

# Bacterial Population Kinetics and Physicochemical Profiles in Fermented Goat Milks: Roles of *Streptococcus thermophiles* ATCC19258 and *Lactobacillus bulgaricus* ATCC11842

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## Abstract

**Background and Objective:** The fermentation of Algerian goat milk, a process for the production of valuable dairy products, relies on the synergistic activity of *Streptococcus (S.) thermophilus* and *Lactobacillus (L.) bulgaricus*. However, a significant knowledge gap is seen regarding the precise dynamics of these starter cultures within the unique matrix of Algerian goat milks. Specifically, the intricate relationships between their growth patterns and the resulting physicochemical changes, which regulate the distinct biochemical characteristics of fermented products, are poorly understood. So, this study addressed this problem by studying specific contributions of *S. thermophilus* and *L. bulgaricus* to goat milk fermentation.

**Material and Methods:** Goat milk was fermented by starter cultures of *S. thermophilus* and *L. bulgaricus* (8 h). Bacterial growth and physicochemical parameters, including pH, titratable acidity, viscosity and syneresis, were assessed. Mixed-effects models were used for statistical analysis to assess the relationship between physicochemical changes and bacterial growth.

**Results and Conclusion:** The results showed a strong relationship between *L. bulgaricus* and the control of acidification, viscosity and syneresis ( $r = 0.979$  for titratable acidity,  $p < 0.0001$ ). *S. thermophilus* contributed significantly, particularly to the increases in viscosity ( $r = 0.773$ ,  $p < 0.01$ ). The two species significantly decreased the pH, with *L. bulgaricus* having twice the acidifying effects. By the end of the fermentation process, pH reached  $4.12 \pm 0.20$ , titratable acidity increased to  $84.75 \pm 2.19$  °D and viscosity increased to  $6425.00 \text{ mPa.s} \pm 638.64$ . The final bacterial counts of *S. thermophilus* and *L. bulgaricus* were  $519.00 \pm 115.29 \times 10^7$  and  $65.54 \pm 6.89 \times 10^7$  CFU.ml<sup>-1</sup>, respectively. In addition to providing a robust statistical framework for process control and quality assurance in fermented milk manufacture, this study highlighted the critical role of *L. bulgaricus* in regulating structural and sensory qualities of fermented goat milks. Results can be used to optimize fermentation processes for goat milk by strategically manipulating the ratio of *L. bulgaricus* to *S. thermophilus*. The strong correlation between *L. bulgaricus* and acidification, viscosity and syneresis ( $r = 0.979$  for titratable acidity,  $p < 0.0001$ ) provides a clear target for controlling key product attributes.

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## 1. Introduction

In Algeria, where goat farming is an essential part of the rural economy particularly in the dry and semi-arid regions with an estimated 4.2 million goats, goat milk processing into fermented products offers significant advantages. This process meets the growing consumer demand for quality-

processed foods while optimizing the use of a readily available but currently underused resource. Because of their synergistic effects on the physicochemical and sensory characteristics of fermented dairy products, *Streptococcus (S.) thermophilus* and *Lactobacillus (L.) bulgaricus* are freq-



uently used as starter cultures in cow milk fermentation. In this study, these strains were used in the fermentation of goat milk, which is novel for facilitating investment in the industrialization of this milk and guaranteeing its organoleptic and nutritional quality. This focus is critical, especially considering that factors such as milking frequency have been shown to significantly affect the nutritional and microbiological quality of cow milk in Algeria [1]. However, the precise dynamics of these cultures in goat milk, particularly the distinct biochemical characteristics of Algerian goat milk, are not fully understood. These biochemical features can directly affect fermentation kinetics and finished product characteristics, such as a higher concentration of short and medium-chain fatty acids (SCFA and MCFA, respectively). In addition, studies on conventional dairy production systems have demonstrated the importance of endogenous strains in regulating the unique qualities of local products [2]. These microorganisms contribute to gel formation, improved viscosity, acidification and modification of sensory qualities such as flavor and texture [3].

The use of probiotics to improve metabolic health is a relatively novel indication for probiotic therapy. The potential for probiotics to modulate inflammatory status is particularly interesting, as demonstrated in cell cultures [4]. These two strains show a valuable synergistic relationship, stimulating the other strain growth through the exchange of metabolites in a process known as proto-cooperation [5]. Specifically, *L. bulgaricus* expresses extracellular protease to use milk proteins, providing an abundant nitrogen source for itself and *S. thermophilus* is described to supply *L. bulgaricus* with certain acids (e.g. formic and folic acids) and carbon dioxide [6]. Additionally, *S. thermophilus* synthesizes several amino acids and expresses a cell envelope proteins [5]. The synergistic effect between *L. bulgaricus* and *S. thermophilus* accelerates milk fermentation and enhance microbial growth. Therefore, the primary objective was to investigate the milk fermentation process with particular emphasis on the interactions between *S. thermophilus* and *L. bulgaricus*. A key question is if each bacterium significantly contributes to physicochemical changes in milk, specifically acidification, viscosity and syneresis, critical factors in product innovation. While bacteria such as *Streptococcus*, *Lactobacillus* and *Bifidobacterium* Sp. have extensively been studied in various milk types, research on their interactions in goat milk is limited despite the unique characteristics of goat milks [7].

Fermented dairy products, particularly those derived from goat milks, are significant components of traditional and modern diets, offering valuable nutritional and probiotic benefits. The fermentation process, driven by bacterial cultures such as *S. thermophilus* and *L. bulgaricus*, involves a complex interaction of biological and physicochemical

factors. Understanding the dynamics of these bacterial populations and their interactions with their surrounding environment is critical for ensuring consistent product quality and safety. Existing research has identified key physicochemical parameters affecting bacterial growth during fermentation, including temperature, pH and substrate availability. However, a significant gap is seen in quantifying these interactions. While qualitative observations are abundant, lack of robust predictive models that can accurately describe the relationships between bacterial growth kinetics and these parameters, particularly within the unique matrix of Algerian goat milks, is addressed. This limitation delays the precise control and optimization of industrial fermentation processes. Furthermore, the specific population kinetics of *S. thermophilus* and *L. bulgaricus* in Algerian goat milks, regarding its unique composition affected by local breeds and environmental factors, requires in-depth investigation. The current knowledge may not fully capture dynamics of these bacteria in this specific context. The lack of understanding can lead to variability in product qualities, inconsistent fermentation outcomes and safety concerns.

This study addressed gaps in understanding of lactic acid fermentation in Algerian goat milks. It was beyond general explanations by providing a statistically rigorous analysis that quantified specific roles of *S. thermophilus* and *L. bulgaricus*. Unlike previous studies that often treated starter cultures as single entities, the present study highlighted the individual contributions of each bacterium to acidification, viscosity and syneresis. Mixed-effects models were used to establish strong correlations between bacterial growth and physicochemical variations. This study on Algerian goat milk challenged the conventional emphasis on cow milk in fermentation research. This alternative substrate offers potential advantages for consumers pursuing less allergenic dairies. The experimental approach reveals the development of distinct microbial strains and flavors associated with *L. bulgaricus*. Advanced statistical modelling combined with detailed bioanalysis and industrial uses represents a significant advancement in the current knowledge. Practical recommendations emerging from the results can optimize local transformation processes and promote sustainable goat farming, effectively bridging the gap between fundamental research and real-world effects.

## 2. Materials and Methods

The raw milk used in the experiment was purchased from local goats in a dairy farm in Tissemsilt, Algeria. This was stored at 4 °C before fermentation. The *L. bulgaricus* ATCC 11842 and *S. thermophilus* ATCC 19258 were purchased from a specialized food biotechnology supplier (Fly Chemicals). To avoid contamination, these cultures were



used to inoculate the milk at a concentration of  $10^7$  CFU.ml<sup>-1</sup> under aseptic conditions in the laboratory. Raw milk was inoculated with *L. bulgaricus* and *S. thermophilus* at a concentration of  $10^7$  CFU.ml<sup>-1</sup>, respectively, and then incubated at 42 °C for 8 h. Samples were collected every 2 h to assess pH, acidity, viscosity and syneresis.

### 2.1. Physicochemical analyses

The pH was assessed regularly using calibrated pH meter (Hanna HI-2211, Romania) based on AFNOR standards. The acidity was assessed using titration with 0.1 N NaOH and ISO 6091:2010 method [8]. Acidity was expressed in Dornic degrees (°D), where 1 °D included 0.1 g of lactic acid in 100 ml of milk. The viscosity was assessed using viscometer (Fungilab, Alpha series, Spain). Syneresis was assessed using centrifuge (Sigma D-37520, 3-18KS, Germany). The syneresis rate was the percentage of whey separation with the total volume of the product and centrifuged at 1,125 and 3,125 g.

### 2.2. Microbiological Analyses

The microbiological analyses aimed to quantify the number of lactic acid bacteria (LAB) and pathogens. For the bacterial enumeration, samples were inoculated onto MRS (Man, Rogosa, Sharpe) agar and incubated at 37 °C for 48 h. Results were expressed as colony-forming units (CFU.ml<sup>-1</sup>).

### 2.3. Identification of Pathogenic Bacteria

Potential pathogens, including *Escherichia coli* and *Listeria monocytogenes*, were detected using ISO [9] method for *E. coli* and ISO 11290-1, 2017[10] method for *L. monocytogenes*.

### 2.4. Data Analysis

Data analysis was carried out using JMP Pro 17 software. Bacterial concentrations were log<sub>10</sub>-transformed to normalize the distributions and stabilize the variance. The initial concentrations of *S. thermophilus* and *L. bulgaricus* ranged  $0.56\text{--}682 \times 10^7$  and  $0.19\text{--}594 \times 10^7$  CFU.ml<sup>-1</sup>, respectively. Linear mixed-effects models were used to analyze the dependent variables (pH, titratable acidity, viscosity and syneresis), considering fixed effects of the transformed concentrations and the random effects of time measurements. Although indications suggested nonlinear relationships, a linear model was chosen to avoid overfitting the data. The model validity was verified using several diagnostic procedures such as tests for the normality of residuals and assessment of homoscedasticity. The AIC and BIC criteria were used to assess model fit and R<sup>2</sup> statistics were used to assess the model explanatory power. These analyses verified the robustness of the model in assessing effects of lactic acid fermentation on the physicochemical characteristics of raw milks.

## 3. Results and Discussion

During the early fermentation phase (2h), pH decreased to  $5.61 \pm 0.05$ , whereas the titratable acidity increased significantly to  $40.25 \text{ }^\circ\text{D} \pm 1.58$ . The first measurable viscosity readings were recorded as  $171.63 \text{ mPa}\cdot\text{s} \pm 49.32$  ( $120\text{--}270 \text{ mPa}\cdot\text{s}$ ). Bacterial populations showed early growth, with *S. thermophilus* increasing to  $6.41 \pm 8.33 \times 10^7$  CFU.ml<sup>-1</sup> and *L. bulgaricus* reaching  $1.73 \pm 0.05 \times 10^7$  CFU.ml<sup>-1</sup> (Table 1). By mid-fermentation (4 h), significant changes were observed in all parameters (Table 1). The pH decreased to  $4.77 \pm 0.10$ , accompanied by increased titratable acidity ( $66.75 \text{ }^\circ\text{D} \pm 1.75$ ). Initial syneresis was observed ( $4.78\% \pm 0.71$ ) and the viscosity increased substantially to  $2562.50 \text{ mPa}\cdot\text{s} \pm 1263.71$ . The *S. thermophilus* showed exponential growth, reaching  $386.50 \pm 166.36 \times 10^7$  CFU.ml<sup>-1</sup>, whereas *L. bulgaricus* increased to  $5.72 \pm 0.13 \times 10^7$  CFU.ml<sup>-1</sup>. Within 6 h (Table 1), fermentation progressed with the pH decreasing to  $4.50 \pm 0.02$  and the titratable acidity increasing to  $84.75 \text{ }^\circ\text{D} \pm 2.19$ . Syneresis increased to  $7.50\% \pm 0.51$  and the viscosity reached  $4876.25 \text{ mPa}\cdot\text{s} \pm 708.88$ . The *S. thermophilus* showed a slight decrease to  $361.38 \pm 85.41 \times 10^7$  CFU.ml<sup>-1</sup>, whereas *L. bulgaricus* showed continues growth, reaching  $51.58 \pm 6.04 \times 10^7$  CFU.ml<sup>-1</sup>. At the end of fermentation (8 h), the samples reached their lowest pH ( $4.12 \pm 0.20$ , ranging  $3.83\text{--}4.32$ ) with maximum syneresis ( $12.75\% \pm 0.88$ ) and viscosity ( $6425.00 \text{ mPa}\cdot\text{s} \pm 638.64$ ). The final bacterial counts showed that *S. thermophilus* and *L. bulgaricus* increased to  $519.00 \pm 115.29 \times 10^7$  and  $65.54 \pm 6.89 \times 10^7$  CFU.ml<sup>-1</sup>, respectively (Table 1).

### 3.1. Bacterial Population Dynamics

Critical shifts and variations were observed in the population patterns of *L. bulgaricus* and *S. thermophilus* over the fermentation time. Similar to the findings of Moghadam et al. *S. thermophiles* and *L. bulgaricus* experienced significant growth during fermentation [11]. This phase of growth is critical for acidification. Data indicated that the *L. bulgaricus* population reached  $1.73 \pm 0.05 \times 10^7$  CFU.ml<sup>-1</sup>, while *S. thermophilus* increased to  $6.41 \pm 8.33 \times 10^7$  over the first 2 h [12]. This advanced phase, in which *S. thermophiles* increased in quantity, boosted deacidification of the liquid media. The study highlighted the need of *S. thermophilus* to help acidification process while allowing the remaining environment appropriate for *L. bulgaricus* to thrive. The growth patterns of the two species differed at 4-h point. During this time, the population of *S. thermophilus* increased significantly, reaching  $386.50 \pm 166.36 \times 10^7$  CFU.ml<sup>-1</sup>.

In contrast, the population of *L. bulgaricus* increased, showing a modest increase to  $5.72 \pm 0.13 \times 10^7$  CFU.ml<sup>-1</sup>. Moreover, *S. thermophilus* showed a stable population at



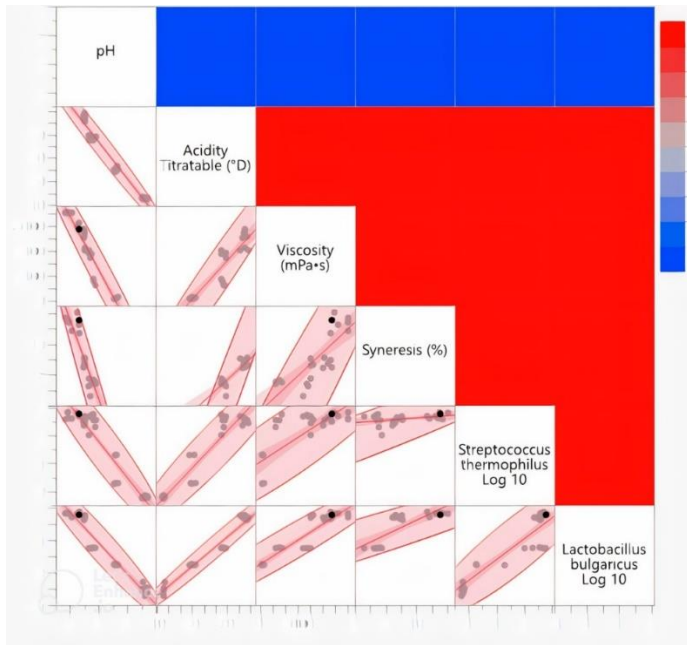
and after the 6-h point, while the *L. bulgaricus* count increased, achieving a maximum  $65.54 \pm 6.89 \times 10^7$  CFU.mL<sup>-1</sup> at 8-h time point. These findings were similar to those by Meng et al., who detected synergistic interspecific cooperation between the two bacterial species through omics approaches[13]. This verified that *S. thermophiles* initiated the fermentation process, whereas *L. bulgaricus* continued to thrive during the later stages of fermentation. Previously, Ahsan et al. (2022) have shown a broader range of food matrices; to which, these bacteria could be adapted. They reported that *S. thermophilus* and *L. bulgaricus* could be used in the fermentation of soy milks [14]. As stated, this study illustrated the changes in population sizes at various stages of fermentation and those at the final stage. The authors previously verified the established claims that the two strains included a synergistic relationship; in which, *S. thermophilus* was the first to initiate acid production for improving conditions of *L. bulgaricus* growth. Supporting evidence has further been clarified at the molecular level.

Correlation analysis revealed intricate relationships between bacterial growth and the physicochemical parameters during fermentation (Fig. 1). The pH demonstrated significant negative correlations with all assessed variables ( $p < 0.01$ ), showing particularly strong negative relationships with titratable acidity ( $r = -0.982$ ) and *L. bulgaricus* growth ( $r = -0.958$ ). This underscored the fundamental role of pH in modulating the fermentation environment. The *L. bulgaricus* showed significantly stronger correlations with physicochemical parameters than those *S. thermophiles* did, suggesting its predominant effects on the product characteristics. Specifically, *L. bulgaricus* showed strong positive correlations with titratable acidity ( $r = 0.979$ ) and viscosity ( $r = 0.929$ ) while maintaining a strong positive relationship with *S. thermophiles* growth ( $r = 0.898$ ). In contrast, *S. thermophilus* showed a moderately strong correlation with viscosity ( $r = 0.773$ ) and a significantly weaker correlation with syneresis ( $r = 0.405$ ), indicating its secondary role in texture development.

**Table 1.** Descriptive statistics of the physicochemical characteristics and bacterial counts during 8 h of fermentation at various time points

Time	Parameter	N	Range	Min	Max	Mean	Standard deviation
0 h	pH	8	0.21	6.52	6.73	6.62	0.07
	Titrable acidity (mPa*s)	8	2	15	17	15.88	0.64
	<i>S. thermophiles</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	0.15	0.56	0.71	0.62	0.04
	<i>L. bulgaricus</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	0.32	0.19	0.51	0.30	0.09
2 h	pH	8	0.14	5.54	5.68	5.60	0.04
	Titrable acidity °D	8	5	38	43	40.25	1.58
	Viscosity (mPa*s )	8	150	120	270	171.63	49.3
	<i>S. thermophiles</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	18.15	1.85	20.00	6.40	8.32
4 h	<i>L. bulgaricus</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	0.15	1.67	1.82	1.73	0.04
	pH	8	0.25	4.65	4.90	4.77	0.09
	Titrable acidity °D	8	4	65	69	66.75	1.75
	Syneresis (%)	8	2.30	3.20	5.50	4.77	0.70
	Viscosity	8	2800	1200	4000	2562.50	1263.71
	<i>S. thermophiles</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	472.00	103.00	575.00	386.50	166.36
	<i>L. bulgaricus</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	0.39	5.61	6.00	5.71	0.12
	pH	8	0.05	4.47	4.52	4.49	0.018
6 h	Titrable acidity °D	8	7	81	88	84.75	2.188
	Syneresis (%)	8	1.32	6.88	8.20	7.49	0.511
	Viscosity	8	1700	4000	5700	4876.25	708.88
	<i>S. thermophiles</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	258.00	230.00	488.00	361.37	85.40
8 h	<i>L. bulgaricus</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	17.00	42.40	59.40	51.57	6.04
	pH	8	0.49	3.83	4.32	4.11	0.20
	Syneresis (%)	8	2.70	11.40	14.10	12.75	0.87
	Viscosity	8	1400	5600	7000	6425.00	63.63
	<i>S. thermophiles</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	301.00	381.00	682.00	519.00	115.28
	<i>L. bulgaricus</i> (10 <sup>7</sup> CFU.mL <sup>-1</sup> )	8	23.60	51.50	75.10	65.53	6.88





**Figure 1.** Correlation matrix of the physicochemical characteristics and bacterial growth parameters in fermented dairy products

The textural characteristics demonstrated distinct correlation patterns, with viscosity showing stronger associations with *L. bulgaricus* ( $r = 0.929$ ) than *S. thermophilus* ( $r = 0.773$ ). Syneresis demonstrated a similar issue, correlating stronger with *L. bulgaricus* ( $r = 0.798$ ) than *S. thermophilus* ( $r = 0.405$ ). These relationships suggested that *L. bulgaricus* played a further important role in texture development, possibly through enhanced exopolysaccharide production and/or proteolytic activity. The strong correlation between bacterial growth and physicochemical parameters was further validated by non-parametric analyses (Kendall tau-B and Spearman rho), verifying the robustness of these relationships regardless of the statistical approach used. These findings correlated with those by Nadirova and Sinyavskiy (2023), who reported that *L. bulgaricus* could grow despite decreased pH levels, owing to its intrinsic adaptability to acidic conditions [15].

Data collected at 6 and 8-h intervals further illustrated the growth patterns of these bacteria. At 6-h point, population of *S. thermophilus* slightly stabilized, measuring  $361.38 \pm 85.41 \times 10^7$  CFU.ml<sup>-1</sup>. In contrast, *L. bulgaricus* demonstrated a further vigorous growth rate, reaching a population of  $51.58 \pm 6.04 \times 10^7$  CFU.ml<sup>-1</sup>. At 8-h point, *S. thermophilus* population showed slight stability, with an increased count of  $519.00 \pm 115.29 \times 10^7$  CFU.ml<sup>-1</sup>, while *L. bulgaricus* demonstrated strong growth, achieving a population of  $65.54 \pm 6.89 \times 10^7$  CFU.ml<sup>-1</sup>. The present results have been verified in other products such as yoghurt, where *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* multiplied significantly during fermentation [16].

These findings addressed those of Siewuerts et al. (2010), whose transcriptome analysis revealed that mixed-culture growth involved upregulation of biosynthesis pathways for nucleotides and amino acids that were vital for the growth of the two bacteria [17]. Furthermore, Nadirova and Sinyavskiy (2023) highlighted the complementary roles of these two bacterial species in the fermentation process. The *S. thermophilus* was essential in initiating acidification, whereas *L. bulgaricus* proliferated in acidic environments created by the other strain. This complex interaction not only enhanced fermentation efficiency but also contributed to development of the desired flavor and texture profiles in fermented dairy products. In contrast to the findings of Picon et al. (2016), the present findings demonstrated that acidification occurred at a slower further gradual rate [18]. This was primarily attributed to the diverse range of naturally occurring LAB strains that affected lactic acid production efficiency. Conversely, optimized interactions between *S. thermophilus* and *L. bulgaricus* strains led to further rapid acidification, increased lactic acid production and consistent acidification profile.

### 3.2. PH

The linear mixed-effects model analysis of bacterial fermentation dynamics revealed a significant effect of bacterial growth on pH regulation during fermentation (Fig. 2). This model, showing excellent fit characteristics (AICc = -6.299, BIC = -0.686), verified a baseline pH of  $6.087 \pm 0.058$  (SE,  $p < 0.0001$ ) in goat milks. A significant rapid acidification of milk with pH decreasing from  $6.62 \pm 0.08$  to  $4.12 \pm 0.20$  over 8 h. This pH decrease, critical for flavor development and microbial stability, was primarily driven by the action of the two bacterial species with *L. bulgaricus* showing a stronger effect ( $-0.589 \pm 0.083$  pH units per log<sub>10</sub> CFU.ml<sup>-1</sup>,  $p < 0.0001$ ), compared to *S. thermophilus* ( $-0.304 \pm 0.060$  pH units per log<sub>10</sub> CFU.ml<sup>-1</sup>,  $p < 0.0001$ ). This approximately two-fold difference in acidification capacity was statistically supported by highly significant F-ratios for *S. thermophilus* ( $F_{1,37} = 25.66$ ,  $p < 0.0001$ ) and *L. bulgaricus* ( $F_{1,37} = 49.74$ ,  $p < 0.0001$ ), demonstrating their differential contributions to fermentation. The model random effects structure indicated negligible temporal variances, suggesting consistent acidification patterns over time. The residual variance was small ( $0.043 \pm 0.010$ ), supporting the model precision and reliability. A significant negative correlation ( $r = -0.898$ ) between the effects of *S. thermophilus* and *L. bulgaricus* underscored the complex interactive dynamics between these species rather than a simple additive effect on pH decrease. These findings were similar to those of previous studies, which identified *L. bulgaricus* as a primary driver of acidification in milk fermentation systems [7,19,20]. Similarly, another study showed that *S. thermophilus* promoted rapid bacterial growth and enhances fermentation efficiency, creating a favourable environment



for *L. bulgaricus*. This synergistic relationship led to improved metabolite profiles, with *S. thermophilus* facilitating the production of flavor compounds during milk fermentation at optimal temperatures [19]. A recent study using omics analyses has revealed the molecular mechanisms driving bacterial synergy, driving decreases in pH [12]. This intricate relationship between bacterial interactions and pH dynamics was further supported by Wu et al., whose study linked bacterial population ratios and fermentation times to the sensory profile, including acidity [19]. This revealed the broader implications of these pH changes, as they resulted from bacterial metabolic activity and affected the final quality and sensory characteristics of the product. Thus, bacterial populations strongly affected pH dynamics, particularly *L. bulgaricus*, which showed a greater acidifying capacity, verifying its key roles in the acidification process.

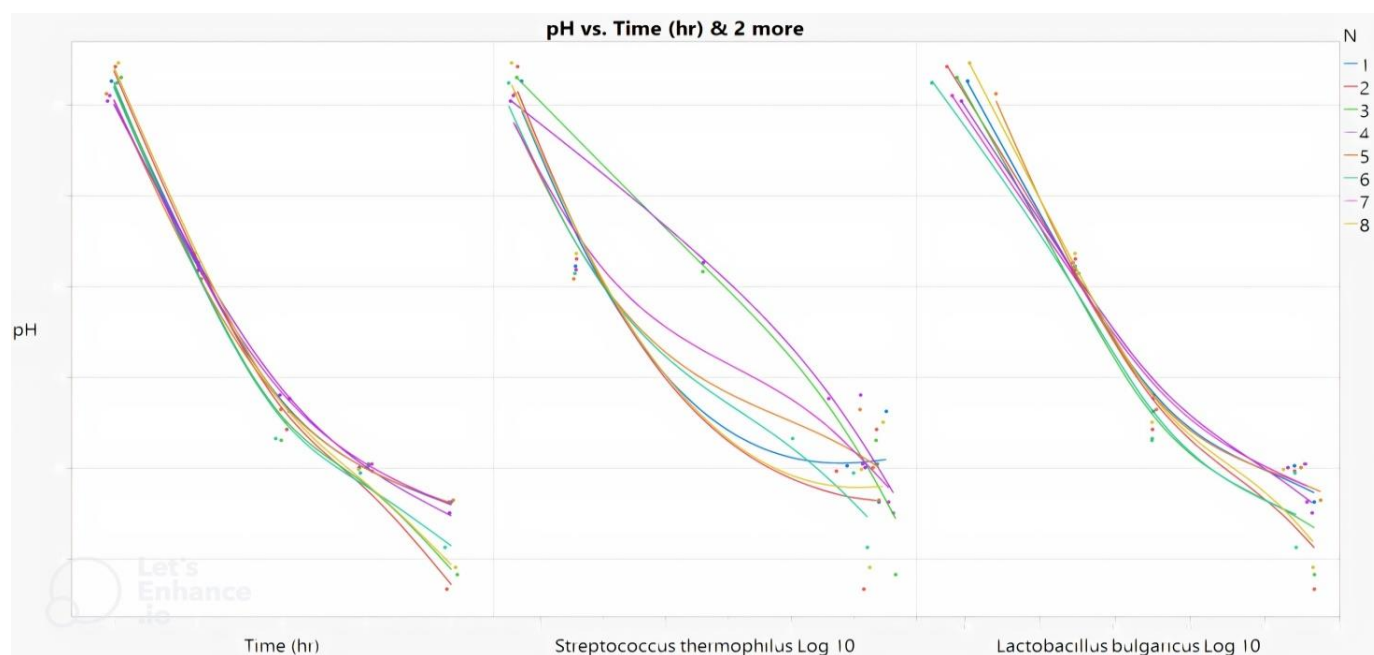
### 3.3. Titratable Acidity

A linear mixed-effects model analyzed the relationship between bacterial populations and titratable acidity (Fig. 3). The model demonstrated satisfactory fit indices (-2 residual log likelihood = 159.71, AICc = 172.84, BIC = 177.22). Fixed-effects analysis demonstrated highly significant positive relationships between the titratable acidity and logarithmic populations of the bacterial species. The *L. bulgaricus* showed a stronger effect ( $\beta = 21.44 \pm 1.47$  D.log<sub>10</sub> CFU<sup>-1</sup>,  $t(29) = 14.55$ ,  $p < 0.0001$ ), compared to *S. thermophiles* ( $\beta = 7.43 \pm 0.97$  °D.log<sub>10</sub> CFU<sup>-1</sup>,  $t(29) = 7.67$ ,  $p < 0.0001$ ). This approximately three-fold difference in

effect size indicated that *L. bulgaricus* was the primary driver of acid production in the fermentation system. The model intercept of  $30.20 \pm 0.92$  °D ( $t(29) = 32.85$ ,  $p < 0.0001$ ) represented the baseline acidity when controlling for bacterial populations.

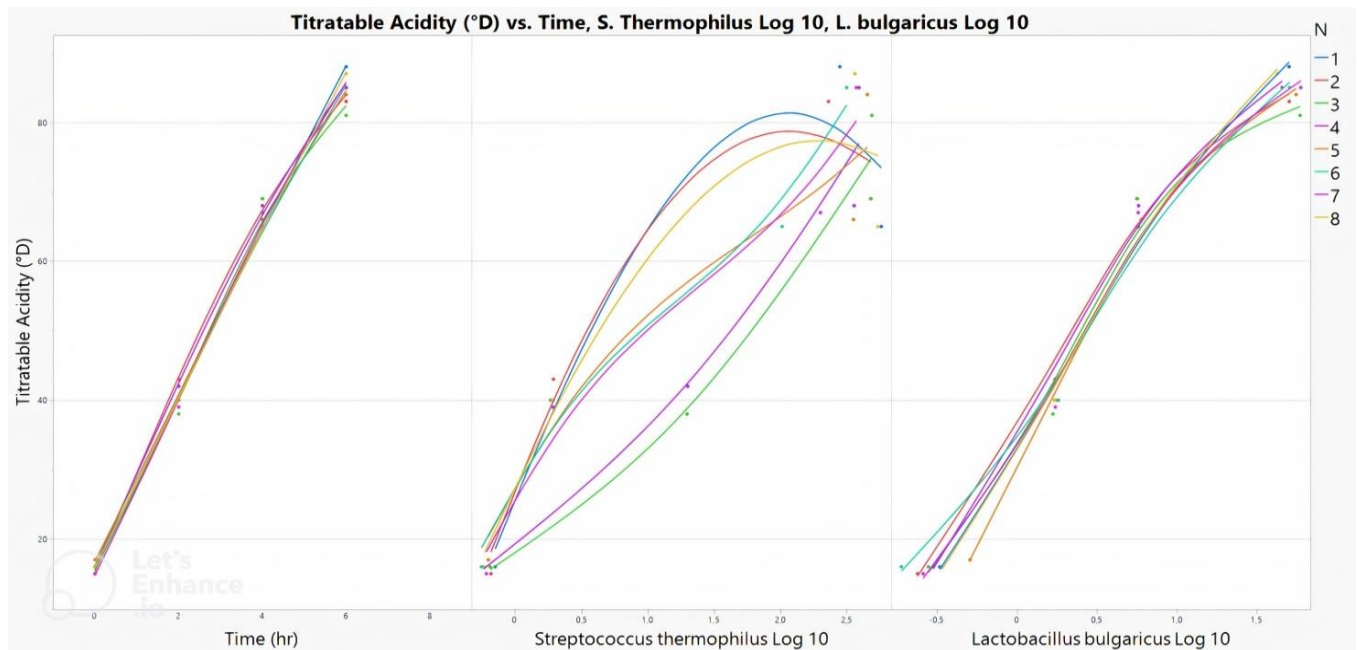
The significance of these relationships was further supported by fixed-effects tests, which showed strong evidence of the effect of *S. thermophilus* [ $F(1,29) = 58.83$ ,  $p < 0.0001$ ] and *L. bulgaricus* [ $F(1,29) = 211.58$ ,  $p < 0.0001$ ]. The substantially larger F-statistic for *L. bulgaricus* verified its dominant role in acid production, with direct implications for starter culture formulations in fermented dairy products. The random effects structure analysis revealed that the time component was confounded with residual variance, resulting in a residual variance estimate of  $10.60 \pm 2.78$  °D (95% CI: 6.72–19.16 °D). This confounding factor suggested that bacterial population dynamics, rather than time-dependent factors, were the primary determinants of acid development in this system, which is an important consideration for process control in industrial settings.

Model diagnostics supported the validity of these statistical assumptions. The actual by predicted plots demonstrated a strong linear relationship between the observed and predicted values across the full range of measurements (10–90 °D). Residual analysis revealed a generally symmetric distribution near zero (-6 to +6 °D), with the residual quantile plot indicating approximate normality. The model strong predictive capability suggested that it could be a reliable tool for controlling acidification processes in fermented dairy production.



**Figure 2.** The pH evolution as a function of time and bacterial population (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) during fermentation





**Figure 3.** Titratable acidity evolution as a function of time and bacterial population (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) during fermentation

While goat milk (19.05 °D) and cow milk (17 °D) showed distinct initial acidity levels, the titratable acidity significantly varied during the fermentation process [20]. A significant increase was observed, rising from 15.88 °D  $\pm$ 0.64 to 84.75 °D  $\pm$ 2.19 within 8 h. This increase strongly correlated with bacterial growth, particularly that of *L. bulgaricus*, whose metabolic activity significantly contributed to milk acidification through lactic acid production. These verified the findings that the proliferation of *L. bulgaricus* directly affects titratable acidity and that lactic acid production is essential for controlling final characteristics of the fermented product [21,22]. Andrew et al. emphasized that titratable acidity is a relevant indicator of the progression of fermentation process in *L. bulgaricus* based products. The results of Abbasalizadeh et al. indicated that the maximum lactic acid production in the Media12 media reached 35.01 g.l<sup>-1</sup>) [23]. This result verified the current results as the titratable acidity reached 84.75 °D  $\pm$ 2.19 after 6 h of fermentation. These results demonstrated the importance of acidification in fermentation processes.

Further supporting the present results, Sonnier et al. showed the synergistic actions of *S. thermophilus* and *L. bulgaricus* that promoted rapid acidification of the media [24]. More specifically, Qiu et al. provided mechanistic insights into the current findings by revealing the metabolic pathways and metabolites involved in acid production, particularly the role of lactic acid production by *L. bulgaricus*. According to Wu et al., the link between bacterial ratios and acidity strengthened the connection to product sensory attributes and verified that specific bacterial ratios affected acidity and sensory profile. The present study

highlighted the strong acidifying capacity of *L. bulgaricus*, which was twice that of *S. thermophilus*. Although the study on *L. plantarum* SU-KC1a did not directly assess acid production in fermentation, it demonstrated robust tolerance to pH variations [21]. This suggested that *L. plantarum* SU-KC1a might contribute to acidification during fermentation, although not as strongly as *L. bulgaricus*. These findings underscored the role of *L. bulgaricus* as the primary contributor to acidity and the importance of understanding dynamics of bacterial populations for process control, while reinforcing these conclusions by contextualizing them within the current understanding of these mechanisms and their effects on organoleptic characteristics of the final product.

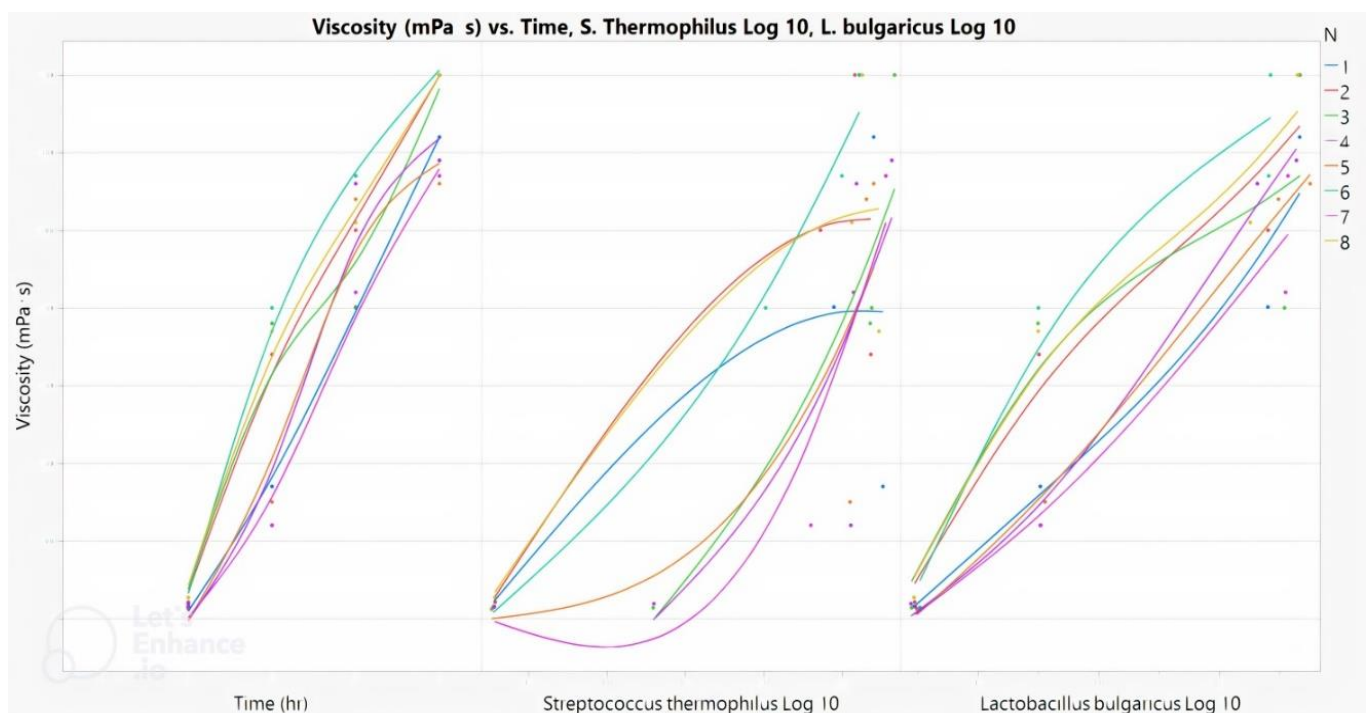
### 3.4. Viscosity

Analysis of the fixed effects revealed a significant difference in the effect of the two bacterial species on viscosity development (Fig.4), with *L. bulgaricus* demonstrating dominant effects and coefficient of 3106.28  $\pm$ 397.51 mPa·s per log unit increase in cell density ( $p < 0.0001$ ), approximately 8.84 times greater than the effect observed for *S. thermophilus* (351.37  $\pm$ 281.73 mPa·s per log unit,  $p = 0.2223$ ). This difference demonstrated that *L. bulgaricus* largely explained viscosity of the fermented media. The random effects structure revealed negligible temporal variance [ $t$  (h) = 0], indicating that viscosity changes were not significantly affected by the duration of the experiment. The residual variance was significant (881,027.22), indicating that variations in viscosity were predominantly explained by the bacterial concentrations



rather than temporal patterns. Model diagnostics supported these findings; the actual-by-predicted plot demonstrated a generally linear relationship with increased variability at higher viscosity levels (0–7000 mPa·s). The model strong statistical characteristics (F-ratio for *L. bulgaricus*= 61.06,  $p < 0.0001$ ) provided robust evidence for the differential effects of these bacterial species on viscosity development. However, several considerations guaranteed further attentions. First, the negative intercept (-731.81 mPa·s) represented a theoretical value outside biologically relevant conditions and should be interpreted cautiously. Furthermore, although the model demonstrated heteroscedasticity (unequal variance of the residuals) at higher viscosity values, this did not invalidate the primary findings regarding the relative effect of each species. Of the changes in viscosity, the most significant was observed after 8 h when the viscosity reached 6425.00 mPa·s  $\pm 638.64$ , which was more than two times higher than the initial value. These results were similar to those of Qiu et al., who identified *L. bulgaricus* with its primary activity of exopolysaccharides (EPS) production as the major reason for the improvement in viscosity. In other words, data were similar to those that addressed *L. bulgaricus* as an essential component in boosting the product viscosity, predominantly through the

production of EPS. A highly positive association ( $r = 0.929$ ) was detected between the abundance of *L. bulgaricus* and the magnitude of viscosity, which provided further evidence for this microbial leading role in improving the rheological characteristics of the final product. The importance of this finding is that viscosity is a critical characteristic that dictated the degree of product acceptance; thus, texture and mouthfeel were directly affected by viscosity. The present results were similar to those of Nadirova and Sinyavskiy, who underlined that the increase in viscosity was necessary not only for improving the texture and stability of fermented products during storage but also for providing favorable conditions for the growth of relevant bacteria. This is a further step and a part of bacteriophage resistance of these isolates. Moreover, a project by Afzal et al. on the specific structure of the EPS produced by *L. bulgaricus* and how researchers verified that the produced EPS could make a difference in the viscosity levels of the final product of fermented milk were significantly verified [25]. Through the interactions of *L. bulgaricus* EPS with indigenous strains or natural ingredients, viscosity and texture could be optimized; thereby, extending the shelf life of quality products.



**Figure 4.** Viscosity evolution as a function of time and bacterial population (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) during fermentation

### 3.5. Syneresis

Mixed-effects model analysis revealed significant relationships between the bacterial strain concentrations and

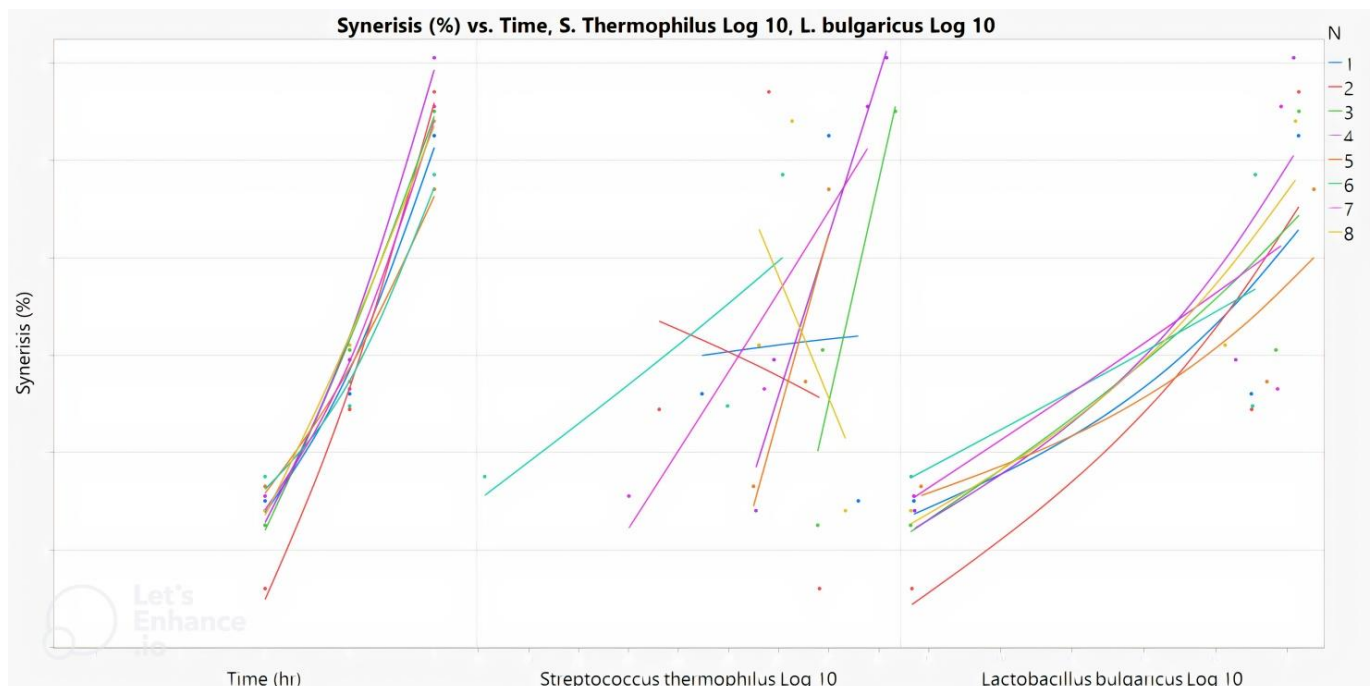


syneresis in fermented dairy products (Fig. 5). The *L. bulgaricus* demonstrated a strong positive association with syneresis ( $\beta = 5.26 \pm 0.92$ ,  $p < 0.0001$ ), indicating that higher concentrations of this strain significantly increased water expulsion from the gel matrix. While *S. thermophilus* showed a positive development ( $\beta = 3.67 \pm 2.49$ ,  $p = 0.1561$ ), its larger standard error (SE) and non-significant  $p$ -value suggested significant variability in its effects on syneresis. The intercept of the model ( $\beta = -8.68 \pm 6.23$ ,  $p = 0.1786$ ) was not significantly different from zero, suggesting minimal baseline syneresis in the absence of bacterial activity. Model diagnostics supported the validity of the present findings, with residual analyses showing appropriate distribution patterns and no substantial violations of the model assumptions. The significantly positive coefficient for *L. bulgaricus* was strong across multiple diagnostic assessments, reinforcing its critical role in controlling syneresis. However, significant residual variances and wide confidence intervals for some parameters suggested that additional factors such as protein concentration, pH dynamics and temperature fluctuations might contribute to syneresis variation in ways that were not captured by this model.

The fermentation phase was highlighted by increases in syneresis of  $4.78\% \pm 0.71$  and a significant separation of lactoserum from the gels with syneresis increasing to as high as  $12.75\% \pm 0.88$ . The correlation coefficient between *L. bulgaricus* and syneresis ( $r = 0.798$ ) was confusing with that presented by Nadirova, who argued that *L. bulgaricus* might

improve gel syneresis and increase gel strength. This is an important question. Although (EPS) are known to originate from *L. bulgaricus* and alter gel structures favorably. Two studies showed that specific EPS structures antagonized syneresis, highlighting that *L. bulgaricus* produced EPS that improved water retention [26]. One interpretation of the present results is that *L. bulgaricus* under specific fermentation conditions changes the balance of syneresis positively. Certain conditions of the present experiment and a certain strain of *L. bulgaricus* used in this experiment may need further investigations. However, it is possible that high concentrations of *L. bulgaricus* and its byproducts facilitated whey removal and gel retention was compromised. Qiu et al. reported that while the EPS synthesized by *L. bulgaricus* helped lessen syneresis, the effect was incomplete [12].

The large residual variances and broad ranges of confidence intervals in the present model reveal that various other components such as protein concentrations, pH shifts and temperature changes, which were not seen in the present model, might include effects on syneresis. Overall, the study provided evidence of a further complex relationship between *L. bulgaricus*, EPS formation and syneresis and results indicated needs of further integrated understanding to enhance the fermentation process. Additional studies are needed to investigate which specific strains lead to decreased syneresis under what conditions, modelling and Optimization.



**Figure 5.** Syneresis evolution as a function of time and bacterial population (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) during fermentation



During fermentation, the population dynamics of *S. thermophilus* and *L. bulgaricus* showed complex significant fluctuations (Table 2). At early stages, the two bacterial species showed exponential growth, aligning with the findings of [15], which highlighted the significant growth of *S. thermophilus* and *L. bulgaricus* during fermentation. This rapid growth is critical for acidification. The present data indicated that within the first 2 h of fermentation, the population of *S. thermophilus* increased to  $6.41 \pm 8.33 \times 10^7$  CFU.ml<sup>-1</sup>, while *L. bulgaricus* reached  $1.73 \pm 0.05 \times 10^7$  CFU.ml<sup>-1</sup>. This initial phase, highlighted by the swift growth of *S. thermophilus*, was vital for the starting of the acidification of liquid media, similar to other findings by Qiu et al. Their study emphasized the significant role of *S. thermophilus* in initiating acidification; thereby, creating a further favorable environment for the growth of *L. bulgaricus*. After 4 h, growth rates of the two species were shifted. At this point, population of *S. thermophilus* significantly increased to  $386.50 \pm 166.36 \times 10^7$  CFU.ml<sup>-1</sup>, while *L. bulgaricus* showed a further modest increase to  $5.72 \pm 0.13 \times 10^7$  CFU.ml<sup>-1</sup>. As fermentation continued, *S. thermophilus* included a relatively stable population after 6 h, whereas *L. bulgaricus* grew, reaching a final count of  $65.54 \pm 6.89 \times 10^7$  CFU.ml<sup>-1</sup> after 8 h. These findings were similar with those of Hansen et al., who verified synergistic growth of the two bacterial species through omics analyses [12]. This supported the idea that *S. thermophilus* initiated fermentation, whereas *L. bulgaricus* increased as fermentation advanced. Additionally, adaptability of these bacteria to various matrices was demonstrated by Nadeem et al., who showed that *S. thermophilus* and *L. bulgaricus*

could effectively ferment plant-based milk alternatives. The current study highlighted changes in population sizes during fermentation and illustrated how the two bacteria acted together, with *S. thermophilus* starting the acidification process and creating conditions that allowing *L. bulgaricus* to increase, similar to previous studies.

#### 4. Conclusion

This study provides a broader understanding of fermentation in goat milks. It underscores the complementarity of *S. thermophilus* and *L. bulgaricus*. Generally, *S. thermophilus* contributes to the rapid acidification process, leading to the proliferation of *L. bulgaricus*, which is a significant contributor to the texture, stability and organoleptic characteristics of the final product. The synergistic interaction between these two species results in desirable texture and decreased syneresis. This improves quality of the fermented dairy products by enhancing the unique characteristics of goat milks. These findings are invaluable for optimizing production processes in the agricultural food industry, where the local context is critical particularly in use of natural resources, as exemplified by the use of Algerian goat milks. These advancements have further contributed to increasing demands for functional and healthy foods. Future studies should investigate interactions between these bacterial strains and other environmental parameters or natural ingredients. The major aim is to optimize nutritional and sensory qualities of the final products, while minimizing challenges such as excessive syneresis.

**Table 2.** Estimation of fixed effects parameters for physicochemical characteristics as functions of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* populations

Response Variables	Term	Estimate	Std Error	DFDen	t Ratio	P-value	Confidence Limit (95%)	
							Lower	Upper
pH	Constant	6.08	0.05	37.0	105.07	<0.0001	5.96	6.20
	<i>S. thermophilus</i> Log 10	-0.30	0.06	37.0	-5.07	<0.0001	-0.42	-0.18
	<i>L. bulgaricus</i> Log 10	-0.58	0.08	37.0	-7.05	<0.0001	-0.75	-0.41
acidity	Constant	30.20	0.91	29.0	32.85	<0.0001	28.32	32.08
	<i>S. thermophilus</i> Log 10	7.42	0.96	29.0	7.67	<0.0001	5.44	9.40
	<i>L. bulgaricus</i> Log 10	21.43	1.47	29.0	14.55	<0.0001	18.42	24.45
Viscosity	Constant	-731.80	406.11	29.0	-1.80	0.08	-1562.4	98.78
	<i>S. thermophilus</i> Log 10	351.37	281.73	29.0	1.25	0.22	-224.83	927.58
	<i>L. bulgaricus</i> Log 10	3106.27	397.51	29.0	7.81	<0.0001	2293.27	3919.28
Syneresis	Constant	-8.67	6.23	21.0	-1.39	0.17	-21.64	4.28
	<i>S. thermophilus</i> Log 10	3.66	2.49	21.0	1.47	0.15	-1.51	8.84
	<i>L. bulgaricus</i> Log 10	5.25	0.92	21.0	5.69	<0.0001	3.33	7.18



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## سرعت تغییر جمعیت باکتریایی و ویژگی‌های فیزیکی‌شیمیایی در شیر تخمیر شده بز: نقش استرپتوکوک ترموفیلوس ATCC19258 و لاکتوباسیلوس بولگاریکوس ATCC11842

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- لاکتوباسیلوس بولگاریکوس
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### چکیده

**سابقه و هدف:** تخمیر شیر بز الجزایری، فرآیندی برای تولید فرآورده‌های شیری با ارزش، بر فعالیت هم‌افزایی استرپتوکوکوس ترموفیلوس و لاکتوباسیلوس بولگاریکوس متکی است. با این حال، یک شکاف علمی قابل توجه در مورد دینامیک دقیق این کشت آغازگر در زمینه منحصر به فرد شیر بز الجزایری دیده می‌شود. به‌ویژه، روابط پیچیده بین الگوهای رشد آن‌ها و تغییرات فیزیکی‌شیمیایی حاصل که ویژگی‌های بیوشیمیایی متمایز محصولات تخمیری را موجب می‌شود به خوبی شناخته نشده است. بنابراین، مطالعه حاضر با بررسی میزان مشارکت / استرپتوکوکوس ترموفیلوس و لاکتوباسیلوس بولگاریکوس در تخمیر شیر بز به این مشکل پرداخت.

**مواد و روش‌ها:** شیر بز با استفاده از کشت آغازگر استرپتوکوکوس ترموفیلوس و لاکتوباسیلوس بولگاریکوس (۸ ساعت) تخمیر شد. رشد باکتری و پارامترهای فیزیکی‌شیمیایی (pH، اسیدیته قابل تیتراژ، گرانروی<sup>۱</sup> و آب‌اندازی) ارزیابی شد. از مدل‌های اثرات مختلط برای بررسی آماری رابطه بین تغییرات فیزیکی‌شیمیایی و رشد باکتری استفاده شد.

**یافته‌ها و نتیجه‌گیری:** نتایج یک رابطه قوی بین لاکتوباسیلوس بولگاریکوس و کنترل اسیدی شدن، گرانروی، آب‌اندازی را نشان داد ( $r = 0.979$  برای اسیدیته قابل تیتراژ  $P < 0.0001$ ). استرپتوکوکوس ترموفیلوس به‌طور قابل توجهی در افزایش گرانروی ( $r = 0.773$ ،  $P < 0.01$ ) کمک کرد. این دو گونه به‌طور قابل توجهی pH را کاهش دادند و اثر اسیدی‌کنندگی لاکتوباسیلوس بولگاریکوس دو برابر بود. در پایان فرآیند تخمیر pH به  $4.12 \pm 0.20$  رسید، اسیدیته قابل تیتراژ به  $84.75 \pm 2.19^\circ\text{D}$  و گرانروی به  $638.64 \text{ mPa.s} \pm 64.25/0.00$  افزایش یافت. تعداد استرپتوکوکوس ترموفیلوس و لاکتوباسیلوس بولگاریکوس به ترتیب  $1.0^7 \text{ CFU.ml}^{-1} \pm 115.29$  و  $5.19/0.00$   $\times 10^7$   $\times 6/89 \pm 65/54$  بود. این مطالعه علاوه بر ارائه یک چارچوب آماری قوی برای کنترل فرآیند و تضمین کیفیت در تولید شیر تخمیری، نقش حیاتی لاکتوباسیلوس بولگاریکوس را در تنظیم کیفیت‌های ساختاری و حسی شیر بز تخمیری نشان داد. این یافته‌ها را می‌توان برای بهینه‌سازی فرآیندهای تخمیر شیر بز و سایر فرآورده‌های شیری با دستکاری راهبردی نسبت لاکتوباسیلوس بولگاریکوس به استرپتوکوکوس ترموفیلوس استفاده کرد. همبستگی قوی بین لاکتوباسیلوس بولگاریکوس و اسیدی شدن، گرانروی و آب‌اندازی ( $r = 0.979$  برای اسیدیته قابل تیتراژ،  $P < 0.0001$ ) یک هدف شفاف برای کنترل ویژگی‌های کلیدی محصول فراهم می‌کند.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.

<sup>۱</sup> viscosity

