fermentation

MDPI

Article

Impact of a Biopreservative Derived from Lactic Fermentation on Quality after Food Processing: A Case Study on Sliced Cooked Ham

André Fioravante Guerra [1,*], Angela Gava Barreto [1], Isabella Rodrigues Viviani [1], Lucas Marques Costa [2], Carlos Alberto Guerra [2], Viviana Corich [3,4], Alessio Giacomini [3] and Wilson José Fernandes Lemos Junior [5,*]

[1] Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (CEFET/RJ), Valença 27600 000, Rio de Janeiro, Brazil
[2] BRC Ingredientes Ltd., Rio Claro 13505-600, São Paulo, Brazil
[3] Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, 35020 Padua, Italy
[4] Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, 35020 Padua, Italy
[5] Department of Biology, University of Padova, 35020 Padua, Italy
* Correspondence: andrefioravanteguerra@gmail.com (A.F.G.); juniorjflemos@gmail.com (W.J.F.L.J.)

Abstract: This study presents an innovative approach to enhancing the shelf life and maintaining the quality of sliced cooked ham through the application of a natural biopreservative derived from lactic fermentation. The biopreservative, at concentrations ranging from 1% to 3.5%, demonstrated substantial efficacy in microbial inhibition, keeping the microbial density low and relatively constant over time ($p < 0.05$). Remarkably, even at the lower concentration of 1%, the microbial growth rates were significantly reduced, with the treated samples showing notable stability over 24 days at both 7 °C and 25 °C. The microbial count in the treated with biopreservative group was significantly lower (3.19 log cfu/g) compared to the blank (4.59 log cfu/g) and control (5.01 log cfu/g) over 4 days at 7 °C. The shelf life of the ham was 24 days for the blank, 20 days for the control, and 101 days for the treated group at 7 °C. Moreover, colorimetric analysis revealed that the treated samples maintained better color stability, experiencing less variation in the hue angle and chroma, suggesting a protective effect against quality degradation over time. The successful application of the biopreservative aligns with the growing consumer demand for natural food additives and underscores the movement toward sustainable, health-conscious food preservation practices. The findings of this study indicate a promising avenue for the food industry to adopt environmentally friendly alternatives to synthetic additives, which could significantly influence future standards in food processing and preservation.

Keywords: biopreservative; lactic fermentation; sliced cooked ham; shelf life; microbial inhibition; color stability; sustainable food practices

Citation: Fioravante Guerra, A.; Gava Barreto, A.; Rodrigues Viviani, I.; Marques Costa, L.; Alberto Guerra, C.; Corich, V.; Giacomini, A.; Lemos Junior, W.J.F. Impact of a Biopreservative Derived from Lactic Fermentation on Quality after Food Processing: A Case Study on Sliced Cooked Ham. *Fermentation* **2024**, *10*, 336. https://doi.org/10.3390/fermentation10070336

Academic Editor: Antonio Morata

Received: 20 April 2024
Revised: 15 May 2024
Accepted: 26 June 2024
Published: 27 June 2024

1. Introduction

The consumption of processed meats like cooked ham is widespread due to their convenience, taste, and long shelf life. However, the preservation of these meats typically involves synthetic chemicals that can have negative health implications and contribute to environmental degradation [1]. As consumer awareness increases and regulatory bodies tighten the rules, the food industry faces significant pressure to find greener and safer preservation methods [2]. Lactic fermentation involves lactic acid bacteria fermenting sugars like glucose to produce lactic acid, which acts as a natural preservative [3]. This approach not only enhances food quality by lowering the pH and creating conditions unfavorable for pathogenic microorganisms but also improves the nutritional and sensory properties of the food products [3].

Traditionally, ham was manufactured by injecting pork raw materials with a brine solution, followed by a series of controlled cooking and cooling steps [4]. The innovative part of the process involved the incorporation of a biopreservative produced through an axenic lactic fermentation of glucose. This process was optimized to achieve a biopreservative that is effective at extending the shelf life and maintaining the color and texture of the ham, which are critical quality attributes that influence consumer acceptance [5].

In-depth physicochemical and microbiological analyses were conducted to ensure that the biopreservative meets safety and quality standards [6]. Color degradation not only affects the product's appearance but can also underlie chemical changes that may affect safety and palatability [7].

The use of fermentation products as natural biopreservatives offers several advantages over synthetic alternatives. Firstly, the involved bacteria are generally regarded as safe (GRAS) by regulatory authorities, reducing the health risks associated with synthetic preservatives. Secondly, natural fermentation processes can enhance the potential addition of health benefits, such as probiotics, to the food. The environmental impact of producing natural preservatives is typically lower, contributing to a more sustainable food production system [8], given the pressing need for sustainable food production and preservation methods [9].

This study explores an innovative approach to extend the shelf life of sliced cooked ham using a natural biopreservative (fermented solution) produced from the lactic fermentation of glucose. Therefore, the aim of this study is to move beyond traditional food microbiology practices and storage settings, demonstrating the feasibility and benefits of using a natural biopreservative in meat products. Specifically, this study contributes to the broader goals of sustainable food processing. Initially, this study focuses on optimizing the best concentration of biopreservative to identify the most effective conditions. Subsequently, assays are conducted on hams, considering various storage temperatures and their fluctuations. Additionally, the quality parameter of color is evaluated to understand the impact of the biopreservative on ham preservation.

2. Materials and Methods

2.1. Biopreservative Assays

2.1.1. Biopreservative Manufacturing

The lactic fermentation was conducted in the presence of 0.1% of glucose, 0.5% of yeast extract, 0.1% of magnesium sulphate, 0.05% of ammonium citrate tribasic, and 0.05% of dipotassium phosphate by adding *Lacticaseibacillus paracasei* DTA-83. The fermentation was carried out at BRC Ingredientes Ltda., located in the city of Rio Claro, in the state of São Paulo, Brazil. In the production, the medium was prepared with food-grade ingredients in a stirred-tank bioreactor with a nominal load to 2000 L. The powdered ingredients were dissolved in drinking water in the vessel of the tank with gentle agitation at about 80 rpm. After complete dissolution, the temperature of the medium was heated up (1 °C per minute) to 75 °C for 2 h by an indirect injection of steam provided by a steam-boiler at 115 °C in the tank jacket. During heating, the medium was axially agitated at 84 rpm using a mechanical stirrer with a four-blade propeller (50 × 15 mm, length × width) and a pitch coupled to the bioreactor. After the heat treatment, the temperature of the medium was reduced to 36 °C (heating down by 1.0 °C per minute) by an indirect circulation of cold water in the tank jacket. In the starter solution, *L. paracasei* DTA-83 was growth in sterile modified MRS broth. An 18 h culture, at the log-phase stage, was added (1/100 of inoculum) into the bioreactor containing 2000 L of medium to obtain a final inoculum concentration of ca. 7 log cfu/mL. After 15 h of fermentation coupled with a pH decay to around 3.5, the medium was heat-treated at 95 °C for 5 min. The biopreservative was hot-bottled in polypropylene containers of 50 L.

2.1.2. The Minimum Concentration Assays

The in vitro antimicrobial activity of the biopreservative to inhibit the growth of the microbiota isolated from the cooked ham was assessed by the minimum inhibitory concentration test [10].

Serial dilutions of the biopreservative were prepared to achieve final concentrations ranging from 0.05% to 3.5%. The broth microdilution method in 96-well microtiter plates was performed according to the Clinical and Laboratory Standards Institute consensus process [11]. A positive control was included, consisting of microbial cultures grown in the absence of the inhibitory substance.

Microbial growth was monitored by measuring the optical density at 600 nm (OD600) using a spectrophotometer. Measurements were taken at predetermined intervals over a period of 30 days. All the tests were performed in triplicate. The mean and standard deviation were calculated for each time point and concentration.

The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the inhibitory substance that resulted in no visible growth of the microbe compared to the positive control. Growth was assessed by measuring the OD600 after 24 days of incubation.

2.2. Sliced Cooked Production

Sliced Cooked Ham Manufacturing

The cooked ham was produced at an agroindustry producer located in the city of Patos de Minas, in the state of Minas Gerais, Brazil, by injecting the pork raw materials with 50% brine injection at −1 to −5 °C.

The raw materials were placed in a vacuum meat tumbler for 6 h at 12 rpm (40 min clockwise and for 20 min counterclockwise). The mass obtained was packed in stainless steel boxes and taken to the curing chamber at 0 °C from 12 to 24 h. After the curing process, the product was placed in cook-in packaging and placed in molds, which were closed under pressure and taken to cooking tanks with water with the following binomials of time and temperature: 60 °C for 40 min, 65 °C for 40 min, 70 °C for 40 min, 75 °C for 40 min and 80 °C until the coldest point of the piece achieve 72 °C. Then, the product was immersed in another tank containing ice water to cool it and stored in a refrigerated chamber at 0 °C. Upon reaching 15 °C, the ham was unmolded, visually inspected and stored in chambers for 24 h.

2.3. Sliced Cooked Characterization

2.3.1. Physicochemical Analyses

General Physicochemical Analyses

The protocol reported in [12] was performed to determine the moisture (%w/v) in an oven-drying process at 105 °C until constant weight, ash (%w/v) by incinerating in a muffle furnace at 550 °C for 4 h, protein (%w/v) by the Kjeldahl method, total fat content (%w/v) by the Soxhlet extraction method with hexane (%w/v), and carbohydrate as the difference between a 100% and the sum of the percentages of moisture, ash, lipid, and protein [2].

Water Activity Measurement

The protocol reported in ISO 18787:2017 [13] was performed for the water activity measurements. An AcquaLab Lite device (Decagon, Pullman, WA, USA) equipped with a dielectric humidity sensor and infrared sample surface temperature sensor, which determine water activity by the electrical conductivity of an electrolyte, was used for the water activity measurements. The equipment was calibrated using standard solutions of K_2SO_4 (*aw* 0.973, CAS 7778–80–5) and KCl (*aw* 0.843, CAS 7447–40–7)). The sample was cut into small pieces approximately 0.3 mm in size with the help of a knife. The sample portion was positioned in a dish so that there was no empty space at the bottom. The equipment's reading stability was checked during the analytical series.

pH Values

The protocol reported in ISO 2917:1999(E) was performed for the pH determination [14]. A potentiometer device equipped with a calibrated surface pH probe (2015PC, Akso, Brazil) was used for the measurements. The pH value was calculated as the mean value of the measurements at 15 positions in a slice, which included the central and peripheral sections.

2.3.2. Texture Profile Analyses

The protocol reported in ISO 11036:2020 was performed for the texture profile analysis [15]. A texture analyzer (TA.XT, Stable Micro Systems, USA) and TA-XT+ software (TA.XT, Stable Micro Systems, USA) were used for the measurements. The sample was sectioned axially (2.0 cm) using a sampler. Measurements were carried out at six positions in the sample, covering the central and peripheral sections. All the measurements were performed at room temperature (about 23 °C). A 9.1 cm diameter circular plate was clamped onto a 500 N load cell and the sample was compressed to 60% of its original height at a crosshead speed of 200 mm/min in two cycles. The TPA parameters of hardness-1, hardness-2, cohesiveness, elasticity, and chewability were determined. Briefly, hardness-1 is the force required for the first compression and hardness-2 is the peak force for the second compression. Elasticity is the distance that the sample recovers in height after the first compression. Cohesiveness is the relationship between the two total areas under the compression curves (A1/A2). Chewing is the product of hardness-1, elasticity and cohesion [15].These parameters, hardness-1 and -2, were expressed in N/cm^2; elasticity was expressed in m/cm^2; and chewiness in J/cm.

2.4. Slice Cooked Ham Preservation

2.4.1. Microbial Load

The protocol reported in the method of MicroLab_ShelfLife [16] was performed to estimate the durability of sliced cooked ham at three temperature profiles (Figure 1). The end of the shelf life was determined when the total microbial count achieved the stationary growth phase (*ca.* 9.3 log cfu/g). The horizontal method for enumeration of microorganisms [17] was performed to determine the total microbial load, using plate count agar medium (HiMedia, Mumbai, India), at the zero time and after stimulating the microbial growth by incubation of the samples at 7 and 25 °C. The microbial load was counted at time zero and on days 2 and 4 of the incubation. The MicroLab_ShelfLife software [16] was used to estimate the microbial growth parameters and to model the microbial growth (log) phase, deceleration phase and the entrance of the microbiota in the stationary phase. Three simulations were performed for each sample group to mimic various conditions that the product may experience when for sale in markets.

2.4.2. Minimal Inhibitory Concentration

Concomitantly with the sample incubation in the durability study, spoilage microorganisms were obtained from five packages. Aliquots (0.1 mL) from the initial decimal suspension was transferred to tubes containing 5 mL of brain–heart infusion (BHI) broth or deMan–Rogosa–Sharpe (MRS) broth or yeast extract–peptone–dextrose (YPD) broth. The tubes were incubated at 30 °C for 24 h. The inoculum was obtained separately from each culture medium by transferring 1 mL of the tube content, with expressive growth (turbidity above 0.5 MacFarland standard), to an empty sterile screw-cap tube. Cells free of toxic compounds were obtained by twice washing the biomass cell pellets with a routine of centrifugation at 6000× *g* for 6 min for pellet sedimentation at the bottom of the tube, discarding the liquid fraction, adding 2 mL of phosphate buffer pH 7.2, and homogenizing in a vortex. The turbidity of the microbial suspension was adjusted to 0.5 McFarland standard and 2-fold diluted. The biopreservative was outlined to the final concentrations from 0.0 to 3.5% (*v*/*v*) in the brain–heart infusion broth. The dilutions were prepared in the same media used in the test to avoid a shortage of nutrients for microbial growth. Finally,

10 µL of the microbial suspension was added into the tubes to achieve a final microbial concentration of ca. 5 log cfu/mL.

The microbial density was measured in an automated microplate reader (Epoch2, Biotek, Agilent, Santa Clara, CA, USA) and Gen5 software (Biotek, Agilent, Santa Clara, CA, USA) for 24 h. The reader was equipped with an automated temperature control system and automated for homogenization, as activated before each reading record. It was adjusted to an incubation temperature of 30 °C, with a monochromatic wavelength of 620 nm, without a temperature gradient, with a recording of the optical density values, in absorbance, at regular intervals of 30 min, for 24 h. To homogenize the cell biomass before each reading, the microplate was agitated automatically by orbital movements, with a frequency of 282 cpm (3 mm) for 5 s. The absorbance values were plotted on a scatter plot with the variable time on the abscissa axis (x) and the absorbance values on the coordinate axis (y).

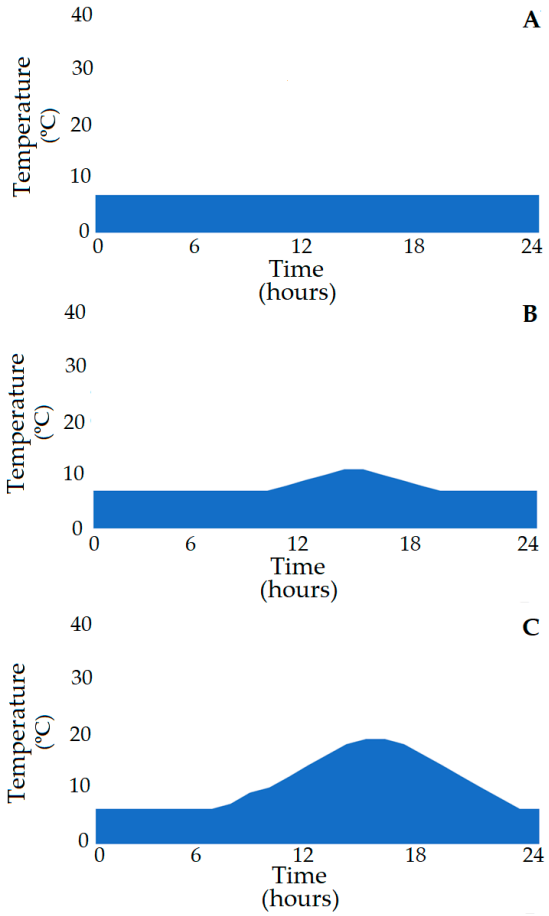

Figure 1. Dynamic temperature profiles based on the hourly variation over a period of one day to simulate realistic temperature conditions during the distribution of the products for sale in markets: (**A**)—cold storage at 7 °C; (**B**)—cold storage with a small abuse; and (**C**)—cold storage with a large abuse.

2.5. Quality Evaluation

Color Assays

A piece of ham was sliced in an electric slicer machine (BM 06 NR, Bermar, Brazil) with a thickness of 0.4 mm and placed in expanded polypropylene trays (N = 18), with one slice per tray. The packages were covered with polyvinyl chloride (PVC) film and divided into three groups: treatment (with 2.0% biopreservative added), control (with 2.0% sterile deionized water added) and white (no addition of biopreservative or water). All the groups were stored at 7 °C and 25 °C as follows:

B1—Blank cold storage at 7 °C;
B2—Blank storage at 25 °C;
C1—Control cold storage at 7 °C;
C2—Control storage at 25 °C;
T1—Treatment cold storage at 7 °C;
T2—Control storage at 25 °C;

The color was analyzed with a colorimeter (450 G, Delta Color, Brazil) provided with an 8 mm diameter measuring area and a 50 mm diameter illumination area. Illuminant A (average incandescent, tungsten-filament lighting, 2857 K) and a 10° standard observer were used to place more emphasis on the proportion of red wavelengths and captures# a larger portion of the scanned sample [18,19]. The readings were performed randomly for each treatment (n = 15), before and after cooking. Raw and cooked sausages with no addition of nitrite (T1) were used as the control. The color values were expressed as the L* (100 = white, 0 = black), A* (positive values = redness, negative values = greenness), and B* (positive values = yellowness, negative values = blueness).

2.6. Statistical Analysis

The samples were described using the microbial growth predictor MicroLab_ShelfLife, with a natural microbial load. All the data were evaluated by parametric and non-parametric tests using XLSTAT statistical and R 3.6.

3. Results and Discussion

3.1. Screening Biopreservative Concentration

Initially, we evaluate the MIC values to consider the best concentration of biopreservative at 10 different points from 0.03 up to 3.5%.

The growth curves (Figure 2) indicate that the microbial density increases over time in all the samples, including the positive control. The positive control shows a robust growth pattern, reaching a high optical density, suggesting that under normal conditions, the microbial population proliferates effectively, as accords with [20].

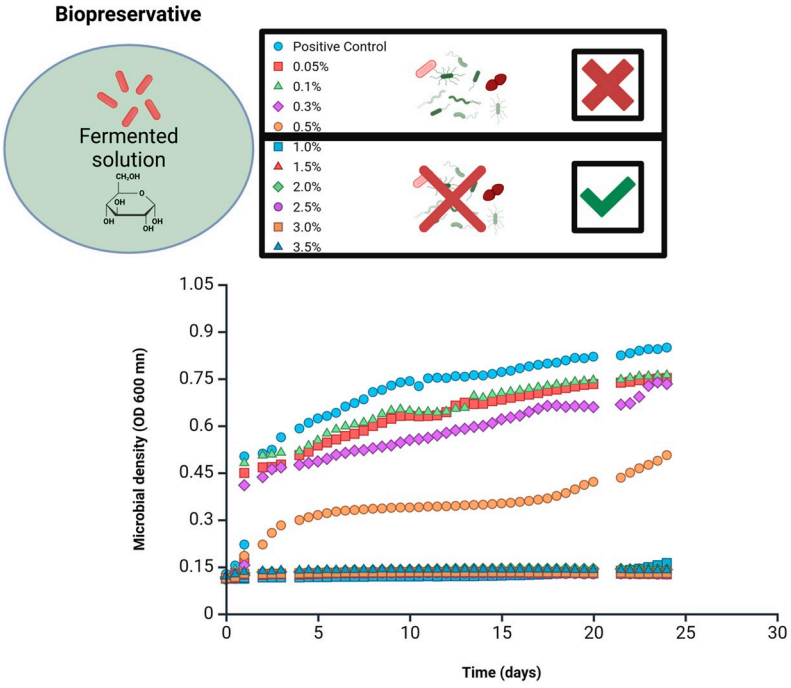

Figure 2. Microbial density at OD 600 nm over a 24-day period for 10 different concentrations of biopreservative (0.03% up to 3.5%).

At lower concentrations of the inhibitory substance (0.05% to 0.5%), there is a noticeable increase in the microbial density, although it is less than the positive control. At a 1% concentration, microbial growth is significantly suppressed, suggesting that this concentration is the minimum required to achieve good inhibition. It is at this concentration that the microbial density remains relatively flat, indicating effective inhibition of growth over 24 days.

At higher concentrations (1% to 3.5%), the microbial density remains low and relatively constant over time ($p < 0.05$), suggesting that these concentrations maintain strong inhibition and potentially bactericidal activity, preventing any significant increase in the microbial population.

Therefore, we selected for the next steps the minimum percentage of biopreservative (1%) to be applied as treatment in the ham samples.

3.2. Physicochemical Characterization of the Ham

The analysis of the physicochemical properties (Table 1) of the ham sample reveals a high moisture content (77.46 ± 0.021%), which is indicative of a water-rich food matrix. The protein content is substantial (16.87 ± 0.247%), suggesting the sample is a significant source of protein. The fat content is relatively low (3.61 ± 0.091%), aligning with leaner food products, and the carbohydrate content is minimal (0.84%), which could be beneficial for low-carbohydrate dietary needs. The ash content, representing the total mineral content, is modest (1.22 ± 0.080%). The pH is slightly acidic (6.49 ± 0.021), and the water activity (aw) is high (0.976 ± 0.002), potentially affecting the microbial stability and shelf life.

Table 1. Physico-chemical parameters of the cooked ham (mean ± standard deviation).

Parameter	Test	Quantity
Physico-chemical	Moisture (%)	77.46 ± 0.021
	Protein (%)	16.87 ± 0.247
	Fat (%)	3.61 ± 0.091
	Ash (%)	1.22 ± 0.080
	Carbohydrate (%)	0.84
	Potential of hydrogen (pH)	6.49 ± 0.021
	Water activity (*aw*)	0.976 ± 0.002
Texture profile	Fracturability (N/cm^2)	19.453 ± 0.983
	Hardness-1	21.366 ± 1.001
	Hardness-2	19.753 ± 0.894
	Cohesiveness	1.205 ± 0.222
	Springiness (mm)	13.600 ± 0.432
	Chewiness (J/cm^2)	35.015 ± 0.567

The texture profile analysis shows considerable fracturability (19.453 ± 0.983 N/cm^2), indicating that the sample requires a significant force to fracture, suggesting a firm structure. The hardness is also significant (hardness-1: 21.366 ± 1.001 N; hardness-2: 19.753 ± 0.894 N), which correlates with the fracturability results. The cohesiveness (1.205 ± 0.222) and springiness (13.600 ± 0.432 mm) values suggest that the sample has a notable internal binding and elastic nature. The chewiness (35.015 ± 0.567 J/cm^2), which combines the hardness, cohesiveness, and springiness, indicates that the sample has a substantial chew, which could contribute to consumer satiety and mouthfeel during consumption.

In this study, we developed the experimental design by considering a ham with a high moisture content coupled with high water activity, which suggests that while the sample may offer a desirable mouthfeel, due to its composition, it might be prone to microbial proliferation, potentially reducing the shelf life unless properly stored. The low fat and carbohydrate contents are favorable for those seeking healthier food options with reduced caloric intake [21].

From a textural perspective, the high fracturability and hardness reflect a firm and dense food product, possibly requiring sturdy packaging to prevent damage during transportation. The texture properties measured, including the cohesiveness and chewiness, provide insight into the potential sensory experience of the product, which is an important consideration for consumer acceptance [22].

After this characterization the potential for microbial growth was evaluated, considering the samples with water as a preservative (blank), the traditional method (control), and with the biopreservative (1%), exploring the use of natural preservatives or modified atmosphere packaging on enhancing the shelf life.

3.3. Impact of Storage Temperature on Microbial Growth in Sliced Cooked Ham

A concentration of 1.0% biopreservative was applied to sliced cooked hams and the impact of the storage temperature was evaluated in comparison with hams treated with 1.0% sterile deionized water (control) and hams without any application (blank).

The investigation assessed the efficacy of a treatment on the microbial inhibition at 7 °C and 25 °C over 4 days. Initially, the treatment started with a lower microbial count (3 log cfu/g) compared to the blank (3.82 log cfu/g) and control (3.65 log cfu/g).

After 2 and 4 days at 7 °C, the microbial count in the treated group was significantly lower (3.02 and 3.19 log cfu/g, respectively) compared to the blank (4.02 and 4.59 log cfu/g) and control (4.13 and 5.01 log cfu/g), suggesting effective microbial inhibition by the treatment.

At the higher temperature of 25 °C, the microbial counts increased across all the conditions. However, the treated group maintained lower counts (3.81 log cfu/g after 1 day and 4.13 log cfu/g after 3 days) compared to the blank (6.24 log cfu/g after 1 day and 7 log cfu/g after 3 days) and the control (6.99 log cfu/g after 1 day and 8.01 log cfu/g after 3 days), indicating that the treatment remained effective even at elevated temperatures.

In Figure 3A,C, we can observe the overall microbial growth across the different treatments at both temperatures. At 7 °C, all the treatment groups show a lower microbial count compared to the control and blank, with the treated group t exhibiting the most significant reduction ($p < 0.001$). This suggests that treatment is particularly effective at this refrigeration temperature over 4 days.

In Figure 3B,D, the box plots provide a distributional view of the microbial counts for the blank, control, and treated groups at both temperatures. At 7 °C, the treated group shows a broad distribution but a lower median log UFC/g.

The rapid growth in the control at 25 °C highlights the role of temperature in accelerating microbial proliferation. There is a hypothesis to explain this effect and these differences regarding the microbial densities at 7 °C. For instance, CO_2 is dissolved in the samples in a temperature-dependent manner, while increasing the temperature might lead to CO_2 release, thereby increasing the pH, as reported by Lamichhane et al. [23]. These authors described a linear decrease in the CO_2 concentration with the increase of both the temperature and the salt-in-moisture content, whereas the solubility of CO_2 increased, increasing the pH. In addition, *L. paracasei* DTA-83 showed high performance as heterofermentative bacteria, as described in previous studies [19,24,25]; therefore, its capability to produce organic acids as well as lactic acid can interfere with the ionic strength of the preservative liquid. When this biopreservative is exposed to high temperatures, the molecular ionization may decrease, resulting in a higher pH [26].

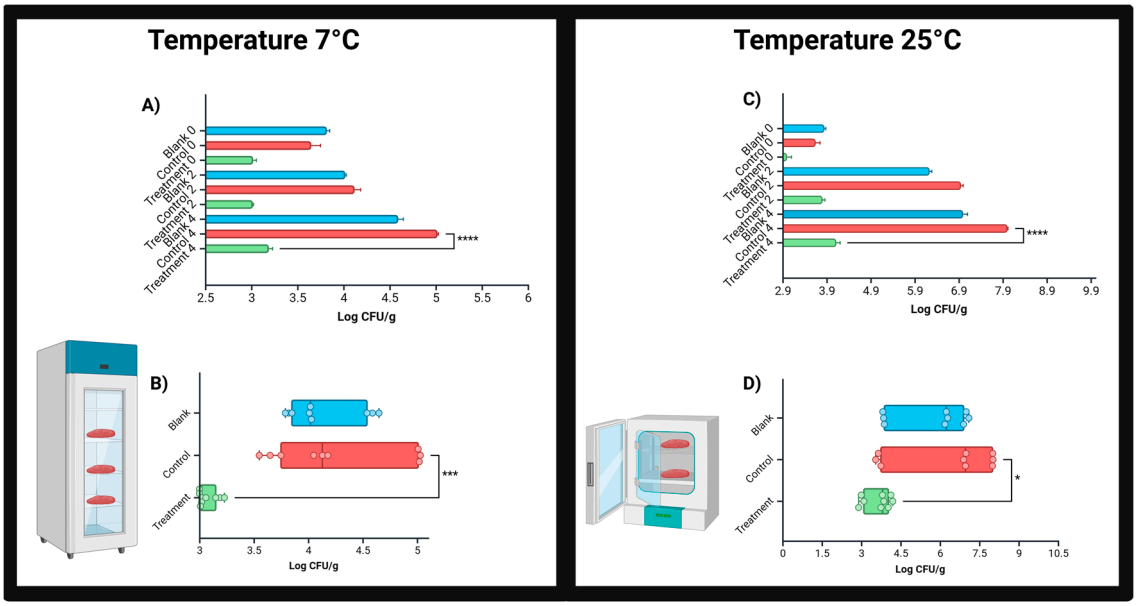

Figure 3. Impact of storage temperature on microbial growth in sliced cooked ham. (**A**) Bar graph depicting the log CFU/g of microbial populations at 7 °C for different times 0, 2 and 4 days (****) means $p < 0.001$ generated by the Mann–Whitney test). (**B**) Box plot representation of the microbial count variability at 7 °C considering 0, 2 and 4 days (***) means $p < 0.05$ generated by the *t* test). (**C**) Bar graph depicting the log CFU/g of microbial populations at 25 °C for different times 0, 2 and 4 days (****) means $p < 0.001$ generated by the Mann–Whitney test). (**D**) Box plot representation of the microbial count variability at 7 °C considering 0, 2 and 4 days (* means $p < 0.05$ generated by the *t* test).

3.4. Shelf-Life Evaluation

The durability of sliced cooked hams was assessed by applying a 1.0% biopreservative treatment and comparing with hams treated with 1.0% sterile deionized water (control) and hams without any application (blank). The specific microbial growth rates (log cfu/g/day) were measured under various temperature conditions (Table 2).

Table 2. MicroLab test.

Temperature Profile		Blank (Log cfu/g/Day)	Control (Log cfu/g/Day)	Treatment (Log cfu/g/Day)
Cold storage at 7 °C	*N (Tgrowth)*	0.2562	0.3124	0.0677
	N (Tdeceleration)	0.1693	0.2235	0.0447
Cold storage with soft abuse	*N (Tgrowth)*	0.302	0.3454	0.0839
	N (Tdeceleration)	0.1903	0.2145	0.0555
Cold storage with large abuse	*N (Tgrowth)*	1.018	1.111	0.4947
	N (Tdeceleration)	0.3726	0.4532	0.181

At 7 °C, the growth rate during the growth phase (N(T_growth)) was lowest in the treated group (0.0677) compared to the control (0.3124) and the blank (0.2562). During the deceleration phase (N(T_deceleration)), the treated group also showed a lower rate (0.0447) compared to the control (0.2235) and the blank (0.1693).

Under soft-abuse conditions, the treated group maintained a lower growth rates during both the growth (0.0839) and deceleration (0.0555) phases than the control (0.3454 and 0.2145, respectively) and the blank (0.302 and 0.1903, respectively).

When subjected to large abuse, the treated group's growth rates were significantly lower during the growth (0.4947) and deceleration (0.181) phases compared to the control (1.111 and 0.4532, respectively) and the blank (1.018 and 0.3726, respectively).

The application of a 1.0% biopreservative significantly inhibited microbial growth on the sliced cooked hams under all the storage conditions. The efficacy of the biopreserva-

tive was particularly notable under abusive temperature conditions, which are known to accelerate microbial proliferation.

The results at 7 °C suggest that the biopreservative is effective and the data also indicate that even under mild temperature fluctuations (soft abuse), the biopreservative continues to inhibit microbial growth effectively, although the rate of growth was higher compared to the ideal storage temperature.

However, the substantial increase in the microbial growth rate under large-abuse conditions, despite the presence of the biopreservative, highlights the challenges in maintaining food quality when storage temperatures are not strictly controlled.

The shelf-life assays were evaluated on sliced cooked hams stored under three different conditions (Figure 4).

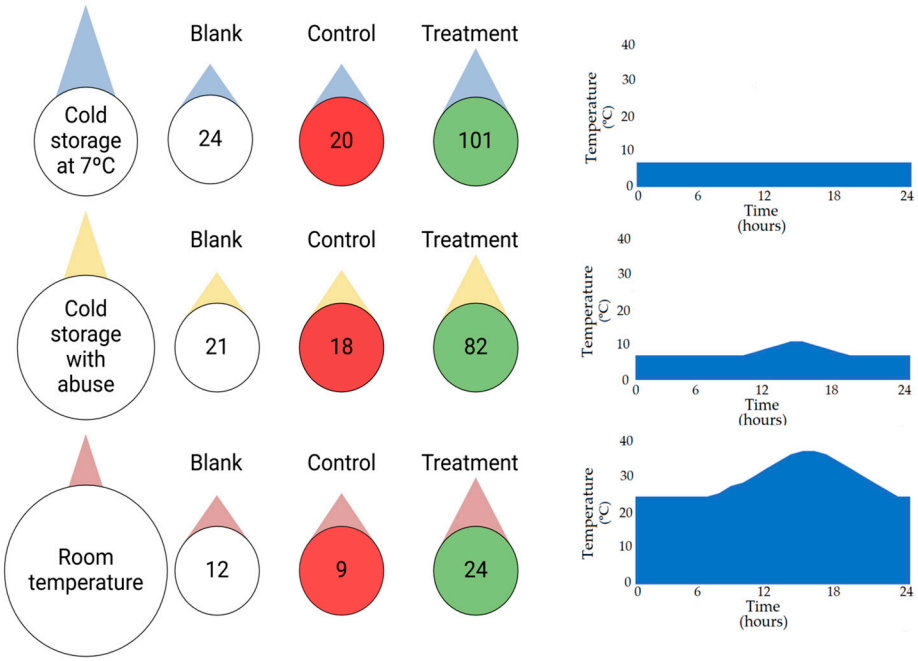

Figure 4. Shelf life of samples evaluated by MicroLab_ShelfLife.

Under cold storage at 7 °C, the durability of the hams was extended to 24 days for the blank, 20 days for the control, and markedly higher at 101 days for the treated group (Figure 4).

When exposed to cold storage with abuse, which likely represents temperature fluctuations, the durability decreased to 21 days for the blank, 18 days for the control, and 82 days for the treated group.

At room temperature, the durability was significantly reduced, with the blank lasting only 12 days, the control 9 days, and the treated group showing a durability of 24 days.

The application of 1.0% biopreservative (treated group) substantially increased the durability of the sliced cooked hams across all the storage conditions. Notably, at 7 °C, the treatment prolonged more than four times the durability comparted to the blank and five times compared to the control, highlighting the effectiveness of the biopreservative under optimal cold storage conditions. It is important to note that the promising effects of the biopreservative treatment observed in this study are contingent upon the initial microbial load being relatively low. In our case, the initial microbial load was around 3 log CFU/g, which significantly influenced the outcome of the treatment.

The reduced durability assessed in the abuse conditions suggests that although the biopreservative enhances the shelf life, the treatment is not a substitute for proper storage practices. However, the treatment still provided substantial durability benefits under these less-than-ideal conditions.

The results at room temperature are particularly compelling, as they indicate that the biopreservative still had a protective effect even when the hams were stored outside of the recommended temperatures, where spoilage typically accelerates.

The increased shelf life of the treated samples emphasizes the potential of biopreservatives in storing meat products [27].

3.5. Quality Impact Assays

After storage, the evaluation of some important quality parameters was considered. Color was selected to understand the changes in quality aspects, and at the same time, the test was carried out following the same experimental design. As shown in Table 3, the treated group and controls recorded no significant differences as storage at both temperatures T1 (7 °C) and T2 (25 °C) recorded similar lightness values of 68.650 ± 0.240 and 68.261 ± 0.245, respectively. This similarity suggests that the range of temperature conditions studied had a negligible impact on the lightness parameter of the samples.

Table 3. Colorimetric properties determined over a period of seven days. Two temperature-controlled groups, T1 (7 °C) and T2 (25 °C), were assessed alongside their counterparts, B1, C1, and B2, C2. The parameters examined included *L** (lightness), *a** (red–green coordinate), *b** (yellow–blue coordinate), C* (chroma), hab (hue angle), and ΔE (total color difference). The results are expressed as the mean ± standard deviation, with statistical significance denoted by superscript letters.

Groups	*L**	*a**	*b**	C*	hab	ΔE
B1	65.198 ± 4.358 ^{bcd}	6.626 ± 0.067 ^{a}	10.890 ± 0.050 ^{ab}	12.748 ± 0.007 ^{bc}	58.680 ± 0.375 ^{ab}	7.092 ± 0.100 ^{b}
C1	68.652 ± 0.401 ^{a}	6.637 ± 0.058 ^{a}	11.647 ± 0.132 ^{a}	13.405 ± 0.143 ^{a}	60.322 ± 0.065 ^{a}	8.402 ± 0.575 ^{a}
T1	68.650 ± 0.240 ^{a}	8.115 ± 0.106 ^{c}	9.585 ± 0.474 ^{c}	12.562 ± 0.293 ^{bc}	49.727 ± 1.766 ^{c}	6.549 ± 1.509 ^{b}
B2	68.579 ± 0.550 ^{a}	6.887 ± 0.219 ^{b}	11.020 ± 0.669 ^{d}	12.995 ± 0.684 ^{bc}	57.978 ± 0.747 ^{ab}	8.417 ± 0.934 ^{a}
C2	68.261 ± 0.245 ^{ab}	7.057 ± 0.460 ^{b}	10.660 ± 0.160 ^{b}	12.786 ± 0.388 ^{bc}	56.514 ± 1.323 ^{b}	6.589 ± 0.317 ^{b}
T2	68.261 ± 0.245 ^{ab}	8.480 ± 0.028 ^{c}	9.130 ± 0.948 ^{c}	12.468 ± 0.713 ^{cd}	47.032 ± 2.873 ^{c}	6.877 ± 0.061 ^{b}

A marked distinction was observed in the *a** parameter, with a T1 value of 8.115 ± 0.106, which was not significantly different from T2 (8.480 ± 0.028).

The *b** values for T1 and T2 (9.585 ± 0.474 and 9.130 ± 0.948, respectively) were lower than the controls. The lower values at both T1 and T2 may be due to the fact that temperature variations might cause a decrease in the perceived yellowness of the samples.

T1 had a chroma value of 12.562 ± 0.293, whereas T2 displayed a lower value (12.468 ± 0.713). This finding suggests that the low intensity of color at a higher temperature is potentially due to pigment or structural changes within the samples.

The hab presented notable differences between the two temperatures, with T1 having a higher hue angle of 49.727 ± 1.766 compared to T2 (47.032 ± 2.873).

The color difference for T1 was 6.549 ± 1.509, in contrast to T2, which had a ΔE of 6.877 ± 0.061. Although they exhibited relatively lower ΔE values compared to the control groups, the small increase in the ΔE at the higher temperature points to a more discernible change in color from the standard or initial state.

Based on these results, we can conclude that the treatment do not influence the color. Additionally, *L. paracasei* DTA-83 can influence biochemical processes, such as lactic acid production, that can be modulated by temperature [19,24].

4. Conclusions

Our research validated the application of a natural biopreservative derived from lactic acid bacteria as an effective means of extending the shelf life and preserving the quality of sliced cooked ham. Our study revealed that even at a low concentration of 1%, the biopreservative significantly curtailed microbial growth, showcasing its potency in both optimal and fluctuating temperature conditions over an extended period of 24 days. The

treated samples demonstrated superior color stability, indicating the biopreservative's role in mitigating quality degradation.

Our outcomes resonate with the increasing consumer preference for natural food additives and can support a shift of the food industry toward more sustainable and health-conscious preservation methods. The results underline the potential of leveraging bioactive compounds such as lactic acid produced by lactic fermentations to develop natural preservation solutions that not only meet quality standards but also align with the global drive for environmentally responsible food processing following sustainable practices.

Author Contributions: Conceptualization, A.F.G. and W.J.F.L.J.; Formal analysis, A.F.G.; Investigation, A.F.G., L.M.C. and C.A.G.; Data methodology, L.M.C., A.G.B., I.R.V. and C.A.G.; statistical W.J.F.L.J.; writing—original draft preparation, W.J.F.L.J.; writing—review and editing, W.J.F.L.J., V.C. and A.G.; visualization, A.F.G.; coordination and supervision, W.J.F.L.J.; project administration, A.F.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

Acknowledgments: The authors thank the Brazilian National Council for Scientific and Technological Development (CNPq) and meat industries, which enabled this work through their collaboration.

Conflicts of Interest: Author Lucas Marques Costa was employed by the company BRC Ingredientes Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Leroy, F.; Degreef, F. Convenient Meat and Meat Products. Societal and Technological Issues. *Appetite* **2015**, *94*, 40–46. [CrossRef] [PubMed]
2. King, T.; Cole, M.; Farber, J.M.; Eisenbrand, G.; Zabaras, D.; Fox, E.M.; Hill, J.P. Food safety for food security: Relationship between global megatrends and developments in food safety. *Trends Food Sci. Technol.* **2017**, *68*, 160–175. [CrossRef]
3. De Souza, E.L.; De Oliveira, K.Á.; De Oliveira, M.E. Influence of lactic acid bacteria metabolites on physical and chemical food properties. *Curr. Opin. Food Sci.* **2023**, *49*, 100981. [CrossRef]
4. Bosse (Née Danz), R.; Müller, A.; Gibis, M.; Weiss, A.; Schmidt, H.; Weiss, J. Recent advances in cured raw ham manufacture. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 610–630. [CrossRef] [PubMed]
5. Vasilopoulos, C.; De Vuyst, L.; Leroy, F. Shelf-life Reduction as an Emerging Problem in Cooked Hams Underlines the Need for Improved Preservation Strategies. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1425–1443. [CrossRef] [PubMed]
6. Heras, M.; Huang, C.-C.; Chang, C.-W.; Lu, K.-H. Trends in chitosan-based films and coatings: A systematic review of the incorporated biopreservatives, biological properties, and nanotechnology applications in meat preservation. *Food Packag. Shelf Life* **2024**, *42*, 101259. [CrossRef]
7. Oey, I.; Lille, M.; Van Loey, A.; Hendrickx, M. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: A review. *Trends Food Sci. Technol.* **2008**, *19*, 320–328. [CrossRef]
8. Nisa, M.; Dar, R.A.; Fomda, B.A.; Nazir, R. Combating food spoilage and pathogenic microbes via bacteriocins: A natural and eco-friendly substitute to antibiotics. *Food Control* **2023**, *149*, 109710. [CrossRef]
9. Chemat, F.; Rombaut, N.; Meullemiestre, A.; Turk, M.; Perino, S.; Fabiano-Tixier, A.-S.; Abert-Vian, M. Review of Green Food Processing techniques. Preservation, transformation, and extraction. *Innov. Food Sci. Emerg. Technol.* **2017**, *41*, 357–377. [CrossRef]
10. *ISO 20776-2*; Clinical Laboratory Testing and In Vitro Diagnostic Test Systems—Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices—Part 2: Evaluation of Performance of Antimicrobial Susceptibility Test Devices against Reference Broth Micro-Dilution. ISO: Geneva, Switzerland, 2021.
11. CLSI. Clinical and Laboratory Standards Institute consensus process. In *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 2nd ed.; Approved Standard—Ninth. M07-A9; Clinical and Laboratory Standards Institute: Malvern, PA, USA, 2012; Volume 32.
12. AOAC (Ed.) *Official Method of Analysis*; AOAC: Rockville, MD, USA, 2005.
13. *ISO 18787:2017*; Foodstuffs—Determination of Water Activity 15. ISO: Geneva, Switzerland, 2017.
14. *ISO 2917:1999(E)*; Meat and Meat Products—Measurement of pH—Reference Method 9. ITEH Standards: Toronto, ON, Canada, 1999.

15. *ISO 11036:2020. (n.d.)*; Sensory Analysis—Methodology—Texture Profile. ISO: Geneva, Switzerland, 2020.
16. Guerra, C.A.; Costa, L.M.; De Oliveira, V.S.; De Paula, B.P.; Junior, W.J.F.L.; Luchese, R.H.; Corich, V.; Giacomini, A.; Guerra, A.F. Correlation between natural microbial load and formation of ropy slime affecting the superficial color of vacuum-packaged cooked sausage. *Meat Sci.* **2023**, *201*, 109197. [CrossRef] [PubMed]
17. *ISO 4833-1:2013. (n.d.)*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1: Colony Count at 30 °C by the Pour Plate Technique. ISO: Geneva, Switzerland, 2013.
18. Biffin, T.E.; Smith, M.A.; Bush, R.D.; Collins, D.; Hopkins, D.L. The effect of electrical stimulation and tenderstretching on colour and oxidation traits of alpaca (Vicunga pacos) meat. *Meat Sci.* **2019**, *156*, 125–130. [CrossRef] [PubMed]
19. De Lima, A.L.; Guerra, C.A.; Costa, L.M.; De Oliveira, V.S.; Lemos Junior, W.J.F.; Luchese, R.H.; Guerra, A.F. A Natural Technology for Vacuum-Packaged Cooked Sausage Preservation with Potentially Postbiotic-Containing Preservative. *Fermentation* **2022**, *8*, 106. [CrossRef]
20. Iseppi, R.; Camellini, S.; Sabia, C.; Messi, P. Combined Antimicrobial Use of Essential Oils and Bacteriocin bacLP17 as Seafood Biopreservative to Control Listeria Monocytogenes Both in Planktonic and in Sessile Forms. *Res. Microbiol* **2020**, *171*, 351–356. [CrossRef] [PubMed]
21. Clarke, C.; Best, T. Low-carbohydrate, high-fat dieters: Characteristic food choice motivations, health perceptions and behaviours. *Food Qual. Prefer.* **2017**, *62*, 162–171. [CrossRef]
22. Guerrero, L.; Gou, P.; Arnau, J. The influence of meat pH on mechanical and sensory textural properties of dry-cured ham. *Meat Sci.* **1999**, *52*, 267–273. [CrossRef] [PubMed]
23. Lamichhane, P.; Sharma, P.; Kelly, A.L.; Risbo, J.; Rattray, F.P.; Sheehan, J.J. Solubility of carbon dioxide in renneted casein matrices: Effect of pH, salt, temperature, partial pressure, and moisture to protein ratio. *Food Chem.* **2021**, *336*, 127625. [CrossRef] [PubMed]
24. Lemos Junior, W.J.F.; Fioravante Guerra, A.; Da Silva Duarte, V.; Treu, L.; Tarrah, A.; Campanaro, S.; Luchese, R.H.; Giacomini, A.; Corich, V. Draft genome sequence data of Lactobacillus paracasei strain DTA83 isolated from infant stools. *Data Brief* **2019**, *22*, 1064–1067. [CrossRef] [PubMed]
25. Lemos Junior, W.J.F.; Guerra, A.F.; Tarrah, A.; Da Silva Duarte, V.; Giacomini, A.; Luchese, R.H.; Corich, V. Safety and Stability of Two Potentially Probiotic Lactobacillus Strains After In Vitro Gastrointestinal Transit. *Probiotics Antimicrob. Proteins* **2020**, *12*, 657–666. [CrossRef] [PubMed]
26. Andrés-Bello, A.; Barreto-Palacios, V.; García-Segovia, P.; Mir-Bel, J.; Martínez-Monzó, J. Effect of pH on Color and Texture of Food Products. *Food Eng. Rev.* **2013**, *5*, 158–170. [CrossRef]
27. Santiesteban-López, N.A.; Gómez-Salazar, J.A.; Santos, E.M.; Campagnol, P.C.B.; Teixeira, A.; Lorenzo, J.M.; Sosa-Morales, M.E.; Domínguez, R. Natural Antimicrobials: A Clean Label Strategy to Improve the Shelf Life and Safety of Reformulated Meat Products. *Foods* **2022**, *11*, 2613. [CrossRef]