

Article



# Evaluation of the Growth Kinetics of Lactobacillus Plantarum ATCC 8014 on a Medium Based on Hydrolyzed Bovine Blood Plasma at Laboratory and Bench-Scale Levels and Its Application as a Starter Culture in a Meat Product

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**Abstract:** Lactobacilli are used in food because of their beneficial effect on human health and their biopreservative activity in matured meat products. The objective of this work was to study the growth kinetics of *Lactobacillus plantarum* ATCC 8014 by submerged fermentation at laboratory and bench scales, using a culture medium based on bovine blood plasma (BBP) with hydrolyzed proteins, and to evaluate the maturational effects and sensory properties conferred by the obtained biomass on a matured meat product (pepperoni). At bench scale, it was found that the maximum viable biomass concentration of *L. plantarum* was 9.58 log CFU/mL, which was higher than what was found in the MRS culture medium (9.53 log CFU/mL). The mathematical model proposed appropriately described the *L. plantarum* growth kinetics and carbohydrate dynamics during fermentation at laboratory and bench scales in hydrolyzed BBP medium. The application of viable *L. plantarum* biomass propagated on this medium did not show statistically significant differences during pepperoni maturation compared to the product made with the commercial starter culture. The sensory panel found no differences in the evaluated sensory attributes between these two products. The *L. plantarum* biomass obtained on this medium can be used successfully in maturation processes in different meat matrices.

**Keywords:** enzymatic hydrolysis; growth kinetics; mathematical modeling; *pepperoni*; submerged fermentation

# 1. Introduction

A limited number of lactic acid bacteria (LAB) species are used as a starter culture in the meat industry. The main species used are *Lactobacillus sakei*, *L. curvatus*, *L. plantarum*, *Pediococcus pentosaceus*, and *P. acidilactic* [1]. They contribute to food preservation, prolonging shelf-life, improving nutritional and therapeutic value, and promoting changes in aroma, flavor, and texture [2–4]. According to the species, they have complex nutritional requirements, but in general, for lactobacilli, nitrogen should be organic in the form of amino acids or peptides. Within this group, glutamic acid, isoleucine, and valine are considered growth factors that must be present in the culture medium [5,6]. LAB, thanks to their fermentative metabolism in which lactic acid is produced as the main product [7], exhibit an inhibitory potential against the development of altering microorganisms and pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Clostridium* spp., *Staphylococcus aureus*, *Shigella flexneri*, *S. sonnei*, *Salmonella enteritidis*, and *Yersinia enterocolitica* in food [8–11]. This inhibitory potential of LAB is

mainly due to the production of peptides with antimicrobial activity of ribosomal synthesis called bacteriocins [12–15]. Therefore, the application of starter cultures is a technique widely used in the food industry. This practice consists in the inoculation of one or several strains of active bacteria capable of multiplying in the food matrix (raw, cured, and matured meat products) promoting its rapid acidification and producing specific changes in its aroma, flavor, texture, body, acidity, moisture, and digestibility, which allows food preservation [16].

On the other hand, bovine blood is considered as an important waste stream in most slaughterhouses due to its high organic matter content, so alternative ways for its valorization are needed. The blood and protein hydrolyzates of bovine blood plasma (BBP) have been used as a source of nitrogen in the medium preparation for the growth of homolactic starter cultures and production of probiotics and LAB [17–19], although this industrial alternative has been little explored in the food industry. For the use of this waste, the partial enzymatic hydrolysis of its proteins has great potential in the preparation of culture media for microorganisms of industrial interest [20,21] such as LAB employed as starter cultures in the food industry. Nevertheless, submerged fermentation studies at bench scale and pilot level for LAB production from BBP-based media have not yet been reported in the available literature. This kind of study is required in order to get insight on the regularities of this cultivation process and its scaling-up possibilities. Therefore, the objective of this work was to study the growth kinetics of *Lactobacillus plantarum* ATCC 8014 by batch submerged fermentation at laboratory and bench scales, using a culture medium based on bovine blood plasma (BBP) with hydrolyzed proteins, and to evaluate the maturational effects and sensory properties conferred by the obtained biomass on a matured meat product (pepperoni).

# 2. Materials and Methods

#### 2.1. Bovine Blood Plasma

The BBP was supplied by Frigodán Ltd.a. (Bogotá, Colombia), in the form of a fine particle powder. The BBP was reconstituted with distilled water up to a protein content of  $70 \pm 2$  g/L. This substrate underwent physical-chemical (moisture content by gravimetry, fat content by Soxhlet method, minerals by atomic absorption, and pH) and microbiological analyses (counts of coagulase-positive staphylococci, spores of sulfite-reducing clostridia, and *Escherichia coli*, and detection of *Salmonella* spp.) before its use. The protein estimations of the supplied bovine blood plasma powder concentrate and the protein preparations made from it were performed by the Biuret method [22].

#### 2.2. Enzymatic Hydrolysis of Bovine Blood Plasma

For the hydrolysis of bovine blood plasma proteins, the procedure described by Barragán et al. [23] was followed. For this, a 3-L glass bioreactor with water recirculation jacket was used. Alcalase 2.4 L  $FG^{\mathbb{R}}$  (Novozymes, Denmark), a commercial preparation of Subtilisin A, a bacterial serine endoproteasa (EC 3.4.21.62), was added to the BBP at a dosage of 0.10 g/g protein. Protein hydrolysis was performed at 61 °C and pH of 8.5 during 1 h. Enzyme inactivation was conducted at 90 °C.

Assimilable nitrogen (amino nitrogen) was determined in triplicate in the hydrolyzates of BBP proteins obtained to prepare the media, using the potentiometric method described in the AOAC methods [24].

#### 2.3. Strain Activation and Preservation

*L. plantarum* ATCC 8014 strain obtained from the American Type Culture Collection bank was used. For strain activation, lyophilized suspensions were made in sterile saline (1% p/v) for subsequent seeding on Man, Rogosa and Sharpe (MRS) agar (Oxoid, England) and incubation at 37 °C for 48 h in an incubation chamber; this medium is specifically intended for lactobacilli propagation. After this incubation period, bacterial colonies of these cultures were transferred with an inoculation loop to 1.5-mL cryotubes with soy triptone broth supplemented with glycerol and sucrose as a cryoprotectant,

for later freezing at -70 °C until the time of use. These procedures were performed at the Food Microbiology Laboratory of the Food Technology Unit, at the Universidad de Caldas.

# 2.4. Lactic Acid Bacteria Count and Identification

For LAB count at the established sampling times, the direct plate count technique was used. For this purpose, MRS agar with 3% aniline blue contrast dye was used, and its result was expressed as Colony Forming Units per milliliter (CFU/mL) [25]. For their identification, biochemical tests of microscopic observation (determination of cytochrome, oxidase and catalase enzymes) were performed. For the catalase test, part of the center of a colony was transferred to the surface of a glass slide. Subsequently, a drop of 3% hydrogen peroxide was added and bubble formation was observed. For the cytochrome oxidase test, a colony was dispersed with the inoculation loop on Bactident<sup>®</sup> (Darmstadt, Germany) Oxidase commercial strips [26,27].

# 2.5. Preparation of Culture Media

The composition of the culture medium used for the fermentation tests is presented in Table 1. For its preparation, a bovine blood plasma hydrolyzate with an amino nitrogen content of 825 mg/L was used to replace the nitrogen sources of the MRS commercial medium (peptone, meat extract, and yeast extract). The other micronutrients in the MRS broth were added according to the recommendations of Man et al. [28] for lactobacilli culture. In the original MRS medium, the carbon source is glucose at a concentration of 20 g/L; in this work, sucrose was used as a carbon source in order to reduce the cost of the culture medium based on a waste stream from meat industry. For laboratory tests, 20 g/L sucrose was used for preparing the medium, while 80 g/L sucrose was used for bench-scale tests, considering the need to obtain high concentrations of LAB biomass. The media were adjusted to a pH of 6.2 with citric acid and finally sterilized in a vertical autoclave (Tecnik, Colombia) at 120 °C for 15 min under 14 psi.

Components	Quantity		
BBP with hydrolyzed proteins (corresponding to an AMN content of 825 mg/L)	750 mL/L		
Cuerose -	20.00 g/L (lab scale)		
Sucrose	80.00 g/L (bench scale)		
Potassium diacid phosphate	2.00 g/L		
Sodium acetate	5.00 g/L		
Ammonium citrate	2.00 g/L		
Magnesium sulfate	0.20 g/L		
Manganese sulfate	0.05 g/L		
pH: 6.4 ± 0.2 at 25 °C			

Table 1. Composition of the bovine blood plasma-based medium.

AMN: Amino nitrogen; BBP: Bovine blood plasma.

# 2.6. Fermentation Kinetics of L. plantarum at the Laboratory Level

*Lactobacillus* plantarum ATCC 8014 fermentations were carried out in triplicate in the liquid medium presented in Table 1 in 250-mL flasks. The media were adjusted to a pH of 6.2 with citric acid and finally sterilized in a vertical autoclave (Bogotá, Colombia) at 120 °C for 15 min at a pressure of 14 psi. 250-mL Erlenmeyer flasks with an effective volume of 150 mL were used and seeded with an inoculum level of 2.5% (v/v). These inoculated media were incubated at 37 °C in a chamber (Binder, Germany) without shaking for 60 h with sampling at 12-h intervals to monitor cell growth as CFU/mL and substrate consumption in g/L.

#### 2.7. Fermentation Kinetics of L. plantarum at Bench Scale

The kinetic study at bench scale, using the culture medium described in Table 1, was carried out in a 3 L bioreactor (Cali, Colombia) with stirring at 40 rpm to homogenize it without an air supply; this culture was performed in triplicate. Additionally, a reference culture was carried out, also in triplicate, using the commercial MRS medium in 250-mL-Erlenmeyer flasks with an effective volume of 150 mL without stirring. All cultures were seeded with 2.5 % (v/v) inocula and incubated at 37 °C for 60 h with sampling at 12-h intervals. The concentrations of viable cell biomass per milliliter of medium (in CFU/mL) and total carbohydrates were measured according to the method of Dubois et al. [29].

# 2.8. Mathematical Modeling of L. plantarum Culture

A deterministic, non-segregated and dynamic model was used for the mathematical modeling of the growth kinetics of *L. plantarum* ATCC 8014 culture. For this purpose, experimental data on cell growth (in log CFU/mL), and total carbohydrate concentration (in g/L), obtained at the laboratory (150 mL) and bench-scale (3 L) levels were used.

The mathematical model proposed for both scales (laboratory and bench-scale) included the modified logistic equation [30] for describing the formation of *L. plantarum* ATCC 8014 cellular biomass in time, according to the following expression:

$$\frac{dX}{dt} = \mu_{max} X \left[ 1 - \left( \frac{X}{X_{max}} \right)^n \right] \tag{1}$$

where  $\mu_{max}$  is the maximum specific growth rate (in h<sup>-1</sup>),  $X_{max}$  is the maximum biomass concentration (in log CFU/mL), X is the biomass concentration evaluated at a given time (in log CFU/mL), and n is an inhibition factor, such that if n < 1, the organism is relatively sensitive to self-inhibition and this occurs for very low values of X. When n = 1, it is the conventional logistic equation, and when n > 1, the organism is relatively resistant to self-inhibition and this occurs only when X tends to  $X_{max}$ .

To represent the behavior of carbohydrate dynamics over time at the laboratory and bench-scale levels, the equation proposed in a previous work by Montoya, et al. [31] was used:

$$\frac{dCHO}{dt} = q_p \cdot \mu_{max} \cdot \frac{dX}{dt} \cdot \left[ 1 - (n+1) \cdot \left(\frac{X}{X_{max}}\right)^n \right]$$
(2)

where  $q_p$  is the constant rate of carbohydrate production (in g carbohydrates/(log CFU × h<sup>-1</sup>)) and  $\mu_{max}$  is the specific growth rate (in h<sup>-1</sup>).

The software Matlab<sup>®</sup> R2013 (MathWorks, Natick, MA, USA) was used for solving the mathematical model. A non-linear regression algorithm was used to obtain the kinetic parameters of the model equations using the lsqcurvefit function. For its resolution, the ode45 function was used based on the numerical method that applies an explicit Runge–Kutta formula.

#### 2.9. Validation of the Use of Biomass Obtained in the Elaboration of a Meat Product

For the validation of the maturation activity of the *L. plantarum* ATCC 8014 biomass obtained in the fermentation at bench scale, it was separated from the fermentation broth by centrifugation, followed by continuous washing after centrifugations with sterile distilled water, to eliminate interference from the medium constituents. Subsequently, the amount of biomass required for the production of the meat product was transferred to vials with tryptic soy broth supplemented with glycerol and sucrose for its preservation at -20 °C, until the time of use.

The temperature and pH of the initial meat raw material were recorded. At this stage, the temperature did not exceed 7 °C and the pH varied between 5.4 and 5.8. Subsequently, the meat raw material was stored at a temperature below 0 °C for 24 h to favor obtaining clean cuts at the time of cleaning and chopping. The connective tissue and other undesirable tissues were removed from the meat, which was then frozen

at -18 °C for 24 h. For the production of pepperoni, 72.80% lean pork and 23.80% bacon were used. In the case of the control product, the meat was chopped in a cutter (Javar-CTT15, Cundinamarca, Colombia) to a size of approximately 1 cm in diameter, while the commercial culture (Lyocarni SHI-59, Sacco, Italy) consisting of *Staphylococcus xilosus*, *Pediococcus pentosaceus*, and *L. plantarum* was added. At the same time, a product with the same formulation was prepared, but using *L. plantarum* ATCC 8014 cell biomass obtained in the fermentation at bench scale which was previously preserved. The formulation was prepared by reconstituting the *L. plantarum* ATCC 8014 cell biomass in water, salts, nitrates, condiments, and commercial sucrose (see Table 2). Bacon was then added and combined until a homogeneous mixture of 1–2 mm in diameter was obtained. The mixture was stuffed with a CAEH20 hydraulic filler (Bogotá, Colombia) in 26-mm beef collagen casings (Medellín, Colombia). Sausages were taken to storage at 3 °C for 24 h to condition the starter strain in the meat matrix.

Ingredient	Percentage (%)	Quantity (kg)
Lean pork 90/10	72.80	3.640
Bacon	23.80	1.190
Polyphosphate mix (801)	0.36	0.018
Garlic powder	0.07	0.004
Refined salt	1.40	0.070
Nitral-curing salt (5700)	0.33	0.017
Ascorbic acid	0.14	0.007
Commercial sucrose	0.50	0.025
White pepper	0.28	0.014
Black pepper	0.28	0.014
Starter culture (L. plantarum ATCC 8014 and/or Lyocarni SHI-59 commercial culture)	0.03	0.002
Total	100.00	5.000

Tab	le 2	2. L	ow	acidity	pepperoni	formu	lation.
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Once the storage was finished, the product was transferred to a heated chamber (Büchenbach, Germany) for its ripening and drying, which started with a temperature from 20 to 25 °C and relative humidity of 80–85% for 8 days. Then, the temperature was lowered down to 14 °C with 84–90% relative humidity for 2 days, and finally the relative humidity was modified between 70% and 75% for three days to avoid the shortening of the mixture until achieving a weight decrease from 20 to 25%. After ripening, the *pepperoni* sausages were cut in a chopper (Milano, Italy) into 3 mm thick slices arranged in rows of 10 and vacuum packed (Plochingen, Germany) in flexible polyamide bags and sealing layer of 70- $\mu$ m-thick low-density polyethylene. This film has a water vapor transmission of 15 g/m<sup>2</sup>/day/atm at 38 °C and 100% relative humidity, as well as an oxygen permeability of 60 cc/m<sup>2</sup>/day/atm at 23 °C and 0% relative humidity.

The percentage of pepperoni moisture obtained was determined by gravimetry in a halogen moisture lamp (Kyoto, Japan). For the evaluation of the fermentation capacity of the studied strain during the processes of resting, fermentation, and drying, the pH was determined by potentiometry (Herisau, Switzerland) on a crushed and homogenized sample of both study and control pepperoni. Likewise, the determination of titratable acidity expressed as a percentage of lactic acid was performed in duplicate by taking 10 g of pepperoni sample and homogenizing it with 200 mL of distilled water. This sample was filtered and titrated with a 0.1 N NaOH solution using phenolphthalein as an indicator and 100 mL of distilled water as a blank [32]. The percentage of lactic acid was expressed as follows:

% lactic acid = 
$$\left(\frac{V \, NaOH * N \, NaOH * meq \, of \, lactic \, ac * f}{Sample \, weight}\right) \times 100$$
 (3)

where *f* represents the dilution factor of the sample.

Microbiological analyzes of both, pepperoni with the conventional formula and pepperoni obtained using *L. plantarum* ATCC 8014, were performed according to the microbiological requirements

for matured or fermented meat products required by Colombian standard NTC 1325 [33] for non-canned processed meat products.

# 2.10. Sensory Analysis of Fermented Products

For the sensory evaluation, a triangle taste test was applied, according to the methodology proposed by Larmond [34,35]. In this test, a panel of 10 semi-trained testers was presented with three samples; two of them were the same sample and testers were asked to identify the different one. Finally, to evaluate the general quality of the product as high, medium, or low, descriptive and quantitative tests described by Colombian standards NTC 5328 and 3932 were applied. A questionnaire was structured for the studied pepperoni and control product. The non-parametric method of Kruskal and Wallis was used [36] for the analysis of the data obtained in the sensory evaluation because they are continuous qualitative variables.

# 3. Results and Discussion

# 3.1. Kinetic Analysis of L. plantarum Culture on Plasma-Based Media at Laboratory and Bench Scale

Figure 1a,b show the experimental data of L. plantarum ATCC 8014 cell growth on two media based on bovine blood plasma with hydrolyzed proteins at the laboratory and bench-scale levels, respectively. It is observed that cellular biomass increases significantly in the first 36 h of culture for the laboratory scale, while at bench scale the biomass steadily increases in the first 26 h reaching higher values. Regarding the concentration of total carbohydrates in both fermentations, it is observed that their concentration at the laboratory-scale fermentation takes up to 10 h to begin the reduction and to subsequently exhibit a traditional decreasing substrate behavior. For bench-scale fermentation, the carbohydrate content increases from the beginning until 12 h and then decreases until 36 h. From this moment, the concentration of carbohydrates is stabilized, which coincides with the beginning of the stationary phase of lactobacilli growth. It is important to note that the method used to determine the concentration of the carbon source corresponded to the quantification of total carbohydrates [29]. This method not only quantifies the sucrose content, but also includes the content of different types of carbohydrates including monosaccharides, oligosaccharides, and polysaccharides. The above explains the atypical behavior of carbon source kinetics at bench scale since different researchers have demonstrated the formation of exopolysaccharides during lactic fermentation [37–39], which represent a mechanism of defense of bacteria against desiccation, phagocytosis, phage attack, antibiotics, toxic compounds, protozoan predation, and osmotic pressure, among other factors, in addition to playing a role in cell recognition. This ability of lactobacilli to synthesize exopolysaccharides may explain the increase in carbohydrate concentration at the beginning of fermentation, especially if considering that the culture conditions in a bioreactor are more severe and controlled than in a laboratory flask.

With the experimental data from Figure 1, the non-linear regression algorithm was applied to find the four parameters of the mathematical model proposed (see Table 3). The specific cell growth rate obtained for the culture model at bench scale  $(0.0545 h^{-1})$  was higher than the average of the  $\mu_{max}$  values for the laboratory scale  $(0.0111 h^{-1})$ . The maximum biomass  $X_{max}$  also showed higher values at bench scale (9.4136 CFU/mL) than at the laboratory scale (7.44 log CFU/mL). The above is explained by the particularities of the technological system of fermentation at the laboratory and bench-scale levels, since in the latter the conditions of the environment are more homogeneous, which translates into better availability of nutrients, substrates, and dispersion of products. On the other hand, the  $q_p$  coefficient showed substantially higher absolute values at the laboratory scale produces a more tangible decrease in the concentration of total carbohydrates in the medium, considering that it does not seem to indicate a greater production of exopolysaccharides than the consumption of carbohydrates, a phenomenon

n



that can be seen in the fermentation at bench scale. On the other hand, the values of the n factor indicate that the microorganism is relatively resistant to self-inhibition in both cultivation scales.

**Figure 1.** Time-profiles of *L. plantarum* ATCC 8014 biomass (blue line) and total carbohydrates (red line) concentrations obtained during batch submerged fermentation on media based on bovine blood plasma with hydrolyzed proteins. Discrete markers (**x**) correspond to the experimental data; the continuous lines were calculated by the kinetic model proposed. (**a**) Laboratory level (150 mL of working volume); (**b**) bench-scale level (2.5 L of working volume).

manual and Thee solf culture on a plasma based mediani. Source. Own elaboration.						
Parameter	Bench-Scale Fermentation	Lab-Scale Fermentation	Units			
μ <sub>max</sub>	0.0545	0.0111	h <sup>-1</sup>			
$X_{max}$	9.4136	7.4410	log CFU/mL			
$q_p$	-102.1780	-478.7656	$g \times \log CFU^{-1} \times h^{-1}$			

9.002

Dimensionless

4.6357

**Table 3.** Kinetic parameters of the kinetic model of submerged fermentation at the bench scale for *L*. *plantarum* ATCC 8014 culture on a plasma-based medium. Source: Own elaboration.

When solving the system of two ordinary differential Equations (1) and (2) that make up the mathematical model with these parameters values, the kinetic curves were generated as depicted in Figure 1 through continuous lines. The proposed deterministic, non-segregated, and dynamic model adequately described the growth kinetics of L. plantarum ATCC 8014 on a discontinuous culture at bench scale and laboratory level, as well as the dynamics of carbohydrates for these two cases. Considering the above, the proposed kinetic model captures well the complexity of these phenomena. In particular, it is suggested that the sucrose present in the medium is hydrolyzed into its two constituent monosaccharides (glucose and fructose). These monosaccharides are absorbed by bacterial cells for their energy metabolism and, subsequently, for the synthesis of exopolysaccharides, which leads to a complex balance of total carbohydrates present in the culture broth. Equation (2), proposed in a previous work [31], applied a kinetic relationship that describes the formation and consumption of reducing sugars during the degradation of lignocellulosic materials by white rot fungi. In this work, the aforementioned equation was applied to model this carbohydrate balance based on the growth rate of cells and, to a lesser extent, on their concentration. Regarding the biomass concentration, the obtained adjustment allows us to conclude that, at both, laboratory and bench-scale levels, the logistic equation appropriately captures growth behavior at its exponential and stationary phase for the fermentation systems studied.

A submerged fermentation process was implemented at bench scale using the proposed medium for *L. plantarum* growth based on bovine blood plasma with enzymatically hydrolyzed proteins (see Table 1). The studied medium was then compared to a commercial medium (MRS) for *L. plantarum* propagation as can be observed in Figure 2. It is evident that the cell biomass curve during fermentation

at bench scale has a behavior similar to that obtained at the laboratory level (150-mL working volume). However, the comparison with the biomass formation curve in the MRS medium indicates that there is an appreciable latent phase in the plasma-based medium. This latency is due to the period of adaptation required by lactobacilli to assimilate the carbon and nitrogen sources available in the plasma-based medium, which are different from the MRS commercial medium: glucose as a source of carbon and peptone, yeast and meat extracts as nitrogen sources. This means that time required for reaching the maximum values of cell biomass, increases from 24 h in the commercial MRS medium to 52 h in the plasma-based medium. Although it is evident that the growth rate in the medium based on the hydrolyzate of BBP proteins at bench scale has a lower rate than that of the MRS medium, it achieves higher concentrations of viable biomass of *L. plantarum* (9.58 log CFU/mL or  $3.80 \times 10^9$  CFU/mL versus 9.53 log CFU/mL or  $3.39 \times 10^9$  CFU/mL of the MRS medium). This result is relatively similar to that reported by Hyun and Shin [19] for a medium based on an enzymatic hydrolyzate of bovine blood plasma mixed with MRS broth, with a protein content close to that of this study of 30.2 g/L, in which a maximum concentration of 9.71 log CFU/mL ( $5.13 \times 10^9$  CFU/mL) was obtained at 24 h of fermentation.



**Figure 2.** Cell growth of *L. plantarum* ATCC 8014 in the medium based on bovine blood plasma with hydrolyzed proteins at bench scale and its comparison with a laboratory level culture in the MRS reference medium.

It should be noted that the MRS medium is already optimized for the proliferation of lactobacilli in general at the laboratory, but its use at bench, pilot, or industrial levels is limited by its high costs. It is precisely for this reason that a plasma-based medium from a residual effluent, such as bovine blood from slaughterhouses, is an attractive alternative to use a waste from the meat industry, especially when the final biomass concentrations reached are comparable to those of a medium used for laboratory level research.

#### 3.2. Validation of the Use of the Biomass Obtained for the Elaboration of a Meat Product

The cell biomass of *L. plantarum* obtained during bench-scale fermentations was recovered from the culture broth and washed in order to be used for preparing pepperoni, a maturated meat product. For comparison, pepperoni was prepared by using a commercial starter culture widely employed in meat industry. The results of the physical-chemical and microbiological requirements of both the meat raw material and finished products are consolidated in Tables 4 and 5.

Physical-Chemical Analysis					
рН	5.8-6.2				
Acidity	1.745%				
Moisture	72.30%				
Microbiological analysis					
Coagulase-positive staphylococci count, CFU/g	<100				
Sulfite-reducing clostridia spore count, CFU/g	<100				
Detection of Salmonella spp., /25 g	Absence				
<i>Escherichia coli</i> count, /g	<3 bacteria/g				

Table 4. Physical-chemical and microbiological analyses for pork.

**Table 5.** Physical-chemical and microbiological parameters of *pepperoni* with *L. plantarum* and control with commercial culture.

Physical-Chemical Analysis						
Pepperoni with L. plantarum		Pepperoni with commercial culture				
pH	5.56	pH	5.28			
Acidity	0.3843%	Acidity	0.4240%			
Moisture	23%	Moisture	22.46%			
1	Microbiologi	cal Analysis				
Pepperoni with L. plantarum		Pepperoni with commercial culture				
Coliform count /g	<10	Coliform count /g	<10			
Coagulase-positive Staphylococcus aureus count, CFU/g	<100	Coagulase-positive Staphylococcus aureus count, CFU/g	<100			
Sulfite-reducing clostridia spore count, CFU/g	<10	Sulfite-reducing Clostridium spore count, CFU/g	<10			
Detection of Salmonella spp, /25 g Absence		Detection of Salmonella spp, /25 g	Absence			
Detection of Listeria monocytogenes, /25 g	Absence	Detection of Listeria monocytogenes, /25 g	Absence			
E. coli/g count	<10	E. coli/g count	<10			

The data presented in Tables 4 and 5 were within the indices allowed by the Colombian standard NTC 1325, which indicates adequate management of hygienic conditions in pre-processing and subsequent stages of pepperoni preparation, including cutting and packing. In this way, raw materials have the appropriate sanitary conditions to be subjected to the maturation process, and they can also go through the stages of conditioning, drying, and ripening adequately to obtain a product that meets the sanitary requirements for its consumption. The values of pH, acidity, moisture, and count of altering microorganisms correspond to a level of good quality and absence of pathogens and provide a suitable medium for the development of starter cultures (LAB), allowing these bacteria to more easily colonize the pepperoni mixture and develop its characteristic fermentative metabolism without competing with other microbial flora. The comparative results of the physical-chemical and microbiological analyses of pepperoni made with the studied strain and the control pepperoni made with the commercial starter culture are shown in Table 5. It is observed that the control product and the pepperoni made with *L. plantarum* have a good quality level according to NTC 1325. In addition, moisture and pH meet the composition and formulation requirements for matured or fermented products.

The results obtained for the triangle sensory analysis of pepperoni using *L. plantarum* ATCC 1084 and the control with commercial culture are presented in Table 6. These outcomes show that 70% of the testers identified pepperoni made with *L. plantarum* as a different sample. However, 50% of the testers rated the samples as slightly different and 20% of them thought the differences were moderate.

Table 7 shows the results of the Kruskal Wallis test for multidimensional profile analysis obtained from the sensory evaluation of the nine descriptors applied to pepperoni and performed by ten semi-trained testers. It can be observed that there are no significant differences in the perception of the intensity of each descriptor for pepperoni made with *L. plantarum* and with the commercial culture, given that there are no differences between the means.

Figure 3 shows the multidimensional sensory profile obtained for the product inoculated with *L. plantarum* and for the product inoculated with the commercial culture. The result shows that the

sensory profile of pepperoni in the evaluation overlaps for all descriptors, except for the color and characteristic odor or aroma, which are still close.



**Figure 3.** Sensory profile of *pepperoni* prepared with commercial culture vs. *pepperoni* with *L. plantarum* propagated at bench scale.

**Table 6.** Triangle test results of pepperoni with commercial culture and with *L. plantarum* propagated at bench scale.

Triangle Test: (Pepperoni Control with Commercial Culture (622, 768) and Pepperoni with L. Plantarum (577)						
Panel	Sample Code: 577			Sample Code: 622, 768		
Tester	Slight Difference	Moderate Difference	Large Difference	Slight Difference	Moderate Difference	Large Difference
Tester 1	1					
Tester 2				1		
Tester 3	1					
Tester 4	1					
Tester 5	1					
Tester 6		2				
Tester 7					2	
Tester 8	1					
Tester 9		2				
Tester				1		
10				1		

The appearance of products formulated with the commercial culture and with *L. plantarum* is shown in Figure 4. It is evident that the color and general appearance of the control product and that made with *L. plantarum* do not show an appreciable visual difference.



**Figure 4.** Appearance of ripened *pepperoni* with commercial culture (**a**) vs. appearance of ripened *pepperoni* with *L. plantarum* ATCC 8014 (**b**) propagated on a medium based on the enzymatic hydrolyzate of bovine blood plasma.

Pepperoni Type with	Descriptor	Mean	Standard Deviation	Coefficient of Variation	<i>p</i> -Value Kruskal-Wallis
Commercial culture	Color	4.1	±1.37032	33.42%	0 310933
L. plantarum	Color	4.7	±1.25167	26.63%	0.010900
Commercial culture	Characteristic odor/aroma	4.6	±1.26491	27.50%	
L. plantarum	Characteristic odor/aroma	4.4	±1.3499	30.68%	0.785341
Commercial culture	Objectionable odor/aroma	0.9	±1.52388	169.32%	
L. plantarum	Objectionable odor/aroma	0.6	±0.699206	116.53%	0.966559
Commercial culture	Characteristic meat flavor	5.8	±0.918937	15.84%	
L. plantarum	Characteristic meat flavor	5.8	±1.0328	17.81%	1
Commercial culture	Salty taste	2.4	±2.1187	88.28%	
L. plantarum	Salty taste	2.4	±1.7127	71.36%	0.843768
Commercial culture	Sour taste	2.8	±1.75119	62.54%	
L. plantarum	Sour taste	2.7	±1.56702	58.04%	0.938339
Commercial culture	Objectionable taste	0.3	$\pm 0.483046$	161.02%	0.887537
L. plantarum	Objectionable taste	0.4	±0.699206	174.80%	0.007007
Commercial culture	Fatty taste	3.7	±1.49443	40.39%	0.815117
L. plantarum	Fatty taste	3.8	±1.54919	40.77%	0.010117
Commercial culture	Juiciness	4.4	±1.07497	24.43%	0.874162
L. plantarum	Juiciness	4.3	$\pm 0.948683$	22.06%	. 0.071102

**Table 7.** Results of the Kruskal Wallis test for the sensory evaluation of *pepperoni* from the semi-trained panel of ten testers.

0 = absence, 1 and 2 = mild, 3 and 4 = moderate, 5, 6 and 7 = intense.

Regarding the overall quality, the results of the semi-trained panel were as follows: 70% of the testers considered pepperoni made with *L. plantarum* with a high-quality level and the remaining 30% evaluated it with medium quality. For the control product, 80% of the testers evaluated it with a high-quality level and 20% with medium quality.

# 4. Conclusions

It was found that the maximum viable biomass concentration of *L. plantarum* ATCC 8014 at bench scale in a submerged batch fermentation reached 3802 million CFU/mL in a medium based on bovine blood plasma medium with hydrolyzed proteins with an amino nitrogen content of 825 mg/L and 80 g/L sucrose, which corresponds to a concentration 12.2% higher than that obtained in the MRS medium. The mathematical model proposed to find the growth profile of *L. plantarum* ATCC 8014 can be used to predict the growth kinetics behavior of viable lactobacilli biomass at the bench scale and laboratory level. It can also predict the dynamics of carbohydrates in media that use protein hydrolyzates of BBP as a nitrogen source.

Pepperoni made using *L. plantarum* ATCC 8014 cell biomass propagated at bench scale on the BBP-based medium did not show a statistically significant difference for pH, water activity and sensory properties during the maturation, compared to the control pepperoni made with the commercial starter culture. Thus, the viability of the production of LAB starter cultures (in the case of *L. plantarum*) was demonstrated, using media based on hydrolyzed bovine plasma proteins. Its application as starter cultures in meat matrices of matured products allows reaching a maturation capacity, sensory stability and general quality similar to that obtained with commercial starter cultures.

With this work, the usefulness of a novel process for the utilization of a waste from the meat supply chain was explored and demonstrated. This process is an attractive alternative to obtain products with greater added value, such as ripening starter cultures for the food industry. In addition, the fermentation process disclosed in the present work has the potential to decrease the environmental impact caused by the discharges of wastewater in slaughterhouses and related facilities.

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