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Viability of *Lactobacillus acidophilus* in whole goat milk yogurt during fermentation and storage stages: a predictive modeling study

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Abstract

The incorporation of probiotics into fermented milk is beneficial for the consumer's health and it is important to know the viability of these microorganisms in the product, from production to final consumption. This research carried out a predictive study on the behavior of *Lactobacillus acidophilus* LAFTI L10 probiotic strain during the fermentation and storage stages of whole goat milk yogurt. Selective counts of *L. acidophilus* were performed with MRS medium added with clindamycin. The primary model of nonlinear regression by Baranyi and Roberts was fit to the experimental data in DMFit 3.5 software and statistically evaluated. The total fermentation time of the yogurt was 6.5 h and 3 logarithmic cycles of probiotic cell growth were observed. During storage, time influenced the concentration of *L. acidophilus* from the 30th day onwards, maintaining a minimum count that allows the denomination of probiotic yogurt to the product and certifying its quality. The Baranyi and Roberts model fit satisfactorily the experimental data. The results can benefit the dairy industry in the development, optimization, and reliability of their processes for dairy products.

Keywords: dairy products; predictive microbiology; probiotics modeling; probiotics viability.

Practical applications: *Lactobacillus acidophilus* can be applied and predicted in goat milk yogurt matrices.

1 Introduction

There is a growing development of dairy goat products, such as yogurts, dairy drinks, and cheeses, as a way of masking the characteristic goat milk flavor and turning its derivative goods more attractive. Studies have evaluated, for example, the use of plant extracts such as quinoa extract (El-Shafei et al., 2020), *Pistacia atlantica* resin (Hadjimbei et al., 2020) and *Moringa oleifera* leaf powder (Wulansari et al., 2022), to improve the sensory and technological properties of fermented goat milk, as well as the addition of probiotic microorganisms, which, in addition to making the food functional, can release compounds that contribute to the aroma and flavor of the final product (Ranadheera et al., 2019). In addition to it, the increased demand for hypoallergenic and functional food is also contributing to the expansion of this field, mainly for yogurt production (Ranadheera et al., 2019; Ranadheera et al., 2018).

In the context of functional foods, probiotics are one of the main factors of interest. These are defined as microbial cells (viable or not) that, when consumed, are potentially beneficial to the consumer's health. *Lactobacillus acidophilus* is one of the main microorganisms exploited as probiotic by the food industry and stands out for surviving in more acidic media when compared to other probiotics, so, it can be considered a true probiotic, which is viable and active cells acting in the host (Zendeboodi et al., 2020). One of its most desirable properties is the production of antimicrobial metabolites, probioactives, great allies in the treatment and prevention of infection by food pathogens, which makes this microorganism to be studied in several products, especially dairy products (Parvarei et al., 2021; Ozcan & Eroglu, 2022; Ryan et al., 2020; Moghaddam et al., 2020). In addition, it is noteworthy that dairy goat products are potential food matrices for delivery of probiotic bacteria (Ranadheera et al., 2019)

In recent years, interest has grown in the study of the survival kinetics of true probiotic species, applied to different environmental conditions and food matrices, highlighting the fact that fermentation is one of the main strategies for probiotics to synthesize compounds of interest, the probioactives (Champagne et al., 2018; Ryan et al., 2020). Predictive microbiology is a science that investigate parameters for predicting the behavior of microorganisms in food, which provides a fast and reliable path to knowledge about microbial growth, inactivation, and survival under specific conditions (Gonçalves et al., 2018; Ross & McMeekin, 1994).

Considering that growth of microoganisms in food are real and complex systems that do not follow a simple mechanism, there

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is interest in the knowledge of the concentration profile of many microorganisms in dairy foods over time, even more in relation to probiotics during storage, in this sense, mathematical models are being widely used to describe their behavior (Possas et al., 2022; Shori, 2022). Baranyi and Roberts' mathematical model (Baranyi & Roberts, 1994) is classified as empirical, kinetic, and primary. Moreover, it is widely applied in predictive microbiology. This model is favored due to some reasons: it is easy to apply; it applies to conditions with dynamic variations in the environment; it has a good fit and most of the model parameters are biologically interpretable (Van Impe et al., 2005).

Predictive microbiology is more associated with microbiological quality and risk of food contamination by predicting the growth of pathogens and deteriorators (Possas et al., 2022). In addition to this, it can also be an important ally in the study of the behavior of probiotic cells, such as lactic bacteria, present in milk and its derivatives. Mathematical modeling of the experimental data aids to obtain important data on the growth dynamics of probiotics under the studied conditions, which contributes to the optimization of the food manufacturing process, as well as to the estimation of their functional viability (Gonçalves et al., 2018; Stavropoulou & Bezirtzoglou, 2019).

This research aimed to study the viability of the probiotic strain *Lactobacillus acidophilus* LAFTI L10 at a fixed temperature, during the fermentation of whole goat milk yogurt at 43 ± 1 °C, and during its storage at 4 ± 1 ° C for 35 days from the Baranyi and Roberts' primary model adjustment.

2 Materials and methods

2.1 Whole goat milk yogurt production

The whole goat milk yogurt production followed the methodology adapted by Costa et al. (2014). Powdered whole goat milk was reconstituted according to the manufacturer's instructions: 250 ± 0.005 g was weighed on a semi-analytical scale (Ramuza®) and diluted in mineral water up to 2000 mL of total volume. The goat milk was transferred to another glass container and sugar was added at a concentration of 8% (w/w), followed by homogenization.

Heat treatment was carried out on an industrial stove for 30 min and at a temperature of 80 ºC ± 2 ºC (slow pasteurization). The next stage was refrigeration, carried out in a stainless-steel vat with water and ice cubes, with thermal monitoring of pasteurized milk, until it reached 42 ºC ± 1 ºC. Then, the direct vat set mixed starter culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* DELVO YOG CY 340 (Globalfood, Brazil) and the probiotic culture *Lactobacillus acidophilus* LAFTI L10 (Globalfood, Brazil) were added, both at a concentration of 1% (v/v), in the form of suspensions previously prepared in a concentration of 10^{12} CFU/mL.

Finally, the content was equally divided into 6 sterile glass jars and subjected to 43 ºC ± 1 ºC for fermentation in a bacteriological oven (Fanem®). During this stage, the pH and acidity (% of lactic acid) of the samples were monitored at 1 h intervals. Each measure was taken in triplicate up to reaching values of 4.6 and 0.65, respectively. Then, the products were removed from the oven and stored in a refrigerator at 4 ºC ± 1 ºC.

2.2 *Lactobacillus acidophilus LAFT-L10 count*

For the study of the growth and viability of probiotic culture in whole goat milk yogurt, selective counting of *L. acidophilus* LAFT-L10 was performed during the fermentation stage at each 1 h and during the 35-day storage under refrigeration every 5 days. The counts were performed in triplicate.

The selective counting of *L. acidophilus* LAFTI-L10 (Globalfood) was performed according to the method described by Van de Casteele (2006). The pour plate technique was used in a DeMan-Rogosa-Sharpe Agar medium added with the antibiotic clindamycin hydrochloride (Merck) at a concentration of 5 ppm (MRS-CL). The antibiotic was added to the MRS Agar medium (Merck) immediately before plating at the defined concentration and with an average temperature of 42 °C.

Sample preparation was carried out according to the methodology described by Silva et al. (2017) to obtain serial dilutions. Aliquots of 1.0 g from the dilutions were transferred to sterile Petri dishes and approximately 20.0 mL of MRS-CL culture medium was poured into each, followed by homogenization. After solidification of the mediums, the Petri dishes were incubated in a BOD chamber (Tecnal®) in the absence of oxygen at 37 ºC ± 2 ºC for 72 ± 1 h. The quantification of typical colonies (creamy white with irregular edges) was performed with the aid of a mechanical colony counter (Phoenix Luferco®) and the bacterial concentration was expressed in the 10-base logarithm of Colony Forming Units per gram of sample (log CFU/g).

2.3 Mathematical modeling of data

The experimental data of *L. acidophilus* counts during the fermentation and storage stages were adjusted to the Baranyi and Roberts' model, which relates the variation in microbial concentration with the storage time at a set temperature. Model fitting was performed using the DMFit tool from Microsoft Excel© developed by the Institute of Food Research (United Kingdom) that allows one to estimate the following kinetic parameters: maximum growth rate (μ, expressed in h^{-1}); lag phase time (λ, expressed in hours); initial population (y_0, expressed in log CFU/g); and maximum population ($y_{max,}$ expressed in log CFU/g).

2.4 Statistical analysis

The data were evaluated using ANOVA and compared using the Tukey test at a significance level of 5% with aid of Statistic 7.0. The fit of the Baranyi and Roberts model was evaluated by the calculation of the statistical indexes R^2, RMSE, bias factor, and accuracy factor and by comparing the experimental data with the values predicted by the non-linear regression model, according to Ross (1996).

3 Results and discussion

The data for pH, acidity, and concentration of L. acidophilus LAFTI L10 that were obtained during the fermentation stage (Table 1) varied significantly over time ($p \leq 0.05$). In 6.5 h, the

Table 1. Concentration of *L. acidophilus* LAFTI L10, pH, and acidity during fermentation of whole goat milk yogurt between 0 and 6.5 h at 43 °C ± 1 °C.

Time (hours)	Concentration of *L. acidophilus* (logCFU/g)	pH	Acidity (% lactic acid)
0	4.12 ± 0.02^g	6.34 ± 0.08^a	0.18 ± 0.01^h
1	4.47 ± 0.01^f	6.22 ± 0.04^a	0.18 ± 0.01^h
2	5.07 ± 0.01^e	6.10 ± 0.01^b	0.21 ± 0.01^g
3	5.36 ± 0.02^d	5.49 ± 0.03^c	0.38 ± 0.01^f
4	5.90 ± 0.03^c	4.97 ± 0.01^d	0.55 ± 0.01^e
4.5	5.89 ± 0.03^c	4.80 ± 0.01^e	0.60 ± 0.01^d
5	-	4.78 ± 0.01^e	0.64 ± 0.01^b
5.5	6.18 ± 0.10^b	4.74 ± 0.02^{ef}	0.66 ± 0.01^a
6	-	4.72 ± 0.01^{ef}	0.63 ± 0.01^c
6.5	7.05 ± 0.01^a	4.64 ± 0.01^f	0.65 ± 0.01^a

Mean values vertically followed by equal letters do not differ statistically from each other according to the Tukey test at 5%.

probiotic count ranged from 4.12 log CFU/g to 7.05 log CFU/g, the pH decreased from 6.34 to 4.64 and the acidity increased from 0.18 to 0. 65 (% lactic acid).

The fermentation stage is characterized by intense microbial metabolic activity, in which lactic cultures, mainly *S. thermophillus*, in optimal pH and temperature conditions, use the lactose available in goat milk for the production of lactic acid (lactic fermentation). This fermentative process causes a reduction in pH and an increase in the acidity of the medium, as can be observed in this research (Parvarei et al., 2021; Rosyidi et al., 2021). According to Gopal (2011), the growth of *L. acidophilus* in yogurt is favored by the presence of metabolites such as formic acid and possibly by carbon dioxide and pyruvate, both produced by the colonies of *S. thermophilus* present in the medium. Similar results were found by Shu et al. (2018) in a study with goat milk yogurt with the addition of *Lactobacillus acidophilus*. The authors obtained a pH reduction from 5.4 to 4.5 by varying the concentration of *L. acidophilus* from 6.48 to 7.18 log CFU/g in a 3-hour fermentation.

Figure 1 shows the fitting of Baranyi and Roberts' model for the growth of *L. acidophilus* LAFTI L10 during the yogurt fermentation stage. The kinetic parameters were obtained from the profile and provided information on microbial growth during the studied fermentation time (Table 2).

According to the results, no lag time (lag phase) was observed, the maximum specific growth rate (μ) was 0.4214 h^{-1}, the initial population (y_0) was 4.124 log CFU/g and the final fermentation population (y_{max}) was 6.8516 log CFU/g. According to the statistical parameters displayed in Table 2, the model presented a satisfactory fit to the experimental data, considering that the regression coefficient (R^2) was 0.9742 and the root mean squared error value was at around 0.0144. The bias factor (Fb) and accuracy factor (Fe) were both close to 1, more precisely 1.0037 and 1.0148, respectively. According to Ross (1996), the Fb > 1 indicates a maximization of the data prediction, therefore, the predicted value is above the observed value, while Fe indicates the average deviation from the experimental value

Table 2. Kinetic parameters and statistical indexes estimated according to the Baranyi and Roberts' model referring to the fermentation stage of whole goat milk yogurt at 43 °C ± 1 °C.

Kinetic parameters				Statistical indexes			
μ	λ	y_0	$y_{máx}$	RMSE	bias	R^2	accuracy
0.4214	-	4.1124	6.8516	0.0144	1.0037	0.9742	1.0148

y_0: log of the initial count (logCFU/g); μ: maximum growth rate (h^{-1}); λ: time of the lag phase (h); $y_{máx}$: log of the final count (logCFU/g); RMSE: root mean squared error; bias: bias factor; R^2: regression coefficient; accuracy: accuracy factor.

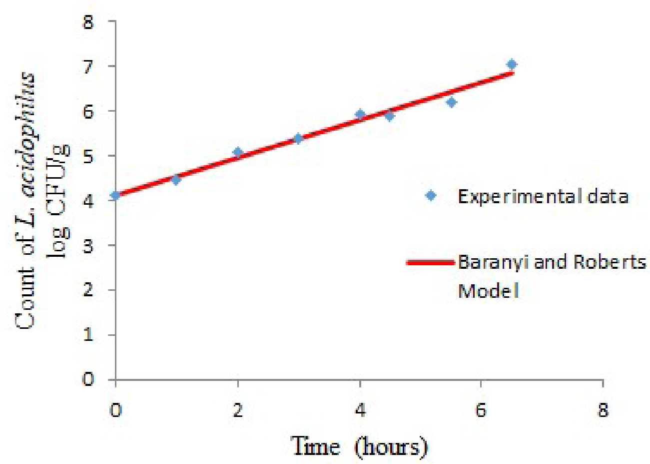

Figure 1. Model fitting of *L. acidophilus* concentration profile during the fermentation stage of whole R^2: regression coefficient; accuracy: accuracy factor goat milk yogurt at 43 °C ± 1 °C by Baranyi and Roberts' model.

to the predicted value. In the present case, the accuracy factor indicated a forecast error of approximately 1.5% of the real value.

Regarding the monitoring of *L. acidophilus* viability in whole goat milk yogurt for 35 days of storage, the concentration values, as well as the pH and acidity are presented in Table 3.

The counts of *Lactobacillus acidophilus* LAFTI L10 in the goat milk yogurt produced throughout the storage period (Table 3) remained in concentrations greater than 6 log CFU/g, which is a generally recommended concentration of probiotics for functional foods to promote health and other benefits to the final consumer (Parker et al., 2018; Sangami & Sri, 2017; Shori, 2022).

Regarding the population of *L. acidophilus* LAFTI L10, stability was observed during the first 25 days of storage ($p > 0.05$), followed by a significant decrease from the 30th day ($p \leq 0.05$). The initial stability of the probiotic concentration can be related to the excellent nutritional and environmental conditions of the mediums, since cultures of *L. acidophilus* have displayed growth affinity at the refrigeration temperature and also at pH typical for fermented milk (Gocer et al., 2021; Gopal, 2011).

The reduction in the concentration of *L. acidophilus* in the last weeks of storage (Table 3) may have occurred due to the intense metabolic activity of the other cultures present in the medium, mainly *L. bulgaricus*, which results in the production of organic

Table 3. Concentration of *L. acidophilus*, pH, and acidity during the storage of whole goat milk yogurt for 35 days at 4 °C ± 1 °C.

Time (days)	Concentration of *L. acidophilus* (logCFU/g)	pH	Acidity (% lactic acid)
0	7.15 ± 0.11[a]	4.63 ± 0.01[a]	0.66 ± 0.01[b]
5	7.15 ± 0.21[ab]	4.40 ± 0.04[b]	0.70 ± 0.01[a]
10	7.14 ± 0.04[ab]	4.44 ± 0.01[b]	0.70 ± 0.01[a]
15	7.09 ± 0.07[ab]	4.43 ± 0.02[b]	0.70 ± 0.01[a]
20	7.04 ± 0.06[abc]	4.45 ± 0.01[b]	0.70 ± 0.01[a]
25	6.95 ± 0.07[abc]	4.44 ± 0.02[b]	0.70 ± 0.01[a]
30	6.76 ± 0.03[bc]	4.44 ± 0.01[b]	0.70 ± 0.02[a]
35	6.65 ± 0.08[c]	4.42 ± 0.01[b]	0.71 ± 0.01[a]

Means vertically followed by equal letters do not differ statistically from each other by the Tukey test at 5%.

acids, that can decrease the pH, and hydrogen peroxide. These byproducts can affect the growth stability of the *starting* probiotic strain (Gopal, 2011; Ozcan & Eroglu, 2022; Ryan et al., 2020).

Regarding the pH (Table 3), a significant decrease ($p \leq 0.05$) could be expected over the weeks due to the fermentative activity of the lactic acid bacteria converting the lactose to lactic acid, even at the refrigeration temperature (Ryan et al., 2020). An undesirable phenomenon known as post-acidification is caused by lactic bacteria of the species *L. bulgaricus*, resulting from their metabolic activity. In spite of that, the presence of these microorganisms is important to attribute sensorial characteristics to the final product, mainly due to the formation of aromatic compounds (Dan et al., 2019). The results are in agreement with the increase in acidity levels.

The results obtained in this research corroborate the values found by Machado et al. (2017), who noticed a reduction of approximately 6% in pH and a 7% increase in acidity over 28 days of storage for a goat milk yogurt formulation. Furthermore, they observed a decline of two logarithmic cycles of the concentration of *L. acidophilus* in the same period. Parvarei et al. (2021) also observed a reduction in pH and an increase in acidity in yogurt samples with *L. acidophilus* during 28 days of storage at 4 °C.

Some research has investigated alternatives to increase the viability of *L. acidophilus* in goat milk yogurts, such as the addition of honey (Machado et al., 2017) and the incorporation of prebiotics, such as stachyose (Shu et al., 2018). These ingredients are favorable to the growth of the probiotic strain, increasing the functional character of fermented milk products.

To describe the experimental data regarding the concentration profiles of *Lactobacillus acidophilus* LAFTI L10 (in log CFU/g) in whole goat milk yogurt refrigerated at 4 °C ± 1 °C, the primary model of Baranyi & Roberts was used (Figure 2). The kinetic parameters and statistical indexes are shown in Table 4.

The kinetic parameters (Table 4) describe the microbial behavior during storage. The specific growth rate (μ) was estimated at -0.0012 h^{-1}, indicating that there was a decline in the concentration of *L. acidophilus*. The lag phase time (λ) lasted for 409.34 h (about 17 days), a period of stable microorganism

Table 4. Kinetic parameters and statistical indexes obtained for the modeling of *L. acidophilus* profile over the storage stage of whole goat milk yogurt at 4 °C ± 1 °C according to Baranyi and Roberts model.

Kinetic parameters				Statistical indexes			
μ	λ	y_0	$y_{máx}$	RMSE	bias	R^2	accuracy
-0.0012	409.34	7.1420	-	0.0004	1.0001	0.9848	1.0022

y_0: log of the initial count (logCFU/g); μ: maximum growth rate (h^{-1}); λ: time of the lag phase (h); $y_{máx}$: log of the final count (logCFU/g); RMSE: root mean squared error; bias: bias factor.

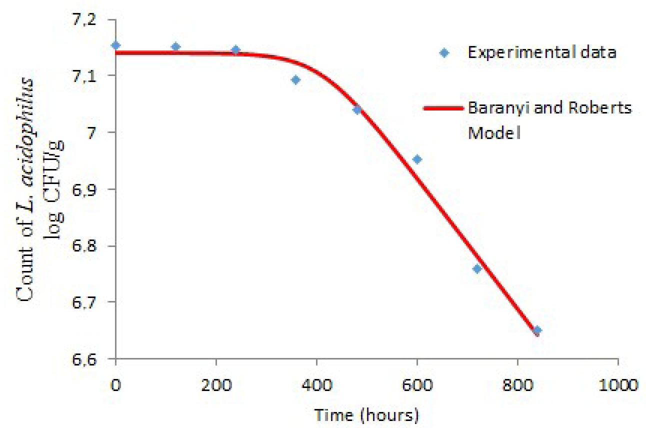

Figure 2. Model fitting of *L. acidophilus* concentration profile during storage of whole goat milk yogurt at 4 °C ± 1 °C.

concentration in the product, characterizing the adaptation to the environmental conditions of the food.

The statistical indexes (Table 4) demonstrated that the observed values were well described by the primary mathematical model of Baranyi & Roberts. The low value of the root mean squared error (RMSE = 0.0004) and the regression coefficient close to 1 ($R^2 = 0.9848$) show a small probability of errors and, consequently, a suitable fitting of the model. The bias factor greater than 1 (Fb = 1,0001) indicates that the results found experimentally are, in general, lower than those predicted by the model. The accuracy factor was also greater than 1 (Fe = 1.0022), indicating a forecast error of less than 1%, which further reinforces the agreement of the model for predicting the behavior of *L. acidophilus* LAFTI L10 cells under the evaluated conditions.

Despite the vast knowledge of predictive microbiology, there is still a lack of research on investigating the viability of probiotic strains through mathematical modeling, especially in goat-sourced dairy products. Nikmaram et al. (2016) carried out a predictive study of the viability of *L. acidophilus* La-5 during 21 days of storage at 4 °C in samples of pomegranate yogurt, in which they observed a good fit of experimental data to the Monte Carlo model, with $R^2 = 0.96$. They emphasized the importance of this tool to estimate the concentration of probiotics during the storage of the final product.

In addition, it is emphasized that in addition to the studies of microbiological and physicochemical behavior, it is necessary to perform additional sensory studies, through different methods, as a way of ratifying the consumer's perception of the probiotic yogurt produced (Mamede et al., 2020; Portela et al., 2022; Rodrigues et al., 2021a; Rodrigues et al., 2021b; Rodrigues et al., 2021c)

4 Conclusion

Whole goat milk yogurt proved to be a food matrix favorable to the growth and viability of the probiotic strain *Lactobacillus acidophilus* LAFTI L10 (Globalfood). Mathematical modeling provided values of kinetic parameters that allowed a better understanding of the behavior of probiotic cells during the stages of fermentation and storage of samples, being able to accurately and exactly describe the counting of probiotic microorganisms over time. The calculated statistical indexes demonstrated that the primary model of Baranyi and Roberts can be used to predict the growth and death of *L. acidophilus* culture in the studied conditions with high reliability. This knowledge displays great relevance to dairy industries since they can apply predictive modeling to optimize the process and control the probiotic viability of their products.

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