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Article

Isolation and identification of *Staphylococcus aureus*, *Salmonella* spp., and coliforms in artisanal cheeses from Aculco, State of Mexico

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Abstract:

The consumption of milk-based products is of great economic and social importance in Mexico. Artisanal cheeses are the economic basis of many small producers in the country; nevertheless, one of the major threats to this sector is the manifestation of foodborne diseases (FBDs) due to poor quality and safety during the production process. Three cow's milk cheese production units were sampled in the municipality of Aculco, State of Mexico, Mexico, with the aim of detecting the presence of *Staphylococcus aureus*, *Salmonella* spp, and total coliforms on plate in artisanal cheeses. The methodology of NOM-210-SSA1-2014 and the methodology of NOM-113-SSA1-1994 were used, obtaining results for the three production units above the limits allowed by the regulations for *S. aureus* and total coliforms, but demonstrating the absence of *Salmonella* spp. An evaluation was made by means of questionnaires and observation of the production, sanitization, and storage processes of the products, as well as descriptive statistics of the results, to establish the main causes of the high counts in the microbiological analyses, determining that the main cause is the lack of

implementation of good manufacturing practices, such as hand washing, specific clothing for the work area, and sanitization of the different areas of the cheese factories and the tools used during the manufacturing process.

Keywords: Dairy products, Artisanal cheeses, *Staphylococcus aureus*, *Salmonella* spp, Coliform bacteria.

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Introduction

Dairy farming represents a primary activity of great relevance in Mexico, not only because of its economic impact, but also because of its ability to generate value-added products[1]. According to the Agrifood and Fisheries Information Service (SIAP, for its acronym in Spanish), cow's milk production increased by 2.3 % between 2019 and 2020, reaching 12,563 million liters, with an average annual growth rate of 1.5 % since 1980[1,2]. This growth reflects a sustained demand for milk both for direct consumption and for its industrialization and import[2]. Despite this increase, a deficit between national production and domestic demand persists, which is attributed to factors such as limited access to technology, lack of training, and inequality in access to government services[3,4]. Faced with this situation, artisanal cheese production continues in regions such as Aculco, State of Mexico, where traditional practices are preserved within the so-called cheese-dairy basins[5]. Data from the 2020 National Household Income and Expenditure Survey by INEGI show that 28 % of family spending is allocated to food and beverages, and of this percentage, 9 % corresponds to the consumption of milk and dairy products, including a monthly average of $289.00 in milk and $254.00 in other dairy products, such as cheeses[6]. Although a significant part of consumption comes from industrialized products, artisanal cheeses maintain a relevant presence in the national market[6].

The growing preference for traditional and organic products has driven the purchase of artisanal foods[6]. In this context, two types of cheese factories are recognized: the artisanal one, which is characterized by its dependence on manual dexterity and minimal technology; and the traditional or family factory, where inherited recipes and unique, locally recognized characteristics are preserved[5]. Both promote the local economy and have a positive impact on regional tourism[6]. Despite the advance of agribusiness, the demand for artisanal cheeses

has increased due to the symbolic value they provide: cultural identity, family history, and unique sensory characteristics[7]. These factors have become marketing tools in the face of industrialized products[7].

In Mexico, about 75 % of cheese production corresponds to small-scale plants engaged in artisanal production, with soft cheeses being the main destination for milk, with a proportion of 75 to 80 % compared to 20-25 % for hard or semi-hard cheeses[8,9]. Nonetheless, this type of production also carries risks. The absence of standardized processes can compromise the quality and safety of products, increasing the risk of foodborne diseases[10]. Among the main challenges faced by genuine Mexican cheeses are variability in quality and health, high production costs, marketing problems, lack of regulatory compliance, and an uncertain future for many producers[11].

Although there are efforts to standardize the production of artisanal cheeses, the varying conditions of each production unit make them difficult to implement. However, improving safety is a priority objective to reduce health risks[10]. Every producer has the responsibility to guarantee the quality of their food. To do this, they must consider Good Manufacturing Practices and comply with current regulations, especially with NOM-210-SSA1-2014, which regulates microbiological test methods, and NOM-113-SSA1-1994, which establishes the method for the total coliform count[12,13].

Material and methods

The study site was the municipality of Aculco, State of Mexico, Mexico, which is recognized for the production and sale of artisanal cheeses, located at an altitude of 2,400 m, and characterized by an average temperature of 14 °C and a subhumid mountain climate. Four samplings were performed on different dates: September 25, November 8, January 8, and March 8; they were carried out in three cheese factories, coded as follows:

Cheese factory A (CFA); Cheese factory B (CFB); Cheese factory C (CFC), which produce Manchego, ranchero, string cheese, botanero, panela, and morral cheeses. In order to characterize the artisanal process of each of the cheese factories, observations were made during the process considering the following items of Good Manufacturing Practices (GMPs): GMPs related to staff activities; GMPs related to facilities; and GMPs related to the use of tools and utensils before, during and after the process.

In order to obtain a diagnosis of the situation of each of the cheese factories and with the aim of analyzing the information to conclude whether the contamination of the products was related to poor manufacturing practices, dichotomous questionnaires were applied, which were prepared based on the items of the FAO GMP Manual for the production of dairy products[9], and the studies carried out by other authors[10], for the characterization of the cheese factories evaluated. Likewise, the production process was observed to obtain tests during the production of the different cheeses for all the cheese factories; we also participated in the production of ranchero cheese, string cheese, and morral cheese in the three cheese factories, to facilitate the application of the questionnaire with the cheesemakers in charge and to observe the routine of the manufacturing and packaging processes.

Sample collection and shipment

The cheese samples were obtained based on the reference of PROPOSED NOM-109-SSA1-1994 "Proposed Official Mexican Standard, Goods and Services, Procedures for the Taking, Handling, and Transport of Food Samples for Microbiological Analysis", for foods exposed to the open air and other contaminants, performing the correct hand washing and wearing a gown, cap, face mask, and sterile gloves. Sterile polyethylene bags were used to store the samples, clearly identifying each of them, and they were stored in polystyrene coolers with refrigerants to preserve them at a temperature of approximately 4 °C until they were transferred to the laboratory in a period of no more than 12 h for immediate microbiological processing, not exceeding 24 h after collection[14].

Estimation of *S. aureus* count

The Reference Method was used for estimating the *Staphylococcus aureus* count in accordance with the normative appendix B of the Official Mexican Standard NOM-210-SSA1-2014, products and services. Microbiological test methods. Determination of indicator microorganisms. Determination of pathogenic microorganisms[12].

For each cheese, 25 g was transferred to dilution bottles with 225 ml of sterile phosphate buffer solution, to prepare a 1:10 dilution, 0.1 ml of the initial suspension (10^{-1} dilution) was transferred with a sterile pipette in duplicate to Baird-Parker agar plates and the procedure was repeated for the following dilutions. The inoculum was distributed over the surface of the agar with sterile glass rods at right angles, using one for each plate and dilution. The plates were kept with the lids facing up until the inoculum was fully absorbed by the agar.

The plates were inverted and incubated for 48 h at 37 °C; after this time, colonies with typical colonial morphology were sought, that is, colonies that were black, circular, shiny, convex, smooth, with a diameter of 1 to 2 mm and that showed an opaque, moist area with a clear halo (due to the activity of lecithinase) around the colony. Plates with between 15 and 150 typical and atypical colonies of *S. aureus* were selected; five typical colonies were selected per sample for confirmation for Gram staining; coagulase, catalase, and anaerobic use of glucose and mannitol tests were performed to confirm the presence of *S. aureus*[12].

Presence of *Salmonella* spp.

The Reference Method for the isolation of *Salmonella* spp. was used in accordance with the normative Appendix A: Reference method for the isolation of *Salmonella* spp. of the Official Mexican Standard NOM-210-SSA1-2014, Products and Services. Microbiological test methods. Determination of indicator microorganisms. Determination of pathogenic microorganisms[12].

An initial suspension was performed with 25 g of each cheese sample in 225 ml of sterile peptone water as a pre-enrichment medium to obtain a 1:10 dilution, and the initial dilution was incubated at 37 °C for 18 h. For selective enrichment, 0.1 ml of the pre-enrichment culture was transferred to a 10 ml tube of Rappaport-Vassiliadis Soya (RVS) broth and 1 ml to a tube containing 10 ml of Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth. The RVS broth was incubated at 41.5 °C for 24 h and the MKTTn broth at 36 °C for 24 h. For isolation, a set of agar plates was used, consisting of a xylose-lysine-deoxycholate (XLD) agar plate, a brilliant green agar plate, and a bismuth sulfite agar plate. Using a bacteriological loop, the agar plates were seeded using the pure culture technique. Once this process was completed, the plates were incubated at 36 °C for 24 h; once the incubation time had elapsed, each of the agar plates was checked for suspect colonies of *Salmonella* spp. To confirm the presence of *Salmonella* spp, at least one suspect colony was taken from the culture media for inoculation into the nutrient agar by streaking and incubated at 36 °C for 24 h; after this time, lysine iron agar (LIA) and triple sugar iron (TSI) agar were seeded. For the LIA agar, it was seeded by puncturing 1.5 cm from the bottom of the medium; on the TSI agar, the medium was seeded by puncturing the bottom and streaking on an inclined surface; once inoculation was performed, both tests were incubated at 36 °C for 24 h, and after the incubation time had elapsed, the biochemical tests were read[12].

Estimation of total coliforms on plate

The method for the plate count of total coliform microorganisms was used in accordance with the Official Mexican Standard NOM-113-SSA1-1994, Goods and Services. Method for the plate count of total coliform microorganisms[13].

First, the violet red bile agar culture medium was prepared according to the manufacturer's instructions; once the medium was homogenized, it was autoclaved. At the end of this process, the culture medium was removed and kept at a temperature of 45 °C until used in the procedure. A primary dilution of 10 g of sample was prepared and added to 90 ml of diluent (sterile peptone water), then the dilution was placed in the blender for 2.5 min until a homogeneous mixture was obtained; after this, 1 ml of the initial dilution was transferred to the first test tube, homogenizing and transferring one milliliter to the next tube until five dilutions were obtained. A Petri dish was used in duplicate for each of the samples, one milliliter was added to each Petri dish for each of the dilutions and 13 ml of the culture medium was added; immediately, the inoculum was mixed with the medium with six movements from right to left and six movements in a clockwise direction and six movements in the opposite direction, all on a flat surface[13]. Once the medium was solidified, the plates were inverted and incubated at 35 °C for 24 h; after this time, the dishes were removed from the incubator for observation, selecting the plates that had between 15 and 150 typical colonies, which are red with a precipitation halo due to the light red or pink bile salts, with colonial morphology of biconvex lenses with a diameter of 0.5 to 2 mm[13].

Data analysis

The samples were operationalized by type of cheese and by cheese factory. The Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used to determine whether the data followed a normal trend. The Kruskal-Wallis test was used to compare a distribution between different groups, in this case, the types of cheese. For the application of the statistical tests, Excel and SPSS version 29.0 were used. Scores were given to the responses to the questionnaires and to what was observed during the preparation process to quantify compliance with the GMPs as follows:

Score of 0, requirement not met. Score of 1, requirement partially met. Score of 2, the requirement is met.

To characterize the cheese factories, the García-Rincón[15] model was used [%GMP Compliance= Score obtained (SOB)/Maximum score (SMX)*100], placing them in three possible categories depending on their percentage of compliance:

Favorable: score of 90-100 %; favorable with requirements: score of 60-89 %; unfavorable: score less than 59 %.

Results

A total of 36 samples of cheeses were obtained from the producers, which are distributed as follows:
Cheese Factory A (CFA), where the following were sampled: 5 string cheeses, 4 ranchero cheeses, 1 Manchego cheese.
Cheese Factory B (CFB), where the following were sampled: 4 ranchero cheeses, 4 string cheeses, 3 morral cheeses, 1 panela cheese, 1 Manchego cheese.
Cheese Factory C (CFC), where the following were sampled: 4 ranchero cheeses, 4 botanero cheeses, 4 Manchego cheese, 1 string cheese.

Observations were made and questionnaires were filled out during the stay in the cheese factories; it was possible to observe the entire process for the varieties of cheeses sampled, from the reception of the milk to the packaging and storage of the products. The results obtained from the observations and questionnaires applied were expressed (Tables 1 and 2). The total score obtained in the questionnaires and their percentage of compliance with GMPs were determined, characterizing them as follows:

CFA: favorable with requirements (79.3 %); CFB: unfavorable (45.7 %); CFC: unfavorable (13 %).

Table 1: Score obtained in each of the cheese factories according to the GMPs that were evaluated

	Cheese factory		
GMPs related staff activities	**CFC**	**CFB**	**CFA**
Personal hygiene	0	1	2
Correct use of the uniform	1	1	0
Hand washing	0	1	1
No jewelry	0	2	2
Correct use of caps and face masks	0	0	0
No evidence of eating, chewing gum, drinking beverages, or smoking during the process	0	0	2
Personal belongings in places defined for this purpose	0	1	2
GMP training	0	0	0
Staff registration and hygiene control	0	0	0
GMPs related to facilities	**CFC**	**CFB**	**CFA**
Use of exclusive facilities for cheese making	0	2	2
The process area is set apart from other areas that could cause cross-contamination	0	0	2
The design of the cheese factory allows for the correct cleaning of the cheese factory (floors, walls, and ceilings)	0	1	2
Proper ventilation	0	1	2
Pest free	0	0	0
Adequate lighting	1	2	2
Correct storage of raw materials	0	2	2
Correct storage of the finished product	2	1	2
Correct storage of chemicals	1	2	2
The exterior of the establishment is in good conditions of maintenance and cleanliness	0	0	2
GMPs related to utensils during the process	**CFC**	**CFB**	**CFA**
Design of equipment and utensils that allow easy cleaning and disinfection	1	2	2
A routine of cleaning and disinfecting utensils is established	0	2	2
There is a cleaning routine prior to the process development	0	0	1
Areas free of unused utensils and equipment	0	0	2

Table 2: Observations of the production process

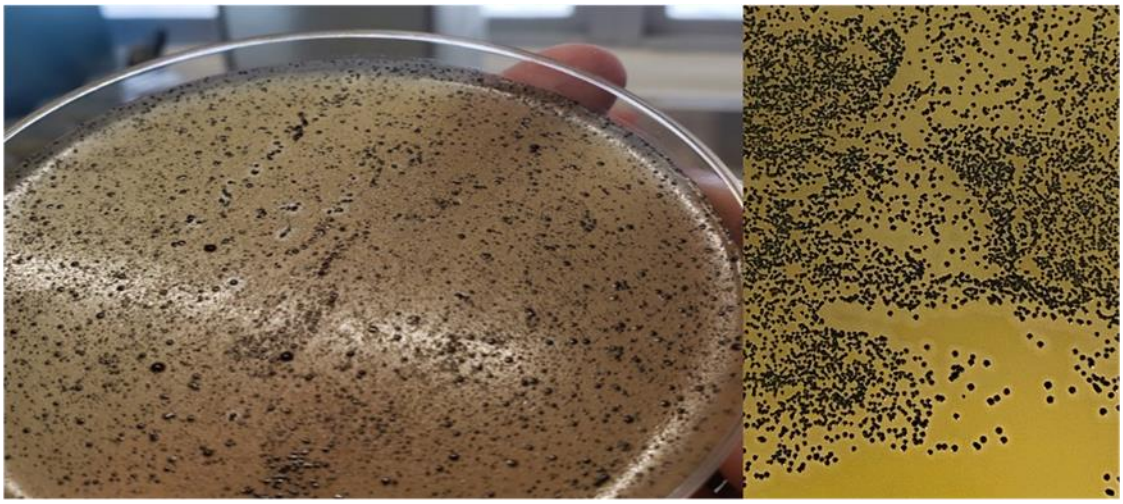
	Cheese factory A	**Cheese factory B**	**Cheese factory C**
Family history and structure	Founded by the father of the current owner. Family members and five external people work.	The work is carried out by family members (grandfather and granddaughters) and two cheesemakers from outside the family.	Two parents, daughters, and grandchildren work, as well as two external workers.
Cleansing routine	Containers are washed with soap and water; however, it is insufficient as there were traces of curd from previous days in the cheese factory's mill.	Worn containers and tables are washed with soap and water.	Surface washing of work tables with water without soap and detergent. Buckets are washed with water and whey resulting from the manufacturing process. Containers are washed with water and soap powder.
Cheese factory and GMPs	They wear work boots and aprons; they do not accept the use of caps and face masks; they do not use the sink to wash their hands. Doors of the cheese factory are open while making the cheese; therefore, there are airflows and flies on the curd.	They wear boots, aprons, and caps, but do not use face masks. Strip curtains at the entrance of the cheese factory, absence of hand washing, which is done in a bucket. Storage refrigerator with broken door.	Door of the cheese factory is open and the glass is broken, which allows flies to enter. They do not have work clothes, and do not wear caps, aprons, face masks, and work boots. Buckets are contaminated with bovine feces. They lack a handwashing area; they only rinse them with the whey.

Counting, isolating and identifying *S. aureus*

In the tests for the determination of CFU/g of cheese for *S. aureus*, typical colonies were observed on the Baird-Parker agar, which were round, smooth-edged, convex, 1-2 mm in diameter, moist, shiny and black, with the formation of the clear halo around the colony due to the action of lecithinase (Figure 1). Confirmatory testing for the presence of *S. aureus* showed positive results for the coagulase test, determined by the formation of the clot at 24 h; likewise, a positive reaction to the catalase test was observed, and gram-positive cocci grouped in clusters were observed with Gram staining of the suspect colonies. When counting

(Table 3), it was determined that most of the sampled cheeses do not comply with the limits allowed by NOM-210-SSA1-2014[12].

Figure 1: *Staphylococcus aureus* colonies on Baird-Parker agar



Colonies 1-2 mm in diameter, moist, shiny, and black with the formation of the clear halo around the colony due to the action of lecithinase.

Table 3: *S. aureus* count in the 36 cheeses sampled

Name of the cheese factory	Type of cheese	CFU/g Sept 25	CFU/g Nov 08	CFU/g Jan 08	CFU/g Mar 07
CFA	String cheese	54	10,000	4000	150,000
CFA	Ranchero	150,000	10,000	280,000	150,000
CFA	Aged and smoked string cheese	NA	10,000	NA	NA
CFA	Manchego	NA	NA	NA	150,000
CFB	Ranchero	162	10,000	220,000	150,000
CFB	Morral cheese	173	10,000	NA	150,000
CFB	String cheese	150,000	10,000	100,0000	150,000
CFB	Panela cheese	NA	NA	1,100,000	NA
CFB	Manchego	NA	NA	300,000	NA
CFC	Ranchero	150,000	10,000	3’900,000	150,000
CFC	String cheese	150,000	NA	NA	NA
CFC	Botanero	150,000	10,000	9’400,000	150,000
CFC	Manchego	150,000	10,000	8’500,000	NA
CFC	Habanero manchego	NA	NA	NA	150,000

NA= not applicable. No statistically significant differences were observed between the different sampling dates ($P>0.05$).

When performing the descriptive statistics, was worked with median, PE25 and PE75 as a measure of central tendency and dispersion measures. The Kruskal-Wallis test was applied to compare the distribution of colony-forming units (CFUs) of *S. aureus* with respect to the different types of cheese on the different sampling days, resulting in the conservation of the null hypothesis for each of the cases, that is, all types of cheese have the same CFU distribution on the different sampling days.

Determination of the presence or absence of *Salmonella* spp.

Tests for the presence of *Salmonella* spp. did not show colonies in the selective XLD agar media or in brilliant green agar; nevertheless, 25 samples with suspect colonies were found in the bismuth sulfite agar (Figure 2). When confirmatory tests were performed for suspect colonies, no change in coloration was observed in the TSI and LIA media that could indicate the presence of *Salmonella* spp. Therefore, it was determined that there was an absence of *Salmonella* spp. in all cheeses sampled for all production units (Table 4) and that the requirement set out in NOM-210-SSA1-2014, Products and services, was met.

Figure 2: Suspect colonies of *Salmonella* spp. on bismuth sulfite agar

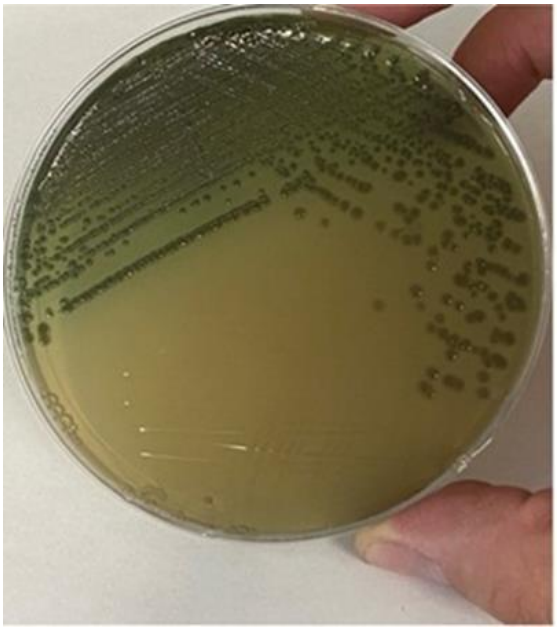


Table 4: Presence/absence of *Salmonella* spp. in the 36 cheeses sampled

Name of the cheese factory	Type of cheese	CFU/g Sept 25	CFU/g Nov 08	CFU/g Jan 08	CFU/g Mar 07
CFA	String cheese	Absent	Absent	Absent	Absent
CFA	Ranchero	Absent	Absent	Absent	Absent
CFA	Aged and smoked string cheese	NA	Absent	NA	NA
CFA	Manchego	NA	NA	NA	Absent
CFB	Ranchero	Absent	Absent	Absent	Absent
CFB	Morral cheese	Absent	Absent	NA	Absent
CFB	String cheese	Absent	Absent	Absent	Absent
CFB	Panela cheese	NA	NA	Absent	NA
CFB	Manchego	NA	NA	Absent	NA
CFC	Ranchero	Absent	Absent	Absent	Absent
CFC	String cheese	Absent	NA	NA	NA
CFC	Botanero	Absent	Absent	Absent	Absent
CFC	Manchego	Absent	Absent	Absent	NA
CFC	Habanero manchego	NA	NA	NA	Absent

NA= not applicable. No statistically significant differences were observed between the different dates of the samplings ($P>0.05$).

Determination of total coliforms on plate

Typical red colonies with a precipitation halo were detected with the typical characteristics of coliforms in the violet red bile agar (Figure 3) and when counting (Table 5), it was determined that most of the sampled cheeses do not comply with the limits allowed by NOM-113-SSA1-1994, which establishes that the maximum limit of total coliform organisms is <100 CFU/g[13]. When performing the descriptive statistics, it was worked with median, PE25 and PE75 as a measure of central tendency and dispersion measures. The Kruskal-Wallis test was applied to compare the distribution of total coliforms with respect to the different types of cheese on the different sampling days, which resulted in all types of cheese having the same CFU distribution on the different sampling days. The Friedman test was performed for the tests that had a complete record, and it was established that the distributions of September 25, November 8, January 8, and March 7 obtained a significance where ($P<0.05$), that is, there was a statistical difference. As there was a statistical difference, as a result of the Friedman test, a comparison was made in pairs with an adjusted significance between the different dates of the sampling, resulting in the quantification of CFUs for coliforms from the November 8 sampling being higher than the one carried out on September 25.

Figure 3: Colonies with the typical characteristics of coliforms on violet red bile agar

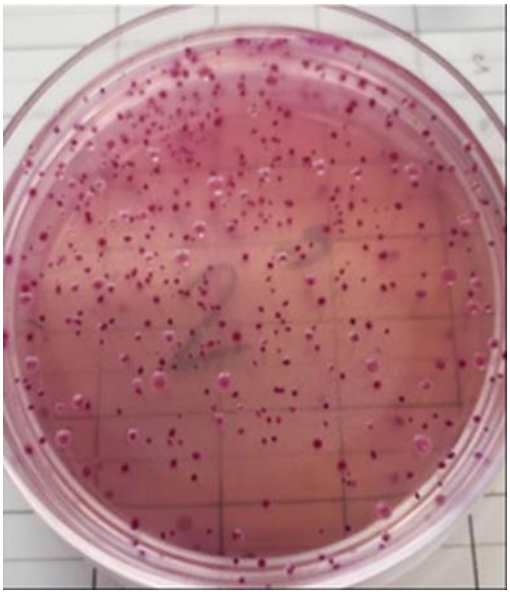


Table 5: Total coliform plate count in the 36 cheeses sampled

Name of the cheese factory	Type of cheese	CFU/g Sept 25*	CFU/g Nov 08*	CFU/g Jan 08*	CFU/g Mar 07*
CFA	String cheese	700,000	5’900,000	12,000	1,000
CFA	Ranchero	250	15’000,000	10,000	8’000,000
CFA	Aged and smoked string cheese	NA	60	NA	NA
CFA	Manchego	NA	NA	NA	15’000,000
CFB	Ranchero	Absent	200,000	Absent	15’000,000
CFB	Morral cheese	160	200,000	NA	7’300,000
CFB	String cheese	Absent	1’100,000	8’500,000	15’000,000
CFB	Panela cheese	NA	NA	70,000	NA
CFB	Manchego	NA	NA	700,000	NA
CFC	Ranchero	43	15’000,000	100,000	3’500,000
CFC	String cheese	20	NA	NA	NA
CFC	Botanero	45,000	15’000,000	6’600,000	1’900,000
CFC	Manchego	300	15’000,000	11’200,000	NA
CFC	Habanero manchego	NA	NA	NA	12’000,000

NA= not applicable. Dates marked with an asterisk indicate significant differences ($P<0.05$) between samplings.

Discussion

According to the results of the microbiological analyses, it can be established that the artisanal cheeses from the production units that were sampled in the municipality of Aculco, State of Mexico, Mexico, exceed the limits allowed by the regulations regarding *S. aureus* and total coliforms; however, *Salmonella* spp. is absent in all the samples processed. The absence of *Salmonella* spp. in the results obtained may indicate a limited contamination or a very low load of the bacteria in the products evaluated in the three cheese factories; nevertheless, these results differ from those reported in other research studies[15,16], which obtained positive results for the presence of *Salmonella* spp, of 50 % and 33 %, respectively, of the total number of sampled cheese factories whose conditions were not favorable with respect to the safety of the manufacturing processes, similar to those of the present work. Because of this, the presence of *Salmonella* spp. in the sampled cheeses cannot be totally ruled out, so molecular tests and immuneenzymatic methods would be recommended to confirm the results[16,17,18].

The results of the microbiological analyses for *S. aureus* show that most of the cheeses have limits higher than those allowed by the NOM (1,000 CFU/g); only three cheeses out of the 36 falls within the established limits, that is, 8.33 %. The highest average recorded was in Cheese factory C, with a value of 1’749,615 CFU/g, whereas Cheese factory A has the lowest average value of 91,405 CFU/g. According to the statistical analysis, it was determined that there is the same distribution in the quantification of CFUs for *S. aureus* and total coliforms regardless of the type of cheese that was processed, so that high bacteriological counts can be associated directly with the manufacturing process, considering that the cheese factories were characterized by having a percentage of compliance with GMPs that was unfavorable or favorable with requirements.

Among the findings of this statistical analysis, it was determined that, in the case of total coliforms, there was a statistical difference in the results of the September sample compared to the November sampling, with the quantification of CFUs being lower in the September sampling. This may be due to the fact that the producers were notified that analyses of the products and a possible review of the manufacturing process would be carried out, so practices different from those common in the cheese factories were reported, implementing an improvement in GMPs for the first of the samplings, whereas the other samplings were done without prior notice of the day on which the products would be sampled. Some of those changes were: proper use of face masks, sanitization of utensils and work tables with clean water and soap, and more constant hand washing.

The results of the microbiological analysis for total coliforms on plate show that most cheeses (88.89 %) have a bigger number of CFU/g than the limits allowed by NOM-113-SSA1-1994. Only four cheeses are below the established limits, that is, 11.11 %. The highest data recorded was in Cheese factory C, obtaining 6'180,412 CFU/g on average of the sampled products, whereas Cheese factory B had the lowest average CFU/g of cheese with 3'697,704. Despite the fact that Cheese factory B is categorized as unfavorable, the application of practices to control airflows and greater control of flies through the use of mosquito nets and strip curtains at the entrance of the cheese factory imply greater control of coliform contamination, which differs from the other two cheese factories. In the case of Cheese factory A, it obtained the lowest counts in CFU load for *S. aureus*, which may be due to the fact that, in their cheese factory, there is more control over the manufacturing process and the activities of the workers, giving them masks before entering the process, allocating an area for hand washing, monitoring the hygiene of workers upon entering the process, and avoiding the use of personal items, such as bracelets, watches, or rings. Nonetheless, some of these practices were partially complied with during the process and according to other authors[10], minimal factors such as the inappropriate use of face masks, the continuous opening of doors for the reception of milk, the lack of participation by workers in the use of sinks, and inadequate hand washing end up in a product that, despite not being the highest in total coliform plate counts and being the lowest in the CFU count for *S. aureus*, still does not meet the limits established by the regulations.

Within the process, it is important to consider the conservation of the product after packaging; the products were not kept refrigerated for more than two days before offering them to the public or taking them to the point of sale in the case of all cheese factories; nevertheless, at the point of sale of Cheese factory B, it was found that there were cheeses that had been refrigerated for more than 5 days. Despite having GMPs that the other two cheese factories did not have, it was observed that the refrigerator where the products for sale were stored did not close correctly; according to Aguirre-Alcántara[19], the shelf life of food will depend on the formulation or elements that make up the product, the proper processing, packaging and the conditions after it, including its conservation; in her study, it was determined that when products are kept at room temperature or refrigerated having high bacterial loads (greater than 1,100 CFU/g of cheese for total coliforms and greater than 5,000 CFU/g for *S. aureus*), there is a change in the organoleptic characteristics, resulting in a product with a shelf life of no more than four days due to the health risk. However, points of sale are not exempt from the presence of vectors during the production process, airflows from open windows, and the lack of sanitization of some tools for cheese production, as mentioned by other studies[10].

In contrast, Cheese factory C showed the highest CFU counts for *S. aureus* and for total coliforms on plate; if the averages obtained are compared to the results of the diagnosis of the situation of the cheese factories, it can be concluded that being a production unit that has just begun the production of artisanal cheeses, it has not had an improvement in terms of the standardization of its manufacturing process or in the development of a protocol for cleaning, product conservation, and safety during processing; likewise, there are risk factors, such as the fact that the cheese factory shares its area with cattle, poultry and dogs from the area, together with the fact that the facilities available to carry out the activity are inadequate due to the type of floor, problems of temperature increase that force the cheesemakers to work with the door open and that the only area that would allow adequate hand washing is found outside the cheese factory. Unlike Cheese factories A and B, the products of Cheese factory C do not have the same reach due to the productive capacity in terms of facilities and labor available for the work, being limited to the production of a few pieces and to the sale in the vicinity of Mexico City. According to other authors[19], the physical-chemical and microbiological characteristics of products facilitate their commercialization in different market niches by offering a higher quality product and longer shelf life, so a more standardized process, good hygiene practices by workers, and reduction of the constant flow of workers outside and inside the cheese factory could translate into a significant improvement in the quality and safety of the products, initially avoiding a considerable investment in the technification of its process.

The results obtained are similar to those obtained in other studies[20], which determined that 97.4 % of the cheeses sampled for their study were outside normal values, with the absence of *Salmonella* spp, coinciding with what has been reported by other studies[21], under similar conditions for the production of fresh cheeses. Both cases share the trend of artisanal cheese factories in which there is contamination due to the absence of hygienic measures or their poor implementation. Despite not having the necessary infrastructure to carry out a more technical process, in all cases there are certain elements and tools that have the appropriate characteristics for the production of cheese; nevertheless, regardless of the degree of technification that each cheese factory has, contamination of certain work tools and supplies before the start of the process and adequate sanitization of the utensils and work tables at the end of it were observed in all processes. According to some authors[21], high counts in microbiological results may be derived from cross-contamination with the utensils and tools used during the manufacturing process, and there is a relationship between what is obtained in the results of the diagnosis of the situation of the cheese factories and the bacteriological counts. Some authors report similar results regarding the levels of contamination by *S. aureus* and coliforms in cheese factories that share similar conditions of facilities and processes; nonetheless, they mention that producers show interest and openness to the implementation of techniques that improve the quality of their products, something similar to what was observed during the evaluation of cheese factories[22].

The producers showed genuine interest in the observations made during the evaluation of their process, mainly in the case of the Cheese factory A, which resulted with the lowest average in bacteriological counts and which was associated with a more marked presence in the implementation of GMPs. In line with what has been reported by other authors[10,22], basic changes can be suggested during manufacturing, such as proper nail trimming, having exclusive work clothes for the cheese factory, cleaning and disinfection of the areas, as well as always keeping the material that will be used for production clean and avoiding its use in other work areas, so that, once the general cleaning, GMP, SSOPs, and supplier control programs have been standardized, a HACCP program can be carried out to improve the characteristics of the product and even be able to reach a certification. The production units where the study was carried out are entirely artisanal and family-owned, with processes that have been followed over the years in the case of Cheese factories A and B; however, in the case of Cheese factory C, there is a great opportunity to improve and adopt new hygiene practices that lead to the production of higher quality and more economically profitable products. Likewise, in the case of the three production units, there is the participation of the next family generation, with willingness to learn and put into practice activities and processes for other dairy products for which workshops have been held by the project; so it could be a teaching opportunity to implement better manufacturing practices within cheese factories and modify the quality of the product for the benefit of producers and risk prevention for consumers.

Conclusions and implications

None of the production units evaluated complies with the microbiological standards established by the NOM for *S. aureus* or total coliforms, which shows that the absence of GMPs during cheese production compromises their quality and implies a health risk for the consumer, considering the minimum infective doses of *S. aureus* (100,000 CFU/g) and *E. coli* (100–1000 CFU/g). The situation diagnosis and microbiological results show a direct relationship between the lack of GMPs and high levels of contamination; nevertheless, it was observed that their implementation, even without infrastructure improvements, can increase the safety of the product, as suggested by the significant difference between the notified and unnotified sampling days. Although economic constraints make immediate improvements to facilities difficult, awareness-raising actions can be promoted to reduce sources of pollution, and the interest shown by new generations of producers offers an opportunity to introduce progressive changes in production practices towards sustainable improvement.

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Conflict of interest

The authors declare that there is no conflict of interest in relation to the publication of this article.

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