significant differences were found between treatments ($p = 0.904$). The chroma (C*) depends on the values of a* and b*; therefore, the presence of the probiotic *L. plantarum* BFL was able to enhance the color of the salamines, mainly after the 30th day of conservation, at both temperatures ($p < 0.001$).

Table 1. Luminosity (L*), redness (a*), yellowness (b*), hue (h*), and Chroma (C*) (means ± standard deviations) of Salamines Criollos in the control group, in the free *L. plantarum* BFL treatment (P), and in the encapsulated *L. plantarum* BFL treatment (E) during 60 d of storage at 20 °C and 5 °C.

		Color Measurement				
20 °C	Treatment	L*	a*	b*	h*	C*
0 d	C	31.38 ± 1.3 ^{c}	17.06 ± 0.8 ^{a}	7.55 ± 0.5 ^{a}	0.42 ± 0.01 ^{a}	18.66 ± 0.89 ^{a}
	P	39.20 ± 0.8 ^{a}	17.34 ± 0.6 ^{a}	7.60 ± 0.4 ^{a}	0.41 ± 0.01 ^{a}	18.91 ± 0.71 ^{a}
	E	37.67 ± 1.0 ^{b}	17.83 ± 0.2 ^{a}	10.91 ± 7.3 ^{a}	0.51 ± 0.23 ^{a}	21.50 ± 4.70 ^{a}
15 d	C	31.52 ± 2.9 ^{c}	14.13 ± 0.5 ^{b}	7.16 ± 0.7 ^{b,c}	0.47 ± 0.03 ^{a}	15.84 ± 0.59 ^{a}
	P	38.06 ± 0.3 ^{a}	16.35 ± 2.0 ^{a}	8.01 ± 0.4 ^{a}	0.46 ± 0.04 ^{a}	18.22 ± 1.93 ^{a}
	E	34.78 ± 0.5 ^{b}	17.00 ± 1.2 ^{a}	7.31 ± 0.4 ^{b}	0.41 ± 0.01 ^{a}	18.50 ± 1.26 ^{a}
30 d	C	29.99 ± 0.7 ^{b}	14.76 ± 0.5 ^{c}	7.11 ± 0.2 ^{b}	0.45 ± 0.02 ^{a}	16.38 ± 0.46 ^{b}
	P	37.77 ± 0.6 ^{a}	15.96 ± 0.2 ^{b}	7.65 ± 0.2 ^{a}	0.47 ± 0.01 ^{a}	17.70 ± 0.12 ^{a,b}
	E	36.40 ± 0.9 ^{a}	16.55 ± 1.2 ^{a}	7.64 ± 0.7 ^{a}	0.43 ± 0.07 ^{a}	18.23 ± 1.34 ^{a}
45 d	C	30.5 ± 0.4 ^{a}	15.683 ± 0.2 ^{c}	7.65 ± 0.5 ^{a}	0.45 ± 0.02 ^{a}	17.45 ± 0.37 ^{b}
	P	38.2 ± 0.21 ^{b}	16.46 ± 0.2 ^{b}	7.66 ± 0.3 ^{a}	0.43 ± 0.02 ^{a}	18.16 ± 0.22 ^{a}
	E	36.31 ± 1.6 ^{b}	17.04 ± 0.3 ^{a}	7.76 ± 0.1 ^{a}	0.43 ± 0.02 ^{a}	18.73 ± 0.33 ^{b}

Table 1. *Cont.*

		Color Measurement				
60 d	C	30.42 ± 0.9 [b]	15.33 ± 0.4 [b]	7.39 ±0.4 [a]	0.45 ± 0.02 [a]	17.03 ± 0.53 [b]
	P	37.15 ± 0.2 [a]	16.92 ± 0.5 [a]	7.80 ± 0.2 [a]	0.43 ± 0.01 [a]	18.63 ± 0.55 [a]
	E	36.70 ± 0.8 [a]	16.19± 0.7 [a]	7.48 ± 1.5 [a]	0.43 ± 0.02 [a]	17.84 ± 0.91 [a,b]
5 °C	Treatment	L*	a*	b*	h*	C*
0 d	C	31.38 ± 1.3[c]	17.06 ± 0.8 [a]	7.55 ± 0.5 [a]	0.42 ± 0.01 [a]	18.66 ± 0.9 [a]
	P	39.20 ± 0.8 [a]	17.32 ± 0.6 [a]	7.60 ± 0.4 [a]	0.41 ± 0.02 [a]	18.92 ± 0.71 [a]
	E	37.67 ± 1.0 [b]	17.83 ± 0.2 [a]	10.91 ± 7.3 [a]	0.51 ± 0.23 [a]	21.50 ± 4.7 [a]
15 d	C	32.95 ± 0.9 [b]	14.30 ± 0.8 [a]	6.66 ± 1.2 [b]	0.82 ± 0.64 [a]	20.55 ± 0.3 [a]
	P	37.60 ± 0.7 [a]	16.45 ± 0.8 [a]	7.56 ± 0.4 [a]	0.43 ± 0.02 [a]	18.13 ± 0.86 [a]
	E	36.84 ± 1.6 [a]	15.12 ± 2.9 [a]	7.59 ± 0.1 [a]	0.47 ± 0.08 [a]	16.96 ± 2.7 [a]
30 d	C	34.02 ± 1.54 [b]	14.36 ± 0.7 [c]	6.69 ± 0.3 [b]	0.42 ± 0.02 [a]	15.76 ± 0.7 [b]
	P	38.33 ± 0.8 [a]	16.09 ± 0.4 [b]	7.26 ± 0.6 [a]	0.42 ± 0.02 [a]	17.66 ± 0.6 [a]
	E	37.81 ± 1.6 [a]	17.07 ± 0.4 [a]	7.70 ± 0.3 [a]	0.42 ± 0.02 [a]	18.73 ± 0.4 [a]
45 d	C	30.80 ± 1.0 [c]	15.38 ± 1.0 [c]	6.77 ± 0.3 [b]	0.28 ± 0.24 [a]	17.60 ± 0.9 [b]
	P	38.09 ± 0.6 [a]	16.82 ± 0.3 [b]	7.52 ± 0.3 [a]	0.42 ± 0.01 [a]	18.42 ± 0.4 [a,b]
	E	36.28 ± 0.2 [b]	18.05 ± 0.3 [a]	7.80 ± 0.4 [a]	0.41 ± 0.01 [a]	19.67 ± 0.4 [a]
60 d	C	29.56 ± 1.3 [b]	15. 23 ± 0.3 [b]	6.61 ± 0.4 [c]	0.42 ± 0.02 [a]	16.61 ± 0.40 [a]
	P	37.16 ± 2.1 [a]	16.00 ±1.0 [a]	7.18 ± 0.2 [b]	0.42 ± 0.03 [a]	17.54 ± 0.8 [a,b]
	E	36.66 ± 0.3 [a]	16.40 ± 0.5 [a]	7.86 ± 0.3 [a]	0.43 ± 0.01 [a]	18.18 ± 0.6 [a]

The different letters indicate significant differences ($p < 0.05$) between treatments on the same day under the same storage temperature.

3.7. Texture Profile Analysis (TPA)

The evaluation of the Salamines Criollos texture profile (TPA) during the 60 days of storage at 5 °C and at 20 °C showed that the presence of *L. plantarum* (P and E) caused higher values in hardness and chewiness compared to the control ($p < 0.001$) (Table 2). However, the reported hardness values for the P and E treatments at both temperatures ranged between 41 N and 66 N and were in the range reported by other authors due to the loss of moisture values in probiotic sausages [30,31]. The increase in hardness and chewiness in P and E could be due to a higher content of lactic acid that leads to a greater loss of moisture and, therefore, greater hardness. On the other hand, higher hardness values were observed in treatment E, where the pH was not the highest. This could indicate that the hardness increase in Salamines Criollos could be due to more than one unique factor. Another cause may be that the incorporation of the WPI-MTX microcapsules had an impact on the textural properties of sausages. According to the sensory aspects, in order to avoid textural modification on the food carrier (coarseness and graininess), the particle size should be kept as small as possible while providing maximum protection to the probiotic [32]. In addition, regular, spherical, and soft microcapsules are less detectable than irregular and firm microcapsules with the same diameter. In this sense, the encapsulation technique used greatly influences the shape of the obtained microcapsules. While spray drying allows microcapsules with diameters between 10 and 500 µm and a relatively spherical shape to be obtained, the extrusion technique provides microcapsules with diameters between 200 and more than 1000 µm [32]. The optimal size for probiotics microcapsules depends on the intended purpose and the type of food. For example, 1% capsules with diameters between 2 and 3 mm could not be sensorily detected in salami [8]; capsules of that size range give an adverse sensorial effect in dairy

matrices [33]. In this work, the microscopic characterization by SEM showed microcapsule sizes and shapes in accordance with the spray-drying technique used, which could not generate changes in texture (Figure 1). Therefore, the generated changes in hardness could have been due to molecular interactions between the WPI-MTX microcapsules and the gelling properties of the meat matrix. Similar results have been demonstrated by other authors. Cavalheiro et al. [30] found that the addition of an encapsulated probiotic to fermented sausages led to an increase in hardness, probably due to encapsulation by extrusion. Zhu et al. [34] performed a dynamic rheological analysis on probiotic sausages inoculated with *L. plantarum*, finding that the rheological character and viscosity of sausages were improved (G′ and G″ gave values higher than the uninoculated samples). On the other hand, no differences were observed in the elasticity of samples during 60 days of storage for any of the storage temperatures studied ($p > 0.05$) [30]. Similar results were observed in the case of the cohesiveness parameter when the measurements were made on sausages preserved under refrigeration ($p > 0.05$). However, lower cohesiveness values were reached at room temperature in treatment E. Since cohesion is defined as the degree to which the sample could deform before breaking, these differences could be related to changes in the molecular grade caused by gelation in the E treatment. More studies must be carried out to determine what effect WPI-MTX microcapsules have on the texture of Salamines Criollos.

Table 2. Texture profile analysis (means ± standard deviations) of Salamines Criollos in the control group, in the free *L. plantarum* BFL treatment (P), and in the encapsulated *L. plantarum* BFL treatment (E) during 60 d of storage at 20 °C and 5 °C.

		TPA			
5 °C	Treatment	Hardness	Cohesiveness	Springiness	Chewiness
0	C	30.44 ± 8.4 ^{b}	0.58 ± 0.05 ^{a}	1.7 ± 0.07 ^{a}	13.93 ± 6.1 ^{b}
	P	41.07 ± 14.6 ^{b}	0.53 ± 0.08 ^{a}	1.7 ± 0.07 ^{a}	14.90 ± 6.7 ^{a,b}
	E	55.58 ± 9.9 ^{a}	0.58 ± 0.03 ^{a}	1.79 ± 0.1 ^{a}	23.00 ± 4.1 ^{a}
15	C	23.0 ± 3.6 ^{b}	0.58 ± 0.05 ^{a}	1.7 ± 0.03 ^{a}	8.95 ± 2.2 ^{b}
	P	49.23 ± 11.0 ^{a}	0.57 ± 0.07 ^{a}	1.4 ± 0.6 ^{a}	19.30 ± 6.4 ^{a}
	E	47.44 ± 16.6 ^{a}	0.59 ± 0.03 ^{a}	1.68 ± 0.02 ^{a}	18.00 ± 7.5 ^{a}
30	C	28.51 ± 1.5 ^{b}	0.56 ± 0.07 ^{a}	1.6 ± 0.06 ^{a}	10.49 ± 1.0 ^{b}
	P	44.42 ± 18.5 ^{a}	0.49 ± 0.03 ^{a}	1.3 ± 0.6 ^{a}	14.51 ± 6.6 ^{a}
	E	60.24 ± 7 ^{a}	0.53 ± 0.05 ^{a}	1.67 ± 0.01 ^{a}	20.40 ± 3.0 ^{a}
45	C	26.98 ± 7.0 ^{a}	0.53 ± 0.03 ^{a}	1.6 ± 0.01 ^{a}	8.71 ± 2.2 ^{c}
	P	44.37 ± 8.7 ^{b}	0.54 ± 0.08 ^{a}	1.36 ± 0.6 ^{a}	16.30 ± 4.7 ^{b}
	E	64.61 ± 4.9 ^{c}	0.55 ± 0.03 ^{a}	1.67 ± 0.01 ^{a}	22.70 ± 0.3 ^{a}
60	C	24.97 ± 1.9 ^{a}	0.55 ± 0.02 ^{a}	1.6 ± 0.01 ^{a}	7.91 ± 0.95 ^{b}
	P	47.32 ± 11.3 ^{b}	0.51 ± 0.1 ^{a}	1.00 ± 0.6 ^{b}	15.01 ± 8.3 ^{a}
	E	62.05 ± 5.7 ^{c}	0.52 ± 0.03 ^{a}	1.67 ± 0.07 ^{a}	20.30 ± 0.7 ^{a}
20 °C	Treatment	Hardness	Cohesiveness	Springiness	Chewiness
0	C	30.43 ± 8.45 ^{b}	0.59 ± 0.06 ^{a}	1.72 ± 0.07 ^{a}	13.93 ± 6.13 ^{b}
	P	41.07 ± 14.7 ^{b}	0.54 ± 0.08 ^{a}	1.75 ± 0.07 ^{a}	14.95 ± 6.7 ^{a,b}
	E	55.58 ± 9.9 ^{a}	0.58 ± 0.04 ^{a}	1.79 ± 0.14 ^{a}	23.84 ± 4.17 ^{a}
15	C	27.18 ± 7.5 ^{c}	0.62 ± 0.04 ^{a}	1.74 ± 0.1 ^{a}	11.61 ± 2.6 ^{b}
	P	47.91 ± 11.34 ^{b}	0.59 ± 0.009 ^{a}	1.73 ± 0.02 ^{a}	19.06 ± 4.2
	E	66.18 ± 5.5 ^{a}	0.55 ± 0.042 ^{b}	1.7 ± 0.01 ^{a}	25.08 ± 2 ^{a}

Table 2. *Cont.*

		TPA			
30	C	19.09 ± 8.64 [b]	0.60 ± 0.05 [a]	1.69 ± 0.06 [a]	7.73 ± 3.6 [b]
	P	57.12 ± 5.81 [a]	0.6 ± 0.02 [a]	1.68 ± 0.02 [a]	23.11 ± 1.7 [a]
	E	53.83 ± 2.5 [a]	0.53 ± 0.04 [b]	1.72 ± 0.02 [a]	20.30 ± 0.4 [a]
45	C	33.86 ± 5.34 [b]	0.56 ± 0.07 [a]	1.7 ± 0.02 [a]	12.70 ± 3.9 [b]
	P	55.99 ± 10.9 [a]	0.53 ± 0.032 [a]	1.70 ± 0.02 [a]	20.27 ± 5.82 [a]
	E	55.91 ± 4.6 [a]	0.56 ± 0.06 [a]	1.72 ± 0.01 [a]	21.63 ± 1.8 [a]
60	C	24.78 ± 3.07 [b]	0.58 ± 0.04 [a]	1.69 ± 0.02 [a]	9.47 ± 0.72 [c]
	P	58.94 ± 4.69 [a]	0.56 ± 0.05 [a]	2.75 ± 1.8 [a]	24.18 ± 0.74 [a]
	E	54.18 ± 0.13 [a]	0.47 ± 0.03 [b]	1.75 ± 0.04 [a]	17.35 ± 1.90 [b]

The different letters indicate significant differences ($p < 0.05$) between treatments on the same day under the same storage temperature.

4. Conclusions

The viability studies of the encapsulated (E) and unencapsulated (P) probiotic *L. plantarum* BFL in Salamines Criollos for 60 days allow us to conclude that the strain generally tolerated the conditions of the studied meat matrix. The presence of *L. plantarum* BFL in Salamines Criollos had a positive effect on the hygienic qualities of the product since it decreased the Enterobacteria, *E. coli*, and mannitol-salt-positive *Staphylococcus* bacterial counts. In addition, encapsulated *L. plantarum* BFL (E) could have an antioxidant effect at 20 °C and at 5 °C during the end of storage ($p < 0.05$). On the other hand, spray-drying encapsulation of *L. plantarum* BFL in this food system as an enhancement of viability is not recommended. This is because encapsulation entails high input costs and previous production steps that do not represent a significant improvement in the viability of the probiotic, which showed a high viability rate as a free cell. The means of the *L. plantarum* BFL counts in the Salamines Criollos storage period for the free probiotic (P) treatment at room temperature and under refrigeration were 7.63 log CFU/g and 8.56 log CFU/g, respectively. On the other hand, the means of the *L. plantarum* BFL counts in Salamines Criollos for the encapsulated probiotic treatment (E) at room temperature and under refrigeration were 7.20 log CFU/g and 7.82 CFU/g, respectively. The recommended daily dose of probiotics with beneficial effects for health is between 10^6 and 10^{12} live cells. Thus, a medium portion (approximately 15 g or two slices) of Salamines Criollos with *L. plantarum* BFL could provide consumers with an adequate dose of probiotics to exert a healthy effect. This recommended consumption must be perfectly balanced with a balanced diet; therefore, its contribution would be added to the rest of the beneficial bacteria incorporated from other fermented foods.

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References

1. Marco, M.L.; Sanders, M.E.; Gänzle, M.; Arrieta, M.C.; Cotter, P.D.; De Vuyst, L.; Hill, C.; Holzapfel, W.; Lebeer, S.; Merenstein, D.; et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) Consensus Statement on Fermented Foods. *Nat. Rev. Gastroenterol. Hepatol.* **2021**, *18*, 196–208. [CrossRef]
2. Zhao, B.; Zhou, H.; Zhang, S.; Pan, X.; Li, S.; Zhu, N.; Wu, Q.; Wang, S.; Qiao, X.; Chen, W. Changes of Protein Oxidation, Lipid Oxidation and Lipolysis in Chinese Dry Sausage with Different Sodium Chloride Curing Salt Content. *Food Sci. Hum. Wellness* **2020**, *9*, 328–337. [CrossRef]
3. Sirini, N.; Lucas-González, R.; Fernández-López, J.; Viuda-Martos, M.; Pérez-Álvarez, J.A.; Frizzo, L.S.; Signorini, M.L.; Zbrun, M.V.; Rosmini, M.R. Effect of Probiotic *Lactiplantibacillus plantarum* and Chestnut Flour (*Castanea Sativa Mill*) on Microbiological and Physicochemical Characteristics of Dry-Cured Sausages during Storage. *Meat Sci.* **2022**, *184*, 108691. [CrossRef]
4. Sirini, N.; Roldán, A.; Lucas-González, R.; Fernández-López, J.; Viuda-Martos, M.; Pérez-Álvarez, J.A.; Frizzo, L.S.; Rosmini, M.R. Effect of Chestnut Flour and Probiotic Microorganism on the Functionality of Dry-Cured Meat Sausages. *LWT* **2020**, *134*, 110197. [CrossRef]
5. Klingberg, T.D.; Axelsson, L.; Naterstad, K.; Elsser, D.; Budde, B.B. Identification of Potential Probiotic Starter Cultures for Scandinavian-Type Fermented Sausages. *Int. J. Food Microbiol.* **2005**, *105*, 419–431. [CrossRef]
6. Stadnik, J.; Kęska, P. Meat and Fermented Meat Products as a Source of Bioactive Peptides. *Acta Sci. Pol. Technol. Aliment.* **2015**, *14*, 181–190. [CrossRef]
7. Sirini, N.; Frizzo, L.S.; Aleu, G.; Soto, L.P.; Rosmini, M.R. Use of Probiotic Microorganisms in the Formulation of Healthy Meat Products. *Curr. Opin. Food Sci.* **2021**, *38*, 141–146. [CrossRef]
8. Muthukumarasamy, P.; Holley, R.A. Microbiological and Sensory Quality of Dry Fermented Sausages Containing Alginate-Microencapsulated Lactobacillus Reuteri. *Int. J. Food Microbiol.* **2006**, *111*, 164–169. [CrossRef] [PubMed]
9. de Oliveira Gomes, B.; de Mesquita Oliveira, C.; de Marins, A.R.; Gomes, R.G.; Feihrmann, A.C. Application of microencapsulated probiotic *Bifidobacterium animalis* ssp. lactis BB-12 in Italian salami. *J. Food Processing Preserv.* **2021**, *45*, e15841.
10. Bujalance, C.; Jiménez-Valera, M.; Moreno, E.; Ruiz-Bravo, A. A Selective Differential Medium for *Lactobacillus plantarum*. *J. Microbiol. Methods* **2006**, *66*, 572–575. [CrossRef]
11. Rosmini, M.R.; Perlo, F.; Pérez-Alvarez, J.A.; Pagán-Moreno, M.J.; Gago-Gago, A.; López-Santoveña, F.; Aranda-Catalá, V. TBA Test by an Extractive Method Applied to 'Paté'. *Meat Sci.* **1996**, *42*, 103–110. [CrossRef]
12. Herrero, A.M.; Ordóñez, J.A.; de Avila, R.; Herranz, B.; de la Hoz, L.; Cambero, M.I. Breaking Strength of Dry Fermented Sausages and Their Correlation with Texture Profile Analysis (TPA) and Physico-Chemical Characteristics. *Meat Sci.* **2007**, *77*, 331–338. [CrossRef] [PubMed]
13. Tao, T.; Ding, Z.; Hou, D.; Prakash, S.; Zhao, Y.; Fan, Z.; Zhang, D.; Wang, Z.; Liu, M.; Han, J. Influence of Polysaccharide as Co-Encapsulant on Powder Characteristics, Survival and Viability of Microencapsulated *Lactobacillus paracasei* Lpc-37 by Spray Drying. *J. Food Eng.* **2019**, *252*, 10–17. [CrossRef]
14. Nunes, G.L.; de Araújo Etchepare, M.; Cichoski, A.J.; Zepka, L.Q.; Jacob Lopes, E.; Barin, J.S.; de Moraes Flores, É.M.; de Bona da Silva, C.; de Menezes, C.R. Inulin, Hi-Maize, and Trehalose as Thermal Protectants for Increasing Viability of *Lactobacillus acidophilus* Encapsulated by Spray Drying. *LWT* **2018**, *89*, 128–133. [CrossRef]
15. Barajas-Álvarez, P.; González-Ávila, M.; Espinosa-Andrews, H. Microencapsulation of Lactobacillus Rhamnosus HN001 by Spray Drying and Its Evaluation under Gastrointestinal and Storage Conditions. *LWT* **2022**, *153*, 112485. [CrossRef]
16. Trząskowska, M.; Kołożyn-Krajewska, D.; Wójciak, K.; Dolatowski, Z. Microbiological Quality of Raw-Fermented Sausages with Lactobacillus Casei LOCK 0900 Probiotic Strain. *Food Control* **2014**, *35*, 184–191. [CrossRef]
17. Gardiner, G.E.; O'sullivan, E.; Kelly, J.; Auty, M.A.E.; Fitzgerald, G.F.; Collins, J.K.; Stanton, C. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. *Appl. Environ. Microbiol.* **2000**, *66*, 2605–2612. [CrossRef] [PubMed]
18. Shori, A.B. Microencapsulation Improved Probiotics Survival During Gastric Transit. *HAYATI J. Biosci.* **2017**, *24*, 1–5. [CrossRef]
19. Ben Slima, S.; Ktari, N.; Trabelsi, I.; Triki, M.; Feki-Tounsi, M.; Moussa, H.; Makni, I.; Herrero, A.; Jiménez-Colmenero, F.; Ruiz-Capillas Perez, C.; et al. Effect of Partial Replacement of Nitrite with a Novel Probiotic *Lactobacillus plantarum* TN8 on Color, Physico-Chemical, Texture and Microbiological Properties of Beef Sausages. *LWT* **2017**, *86*, 219–226. [CrossRef]
20. Blaiotta, G.; Murru, N.; Di Cerbo, A.; Romano, R.; Aponte, M. Production of Probiotic Bovine Salami Using *Lactobacillus plantarum* 299v as Adjunct. *J. Food Sci.* **2018**, *98*, 2285–2294. [CrossRef]
21. Pavli, F.G.; Argyri, A.A.; Chorianopoulos, N.G.; Nychas, G.-J.E.; Tassou, C.C. Effect of *Lactobacillus plantarum* L125 Strain with Probiotic Potential on Physicochemical, Microbiological and Sensorial Characteristics of Dry-Fermented Sausages. *LWT* **2020**, *118*, 108810. [CrossRef]

22. Soto, L.P.; Sirini, N.E.; Frizzo, L.S.; Zbrun, M.V.; Zimmermann, J.A.; Ruiz, M.J.; Rosmini, M.R.; Sequeira, G.J.; Miotti, C.; Signorini, M.L. Lactic Acid Bacteria Viability in Different Refrigerated Food Matrices: A Systematic Review and Meta-analysis. *Crit. Rev. Food Sci. Nutr.* **2022**. [CrossRef] [PubMed]
23. Nissen, H.; Holck, A. Survival of *Escherichia Coli* O157:H7, *Listeria monocytogenes* and *Salmonella kentucky* in Norwegian Fermented, Dry Sausage. *Food Microbiol.* **1998**, *15*, 273–279. [CrossRef]
24. Pinto, R.A.; de Paula, A.V.; Rossi, E.A.; de Valdez, G.F.; Roselino, M.N.; Cavallini, D.C.U.; Ract, J.N.R.; de Almeida, J.F.; Canaan, J.M.M. Safety of a Low-Fat Fermented Sausage Produced with Enterococcus Faecium CRL 183 and *Lactobacillus acidophilus* CRL1014 Probiotic Strains. *Int. Food Res. J.* **2017**, *24*, 2694–2704.
25. Yu, D.; Feng, M.; Sun, J. Influence of Mixed Starters on the Degradation of Proteins and the Formation of Peptides with Antioxidant Activities in Dry Fermented Sausages. *Food Control* **2021**, *123*, 107743. [CrossRef]
26. Aksu, M.İ.; Kaya, M.; Ockerman, H.W. Effect of Modified Atmosphere Packaging, Storage Period, and Storage Temperature on the Residual Nitrate of Sliced-Pastırma, Dry Meat Product, Produced from Fresh Meat and Frozen/Thawed Meat. *Food Chem.* **2005**, *93*, 237–242. [CrossRef]
27. Dong, C.; Wang, B.; Li, F.; Zhong, Q.; Xia, X.; Kong, B. Effects of Edible Chitosan Coating on Harbin Red Sausage Storage Stability at Room Temperature. *Meat Sci.* **2020**, *159*, 107919. [CrossRef] [PubMed]
28. Pérez-Alvarez, J.A.; Sayas-Barberá, M.E.; Fernández-López, J.; Aranda-Catalá, V. Physicochemical Characteristics of Spanish-Type Dry-Cured Sausage. *Food Res. Int.* **1999**, *32*, 599–607. [CrossRef]
29. López, J.F. Estudio del Color Por Metodos Objetivos en Sistemas Modelo de Pastas de Embutidos Crudo-Curados. Universidad de Murcia. 1998. Available online: http://purl.org/dc/dcmitype/Text (accessed on 5 September 2022).
30. Cavalheiro, C.P.; Ruiz-Capillas, C.; Herrero, A.M.; Jiménez-Colmenero, F.; Pintado, T.; de Menezes, C.R.; Fries, L.L.M. Effect of Encapsulated *Lactobacillus plantarum* as Probiotic on Dry-Sausages during Chilled Storage. *IJFST* **2020**, *55*, 3613–3621. [CrossRef]
31. Hu, Y.; Zhang, L.; Zhang, H.; Wang, Y.; Chen, Q.; Kong, B. Physicochemical Properties and Flavour Profile of Fermented Dry Sausages with a Reduction of Sodium Chloride. *LWT* **2020**, *124*, 109061. [CrossRef]
32. Burgain, J.; Gaiani, C.; Cailliez-Grimal, C.; Jeandel, C.; Scher, J. Encapsulation of Lactobacillus Rhamnosus GG in Microparticles: Influence of Casein to Whey Protein Ratio on Bacterial Survival during Digestion. *Food Sci. Technol.* **2013**, *19*, 233–242. [CrossRef]
33. Hansen, L.T.; Allan-Wojtas, P.M.; Jin, Y.-L.; Paulson, A.T. Survival of Ca-Alginate Microencapsulated *Bifidobacterium* spp. in Milk and Simulated Gastrointestinal Conditions. *Food Microbiol.* **2002**, *19*, 35–45. [CrossRef]
34. Zhu, Y.; Guo, L.; Yang, Q. Partial Replacement of Nitrite with a Novel Probiotic *Lactobacillus plantarum* on Nitrate, Color, Biogenic Amines and Gel Properties of Chinese Fermented Sausages. *Food Res. Int.* **2020**, *137*, 109351. [CrossRef] [PubMed]