Modeling The Kinetics of Lactic Initiators Using Lactobacillus Delbrueckii Ssp Bulgaricus and Streptococcus Thermophilus in Jora Corn Germinated Grain Hydrolysate

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Abstracts: The objective of this research was to determine the growth kinetics of lactic acid starter bacteria such as Lactobacillus delbrueckii ssp bulgaricus and Streptococcus thermophilus, experimentally using the Gompertz and Baranyi-Roberts mathematical models. As part of the method, lactic bacteria were inoculated in a germinated jora maize hydrolyzate at temperatures of 40°C, 41°C and 42°C. The Log10 CFU/ml growth initiators will be finished, for 12 hours with iterations of 1 hour each. The growth parameters and lactic initiated strains were adjusted with the Gompertz and Baranyi-Roberts models using the Curveexpert 1.4 and DMfit programs from Combase, which were validated through the mathematical indices R2, root mean square error (RMSE), bias factor (Bf) and precision (Af). The results showed that increasing temperatures decreased with lag time, while generation time slightly increased growth velocity based on logarithmic levels of the initial population. The adjustment of the kinetic growth curves showed that the initial population (N0) for the Gompertz model at temperatures of 40°C, 41°C and 42°C are 5.28, 5.46 and 5.36 Log CFU/ml, with no significant difference between them (p> 0.05), it will increase the logarithmic growth levels of 2.0, 2.37, 2.99 Log CFU/ml, with µmax speeds of 0.53, 0.49 and 0.45 Log CFU/ml/h and for the Baranyi-Roberts model the initial population at the same temperatures. of study are 5.25, 5.39 and 5.37 Log CFU/ml, with logarithmic levels of 1.93, 2.46, 3.02 Log CFU/ml and µmax growth rates of 0.33, 0.36 and 0.48 Log CFU/ml/h corresponding to Lbd.

Keywords: Predictive, Microbiology, Lactic Acid, Bacteria. Lactic Initiators.

1. INTRODUCTION

The racial diversity of maize in countries such as Peru and Mexico has been conserved over time as a primary center of genetic diversity of this species [1]. Furthermore, in Mexico and Latin America, maize is considered a staple food in the diet of the population and is consumed in a wide variety of forms and preparations [2]. Worldwide, maize (Zea mays L.) plays a significant role as a source of energy and protein in the human diet [3]. In addition, it has an outstanding antioxidant capacity due to its high concentrations of secondary metabolites, such as phenolic compounds, flavonoids and carotenoids that contribute to its antioxidant properties, which is beneficial to the consumer's health [4] and physicochemical and functional properties of corn (*Zea mays L*.) itself, which are closely related to the structure of the starch whose molecules are formed by two glucose polymers: amylose and amylopectin [5]

The way to take advantage of the starch structure or matrix and for it to develop efficiently is through its germination process. [6] and that it goes through a process that allows having a substrate with an abundance of nutritional monomers such as starch hydrolysis [7]. In this process, acid is added to the concentrated starch suspension (36% and 40% solids) and the temperature is controlled to avoid its gelatinization (40°C and 60°C), with

mineral acid [8]. However, phenolic type inhibitors can be generated when the pH condition is low and can inhibit the survival conditions of bacteria, molds or yeasts [9]. Therefore, the acid hydrolysis of starch is the most widely used as a pretreatment step to obtain fermentable sugars [10].

In the same way, another of the most widely used processes to improve food quality and food preservation is fermentation [11]. These fermentations involve the use of microorganisms such as bacteria and yeasts to process cereals and transform their components, resulting in foods with improved organoleptic characteristics and longer shelf life [12]. On the other hand, fermentation requires anaerobic environments, allowing carbohydrates to be good substrates in fermentative processes [13].

Lactic acid bacteria are widely distributed in various media and are used in food production and processing as starter cultures, in order to monitor fermentative processes [14] and their ability to produce lactic acid and inhibit the growth of pathogens [15]. Likewise, a starter culture can be defined as a preparation containing a high number of microorganisms that are added to a raw or pasteurized feed to initiate, accelerate and direct its rapid fermentation [16]. Starter cultures for the production of fermented products generally consist of lactic acid bacteria selected for their ability to grow at the beginning of the technological process [17]. They also produce an abundant amount of lactic acid to lower the pH, giving a pleasant taste and aroma to the final product [18]. It is currently accepted the possibility of preserving food by adding starter cultures or by incorporating antimicrobial metabolites produced by lactic acid bacteria [19] that are involved in a fermentation process whose capacity to generate various compounds with inhibitory properties lies in the synergy of action of their metabolites on unwanted bacteria [20].

Lactic starter cultures can trigger fermentation processes using simple substrates. [21] used as substrate a mixture of water and bread and inoculated starter cultures (*Lactobacillus rhamnosus*) at a concentration of 6-7ufc/g fermenting them at 38ºC for 24 hours, where he showed a very high acidification rate (0.36±0.04) and better viability. [22] studied the lactic fermentation of whey and potato starch as substrate. They inoculated the whey with 100µL of *lactobacillus bulgaricus*. They obtained an average value of 6.91 ± 0.3769% of titratable acidity for 24 hours concluding as optimal for obtaining lactic acid.

Although the substrate factor is important, the temperature factor is also important and is considered to be responsible for the activation of metabolic processes in the starter bacteria and the optimal maintenance of the substrates. The optimal temperature range used in the dairy industry for the growth of thermophilic cultures is between 40-45ºC, such as some species of the genera Streptococcus, *Lactobacillus Str. thermophilus* and L. *delbrueckii ssp. bulgaricus* used for the production of yogurt [23].

According to the studies of [24] determined that the appropriate growth conditions of *Lactobacillus brueckii ssp. bulgaricus* and *Streptococcus thermophilus* for the production of lactic acid in whey substrate. They obtained lactic acid with 78.0% purity (36.7 g/L), at temperatures of 40 ºC, considering that it is necessary to supplement the whey with lactose in order to provide fundamental nutrients for their growth and development.

[25] determined that the strain *L. plantarum* ATCC 8014 produced exopolysaccharides (EPS) using corn stover hydrolysate (1.31 g/L), at temperature 30°C, pH 6.5 and 48 hours of incubation and at 37°C, it was 1.63 g/L, while the EPS production by *L. rhamnosus* was 1.63 g/L at 37°C. The total carbohydrate EPS obtained under the above conditions were 83.18% and 80.73% for *L. rhamnosus* and L. plantarum ATCC 8014, respectively.

It is important to recognize the conditions offered by Jora corn as a substrate as mentioned by [26] where they elaborated a fermented beverage based on wheat flour, chickpea and Jora corn (Zea mays L.) obtaining a proximate analysis in protein values of 10.52 %, humidity 13.5 %, fat 3.7 %, carbohydrates 73.4 % and ashes 1.7 % made with Jora corn. The beverage presented a decrease in pH, fluctuating at the beginning of fermentation with 4.27- 4.80 and ending with 3.11- 3.44. The acidity (expressed in g/l lactic acid) registered an increase of this parameter of 0.08-0.93 allowing to cover the purpose of obtaining the fermented beverage. [27] elaborated a fermented beverage using lactic acid bacteria with quinoa as fermentable substrate. The final product had a pH value of 4.2, acidity 0.308% and 10°Bx. The selected product was evaluated by 70 consumers, comparing an unsweetened fermented quinoa beverage, a sweetened fermented quinoa beverage and a commercial fermented

rice beverage. Eighty-three percent of the consumers expressed acceptance of the sweetened quinoa drink.

In turn [28] studied the responsibility of lactic acid bacteria in fermentation processes, for such reason that the lack of control caused compounds such as lactates, acetates, ethanol and carbon dioxide to be obtained. The fermentation was stopped by destroying these bacteria by means of thermal treatments, for which they subjected the “chicha de Jora” to different thermal profiles (46 ºC, 48 ºC, 50 ºC, 52 ºC, 53 ºC, 55 ºC, 60 ºC, 65 ºC and 70 ºC) and each of them to different exposure times (0, 5, 10, 15 and 20 minutes). It was concluded that the thermal death time of the lactic acid bacteria in the chicha de Jora is 15 minutes exposed to 53ºC.

Lactic acid bacteria have the capacity to use starchy substrates, however, their own nature can condition their growth. [29] elaborated fresh tocosh , potato-based food, showing that the number of lactic acid bacteria colonies during fermentation for the treatment of (potato + water) in the first 5 days was 9×10^5 cfu/g, for 30 days increased to 74×10^5 cfu/g; while, for (potato + water + carbon source (CF): 2.5 % + nitrogen source (NF): 1.5 g/L) the number of colonies at 5 days was 29×10^4 cfu/g increasing significantly at 30 days to 19×10^6 cfu/g; and for the treatment (potato without peel + water + FC: 2.5 % + FN: 1.5 g/L) in the 5 days was 11×10^3 cfu/g and for the 30 days was at 73×10^5 cfu/g in this last treatment it was observed that the growth is low compared to the other treatments, due to the fact that the peel was removed and with it the microbial load. It is concluded that the percentage of viability for these treatments decreased.

[30] evaluated soybean, quinoa and barley hydrolysates separately as the most favorable substrate for the growth viability of the probiotic *Lb. Acidophilus* .The results showed that its growth in soybean hydrolysate had latency time of 5.03 hours, maximum growth rate 0.40 Log CFU/ml/h and generational time 1.71 hours; whereas, those obtained in quinoa hydrolysates latency time was 6.08 hours, growth rate 0.37UFC/ml/h, generation time 1.84 hours and the barley hydrolysate latency time was 7.02 hours, growth rate 0.33 Log UFC/ml/h and generation time 2.08 hours. It was concluded that soybean hydrolysate is the best substrate for the growth of *Lb. Acidophyllus*. [31] conducted a starch hydrolyzate of chulpi corn (*Zea mays sacchara*), to convert it into fermentable sugars, followed by the fermentation process with *Lactobacillus rudii* and Fermento R-703 at 30 and 40 °C, constant pH of 6.5 ± 0.03 and 120 hours. The fermentation product was vacuum distilled at 61 °C to finally obtain a maximum of 2.27 % lactic acid using *Lactobacillus rudii* at 40 °C, final pH was 5.95. The lowest yield was obtained with Ferment R-703 at 30 °C with a lactic acid concentration of 0.38% and pH of 4.7. From the results obtained, it is concluded that the lactic acid obtained by *Lactobacillus rudii* and Ferment R-703 at 40°C and *Lactobacillus rudii* at 30°C can be used in moisturizing cosmetic products.

[32] Isolated and identified lactic acid bacteria from "Chicha de Siete Semillas" and their preliminary results show a large variation in viable bacteria counts among 6 producers, ranging from 7.7×10^4 to 1.1×10^8 cfu/ml, as well as the presence of a high number of isolates (approximately 70) of lactic acid bacteria. The identification of the isolates shows the presence of *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Enterococcus species*.

Currently, the good adaptation of lactic acid bacteria in cereals has been proven, which suggests that the use of a probiotic strain as a lactic starter culture produces a fermented food with defined characteristics and health-promoting properties [33]. Several technological aspects must be considered in a food fermentation process such as composition, substrate processing, growth capacity and productivity of the starter culture, and stability of the final product during storage [34].

On the other hand, advances in technology and the growing interest in predicting bacterial growth have led to the development of mathematical models that help to understand microbial growth under different conditions [35]. Likewise, mathematical modeling is used to evaluate the quality of foods that are susceptible to spoilage due to spoilage microorganisms [36]. Mathematical models of microbial growth characteristic of a product depend on the physicochemical properties of the food such as pH, Aw, storage temperature and relative humidity [37] [38]. This allows predicting the behavior of the microbial population and its effect on the environment [39]. Therefore, they can help to determine the shelf life of foodstuffs [40]. Predictive modeling for bacteria in food has experienced an exponential growth from simple to complex models for the analysis of growth patterns and the prediction of

microbial activity in food [41]. Frequently, mathematical models are used to perform data fitting related to bacterial growth such as the Baranyi-Roberts model that predicts microbial growth, combined with a fitting function A(t) that depends on the physiological state of microbial cells [42]. On the other hand, sigmoidal models, such as the modified Gompertz and logistic models, are used to fit microbial growth data [43].

In our country there are many fermentable substrates that do not require chemical additives and that present very accessible and low cost fermentation processes. However, the physiological behavior of these measurable additives, such as lactic acid bacteria that act naturally and guarantee innocuous and highly nutritious foods, is unknown, which allows giving added value to such an important substrate in our country. Since years ago, there has been a growing interest in taking advantage of their potential to promote health, recommending their inclusion in national dietary recommendations. Recent clinical studies in humans have supported that fermentative processes and the microorganisms involved can provide additional properties to basic nutrition [44]. These benefits include the production of lactic acid, as well as the presence of acidifying bacteria known as starter bacteria where they contribute significantly to the flavor, aroma, texture, and nutritional value of fermented foods [45]. In addition, these microorganisms can also influence the production of exopolysaccharides and protein modification, which further enriches the characteristics and benefits of these fermented foods [46].

The fermentation processes are not properly controlled according to the physiological behavior of the growth of the microorganisms and, likewise, their growth parameters and times that can optimize the process either by accelerating or delaying it, as appropriate, are unknown. Likewise, it is necessary to know the action of lactic acid bacteria fermenting in substrate whose complexity is new in starchy fermentable products as is the case of hydrolyzed products that come from a process from grain germination, flour production and physical-chemical hydrolyzation, obtaining a product whose complexity affects, to some extent, the growth phases and therefore it is necessary to know their respective parameters and especially the times in which they occur. The presentation of the fermented hydrolyzed corn Jora provides a final product considered as a modified substrate that can enhance the nutritional and regulatory properties for the consumer's health due to its chemical components. It also incorporates other nutrients and vitamins from starter lactic acid bacteria, which in turn are phylogenetically related to probiotic strains, becoming a food source of live microorganisms [47].

The objective of the present study is to determine the growth and kinetic parameters of lactic starter bacteria in the hydrolyzate of Jora corn grain, using mathematical models (Gompertz and Baranyi-Roberts models) to determine and evaluate the kinetic growth of lactic starters such as *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* grown in the hydrolyzate of germinated Jora corn grain at temperatures of 40, 41 and 42 °C.

2. MATERIEL AND METHODS

2.1. Type And Design of Research

The research carried out is prospective, due to the characterization of the variables as a function of time and is experimental because the experimental variable was manipulated under strictly controlled conditions, in order to describe the mode or cause of the growth of the lactic starter bacteria in the hydrolyzed germinated corn germinated grain of Jora corn. The design was conducted completely at random with a 2^3 factorial arrangement with three replicates. The hydrolyzed germinated corn sprouts were separately inoculated with the lactic starter bacteria *Lactobacillus delbrueckii ssp*. bulgaricus (Ldb) and Streptococcus thermophilus (St) and then subjected to thermotolerance conditions of 40°C, 41°C and 42°C for a period of time that it took to reach the stationary phase. The experimental parameters obtained were adjusted by the Gompertz and Baranyi-Roberts models and statistical parameters were obtained and compared and evaluated in relation to the experimental parameters.

2.2. Research Method

The numbers of Log CFU/ml of Ldb and St for each hourly iteration and temperature were used to elaborate a graph and find the growth and kinetic parameters. Likewise, the same data were adjusted by means of the

mathematical models of Gompertz and Baranyi-Roberts which report the growth and kinetic parameters, with their respective statistical value attached. The adjustment of the Lbd and St growth values and their respective kinetic parameters as a function of time, provided by the Gompertz and Baranyi-Roberts models, were validated to show that they are not very different from those measured experimentally. When bacterial growth used germinated Jora corn hydrolysate under thermotolerance conditions, the mathematical indicators Coefficient of Determination (R^2), Root Mean Square Error (RMSE), Bias Factor (Bf) and Precision Factor (Af) were considered.

2.3. Population and Sample

The population is represented by 6 liters of germinated grain hydrolysate that will serve as substrate for the experimental purposes of this study. The sample to be obtained will be of the same size as the population where the germinated grain hydrolysate will have the possibility of being tested by maintaining microbial populations conveniently distributed that allowed generalizations to be made from the results of the sample with respect to the microbial population.

2.4. Place Of Study and Period Developed

The project was carried out in the facilities of the Laboratory of the Faculty of Natural Sciences of the Universidad Nacional del Callao, during the period 2022-2023.

2.5. Techniques and Instruments for The Collection of Information

The present study used the experimental observation technique, because data were obtained under conditions controlled by the researcher, manipulating the variable time and temperature that allowed to know the growth of the starter bacteria in a direct and simple way without depending on an intermediary. With this technique, the growth and kinetic parameters of natural occurrence were obtained avoiding, as far as possible, external biases or prejudices, which allowed making adjustments with Gompertz and Baranyi-Roberts models, obtaining clear statistical growth parameters that in turn allowed validating these models and making better decisions. The tools used were record cards appropriately prepared in accordance with the objectives of the study.

2.5.1. Microbiological Quality of The Hydrolyzed Fermentate

The microbiological quality of the amylase hydrolyzed fermented sprouted grains produced by hand was determined according to the standard proposed by NTS N°071-MINSA/DIGESA-V,01. The microbial agents analyzed were aerobic mesophiles, molds and yeasts. Ten ml of hydrolyzed fermented sample were used, which were diluted in 90 ml of a 0.1% peptone water solution. The sample was then homogenized, and the corresponding serial dilutions were made.

2.5.2. Determination of pH

The determination was carried out using a pH meter with a resolution of 0.01 of the Hanna Instruments brand, model HI2211-01, where the initial pH of the prepared samples was measured. The measurements were taken at the same time as the samples were taken to measure the concentration of lactic acid and number of CFU/ml. [48].

2.5.3. Brix Determination

Brix determination was carried out using a specific refractometer for this scale [48].

2.5.4. Determination of Lactic Acid Production

It was determined by acid-base titration according to AOAC method 947.05, using a standardized solution of sodium hydroxide NaOH 0.1N in the presence of the indicator phenolphthalein as an indicator [49].

2.5.5. Baranyi's Primary Model

The growth data for each temperature are fitted by nonlinear regression analysis to the primary Baranyi model using DMFit 2.1 software to obtain the kinetic parameters: lag phase (λ), maximum growth velocity ($\mu_{máx}$) and maximum population density (N_{max}). The model described by [42] is defined by the following equation (1):

$$\text{Ln}\,(N) = \ln\,(N_0) + \mu_{max}\ A_{(t)} - \ln\left|1 + \frac{e^{\mu_{max} A_{(t)}} - 1}{e^{N_{max} - N0}}\right| \qquad (1)$$

Where $\ln(N_{(t)})$ is the log of the cell concentration at time t [$d_{(day)}$] (CFU/g); ln (N_0) is the log of the initial cell concentration (CFU/g); μ_{max} is the exponential growth rate (log CFU/g day); ln (N_{max}) is the log of the final cell concentration (CFU/g); and A is a parameter representing the logarithmic increase of the population related to the physiological cell state, and is determined by equation 2:

$$A_{(t)} = t + \left(\frac{1}{\ldots}\right) \ln\left(e^{\mu_{max} t} + e^{-\mu_{max}\lambda} - e^{-\mu_{max}(t+\lambda)}\right) \quad (2)$$

The model proposed by [42] described in equation (2), is one of the most widespread models currently used to model microbial growth. This model describes growth as a first order kinetic of ratio $\mu_{(t)}$, which varies according to environmental conditions and the phase of the population. During the exponential phase this ratio is equal to μmax, while during the adaptive and stationary phases it is reduced by the coefficients $\alpha_{(t)}$ and $\gamma_{(t)}$, both comprised between zero and one. In this model, the adaptation phase is described by assuming that there is a fictitious substance $P_{(t)}$ that acts as a bottleneck. The growth of this substance follows Michaelis-Menten kinetics, which is reflected in the parameter $\alpha_{(t)}$ as defined by equation (3).

$$\frac{dN}{dt} = \alpha_{(t)} \cdot \mu_{max} \cdot y_{(t)} \cdot N_{(t)} \quad (3)$$

2.5.6. Gompertz Primary Model

This model is defined as a kinetic model based on the response of the study agent, growth or survival under given conditions [50] and primary because it describes, fundamentally, the number of microorganisms as a function of time. Likewise, they are quantified in colony forming units (CFU) / ml or g, toxin formation, metabolite formation or as a function of absorbance, e.g. [51].

Derivation of the Gompertz Model: The derivation for food microbiology of the modified Gompertz equation can be synthesized in the following expression:

$$\log N = A + C \exp[-\exp[-B\,(t - M)]]$$

Where:

Log N = Decimal logarithm of microbial counts [log [UCF/ml)] at time t.

A = Logarithm of the counts when time decreases indefinitely.

C = Logarithm of the counts when time is increased indefinitely.

M = Time required to reach maximum growth rate [hs].

B = Growth rate relative to time M $[hs]^{-1}$.

From these parameters we derive:

Specific growth rate (μ).

$$\mu = B \times \frac{C}{\quad} \qquad (5)$$

Duration of latency phase

$$(LPD).LPD = \frac{(M-1)}{B} \qquad (6)$$

Maximum population density (MPD)

$$MPD = A + C \qquad (7)$$

2.5.7. Validation of Predictive Models in Food Microbiology

At present, there is no published and internationally accepted criterion that can establish when a model is valid, i.e., whose application is accurate and whose results can be inferred to the real world, and when it is not. Traditionally, statistics such as the coefficient of determination or the MSE (mean square error) have been used to get an idea of the validity of the model being evaluated [52] [53].

2.5.8. Preparation Of the Starter Strain

Lyofast starter cultures composed of *Lactobacillus acidophilus* and *Streptococcus thermophillus*, marketed and distributed to all industries and combined dairies in the country, were used. Subsequently, the lactic cultures were reactivated with powdered whole milk. The activation of the starter strains was continued by applying the method described by [54] where they were transferred to 100 mL of MRS broth incubating at 37°C for 24 h, after which period 1 mL was taken and added to 49 mL of MRS broth and incubated at the same temperature for 6 h. Immediately 1 mL was taken from the broth and 10 serial dilutions were made in duplicate from 1 to 1 x 10^{-9}. Each dilution was streak-seeded in the MRS agar culture medium and incubated in a bacteriological oven at a temperature of 40°C for 24 h to obtain typical colonies of Ldb and St, confirmed by Gram staining and phenotypic responses using API50 and API 20 systems [54].

2.5.9. Fermentable Substrate: Hydrolyzed Germinated Grain Of "Zea Mays" Corn

A total of 6.0 liters of neutralized hydrolyzate obtained by physicochemical techniques from germinated corn germinated grain (HGM) from a company that sells various grain flours was used. The product was diluted to 75 % with sterile distilled water, which was then dispensed individually in quantities of 99.9 ml in 6 sterile bottles with a capacity of 500 ml.

The flasks were divided into 3 groups (40°C, 41°C and 42°C) and each group consisted of 2 flasks with 999 ml of hydrolysate. One flask of each group was inoculated with 1.0 ml of activated *Ldb culture* (10^8 cfu/ml) with n=3, and the other flask with 1.0 ml of activated St, (10^8 cfu/ml) with n=3; and then incubated at temperatures 40°C, 41°C and 42°C in a water bath with built-in stirrer to mix the inocula constantly at a static temperature.

2.5.10. Obtaining Experimental Data

From the bottle of Ldb incubated at 40 °C, 1.0 ml of the hydrolysate was obtained under sterile conditions, to apply a serial dilution to the tenth to 10^{-3}. Then, 1.0 ml of each dilution was taken to inoculate it in a sterile Petri dish and MRS medium was added immediately with an amount of 12 ml over the inoculum, shaking it with rotating movements to distribute the inoculum over the whole area of the Petri dish. The culture medium was allowed to solidify and incubate at 40°C for 24 to 48 hours.

2.5.11. Colony Count on Plate

Step 1:

The numbering of colonies on the plate was performed after 24 or 48 hours using a BOECO electric colony counter. The results were expressed in colony-forming units per milliliter (cfu/ml) according to [55]. Then the result obtained was interpreted as Log Ufc/ml corresponds to the initial time or Tempo zero (T0), of the sample (n1) coming from the first Ldb flask cultured at 40°C. The flask continued to incubate and after 1 hour another sample was obtained (T1) and so on until completing 12 hours or reaching the stationary phase of growth.

Step 2:

The same procedure used in step 1 was applied to all the samples from the flasks intended for fermentation at 40°C, 41°C and 42°C.

2.5.12. Growth Curves Graphical Method

The experimental data of Ldb and St, in logarithmic function (log_{10} cfu/ml) were used to construct the experimental growth curves using the graphical method that allowed to obtain the growth parameters: initial population (N0 = Log cfu/ml), final population (Nf= Log cfu/ml), population variation (Log cfu/ml), maximum growth rate (μ_{max} = Log cfu/ml/h), Time to reach μ_{max} (hours), Latency phase (λ= hours). The determination of the Generational time (Tg=hours) and physiological state was performed using the corresponding formulas.

2.5.13. Obtaining Growth and Kinetic Parameters Using The Gompertz And Baranyi-Roberts Models

The variables maintained by the Gompertz model are A, B and M. Log N being the decimal logarithm of the microbial count at time t, N_0 is the value of the lower asymptote (equivalent to the log of the initial microbial count), A is the logarithmic increase of the microbial count (equivalent to the log of the maximum microbial count during the stationary phase minus the log of the initial count), B is the relative growth rate over time (h^{-1}) and M is the time required to reach the maximum growth rate (h), $Nmax$ is the maximum microbial count ,being microbiological descriptors to be obtained by the Gompertz and Baranyi-Roberts models respectively.

2.5.14. Validation of The Models

Validation of the predictive models of bacterial growth will be carried out according to [56]. comparing the observed and modeled growth responses. This was done by means of graphs and a series of mathematical statistics such as: the regression coefficient R^2, RMSE, the BIAS factor (Bf) and the accuracy factor (Af).

2.6. Data Analysis and Processing

2.6.1. Data Analysis

The growth curves of Ldb and St were fitted by the Gompertz and Baranyi-Roberts models and the fit parameters, for each of them, was interpreted by the statistical indicator Coefficient of Determination (R2). The measurement of the average deviation between the fitted and observed value was performed according to the root mean square error (RMSE) which represents the "standard error of the model". Significant differences between the growth parameters between the fermentative and modeled process were estimated using the ANOVA statistic.

The validation of the models was carried out by calculating the mathematical indicators Af and Bf, whose differential stocks were assessed using the ANOVA statistic.

2.6.2. Data Processing

For the purpose of comparing the growth parameters obtained under experimental and predictive thermotolerance conditions, analysis of variance was applied to establish statistically significant differences.

3. RESULTS

3.1. Characterization of corn germinated hydrolysate (CGH)

The characteristics of (HGM), sugar concentration expressed as degrees °Brix, lactic acid percentage and pH units were estimated, with average values of 22.40 ± 0.70, 0.92 ± 0.02 and 6.74 ± 0.02, as described in Table **1**, respectively. To determine the assumptions of normality and homogeneity, the sample replicates of each characteristic were subjected to the Kruskall Wallis nonparametric test, which showed that the characteristics considered comply with these assumptions and that the data are normally distributed; likewise, the significance values are greater than 0.05, which determines the acceptance of the null hypothesis (h_0) meaning that all the samples of each of the properties of the HGM are equal.

Table 1: Assessment of the properties of germinated jora corn hydrolysate and their level of statistical significance

Properties	Mean (n=3)	Standard deviation	Significant deviation normality W(n=3)	Significance level (α<0.05)
°Brix	22.4	0.7	0.987	0.173
Lactic acid (%)	0.92	0.02	0.964	0.989
pH	6.74	0.02	0.964	0.989

The microbiological quality of the HGM was evaluated for the presence of spoilage microorganisms, according to Peruvian standard NTS 071-2008, belonging to categories 1, 2 and 3. Table **2** shows the following microbial agents quantified Mesophilic aerobes (<10 CFU/ml), yeasts (<10 CFU/ml) and molds (<10 CFU/ml). The results show that HGM is ideal for modeling the growth of starter bacteria, without metabolic competition, which is explained by the heat treatment applied during hydrolysis (110°C, 10 minutes); as well as the relationship with the acidity of the hydrolysate.

Table 2: Microbiological quality of hydrolyzed flour hydrolysate from germinated jora maize

Microbial agent	Quantity CFU/ml	Permissible limits	
		m	M
Mesophilic aerobes	<10	102	$2x10^3$
Molds	<10	<10	10
Yeast	<10	<50	50

3.2. Determination Of Growth and Kinetic Parameters of Lactic Starters: Lactobacillus Delbrueckii Subsp Bulgaricus and Streptococcus Thermophilus Cultured on The Hydrolyzed Substrate Of Germinated Corn Kernels At Temperatures Of 40°C, 41°C And 42 °C

Table **3**. Shows the growth parameters of Ldb on the HGM substrate from the growth that occurred during 12 hours at temperatures of 40°C, 41°C and 42°C. The numbering of the CFU/ml for each hourly iteration and temperatures were translated into a growth curve for each of the strains studied, relating them to the proposed temperatures. In the case of St, we started with an experimental population (N_0) of 5.15, 5.06 and 5.04 Log CFU/ml, for temperatures of 40, 41 and 42°C respectively, ($p>0.05$), populations that after 12 hours of incubation reach a final population increase of 2.14, 2.46 and 3.13 Log CFU/ml levels, with respect to the same temperatures ($p<0.05$).

Table **3** shows the values of the kinetic parameters of Ldb growth, characterized by the highest acceptance values at 42 °C in which the adaptation time or Lag phase (λ) to the HGM substrate is 2.12 hours, to then continue

a logarithmic growth at a maximum speed (μ_{max}) of 0.49 Log CFU/ml/h recorded at 5.58 hours. Compared to the temperatures of 40 and 41°C, it is evident that the aforementioned parameters offer lower values, establishing significant differences between them ($p<0.05$). On the other hand, the maximum speed (μ_{max}) is an indicator of the logarithmic growth that influenced the doubling time of 0.87 hours, in which the physiological state (h_0) quantified was 0.003 Log CFU/ml which quantifies the initial physiological state of the cells and indicates that the physical adaptation of the cells to their environment is low due to the effect of the temperature of 42 °C; similarly, the generation time (Tg) was 0.80 hours (48 minutes), shorter time compared to the temperatures of 40 and 41 °C, with significant differences (p=<0.05).

Table 3: Microbiological quality of hydrolyzed flour hydrolysate from germinated jora maize

Parameters	Lactobacillus delbrueckii subsp bulgaricus			Streptococcus thermophilus		
	Temperatures			Temperatures		
	40 °C	41 °C	42 °C	40°C	41°C	42°C
Growth parameters						
N_0	5.21	5.33	5.37	5.15	5.06	5.04
C	1.98	2.36	3.11	2.14	2.46	3.13
Growth statistics						
R^2	0.98	0.99	0.99	0.97	0.98	0.96
DS	0.06	0.01	0.09	0.09	0.05	0.04
Kinetic parameters of growth						
μ_{max}	0.39	0.42	0.49	0.3	0.45	0.5
λ	3.62	2.82	2.12	4.18	3.05	2.4
M	6.09	5.98	5.58	7.45	6	5.7
Tg	1.26	0.87	0.8	1.08	0.91	0.55
h_0	0.05	0.02	0.01	0.05	0.11	0.17

Note: No Initial population (Log CFU/ml), C Population variation (Log CFU/ml), μmax Growth rate (Log CFU/mL/h), λ adaptation phase (hours), M Time to reach μmax (hours) , Tg Generational time (h), h0 Physiological state, R2 Coefficient of determination, SD Standard error.

Table **3** presents the growth behavior of the lactic starter strain *Streptococcus thermophilus* according to the Gompertz model using as substrate the hydrolyzate of germinated corn germ (HMG) at temperatures of 40°C, 41°C and 42°C for a period of 12 hours. The log CFU/ml numbered in each hour of culture generated a sigmoid type curve on which the growth and kinetic parameters were determined. The initial inoculum (N0) for each culture was 5.15, 5.06, 5.04 Log CFU/ml, for temperatures of 40°C, 41°C and 42°C, respectively; there were no significant differences between these inocula ($P(p<0.05) =0.074$); at these temperatures the initial populations and after 12 hours of incubation reached a final population increase (N_f) of 2.14, 2.46 and 3.13 Log CFU/ml levels.

Before presenting the logarithmic growth phase St requires an adaptation time or lag phase according to the temperature of 40°C, 41°C and 42°C, it was 4.18, 3.05 and 2.40 hours, respectively ($P_{(p<0.05)} = 0.038$), noting that the shortest adaptation time occurs at 42 °C in which the physiological state (h0) bacterial is 0.173 log CFU/ml compared to those presented at 40 and 41 °C whose values are 0.047 and 0.107 Log CFU/ml.

At the end of the adaptation phase, the logarithmic phase occurs, where the initial population shows a constant exponential increase at a speed (μ_{max}) of 0.35, 0.37 and 0.47 log CFU/ml/h, depending on the time and temperatures experienced, establishing the generational time (Tg) of 1.08, 0.91 and 0.55 hours, for temperatures of 40°C, 41°C and 42°C, respectively, highlighting the incubation temperature of 42°C in which the highest values of maximum growth rate and the shortest time to achieve a logarithmic doubling are obtained.

Table **4** shows the growth and kinetic parameters of Ldb obtained by fitting the experimental data to the Gompertz model, its variables being A (Initial population, N_0), C (Variation of logarithmic levels of growth), B (Growth rate (μ_{max}) and M (time to reach μ). The kinetic responses of the model were calculated using equations 4, 5 and 6

and in this way the values of λ, μ_{max}, h_0 and Tg were obtained, which are the variables in which the effects of the intrinsic factors of the HGM and extrinsic factors such as temperature and acidity on the lactic starters are observed.

Table 4: Growth and kinetic parameters according to Gompertz model of lactic starters in hydrolysate of germinated Jora corn under thermo-tolerance conditions

Parameters	Lactobacillus delbrueckii subsp bulgaricus			Streptococcus thermophilus		
	Temperatures			Temperatures		
	40 °C	41 °C	42 °C	40°C	41°C	42°C
Growth parameters						
N_0	5.28	5.46	5.36	5.18	5.12	5.09
C	2.01	2.37	2.99	2.19	3.58	4.29
Growth statistics						
R^2	0.99	0.99	0.99	0.99	0.99	0.99
DS	0.05	0.01	0.08	0.04	0.06	0.07
Kinetic parameters						
μ_{max}	0.53	0.49	0.45	0.38	0.35	0.32
λ	3.76	3.16	1.78	4.55	3.51	2.95
M	5.64	5.18	4.97	7.18	6.37	6.07
Tg	0.8	0.7	0.61	0.99	0.75	0.69
h_0	0.01	0.03	0.16	0.02	0.06	0.11

Note: No Initial population (Log CFU/ml), C Population variation (Log CFU/ml), μmax Growth rate (Log CFU/mL/h), λ adaptation phase (hours), M Time to reach μmax (hours) , Tg Generational time (h), h0 Physiological state, R2 Coefficient of determination, SD Standard error.

The adjustment of the growth curve with the Gompertz model indicates that the initial population (A) for the temperatures of 40°C, 41°C and 42°C are 5.28, 5.46 and 5.36 Log CFU/ml, respectively, with no significant difference between them ($p>0.05$), generating logarithmic level increments of 2.0, 2.37, 2.99 Log CFU/ml, with growth rates μmax of 0.53, 0.49 and 0.45 Log CFU/ml/h, corresponding to the same study temperatures.

The analysis of the growth parameters indicates that as the temperature increases from 41°C to 42°C the parameters tend to vary their values, thus we have that the initial population (N_0) are similar, with no significant differences ($p> 0.05$). As growth develops, the dormancy phase decreases as a function of temperature, being 42 °C the one that presents the shortest time of 1.78 hours to adapt to the HGM substrate and, within the same context of the parameter, the initial physiological state (h0) of the cells was 0.16 Log CFU/ml, being this a value considered low, to be able to quickly initiate the exponential phase.

The logarithmic growth is characterized for being constant and for a period of time of 5.64, 5.18 and 4.00 hours, in which the maximum velocities associated to the generation times (Tg) 0.80, 0.70 and 0.61 hours are reached respectively for the temperatures of 40°C, 41°C and 42°C.These results show that at the temperature of 42°C it doubles rapidly and in a very short time in comparison with what occurs at the temperatures of 40 and 41°C, there being significant differences between the parameters analyzed and their respective temperatures ($P_{(p<0.05)}$=0.007).

Another of the models proposed to validate their response by showing the growth and kinetic parameters of LDB is the one by [42]. The model incorporates the variable physiological state of the cells h0, considered as a dimensionless parameter difficult to observe, validate and quantify experimentally. The duration of the adaptation phase is determined by the value of this variable at the time of inoculation and by the new environment in which it is found [42].

Table **5** shows the data of the experimental growth curve of Lbd, which is adjusted by the Baranyi-Roberts model

showing initial populations (N_0) of 5.25, 5.39, 5.37 Log CFU/ml, considered similar among them ($P_{(p<0.05)}$ = 0.062) and favorable for the purposes of this research, which were incubated at temperatures of 40°C, 41°C and 42°C, respectively. The results obtained were population increases of 1.93, 2.46 and 3.02 Log CFU/ml. The latency or adaptation phase (λ) for the same temperatures used were 3.27, 2.44 and 1.19 hours; there were significant differences between them ($P_{(p<0.05)}$ = 0.021), so that the temperature of 42 °C is the one that provides an adaptation to the environment in a shorter time than the other temperatures. In this circumstance the initial physiological state of the Lbd cells showed values of 0.05, 0.06, 0.031 LogUFC/ml, which predisposed the logarithmic growth at a maximum rate of (μ_{max}) of 0.33, 0.36, 0.48 Log UFC/ml/h, with respect to the temperatures experienced; noting that at the temperature of 42 °C the growth develops with greater speed.

Table 5: Growth parameters and kinetics of starter bacteria in hydrolysate of germinated corn kernel under thermotolerant conditions according to Baranyi-Roberts model

Parameters	Lactobacillus delbrueckii subsp bulgaricus			Streptococcus thermophilus		
	Temperatures			Temperatures		
	40 °C	41 °C	42 °C	40°C	41°C	42°C
Growth parameters						
N_0	5.25	5.39	5.37	5.17	5.12	5.1
C	1.93	2.46	3.02	2.22	3.08	3.44
Growth statistics						
R^2	0.99	0.99	0.99	0.99	0.99	0.99
DS	0.043	0.05	0.05	0.038	0.035	0.054
Kinetic parameters						
μ_{max}	0.33	0.36	0.48	0.32	0.45	0.49
λ	3.27	2.44	1.19	4.32	3.49	2.88
M	6.96	6.24	5.28	7.44	7. 29	6.72
Tg	1.31	0.93	0.57	1.16	0.59	0.486
h_0	0.05	0.06	0.031	0.032	0.027	0.039

Note: No Initial population (Log CFU/ml), C Population variation (Log CFU/ml), μmax Growth rate (Log CFU/mL/h), λ adaptation phase (hours), M Time to reach μmax (hours) , Tg Generational time (h), h0 Physiological state, R2 Coefficient of determination, SD Standard error.

Based on the maximum velocity increment the Generational time (Tg) was 1.31, 0.93 and 0.57 hours, correspondingly at temperatures of 40°C, 41°C and 42 °C, the latter favoring a shorter Tg value. It is evident that the temperature of 42 °C is the one that presents the best values for the growth and kinetic parameters that allow a good growth of Ldb.

Table **5** presents the data of the experimental growth curve of Streptococcus thermophilus adjusted by the Baranyi-Roberts model. The model fit reports that the initial populations (N_0) were 5.17, 5.12 and 5.10 Log CFU/ml, considering them as similar ($P_{(p<0.05)}$ = 0.071), which after being inoculated were incubated at 40, 41 and 42 °C, respectively; under these conditions, population increases of 2.22, 3.08 and 3.44Log CFU/ml, with a latency or adaptation phase (λ) of 4.32, 3.49 and 2.88hours, with significant differences between them ($P_{(p<0.05)}$ = 0.031), being the temperature of 42 °C the one that provides an adaptation to the environment in a shorter time than the other temperatures. In these circumstances the values of the initial physiological state (h_0) of the St cells were 0.05, 0.06, 0.031 LogUFC/ml, which predispose logarithmic growth, where the increase in the number of cells per unit of time was at a maximum speed (μ_{max}) of 0.32, 0.45 and 0.49 Log UFC/ml/h, with respect to the temperatures experienced, the highest speeds were achieved at 41 and 42 °C.

Regarding the generation time (Tg), the model reports values of 1.16, 0.59 and 0.49, for temperatures of 40°C, 41°C and 42°C, respectively; however, for this parameter it is the 41°C temperature that favors a shorter Tg value. It becomes evident that the optimum parameter for the growth of *Streptococcus thermophilus* is the temperature of

41°C.

3.3. Model Validation

After the development of the Gompertz and Baranyi-Roberts models using experimental data, these models must be validated in real situations. This is critical in order to have confidence in them. Validation showed that microorganisms behave similarly in both laboratory and real systems. Validation of the predictive models was performed using a hydrolyzed germinated corn stover whose complex chemical matrix has the nutrient capacity to sustain high population densities of starter lactic acid bacteria, which are very demanding in bioavailable nutrients for short periods of time and at non-mesophilic temperatures such as 40°C, 41°C and 42°C, and in these situations the model simulates the experimental conditions.

The data input to the model came from the iteration of colony forming units (Log CFU/ml) obtained by periodic counts and then plotted the growth of lactic acid starter strains of fermented products. Finally, the values observed in the experimental runs and in the validation, trials were compared with the values predicted by the Gompertz and Baranyi-Roberts model. Bias and precision indices were also determined to numerically verify the fit of the developed model.

Table **6** shows the mathematical indices used for the validation of the models describing the effect of temperature on the growth of *Streptococcus salivarius subsp thermophilus* in hydrolyzed germinated corn kernels at temperatures of 40°C, 41°C and 42°C, where the coefficients of determination of TSS growth were obtained from the adjustment of the experimental data by the Gompertz and Baranyi-Roberts models. The coefficients obtained are representative at 0.98 for the Gompertz model and 0.99 for the Baranyi-Roberts model when growth was carried out at temperatures between 40 and 42ºC; it being understood that 98% and 99% of the variance of the dependent variable under study is explained by the variance of the independent variable of each model, respectively. According to the values obtained, there is a high goodness of fit, therefore, they are very reliable models for future forecasts.

Another indicator of goodness of fit is the root mean square error (RMSE), which determined the average difference between the values predicted by the same models used and the actual values; it is a measure of how dispersed these residuals are. In other words, it indicates how concentrated the data are around the line of best fit. The lower the value of this index the better the fit of the model to the experimental data. In this study the Gompertz model shows RMSE values of 0.04, 0.04 and 0.25 much lower than the Baranyi-Roberts model whose values are 0.15, 0.09, 0.11 ($P_{(p<0.05)}$ = 0.032), for temperatures of 40°C, 41°C and 42°C, respectively.

These results show that the Gompertz model can predict the value of the response variable in absolute terms better than the Baranyi-Roberts model. However, both RMSE and R^2 quantify how well a regression model fits a data set with the difference that RMSE tells us how well a regression model can predict the value of the response variable in absolute terms, R2 tells us how well a model can predict the value of the response variable in percentage terms.

Table **6** also shows the indices that refer to the relationship between the predictions that a model can produce and the model's verifications or validations; two widely used indices were determined: the BIAS factor (Bf) and the accuracy factor (Af).

The BIAS factor or accuracy factor (Bf) for TSS growth with the Gompertz model had averages of 1.01, 0.79 and 0.82 ($P_{(p<0.05)}$ =0.0008), when cultures are grown at temperatures of 40°C, 41°C and 42°C, respectively; indicating that the model reliably predicts the growth rate only at 40°C; while at temperatures of 41°C and 42°C it is not safe for estimating that parameter.

Table 6: Mathematical indices used for the validation of models describing the effect of temperature on the growth of Streptococcus thermophilus in hydrolysate of germinated corn kernels at temperatures of 40°C, 41°C and 42°C

Indexes	Gompertz model			Baranyi-Roberts Model		
	Temperatures			Temperatures		
	40°C	41°C	42°C	40°C	41°C	42 °C
R^2	0.999	0.999	0.999	0.98	0.98	0.98
RMSE	0.035	0.041	0.25	0.149	0.093	0.109
Bf	1.01	0.785	0.82	0.856	0.777	0.856
Af	1.172	1.192	1.188	0.972	0.998	0.972

Note: R^2 Coefficient of determination, RMSE Root mean square error, Bf Bias factor; Af Accuracy factor.

When it comes to the Baranyi-Roberts model, the BIAS factor for the reliable prediction of the TSS growth rate reaches values of 0.86, 0.78 and 0.86 at temperatures of 40°C, 41°C and 42°C, respectively. These values show that the expression of the average growth parameter given by the predictive model on the same substrate is <1 inferring that the model prediction is not confident, it predicts below the experimentally obtained values.

The accuracy factor of the models, Af, shows how much the estimated values of the TSS growth rate differ from the observed ones. Table **6** shows the accuracy values for the Gompertz model: 1.17, 1.19 and 1.19; for the Baranyi-Roberts model: 0.97, 0.99 and 0.9. Both models show statistically significant differences, which allow inferring that, although both models allow a high coincidence between the observed values and the estimated values, the Baranyi model gives a better quality of its products.

Table **7** shows the mathematical indices used for the validation of the models describing the effect of temperature on the growth of *Lactobacillus delbruckii ssp. bulgaricus* in hydrolysate of germinated corn kernels at temperatures of 40, 41 and 42 °C.

Table 7: Mathematical indices used for the validation of models describing the growth of Lactobacillus delbrueckii subsp. bulgaricus in hydrolysate of germinated corn kernels

Indexes	Gompertz model			Baranyi-Roberts Model		
	Temperatures			Temperatures		
	40°C	41°C	42°C	40°C	41°C	42 °C
R^2	0.99	0.99	0.99	0.99	0.99	0.99
RMSE	0.05	0.11	0.21	0.04	0.04	0.08
Bf	0.92	0.51	0.69	0.91	0.79	0.77
Af	1.12	1.24	1.21	1.17	1.19	1.20

Note: R^2 Coefficient of determination, RMSE Root mean square error, Bf Bias factor; Af Accuracy factor.

The simple linear regression technique was used and the maximum growth rate (μ_{max} =Log CFU/ml/h) was contrasted as a function of temperature at 40°C, 41°C and 42°C using the Gompertz and Baranyi-Roberts models, which reported the determination index (R^2). The coefficients of determination of LSB growth in attention to μmax were obtained from the fit of the experimental data by the Gompertz and Baranyi-Roberts models. The coefficients for the Gompertz model are 0.999, 0.997 and 0.999 and for the Baranyi-Roberts model 0.996, 0.997 and 0.998 in relation to the temperatures of 40, 41 and 42 ºC. This index is an indication of the quality of the adjustment of the models to the experimental data and from the values obtained it can be observed that the goodness of fit of the two models is high, in such a way that it allows estimating the proportion or percentage of variability present in the experimental data and explained by the model; it also allows estimating the variation of the response variable at different values of the temperature factor, with an acceptable precision.

Another indicator of goodness of fit is the root mean square error (RMSE), which is used to measure the

distance between the predicted and actual values. In other words, it tells how concentrated the data are around the line of best fit which determined the average difference between the values predicted by the same models used and the actual values. The lower the value of this index the better the fit of the model to the experimental data.

In this study, the Gompertz model shows RMSE values of 0.05, 0.11 and 0.21 and the Baranyi-Roberts model 0.04, 0.04 and 0.08 ($P_{(p<0.05)}$ =0.012), for temperatures of 40°C, 41°C and 42°C, respectively. These results demonstrate that the Gompertz model can predict the value of the response variable in absolute terms better than the Baranyi-Roberts model.

Table **8** also shows the indices that refer to the relationship found between the predictions that a model can produce and the checks or validations of the model; the indices determined were the BIAS factor (Bf) and the accuracy factor (Af).

The BIAS factor (Bf) for the Gompertz model for LSB growth averaged 0.92, 0.51 and 0.69 ($P_{(p<0.05)}$ = 0.014), when cultures are grown at temperatures of 40°C, 41°C and 42°C, respectively; indicating that the model does not reliably predict the value of growth rate at these temperatures and the observed values are higher than those estimated by the predictive model.

In relation to the Baranyi-Roberts model, the BIAS factor for the safe prediction of the LSB growth rate reaches values of 0.91, 0.79 and 0.77 when the culture is carried out at temperatures of 40°C, 41°C and 42°C, respectively. These values demonstrate that the expression of the average growth parameter provided by the predictive model is not certain, and the predicted values are below the experimentally obtained values.

The accuracy factor of the models (Af) shows how much the estimated values differ from the observed values in relation to the growth rate of St. John's wort. Table **7** shows the accuracy values for the Gompertz model: 1.18, 1.20 and 1.21; as well as for the Baranyi-Roberts model: 1.17, 1.19, 1.20. Both models do not show significant differences, which allows inferring that one or the other model fits the experimental data with high coincidence of the observed values and the estimated values.

4. DISCUSSION OF RESULTS

4.1. Physicochemical and Microbiological Properties of Corn Germinated Grain Hydrolysate

HGM was considered to come from a grain whose carbohydrate and protein content depends on the endosperm; crude fat and, to a lesser extent, protein and minerals, on the germ. [57] mentioned that these conditions allowed the product to be considered as a nutritious substrate to sustain Ldb and St. growth for a prolonged period of time.

The HGM was characterized by soluble solids concentrations of 22.40°brix, pH 6.74 and % acidity 0.923, values considered sufficient and appropriate for its usefulness for the fermentation process with inoculums of Ldb and St. John's wort. On the other hand, the microbiological quality of the HGM indicates the absence of indicators of contamination by aerobic mesophilic bacteria and fecal indicators; in this way, it was guaranteed that the growth of the lactic starters was carried out without competitors for the substrate, within a thermotolerance environment of 40°C, 41°C and 41°C. These values are similar to those found by [31] who used lactic batteries of the Lactobacillus genus for the fermentation of corn starch and performed the acid hydrolysis process at temperatures of 30°C and 40°C. The hydrolysate presented an initial pH of 6.5 + 0.03 and was characterized by having 2.27% lactic acid at 40°C with a final pH of 5.95.

4.2. Growth Curve Fitting

The growth and kinetic parameters determined experimentally in HGM were quantified from the graphic representation that adopted a sigmoid type curve in which it was possible to visualize growth segments that could be unsafe and unreliable in practice, which motivated the analysis of the coefficient of determination (R^2) that was

very useful. Likewise, the CFU/ml were expressed as Log CFU/ml. Expressing in this way means that the point spreads are evenly distributed over a wider range. However, trends in the data may go unnoticed in this type of logarithmic representation. "The logarithmic transformation representation of the kinetic parameters is more advisable as the distribution error is homogeneous" [58].

The fermentation process with Ldb started with a population between 5.21 and 5.37, while St. obtained 5.04 and 5.15 Log CFU/ml; values that are approximately very close, for both strains; a situation that contributed to the responses of other parameters reflecting the use of the substrates and that the significant differences that were found were produced by the cellular metabolic activity, the main cause being the applied temperature, especially between 40°C and 41°C. The highest population increase was at 42°C for both bacteria, which expressed in logarithmic levels represent low growth (3.11 for Ldb and 3.13 for St.). If we compare the growth in milk to produce yogurt we will notice that the difference is similar as those obtained, in yogurt fermentations at 42 °C the viable cell counts of Lb bulgaricus increased from 6 to 7.88 log units after 6 h of fermentation remaining constant until the end of the first week of storage, decreasing to 5 log units at the end of the third week; while the colony counts of S. thermophilus increased exponentially during the first hours of fermentation from 6 to a maximum of 9 log units, to then decrease markedly after 24 h. Likewise, [59] observed the correlation of the CFU of L. casei growth up to 75h, in whey, counts between 10^9 and 10^{10} CFU/mL, which are recommended for lyophilization.

In all cases, the presence of sufficiently high values of lactic acid bacteria (10 -10^{78} CFU/ g) to guarantee their functionality has been demonstrated [60]. In addition, in products containing bifidobacteria and the typical yogurt starters (*L. delbrueckii ssp bulgaricus and S. thermophilus*) it is observed that the flora is composed of 6-10% bifidobacteria (10 -10^{67} CFU/g), 70-90% Streptococcus (10^7 -10^8 CFU/g) and 5-10% *Lactobacillus* (10^6 -10^7 CFU/g). In yogurts with conventional ferments, streptococci constitute between 80 and 90% (10^8 CFU/g), and lactobacilli between 10 and 20% (10^7 CFU/g) [61].

After inoculation, the Lag phase occurs, which is measured in hours and is obtained by extrapolating the tangent on the exponential part of the growth curve back to the inoculum level [62]. The values found in Table **3** on the kinetic growth of Ldb and St on HMG substrate. It is observed that the behavior for bacterial growth is different as a function of temperature using the same substrate. The lowest substrate adaptation phase (lag phase) for Ldb and St is at 42°C being 2.12 h and 2.40 h respectively. From previous experience, the study considered it convenient to start with a population of approximately 5.0 log CFU/ml, a very convenient level to achieve CFU/ml numbers and to clearly visualize the population increase; however, this level was not sufficient to achieve a population increase. [63] indicated that "The duration of the lag phase also depends on the initial concentration of microorganisms (inoculation level); if the concentration is high (log CFU/ml), the duration of this phase is shorter". Under these concepts, it was evidenced that the highest logarithmic levels of growth were reached at 42°C (Ldb 2.14 and St 3.13 logarithmic units), results that indicate that St adapts as well as Ldb, but grows generating a higher logarithmic level and whose population ratio St/Ldb is 1.46.

[64] compared "the levels of populations between Bb and St, during the fermentation of yogurt enriched with quinoa ("Chenopodium quinoa" Willd), being able to notice that practically in all the period of time of study, St presents higher populations than Ldb. The St/Ldb ratio persists with values ranging from 1.06 to 1.14".

[65] mentions that "St initiates lactose fermentation towards the lactic acid side and grows fast, up to pH 5.5 forming in addition amino substances originating from whey proteins that, in turn, stimulate the growth of Lbd that will continue to reduce pH and form more amino acids that will again stimulate St. Over time, its following pH drops will initiate a gradual inhibition of St".

Lactic acid bacteria after having achieved their adaptation to the HGM substrate continued their growths during the logarithmic phase (Table **3**), where the maximum growth rate (μmax), the time required to reach it (M) are similar for Ldb and St, ($p>0.05$), within the incubation environment at 42°C; while at the same temperature the generational time and physiological state of Ldb are significantly different ($p<0.05$) to St, ($p<0.05$), being this the one that presents a lower generation time and higher physiological state at 42°C. In this regard, we have the study

of [66] who reports that being the substrate a fermented beverage using Chonta, at 28°C, lactic acid bacteria begin their growth with 5.42 log CFU/mL until 30 hours with 7.45 log CFU/mL in this time the maximum growth rate (μmax) was established at 0.1369 (h^{-1}), with generation time of 5.06 hours; at 36 hours a quiescence of the cells was observed with a value of 7.46 log CFU/mL and at 42 hours there is a decrease of the microorganisms of 7.42 log CFU/mL.

The experimental evidence of these results shows that even though the lactic acid bacteria may be the same, but the fermentable matrices are different, it is not enough to know the initial and final state of growth; it is also necessary to know the kinetic parameters that are related to the concentration of nutrient supply in bioavailable conditions for the bacterial metabolic process.

The results of this study are important to describe the fermentation process of the hydrolyzate of germinated Jora corn, obtaining details of the growth and kinetic parameters of Lactobacillus delbrueckii ssp bulgaricus and Streptococcus thermophilus, demonstrating the possibility of obtaining lactic acid and other acids to formulate probiotic drinks and sauces.

The experimental results for the growth of Ldb and St were fitted with the primary models of Gompertz and Baranyi-Roberts in order to know if the relationship of the experimentally obtained time-dependent growth values and their respective generated parameters can be of the same magnitude, for which the kinetic models used represent a better approximation to the experimental data.

Consequently, the hypothesis that the adjustment of the growth of lactic starter bacteria in the hydrolysate of germinated grain of Jora corn by the Gompertz and Baranyi-Roberts models established the growth and kinetic parameters under thermo-tolerance conditions is accepted, with no significant differences ($p>0.05$) between the two models.

4.3. Validation of Predictive Growth Models Developed on Geminate Maize Hydrolysate (GMH).

. The validation of the models consists of a comparison between the kinetic parameters obtained by the models and those observed in the experiments for Ldb and St, using mathematical indexes of adjustment to the fermentation conditions of hydrolyzed corn germinated at temperatures of 40°C, 41°C and 42°C.

Due to the importance of the hydrolysates of germinated grains, the predictive models are required to obtain the growth and kinetic parameters of the starter bacteria; for this reason, they must have validity through some parameters of judgment, which indicate their performance and adjustment.

Of all the parameters obtained experimentally, it is the maximum growth rate (μ_{max}. Log CFU/ml/h) that simulates the slope of the growth curve when the microorganism grows exponentially. This parameter represents the part of the curve that is approximately linear and the value of its slope is determined by linear regression and is a first order relationship, so it will be the parameter that will be used to estimate the valuation of the model.

According to the results of R^2 shown in Table **6**, the Gompertz and Baranyi-Roberts models reach high values, very close to 1.00, for Ldb and St indicating a low proportion of variability in the experimental data that is explained by the models. We agree with the interpretation of this indicator with [67] who confirm that the R^2 "measures the percentage of the variability of the response that is explained by the model over the total variability of the results". These results, representing a relationship of the linearity between the quantitative variable (μ_{max}) at experienced temperatures, of each model, describe that the higher the value of R^2, the better the results obtained fit the experimental data.

The results found allow accepting the hypothesis that the validation of the Gompertz and Baranyi-Roberts models allow estimating the development of lactic starter bacteria, under specific thermotolerance conditions, in the hydrolyzate of germinated Jora corn grain, providing conditions of high goodness of fit or precision of the model, together with its high accuracy factors and low bias values.

4.4. Contrasting the Results with Other Similar Studies

Similar studies on the physicochemical and microbiological properties of corn germinated grain hydrolysate to be used in fermentation processes are found in the study conducted by [31] where he shows "the most appropriate conditions for the fermentation process being a temperature of 40°C and an initial pH of 6.5 ± 0.03". [68] [69] consider that "most of the species belonging to these genera have tolerance to pH below 5 and the optimum growth temperature is 40°C". Likewise, [70] established that the most suitable conditions for fermentation with *Lactobacillus rudii* and Ferment R-703 were at a temperature of 40°C, pH 6.5 ± 0.03 and 120 hours.

The initial chemical conditions offered by the HGM varied after the fermentation process with inoculums of Ldb and St for the chemical indicators °Brix, pH and lactic acid concentration as a function of temperature, in such a way that at 40°C and 41°C the tendency was to increase, while at 42°C it was to decrease, considering that at this temperature the effect is on the metabolic function that somehow affects the enzymatic reactions of the microorganisms, even when the general growth increases. The behavior of Ldb stands out, which above 41°C decreases the production of lactic acid, reaching a lower production at 42°C, while for St it increased steadily until the end of the fermentation process.

The scientific evidence demonstrates that there is an effect of thermotolerance on the growth of starter bacteria; an opinion in agreement with [71] who "*isolated two strains of Lactobacillus plantarum,* Pro 1b and Pro 2b, which when cultured at 45°C for 24 hours, showed a growth of 1.0 x 10^7 CFU/ml and 3.0 x 10^{11} CFU/ml respectively. Finally, at 38°C for 24 hours, Pro 1b and Pro 2b showed a growth of 4.0 x 10^8, 5.0 x 10^9 CFU/ml, and 1.0 x 10^8 6.0 x 10^8 and 1.0 x 10^9 CFU/ml respectively. Likewise, [24] report that "Ldb and St grown in whey at 42°C have higher lactose consumption and, therefore, higher lactic acid production. However, they were able to appreciate that there is a higher yield for lower levels of CFU/mL".

It is necessary to consider that the fermentation matrix is a hydrolyzate of germinated corn grain flour that provides nutrients, such as bioavailable amino acids and large portions of starchy nutrients, for the lactic acid bacteria, but in limited quantities that affect growth and force the formation of products of secondary metabolism; therefore, the starter lactic acid bacteria must condition their metabolic action to a new environment.

The HGM offers a variety of soluble solids (°Brix), which represents the amount of sugars released (22%) from the acid hydrolysis of starch and considered as an energy source to allow the growth of lactic acid bacteria, being the case of Ldb that increases the percentage of soluble solids consumption when the fermentation is carried out at 40°C (38%) and 41°C (43%) and then decreases at 42°C (29%) ($p<0.05$); while, for St the consumption increases through temperatures, obtaining the highest value at 42°C (46%).05); whereas, for St the consumption increases through the temperatures obtaining the highest value at 42°C (46%). Similar values were found in the study by [72] who indicate that "the carbohydrate content is related to the germination time and that the final carbohydrates provided, after 96 hours, was 69%, which used in fermentation processes (20%) sustains high growth of lactic bacteria"; however, the fact that Ldb decreases the use of soluble solids at 42°C may be attributed to the fact that the rate of survivors at this temperature has a greater effect due to nutrient depletion or due to antimicrobial substances present in the corn. In this regard, [73] investigated the viability of commercial yogurt starter bacteria at temperatures of 4°C, Ldb and St, in rice-based beverages and noted that "the survival rate of lactobacilli was worse than that of streptococci, which may be due to the negative influence of antimicrobial substances derived from the plant matrix, low pH and inadequate refrigerated storage conditions; however, the survival rate of lactobacilli was worse than that of streptococci, which may be due to the negative influence of antimicrobial substances derived from the plant matrix, low pH and inadequate refrigerated storage conditions, [74] observed that "during the fermentation of malted corn variety INIA 603 the Brix degree decreases up to 5 Brix degrees evaluated during 6 days of fermentation. The result obtained in the present study showed that the intrinsic and extrinsic environment is favorable to sustain growth of starter bacteria that translate into growths of the same which deserved to adjust it with the Gompertz and Baranyi-Roberts models which reported growth and kinetic parameters. Thus, we have studies carried out on the adjustment of growth curves, such as the one by [21] who demonstrated that "during the fermentation of a bread flour-based beverage, with and without the addition of an enzyme preparation (α-amylase

and glucoamylase). The growth rate of Lb. plantarum was 0.10±0.01 - 0.12±0.01 and was lower than that shown by [75] in the fermentation of a malt flour-based substrate (0.41±0.03). This fact could be due to a lower content of monosaccharides (glucose and fructose) and disaccharides (maltose and sucrose) of the beverages elaborated in the present work, which seem to be necessary for an optimal growth of this bacterium [75].

Of special interest are the growth and kinetic parameters generated by the fermentation of HGM at 42°C by Ldb and St which were adjusted by the Gompertz model. The comparative analysis of their parameters indicates that the growth of Ldb is more effective than St. One of the reasons for this appreciation is explained in the metabolic behavior of Ldb and St fermenting bacteria, starting with the pH of HGM which was initially set at 6.5 as it was considered appropriate for both the substrate and the bacterial cell. [76] consider that "often self-metabolism is influenced by pH. " Lactic acid bacteria grow best in near neutral conditions; e.g., Streptococcus thermophilus (pH 6.5), L. delbrueckii ssp bulgaricus (pH 5.8 to 6).

[77] investigated that several growth characteristics such as maximum biomass concentration, specific growth rate, doubling time, substrate consumption and product yields are influenced by pH value. In addition, research conducted by [78] considered pH as an important factor in biomass and lactic acid production of Lactobacillus delbrueckii ssp, in this study they observed that at pH 6, the lag phase lasted only four hours, significantly smaller time than those evaluated at lower pH conditions, derived from this study, they claim that the cells started to utilize the substrate in an accelerated manner at the beginning of fermentation, contributing this to high cell growth rates.

Similarly [78] "consider pH as an important factor in biomass and lactic acid production of Lactobacillus delbrueckii ssp, they observed that at pH 6, the lag phase lasted only four hours"; in the present study using the substrate HGM fermented by Ldb and St the pH was decreasing from 6.5 to 5.0 and the lag phase reached times of 1.78 h and 2.95 respectively, at 42°C. Likewise, within the physiological characteristics of St it is reported that it produces between 0.7 -0.8% of lactic acid, thermo resistant, it develops optimally at temperatures between 42°C - 45ºC, the minimum is 1ºC and the maximum is 50ºC and even 65ºC for half an hour, it has less acidification power than Lactobacillus [31]. On the other hand, "the proteolytic activity in milk is small since the amino acids released are consumed during its logarithmic growth phase [77].

In the same way that Ldb has metabolic properties to develop growths in HGM, so does St and that the characterization of its parameters can be performed with the Gompertz model, appreciating that the values of the coefficient of determination R^2 are high (0.99) and that justifies the goodness of fit of the Gompertz model for the data analyzed; however, the coefficient of determination alone is not sufficient for the analysis of this type of model, which represents an important difference with the analysis of linear regression models, which is why the analysis of residuals is also included.

[79] modeled the individual growth of two LAB species, one as a bacteriocin producer (*Lactobacillus plantarum*) and the other as a non-bacteriocin producer (*Lactobacillus paracasei*), in MRS broth. The fit of these models and the kinetic parameters estimated for each of the replicates of four microorganisms (L. plantarum and E. coli, L. plantarum and L. monocytogenes, L. paracasei and E. coli, and L. paracasei and L. monocytogenes): the MRS phase, the MRS phase, and the MRS phase. monocytogenes): lag phase, growth rate, initial (minimum) and maximum cell concentration, based on nonlinear regression analysis it could be concluded that the two models, modified Gompertz and Baranyi, presented a good fit. The SE and R2 for each of the models showed that both models fitted the experimental data adequately, with 0.420 being the highest SE and 0.971 the lowest R^2 for all 16 individual growth curves modeled.

The Gompertz model is based on changes in specific growth velocity [80]. This is the most widely used empirical primary model and generates an asymmetric curve that simulates the latent, exponential and stationary phases. The drawback is that it underestimates the growth velocity, requires data throughout the latency, exponential and stationary phases for a good prediction [81].

The dynamic model of [42] is a mathematical model with a mechanistic part based on the principle that there are substances that limit bacterial growth. It assumes that the state of a homogeneous population of bacteria can be

characterized by physicochemical factors, the extracellular medium and intracellular conditions. This model describes a sigmoidal bacterial curve. It presents 4 main parameters: initial value, latency or lag/interphase phase, maximum rate, final value and 2 curvature parameters: mCurv and nCurv, which describe the curvature of the sigmoid curve respectively at the beginning and at the end of the growth phase. It allows to quantify the microbial growth kinetics and to obtain, for example, the maximum specific growth rate.

The Baranyi model was fitted to the growth curves generated from the experimental data of Ldb and St growth in HGM which are shown in Table **3**. By analyzing the results of the nonlinear regression process the Baranyi model appropriately describes the growth of the two strains in separate cultures. The standard deviations and the coefficient of determination R^2, were obtained, the highest value being 0.087 and the R2 0.987 for Lbt and for St. 0.04 and R^2 0.96 at 42°C incubation for 12 hours. There were no significant differences ($p>0.05$) between the model fit and the experimental data. Similarly, the comparison of the adaptation phase, physiological stage and growth rate, no significant differences were found between Ldb and St. ($p>0.05$). These results demonstrated that the behavior of these lactic acid bacteria in the HGM is similar.

The validation of the predictive growth models developed for the hydrolyzed geminate maize (HGM) currently has little information due to the use of models formulated for other fermentative matrices, which is why it is necessary to know if the models used to obtain kinetic growth parameters can provide confidence, precision and low probability of biases.

Similar studies on modeling fermentations on similar substrates and lactic acid bacteria can be found in. [82], who "proposed the adjustment of a model for the growth of *Lactobacillus acidophilus* in complex substrate fermentation, comparing parameters such as specific growth rate, to the Gompertz model that suggests a specific growth rate ($\mu_{máx}$) 0.12964. The reliability of the fit determined a correlation coefficient (R^2) equal to 0.982. This describes a reliable and high fit of the parameters using the Gompertz model.

[83] reports that, "after calculating the respective residuals between the experimental values of the growth of S. aureus in trout meat, according to the Gompertz model, and the predicted values, it was found that the coefficient of determination is 0.9814, which is close to unity, being able to establish with 95% confidence that a good prediction was reached using the modified Gompertz model for the present investigation".

[84] determined "the kinetic parameters μ_{max} and N_{max} for lactic acid bacteria and Listeria monocytogenes were estimated by fitting the Baranyi and Roberts model to the data. Growth rate was expressed as maximum growth rate (μmax , log CFU/h). The model showed an excellent fit, with an $R^2 \geq 0.98$ and low RMSE values (< 0.20 log CFU/mL). As expected, an increase in temperature from 4 to 20 °C led to an increase in μ_{max} of Lb. sakei and L. monocytogenes from 0.021±0.001 and 0.037±0.001 log CFU/h to 0.0275±0.018 and 0.328±0.028 log CFU/h, respectively."

[85] mentioned that the Gompertz kinetic model applied to the growth of Lactococcus lactis subsp. lactis in milk determined that the coefficient of determination R2 obtained for this model was 0.991, which makes it quite clear that there was a good fit. On the other hand, it is important to emphasize that both the minimum generation time and the maximum exponential growth rate represent microbiological parameters descriptive of the growth rate of the microorganism in the medium or food, in this case milk, both being mathematically related, so it is sufficient to model one of them to achieve a predictive function".

[86] "Evaluated the effect of temperature on starter culture growth during ripening of Edam-type cheese. Experimental data fitted by Baranyi-Roberts and Gompertz mathematical models on the maximum exponential growth rate (μ_{max}) determined the coefficient of determination (R^2) at temperatures 30°C, 38°C and 45°C. In this case the determinant factor in starter culture growth is higher at temperature 30°C decreasing the μmax, as the temperature increases the goodness of fit is $R^2 = 0.970$, followed for temperature 38°C R^2 =0.948, finally at 45°C R^2 = 0.889 for the Baranyi and Roberts model; while; for the Gompertz model it is at 30°C R^2 0.970, 38°C R^2 0.948 and 45°C R^2 0.889. From the validation at 30, 38 and 95°C it was determined that the Gompertz model predicts better (bias factor indexes (βf) = accuracy factor (Af)= 1.00), followed by Baranyi-Roberts (Bf = Af= 0.01); therefore, the

validated models correspond to Baranyi &Roberts and Gompertz.

Tables **6** and **7** contain the results obtained for the root mean square error (RMSE) statistic, which show reduced deviations of the experimental data from the fit of the models. Thus, for St the Gompertz model the RMSE value is higher at 42°C and the Baranyi-Roberts model gives similar values for the three temperatures to which it was subjected; while for the case of Ldb the Gompertz model presents values that increased proportionally to the increase in temperature; whereas, the Baranyi-Roberts model has the highest value at 42°C. These results show that temperature affects the growth rate under thermo-tolerance conditions, a situation in which the physiological state (h_0) is very influential on the metabolic state that conditions enzymatic reactions that would be functioning in a suboptimal state, thus initiating the entry into the stationary phase.

The bias and accuracy indices were used, which express the reality as it is of the good performance of a model [52]. These indices are not based on the deviation between the observed response and the mean, as traditional statistical methods do. This statement causes a difficulty when it comes to assessing the reliability of models with new data, since the main response is unknown. It is recognized that these factors are useful tools for measuring the reliability of predictive models [87].

The Bias or bias factor (Bf) indicates whether the observed values were found above or below the equivalence line and thus provides a measure of the structural deviation of the models used, where $\mu_{predictive}$ is the value of the maximum specific growth rate predicted (h^{-1}) and $\mu_{observed}$ is the value of the maximum specific growth rate observed (h^{1}); n is the number of data or values.

In relation to the values of Bf it is observed that the Gompertz and Baranyi-Roberts model estimated better the maximum exponential growth rate (μ_{max}) of Ldb and St in HGM, since they present values lower than 1.00, for temperatures of 40°C, 41°C and 42°C (Table **6** and **7**). This index indicates that the predictions obtained are below what they should be and that they are in the equivalence line. There is not a large difference between the values, i.e., they are very close to unity.

The proximity of the predicted values to the observed values was estimated by the Accuracy Factor (Af) index of the Gompertz and Baranyi-Roberts models. The results show that the models developed for the growth rate of St in HGM, under the experimental conditions evaluated, the Gompertz model presents values of Af between 1.17 and 1.19; on the other hand, the Baranyi-Roberts model presents values very close to 1.00 and for all the temperatures tested (Table **6**), Likewise, for Ldb the models presented values no greater than 1.24 in relation to the temperatures tested (Table **7**). These facts indicate that the adjustments of the experimental points to the values predicted by the models are adequate, reaching appropriate estimations to the growth of the Ldb and St population as a function of time and to the intrinsic conditions of the HGM substrate and the temperatures of 40°C, 41°C and 42°C.

The results obtained on the validations of the models used are in accordance with the criteria of [52] who states that, "Bf values between 0.9 to 1.0 or 1.0 to 1.05 are considered adequate, while Bf values between 0.7 to 0.9 or 1.06 to 1.15 are considered acceptable when predicting the estimation of the kinetic parameters of growth of microorganisms".

The results obtained are similar to those obtained by [88] who in their study on Listeria monocytogenes in fermented sausages supported by stochastic modeling and meta-analysis reported Af values of 0.98 and 1.19 as well as Bf of 1.05 and 1.22; considering them as appropriate to describe the growth of the pathogen in fermentative environments. [89] indicates that L. mesenteroides, as the responsible for deteriorations in vacuum-packed sliced cooked hams, determining its growth parameters adjusted to the Gompertz models and using the growth rate, explained by the model, a lower Bf (0.0004) is observed, that is, the deviation of the model to the experimental data is very close to zero indicating a better fit between observations and estimations. The accuracy of the model is represented by a value of Af (1.06) very close to unity, which will allow more accurate predictions of the behavior of L. mesenteroides as a function of storage temperature, this being the only parameter that significantly affects ($p \leq 0.05$) the growth of this microorganism during storage.

CONCLUSION

The hydrolyzed germinated grain of Jora corn is a nutritional substrate used by the lactic starter bacteria *Lactobacillus delbrueckii ssp* bulgaricus and *Streptococcus thermophilus* where it uses its physical-chemical and microbiological characteristics for its growth and development of the fermentation process at thermo-tolerant temperatures.

The experimental growth parameters of lactic starter bacteria when using the hydrolysate of germinated grain of Jora corn at thermotolerant temperatures can be adjusted by the Gompertz and Baranyi-Roberts models obtaining growth parameters and statistical kinetics that are comparatively similar to the experimental ones.

Validation of the Gompertz and Baranyi-Roberts models indicate that both models have a high goodness of fit to describe the growth of *Lactobacillus delbrueckii* and *Streptococcus thermophilus* in the hydrolyzate of germinated Jora corn grain, and from their accuracy and bias values it can be inferred that both can correctly predict growth under thermotolerance conditions, being considered as a good tool for the food industry and consumers.

REFERENCES

[1] Wilkes G. The origin of corn-studies of the last hundred years. Crop Resour 1977;211–23 p.

[2] Paliwal RL, Granados G, Lafitte R, Violic AD, Mathée H. El maíz en los trópicos. Mejoramiento y producción. Colección FAO: producción y protección vegetal N 28. Roma. 2001;

[3] Rehman ZU. Storage effects on nutritional quality of commonly consumed sereals. Food Chem 2006; 95(1):53–7. https://doi.org/10.1016/j.foodchem.2004.12.017

[4] Monroy YM, Rodrigues RAF, Sartoratto A, Cabral FA. Optimization of the extraction of phenolic compounds from purple corn cob (Zea mays L.) by sequential extraction using supercritical carbon dioxide, ethanol and water as solvents. J Supercrit Fluids. 2016;116:10–9. https://doi.org/10.1016/j.supflu.2016.04.011

[5] Tetlow IJ, Morell MK, Emes MJ. Recent developments in understanding the regulation of starch metabolism in higher plants. J Exp Bot. 2004; 55(406):2131–45. https://doi.org/10.1093/jxb/erh248

[6] Valenzuela DP. Utilización de diferentes almidones complementarios y su efecto sobre las características sensoriales de la cerveza 2014.

[7] Rey MC. Hidrolizados caseínicos totales y fraccionados. Evaluación de su acción promotora del crecimiento y estimulante de propiedades tecnológicas de las bacterias ácido lácticas 2009.

[8] Wurzburg OB. Modified starches: properties and uses 1986.

[9] Huapaya CS. Elaboración de una bebida probiótica a partir de la fermentación láctica del almidón hidrolizado de harina de quinua Chenopodium quinoa 2014.

[10] Tovar CRV. El ñame espino (Dioscorea rotundata Poir.): una opción en la producción de jarabes edulcorantes intermedios para la industria alimentaria. Rev Investig Agrar y Ambient. 2010;1(2):19–28. https://doi.org/10.22490/21456453.896

[11] Wacher C. La biotecnología alimentaria antigua: los alimentos fermentados 2014.

[12] Meroth CB, Hammes WP, Hertel C. Characterisation of the microbiota of rice sourdoughs and description of Lactobacillus spicheri sp. nov. Syst Appl Microbiol. 2004;27(2):151–9. https://doi.org/10.1078/072320204322881763

[13] Orosco FG. Producción de ácido láctico por medio de fermentación anaerobia y su polimerización a partir de reacciones de apertura de anillo 2011.

[14] Mayo B, Aleksandrzak-Piekarczyk T, Fernández M, Kowalczyk M, Álvarez-Martín P, Bardowski J. Updates in the metabolism of lactic acid bacteria. Biotechnol Lact acid Bact Nov Appl. 2010;3–33. https://doi.org/10.1002/9780813820866.ch1

[15] Cobo-Monterroza R, Rosas-Quijano R, Gálvez-López D, Adriano-Anaya L, Vázquez-Ovando A. Bacterias ácido lácticas nativas como cultivo iniciador para la elaboración de queso crema mexicano. Agron Mesoam. 2019;30(3):855–70. http://dx.doi.org/10.15517/am.v30i3.34673

[16] Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol. 2004;15(2):67–78. https://doi.org/10.1016/j.tifs.2003.09.004

[17] Cárdenas N. Estudio de las propiedades tecnológicas de bacterias aisladas de leche materna: aplicación para el desarrollo de alimentos funcionales 2019.

[18] Hassam AN, Frank JF. Starter cultures and their use In Applied Dairy Microbiology 2001.

[19] Ramirez JC, Rosas P, Velazquez MY, ULLOA JA, Arce F. Bacterias lácticas: Importancia en alimentos y sus efectos en la salud 2011.

[20] Salomskiene J, Jonkuviene D, Macioniene I, Abraitiene A, Zeime J, Repeckiene J, et al. Differences in the occurence and efficiency of antimicrobial compounds produced by lactic acid bacteria. Eur Food Res Technol. 2019;245:569–79. https://doi.org/10.1007/s00217-018-03227-3

[21] Suárez S. Desarrollo de una bebida vegetal fermentada a base de cereales 2021.

[22] Calvopiña CG, Manotoa JC. Obtención de ácido láctico a partir de lactosuero y almidón de papa mediante fermentación láctica 2020.

[23] Hill AR, Kethireddipalli P. Dairy products: cheese and yogurt. In: Biochemistry of foods. Elsevier; 2013. p. 319–62.

[24] Rojas AM, Montaño LP, Bastidas MJ. Producción de ácido láctico a partir del lactosuero utilizando Lactobacillus delbrueckii subsp. bulgaricus y Streptococcus thermophilus. Rev Colomb Química. 2015;44(3):5–10. https://doi.org/10.15446/rev.colomb.quim.v44n3.55604

[25] Roja A, Mariana J. Producción de exopolisacáridos a partir de bacterias acido lacticas utilizando tusa de maiz como fuente de carbono 2020.

[26] Ponte López EA, Urbina Mejía SC. Elaboración y caracterización de una bebida fermentada a base de una mezcla de maíz de jora (zea mays l.), garbanzo (cicer arietinum l.) y harina de trigo 2010.

[27] Chilo DL. Evaluación de las condiciones de proceso para la elaboración de una bebida fermentada de quinua (Chenopodium quinoa Wild) con inclusión de bacterias ácido lácticas 2020.

[28] Cacsire GA, Ayma C. Tratamiento Térmico para Estabilizar la Chicha de Jora 2012.

[29] Fernández A, Romero J. Evolución de las bacterias ácido lácticas durante la elaboración del tocosh fresco, aislamiento y concentración por liofilización 2020.

[30] Taipe FA. Evaluación de los hidrolizados de Hordeum vulgare (Cebada), Chenopodium quinoa wildnow (Quinua) y Glycine max (Soya) como sustrato favorable para la elaboración de un producto nutracéutico a base de Lactobacillus acidophilus 2012.

[31] Guamán AM. Obtención de ácido láctico a partir del almidón del maíz chulpi (Zea mays sacchara) utilizando la fermentación de bacterias ácido lácticas, para su uso en la industria cosmética 2022.

[32] Rebaza TD, Montes ND, Ruas-Madiedo P. Aislamiento e identificación de bacterias ácido lácticas provenientes de “Chicha de Siete Semillas”, una bebida fermentada tradicional en Perú 2019.

[33] Guerrero PJ. Evaluación de la calidad microbiológica de los microorganismos probióticos en productos lácteos fermentados comerciales en la ciudad de Ocotlán, Jalisco 2012.

[34] De Vuyst L. Technology aspects related to the application of functional starter cultures. Food Technol Biotechnol. 2000;38(2):105–12.

[35] García RM, Zurera G. Modelización predictiva del desarrollo bacteriano en los alimentos. An la Real Acad Ciencias Vet Andalucía Orient. 2004;(17):61–78.

[36] Salinas RM, González GA, Pirovani ME, Ulín-Montejo F. Modelación del deterioro de productos vegetales frescos cortados. Univ y Cienc. 2007;23(2):183–96.

[37] Schultze KK, Linton RH, Cousin MA, Luchansky JB, Tamplin ML. A predictive model to describe the effects of temperature, sodium lactate, and sodium diacetate on the inactivation of a serotype 4b strain of Listeria monocytogenes in a frankfurter slurry. J Food Prot. 2006;69(7):1552–60. https://doi.org/10.4315/0362-028x-69.7.1552

[38] Wordon BA, Mortimer B, McMaster LD. Comparative real-time analysis of Saccharomyces cerevisiae cell viability, injury and death induced by ultrasound (20 kHz) and heat for the application of hurdle technology. Food Res Int. 2012;47(2):134–9. https://doi.org/10.1016/j.foodres.2011.04.038

[39] Ferrer J, Prats C, López D, Vives-Rego J. Mathematical modelling methodologies in predictive food microbiology: a SWOT analysis. Int J Food Microbiol. 2009;134(1–2):2–8. https://doi.org/10.1016/j.ijfoodmicro.2009.01.016

[40] Mafart P. Food engineering and predictive microbiology: on the necessity to combine biological and physical kinetics. Int J Food Microbiol. 2005;100(1–3):239–51. https://doi.org/10.1016/j.ijfoodmicro.2004.10.023

[41] Rivera D, Toledo V, Reyes-Jara A, Navarrete P, Tamplin M, Kimura B, et al. Approaches to empower the implementation of new tools to detect and prevent foodborne pathogens in food processing. Food Microbiol. 2018;75:126–32. https://doi.org/10.1016/j.fm.2017.07.009

[42] Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol. 1994;23(3–4):277–94. https://doi.org/10.1016/0168-1605(94)90157-0

[43] Juneja VK, Melendres MV, Huang L, Gumudavelli V, Subbiah J, Thippareddi H. Modeling the effect of temperature on growth of Salmonella in chicken. Food Microbiol. 2007;24(4):328–35. https://doi.org/10.1016/j.fm.2006.08.004

[44] Coccia A. Probióticos, una mirada objetiva sobre microorganismos beneficiosos para la salud 2020.

[45] Clavel M. Condiciones microbiológicas y aislamiento de bacterias lácticas en quesos artesanales del estado de Hidalgo 2006.

[46] Moreno MV, Polo MC. Occurrence of lactic acid bacteria and biogenic amines in biologically aged wines. Food Microbiol. 2008;25(7):875–81. https://doi.org/10.1016/j.fm.2008.05.004

[47] Sainz L. Desarrollo de estrategias moleculares que permitan el uso de las bacterias lácticas (BAL) como factorías celulares de producción de péptidos bioactivos de interés en la industria alimentaria 2019.

[48] Fula AG. Desarrollo de una bebida fermentada con adición de cocción de maíz 2010.

[49] Helrich K. Official methods of analysis of the Association of Official Analytical Chemists. Association of official analytical chemists 1990.

[50] McMeekin TA, Olley JN, Ross T, Ratkowsky DA. Predictive microbiology: theory and application. biotechnologia. 1993;2(25):94.

[51] Cardenas FJC, Giannuzzi L, Noia MA, Zaritzky NE. El modelado matemático: Una herramienta útil para la industria alimenticia. Cienc Vet. 2001;3(1):22–8.

[52] Ross T. Indices for performance evaluation of predictive models in food microbiology. J Appl Bacteriol. 1996;81(5):501–8. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x

[53] McClure PJ, Blackburn C de W, Cole MB, Curtis PS, Jones JE, Legan JD, et al. Modelling the growth, survival and death of microorganisms

in foods: the UK Food Micromodel approach. Int J Food Microbiol. 1994;23(3–4):265–75. https://doi.org/10.1016/0168-1605(94)90156-2

[54] García C, Arrázola G. Producción de ácido lactico de lactosuero suplementado utilizando lactobacillus casei. Biotecnol en el Sect Agropecu y agroindustrial. 2013;11(1):136–43.

[55] Sarango NM, Yanchapanta RD. Cinética del crecimiento de Microorganismos durante el Proceso de Fermentación de Tres Bebidas Ancestrales a partir de Yuca (Manihot esculenta) 2019.

[56] Rodríguez MR. Desarrollo y validación de modelos matemáticos para la predicción de vida comercial de productos cárnicos 2004.

[57] Patterson DT, Flint EP. Potential effects of global atmospheric CO2 enrichment on the growth and competitiveness of C3 and C4 weed and crop plants. Weed Sci. 1980;28(1):71–5. https://doi.org/10.1017/S0043174500027806

[58] Te Giffel MC, Zwietering MH. Validation of predictive models describing the growth of Listeria monocytogenes. Int J Food Microbiol. 1999;46(2):135–49. https://doi.org/10.1016/S0168-1605(98)00189-5

[59] Escobar LF, Rojas CA, Giraldo GA, Padilla-Sanabria L. Evaluación del crecimiento de Lactobacillus casei y producción de ácido láctico usando como sustrato el suero de leche de vacuno. Rev Investig Univ del Quindio. 2010;20(1):42–9. https://doi.org/10.33975/riuq.vol20n1.707

[60] Vinderola CG, Reinheimer JA. Enumeration of Lactobacillus casei in the presence of L. acidophilus, bifidobacteria and lactic starter bacteria in fermented dairy products. International Dairy Journal 2000 ;10(4) : 271–275. https://doi.org/10.1016/S0958-6946(00)00045-5

[61] Sanz Y, Collado MC, Dalmau J. Probióticos: criterios de calidad y orientaciones para el consumo. Acta pediátrica española. 2003;61(9):476–82.

[62] Cavallini ER. Bacteriología general: principios y prácticas de laboratorio 2005.

[63] Teleken JT, Robazza W da S, Gomes G de A. Mathematical modeling of microbial growth in milk. Food Sci Technol. 2011;31:891–6.

[64] Coronel MA. Estudio de las características fisico-químicas y sensoriales de yogurt enriquecido con quinua (“ Chenopodium quinoa” Willd) 2019.

[65] Oliveira RPS, Florence ACR, Silva RC, Perego P, Converti A, Gioielli LA, et al. Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. Int J Food Microbiol. 2009;128(3):467–72. https://doi.org/10.1016/j.ijfoodmicro.2008.10.012

[66] Tipantuña NV. Cinética del crecimiento de microorganismos durante el proceso de fermentación de una bebida ancestral elaborada a partir de chonta (Bactris gasipaes) 2020.

[67] Pulido HG, De la Vara R, González PG, Martínez CT, Pérez M del CT. Análisis y diseño de experimentos. McGraw-Hill New York, NY, USA 2012.

[68] Carr FJ, Chill D, Maida N. The lactic acid bacteria: a literature survey. Crit Rev Microbiol. 2002;28(4):281–370. https://doi.org/10.1080/1040-840291046759

[69] Gálvez A, Abriouel H, López RL, Omar NB. Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol. 2007;120(1–2):51–70. https://doi.org/10.1016/j.ijfoodmicro.2007.06.001

[70] Tojo Sierra R, Leis Trabazo R, Barros Velázquez J, Prado Rodríguez M. Productos lácteos fermentados. An pediatr 2006 ;4(1) :54–66.

[71] Jurado H, Aguirre D, Ramírez C. Caracterización de bacterias probióticas aisladas del intestino grueso de cerdos como alternativa al uso de antibióticos. Rev MVZ Córdoba. 2009;14(2):1723–35. https://doi.org/10.21897/rmvz.356

[72] Olivares Y, Ricaldi KG. Efecto del proceso de malteado en las características fisicoquímicas y químicas en la obtención de harina de maíz (Zea mays) 2013.

[73] Zareba D, Ziarno M. The viability of yogurt bacteria in selected plant beverages. Zesz Probl Postępów Nauk Rol 2017;(591) :87–96. https://doi.org/10.22630/ZPPNR.2017.591.46

[74] Bustamante A. Influencia de la temperatura de fermentación en las características fisicoquímicas de la chicha de jora, evaluados en dos variedades de germinados de maíz (Zea mays L.) 2019.

[75] Charalampopoulos D, Pandiella SS, Webb C. Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. J Appl Microbiol. 2002;92(5):851–9. https://doi.org/10.1046/j.1365-2672.2002.01592.x

[76] Abdel-Rahman MA, Tashiro Y, Sonomoto K. Recent advances in lactic acid production by microbial fermentation processes. Biotechnol Adv. 2013;31(6):877–902. https://doi.org/10.1016/j.biotechadv.2013.04.002

[77] Serna-Cock L, Stouvenel AR de. Produccion biotecnologica de acido lactico: estado del arte biotechnological production of lactic acid: state of the art produccion biotecnoloxica de acido lactico: estado do arte. CYTA-Journal Food. 2005;5(1):54–65.

[78] Wahidin S, Idris A, Yusof NM, Kamis NHH, Shaleh SRM. Optimization of the ionic liquid-microwave assisted one-step biodiesel production process from wet microalgal biomass. Energy Convers Manag. 2018;171:1397–404.

https://doi.org/10.1016/j.enconman.2018.06.083

[79] Aguilar C. Modelación del crecimiento en cultivo mixto de bacterias ácido lácticas y patógenas asociadas a alimentos 2007.

[80] Shimoni E, Labuza TP. Modeling pathogen growth in meat products: future challenges. Trends food Sci Technol. 2000;11(11):394–402.

https://doi.org/10.1016/S0924-2244(01)00023-1

[81] Barrera GR. Desarrollo y validación de modelos predictivos dinámicos del crecimiento de staphylococcus aureus en productos cárnicos previo al proceso de cocción 2011.

[82] Calderón JF. Ajuste de un modelo cinético para el crecimiento de Lactobacillus Acidophilus en la fermentación de sustrato complejo.

Fundación Universidad de América 2017.

[83] Chicata M. Formulación de un modelo matemático para determinar la vida util de trucha (oncorhynchus mykiss refrigerada utilizando microbiología predictiva 2015.

[84] Costa JCCP. Desarrollo de modelos predictivos de interacción microbiana para listeria monocytogenes y bacterias acido lácticas y su aplicación en la optimización de cultivos bio-protectores en productos pesqueros 2020.

[85] Valbuena E, Barreiro J, Sánchez E, Castro G, Bríñez W, Tovar A. Modelos cinéticos aplicados al crecimiento de Lactococcus lactis subsp. lactis en leche. Rev Científica. 2005;15(5):464–75.

[86] Hancco ETL, Vizcarra TA, Aro JMA, Cutimbo MC. Evaluación del efecto de la temperatura sobre el crecimiento de cultivos iniciadores durante la maduración del queso tipo Edam. Cienc Desarro. 2014;(17):12–20. https://doi.org/10.33326/26176033.2014.17.365

[87] Dalgaard P, Jørgensen LV. Predicted and observed growth of Listeria monocytogenes in seafood challenge tests and in naturally contaminated cold-smoked salmon. Int J Food Microbiol. 1998;40(1–2):105–15. https://doi.org/10.1016/S0168-1605(98)00019-1

[88] Mataragas M, Alessandria V, Rantsiou K, Cocolin L. Management of Listeria monocytogenes in fermented sausages using the Food Safety Objective concept underpinned by stochastic modeling and meta-analysis. Food Microbiol. 2015;49:33–40. https://doi.org/10.1016/j.fm.2015.01.001

[89] Buelvas GA. Desarrollo y validación de modelos matemáticos predictivos del crecimiento microbiano para estimación de la vida útil en jamón lonchado empacado al vacío 2013.

DOI: https://doi.org/10.15379/ijmst.v10i3.1964