Research article

Physicochemical and microbiological quality of raw cow's milk and artisanal fresh cheeses from Nariño Department, Colombia

Calidad fisicoquímica y microbiológica de la leche cruda de vaca y los quesos frescos artesanales del departamento de Nariño, Colombia

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Abstract

Objective. This study evaluated the physicochemical and microbiological quality of raw cow's milk and artisanal fresh cheeses produced in 22 municipalities of Nariño Department, southwestern Colombia, to assess compliance with national food safety regulations and to identify potential food safety hazards. **Methods.** Physicochemical parameters were determined following AOAC International and Colombian Technical Standards (NTC) methods. Microbiological analyses were performed according to International Organization for Standardization (ISO) procedures for enumeration of coliforms, *Escherichia coli*, and *Staphylococcus aureus*, and detection of *Listeria monocytogenes* and *Salmonella* spp. Selected bacterial isolates were genotypically identified through 16S ribosomal RNA (rRNA) gene sequencing. Additionally, processing plants were evaluated for compliance with Good Manufacturing Practices (GMP). **Results.** The physicochemical characteristics of milk and cheese samples complied with Colombian regulatory limits. However, significant pathogenic contamination persisted: 45.5% of milk samples and 36.3% of cheese samples tested positive for *L. monocytogenes*, while *E. coli* counts exceeded permissible levels in 36.4% of

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milk samples and 22.7% of cheese samples. *Salmonella* spp. was not detected in any sample (100% compliance for this pathogen). Molecular identification through 16S rRNA gene sequencing confirmed the pathogenic isolates initially identified through standard microbiological and biochemical techniques. Processing plant infrastructure compliance ranged from 36% to 93% (mean ± standard deviation = 66.5% ± 17.2%), strongly correlating with microbiological quality outcomes. **Conclusion.** While artisanal cheese production in Nariño Department meets physicochemical standards, there are still significant microbiological hazards, particularly *L. monocytogenes* and *E. coli* contamination. Implementation of comprehensive hygiene protocols, enhanced GMP compliance, and formalized quality control systems are urgently needed to ensure food safety and public health protection.

Keywords: raw milk, fresh cheese, food safety, *Listeria monocytogenes*, *Staphylococcus aureus*, good manufacturing practices, Nariño.

Resumen

Objetivo. Este estudio evaluó la calidad fisicoquímica y microbiológica de la leche cruda de vaca y quesos frescos artesanales producidos en 22 municipios del departamento de Nariño, suroccidente de Colombia, para determinar el cumplimiento de las regulaciones nacionales de inocuidad alimentaria e identificar posibles peligros para la salud pública. **Métodos.** Los parámetros fisicoquímicos se determinaron siguiendo métodos de la Asociación de Químicos Analíticos Oficiales (AOAC) y las Normas Técnicas Colombianas (NTC). Los análisis microbiológicos se realizaron según procedimientos de la Organización Internacional de Normalización (ISO) para enumeración de coliformes, *Escherichia coli*, *Staphylococcus aureus*, y detección de *Listeria monocytogenes* y *Salmonella* spp. Aislamientos bacterianos seleccionados se identificaron genotípicamente mediante secuenciación del gen ARNr 16S. Adicionalmente, se evaluó el cumplimiento de Buenas Prácticas de Manufactura (BPM) en las plantas procesadoras. **Resultados.** Las características fisicoquímicas de la leche y el queso cumplieron con los límites regulatorios colombianos. Sin embargo, persiste contaminación significativa por patógenos: el 45.5% de las muestras de leche y el 36.3% de las muestras de queso resultaron positivas para *L. monocytogenes*, mientras que los recuentos de *E. coli* excedieron los niveles permitidos en el 36.4% de las muestras de leche y el 22.7% de las muestras de queso. No se detectó *Salmonella* spp. en ninguna muestra, representando cumplimiento del 100% para este patógeno. La identificación molecular mediante secuenciación del gen 16S rRNA confirmó y verificó

los aislados patógenos identificados inicialmente mediante técnicas microbiológicas y bioquímicas estándar. El cumplimiento de infraestructura en las plantas procesadoras osciló entre 36% y 93% (media ± DE: 66.5 ± 17.2%), correlacionándose fuertemente con los resultados de calidad microbiológica. **Conclusión.** Aunque la producción artesanal de queso en Nariño cumple con los estándares fisicoquímicos, persisten peligros microbiológicos significativos, particularmente contaminación por *L. monocytogenes* y *E. coli*. Es urgente implementar protocolos integrales de higiene, mejorar el cumplimiento de BPM y formalizar sistemas de control de calidad para garantizar la inocuidad alimentaria y proteger la salud pública.

Palabras clave: leche cruda, queso fresco, inocuidad alimentaria, *Listeria monocytogenes*, *Staphylococcus aureus*, buenas prácticas de manufactura, Nariño.

Introduction

Artisanal ground farmer cheese is widely consumed in Colombia. It is characterized by a high moisture content and a near-neutral pH, which, together with the manual grinding and kneading steps performed before packaging, increases its susceptibility to microbial contamination. Production is mostly carried out in small artisanal facilities that often operate under inadequate hygienic and sanitary conditions. These factors, combined with the physicochemical properties of the product, make this cheese highly vulnerable to the growth of both pathogenic and spoilage microorganisms, which may compromise consumer health and reduce shelf life.

Several studies have linked the consumption of fresh cheeses to outbreaks of foodborne illness (1). In Colombia, high contamination levels with pathogenic bacteria have been reported in cheeses made from both raw and pasteurized milk (2,3). Such contamination is mainly attributed to deficiencies in manufacturing practices, poor hygiene during milking, and improper storage and distribution conditions.

Spoilage microorganisms commonly found in dairy products include gram-negative psychrotrophic bacteria, coliforms, lactic acid bacteria, yeasts, and molds (4,5). Total coliforms and mesophilic aerobic counts are major indicators of sanitary quality in cheeses, while pathogens of public health concern include *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., and pathogenic *Escherichia coli* (6–8). The presence of fecal coliforms indicates fecal contamination and/or inadequate sanitary conditions during production, storage, or distribution (4,9). *S. aureus* infections have

been associated with the use of unpasteurized milk or cross-contamination due to poor handling practices, as this pathogen can produce heat-stable enterotoxins when present at concentrations above 5 log colony-forming units (CFU)/mL. Outbreaks related to *L. monocytogenes* contamination in cheese have also been reported, showing relatively high case-fatality rates (15%–30%) (10), which underscores the public health importance of controlling this pathogen in dairy products.

Despite the wide production and consumption of fresh cheeses in Colombia, where cheese production represents 20% of formal dairy processing and approximately 50% of informal milk production is dedicated to artisanal cheese manufacturing (11,12), few studies have comprehensively evaluated their physicochemical and microbiological quality, particularly in rural regions. Considering their economic relevance and potential public health implications, the objective of this study was to assess the physicochemical characteristics and microbiological profile of raw cow's milk and artisanal fresh cheeses produced in the Nariño Department, southwestern Colombia.

Materials and methods

Study area and sample collection

The study was conducted in Nariño Department, located in the southwestern region of Colombia. Sampling was carried out between March and August 2023 in 22 municipalities representing the main dairy-producing areas of the department (Table S1). Raw cow's milk samples (n = 44) were collected directly from producers and artisanal cheese plants, while artisanal fresh cheese samples (n = 66) were obtained from small-scale local processors and rural markets.

Approximately 500 mL of milk and 500 g of cheese were collected aseptically in sterile containers, stored in insulated coolers containing ice packs, and transported at 4 ± 2 °C to the Food Microbiology Laboratory at the Universidad de Nariño within 6 h of sampling. All samples were analyzed immediately upon arrival.

Physicochemical analysis

The physicochemical properties of the milk and cheese samples were determined following standard analytical methods (13,14). The parameters measured in milk included pH, acidity, density, fat, protein, non-fat solids (NFS), and total solids (TS) (15). In cheese, moisture, fat, protein, salt, and pH were determined. pH was measured using a calibrated pH meter (HI98163, Hanna Instruments) at 25 °C. Acidity by titration with 0.1 N NaOH using phenolphthalein as an indicator and expressed as % lactic acid. Density was measured at 15 °C using a digital lactodensimeter. The fat content

determined by the Gerber method for milk and the Van Gulik method for cheese (16). The protein content was determined by the Kjeldahl method (AOAC 991.20). Moisture and TS were measured after oven drying the samples at 102 °C until they reached a constant weight. The salt content was determined by the Mohr titration method using silver nitrate (17).

Microbial counts were $\log_{10}$-transformed prior to analysis. Descriptive statistics (mean ± standard deviation [SD]) were calculated, and comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$). Normality and homogeneity of variances were verified using Shapiro–Wilk and Levene tests, respectively. Statistical analyses were conducted using R version 4.2.2.

Microbiological analyses

Microbiological quality was assessed following International Organization for Standardization (ISO) and Colombian Technical Standards (NTC) procedures. For each sample, 25 g (cheese) or 25 mL (milk) was aseptically homogenized in 225 mL of sterile 0.1% peptone water using a Stomacher (Seward Stomacher 400). Serial tenfold dilutions were prepared, and 100 µL aliquots were plated in triplicate on the corresponding media as described in Table 1. The results are expressed as $\log_{10}$ colony-forming units (CFU)/mL for milk and $\log_{10}$ CFU/g for cheese. The results for *Salmonella* and *Listeria monocytogenes* are reported as "presence/absence in 25 g."

Table 1. Culture conditions for the analyzed bacteria.

Microorganism	Culture medium	Incubation conditions	Reference
Total mesophilic aerobes	Plate Count Agar (PCA)	30 °C / 48 h	ISO 4833-1:2013 (18)
Total coliforms	Violet Red Bile Agar (VRBA)	37 °C / 24 h	ISO 4832:2006 (19)
Fecal coliforms / *Escherichia coli*	Tryptone Bile X-glucuronide Agar (TBX)	44 °C / 24 h	ISO 16649-2:2001 (20)
Staphylococcus aureus	Baird-Parker Agar + Egg Yolk Tellurite	37 °C / 48 h	ISO 6888-1:2021 (21)
Listeria monocytogenes	ALOA Agar	37 °C / 48 h	ISO 11290-1:2017 (22)
Salmonella spp.	Pre-enrichment in buffered peptone water (37 °C, 24 h), followed by selective enrichment in Rappaport–Vassiliadis broth (42 °C, 24 h) and plating on XLD agar	37 °C / 24 h	ISO 6579-1:2017 (23)

Bacterial DNA extraction

Colonies of each bacterial isolate (representative isolates based on morphology and source municipality) were resuspended and centrifuged at 14000 *g* for 2 min. The precipitate was used for DNA extraction using GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich) (24). DNA concentration and purity were assessed by ultraviolet (UV) spectrophotometry (NanoDrop One C, Thermo Fisher Scientific) and 1% agarose gel electrophoresis, with a 2:1:1 ratio of DNA, TE buffer, and EZ-Vision® DNA Dye (Amresco). The GeneRuler 1 kb Plus DNA Ladder (Thermo Scientific) was used as a molecular weight marker. The gel was run at 100 V for 1 h and then visualized in a UV transilluminator (Thermo Fisher Scientific) at 320 nm.

Genotypic identification of isolates

The 16S ribosomal RNA (rRNA) gene was amplified by polymerase chain reaction (PCR) in SimpliAmp™ thermal cycler (Thermo Fisher Scientific) with the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3') (25). The GoTaq Green Master Mix (Promega) kit was used for PCR. The PCR conditions for *E. coli*, *S. aureus*, and *L. monocytogenes* were described previously (26–28) respectively.

Amplicons were sequenced by the Sanger method, and the sequences obtained were reviewed with the BioEdit V.5.09 and UniPro V.52.0 software (29,30). Then, they were converted to the FASTA format and compared to GenBank using BLASTn. The BLASTn search parameters were percentage of identity >97%, E-value <1e−5, and query cover >95%. Sequences showing >99% identity with those in the database were considered reliable identifications; however, all sequences with identity values above 95% are presented for reference.

Assessment of processing plant infrastructure and Good Manufacturing Practices (GMP)

Processing plant infrastructure and compliance with GMP were systematically evaluated across all 22 municipalities. The assessment was conducted using a structured checklist based on Colombian Resolution 2674 of 2013 (Ministry of Health and Social Protection), which establishes food safety requirements for processing facilities.

The evaluation encompassed five key categories:

- **Physical infrastructure (25%):** Facility location, building conditions, floor and wall materials, separation of production areas, potable water supply, adequate drainage and waste disposal systems.

- **Equipment and utensils (20%):** Use of food-grade materials (stainless ste-

el preferred), equipment maintenance status, calibration of temperature control devices, and design features preventing cross-contamination.

- **Personnel hygiene and training (20%):** Availability of handwashing stations, use of protective clothing (uniforms, hairnets, and gloves), documentation of hygiene training programs, and health certificates for food handlers.

- **Sanitation and pest control (15%):** Written cleaning and disinfection procedures, proper storage and labeling of cleaning chemicals, and documented pest control programs.

- **Process control and documentation (20%):** Raw material reception controls, process flow documentation, temperature and time monitoring records, and product traceability systems.

Each criterion was evaluated through direct observation during site visits, review of available documentation (production records, cleaning logs, and training certificates), and interviews with facility managers and operators. For samples obtained from retail markets where direct plant access was not possible, information was gathered through producer interviews and municipal health authority records when available.

Each aspect was scored and an overall compliance percentage was calculated for each facility or production unit. Plants were categorized as high compliance (≥70%), moderate compliance (50-69%), or low compliance (<50%). Evaluation visits lasted 2–4 h per facility and were conducted by trained food safety professionals.

Results

A total of 22 raw milk samples and 21 artisanal fresh cheese samples, each analyzed in triplicate, were collected from 22 municipalities in Nariño Department, Colombia. According to the findings observed in the technical assessment, three artisanal cheese facilities satisfied all infrastructure criteria required for productive operations, nine satisfied until 80%, six satisfied 53%–73%, and the remainder exhibited <47% compliance. In terms of GMP, 11 facilities demonstrated compliance >83%, one showed 63% compliance, and the rest showed <33% compliance.

Physicochemical characteristics of raw milk and fresh cheese

Tables 2 and 3 summarizes the physicochemical parameters of the raw cow's milk and artisanal fresh cheese samples, respectively, collected from the different municipalities.

For the milk samples, the mean ± SD were as follows: pH 6.58 ± 0.11, titratable acidity 0.16% ± 0.03% lactic acid, fat 3.82% ± 0.14%, protein 3.27% ± 0.08%, TS 12.45% ± 0.32%, and NFS 8.63% ± 0.18%. These values are within the ranges established by Colombian regulations (NTC 750, 2000; Decreto 616, 2006). The mean somatic cell count was 3.4 × 10^5 cells/mL, indicating acceptable udder health and adequate milking hygiene (31).

Table 2. The fat, protein, and total solid contents for the raw milk samples.

Code	Fat (%)	Protein (%)	Total solids (%)
01-CE	3.82 ± 0.010	3.12 ± 0.006	13.08 ± 0.000
02-CE	3.54 ± 0.015	3.37 ± 0.003	12.41 ± 0.002
03-CE	4.14 ± 0.256	3.49 ± 0.038	12.90 ± 0.290
04-CE	4.67 ± 0.001	3.33 ± 0.000	13.40 ± 0.001
05-JU	3.68 ± 0.005	3.16 ± 0.005	12.18 ± 0.000
06-OB	3.43 ± 0.018	3.09 ± 0.001	11.87 ± 0.010
07-OB	3.92 ± 0.017	3.18 ± 0.002	12.55 ± 0.006
08-OB	3.84 ± 0.001	3.31 ± 0.006	12.36 ± 0.001
09-OB	3.82 ± 0.044	3.29 ± 0.050	12.48 ± 0.006
10-OB	3.97 ± 0.001	3.18 ± 0.002	12.51 ± 0.005
11-OB	3.47 ± 0.015	3.05 ± 0.003	11.82 ± 0.001
12-OB	3.92 ± 0.003	3.26 ± 0.004	12.48 ± 0.001
13-OB	3.92 ± 0.005	3.26 ± 0.002	12.57 ± 0.005
14-OB	3.11 ± 0.003	3.18 ± 0.003	11.54 ± 0.001
15-OB	3.46 ± 0.145	3.02 ± 0.049	13.31 ± 1.489
16-OB	4.2 ± 0.003	3.08 ± 0.020	12.57 ± 0.001
17-OB	3.56 ± 0.000	3.08 ± 0.001	11.77 ± 0.003
18-PC	3.15 ± 0.001	2.95 ± 0.042	11.47 ± 0.000
19-SA	3.83 ± 0.005	3.35 ± 0.000	12.54 ± 0.002
20-SA	3.68 ± 0.006	3.21 ± 0.003	12.16 ± 2.176
21-SA	3.48 ± 0.003	3.16 ± 0.001	12.05 ± 0.002
22-SA	3.52 ± 0.002	3.36 ± 0.010	12.34 ± 0.004

In the fresh cheese samples, the mean ± SD were as follows: pH 6.43 ± 0.15, moisture 58.3% ± 1.2%, fat 22.6% ± 0.8%, protein 16.8% ± 0.6%, and salt 1.54% ± 0.09%. These values are consistent with those expected for fresh, high-moisture cheeses.

However, the fat and salt contents exhibited significant variation among the municipalities ($p < 0.05$), which may be attributed to differences in artisanal processing practices, such as the pressing intensity and salting time.

Table 3. Humidity and the fat and protein contents in the fresh cheese samples.

Code	% Humidity	RSD*	% Fat	RSD	% Protein	RSD
01-CE	53.93 ± 1.43	2.66	22.16 ± 1.03	4.67	17.80 ± 0.10	0.56
02-CE	55.57 ± 1.22	2.19	20.99 ± 0.85	4.04	18.97 ± 0.49	2.60
03-CE	60.85 ± 0.25	0.41	18.33 ± 0.30	1.61	16.17 ± 0.40	2.50
04-CE**	56.19 ± 2.26	4.02	22.49 ± 0.49	2.16	17.13 ± 0.32	1.88
05-JU	62.17 ± 1.84	2.96	17.98 ± 2.18	12.1	15.87 ± 0.35	2.21
06-OB	60.26 ± 1.30	2.16	18.99 ± 0.01	0.07	16.73 ± 0.38	2.26
07-OB	55.42 ± 0.70	1.26	20.49 ± 0.52	2.53	17.73 ± 0.38	2.13
08-OB	69.40 ± 3.08	4.44	14.00 ± 1.00	7.12	13.83 ± 0.46	3.34
10-OB	52.18 ± 4.84	7.37	20.67 ± 1.75	8.45	16.10 ± 0.56	3.46
11-OB	59.40 ± 0.95	1.59	18.32 ± 0.30	1.63	17.13 ± 0.32	1.88
12-OB	62.44 ± 1.39	2.23	18.33 ± 1.17	6.37	15.57 ± 0.59	3.76
13-OB	61.12 ± 2.25	3.69	12.16 ± 0.76	6.21	17.40 ± 0.57	3.25
14-OB	61.79 ± 1.30	2.11	18.34 ± 0.55	3.01	17.33 ± 0.32	1.85
15-OB	60.80 ± 1.33	2.19	14.16 ± 0.30	2.11	15.70 ± 0.44	2.78
16-OB	60.61 ± 1.27	2.10	17.66 ± 0.28	1.59	16.37 ± 0.21	1.27
17-OB	54.25 ± 2.85	5.25	20.66 ± 2.02	9.78	17.07 ± 0.45	2.64
18-PC	58.96 ± 5.67	9.62	20.00 ± 1.34	6.71	18.97 ± 0.91	4.78
19-SA	50.60 ± 4.30	8.50	25.66 ± 2.31	8.99	20.60 ± 0.75	3.66
20-SA**	60.97 ± 1.95	3.19	18.81 ± 0.30	1.57	14.67 ± 0.55	3.76
21-SA	59.10 ± 0.25	0.42	18.17 ± 0.30	1.65	15.73 ± 0.29	1.83
22-SA	52.60 ± 2.75	5.23	21.33 ± 0.29	1.36	18.93 ± 0.67	3.52

*Relative standard deviation
**Chesses made with raw milk

Microbiological quality of raw milk

The microbiological counts of raw milk are presented in Table 4. The mean ± SD total mesophilic aerobic bacteria count was 5.84 ± 0.27 $\log_{10}$ CFU/mL, indicating a moderate microbial load consistent with raw milk produced under small-scale conditions. The total coliform count ranged from 2.11 to 4.52 $\log_{10}$ CFU/mL, and the fecal coliform count ranged from 1.70 to 3.80 $\log_{10}$ CFU/mL.

S. aureus was detected in 43% of milk samples, with counts of 2.0–4.3 $\log_{10}$ CFU/mL. *L. monocytogenes* was not detected in any milk sample (absence in 25 mL). *E. coli* was detected in 32% of samples, although the counts were below 10^3 CFU/mL. Compared with the limits established by resolution 00017 (32), 68% of the milk samples did not comply with the microbiological standards for mesophilic aerobic bacteria and coliforms, reflecting deficiencies in hygiene during milking, storage, and transportation (33).

Table 4. Microbiological plate counts and somatic cells in raw milk samples from 22 municipalities in Nariño Department.

Code	Mesophilic aerobes (CFU/mL)	Molds and yeasts (CFU/mL)	Total coliforms (CFU/mL)	Fecal coliforms (CFU/mL)	Somatic cells (cells/mL)
01-CE	5.83 ± 0.04	3.31 ± 0.01	3.04 ± 0.00	1.26 ± 0.09	410081.00 ± 1775.45
02-CE	3.85 ± 0.00	1.00 ± 0.00	3.04 ± 0.00	2.03 ± 0.06	1019777.67 ± 9472.92
03-CE	4.28 ± 2.13	2.32 ± 1.10	2.34 ± 0.77	1.35 ± 0.53	495100.33 ± 37964.61
04-CE	5.17 ± 0.06	2.82 ± 0.10	2.40 ± 0.30	0.48 ± 0.00	789141.33 ± 961.50
05-JU	4.74 ± 0.00	3.08 ± 0.00	2.35 ± 0.03	1.92 ± 0.05	509791.67 ± 652.23
06-OB	5.70 ± 0.58	3.11 ± 0.00	3.04 ± 0.00	2.35 ± 0.03	357012.00 ± 631.95
07-OB	4.04 ± 0.00	3.92 ± 0.01	2.66 ± 0.00	0.48 ± 0.00	382082.67 ± 959.80
08-OB	6.28 ± 0.02	4.11 ± 0.03	3.04 ± 0.00	2.36 ± 0.03	379751.67 ± 468.64
09-OB	3.01 ± 0.13	ND*	3.02 ± 0.03	ND	530329.67 ± 25112.44
10-OB	2.68 ± 0.54	ND	2.90 ± 0.37	ND	415861.33 ± 219.60
11-OB	3.54 ± 0.04	ND	2.99 ± 0.05	3.04 ± 0.05	217985.67 ± 209.63
12-OB	2.29 ± 0.20	ND	2.73 ± 0.53	ND	277905.67 ± 692.61
13-OB	2.69 ± 0.49	ND	2.49 ± 0.56	2.35 ± 0.56	412720.33 ± 180.89
14-OB	2.42 ± 0.62	ND	2.33 ± 0.59	0.75 ± 0.65	730919.33 ± 327.37
15-OB	3.30 ± 0.01	1.62 ± 0.11	3.30 ± 0.01	ND	381555.33 ± 293.44
16-OB	2.08 ± 0.05	ND	3.30 ± 0.03	ND	324424.00 ± 91.15
17-OB	2.94 ± 0.03	ND	3.14 ± 0.55	2.63 ± 0.58	430521.67 ± 183.87
18-PC	2.15 ± 0.57	ND	2.04 ± 0.56	1.28 ± 0.59	104446.33 ± 477.65
19-SA	3.22 ± 0.25	0.65 ± 0.16	3.32 ± 0.09	ND	222687.00 ± 398.82
20-SA	3.10 ± 0.58	ND	2.83 ± 0.01	1.65 ± 0.04	308312.67 ± 300.21
21-SA	1.78 ± 0.64	0.10 ± 0.17	1.20 ± 0.63	1.18 ± 0.61	293717.33 ± 105.67
22-SA	2.49 ± 0.57	0.40 ± 0.17	1.96 ± 0.09	0.98 ± 0.09	433531.00 ± 546.60

*Not detected

Microbiological quality of fresh cheese

Based on the results, there was a high presence of mesophilic aerobes in the samples from all municipalities, probably because of the abundance of lactic acid bacteria in the fresh cheeses. According to resolution 1407 de 2022 (34), 77.2% of the samples exceeded the permitted value of <2 log CFU/g, while 9.1% showed absence of these microorganisms. For total and fecal coliforms, 54.5% and 27.2% of samples exceeded the limiting values of <3 log CFU/g and <1.69 log CFU/g, respectively, and 36.6% exhibited the presence of these microorganisms without exceeding the limits, which indicates widespread contamination in the evaluated cheese samples. Regarding coagulase-positive *S. aureus*, 54.5% of samples exceeded the limit of <2 log CFU/g, and 9.5% were >5 log CFU/g, presenting potential public health risks due to its ability to produce enterotoxins. Fifty percent of the samples were positive for β-D-glucuronidase-positive *E. coli*, which has a permitted value of <1 log CFU/g. *Salmonella* spp., the pathogen of greatest risk, was not detected in any samples, whereas *L. monocytogenes* was detected in 36.3%. These findings highlight the need for strict microbiological controls in the production of fresh cheese, in order to minimize health risks and guarantee the safety of the product (Table 5).

Table 5. Microbiological plate counts analyses in fresh cheeses from 22 municipalities in Nariño Department, Colombia.

Code	Mesophilic aerobes log CFU/g	Molds and yeasts log CFU/g	Total coliforms log CFU/g	Fecal coliforms log CFU/g	Coagulase-positive staphylococci log CFU/g	ß-D-Glucuronidase-positive *Escherichia coli* log CFU/g	*Salmonella* spp. absence in 25 g**	*Listeria monocytogenes* absence in 25 g**
01-CE	5.96 ± 0.61	4.67 ± 0.36	3.04 ± 0.00	1.68 ± 0.90	5.24 ± 0.71	2.95 ± 0.14	Absence	Presence
02-CE	5.35 ± 1.40	3.90 ± 1.50	2.54 ± 0.17	0.44 ± 0.61	ND	ND	Absence	Presence
03-CE	5.26 ±0.10	2.84 ± 0.07	2.92 ± 0.22	1.62 ± 0.26	4.92 ± 0.04	ND	Absence	Absence
04-CE*	6.00 ± 0.54	2.74 ± 0.11	>3.04****	1.12 ± 0.07	4.73 ± 0.04	ND	Absence	Absence
05-JU	4.90 ± 0.55	2.92 ± 0.98	2.78 ± 0.36	2.56 ± 0.66	4.22 ± 1.03	2.77 ± 0.42	Absence	Presence
06-OB	5.01 ± 1.02	2.30 ± 0.05	2.49 ± 0.17	ND*****	ND	1.39 ± 1.27	Absence	Presence
07-OB	5.91 ± 0.54	2.97 ± 0.86	>3.04****	>3.04****	5.85 ± 0.12	4.82 ± 0.24	Absence	Presence
08-OB	3.96 ± 0.05	1.97 ± 0.13	1.43 ± 0.10	ND	ND	ND	Absence	Absence
10-OB	5.28 ± 0.03	2.65 ± 0.06	2.66 ± 0.00	ND	4.27 ± 0.06	ND	Absence	Absence
11-OB	4.29 ± 0.03	2.15 ± 0.07	>3.04****	ND	ND	1.93 ± 0.08	Absence	Presence
12-OB	4.29 ± 0.01	1.91 ± 0.07	>3.04****	0.92 ± 0.10	ND	ND	Absence	Absence
13-OB	4.31 ± 0.03	<1***	2.15 ± 0.07	ND	ND	1.59 ± 0.11	Absence	Absence
14-OB	6.31 ± 0.01	2.41 ± 0.07	>3.04****	0.86 ± 0.00	4.96 ± 0.03	3.82 ± 0.06	Absence	Absence
15-OB	6.34 ± 0.01	4.22 ± 0.09	>3.04****	1.23 ± 0.17	ND	ND	Absence	Absence

Code	Mesophilic aerobes log CFU/g	Molds and yeasts log CFU/g	Total coliforms log CFU/g	Fecal coliforms log CFU/g	Coagulase-positive staphylococci log CFU/g	ß-D-Glucuronidase-positive *Escherichia coli* log CFU/g	*Salmonella* spp. absence in 25 g**	*Listeria monocytogenes* absence in 25 g**
16-OB	5.15 ± 0.03	2.92 ± 0.15	2.69 ± 0.33	2.17 ± 0.21	4.45 ± 0.13	2.29 ± 0.68	Absence	Absence
17-OB	4.23 ± 0.03	<1***	>3.04 ****	0.86 ± 0.00	ND	ND	Absence	Presence
18-PC	5.29 ± 0.01	1.68 ± 0.07	>3.04 ****	1.26 ± 0.07	4.41 ± 0.02	4.01 ± 0.02	Absence	Presence
19-SA	4.43 ± 0.03	2.28 ± 0.02	2.34 ± 0.03	ND	ND	ND	Absence	Absence
20-SA*	4.34 ± 0.01	2.60 ± 0.08	>3.04****	2.11 ± 0.06	4.32 ± 0.07	1.68 ± 1.45	Absence	Absence
21-SA	4.30 ± 0.00	2.05 ± 0.01	>3.04****	ND	4.93 ± 0.01	ND	Absence	Absence
22-SA	5.04 ± 0.02	2.72 ± 0.03	>3.04****	2.08 ± 0.00	4.46 ± 0.09	1.49 ± 0.10	Absence	Absence

*Chesses made with raw milk
** Presence/absence results determined according to ISO 11290-1 (*L. monocytogenes*) and ISO 6579-1 (*Salmonella* spp.)
*** ND = not detected; <1 = below the limit of detection (1 log CFU/g)
**** Values with ">" indicate samples exceeding the maximum quantifiable level (3.04 log CFU/g on the medium used)
***** Not detected

Microbiological analysis revealed substantial contamination in both raw milk and artisanal fresh cheese samples across the 22 evaluated municipalities (Figure 1). *L. monocytogenes* was the most prevalent pathogen, detected in 45.5% of milk samples and persisting in 36.3% of cheese samples, indicating only partial reduction during fermentation. *E. coli* contamination exceeded regulatory limits in 36.4% of milk and 22.7% of cheese samples, suggesting inadequate hygienic practices during production. *S. aureus* was present in 43% of milk samples and 54.5% of cheese samples, indicating greater sensitivity to acidification than *L. monocytogenes*. Notably, *Salmonella* spp. was not detected in any sample, representing 100% compliance with Colombian food safety regulations. These results demonstrate that while artisanal fermentation provides partial microbial control, it does not ensure product safety when raw materials are contaminated.

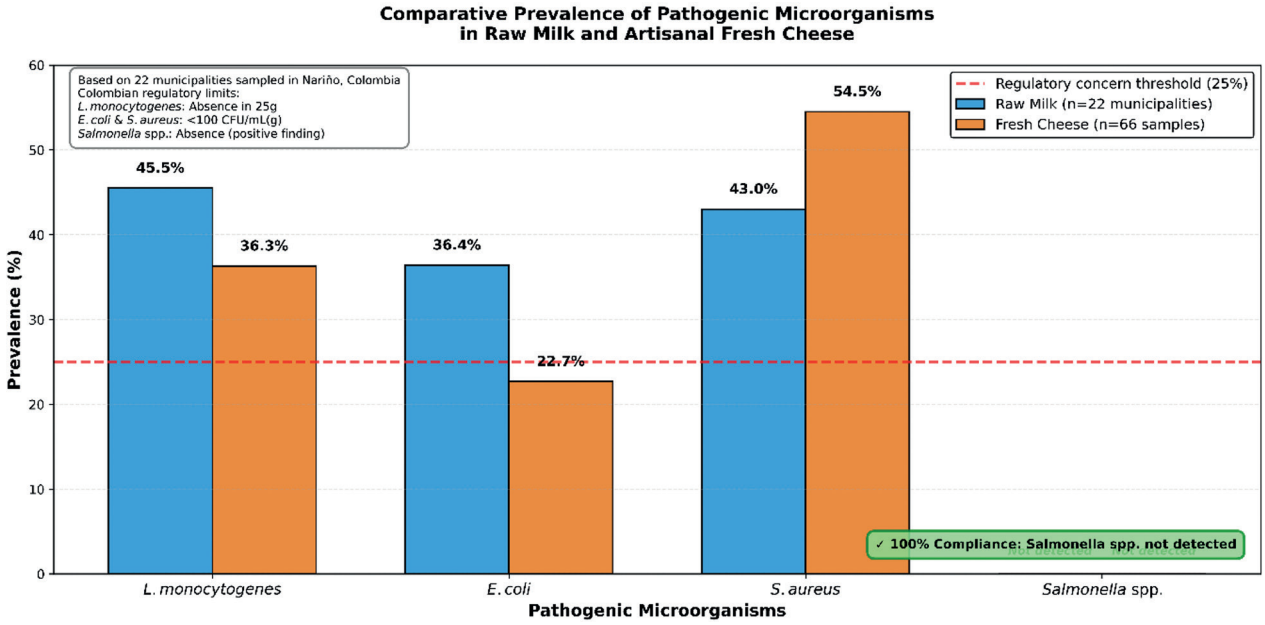

Figure 1. Comparative prevalence of pathogenic microorganisms in raw cow's milk and artisanal fresh cheese samples based on the average contamination rates across 22 municipalities in Nariño Department, Colombia.

Geographic distribution and GMP Compliance

GMP compliance evaluation across 17 sampled municipalities revealed substantial variation in infrastructure quality (Figure 2, Table S3). Overall compliance ranged from 36% to 93% (mean ± SD = 66.5% ± 17.2%). Eight municipalities (47.1%) achieved high compliance (≥70%), seven (41.2%) demonstrated moderate compliance (50%–69%), and two (11.8%) exhibited low compliance (<50%). Five municipalities (22.7%) yielded market-sourced samples without access to the production facilities, so they could not be formally evaluated.

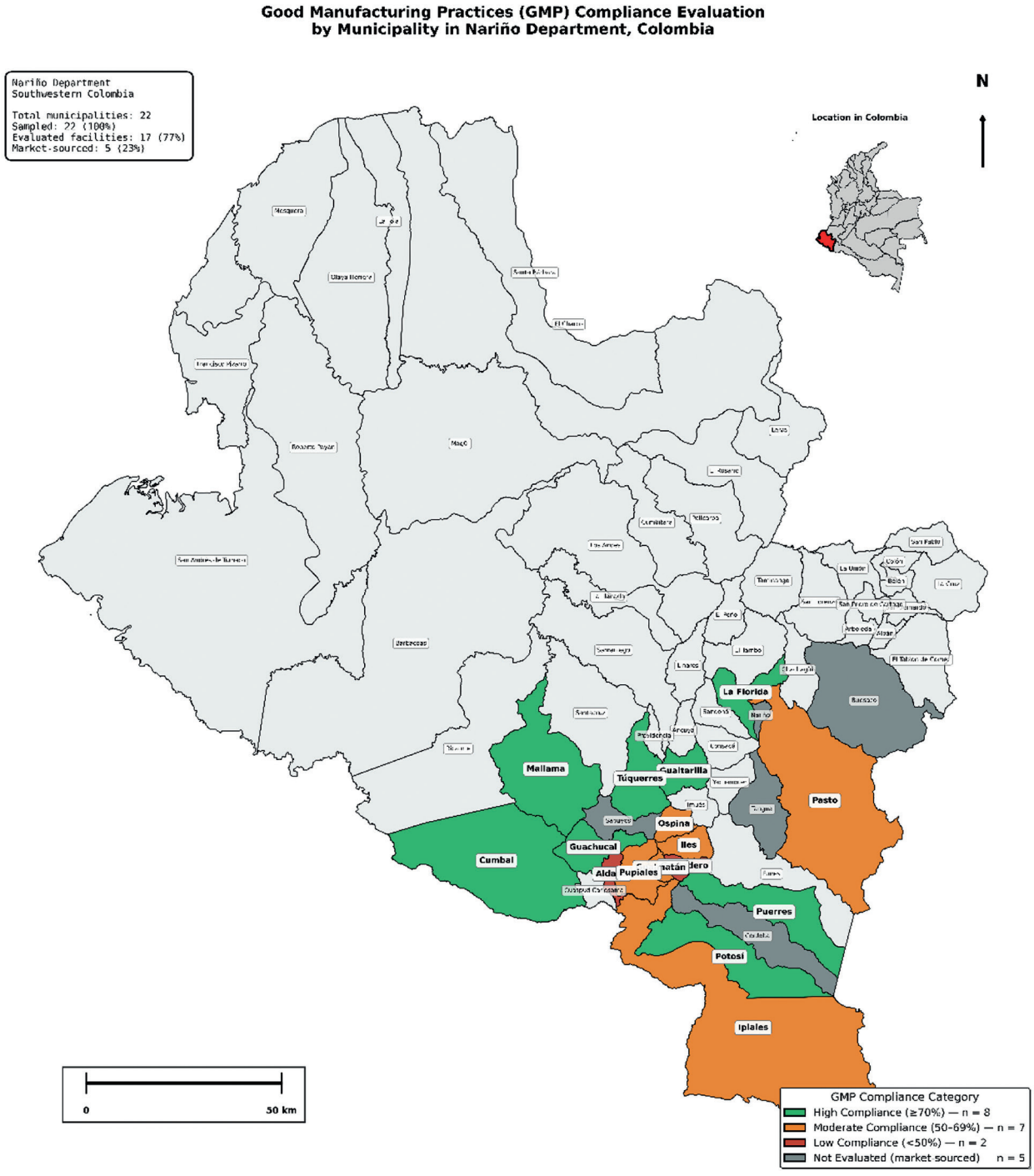

Figure 2. Geographic distribution of the evaluation of compliance with Good Manufacturing Practices (GMP) across 22 municipalities in Nariño Department, southwestern Colombia. Map data source: OCHA Humanitarian Data Exchange (35).

Identification of fresh cheese isolates

Molecular identification of isolates from β-D glucuronidase-positive *E. coli*, coagulase-positive staphylococci, and *L. monocytogenes* confirmed their presence in the municipalities. For *E. coli*, 33 isolates were identified in samples from 12 municipalities, with an identity of 97%–99% and query coverage of 92%–100%. For *L. monocytogenes*, 24 isolates were detected in samples from 8 municipalities, with identity ranging from 96% to 99%, and query coverage ranging from 85% to 100%. For *S. aureus*, 36 isolates were confirmed in 12 municipalities, with identity of 96%–99% and query coverage of 78%–100%. These findings prove the presence of pathogenic bacteria in fresh cheeses produce in Nariño Department (Table S2).

Discussion

This study represents an attempt to characterize the physicochemical and microbiological quality of raw milk and fresh cheeses made in small plants in Nariño Department, Colombia. Although raw milk is not intended for human consumption, its quality directly influences the properties of cheeses, so it is essential to monitor it throughout the production chain (36). In Colombia, the regulations for commercialization of raw milk and the determination of the purchase price from suppliers are governed by Decree 616 of 2006 and Resolution 000017 of 2012 (32). This establishes physicochemical and microbiological parameters that must be met to ensure the quality of the final product. The raw milk analyzed had an average fat content of 3.82%, higher than that reported in previous studies (37,38), indicating a lipid composition in accordance with normative standards. The average protein content was 3.22%, slightly lower than described by Lindmark Månsson (39), but comparable to what Jenkins and McGuire (40) described. Fat and protein are the most appreciated components in the industry because directly influence the cheese production yield. The protein and fat content in milk may be influenced by various factors, including season, animal breed, feed, and environmental factors (41).

Regarding the physicochemical characteristics of cheeses, the samples presented humidity of 56.6%–69.4%, which is within the established range for this type of product in Colombia. These values are comparable to reported in other countries, such as Mexico, Perú, Ecuador, and Brazil (46%–57%) (42). The fat content (12.16%–25.66%) is comparable to that described in México (17%–21%) (43) and Brazil (46.2%–48.2 %) (44). The protein content (13.83%–20.60%) is similar to those of Mexico and Cuba (17%–21%) (43,45). There were high variations in the relative standard deviation of in the evaluated parameters, indicating a lack of standardization in production processes,

which affects the properties of cheeses between the same batch (46). Better controls could improve the quality and homogeneity of the products.

High counts of mesophilic aerobes and total coliforms in raw milk were also found in the United Kingdom (47), Brazil (48), and Ecuador (49). High levels of mesophilic aerobes in raw milk can reduce its quality and shelf life, leading to a decrease in the quality of dairy products. Moreover, the presence of total coliforms is an indicator of environmental and fecal contamination from different sources (41). The results from the present study are similar to studies from other regions in Colombia. For example, in Tunja, 96.7% of the fresh cheese samples exceeded the total coliform limit (5×10^3 CFU/g) (2). In the Colombian Caribbean, 62.9% of the cheeses showed contamination by *Salmonella* spp., and 70.4% showed contamination by *Listeria* spp., demonstrating a high microbiological risk associated with the absence of GMP (50). Likewise, in Hoya del Río Suárez, *Salmonella* spp. was found in 38.3% of samples, *Listeria* spp. was found in 42.6%, and there were high plate counts of *S. aureus* and total and fecal coliforms (51). Previous studies have reported a high prevalence of *L. monocytogenes* in fresh cheese samples in Colombia, reaching levels of 42.6% in the Cundiboyacense highlands, 53.6% in Quindío Department, and 27% in Cali (3,52), indicating a recurrent sanitary problem in the country. The somatic cell count is a key criterion in determining milk quality and setting the purchase price by the dairy industry. Values greater than 400,000 cells/mL, obtained in about 50% of samples, may reflect the presence of subclinical intramammary infections in livestock, affecting the composition and stability of the milk and making it susceptible to contamination (53).

This study identified significant microbiological risks associated with artisanal fresh cheeses produced in Nariño Department, Colombia, highlighting critical implications for food safety. Most of the analyzed samples exceeded microbiological limits established by Resolution 1407 of 2022 from the Colombian Ministry of Health (34), and international standards including those of the Codex Alimentarius (54,55), the European Union Commission (Regulation (EC) No, 2073/2005) (56), the U.S. Food and Drug Administration (57), Canada (58,59), and China (60). Contamination in fresh artisanal cheeses has also been observed in countries such as Cuba and México. In Cuba, *Salmonella* spp. was detected in 17.9% of samples and *S. aureus* in 51.6%, with plate counts greater than 10^5 CFU/g (8). In Mexico, the presence of *Salmonella* spp. was reported in 8.9% of cheeses analyzed, and there were high levels of *E. coli* and coliforms (61). In Ecuador, 100% of the fresh cheeses evaluated in Riobamba were contaminated with *L. monocytogenes* (20.5 CFU/g) (62). Although there are

no international standards for mesophilic aerobes, molds, and yeasts, the high counts found (>10^6 CFU/g) shown inadequate hygiene and poor process control, indicating the need to improved hygienic practices and operator education in Latin America (61,63). In contrast, studies on artisanal cheeses from Italy and Turkey have documented the presence of *E. coli*, coliforms, and *S. aureus*, with a lower prevalence of pathogens, probably because of better hygienic practices and stricter sanitary controls (63,64).

The high prevalence of microorganisms such as *E. coli*, *S. aureus*, and *L. monocytogenes* indicates considerable deficiencies in hygienic practices, as well as inadequate or absent thermal treatments of the milk used (65). Similar microbiological safety concerns have been documented previously in other Colombian regions (2,3), suggesting a broader systemic issue in the Colombian artisanal cheese sector. In Brazil, Camargo et al. (66) reported frequent detection of *L. monocytogenes*, *S. aureus*, and fecal coliforms in cheeses made from raw milk, exceeding the established by the Codex Alimentarius and the European Commission Regulation EC 2073/200. Similar findings in Nariño Department reflect inadequate regulation, a lack of standardization, and a critical situation for dairy production in Colombia and Latin America.

International microbiological standards are particularly stringent concerning pathogens such as *Salmonella* spp. and *L. monocytogenes*: They must be completely absent from food products (57,65,67). The significant prevalence of *L. monocytogenes* found in this study highlights severe non-compliance with international hygiene and safety requirements. Regarding *S. aureus*, over 50% of analyzed samples exceeded the international limits, notably surpassing the threshold of 10^5 CFU/g set by the European Union and the U.S. Food and Drug Administration (FDA) for artisanal fresh cheeses, requiring additional testing for enterotoxins (56–58). These elevated counts represent a significant public health risk due to the potential production of heat-resistant enterotoxins that cause foodborne intoxications (68). Similar issues have been reported in Cuba, with prevalence rates reaching 51.6% (45). The presence of fecal coliforms and *E. coli* indicates direct fecal contamination and poor hygienic practices during cheese production and handling. Guidelines from the European Union and Canada allow limited *E. coli* counts as hygiene indicators, whereas the U,S, FDA provide cheeses unfit for consumption if *E. coli* counts exceed 100 CFU/g (56–58).

GMP compliance directly correlates with pathogenic contamination: Municipalities with high compliance (≥70%) showed substantially lower *L. monocytogenes* (28.1%), *E. coli* (18.4%), and *S. aureus* (23.4%) prevalence

in cheese samples compared with low-compliance facilities (<50%: 47.5%, 45.2%, and 65.1% respectively) (69). Infrastructure improvements—particularly facility design, equipment maintenance, and hygiene training—demonstrably reduce pathogenic loads, consistent with studies from Brazil and Mexico (66,70). Notably, the absence of *Salmonella* spp. across all compliance levels suggests control through initial raw material quality rather than processing (66). Because raw milk quality determined by GMP compliance is the primary determinant of final product safety (69), facilities with scores below 50% require urgent intervention through formalized training, equipment upgrades, and critical control point monitoring (70).

To improve this situation, concrete national interventions should be implemented, starting from primary milk production and continuing through the processing and distribution stages. Training programs in good agricultural practices, implementation of GMP, standardized hygiene protocols in processing plants, regular microbiological monitoring at critical points, and consumer education programs regarding proper cheese handling and storage are strongly recommended because have proven to be effective in reducing microbial loads (71,72). Such interventions could improve microbiological food safety in Colombia and facilitate the access of Colombian artisanal dairy products on global markets.

Conclusions

This study demonstrated that although the physicochemical parameters of raw cow's milk and artisanal fresh cheeses from Nariño Department generally complied with Colombian regulations, their microbiological quality remains a significant concern. High counts of mesophilic aerobes, coliforms, and *S. aureus* were frequently observed, particularly in cheese samples, indicate deficiencies in hygienic practices during milk collection, cheese production, and handling. The detection of *E. coli* and *L. monocytogenes* in several cheese samples highlights potential public health risks associated with the consumption of these artisanal products. Molecular identification confirmed *S. aureus* and *E. coli* as the predominant contaminants, reflecting inadequate sanitary conditions and limited process control within small-scale dairy facilities. Overall, these findings underscore the urgent need to strengthen GMP, to implement regular microbiological surveillance, and enforce national regulatory standards to improve food safety and protect consumer health. Future studies should focus on evaluating intervention strategies such as pasteurization, hygienic handling, and the application of protective cultures, as well as monitoring antimicrobial resistance in isolates to better understand their implications for public health.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Sauders BD, D'Amico DJ. Listeria monocytogenes cross-contamination of cheese: Risk throughout the food supply chain. Epidemiol Infect. 2016;144(13):2693–7.

2. Merchán N, Niño L, Urbano E, Zurymar S. Determinación de la inocuidad microbiológica de quesos. Rev Chil Nutr. 2019;46(3):288–94.

3. Jaramillo-Bedoya E, Trujillo-Alzate YA, Ocampo-Ibáñez ID. Vigilancia de quesos frescos artesanales revela altos niveles de contaminación por Listeria monocytogenes en el Departamento del Quindío, Colombia. Pathogens. 2021;10(10).

4. Blanco-Metzler A, Calvo MTA, Montero-Campos M, Acuña HB, Chacón EC. Vigilancia de los alimentos en Costa Rica. La Salud Pública en Costa Rica. 2015;(July):311–38.

5. Campuzano S, Mejía Flórez D, Madero Ibarra C, Pabón Sánchez P. Determinación de la calidad microbiológica y sanitaria de alimentos preparados vendidos en la vía pública de la ciudad de Bogotá D.C. Nova. 2015;13(23):81–92.

6. Lima M da costa, Da Conceição ML, Schaffner DW, De Souza EL. Intrinsic parameters and bacterial growth prediction in a Brazilian minimally ripened cheese (Coalho) during refrigerated storage. J Food Prot. 2018;81(11):1800–9.

7. Hegab OW, Abdel-Latif EF, Moawad AA. Isolation of enterotoxigenic staphylococcus aureus harboring seb gene and enteropathogenic Escherichia coli (serogroups O18, O114, and O125) from soft and hard artisanal cheeses in Egypt. Open Vet J. 2020;10(3):297–307.

8. Martínez A, Montes De Oca N, Armenteros M, Uffo O, Riverón Y, González D, et al. Identification of bacterial hazards in the production of artisan fresh cheese in Cuba. J Dairy Res. 2020;87(2):263–5.

9. Carrero S, Avila S. Evaluación microbiológica en programas de alimentación escolar en instituciones educativas en el Departamento de Boyacá – Colombia. Nova. 2017;15(28):93–8.

10. Choi, Lee H, Lee S, Kim S, Yoon Y. Cheese microbial risk assessments - A review. Asian-Australas J Anim Sci. 2016;29(3):307–14.

11. MADR, UPRA. Plan de Ordenamiento Productivo, Análisis Situacional de la Cadena Láctea. Minist Agric y Desarro Rural [Internet]. 2021;1–230. Available at: https://upra.gov.co/es-co

12. USDA. An Overview of the Colombian Dairy Market. Report No. CO2024-0013 [Internet]. Bogota D.C; 2024. Available at: https://www.fas.usda.gov/data/colombia-overview-colombian-dairy-market

13. AOAC. Official methods of analysis: 22nd edition. Available at: https://doi.org/10.1093/9780197610145.002.001

14. Instituto Colombiano de Normas Técnicas y Certificación [ICONTEC]. NTC 750. PRODUCTOS LACTEOS, QUESO. Norma Tecnica Colombiana 2009.

15. Experiment A, Dairy N, Hall S, Scientific W, Laboratories C, Cusak E, et al. Method : Collaborative Study. 2001;1499–508.

16. Andrade EHP, da Fonseca LM, de Souza MR, de Andrade Morais Penna CF, Cerqueira MMOP, de Oliveira Leite M. Fat content in fermented milk beverages: Determination by the Gerber method. Semin Agrar. 2022;43(1):441–8.

17. Sulejmani E, Amedi A. Physico-chemical and sensory characterization of fresh cheeses. Lett Food Res. 2025;1(2):1–7.

18. International Organization for Standardization [ISO]. Aerobios mesófilos ISO 4833-1:2013/Amd 1:2022. 2022;47700026.

19. ISO. Coliformes ISO 4832:2006 Recuento de colonias (Revisado y confirmado en 2021). 2021;47700026.

20. ISO. ISO 16649-2. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of b-glucuronidase-positive Escherichia coli. Int Stand. 2001;2001.

21. ISO. ISO 6888-1:2022. Microbiología de la cadena alimentaria. Método horizontal para el recuento de estafilococos coagulasa positivos (Staphylococcus aureus y otras especies). Parte 1: Método que utiliza un medio de agar Baird-Parker. (ISO 6888-1:2021). 2022. Available at: https://www.une.org/encuentra-tu-norma/busca-tu-norma/norma?c=N0069680

22. ISO. ISO 11290-1. Método horizontal para la detección y el recuento de Listeria monocytogenes y de Listeria spp. 2018. Available at: https://tienda.aenor.com/norma-une-en-iso-11290-1-2018-n0059546

23. ISO. ISO 6579-1:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella. 2020;1–11. Available at: https://www.sag.gob.cl/sites/default/files/itanalisis-para-deteccion-de-salmonella-spp.-segun-norma-iso-6579_0.pdf

24. Izquierdo PF, Martínez Muñoz JF, Miramag Yaqueno KM, Ortiz Benavides F. Caracterización molecular de microorganismos silvestres asociados a fermentaciones artesanales de cacao en el municipio de Los Andes, Nariño, Colombia. Nova. 2025;23(44):105–27.

25. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiol Methods. 2007;69(2):330–9.

26. Başkan C, Çelebi G. Evaluation of extended-spectrum β-lactamase production and plasmid-mediated quinolone resistance in Escherichia coli isolated from raw milk. LWT. 2024;207.

27. Bozcal E. Isolation and identification of Staphylococcus aureus obtained from cheese samples. Food Heal. 2020;6(3):151–9.

28. Hellberg RS, Martin KG, Keys AL, Haney CJ, Shen Y, Smiley RD. 16S rRNA partial gene sequencing for the differentiation and molecular subtyping of Listeria species. Food Microbiol. 2013;36(2):231–40. http://dx.doi.org/10.1016/j.fm.2013.06.001

29. Hall T, Biosciences I, Carlsbad C. BioEdit: An important software for molecular biology. GERF Bull Biosci. 2011;2(June):60–1.

30. Okonechnikov K, Golosova O, Fursov M, Varlamov A, Vaskin Y, Efremov I, et al. Unipro UGENE: A unified bioinformatics toolkit. Bioinformatics. 2012;28(8):1166–7.

31. Ministerio de la Protección Social. Decreto Numero 616 de 2006 Reglamento Técnico sobre los requisitos que debe cumplir la leche para el consumo humano. Minist la Protección Soc [Internet]. 2006;32. Available at: https://www.ica.gov.co/getattachment/15425e0f-81fb-4111-b215-63e61e9e9130/2006D616.aspx

32. Ministerio de Agricultura y Desarrollo Rural. Resolución 000017 de 2012. 2012. p. 1–18.

33. Moradi M, Omer AK, Razavi R, Valipour S, Guimarães JT. The relationship between milk somatic cell count and cheese production, quality and safety: A review. Int Dairy J. 2021;113:104884. https://doi.org/10.1016/j.idairyj.2020.104884

34. Ministerio de Salud y Protección Social. Resolución 1407 de 2022: Criterios microbiológicos que deben cumplir los alimentos y bebidas destinados para consumo humano. 2022. p. 1–27.

35. OCHA. Colombia - Subnational administrative boundaries | Humanitarian dataset | HDX [Internet]. 2020 [cited 3 Nov 2025]. Available at: https://data.humdata.org/dataset/cod-ab-col

36. Skeie S. Characteristics in milk influencing the cheese yield and cheese quality. J Anim Feed Sci. 2007;16(Suppl. 1):130–42.

37. Haug A, Høstmark AT, Harstad OM. Bovine milk in human nutrition - A review. Lipids Health Dis. 2007;6:1–16.

38. Surkova A, Shmakova Y, Salukova M, Samokhina N, Kostyuchenko J, Parshina A, et al. LED-based desktop analyzer for fat content determination in milk. Sensors. 2023;23(15):1–13.

39. Lindmark Månsson H. Fatty acids in bovine milk fat. Food Nutr Res. 2008;52:1–4.

40. Jenkins TC, McGuire MA. Major advances in nutrition: Impact on milk composition. J Dairy Sci. 2006;89(4):1302–10. http://dx.doi.org/10.3168/jds.S0022-0302(06)72198-1

41. Kazeminia M, Mahmoudi R, Mousavi S, Mehrabi A. Raw cow milk quality: Physicochemical, microbiological, and seasonal variation. J Microbiol Biotechnol Food Sci. 2023;13(3):1–8.

42. Guzmán L, Mayorga N, Mejía C. Design and development of fresh cheese enriched with soja oil. Apunt Cienc Soc. 2015;05(02):280–6.

43. Ramírez-López, C, Vélez-Ruiz JF. Quesos frescos: Propiedades, métodos de determinaci ón y factores que afectan su calidad. Temas Sel Ing Aliment. 2014;2:18.

44. Silva CB, Ferreira LM, Lima AR, Araújo KGL, Souza RM, Fonseca ABM, et al. Microbiological quality and cultivable bacterial community of fresh and ripened Minas cheeses made from raw and pasteurised milk. Int Dairy J. 2023;143.

45. Martínez A, Villoch A, Ribot A. Evaluación de la calidad e inocuidad de quesos frescos artesanales de tres regiones de una provincia de Cuba Evaluation of quality and safety of fresh artisan cheeses from three regions of a Cuban province. Rev Salud Anim. 2013;35(3):210–3.

46. Fox PF, Guinee TP, Cogan TM, McSweeney PL. Fundamentals of Cheese Science. Second Edi. Milk and Dairy Products in Human Nutrition: Production, Composition and Health. 2017. 477–497 p.

47. Elmoslemany AM, Keefe GP, Dohoo IR, Dingwell RT. Microbiological quality of bulk tank raw milk in prince edward Island dairy herds. J Dairy Sci. 2009;92(9):4239–48. http://dx.doi.org/10.3168/jds.2008-1751

48. Costa Sobrinho PDS, Marçal De Faria CA, Silva Pinheiro J, Gonçalves De Almeida H, Vieira Pires C, Silva Santos A. Bacteriological quality of raw milk used for production of a brazilian farmstead raw milk cheese. Foodborne Pathog Dis. 2012;9(2):138–44.

49. Bustamante–Ordoñez JG, Vintimilla-Rojas AE, Andrade–Guzmán OS, Abad–Quevedo VL, Agurto–Granda DA, López–Espinoza MD, et al. Calidad–inocuidad de la leche cruda de vaca que ingresa a centros de acopio de la provincia Cañar–Ecuador, en el contexto de las normativas Latinoamericanas. Rev Científica la Fac Ciencias Vet. 2023;XXXIII(1):1–8.

50. Soto-Varela ZE, Gutiérrez C, Moya Y, Mattos R, Bolivar-Anillo H, Villareal J. Detección molecular de Salmonella spp., Listeria spp. y Brucella spp. en queso artesanal fresco comercializado en Barranquilla: un estudio piloto. Biomédica. 2018;38:30–6.

51. Alfonso-Vargas NC, Aguilera-Becerra A, Jaimes-Bernal CP, Pulido-Medellín MO, Arias-Mosso JMA. Caracterización microbiológica de queso fresco artesanal distribuidos en la Hoya del Río Suárez, Colombia. Duazary. 2024;21(1):7–18.

52. Ibáñez IDO, González C, Moreno SL, Calderón C, Flórez Elvira LJ, Olaya MB, et al. Presence of Listeria monocytogenes in fresh artisanal cheese marketed in Cali-Colombia. Acta Agron. 2019;68(2):108–14.

53. Albenzio M, Figliola L, Caroprese M, Marino R, Sevi A, Santillo A. Somatic cell count in sheep milk. Small Rumin Res. 2019;176(May):24–30. https://doi.org/10.1016/j.smallrumres.2019.05.013

54. OMS, FAO. GENERAL STANDARD FOR CHEESE CXS 283-1978 Adopted in 1978. Revised in 1999. Codex Aliment. 2024;

55. OMS, FAO. Standart for Fermented Milks CXS 243-2003 Adopted in 2003. Revised in 2008, 2010, 2018. Amended in 2022. CXS 243-2003 2022 Amendment The following Amendment was Made to The Text of The Standard following Decisions Taken at the forty-fifth session of. Fao. 2022;

56. European-Commission. Commission regulation (EC) No 2073/2005 [Internet]. Official Journal of the European Union. 2005. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073

57. FDA. Food Code [Internet]. FDA, 2022. Food Code, US Public health service. 2022 p. 248–53. Available at: https://www.fda.gov/food/fda-food-code/food-code-2022

58. Health Canada. Microbial guidelines for ready-to-eat foods - A guide for the conveyance industry and environmental health officers (EHO). 2013;9. Available at: www.healthcanada.gc.ca/

59. Ganz K, Yamamoto E, Hardie K, Hum C, Hussein H, Locas A, et al. Microbial safety of cheese in Canada. Int J Food Microbiol. 2020;321:108521. Disponible en: https://doi.org/10.1016/j.ijfoodmicro.2020.108521

60. State Administration for Market Regulation. National standards of the People's Republic of China. 29921-2021. China; 2021.

61. Chávez-Martínez A, Paredes-Montoya P, Rentería-Monterrubio AL, Corral-Luna A, Lechuga-Valles R, Dominguez-Viveros J, et al. Microbial quality and prevalence of foodborne pathogens of cheeses commercialized at different retail points in mexico. Food Sci Technol. 2019;39:703–10.

62. Lopez Salazar JR, Rodríguez-Haro CE, Lopez-Ullari VG, Diaz-Monroy BL. Prevalencia de Listeria monocytogenes en quesos frescos artesanales en la ciudad de Riobamba, Ecuador. Polo del Conoc. 2022;7(2):1–12.

63. Pasquali F, Valero A, Possas A, Lucchi A, Crippa C, Gambi L, et al. Occurrence of foodborne pathogens in Italian soft artisanal cheeses displaying different intra- and inter-batch variability of physicochemical and microbiological parameters. Front Microbiol. 2022;13:1–12.

64. Ercan D, Korel F, Orşahin H. Microbiological quality of artisanal Sepet cheese. Int J Dairy Technol. 2014;67(3):384–93.

65. Hickey M. Legislation in relation to cheese [Internet]. Fourth Edi. Vol. 1, Cheese: Chemistry, Physics and Microbiology: Fourth Edition. Elsevier Ltd; 2017. 757–778 p. http://dx.doi.org/10.1016/B978-0-12-417012-4/00030-2

66. Camargo AC, de Araújo JPA, Fusieger A, de Carvalho AF, Nero LA. Microbiological quality and safety of Brazilian artisanal cheeses. Brazilian J Microbiol. 2021;52(1):393–409.

67. OMS, FAO. Codez Alimentarius. Alimentos producidos orgánicamente. Codex Aliment. 2003;2:75.

68. Choi KH, Lee H, Lee S, Kim S, Yoon Y. Cheese microbial risk assessments - A review. Asian-Australasian J Anim Sci. 2016;29(3):307–14.

69. Torres Vargas GM, Cepeda Fonseca HE, Patiño Pacheco DC. Evaluación y correlación de la calidad sanitaria y microbiológica del suero ácido derivado del queso doble crema. Rev Salud Pública. 2022;24(6):1–10.

70. Ibarra-sánchez LA, Olvera-aguilar AL, Caltzontzin-fernández KK, José A. Communications ® Small family cheesemaking in Mexico: Microbial quality and good manufacturing practices. JDSC. 2025;(2018). http://dx.doi.org/10.3168/jdsc.2025-0801

71. FAO, OMS. Cxs 283-1978 Norma General Para El Queso. 2022. Available at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B283-1978%252FCXS_283s.pdf

72. Codex Alimentarius. Code of hygienic practice for milk and milk products (CAC/RCP 57-2004). Code Hyg Pract Milk Milk Prod. 2004;

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