SAÚDE E PESQUISA
e-ISSN 2176-9206

ORIGINAL ARTICLE

https://doi.org/10.17765/2176-9206.2026v19e13391

MICROBIOLOGICAL CHARACTERIZATION OF PINGO AND EVALUATION OF ANTAGONISTIC ACTIVITY AGAINST *LISTERIA MONOCYTOGENES*

CARACTERIZAÇÃO MICROBIOLÓGICA DO PINGO E AVALIAÇÃO DA ATIVIDADE ANTAGONISTA FRENTE A *LISTERIA MONOCYTOGENES*

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Received: 26 dec. 2024
Accepted: 09 jun. 2025

Editors-in-Chief: Dr. Leonardo Pestillo de Oliveira and Dr. Mateus Dias Antunes

ABSTRACT: Pingo is predominantly composed of lactic acid bacteria contributing to coagulation and maturation, inhibiting the presence of undesirable microrganisms, also influencing sensory attributes. The aim of this study was to characterize dripmy and evaluate its antagonistic activity against *Listeria monocytogenes*. At sixty days of maturation, all samples were in accordance with the current legislation for *Escherichia coli* and *Salmonella spp*. Coagulase-positive *Staphylococcus* only treatment with refrigerated raw milk (control) presented results above the legislation. Seventy-five percent of the samples were low moisture cheeses, with a significant difference ($p \leq 0.05$). The values of pH, acidity, ash, chlorides and total solids showed significant differences ($p \leq 0.05$) between 1 and 60 days of maturation. The addition of pingo allowed a decrease in the counts of spoilage and potentially pathogenic, but not the elimination of *Listeria monocytogenes*.
KEYWORDS: Bacteria. Maturation. Microorganisms. Artisanal product.

RESUMO: O pingo é composto predominantemente de bactérias ácido láticas contribuindo na coagulação e maturação, inibindo a presença de microrganismos indesejáveis, influenciando também, atributos sensoriais. O objetivo deste estudo foi caracterizar o pingo e avaliar sua atividade antagonista frente *a Listeria monocytogenes*. Aos sessenta dias de maturação, todas as amostras estavam de acordo com a legislação vigente para *Escherichia coli e Salmonella* spp. *Staphylococcus* coagulase positiva apenas o tratamento com leite cru refrigerado (controle), apresentou resultado acima da legislação. Setenta e cinco por cento das amostras se enquadraram em queijos de baixa umidade apresentando diferença significativa ($p \leq 0{,}05$). Os valores de pH, acidez, cinzas, cloretos e sólidos totais apresentaram diferenças significativas ($p \leq 0{,}05$) entre o 1 e 60 dias de maturação. A adição do pingo possibilitou decréscimo nas contagens de deterioradores e potencialmente patogênicos, mas não a eliminação de *Listeria monocytogenes*.
PALAVRAS-CHAVE: Bactérias. Maturação. Microrganismos. Produto artesanal.

INTRODUCTION

Artisanal cheese made from raw milk must undergo maturation to reduce spoilage and pathogenic microorganisms, such as *Listeria monocytogenes* (LM), thereby ensuring the production of safe, high-quality food. Listeriosis is a rare but serious disease that presents a range of symptoms and may be either non-invasive or invasive, the latter occurring when the infection spreads beyond the intestines. Another significant concern is the contamination of ready-to-eat refrigerated foods with *Listeria*, as the pathogen can multiply even at refrigeration temperatures[1].

Maturation involves a decrease in pH and moisture content, an increase in salt concentration, and contributes to the development of beneficial microorganisms, such as lactic acid bacteria (LAB), which are responsible for key sensory attributes of cheese, including flavor and texture. These bacterial groups have been extensively studied as biocontrol agents in food globally, and researchers have demonstrated that autoxin and bacteriocin production by LAB exhibits antagonistic effects against pathogens present in cheese[2].

Pingo is mostly composed of LAB from the genera *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Lactococcus*. These bacteria positively influence the fermentation process in artisanal cheese production, facilitate coagulation, and inhibit undesirable microorganisms, including spoilage organisms and pathogens, thereby minimizing food losses[3,4]. In this context, this study aimed to incorporate pingo into cheese production to evaluate the antagonistic activity of LAB against LM.

MATERIAL AND METHODS

PINGO AND CHEESE SAMPLE ACQUIREMENT

Five cheese samples (500 g each) were produced from raw milk. The milk was initially heated to 37 °C, and a coagulant (8 mL/10 L milk) was added. After resting for 45 min, the curd was cut and rested for an additional 25 min, then heated to 40 °C. The curd was then transferred to polyethylene molds lined with fabric and salted at a proportion of 2% (10 g/500 g of mass). After 12 h, a second salting was performed. Samples were collected 24 h later and frozen in sterile flasks.

To produce the cheese, 12 L of milk were used to obtain hrree replicate samples of 200 g each, resulting in six treatments: raw milk, raw milk/LM, raw milk/LM/pingo, pasteurized milk, pasteurized milk/LM, and pasteurized milk/LM/pingo.

The milk was divided into three stainless steel containers, each containing 4 L. For treatments with raw milk, the milk was heated to 37 °C, while for pasteurized milk treatments, it was heated to 75°C. Subsequently, coagulant (8 mL/10 L), pingo (25 mL/10 L), and the LM inoculum (2×10^8 CFU/mL) were added as appropriate for each treatment.

LISTERIA MONOCYTOGENES PREPARATION AND INOCULATION

The LM standard strain of LM (ATCC 7644) was reactivated in brain heart infusion supplemented with 0.6% yeast extract and inoculated onto plate count Agar, incubated at 37 °C for 24 h. Colonies were transferred to tubes containing 9 mL of 0.9% sodium chloride solution to achieve a turbidity of 0.5 (10^8 CFU/mL) on the McFarland scale. To assess the reduction in LM growth, 100 μL of this suspension was inoculated at random points in the cheese mass.

MATURATION PROCESS

The samples were matured for 60 days in a glass maturation cabinet at 20 °C and 60% humidity. All analyses were performed in triplicate: pingo was analyzed at 24 h, and cheese samples were analyzed at four time points (T0, T1, T2, and T3, corresponding to 1, 20, 40, and 60 days of production, respectively).

MICROBIOLOGICAL ANALYSIS

A 25 g/mL portion of each sample was transferred to tubes with diluent, followed by serial dilutions in 0.1% peptone water. Enumeration of coagulase-positive staphylococci, LAB, and mesophilic microorganisms was carried out as outlined by the American Public Health Association[5] *Salmonella* spp. detection according to the Food and Drug Administration[6], *Escherichia coli* (EC) detection according to the American Public Health Association[7], and LM detection according to ISO 11290-1:1996 Amendment 1:2004[7].

PHYSICOCHEMICAL ANALYSIS

The method described by the Association of Official Analytical Chemistry[8] was employed. Moisture content was determined by weighing 5 g of sample, drying in an oven at 105 °C, and reweighing until constant weight. Ash content was determined by weighing 5 g of sample, carbonizing over an electric flame, and incinerating in a muffle furnace at 550 °C until constant weight. For pH analysis, 10 g of the sample was mixed with 100 mL of water and measured directly using a pH meter. Total chloride content was measured using the ash content resulting from incineration, titrated with 0.1 N silver nitrate solution. Acidity was determined by titrating 10 g of sample dispersed in 50 mL distilled water with 0.1 N sodium hydroxide solution, using phenolphthalein as indicator.

DATA ANALYSIS AND STATISTICS

All experiments were performed in triplicate. The results were analyzed by analysis of variance, and means were compared using Tukey's test at a 5% significance level.

RESULTS AND DISCUSSION

MICROBIOLOGICAL ANALYSES OF PINGO

The results are presented in Table 1, which shows the absence of *Salmonella* spp. and LM, consistent with findings from previous studies on pingo[9–12]. In the evaluation of EC, a result of 3.96 log CFU/mL^{-1} was observed. Notably, some researchers have reported values of 2.72 and 1.60 log CFU/mL^{-1} [13,14]. For enumeration of coagulase-positive, a count of 3.79 log CFU/mL^{-1} was obtained, while previous studies have reported values of 3.00, 1.68, and 4.12 log CFU/mL^{-1} were observed[13–15]. In this study, the result for LAB was 5.95 log CFU/mL^{-1}, compared to other studies, including values 3.90–7.67 log CFU/mL^{-1} [16], 6.12 log CFU/mL^{-1} [17], 6.27 log CFU/mL^{-1} [18], and 5.81–8.48 log CFU/mL^{-1} [19]. For the mesophilic analysis, the value found was 6.28 log CFU/mL^{-1}, whereas other studies have reported 5.32–8.40 log CFU/mL^{-1} [16], 7.94 log CFU/mL^{-1} [20], and 6.62 log CFU/mL^{-1} [15].

Table 1. Results of the microbiological analyses of endogenous starter culture.

Bacteria (log_{10} CFU/mL^{-1})	Time (1 day)
Mesophilic bacteria	6.28±0.57
Lactic acid bacteria	5.95±0.14
Listeria monocytógenes	Absent
Salmonella spp.	Absent
Coagulase-positive staphylococci	3.79±0.25
Escherichia coli	3.96

Source: survey data.

MICROBIOLOGICAL ANALYSES OF CHEESE

The results for mesophilic bacteria showed an initial concentration of 8 log CFU/g^{-1}, with this count increasing until 40 days of fermentation, followed by a gradual decrease ($p \leq 0.05$), reaching 5, 6, and 7 log CFU/g^{-1} at the end of maturation across all treatments (Table 2). We also observed that LAB exhibited inhibitory activity against mesophilic microorganisms in treatments supplemented with LM and pingo.

Table 2. Microbiological analyses of cheese for mesophilic and lactic acid bacteria.

Bacteria (log CFU$^{\cdot g-1}$)	Treatments	Time (days)			
		1	20	40	60
Mesophilic	Cheese raw milk	8.08±0.06^{bBC}	8.39±0.27^{bB}	7.86±0.07 ^{dC}	9.50±0.07^{aA}
	Cheese raw m./LM	8.74±0.04^{aAB}	9.13±0.05^{aA}	8.28±0.01 ^{bB}	6.87±0.62^{bC}
	Cheese raw m./LM/ES	8.71±0.22^{aAB}	9.13±0.28^{aA}	8.51±0.13 ^{aB}	5.80±0.04^{cC}
	Cheese past. milk	7.06±0.03^{cD}	8.76±0.07^{abB}	8.03±0.06 ^{cdC}	9.57±0.04^{aA}
	Cheese past. milk/LM	8.14±0.02^{bA}	8.38±0.27^{bA}	8.16±0.01 ^{bcA}	7.40±0.04^{bB}
	Cheese past. m./LM/ES	8.56±0.22^{aAB}	8.60±0.05^{abA}	8.22±0.08 ^{bcB}	7.32±0.08^{bC}
Lactic acid bacteria	Cheese raw milk	7.92±0.08 ^{aB}	8.79±0.23^{aA}	7.91±0.04 ^{eB}	7.60±0.07^{aB}
	Cheese raw m./LM	8.61±0.17 ^{aA}	8.82±0.06^{aA}	8.50±0.17 ^{abA}	7.47±0.09^{aB}
	Cheese raw m./LM/ES	8.70±0.18 ^{aA}	8.97±0.17^{aA}	8.62±0.12 ^{aA}	2.22±3.85^{bB}
	Cheese past. milk	6.35±0.65 ^{bC}	9.02±0.06^{aA}	8.18±0.01^{cdAB}	7.53±0.04^{aB}
	Cheese past. milk/LM	8.17±0.02 ^{aB}	8.95±0.12^{aA}	8.02±0.04^{deB}	7.34±0.16^{aC}
	Cheese past.m./LM/ES	8.36±0.08 ^{aB}	9.14±0.03^{aA}	8.34±0.08^{bcB}	7.35±0.15^{aC}

Source: survey data.

For the control treatments, samples prepared from pasteurized milk showed mean mesophilic counts ranging from a minimum of 7.06 log CFU/g^{-1} (1 day) to a maximum of 8.76 log CFU/g (20 days) during maturation. At 60 days, there were significant differences ($p \leq 0.05$) in mesophilic aerobic growth, with counts ranging from 7.06 to 9.57 log CFU/g. Similarly, in the control treatment with refrigerated raw milk, mesophilic counts increased by 2 log CFU/g^{-1} at 60 days of maturation ($p \leq 0.05$). The lowest mesophilic counts were observed in the treatment with pasteurized milk supplemented with pingo at 60 days of maturation, with a value of 5.80 log CFU/g^{-1}. This reduction may be associated with LAB, which acidify the medium and are capable of producing bacteriocins, organic acids, peroxides, and fatty acids[21].

The International Commission on Microbiological Specifications for Foods establishes a maximum acceptable value of 7.0 log CFU/g^{-1} or mL^{-1} [22]. In our study, the treatment with the lowest count (5.80 log CFU/g^{-1}) was the cheese produced with raw milk/LM/pingo, which complies with the standard. Notably, studies have reported values of 8–8.18 log CFU/g^{-1}, 5.80–8.50 log CFU/g^{-1}, and 5.90–10.40 log CFU/g^{-1} [23–25].

Analysis of LAB counts in raw milk treatments with pingo revealed a value of 8.70 log CFU/g^{-1}, which was higher than that of the control treatment on day 1 (7.92 log CFU/g^{-1}) (Table 2). After 60 days of maturation, the trend reversed, with the highest counts observed in the treatment without pingo (control: 7.60 CFU/g^{-1}) compared to the treatment with pingo (7.22 CFU/g^{-1}). A similar pattern was observed in pasteurized milk treatments: on the first day of maturation, the LAB count was 8.36 log CFU/g^{-1} with pingo addition, higher than that of the control (6.35 log CFU/g^{-1}). After 60 days, the LAB count was 7.53 log CFU/g^{-1} in the treatment with pingo and 7.35 log CFU/g^{-1} in the control.

These results indicate that LAB played an important role in reducing mesophilic bacteria after 60 days of maturation, in both raw and pasteurized milk treatments. A reduction in mesophilic counts was observed when comparing control and pingo-treated samples: 9.50–5.80 log CFU/g^{-1} in the raw milk and pingo treatment, and from 9.57–7.32 log CFU/g^{-1} in the pasteurized milk and pingo treatment.

Lactic acid bacteria have the capacity to produce lactic acid and other metabolites, such as hydrogen peroxide, diacetyl, and bacteriocins, which antagonize and inhibit the growth of competitor and pathogenic microorganism[21], thereby contributing to food preservation and safety, as well as enabling physical and sensory changes in the final product. The inhibitory activity of these bacteria is attributed to competition for nutrients and adhesion sites, as well as production of organic acids and bacteriocins[26].

For enumeration of coagulase-positive staphylococci and LM (Table 3), the highest values were observed at 40 days of maturation, with the pasteurized milk control treatment recording 6.88 log CFU/g^{1}. At 60 days of maturation, all samples complied with current legislation (3 log CFU/g^{-1}), except for the control treatment with refrigerated raw milk, which showed a value of 5 log CFU/g^{-1}, exceeding the limit[27].

Table 3. Microbiological analyses of cheese for coagulase-positive staphylococci and *Listeria monocytogenes* (Gram-positive bacteria).

Bacteria (log CFU$^{.g-1}$)	Treatments	Time (days)			
		1	20	40	60
Coagulase positive *staphylococci*	Cheese raw milk	5.12±0.06^{aA}	Absent	Absent	5.00±0.18^{aA}
	Cheese raw m./LM	Absent	4.19±0.22^{aB}	5.87±0.01^{cA}	Absent
	Cheese raw m./LM/ES	Absent	4.42±0.04^{aB}	6.18±0.06 ^{bA}	1.00±0^{cC}
	Cheese past. milk	3.45±0.36^{bB}	Absent	6.88±0.02^{aA}	3.00±0^{bB}
	Cheese past. milk/LM	3.10±0.18^{bB}	Absent	6.79±0.02^{aA}	3.00±0^{bB}
	Cheese past.m./LM/ES	4.69±0.62^{aA}	3.58±0.30^{bB}	5.16±0.13^{dA}	3.00±0^{bB}
Listeria monocytogenes	Cheese raw milk	Absent	2.42±0.10^{dA}	2.92±2.53^{abA}	2.07±0.10^{abA}
	Cheese raw m./LM	4.56±0.54 ^{bA}	4.00±0b^{AB}	Absent	3.07±0.68^{aB}
	Cheese raw m./LM/ES	4.53±0.20 ^{bA}	2.00±0^{eC}	Absent	2.96±0.20^{aB}
	Cheese past. milk	Absent	4.11±0.13^{bA}	3.12±2.70^{abA}	1.67±0.49^{bA}
	Cheese past. milk/LM	5.65±0.03 ^{aA}	3.63±0.12^{cB}	5.77±0.02^{aA}	3.03±0.24^{aC}
	Cheese past.m./LM/ES	5.40±0.04 ^{aA}	5.77±0.16^{aA}	5.78±0.03^{aA}	2.06±0.50^{abB}

Source: survey data.

The presence of staphylococci in cheese is common, as it may originate from milk, the skin and mucous membranes of animals and humans, equipment surfaces, and the milking environment[28]. Due to extensive handling during cheese production, inadequate application of Good Manufacturing Practices (GMP) increases the risk of pathogen transfer from handlers and utensils to the final product[29]. Enumeration of staphylococci is typically utilized as an indicator of handling conditions and GMP compliance in processed foods[30]. Certain strains are capable of causing food poisoning by producing staphylococcal enterotoxins, which are heat-stable and associated with pathogen proliferation in food[31,32]. In fact, values of 5.20 log CFU/g^{-1} [33] and 3.50 log CFU/g^{-1} [25] have been reported.

The behavior of LM was assessed over the 60-day maturation period (Table 3). A more pronounced decrease, with significant variation ($p \leq 0.05$), in pathogen counts was detected in the pasteurized milk and pingo treatment, with values of 5.40 log CFU/g^{-1} (1 day) and 2.06 log CFU/g^{-1} (60 days) of maturation. In the raw milk + LM treatment, counts of 4.56 log CFU/g^{-1} (1 day) and 3.07 log CFU/g^{-1} (60 days) were noted. In the pingo-added treatment, comparable values of 2.96 log CFU/g^{-1} at 60 days were recorded, with no significant variation ($p \geq 0.05$) between these two treatments.

For the pasteurized milk and pingo treatment, a significant reduction ($p \leq 0.05$) of 1 log CFU/g^{-1} was observed at the end of the maturation period (60 days) compared to the treatment without pingo, with values of 3.03 log CFU/g^{-1} (without pingo) and 2.06 log CFU/g^{-1} (with pingo). This reduction is likely associated with the addition of pingo, predominantly composed of LAB, as well as reductions in moisture and pH (Table 6), demonstrating the antimicrobial activity produced by *Lactobacillus* and its action against LM.

The ability to survive and/or proliferate in cheese and within the environment enables persistent biofilm formation by LM[33]. Inefficient sanitization can permit the persistence of LM for years, allowing multiplication via contact with contaminated materials in production environments[33,34]. *L. monocytogenes* is capable of growth across a broad pH range (4.3–9.6), at temperatures between 0–45 °C, and in salt concentrations of 10–20%. Although not resistant to pasteurization, LM exhibits tolerance to successive freeze-thaw cycles, making it a significant public health pathogen associated with infectious diseases[35]. Studies indicate that LAB can inhibit the growth of LM in cheese[36–38]. Researchers found that after 60 days of maturation at 4–5 °C, there was no reduction in LM counts, and similarly, after 120 days at 12 °C, no significant reduction[39].

Furthermore, LM counts in curd mass at 30, 40, 50, and 60 days of maturation at 7.5 °C were 6.5, 8.09, 7.80, and 7.60 log CFU/g^{-1}, respectively[40]. Of ten LAB strains evaluated in milk and cheese, seven demonstrated antagonistic activity against LM as well as diacetyl production[41]. The effect of pingo on *Listeria innocua* was assessed, requiring 32 days of maturation to achieve a one-log reduction in pathogen population compared to 44 days in the absence of pingo[42]. In addition, after 56 days of maturation, the microorganism remained present at counts of 2 log CFU/g^{-1}.

In the present study, *Salmonella* spp. were not detected (Table 4), corroborating the literature[43,45]. Table 4 also presents results of EC analysis, which ranged from 4.63 to 0.48 log MPN/g^{-1}, generally showing a decrease in EC counts over the 60-day maturation period. In the pasteurized milk control treatment, EC counts at 20 and 40 days were higher than in other treatments, measuring 4.63 and 3.56 log MPN/g^{-1}, respectively. At 60 days of maturation, all samples showed decreased counts, falling within the limits established by current legislation (3 log CFU/g^{-1})[27]. For the pasteurized milk plus pingo treatment, a value of 0.48 log MPN/g^{-1} was also observed at 60 days.

EC is commonly utilized as an indicator of gastrointestinal tract contamination in raw foods[32]. When present in foods, EC can cause infection; depending on the serotype, certain species are associated with severe diarrheal diseases, colitis, and enteritis, representing a public health concern[41]. IN 161 sets permissible EC levels for cheese based on moisture content (medium, < 46%; high, ≥ 46%). The detection of EC in products may indicate absence of GMP or possible failures in hygienic processes. This pathogen serves as an indicator of fecal contamination and may reflect the presence of enteric pathogens in cheese[46]. Reported results in the literature range vary at 1.30–3.10 log MPN/g^{-1}, 0.70–3.40 log MPN/g^{-1}, and 0.56–4.87 log MPN/g^{-1} [23,47,48].

Table 4. Microbiological analyses of cheese for *Salmonella* and *Escherichia coli* (Gram-negative bacteria).

Bacteria (log CFU·g-1)	Treatments	Time (days)			
		1	20	40	60
Salmonella spp.	Cheese raw milk	Absent	Absent	Absent	Absent
	Cheese raw milk/LM	Absent	Absent	Absent	Absent
	Cheese raw m./LM/ES	Absent	Absent	Absent	Absent
	Cheese past. milk	Absent	Absent	Absent	Absent
	Cheese past. milk/LM	Absent	Absent	Absent	Absent
	Cheese past. m./LM/ES	Absent	Absent	Absent	Absent
Escherichia coli	Cheese raw milk	<3.48	<3.48	<3.48	0.79
	Cheese ram milk/LM	<3.48	<3.48	<3.48	<0.48
	Cheese raw m./LM/ES	3.96	<3.48	<3.48	<0.48
	Cheese past. milk	<3.48	4.63	3.56	<0.48
	Cheese past. milk/LM	<3.48	<3.48	<3.48	<0.48
	Cheese past. m./LM/FE	3.48	<3.48	<3.48	<0.48

Source: survey data.

PHYSICOCHEMICAL ANALYSES OF PINGO

As shown in Table 5, the measured moisture content was 89.53%. Literature values include 94%[49,50] and 92–93%[51]. For pH, a value of 6.94 was obtained, whereas reported values in the literature are 5.06, 5.49, and 5.72[18,20,52]. The acidity observed in this study was 0.06; in the literature, reported values are 0.4, 1.1, 1.76, and 0.55%[14,16,50]. The determined ash content was 4.28%, while previously reported values are 0.47, 0.51, and 0.57%[49–51]. For chlorides, 0.55% was observed, compared to literature values of 0.19, 5.38, and 5.90%[18,20,51]. Total solids measured 10.46%, with literature values of 6.28 and 6.35%[49,53].

Table 5. Physicochemical analyses of the endogenous starter culture.

Parameter (%)	Time (1 day)
Moisture content	89.53±1.87
Ash content	4.28±1.95
Chlorides	0.55±0.04
pH	6.94±0.08
Total solids	10.46±1.87

Source: survey data.

PHYSICOCHEMICAL ANALYSES OF CHEESE

Moisture analysis (Table 6) revealed values of 50.34% (1 day) and 16.62% (60 days) for refrigerated raw milk cheese, and 56.13% (1 day) and 18.93% (60 days) for pasteurized milk cheese, both exhibiting significant variation ($p \leq 0.05$). Greater moisture loss occurred between days 1 and 20 of maturation when compared across maturation periods. At the start of maturation, two phenomena: surface water evaporation and syneresis, which contribute to cheese moisture loss. Surface evaporation continues as maturation progresses, while whey drainage of the curd diminishes and eventually ceases[17,53]. In this experiment, 75% of analyzed samples qualified as low-moisture cheese, and 12.5% as high- and very high-moisture cheese[54]. A significant increase ($p \leq 0.05$) in ash content, chlorides, acidity, and total solids was also observed, while a significant decrease ($p \leq 0.05$) in moisture and pH was noted.

Table 6. Physicochemical analyses of cheese samples made from refrigerated and pasteurized raw milk cheese.

Samples	Parameters (%)	Time (days)			
		1	20	40	60
Cheese raw milk	Moisture	50.34±0.20^{A}	23.84±0.55^{B}	18.61±1.83^{B}	16.62±0.47^{C}
	pH	6.66±0.20^{A}	5.08±0.04^{B}	5.22±0.04^{B}	5.27±0.01^{B}
	Titratable acidity	0.23±0.04^{B}	0.73±0.13^{A}	0.81±0.0^{A}	0.81±0.01^{A}
	Ash content	3.00±0.02^{C}	4.38±0.03^{B}	5.66±0.17^{A}	5.82±0.01^{A}
	Chlorides	0.11±0.01^{C}	0.15±0.01^{B}	0.24±0.01^{A}	0.23±0.01^{A}
	Total solids	49.65±0.20^{C}	76.15±0.55^{B}	81.38±1.83^{A}	83.38±0.47^{A}
Cheese pasteurized milk	Moisture	56.13±0.17^{A}	23.21±0.93^{B}	16.77±0.15^{D}	18.93±0.75^{C}
	pH	6.63±0.02^{A}	5.55±0.06^{B}	5.50±0.03^{B}	5.26±0.03^{C}
	Titratable acidity	0.06±0.01^{C}	0.54±0.01^{B}	0.67±0^{A}	0.66±0.01^{A}
	Ash content	3.00±0.01^{D}	4.25±0.07^{C}	6.04±0.07^{A}	5.14±0.06^{B}
	Chlorides	0.11±0.01^{D}	0.15±0.01^{C}	0.26±0.02^{A}	0.22±0.01^{B}
	Total solids	43.86±0.17^{D}	76.79±0.93^{C}	83.23±0.15^{A}	81.42±0.75^{B}

Source: survey data.

Production lacked standardization, with variation in salting and pressing methods[55]. During this process, moisture decreases while protein and fat contents increase, resulting in variation in total solids content according to maturation time, due to moisture loss[56]. Moisture loss is an intrinsic phenomenon influenced by the temperature and relative humidity of the maturation chamber[57].

Researchers have reported corresponding moisture results of 56.0–63.70%, 44.22–47.11%, and 30.39–49.92%[58–60]. The pH values were 6.66 (1 day) and 5.27 (60 days) for refrigerated raw milk cheese, and 6.63 (1 day) and 5.26 (60 days) for pasteurized milk cheese; both showed significant variations ($p \leq 0.05$), which is consistent with previous reports[58–60]. When the pH is between 4.0 and 5.5, bacteria are stimulated to produce more decarboxylases as a defense mechanism in acidic environments[14]. Changes in pH and acidity during cheese maturation are presented in Table 6, which shows significant pH reductions ($p \leq 0.05$), with a tendency to stabilize at 20 days of maturation.

The pH reduction results from the action of LAB on the lactose content[61]. Organic acid production may result in a pH decrease, which is important for cheese preservation as it limits the growth of pathogenic and spoilage bacteria, as shown in Table 5. Some LAB may express bacteriocin-encoding genes with antagonistic activity against potentially contaminant microorganisms during cheese production[62,63]. These bacteria ferment lactose, producing lactic, acetic, butyric, and propionic acids, as well as alcohols. This metabolic activity is important for starter cultures, as it acidifies the milk, initiates coagulation, and releases enzymatic compounds that contribute to characteristic flavors[64].

The acidity found in this study was 0.23% (1 day) and 0.81% (60 days) for raw milk cheese, and 0.06% (1 day) and 0.66% (60 days) in pasteurized milk cheese, with both exhibiting significant variations ($p \leq 0.05$). Literature values varied at 0.14–0.57%[58] and 0.27–0.67% 59. For ash content, the results have also varied between 3.00% (1 day) and 5.82% (60 days) for raw milk cheese. For pasteurized milk cheese, the values have fluctuated between 3.00% (1 day) and 5.14% (60 days), both showing significant variations ($p \leq 0.05$). In addition, one study reported ash content between 6.72 and 7.90[60].

Chloride analysis showed values for refrigerated raw milk cheese ranging from 0.11% (1 day) to 0.23% (60 days), and for pasteurized milk cheese from 0.11% (1 day) to 0.26% (40 days); both demonstrating significant variations ($p \leq 0.05$). In the literature, values have spanned 0.7–1.4%[58] and 1.3–2.4%[18]. Total solids in raw milk cheese were 49.65% (1 day) and 83.38% (60 days), and in pasteurized milk cheese, 43.86% (1 day) and 83.23% (40 days); notably, 42.10 and 71.20%[18] and 50.08 and 69.61% have been reported elsewhere[60].

This study provides increased insight into the elucidation of pingo use, which is predominantly composed of LAB and has been extensively studied, in order to contribute to new metrics, standardization efforts, and a better understanding of issues related to foodborne outbreaks, supplementing existing research.

CONCLUSIONS

The absence of *Salmonella* spp. and the inhibitory effects of LAB against mesophilic bacteria were observed. After sixty days of maturation, the samples complied with current legislation regarding *E. coli* and *Salmonella* spp. Regarding coagulase-positive *Staphylococcus*, cheese produced with raw milk showed a count of 5 log CFU/g^{-1}, which does not meet the regulatory standards.

The addition of pingo, combined with the maturation period, resulted in a reduction of *L. monocytogenes* populations; however, this approach was not sufficient to eliminate the pathogen from artisanal cheese. The results demonstrated that pingo exerts a preservative effect during cheese maturation, effectively lowering pH and increasing acidity due to lactic acid production.

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