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Article

Evaluation of microbiological quality in artisanal cheeses in the state of Nuevo León

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Abstract: In most cases, producing and marketing artisanal products needs more hygiene measures in equipment and raw materials before and during production. This research aimed to evaluate the microbiological quality of 31 artisanal fresh, panela, ground, and asadero cheeses packed in various packaging materials marketed in municipalities of the State of Nuevo León. The microbiological analyses carried out were counts of aerobic mesophilic bacteria, psychrophiles, total coliforms, lactic acid bacteria (LAB), fungi, and yeasts. In the aerobic mesophilic count, statistical differences were shown ($p \leq 0.05$) depending on the origin of the cheese and type of packaging, obtaining values ranging from 2.46 - 4.29 Log_{10} CFU/g. No presence of psychrophilic bacteria was detected, while the total coliform count values between 1.97 to 3.45 Log_{10} CFU/g were obtained, presenting statistical differences ($p \leq 0.05$) in the type of cheese. The LAB showed values with statistical differences between the types of cheese ($p \leq 0.05$) with values between 3.31 to 4.84 Log_{10} CFU/g. Finally, fungi and yeasts showed statistical differences ($p \leq 0.05$) for the interaction of origin, type of cheese, and packaging. The results underline the need to implement control measures and good manufacturing practices in producing artisanal cheeses to ensure food safety and consumer health.

Keywords: coliforms; dairy products; packaging; safety.

1. Introduction

The sanitary quality of artisanal cheeses in Mexico is of utmost importance due to the significant public health risks associated with their consumption. Artisanal cheeses, often made with unpasteurized milk and under uncontrolled production conditions, can become vectors of various pathogens, leading to diseases such as hemorrhagic colitis, diarrhea, and meningitis (Martins-Lima *et al*., 2023). A study evaluating the microbiological and toxicological quality of fresh artisanal cheeses in Puebla revealed worrying levels of microbial contamination, with average counts of aerobic mesophilic bacteria of 5.55 log CFU/g and total coliforms of 3.80 log CFU/g, indicating poor sanitary conditions during production and handling (Benítez-Rojas *et al*., 2019).

In Mexico, artisanal cheese production is highly diverse, with at least 40 recognized varieties. Adapted to local conditions, fresh cheeses stand out for their unique flavor, aroma, and texture. Despite being appreciated for their traditional production and nutritional properties, these products often do not comply with hygiene standards or the sanitary standards of dairy farms (Sánchez-Valdés *et al*., 2016). Their production from raw milk, spontaneous fermentation, and rudimentary methods increases the risk of microbial contamination. Fresh cheese has been linked to numerous food poisoning outbreaks (Sánchez-Valdés *et al*., 2016). In addition, improper handling and humidity conditions during the marketing of soft cheeses represent a risk to consumers' health (Rodríguez *et al*., 2009).

On the other hand, the lack of proper labeling and official identification on artisanal cheese packaging, as observed in a study conducted in Dourados, Brazil, underlines the need for strict regulatory oversight and consumer awareness to ensure safety and compliance with sanitary standards (Martins-Lima *et al*., 2023). In this sense, packaging plays a crucial role in preserving and maintaining the quality of artisanal cheeses, significantly affecting their physicochemical properties, shelf life, and economic value (Jafarzadeh *et al*., 2021). Likewise, vacuum packaging has positively influenced the moisture content, color, and rind formation of artisanal fresh goat cheese, resulting in a better visual appearance and higher moisture retention. This method also helps maintain the cheese's rheological behavior over time, making it similar to its initial state, thus extending its shelf life and reducing moisture loss, which is economically beneficial for

producers (Frau *et al*., 2020). Therefore, effective packaging strategies are essential to maintain artisanal cheeses' quality, safety, and economic viability. Cases of alterations in the quality of artisanal cheese in Mexico due to poor packaging have been documented. Studies have shown that the microbial quality of cheeses sold in various retail outlets in Mexico, including supermarkets, street markets, and convenience grocery stores, often need to meet Mexican standards (Guzman-Hernandez *et al*., 2016). Therefore, in the present investigation, the microbiological quality of 31 cheeses of different types (fresh, ground, panela, and asadero) and with different packaging processes (plastic bags, disposable plates, shrink-wrapped or vacuum-packed), marketed in various municipalities of Nuevo León, was evaluated.

2. Materials and Methods

The chemical compounds and reagents used were graded from Sigma Aldrich (St. Louis, Missouri, U.S.A.). The culture media was B.D.'s commercial brand (Becton, Dickinson and Company, New Jersey, U.S.A). All solutions were prepared with double-distilled water from Laboratorios Monterrey, S.A. (Monterrey, Nuevo León, México).

2.1. Sampling of artisanal cheeses

Thirty-one samples of commercial cheeses (fresh, panela, ground, and asadero) were collected in different municipalities of the State of Nuevo León during the summer of 2023. The cheeses were purchased in different commercial establishments and presented in different types of packaging: plastic bag, disposable plate wrapped in plastic bag, vacuum packed, and plastic film. The samples were transported in a cold chain (4 ^{o}C) and processed in the laboratory in less than 24 h.

2.2. Experimental design and microbiological analysis

A 3 x 5 factorial design was used, where each cheese was considered an experimental unit. A composite sample of 10 g was taken from each cheese, from which serial dilutions and seeding in Petri dishes were performed in triplicate.

The preparation and dilution of the artisanal cheese samples followed NOM-110-SSA1-1994, the Mexican standard for preparing and diluting food samples for microbiological analysis. Serial dilutions were performed using homogenizers and sterile tubes containing 90 ml and 9 ml of peptonized water, respectively. These materials were previously sterilized. To obtain the 10^{-1} dilution, 10 g of the sample was added to the homogenizer and processed for four cycles of 30 s each (Castro and Guevara-Muñoz, 2018). In the case of the total aerobic mesophile count, serial dilutions of 10^{-3} a 10^{-6} (Castro and Guevara-Muñoz, 2018). Standard Methods agar was sterilized at 121 °C for 15 min. Seeding was performed by the pour-plate technique, adding 1 mL of each dilution to the Petri dishes to later add the agar. Incubation was performed at 38 °C for 24 h following NOM-092-SSA1-1994. The previous procedure was repeated to determine the psychrophilic bacteria, incubating the plates at 4 °C for 24 h. Total coliform bacteria were determined on Red Violet Bile Agar (RVBA) medium, according to Mexican Official Standard NOM-113-SSA1-1994. Serial dilutions between 10^{-2} and 10^{-4} and the pour-plate technique, as described above, were used. Plates were incubated at 37 °C for 24 h. To determine the concentration of lactic acid bacteria (LAB), serial 10^{-2} and 10^{-3} dilutions of the samples were prepared in peptonized water as described above. One milliliter of each dilution was seeded using the pour-over technique on Petri dishes with MRS agar. The plates were incubated in an inverted position at 37 °C for 48 h in an aerobic atmosphere. Subsequently, the colonies were stabilized and expressed as colony-forming units (CFU/g). The determination of fungi and yeasts was performed according to NOM-111-SSA1-1994. Potato dextrose agar medium supplemented with 10% tartaric acid was used for fungal enumeration. Samples were seeded by pouring in Petri dishes and incubated at 37 °C for 24 h. All analytical results were expressed as a Log_{10} CFU/g sample.

2.3. Statistical analysis

Statistical analysis of the data was performed using IBM SPSS Statistics software for Windows. An analysis of variance (ANOVA) was performed, followed by a Tukey test to compare the means of the groups ($p \leq 0.05$). Correlation between variables was evaluated using Pearson's coefficient ($p \leq 0.05$). Results are expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Microbiological analysis

Figure 1 presents the values obtained for each group of microorganisms in the different types of cheese (ground, panela, fresh, and asadero). Notably, the mesophilic content showed no statistically significant differences between the types of cheese ($p \leq 0.05$), with asadero cheese recording the highest value at 4.12 Log_{10} CFU/g. This finding provides valuable insights into the microbiological characteristics of these cheeses. On the other hand, the total coliform count showed statistically significant differences ($p > 0.05$). The ground cheese had the lowest content (1.97 Log_{10} CFU/g), while the asadero cheese had the highest microbial load (3.45 Log_{10} CFU/g). The panela (2.81 Log_{10} CFU/g) and fresh (3.18 Log_{10} CFU/g) cheeses had intermediate values. Regarding lactic acid bacteria (LAB), the different types presented statistically significant differences ($p > 0.05$). The panela cheese had the highest numerical value (4.84 Log_{10} CFU/g), followed by the asadero cheese (4.37 Log_{10} CFU/g), the fresh cheese (4.02 Log10 CFU/g) and finally the ground cheese (3.31 Log_{10} CFU/g). The analyses showed that the fungi and yeasts had the lowest counts. The asadero cheese had the highest count (2.77 Log_{10} CFU/g), showing a statistically significant difference ($p > 0.05$) concerning the other cheeses. It's worth noting that, in terms of microbiological risk, the Mexican Official Standard NOM-243-SSA1-2010 sets maximum limits for total coliforms, Escherichia coli, Staphylococcus aureus, fungi and yeasts in whey cheeses. The absence of Salmonella spp, Listeria monocytogenes and Vibrio cholerae is also required. However, aerobic mesophiles are not included in this standard, possibly due to the importance of lactic acid bacteria in the cheese ripening process. These bacteria, with their ability to use proteins in the long term, significantly contribute to the development of the aroma, flavour and regional characteristics of the product (Sánchez-Valdés et al., 2022). Several studies have reported high microbial loads in artisanal cheeses, which represents a risk to consumer health. For example, Resendiz et al. (2012) found high total coliform counts in Mexican artisanal cheeses. Similarly, Sánchez-Valdés et al. (2016) and Ercan et al. (2014) reported similar results in their respective studies. De la Rosa-Alcaraz et al. (2020) evaluated the microbiological quality of Poro cheeses from Tabasco and found that total coliform counts were within a specific range. Although Mexican standards do not establish permissible limits of coliforms in fresh cheeses, a high count of these microorganisms often indicates fecal contamination and inadequate manufacturing practices. This contamination can originate at various stages of the manufacturing process, from milk production to product marketing, as several studies have pointed out (De la Rosa-Alcaraz et al., 2020; Ercan et al., 2014; Perin et al., 2017).

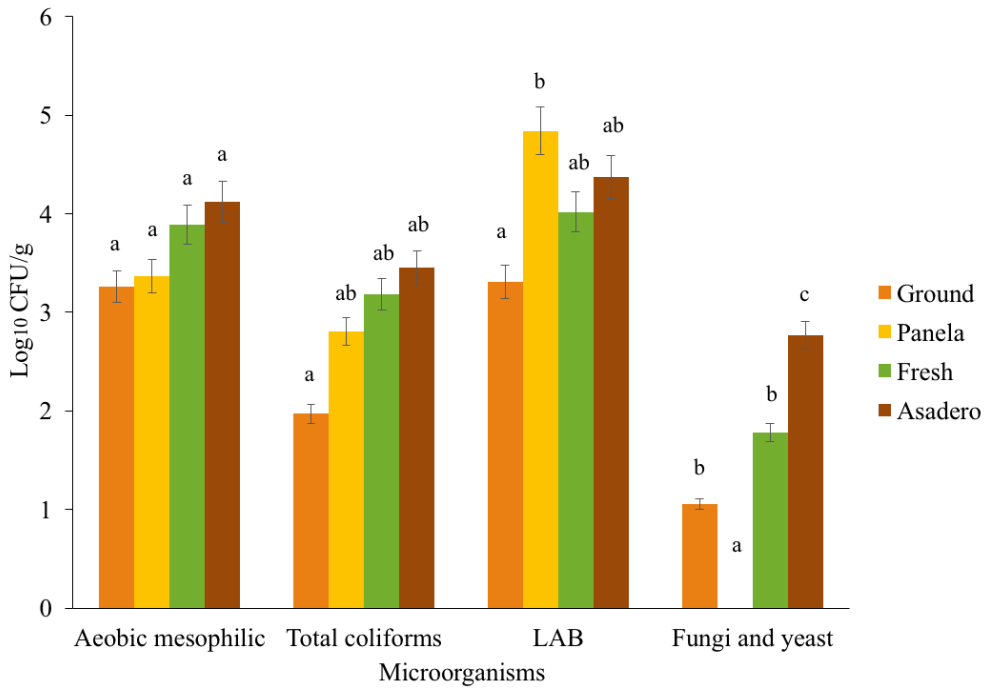

Figure 1. Average number of microorganisms per type of cheese. [a-c] Means with a common letter are not significantly different ($p > 0.05$) by microorganism group. LAB: lactic acid bacteria

It's crucial to understand the role of packaging materials in determining the shelf life of food products. This is why we considered the types of packaging materials commonly used in artisanal cheeses. Figure 2 presents the results for plastic bag, disposable plate in plastic bag, vacuum packaging, and shrink wrap. We observed a statistically significant difference ($p \leq 0.05$) in the content of aerobic mesophiles of the cheese packed in a disposable plate in a plastic bag (2.46 Log_{10} CFU/g), while no significant differences were found between the other types of packaging ($p > 0.05$). The values for total coliforms ranged between 2.25 and 3.75 Log_{10} CFU/g and did not present statistically significant differences between the materials ($p > 0.05$). Similarly, lactic acid bacteria (LAB) had higher values than the other groups of microorganisms analyzed (3.69 to 4.65 Log_{10} CFU/g), without showing significant differences between the different packaging materials ($p > 0.05$). Finally, a statistically significant difference ($p \leq 0.05$) was observed in the content of fungi and yeasts of cheese packed in a disposable plate in a plastic bag, compared to the other packaging materials.Thus, several studies have highlighted the importance of lactic acid bacteria in the quality of artisanal cheeses. Perin et al. (2017) found a high presence of these bacteria in cheeses from Minas Gerais, Brazil, while De la Rosa-Alcaraz et al. (2020) identified different species of lactobacilli in Poro cheeses from Tabasco. These bacteria contribute to the flavor, aroma, and texture of cheeses, in addition to inhibiting the growth of pathogenic microorganisms. The results obtained in this study need to show a clear relationship between the type of packaging material and the microbial load. This suggests that other factors, such as raw materials, hygiene practices, and the cold chain, significantly influence the microbiological quality of artisanal cheeses.

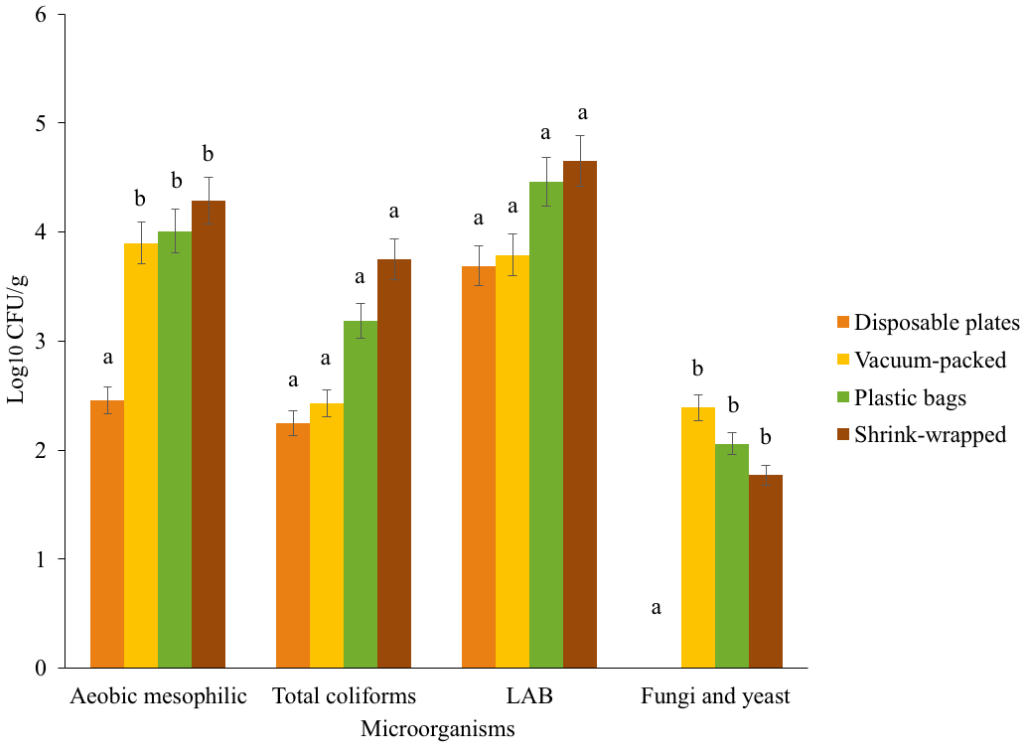

Figure 2. Average number of microorganisms by type of packaging material. a-b Means with a common letter are not significantly different ($p > 0.05$) by microorganism group. LAB: lactic acid bacteria

Figure 3 shows the results by location, where it can be observed that the samples from location B had the lowest content of aerobic mesophiles, with a value of 2.46 Log_{10} CFU/g ($p \leq 0.05$). In contrast, the rest of the locations did not show statistically significant differences ($p > 0.05$), with an average of 4.02 Log_{10} CFU/g. Meanwhile, the values of total coliforms ranged between 2.25 and 3.44 Log_{10} CFU/g without showing statistically significant differences between locations ($p > 0.05$). In the case of lactic acid bacteria (LAB), no statistically significant differences were observed between locations ($p > 0.05$), although location C had the highest concentration of these bacteria, with 5.03 Log_{10} CFU/g.

Regarding fungi and yeasts, locality C presented the highest content of this group of microorganisms (2.91 Log_{10} CFU/g), showing a statistically significant difference ($p \leq 0.05$) concerning the other localities. It is worth mentioning that the Mexican standard NOM-243-SSA1-2010 establishes a maximum limit of 500 CFU/g (2.69 Log_{10} CFU/g) for fungi and yeasts in fresh cheeses. All the products studied, except for asadero cheese, met this specification. De la Rosa-Alcaraz et al. (2020) found re-counts of fungi and yeasts in artisanal Poro cheese from Tabasco that exceeded the limits established by the Mexican standard. Similarly, Chávez-Martínez et al. (2019) reported high counts of fungi and yeasts in different types of cheese marketed in Mexico.

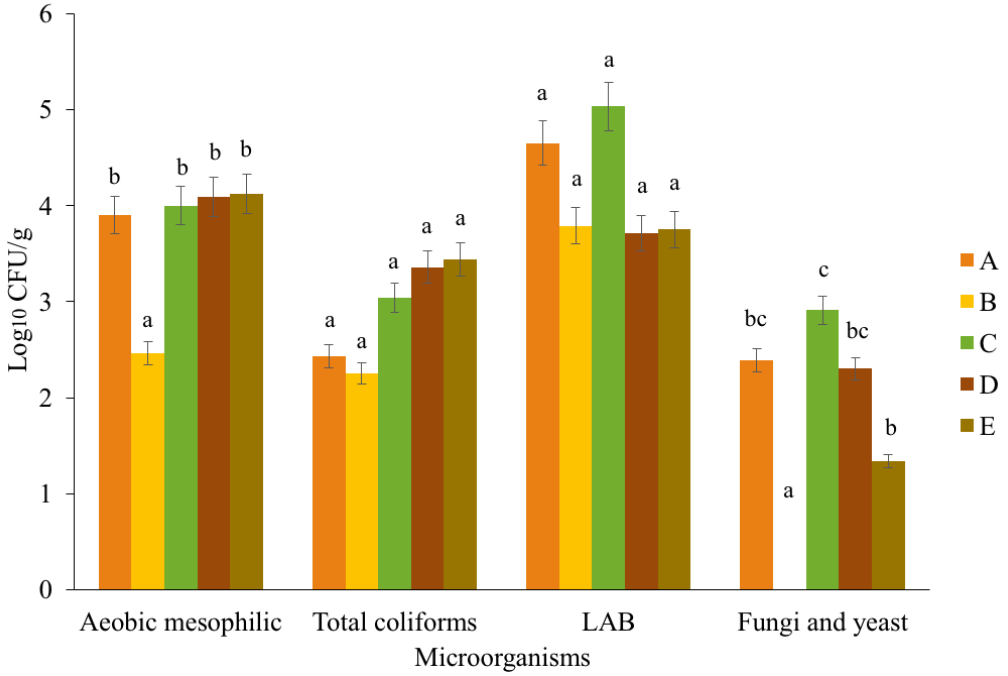

Figure 3. Average number of microorganisms by origin (locality) of the cheese. a-c Means with a common letter are not significantly different (p > 0.05) by microorganism group. LAB: lactic acid bacteria

The correlation analysis, presented in Table 1, shows the relationships between different variables related to the microbiological quality of cheeses, such as aerobic mesophiles, total coliforms, lactic acid bacteria (LAB), and fungi and yeasts. The data suggest that the type of cheese is the variable with the strongest correlation with microbiological quality. Likewise, the type of packaging can influence the levels of microorganisms present in the cheese. A strong correlation was observed between total coliforms and aerobic mesophiles, and a positive correlation was observed between LAB and aerobic mesophiles. These results are consistent with the scientific literature. Montel et al. (2014) highlighted the diversity and richness of the microbiota associated with traditional cheeses, which can confer benefits regarding aroma, flavor, and food safety. Bevilacqua et al. (2012) emphasized the influence of the type of cheese and the production methods on the microbiota and, consequently, on the microbiological quality of the cheese. Gálvez et al. (2007) indicated that lactic acid bacteria are a significant component of the microbiota in dairy products and may benefit food safety. It is essential to clarify that the correlation analysis presented in Table 1 only allows for identifying associations between variables but does not establish causal relationships. Additional studies are required to fully understand how the different variables interact and influence cheeses' microbiological quality.

Table 1. Correlation Analysis

	Origin	**Cheese type**	**Packaging type**	**CT**	**BUT**	**BAL**	**F and Y**
Origin	1.00	$4.5x10^{-6}$	0.00	0.01	0.02	0.02	0.22
Cheese type	0.47	1.00	$1.3x10^{-10}$	$8.6x10^{-4}$	0.02	0.95	$3.8x10^{-4}$
Packaging type	0.83	0.62	1.00	$4.7x10^{-3}$	0.03	0.47	0.07
CT	0.27	0.35	0.30	1.00	$6.9x10^{-8}$	0.03	0.04
AM	0.25	0.25	0.24	0.54	1.00	$1.4x10^{-5}$	0.04
BAL	-0.24	0.01	-0.08	0.24	0.45	1.00	0.94
H y L	0.13	0.37	0.20	0.22	0.22	-0.01	1.00

TC= Total coloforms; AM= Aerobics mesophilic; BAL = lactic acid bacteria; F and Y = Fungi and yeasts

4. Conclusions

The presence of microorganisms, such as total coliforms and aerobic mesophiles, in the region's different artisanal cheeses is a cause for concern. This situation could be related to the need for more quality supervision throughout production and distribution. Therefore, it is necessary to establish training programs for producers to make them aware of the importance of implementing safety measures at all stages of production, from the selection of raw materials to the distribution of the final product. Given the current regulatory vacuum for these products, it is also essential to establish specific Mexican Official Standards to produce artisanal cheese. This will ensure that small producers are aware of the risks associated with non-compliance with sanitary standards and provide them with the information they need to produce safe, quality food.

5. Acknowledgments

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6. Conflicts of Interest:

The authors declare that they have no conflict of interest.

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