

## Determination of the sanitary quality of unpasteurized fresh cheeses for human consumption in Sinaloa, Mexico

## Determinación de la calidad sanitaria de quesos frescos no pasteurizados para consumo humano en Sinaloa, México

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### ABSTRACT

Fresh cheese represents a staple food of great importance for some communities, as well as an income from its commercialization; however, in some cases, the way it is processed could represent a risk to human health. Therefore, the objective of this study was to determine the sanitary quality of unpasteurized fresh cheeses intended for human consumption in Sinaloa. A total of 15, 20, 10, and 15 cheese samples were taken from Mazatlán, Culiacán, Guasave, and Los Mochis in the state of Sinaloa, México, respectively. The microbiological analysis was performed based on the Official Mexican Standard NOM-210-SSA1-2014. In accordance with the standard, the content of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella spp.* was estimated. Of the total samples analyzed, 97% showed *Escherichia coli*, 57% *S. aureus*, and 13% *Salmonella spp.*, above the reference values. The presence of indicator microorganisms and pathogens represents a risk factor for acquiring foodborne diseases and makes evident the need to improve hygienic practices in the preparation and preservation of these products.

**Keywords:** Fresh cheese, Contamination indicators, Foodborne diseases.

### RESUMEN

El queso fresco representa un alimento básico de gran importancia para algunas comunidades, así como un ingreso por su comercialización; sin embargo, en algunos casos, la forma en que se procesa podría representar un riesgo para la salud humana. Por tanto, el objetivo de este estudio fue determinar la calidad sanitaria de quesos frescos sin pasteurizar destinados al consumo humano en Sinaloa. Se tomaron un total de 15, 20, 10 y 15 muestras de queso de Mazatlán, Culiacán, Guasave y Los Mochis en el estado de Sinaloa, México, respectivamente. El análisis microbiológico se realizó con base en la Norma Oficial Mexicana NOM-210-SSA1-2014. De acuerdo con la norma, el contenido de *Escherichia coli*, *Staphylococcus aureus* y *Salmonella spp.*, fue estimado. Del total de muestras analizadas, el 97% presentó *Escherichia coli*, el 57% *S. aureus* y el 13% *Salmonella spp.*, por encima de los valores de referencia. La presencia de microorganismos indicadores y patógenos representa un factor de riesgo para adquirir enfermedades transmitidas por los alimentos, y también hace evidente la necesidad de mejorar las prácticas higiénicas en la elaboración y conservación de estos productos.

**Palabras clave:** Queso fresco, Indicadores de contaminación, Enfermedades transmitidas por alimentos.

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## 1. INTRODUCTION

Globally, around 250 pathogens, comprising bacteria, viruses, fungi, parasites, prions, and toxins, have been reported to be the possible cause for foodborne diseases. *E. coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Clostridium spp.*, *Listeria monocytogenes*, and some fungal species show high incidence (Ostrek et al., 2014). Raw milk and its derivatives are included in the group of foods that could transmit disease-producing pathogens or cause food poisoning (González & Rojas, 2005).

In the United States of America, 1.5% of all diseases have been reported to be caused by the consumption of dairy products, while a 6.8% is reported in the European Union. In Latin America, the frequent consumption of raw milk and milk products represents an important source of microorganisms related to intestinal infections (Philpot & Nickerson, 2002; Yáñez et al., 2008). In 2005, the Federal Commission for Protection against Health Risks (COFEPRIS) analyzed more than 100,000 samples of dairy products and prepared foods, reporting contamination in 43% of the samples, mainly with *Salmonella* and *E. coli* (COFEPRIS, 2007).

US federal regulations permit the manufacture and commercialization of cheese made from unpasteurized milk, provided that the cheese is ripened for at least 60 days at a temperature of no less than 1.7 °C (4 °F). However, several challenge studies have suggested that pathogenic bacteria may survive the ripening process (Bachmann & Spahr, 1995; Leyer & Johnson, 1992; Reitsma & Henning, 1996).

Within the range of processed dairy products, fresh cheese is the most widely produced and consumed in Mexico and Latin America. From January to May 2017, 18% of the production of the cheese industry in Mexico (164,504 tons) corresponded to fresh cheese, according to a report by the Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA, 2017). Furthermore, in Mexico, as in other developing countries, there is an informal economy linked to the use of local resources, whose activities include the sale of this type of food handled in uncontrolled environmental conditions that favor microbiological contamination and reproduction (Caballero-Torres *et al.*, 1998; Cristobal-Delgado & Maurtua-Torres, 2003; González-Montiel & Franco-Fernández, 2015; Reséndiz *et al.*, 2012).

There are some studies in Mexico reporting microbiological contamination in fresh cheese that pose a risk to human health when consumed. Reséndiz et al. (2012) analyzed 100 artisanal fresh cheeses from Tuzupán, Puebla, and informed that these cheeses presented hygienic deficiencies during processing. Soto-Beltrán *et al.* (2015) reported a high incidence of *Listeria monocytogenes*, Shiga

toxin-producing *E. coli*, and coliforms in 75 of the cheese samples retrieved from small stores in Culiacan, Sinaloa. González & Franco (2015) evaluated the composition of the microbiota of Aro cheese from Teotitlán de Flores Magón, Oaxaca, and reported that the number of microorganisms indicative of fecal contamination exceeded the limits established by the Ministry of Health. Sánchez *et al.* (2016) determined the degree of contamination by molds and yeasts, mesophilic aerobic bacteria, total coliforms, and *Salmonella spp.* as indicators of quality and hygiene in fresh cheeses and in the manufacturing environment of Zacazonapan, México, which suggests a lack of hygiene in cheese production and represents a risk to human health when consumed. Estrada & Bernabé (2019) determined and quantified the sanitary quality of raw milk and fresh cheese obtained and distributed in the central area of Loma Bonita, Oaxaca. These products showed a high level of contamination by microorganisms from the group of total coliforms, aerobic mesophiles, molds, and yeasts, as well as *S. aureus*, far exceeding the permissible values, with fresh cheese being the most contaminated product.

For all the aforementioned reasons, a risk assessment of fresh cheese samples collected in different entities of Sinaloa is in order so as to have the technical-scientific support to establish routes for optimal control in the production, storage, and distribution of artisanal fresh cheeses, thus considerably reducing the probability of developing diseases related to this.

## 2. MATERIALS AND METHODS

### 2.1 Samples

Fresh cheeses were purchased directly from different retail outlets originating from different cities of Sinaloa, México: Mazatlán 23°14'29"N 106°24'35"W (n=15), Culiacán 24°47'25"N 107°23'16"O (n=20), Guasave 25°33'56"N 108°28'19"O (n=10), and Los Mochis 25°47'37"N 108°59'49"O (n=15), from January to October 2021. All samples were collected and transported under refrigerated conditions at an average temperature of 5.6 °C. The 60 cheeses tested were produced from unpasteurized raw milk. The samples possessed a complex and diverse microbial population attributed to manufacturing and storage conditions. Only three bacterial species of sanitary importance were studied: *E. coli* as an indicator of fecal contamination, and the pathogens *S. aureus* and *Salmonella spp.*

### 2.2 Determination of *Escherichia coli*

Different dilutions (10-1 to 10-5) were prepared from a 25 g sample of fresh cheese, and 1 mL of each dilution was inoculated into three tubes with lauryl tryptose broth with MUG. All tubes were incubated for 24 to 48 h at 35 °C; gas production and fluorescence were observed by placing the tubes in the dark with a UV light lamp. From the positive tubes (gas and fluorescence producing), a sample was taken and inoculated by cross streak method on Levin eosin methylene blue agar (EMB-L). The inverted plates were incubated at 35 °C for 24 h. Two typical colonies were taken and seeded on nutrient slant agar (standard method or plate count), incubated at 35 °C for 18 to 24 h. Finally, Gram staining and biochemical tests, such as indole, methyl red, Voges-Proskauer, and citrate (NOM-210-SSA1-2014), were performed.

### 2.3 Determination of *Staphylococcus aureus*

25 g of fresh cheese were deposited in 225 mL of peptonized saline solution, and different dilutions (10-1 to 10-3) were prepared. 0.1 mL was transferred in duplicate with a sterile pipette to Baird Parker agar, and the inoculum was distributed over the agar surface with sterile glass rods. The inverted plates were incubated for 44 to 48 h at 36 °C. Plates which contained between 1 and 150 typical and atypical *S. aureus* colonies were selected for Gram staining and biochemical tests, such as catalase, coagulase, and thermonuclease (NOM-210-SSA1-2014).

### 2.4 Determination of *Salmonella* spp.

25 g of cheese sample were added to 225 mL of buffered peptone water and incubated at 36 °C for 18 h. 0.1 mL was transferred to a tube with 10 mL of Rappaport-Vassiliadis broth with soybean (RVS) and 1 mL to a tube with 10 mL of Muller-Kauffman Tetrathionate-Novobiocin (MKTTn) broth, incubated at 41.5 °C and 36 °C for 24 h, respectively. For isolation and colonial morphology, after growth on the two previous media, they were inoculated on Xylose Lysine Deoxycholate (XLD), Hektoen enteric (EH), and Bismuth Sulfite (ASB) agar, incubated at 36 °C for 24 h. The colonies selected as suspicious were incubated on nutrient agar at 36 °C for 24 h. Subsequently, biochemical tests were performed to the strains that showed typical reactions on triple sugar iron agar (TSI), Lysine-iron (LIA), and urea agar. Serological tests were performed with polyvalent antiserum Poly A-I + Vi for final identification (NOM-210-SSA1-2014).

### 2.5 Statistical Analysis

The values obtained from the determinations of *E. coli* and *S. aureus* were transformed to log<sub>10</sub> to normalize the data before performing descriptive statistics; to determine significant differences by bacteria with respect to the sampling sites, one-way analysis of variance (ANOVA) was performed

with a significance level ( $p < 0.05$ ); in the cases that presented significant differences, the Student-Newman-Keuls (SNK) test was applied a posteriori. Analyses were performed using SPSS software ver. 17 (Statistical Package for Social Sciences).

### 3. RESULTS AND DISCUSSION

In general, 97% of the samples far exceeded the permissible value for *E. coli* (NOM-243-SSA1-2010), making these products not suitable for human consumption. Although the aforementioned standard does not establish limits in NMP/g for *E. coli* in fresh cheeses, it establishes them for milk destined for processing ( $\leq 3$  NMP/g) and matured or processed cheeses ( $\leq 10$  NMP/g). This value agrees with that reported by Flores *et al.* (2020), Perdomo *et al.* (2015), Soto-Beltrán *et al.* (2015), and Vásquez *et al.* (2018). 57% exceeded the allowable load of *S. aureus*, a value that coincides with those reported by Araujo *et al.* (2002), Arguello *et al.* (2015) and González & Franco (2015). 13% are non-standard for *Salmonella spp.* (Table 1), a result similar to that reported by González-Montiel & Franco-Fernández (2015) and Sánchez *et al.* (2016).

**Table 1.** Non-standard samples by bacteriological determination.

| Determination            | Minimum value | Maximum value | Above standard | Limits                      |
|--------------------------|---------------|---------------|----------------|-----------------------------|
| <i>E. coli</i> (NMP/g)   | < 3           | > 110 000     | 97%            | <sup>a</sup> $\leq 3$ NMP/g |
| <i>S. aureus</i> (UFC/g) | < 1 000       | 2 200 000     | 57%            | 1000 UFC/g                  |
| <i>Salmonella spp.</i>   | Absent        | Present       | 13%            | Absent                      |

<sup>a</sup>Value for milk used as raw material in cheese production.

When performing an ANOVA to determine differences in the means in log NMP/g for the *E. coli* and log CFU/g for *S. aureus* respectively of the different sampled entities, it was observed that the mean (in log NMP/g) of *E. coli* obtained for Los Mochis ( $2.81 \pm 1.41$ ) was statistically lower compared to Culiacán ( $4.28 \pm 1.16$ ) and Guasave ( $4.23 \pm 1.12$ ):  $F(3,56) = 5.274$ ,  $p < 0.003$  (Table 2); no significant difference was observed for the means in log CFU/g of *S. aureus* in the different populations:  $F(3,56) = 1.149$ ,  $p > 0.05$ .

**Table 2.** Mean  $\pm$  SD of log NMP/g *E. coli* and log UFC/g *S. aureus*, number of positive samples of *Salmonella* spp. and percentage of non-standard samples for the four entities evaluated.

| Determination                | Mazatlán<br>(n=15)            | Culiacán<br>(n=20)           | Guasave<br>(n=10)            | Los Mochis<br>(n=15)         | Total<br>(n=60) |
|------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|-----------------|
| <i>E. coli</i> (log NMP/g)   | 3.24 $\pm$ 1.24 <sup>ab</sup> | 4.28 $\pm$ 1.16 <sup>b</sup> | 4.23 $\pm$ 1.12 <sup>b</sup> | 2.81 $\pm$ 1.41 <sup>a</sup> | 3.64 $\pm$ 1.37 |
|                              | 100%                          | 95%                          | 100%                         | 93%                          |                 |
| <i>S. aureus</i> (log UFC/g) | 3.55 $\pm$ 0.88               | 3.85 $\pm$ 1.02              | 4.23 $\pm$ 0.82              | 3.92 $\pm$ 0.84              | 3.86 $\pm$ 0.92 |
|                              | 33%                           | 50%                          | 80%                          | 73%                          |                 |
| <i>Salmonella</i> spp.       | 0/15                          | 3/20                         | 1/10                         | 4/15                         | 8/60            |
|                              | 0%                            | 15%                          | 10%                          | 27%                          | 13%             |

(a,b) different superscripts indicate statistically different groups ( $p < 0.05$ ) in the sampled populations.

Also, for *E. coli*, 100% of the samples from Mazatlán and Guasave, 95% from Culiacán, and 93% from Los Mochis were found to be above the permissible values according to the Official Mexican Standard NOM-243-SSA1-2010. For *S. aureus*, 80% of the samples from Guasave, 73% from Los Mochis, 50% from Culiacán, and 33% from Mazatlán were found to be non-standard. *Salmonella* spp. was observed in 27% of the samples from Los Mochis, followed by Culiacán with 15%, and Guasave with 10%, while these bacteria were not isolated in the municipality of Mazatlán.

The presence of *E. coli*, *S. aureus*, and *Salmonella* spp. suggests fecal contamination and poor hygiene practices in the processing of fresh cheese. According to the percentage of *S. aureus* (57%), the possibility of finding Staphylococcal enterotoxin in the fresh cheeses analyzed is very high, which can trigger severe intoxications (Hennekinne et al., 2012).

The high percentage of non-standard samples (97% for *E. coli* and 57% for *S. aureus*) in all the entities evaluated suggests deficient sanitary control by the regulatory authorities and poor hygiene and manufacturing practices by the personnel who process these foods, but the most alarming aspect is the presence of *Salmonella* spp. (up to 13%) since all these samples were available for human consumption at different commercial outlets (Table 1 and 2). According to the percentages calculated based on the three determinations, Guasave and Los Mochis had the highest bacterial contamination rates, followed by Culiacán and Mazatlán (Table 2).

The presence of pathogenic microorganisms in cheese depends not only on the health of the cow udder but also on the heat treatment of the milk, the cleanliness of the material used for cheese production, the storage and transport temperature, as well as the handling during the distribution of

the product (Farkye, 2002). In addition to the above, the high humidity levels of fresh cheeses provide a favorable environment for the development of pathogenic microorganisms that cause infections and food poisoning (Ortiz-Hernández *et al.*, 2016).

The high occurrence of *E. coli*, *S. aureus*, and *Salmonella spp.* in all the samples analyzed results in a high risk of diseases transmitted by pathogenic microorganisms present in cheeses, in addition to the possibility that these microorganisms may present resistance or, even more seriously, multi-resistance to antibiotics. Since recent studies have reported the presence of resistance genes in bacteria found in foods including milk and fresh cheeses intended for human consumption, it is imperative to carry out detailed analyses in our entity on the ecology of antibiotic resistance and virulence of these microorganisms to ensure the safety of the final product and of the consumer (Franz *et al.*, 2001; Hammad *et al.*, 2015; Soto-Beltrán *et al.*, 2015; Yesilmen *et al.*, 2014).

#### 4. CONCLUSIONS

According to the results obtained, it becomes evident that the vast majority of the fresh cheeses analyzed presented poor hygiene and did not comply with the permissible limits established in the current sanitary norms and regulations. The presence of indicator and pathogenic microorganisms is a risk factor for the acquisition of foodborne diseases. In addition to this, it is necessary to improve hygiene practices in the preparation, preservation, and transportation of these products, as well as to implement strategies by the health authorities to reduce commercialization of this basic product, which, under these conditions, becomes harmful to the health of consumers.

More research is needed to establish the existing correlation between the non-compliance with good manufacturing practices and their direct effect on the high levels of microorganisms found. It would also be important to establish an integral control strategy with producers with emphasis on risk analysis, since the consumption of fresh artisanal cheese increases the probability of the presence of microorganisms that cause foodborne diseases in the population.

From a hygienic-sanitary point of view, it is important that producers of artisanal cheeses implement milk pasteurization in the process of making fresh cheese in order to eliminate the presence of pathogenic microorganisms; it is also imperative to maintain sanitary control over the personnel involved in making the product. It is recommended that awareness programs be carried out for producers of fresh artisanal cheeses on good manufacturing practices so as to obtain quality products free of microbiological agents that threaten public health.

**Authors' contributions:** Héctor Reyes: carried out the methodological part of the three determinations involved. Julio Montes: wrote and prepared the original draft. Samuel Campista and Luz Isela Peinado: performed the validation and data curation. Esteban Hernández: carried out the translation into English and assisted in the drafting and revision. All authors have read and accepted the published version of the manuscript.

**Conflicts of interest:** "The authors declare that they have no conflicts of interest".

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