

Physicochemical and microbiological quality according to the production systems of bovine milk and fresh cheese (cuajada) in the Isthmus of Tehuantepec, Mexico

Running title: Production and characterization of cow's milk and cheese in Oaxaca, Isthmus of Tehuantepec

Section: Original Research

doi: 10.22201/fmvz.24486760e.2025.1429

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Dates:

Submitted: 2024-10-11

Accepted: 2025-09-13

Published: 2026-01-22

How to cite this:

Ramón-Canul LG, Cuervo-Osorio VD, Santiago-Cabrera R, Cabal-Prieto A, Uribe-Cuauhtzihua F, Hernández-Arzaba JC, Valdivia-Sánchez J, Armida-Lozano J, Hernández-Salinas G, Sosa-Gutiérrez DS, Chay-Canul AJ, Ramírez-Rivera EJ. Physicochemical and microbiological quality according to the production systems of bovine milk and fresh cheese (cuajada) in the Isthmus of Tehuantepec, Mexico. *Veterinaria México OA*. 2026;13. doi: 10.22201/fmvz.24486760e.2025.1429.

Physicochemical and Microbiological Quality According to the Production Systems of Bovine Milk and Fresh Cheese (“Cuajada”) in the Isthmus of Tehuantepec, Mexico

Abstract

The objective of this study was to characterize the physicochemical and microbiological quality of bovine milk and fresh cheese (“cuajada”) produced in the Isthmus of Tehuantepec, Oaxaca, Mexico, as well as to analyze the livestock production systems associated with their manufacture. For each production system, 10 L of raw bovine milk and 10 samples of fresh cheese (500 g each) were evaluated, determining both microbiological and physicochemical parameters. Producers in these systems ranged from 43 to 72 years old and had completed higher education. The cattle herds consisted of 64–187 head, with predominant breeds including Zebu (*Bos indicus*), Swiss, and Indubrasil (*Bos taurus* × *Bos indicus*), yielding 100–168 liters of milk per day. The results showed that both milk and “cuajada” failed to meet the microbiological standards established by Mexican regulations. Regarding physicochemical properties, cheese moisture content was high (50–61 %), while protein ranged from 17–35 % and fat from 8–22 %. Color analysis revealed high lightness (L^* 87–93) and yellowish (b^* 15–20), whereas texture profile indicated significant differences in hardness (5–50 N) and cohesiveness. The findings highlight the importance of providing technical training to improve the quality and safety of these products while preserving their cultural and traditional value.

Keywords: Cheese; Color; Mexico; Cattle; Texture profile.

Study Contribution

In Mexico, the bovine livestock sector plays a major role in the national economy; therefore, it is essential to understand how production systems are structured and what their limitations are. The aim of this study is to contribute holistically to scientific knowledge through a comprehensive characterization of product quality within production systems, analyzing quality standards in accordance with current regulations and integrating an approach that includes both technical and socioeconomic variables. Furthermore, the reported results emphasize the relevance of providing producers with technical assistance to help their products achieve higher quality and safety standards.

Introduction

Cheese production is an important industry worldwide, and a large part of it is carried out through artisanal methods.⁽¹⁾ In Mexico, artisanal cheese production dates back to colonial times. Since then, both the production and consumption of cheese have increased, leading to a wide variety of production methods currently available on the market (2025).⁽²⁾ In small and medium-sized enterprises, cheeses are typically produced using raw milk and, less frequently, pasteurized milk; artisanal and region-specific methods are commonly employed.⁽³⁾ Consequently, the product exhibits heterogeneity in its physicochemical and organoleptic characteristics. Furthermore, because most cheeses are made from raw milk using rudimentary and non-standardized techniques, they may contain pathogenic microorganisms, increasing the risk of foodborne diseases.^(3, 4)

Artisanal cheese is valued for its nutritional characteristics, sensory attributes, and the traditional process involved in its manufacture.⁽²⁾ The physicochemical and sensory properties of these products are determined by geographic location, cultural and natural factors related to traditional know-how, the type of coagulant used, and the inherent properties of the milk.^(2, 3) Among these properties, the natural microbiota of dairy cattle and the type of feed consumed stand out as determining factors.⁽⁵⁾ Moreover, understanding the physicochemical and microbiological characteristics of milk is essential to contribute to the standardization of these products. Artisanal cheese is an integral component of both the Mexican diet and economy.⁽⁶⁾

However, because it is produced from raw milk, it often fails to meet Mexican food safety regulations.⁽¹⁾ Therefore, ensuring the safety of this product is essential to preserve a tradition that has been passed down from generation to generation.^(3, 5) The fresh cheese known as cuajada is made from raw cow's milk in the Mexican municipalities of Juchitán de Zaragoza, San Pedro Comitancillo, Matías Romero, Unión Hidalgo, El Espinal, and Asunción Ixtaltepec— all located in the Isthmus of Tehuantepec region in the state of Oaxaca. This type of cheese is cylindrical, white in color, and constitutes a fundamental element of the diet of consumers in the Isthmus of Tehuantepec, Oaxaca, where it is commonly eaten with totopos (toasted corn tortillas), sauces, or as part of various traditional dishes.

For local cheese producers, this food represents a significant source of income due to its high demand in local markets, contributing directly to their family economies. Unfortunately, scientific studies on this product remain scarce; therefore, this study proposes the characterization and standardization of its entire production process.

Accordingly, the objective of this study was to characterize the production systems and to determine the physicochemical and microbiological quality of milk and fresh cheese produced in the Isthmus of Tehuantepec, Oaxaca.

Materials and Methods

Characterization of Production Systems

Six producers were interviewed to characterize each of the family-based production systems located within the Oaxacan municipalities of San Pedro Comitancillo, Asunción Ixtaltepec, and El Espinal, in the state of Oaxaca. This research was conducted during 2016–2017. Production systems were coded according to the first letters of the municipality name followed by numbers 1 and 2, which indicate the reference coordinates. COMI1 corresponds to San Pedro Comitancillo at 16°43'32" N, 95°12'13" W; COMI2 also corresponds to San Pedro Comitancillo at 16°29'05" N, 95°07'54" W. IXT1 refers to Asunción Ixtaltepec at 16°30'58" N, 95°03'08" W, while IXT2 corresponds to Asunción Ixtaltepec at 16°49'73" N, 95°06'36" W. ESP1 represents El Espinal at 16°49'35" N, 95°04'41" W, and ESP2 to El Espinal at 16°47'23" N, 95°03'35" W.

A structured interview form was used (see supplementary material), including the following sections: (a) personal data, (b) socioeconomic aspects of the producer, (c) feeding and forage management, (d) animal health, (e) milk production, (f) characterization of artisanal cheeses, and (g) conditions of artisanal cheese (cuajada) production.^(7, 8) The interviews were conducted in person with the producers.

Collection and Transport of Milk and Cheese Samples

Samples were collected by simple random sampling following the Mexican Official Standard for sampling, handling, and transportation of food samples.⁽⁹⁾ Six production systems were evaluated, from each one 10 L of raw bovine milk and 10 samples of fresh cheese (500 g each) were collected. The cheese was produced from the same batch of sampled milk. Milk and cheese samples were placed in sterile containers and transported at 4 °C. Upon arrival at the laboratory, the temperature of milk and cheese were recorded and samples were stored under refrigeration (6–7 °C). All samples were processed within eight hours of receipt.

Production of the Cuajada

Milk was obtained by manual milking and filtered through cheesecloth to remove impurities. It was then heated to 37 °C, and commercial rennet was added and left to act for 120 min. Once the cuajada formed, it was cut and whey was manually removed using cheesecloth. Salt was then added directly. The cuajada was placed into plastic molds and, after two hours, the cheeses were removed from these, packed in polyethylene bags, and stored at 7 °C.

Microbiological Analysis of Milk and Cheese

Microbiological counts were performed using 3M™ Petrifilm™ plates, following the procedures described by the *Association of Official Analytical Chemists*.⁽¹⁰⁾ In the milk, the following microorganisms were determined: aerobic mesophilic bacteria,⁽¹¹⁾ total coliforms,⁽¹²⁾ *Escherichia coli*⁽¹³⁾ and *Staphylococcus aureus*.⁽¹⁴⁾ *Salmonella* ssp. and

Brucella abortus were analyzed according to Mexican Official Standards (NOM) and the World Organization for Animal Health guidelines.⁽¹⁵⁻¹⁷⁾ For aerobic mesophiles, 25 g or 25 mL of sample was homogenized with 225 mL of peptone water in serial dilutions (10^{-2} and 10^{-3}). Aliquots were plated on agar by the standard plate count method and incubated at 35 ± 2 °C for 48 ± 2 h.

For total coliforms, 25 g of sample were mixed with 225 mL of peptone water (10^{-2} and 10^{-3} dilutions) They were plated on violet red bile agar and incubated at 35 ± 2 °C for 48 ± 2 h. *Escherichia coli* was determined by homogenizing 25 g of sample in 225 mL of peptone water; aliquots were plated on eosin methylene blue agar and incubated at 35 ± 2 °C for 48 ± 2 h. Suspected colonies were confirmed through biochemical tests (indole, Simmons citrate, methyl red–Voges Proskauer). For *Staphylococcus aureus* 25 g of each sample was homogenized in 225 mL of peptone water, plated on Baird-Parker agar, and incubated at 35 ± 2 °C for 48 ± 2 h. Isolates were confirmed by coagulase, catalase, and thermonuclease tests.

For *Salmonella* identification, 25 g of sample was pre-enriched in lactose broth at 35 ± 2 °C for 24 ± 2 h, then transferred to selenite cystine broth (35 ± 2 °C, 24 ± 2 h), and plated on Xylose Lysine Deoxycholate agar (XLD) and Samonella-Shigella agar under identical incubation conditions. Final identification included Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), urea, and serological tests (agglutination with specific antisera). For *Brucella abortus*, milk samples were homogenized in sterile saline supplemented with penicillin (100 IU/mL) and streptomycin (100 µg/mL), centrifuged at $3\ 000 \times g$ for 15 min. The sediment was plated on dextrose-serum agar and incubated at 37 °C in a 5–10 % CO₂ atmosphere for 21 days.

Isolates were confirmed through oxidase, catalase, urease, and H₂S production tests. All determinations were performed in quintuplicate. Results of microbiological analyses for aerobic mesophiles and total coliforms were expressed as Log₁₀ CFU/mL or grams for statistical analysis, while *Salmonella*, *E. coli*, *B. abortus*, and *S. aureus* results were reported as absence and presence.

Physicochemical Analysis of Milk and Cheese

In milk, protein, fat, lactose, added water, non-fat solids (NFS), salts, freezing point-FP (°C), conductivity (mS/cm³), and density (kg/m³) were determined using an ultrasonic milk analyzer (Lactoscan S, Milkotronic Ltd., 4 Narodni Buditeli Str., 8900 Nova Zagora, Bulgaria). Acidity (g/L of lactic acid) was determined according to AOAC method 947.05.⁽¹⁰⁾ In cheese, the following parameters were analyzed: protein (method 920.123), moisture (method 948.12), ash (method 935.42),⁽¹⁰⁾ and lipids using the Gerber–Van Gulik method.⁽¹⁸⁾ The pH was measured using a potentiometer (Hanna Instruments, model HI 98230, Italy) calibrated with pH 4 and 7 buffer solutions (Sigma México, Mexico), and water activity (A_w) was determined using a Pawkit meter (Decagon Devices Inc., USA). All analyses were carried out in quintuplicate.

Color and Texture Profile of Cheese

Color and texture analyses were conducted using an UltraScan® Vis colorimeter (HunterLab, Hunter Associates Laboratory Inc., Reston, Virginia, USA), calibrated with a white standard to obtain L* (lightness), a* (red–green), and b* (yellow–blue) parameters.

From the latter two values, chroma (C^*) and hue angle (H°) were calculated using equations (1) and (2):

$$C^* = \frac{(a^*)^2 + (b^*)^2}{2} \quad (1)$$

$$H^\circ = \arctan(b^*/a^*) \quad (2)$$

The CIELAB (CIELAB) scale was used with a D65 light source and a 10° observer angle.⁽¹⁹⁾ Texture profile analysis (TPA) was performed using a TA-XTplus texture analyzer (Texture Technologies Corp., Scarsdale, NY / Stable Micro Systems, Haslemere, Surrey, UK). In order to develop the texture profile, cheese samples were cut into cylinders (3 cm diameter \times 5 cm height). Measurements were taken at a constant temperature ($20 \pm 2^\circ\text{C}$) using a compression plate probe (P/75 aluminum, 75 mm diameter). The test consisted of two successive axial compression cycles to 25 % of the probe height without load. Texture parameters evaluated included hardness, fracturability, adhesiveness, cohesiveness, gumminess, springiness, and chewiness.⁽¹⁹⁾ Both color and texture tests were performed five times.

Statistical Analysis

The normality of data distribution was assessed using the Shapiro–Wilk test. For variables meeting this assumption, a one-way analysis of variance (ANOVA) was performed with a significance level of $\alpha = 0.05$. When statistically significant differences were detected, Tukey's test was applied. For variables violating normality assumptions, a 95 % Winsorized transformation was applied to minimize the influence of outliers, and normality

was re-evaluated using the Shapiro–Wilk test. If the transformed data met normality assumptions, ANOVA followed by Tukey’s test was conducted. When normality was not achieved even after transformation, the non-parametric Kruskal–Wallis test was used. All analyses were performed in quintuplicate and statistically processed using “*Statgraphic Centurion*” version XV and XLSTAT 2021.^(20, 21)

Results and Discussion

Characterization of Production Systems

Producers from the municipalities included in this study had an average age of 53 years, with the youngest producer (43 years) and the oldest (72 years) both belonging to the municipality of San Pedro Comitancillo. Producers from Asunción Ixtaltepec and El Espinal had graduated from university. Land tenure in San Pedro Comitancillo and Asunción Ixtaltepec was communal, whereas in El Espinal it was privately owned. The largest cattle inventories were recorded in San Pedro Comitancillo and Asunción Ixtaltepec, with 187 and 149 head of cattle, respectively, while El Espinal had 64 head.

Only San Pedro Comitancillo had the breeds Zebu (*Bos indicus*), Swiss and Indubrasil (*Bos taurus* × *Bos indicus*); whereas the other municipalities had a high proportion of Zebu (*Bos indicus*) and Swiss (*Bos taurus*) cattle. Daily milk production in San Pedro Comitancillo, Asunción Ixtaltepec, and El Espinal was 150, 168, and 100 L/day, respectively. Cattle feeding in Comitancillo was based on agricultural by-products such as maize stover (*Zea mays* L.), grain sorghum stalks (*Sorghum vulgare* L.) and grazing on native grasses such as grama grass *Axonopus affinis* and *Axonopus compressus*, as well as “Guishi Beuu” grass (*Cenchrus* sp.) and roadside grass

(*Bothriochloa pertusa*). In Ixtaltepec, producers used native pasture and sorghum bales, while in El Espinal, native grass was the main feed source.

Microbiological Analysis of Raw Milk

Table 1 presents the results of the microbiological analysis of raw milk; no significant differences were observed among the variables analyzed. According to the sanitary specifications established for raw milk, the results of this study revealed non-compliance with the microbiological limits set by the NOM⁽²²⁾ regarding the aerobic mesophilic bacteria count. The standard establishes a maximum limit of $\leq 100,000$ CFU/mL, a value exceeded by the samples analyzed, indicating microbial contamination. This finding suggests possible deficiencies in milking, storage, or transportation practices, which may promote bacterial proliferation.

Other contributing factors may include environmental and climatic conditions, inadequate post-milking handling, herd health, and the socioeconomic context of the producers — who often lack access to cooling technologies, training in good livestock practices, and the financial resources to implement strict sanitary measures —. Iñiguez-Muñoz et al.⁽²³⁾ reported that cow milk obtained from producers in Tepatitlán de Morelos, Jalisco, exceeded maximum limits (5.8–6.1 Log₁₀ CFU/mL). Regarding total coliforms, milk samples from production systems IXT1, IXT2, ESP1, and ESP2 exhibited the highest microbial loads (5.3, 5.6, 5.4, and 5.2 Log₁₀ CFU/mL, respectively).

Yeasts were detected in all milk samples (3–4.7 Log₁₀ CFU/mL) except for ESP1. This may be due to the presence of yeasts in the production environment, which could act as a natural source of contamination of raw milk.⁽²⁴⁾ However, fungi were absent,

possibly because mesophilic bacteria and yeasts grow rapidly in milk and produce inhibitory metabolites (acids, alcohols, bacteriocins) that create an unfavorable environment for fungi, which grow more slowly and are sensitive to acidification or antimicrobial compounds. All milk samples were negative for *S. aureus* and *Salmonella* spp. Milk samples from COM11, ESPI1, and ESP2 contained *E. coli*, whereas *B. abortus* was detected in COM12.

The levels of aerobic mesophiles, total coliforms, yeasts, and *E. coli* found in this study did not comply with the limits established by the NOM.⁽²⁵⁾ These results demonstrate deficiencies in hygiene and milking routines that lack proper management practices during milk collection and storage.⁽²⁶⁾ Therefore, continuous training of producers in the implementation of good agricultural and livestock practices is essential to improve the microbiological quality of milk.^(26, 27)

Table 1. Microbiological analysis of milk from municipalities in the Isthmus of Tehuantepec, Oaxaca

Milk sample	Aerobic mesophiles	Total coliforms	Molds	Yeasts [†]	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>S. aureus</i>	<i>B. abortus</i>
COMI1	7.6 ^a ±1.75	3.7 ^b ±0.5	0 ±0	4.0 ^b ±1.05	Present	Absent	Absent	Absent
COMI2	7.8 ^a ±1.9	4.1 ^b ±0.5	0 ±0	3.0 ^c ±0.0	Absent	Absent	Absent	Present
IXT1	7.9 ^a ±1.65	5.3 ^a ±0.6	0 ±0	4.4 ^a ±1.5	Absent	Absent	Absent	Absent
IXT2	7.5 ^a ±1.35	5.6 ^a ±1.0	0 ±0	4.7 ^a ±0.6	Absent	Absent	Absent	Absent
ESP1	7.9 ^a ±1.19	5.4 ^a ±1.0	0 ±0	0.0 ^d ±00	Present	Absent	Absent	Absent
ESP2	7.4 ^a ±2.6	5.2 ^a ±1.05	0 ±0	4.4 ^a ±1.5	Present	Absent	Absent	Absent

Microbiological counts are expressed as Log₁₀ CFU/mL.

a, b, c, d Different letters in the same column indicate significant differences according to Tukey's test (n = 5; P ≤ 0.05). Data represent mean ±standard deviation. † = Variables analyzed using the Kruskal–Wallis test.

COMI1 = San Pedro Comitancillo 1; COMI2 = San Pedro Comitancillo 2; IXT1 = Asunción Ixtaltepec 1; IXT2 = Asunción Ixtaltepec 2; ESP1 = El Espinal 1; ESP2 = El Espinal 2.

Physicochemical Analysis of Milk

Significant differences ($P \leq 0.05$) were observed for all physicochemical parameters evaluated (**Table 2**). The protein content (2.66–3.02 %) of the samples was consistent with the values established by the NOM⁽²⁸⁾ (2.8–3.1 %), except for samples ESP1 and ESP2. Milk fat content (4.05–7.24 %) complied with the reference parameter set by the standard (≥ 3.2 %). However, the analyzed milk samples did not meet the NOM⁽²⁸⁾ specifications for milk solids-non-fat (MSNF; 8.3 %). Among all samples, only COMI2 (4.3 %) met the reference range established by the NOM⁽²⁸⁾ (4.3 to 5 %) for lactose content.

Regarding density and freezing point (FP), milk values ranged between 1 023.55–1 027.15 kg/m³ and -0.52 to -0.46 °C, respectively. These results did not meet the NOM⁽²⁸⁾ values, which specify a density of 1 029 kg/m³ and FP range of -0.51 to -0.536 °C. These deviations may be influenced by interrelated factors, such as the high fat content of the samples, which reduces the overall density of milk. Additionally, deviations in FP and density, together with the non-fat solids content, suggest possible dilution with added water.⁽²⁹⁾

Table 2. Physicochemical analysis of milk from municipalities in the Isthmus of Tehuantepec, Oaxaca

Milk sample	Proteins[†] (%)	Fat (%)	SNF[†] (%)	Lactose[†] (%)
COMI1	2.84 ^b ±0.01	4.05 ^b ±0.20	7.66 ^b ±0.02	4.05 ^b ±0.01
COMI2	3.02 ^a ±0.00	6.01 ^c ±0.11	8.11 ^a ±0.01	4.30 ^a ±0.00
IXT1	2.74 ^c ±0.05	7.24 ^a ±0.53	7.34 ^{cd} ±0.13	3.85 ^d ±0.08
IXT2	2.80 ^b ±0.03	5.69 ^b ±0.45	7.48 ^c ±0.11	3.95 ^c ±0.06
ESP1	2.71 ^{cd} ±0.02	6.08 ^b ±0.26	7.26 ^{de} ±0.05	3.81 ^{de} ±0.03
ESP2	2.66 ^d ±0.02	6.23 ^b ±0.37	7.15 ^e ±0.05	3.75 ^e ±0.03

Milk sample	Density (kg/mL)	FP (°C) [†]	Conductivity (mS/cm ³) [†]	Salts (%) [†]
COMI1	1 025.95 ^b ±0.61	-0.48 ^c ±0.00	4.67 ^d ±0.59	0.73 ^d ±0.00
COMI2	1 027.15 ^a ±0.06	-0.52 ^d ±0.00	5.07 ^b ±0.08	0.80 ^a ±0.00
IXT1	1 023.86 ^d ±0.70	-0.48 ^c ±0.01	4.80 ^c ±0.02	0.75 ^b ±0.01
IXT2	1 025.10 ^c ±0.49	-0.48 ^c ±0.00	4.43 ^e ±0.01	0.74 ^c ±0.00
ESP1	1 024.04 ^d ±0.28	-0.47 ^b ±0.01	4.74 ^{cd} ±0.03	0.72 ^e ±0.00
ESP2	1 023.55 ^d ±0.34	-0.46 ^a ±0.00	5.17 ^a ±0.05	0.71 ^f ±0.00

a, b, c, d Different letters in the same column indicate significant differences according to Tukey's test (n = 5; P ≤ 0.05).

Data are expressed as mean ± standard deviation. † = Variables analyzed using the Kruskal–Wallis test. SNF = Solids non-fat; FP = Freezing point.

COMI1 = San Pedro Comitancillo 1; COMI2 = San Pedro Comitancillo 2;
 IXT1 = Asunción Ixtaltepec 1; IXT2 = Asunción Ixtaltepec 2; ESP1 = El Espinal 1;
 ESP2 = El Espinal 2.

The values of conductivity and salt content were within the ranges of 4.43–5.18 mS/cm³ and 0.71–0.80%, respectively (**Table 2**). Regarding acidity, the milk samples COMI2 and ESP1 exhibited the highest values, 1.92 and 1.98 g of lactic acid/L, which exceeded the limits established in the NOM⁽²⁸⁾ (1.3–1.6 g of lactic acid/L). These elevated values may result from several factors, such as failure to maintain milk at adequate refrigeration temperatures or improper handling during storage or transportation, both of which favor microbial contamination. This assumption is supported by the microbiological analyses of the milk samples, which exceeded the limits allowed by Mexican regulations.^(19, 25) Noa-Pérez et al.⁽²⁴⁾ evaluated raw milk in the state of Jalisco, Mexico, and found that 65.8 % of the samples contained between 1.3 and 1.6 g of lactic acid/L.

Microbiological and Physicochemical Analysis of Cheese

Cheeses produced under the different production systems did not comply with the maximum microorganism limits established by the NOM.⁽³⁰⁾ These samples showed a high microbial load (aerobic mesophiles, total coliforms, fungi, yeasts, and *E. coli*) (**Table 3**), which can be attributed to the fact that the milk used for cheese production already exceeded the microbial limits allowed by the NOM.⁽²⁸⁾ These cheeses were highly contaminated, which could cause foodborne diseases (FBD). However, the incidence of FBD associated with the consumption of this type of cheese in these regions is still unknown. It is therefore essential to raise awareness among producers regarding good hygiene and sanitation practices to ensure the production of safe products and enable them to compete commercially in different.

Table 3. Microbiological analysis of cheeses from municipalities in the Isthmus of Tehuantepec, Oaxaca

Cheese	Aerobic mesophiles	Total coliforms [†]	Molds [†]	Yeasts	<i>E. coli</i>	<i>Salmonella</i> spp.
COMI1	7.9 ^a ±0.5	4.4 ^a ±0.3	0.0 ^b ±0.0	3.7 ^a ±1.4	Present	Absent
COMI2	8.1 ^a ±0.0	4.2 ^a ±0.4	0.0 ^b ±0.0	3.3 ^a ±0.0	Absent	Absent
IXT1	7.7 ^a ±1.65	4.5 ^a ±0.8	2.0 ^a ±0.0	2.8 ^b ±0.1	Present	Absent
IXT2	7.8 ^a ±2.65	4.5 ^a ±0.5	2.0 ^a ±0.1	2.3 ^c ±0.3	Absent	Absent
ESP1	7.8 ^a ±0.50	0.0 ^c ±0.0	1.7 ^a ±0.5	3.5 ^a ±0.9	Absent	Absent
ESP2	7.7 ^a ±1.25	2.9 ^b ±0.1	2.0 ^a ±0.0	3.1 ^{ab} ±0.3	Present	Absent

Microbiological data were standardized as Log₁₀ CFU/mL.

a, b, c, d Different letters in the same column indicate significant differences according to Tukey's test (n = 5; P ≤ 0.05). Data represent mean ± standard deviation. † = Variables analyzed using the Kruskal–Wallis test.

COMI1 = San Pedro Comitancillo 1; COMI2 = San Pedro Comitancillo 2; IXT1 = Asunción Ixtaltepec 1; IXT2 = Asunción Ixtaltepec 2; ESP1 = El Espinal 1; ESP2 = El Espinal 2.

Cheeses COMI1 (35.31 %) and COMI2 (27.46%) showed the highest protein levels (P < 0.05) (**Table 4**). These values are consistent with those reported by Silva-Paz et al.⁽²⁷⁾ for fresh cheeses from Ojos Negros, Baja California, Mexico (28.10–32.98%), suggesting that the milk used in San Pedro Comitancillo has a high protein value or that the cheese-making process favors protein retention. In contrast, IXT1 (17.46 %) and ESP1 (17.04 %) cheeses exhibited lower protein contents, similar to those reported by Díaz et al.⁽²⁾ for commercial fresh cheeses (18–25 %), which could be due to differences in cattle feeding or the degree of whey drainage during manufacture.⁽²⁷⁾

ESP1, ESP2, and IXT1 cheeses presented the highest fat levels (22.22, 22.06, and 21.60 %, respectively), indicating greater lipid retention during processing. These values fall within the range reported by Díaz et al.⁽²⁾ for fresh cheeses (12–32 %). The low fat levels observed in COMI1 and COMI2 (10.19 and 7.99 %) may reflect partial skimming or differences in the lipid composition of the raw milk.⁽²⁾ All cheeses exceeded 50 % moisture, with the highest values in COMI2 (61.42 %), followed by IXT1 (58.01 %) and IXT2 (57.80 %). These results are in agreement with those of De la Rosa-Alcaraz et al.⁽³⁾, who reported that fresh cheeses typically contain between 50 % and 65 % moisture. The high moisture content in COMI2 may contribute to its soft texture and shorter shelf life, as it favors microbial growth.⁽³¹⁾

The cheeses IXT2 (4.25 %), ESP1 (3.66 %), and COMI1 (3.60 %) exhibited the highest ash levels, reflecting a higher mineral content, possibly due to salt addition or the natural composition of the milk. These values are comparable to those reported by Díaz et al.⁽²⁾ for Mexican fresh cheeses (3.2–3.8 %).

Table 4. Physicochemical analysis of cheeses from municipalities in the Isthmus of Tehuantepec, Oaxaca

Cheese	Protein (%)[†]	Fat (%)[†]	Moisture (%)[†]	Ash (%)[†]	Aw (%)	pH
COMI1	35.31 ^a ±5.98	10.19 ^{bc} ±5.60	50.89 ^d ±0.33	3.60 ^b ±0.13	0.94 ^a ±0.00	5.25 ^b ±0.1
COMI2	27.46 ^b ±1.55	7.99 ^c ±1.55	61.42 ^a ±0.00	3.11 ^c ±0.03	0.94 ^a ±0.00	5.82 ^a ±0.29
IXT1	17.46 ^c ±1.11	21.60 ^a ±1.01	58.01 ^b ±0.15	2.99 ^c ±0.10	0.95 ^a ±0.00	5.65 ^a ±0.21
IXT2	23.50 ^b ±0.88	14.43 ^b ±2.43	57.80 ^b ±1.71	4.25 ^a ±0.05	0.94 ^a ±0.00	5.48 ^a ±0.14
ESP1	17.04 ^c ±1.45	22.22 ^a ±1.12	57.06 ^{bc} ±0.11	3.66 ^b ±0.11	0.93 ^b ±0.00	5.34 ^{ab} ±0.14
ESP2	18.94 ^c ±0.56	22.06 ^a ±0.32	56.21 ^c ±0.23	3.13 ^d ±0.63	0.93 ^b ±0.00	5.06 ^b ±0.04

a, b, c Different letters within the same column indicate significant differences according to the Least Significant Difference test (n = 5; P ≤ 0.05). ±/= standard deviation. † = variables analyzed using the Kruskal–Wallis test. COMI1 = San Pedro Comitancillo 1; COMI2 = San Pedro Comitancillo 2; IXT1 = Asunción Ixtaltepec 1; IXT2 = Asunción Ixtaltepec 2; ESP1 = El Espinal 1; ESP2 = El Espinal 2.

Regarding water activity (Aw), two groups were identified (P < 0.05). The first group, comprising COMI1, COMI2, IXT1, and IXT2 cheeses, showed Aw values ranging from 0.94 to 0.99, while the second group, ESP1 and ESP2, exhibited lower values (Aw = 0.93). However, no significant differences (P > 0.05) were observed in pH among COMI2, IXT1, IXT2, and ESP1 cheeses, which had values of 5.8, 5.6, 5.4, and 5.3, respectively. The physicochemical quality of the cheeses may be attributed to several factors, primarily the composition of the milk and the manufacturing process involved in the production of fresh “cuajada”-type cheese.^(2, 32)

Instrumental Color Analysis of Cheeses

The results show that the ESP2 cheese exhibited the highest lightness (L^*) value (93.45), whereas COM11 presented the lowest (87.11). This variability in L^* could be attributed to differences in moisture content, as cheeses with higher moisture tend to reflect more light, resulting in greater luminosity and lower color saturation. This finding is consistent with previous studies that associate lightness with cheese microstructure, where greater water retention enhances light scattering, thereby reducing opacity.^(33, 34)

The practical implication of these results lies in quality control and sensory acceptance. Cheeses with higher L^* values may be perceived as fresher and creamier, which is relevant for markets that value visual attributes.⁽³³⁾ However, contrasting with studies such as that of Wendorff et al.,⁽³⁴⁾ who reported high L^* values in ripened cheeses, increased lightness can also be associated with a softer texture due to proteolysis, which alters the cheese structure, making it less cohesive and thus more reflective.

The low a^* values (between -2.42 and -3.04) indicate the absence of perceptible red/green hues, consistent with the findings of García-Simões et al.⁽³⁵⁾ in Brazilian fresh cheeses. In contrast, the b^* values (15.10 – 19.99) confirm a dominance of yellow tones, particularly in COM11, which could be related to the presence of carotenoids or thermal treatment of the milk.⁽³⁵⁾ The chroma (C^*) and hue angle (H°) values near 80° in COM11 and COM12 further reinforce this yellowish tendency, whereas cheeses from Ixtaltepec (IXT1, IXT2) exhibited slightly lower H° values, suggesting differences in composition or processing conditions (**Table 5**).

Table 5. Color analysis of cheeses from municipalities in the Isthmus of Tehuantepec, Oaxaca

Cheese	L*	a*	b*†	Chroma†	H°†
COMI1	87.11 ^d ±0.29	-2.68 ^b ±0.22	19.99 ^a ±0.95	20.16 ^a ±0.98	82.32 ^a ±0.27
COMI2	90.25 ^c ±0.84	-3.04 ^c ±0.17	15.94 ^{bc} ±0.49	16.23 ^{bc} ±0.52	82.83 ^a ±6.19
IXT1	91.85 ^b ±0.33	-2.87 ^{bc} ±0.09	15.50 ^c ±0.26	15.77 ^c ±0.27	79.47 ^a ±0.26
IXT2	92.41 ^{ab} ±0.33	-2.94 ^c ±0.01	16.78 ^b ±0.12	17.03 ^b ±0.12	79.78 ^a ±0.52
ESP1	91.02 ^{bc} ±1.17	-2.63 ^{ab} ±0.04	15.77 ^{bc} ±0.40	15.99 ^{bc} ±0.40	80.52 ^a ±0.11
ESP2	93.45 ^a ±1.01	-2.42 ^a ±0.04	15.10 ^c ±0.82	15.29 ^c ±0.81	80.84 ^a ±0.38

a, b, c, d Different letters within the same column indicate significant differences according to Tukey's test (n = 5; P ≤ 0.05). Data represent the mean ± standard deviation. L* = lightness; a* = variation between green (-a) and red (+a); b* = variation between blue (-b) and yellow (+b); Chroma = color saturation; H° = hue angle. † = variables analyzed using the Kruskal–Wallis test. COMI1 = San Pedro Comitancillo 1; COMI2 = San Pedro Comitancillo 2; IXT1 = Asunción Ixtaltepec 1; IXT2 = Asunción Ixtaltepec 2; ESP1 = El Espinal 1; ESP2 = El Espinal 2.

Instrumental Texture Analysis of Cheeses

The hardness parameter showed a statistically significant difference (P < 0.05) among the evaluated cheeses. The ESP2 sample (50.19 N) exhibited the highest hardness value, which may be attributed to a more rigid protein matrix, possibly resulting from lower proteolytic activity or a higher casein content.^(29, 30) Except for ESP2, the hardness values of all cheeses were lower than those reported by Ballesta-Rodríguez⁽³⁶⁾ and Guzmán et al.,⁽³⁷⁾ who found average hardness values of 26.53 and 16 N, respectively. In this context, cheeses from the municipality of El Espinal (ESP1 and ESP2) showed greater

resistance ($P < 0.05$) to deformation, gumminess, and chewiness; this effect may be associated with their higher moisture content, which contributes to a firmer texture.⁽³⁸⁾

Moreover, the gumminess (0–5.01) and chewiness (0–5.02) values suggest that these cheeses require greater energy to fracture, making them suitable for culinary applications that demand heat resistance. Regarding cohesiveness, only cheeses from San Pedro Comitancillo (COMI1 and COMI2) exhibited high values ($P < 0.05$), a result linked to their moisture content, since higher moisture generally increases the internal binding forces within the cheese matrix.⁽³⁹⁾ The findings highlight the impact of production practices on the quality attributes of fresh “cuajada”-type cheeses. However, one of the limitations of this study is the absence of sensometric analyses that could define sensory attributes and cognitive aspects such as emotions and memories that influence consumer preference or rejection of each artisanal cheese representative of the Isthmus of Tehuantepec region, Oaxaca (**Table 6**).

Table 6. Texture profile analysis of cheeses from municipalities of the Isthmus of Tehuantepec, Oaxaca

Cheeses	Hardness (N) †	Cohesiveness†	Gumminess†	Springiness†	Chewiness†
COM1	7.08 ^c ±2.26	-0.01 ^a ±0.20	2.28 ^a ±0.46	0.573 ^a ±0.51	0.22 ^a ±0.46
COM2	5.74 ^c ±2.35	-0.06 ^a ±0.03	0.46 ^a ±0.63	0.61 ^a ±0.49	0.45 ^a ±0.64
IXT1	10.85 ^{bc} ±4.55	-1.71 ^{ab} ±2.14	1.88 ^a ±1.58	0.79 ^a ±0.44	1.88 ^a ±1.58
IXT2	9.51 ^{bc} ±2.86	-1.39 ^{ab} ±1.79	0.37 ^a ±0.84	0.44 ^a ±0.45	0.37 ^a ±0.84
ESP1	24.43 ^b ±4.61	-3.71 ^a ±3.85	5.01 ^a ±7.11	0.46 ^a ±0.50	5.02 ^a ±7.08
ESP2	50.19 ^a ±0.91	-0.00 ^b ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00

a, b, c Different letters in the same column indicate significant differences according to Tukey's test (n = 5; P ≤ 0.05). Data represent mean ± standard deviation. † = variables analyzed using the Kruskal–Wallis test. COM1 = San Pedro Comitancillo 1; COM2 = San Pedro Comitancillo 2; IXT1 = Asunción Ixtaltepec 1; IXT2 = Asunción Ixtaltepec 2; ESP1 = El Espinal 1; ESP2 = El Espinal 2.

Conclusions

The analyzed production systems are predominantly family-based, involving older producers (average age 53 years) with educational levels that include completed higher education. The cattle herds range from 64 to 187 head, with predominant breeds being Cebu, Brown Swiss, and Indubrasil, and a daily milk production between 100 and 168 liters. Raw milk does not comply with the microbiological limits established by Mexican official standards, showing high loads of aerobic mesophiles, total coliforms, yeasts, and, in some cases, *Escherichia coli* and *Brucella abortus*. This indicates deficiencies in hygiene practices during milking, storage, and transportation.

The cheese is classified as fresh, with a high moisture content (50–61 %) and variability in protein (17–35 %) and fat (8–22 %) contents. These results reflect differences

in milk composition and in processing steps, such as whey drainage and salt addition. Color and texture analyses indicate high luminosity and low cutting resistance. Based on these findings, this study provides a scientific basis for understanding the characteristics and challenges in the production of milk and fresh curd-type cheese in the Isthmus of Tehuantepec region, Oaxaca, Mexico. The results highlight the importance of technical interventions to improve the quality and safety of these products while preserving their cultural and traditional value.

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Data availability

All relevant data are contained within the manuscript.

Acknowledgments

The authors express their gratitude to the producers of the Isthmus of Tehuantepec region for their support and donation of the samples analyzed in this research.

Funding

This study did not receive funding from any agency.

Conflict of interest

The authors declare no conflict of interest regarding this publication.

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