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Sanitary Quality of Milk and Artisan Cheese Produced in Manabi Province, Ecuador

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ABSTRACT

Background: Artisan cheese is part of the nutritional culture of the Ecuadorian people; however, in the north of Manabi there are few studies about the microbiological quality of the cheese made by farmers. **Aim.** To evaluate the sanitary quality of milk and artisan cheese produced in the north of Manabi province. **Materials and methods:** A microbiological analysis was performed to 156 milk and cheese samples using the petrifilm method for counts of mesophile aerobic microorganisms, total coliforms, enterobacteria, fungi and yeasts, and *Staphylococcus aureus*. The procedure was done in compliance with the requisites established in the Ecuadorian standard. The values were expressed in log10 cfu/mL (milk), and cfu/g (cheese). **Results:** The values obtained for the microorganisms were significantly different ($p < 0.001$) between the raw milk and the artisan cheese, which were unsuitable for human consumption according to the following indicators: mesophile aerobics (6.24 and 8.41); total coliforms (5.40 and 7.26), enterobacteria (4.40 and 6.44), fungi and yeasts (3.52 and 5.16), and *S aureus* (4.33 and 5.99), respectively. The correlation coefficient was above 0.65 in terms of milk and cheese contamination by all the microorganisms in the cantons of the province. **Conclusions:** The fresh milk and artisan cheese from the four cantons included in the study did not meet the sanitary quality requirements, exceeding the microbiological limits by the national standards, with a high relevance in canton El Carmen, and a moderate relation between the two items for each indicator microorganism. **Keywords:** milk hygiene, fresh cheese, *Staphylococcus aureus* (Source: AGROVOC)

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INTRODUCTION

Agriculture plays an important role in the economy of Ecuador, contributing with 17.5% of the gross domestic product. Livestocking accounts for 27.3%, with an outstanding production of dairies, beef, pork, chicken, and eggs (Vásquez *et al.*, 2018). Fresh artisan cheese is one of the main bases of people's nutrition in Ecuador for decades. It uses 35% of the milk produced in the country (Arguello, 2015; Vásquez *et al.*, 2018). Dairy production is a complex activity that needs a comprehensive approach with a vision of sustainable agroproductive chain that combines technological, economic, environmental, and social elements (Arguello, 2015).

Therefore, special surveillance is required to maintain the quality and safety of these items, given their nutritional qualities of this food, which is rich in protein, minerals, vitamins, and fats (Komada *et al.*, 2020). The United Nations Organization for Food and Agriculture (FAO) issues warnings reminding that this manufacturing activity is performed in rural areas where the hygienic and sanitary conditions lack the required follow up and control to ensure commercially suitable items (FAO, 2017). Besides, the observance of food quality and safety regulations by farmers is mandatory, which should be controlled by governments to prevent food-transmitted diseases (FTD), with reports of outbreaks in many countries (Arguello, 2015; FAO, 2017; Komada *et al.*, 2020).

Artisan cheese is one of the foods that can transmit etiological agents that cause diseases or food related poisoning; the milk that lacks pasteurization is subject to contamination by pathogenic bacteria (Gutiérrez *et al.*, 2017; Urbano *et al.*, 2017). In turn, Martínez *et al.* (2016) noted that the items manufactured from raw milk, like cheese, must use materials from farms that meet the hygienic and good practices codes.

The cheese that causes these outbreaks have a content of humidity and little or no maturation, which favors the growth of bacteria with a pathogenic potential (Cervantes *et al.*, 2019). Arguello (2015); Ruíz *et al.*, 2017; Idarraga *et al.* (2018), reported pathogenic agents with special importance, such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and in some cases, *Listeria monocytogenes* in some regions of Ecuador and several countries of Latin America.

Cantons, Chone, Pedernales, El Carmen, and Flavio Alfaro, in the north of Manabi province, Ecuador cover the largest cattle breeding area. However, there are no studies associated with the quality of the main raw material (raw milk), or artisan cheeses made by the local farmers.

The aim of this study was to evaluate the hygienic-sanitary quality of milk and artisan cheese produced in north Manabi province.

MATERIALS AND METHODS

Area and type of study

A descriptive transection study was done in artisan cheese factories of four cantons (Chone, Pedernales, EL Carmen, and Flavio Alfaro), located in the north of Manabi province (1°03'08"S 80°27'02").

Selection of manufacturers

A randomized sample selection of manufacturers was done based on the data from every canton recorded in the CONEFA (National Council for the Eradication of Foot and Mouth Disease) database, offered by CORPOGAN (Manabi Cattle Farming Corporation). Table 1 shows the distribution of manufacturers by canton (156 farmers) in the north of Manabi province, all of whom agreed to be part of the study of milk and fresh artisan cheese quality.

Table 1. Distribution of manufacturers by canton

Canton	Number of manufacturers selected
Chone	45
El Carmen	34
Flavio	43
Pedernales	34

Sample collection of milk and artisan cheese

Samples were collected from 100% of milk and cheese from the farms selected during milking and storage, respectively, following a sterilization of the tools by dipping in 70% ethanol (v/v) and flamed with a portable burner (Biotech). The collection consisted of 250 mL of raw milk in a sterile flask, and 150 g of cheese in sterile bags, according to the NTE INEN 1529-2-1999 standard. They were identified and transported at 4-6 °C to the microbiology laboratory of the Technical University of Manabi, the Chone extension.

Determination of microbiological analyses

All the milk and artisan cheese samples were processed. For the cheese, serial dilutions obtained from 25 g of the product were used, in 225 mL dilutant (0.1% peptone water), both according to the NTE INEN-ISO 6887-5- 2014 standard.

The assays used the petrifilm method, acknowledged by the Association of Official Agricultural Chemists - AOAC Official Method 991.14.2002 for counts of mesophile aerobics (aerobics counts 3M™ Petrifilm™), total coliforms, and enterobacteria (coliforms 3M™ Petrifilm™), fungi and yeasts (yeasts and molds 3M™), *S. aureus* (Petrifilm™ Staph Express for *S. aureus* counts) (Silbernagel, *et al.*, 2001).

Interpretation of hygienic-sanitary quality indicators

The mean of the microorganism groups was expressed in Log₁₀ UFC/mL, in milk, and Log₁₀ UFC/g, in cheese, to interpret the results of counts of microorganisms that indicate hygienic-sanitary quality. The results were compared to the limits set for mesophile aerobic microorganisms in the milk, according to standard NTE INEN 9-5-2012, and fresh cheese, following the NTE INEN 1528-1-2012 standard, for the indicators of enterobacteria, *Staphylococcus aureus*, fungi and yeasts. Besides, on-site observation of milk production and cheese manufacturing was performed.

Statistical analysis

The performance of basic assumptions associated with the normality and homogeneity of variances of each sanitary indicator was done through SAS/INSIGHT. Simple analysis of variance was performed to evaluate the effects of milk and cheese and the criteria studied about these indicators. The Tukey's HSD test was performed for multiple comparison.

The Pearson correlation coefficient was determined for the analysis of the level of association between milk and cheese contamination in all the cantons, using GLIMMIX from statistical package SAS v.9.3 (SAS, 2013).

RESULTS AND DISCUSSION

The general tendency (Table 2) showed that the Pedernales canton had the lowest count values in terms of hygienic-sanitary quality indicators evaluated for the raw milk, followed by Chone and Flavio Alfaro, with significant differences ($p \leq 0.001$) for total coliforms, enterobacteria, and *Staphylococcus aureus*, though remained within the 10⁶ (6 Log₁₀) UFC/mL range, respectively. However, El Carmen evidenced a considerable increase of all the indicators evaluated, thus significantly differing ($p \leq 0.001$) from the rest of the cantons.

Table 2. Behavior of logarithmic means (log₁₀ UFC/mL) of the hygienic-sanitary quality indicators of milk from the cantons studied

Hygienic-sanitary quality indicators	Cantons				±SE/ Significance
	Chone	El Carmen	Flavio Alfaro	Pedernales	
Mesophile aerobic microorganisms	6.19b	6.47c	6.29b	6.04a	0.03/0.001
Total coliforms	5.22b	5.82d	5.56c	5.01a	0.08/0.001
Enterobacteria	4.29b	4.72d	4.48c	4.13a	0.03/0.001
<i>Staphylococcus aureus</i>	4.12a	4.76c	4.43b	4.09a	0.04/0.001
Fungi and yeasts	3.47ab	3.77c	3.52b	3.33a	0.04/0.001

Unequal scripts in the columns indicate highly significant differences for $p \leq 0.001$.

The analysis of means of hygienic-sanitary quality indicators evaluated for the fresh artisan cheese (Table 3), produced the same trend as for the milk. Pedernales evidenced the best

performance, and El Carmen showed the highest values of microorganism counts. Chone showed similar results to Pedernales for mesophile aerobics, enterobacteria, *Staphylococcus aureus*, fungi and yeasts. In turn, Flavio Alfaro showed significant differences ($p \leq 0.001$) from the other three cantons in most indicators evaluated.

Table 3. Behavior of logarithmic means (\log_{10} UFC/mL) of the hygienic-sanitary quality indicators of fresh artisan cheese from the cantons studied

Hygienic-sanitary quality indicators	Cantons			
	Chone	El Carmen	Flavio Alfaro	Pedernales
Mesophile aerobic microorganisms	8.18a	8.95c	8.49b	8.10a
Total coliforms	6.94b	7.80d	7.58c	6.74a
Enterobacteria	6.23a	6.79c	6.56b	6.21a
<i>Staphylococcus aureus</i>	5.46a	6.74b	5.46a	5.41a
Fungi and yeasts	4.64a	5.84c	5.62b	4.59a

Unequal scripts in the same row indicate significant differences for $p < 0.001$

The higher values observed in El Carmen canton may have been caused by malpractice in milking hygiene, the utilization of non-pasteurized milk to make cheese, the absence of a cleaning and disinfection plan, and poor staff hygiene, which stood out during the observation of the process of primary production and cheese making. These factors have been reported in several research studies that also refer that milk contamination can be influenced by the hand of milkmen, poor cleaning of tools, poor hygiene in the facilities, inappropriate storage temperature, and transportation (Martínez *et al.*, 2016; Galván and Hernández, 2020; Armenteros *et al.*, 2020).

The Pedernales canton showed better practices in primary production that might be associated with the lower counts observed in milk and cheese. However, it does not exclude the canton from having the same problems observed in the other cantons that compromise the quality and safety of the items evaluated.

Table 4 shows the significant differences ($p < 0.001$) for the counts of all microorganisms indicating hygienic-sanitary quality, evaluated between the raw milk and the fresh artisan cheese, with an increase observed in the latter.

Table 4. Behavior of logarithmic means (\log_{10} UFC/mL) of the hygienic-sanitary quality indicators between raw milk and the fresh artisan cheese

Hygienic-sanitary indicators	Items	
	Milk (\log_{10} UFC/mL)	Cheese (\log_{10} UFC/g)
Mesophile aerobic microorganisms	6.25a	8.42b
Total coliforms	5.40a	7.26b
Enterobacteria	4.40a	6.44b
<i>Staphylococcus aureus</i>	4.33a	5.99b
Fungi and yeasts	3.52a	5.16b

Unequal scripts in the same row indicate significant differences for $p < 0.001$

It is important to note that the count of mesophile aerobic microorganisms exceeds 1.5×10^6 ($6.18 \log_{10}$) UFC/mL for the raw milk graded as D 9 very bad quality), according to the Ecuadorian NTE INEN 9-5-2012 standard. The values found in the fresh artisan cheese exceeded the maximum microbiological limits established by the NTE INEN 1528-1-2012 standard, in relation to enterobacteria 1×10^3 ($3 \log_{10}$) UFC/g.; *S. aureus* 1×10^2 ($2 \log_{10}$); and fungi and yeasts 1×10^2 ($2.3 \log_{10}$) UFC/g.

The high values found in the fresh artisan cheese for all the indicators evaluated, regardless of the canton, were linked to poor quality of raw milk, since it is not pasteurized previously. Additionally, a lack of hygiene was observed in the tools used in the process, along with the presence of total coliform microorganisms, *S. aureus*, fungi and yeasts.

The significant differences of the mean of total count of mesophile aerobic microorganisms were unfavorable in the cheese. They evidence that, as stated before, the hygienic-sanitary quality indicators are above the acceptability limits established, as a result of poor quality of the process and inappropriate manipulation of the milk used as raw material. These results are similar to the ones reported by Arguello (2015) and Idarraga *et al.* (2018), who observed high count values of microorganisms as indicators of hygiene in artisan cheeses in other regions of Ecuador, resulting from deficiencies in the implementation of good manufacturing practices in the production chains of facilities.

The values of coliform microorganisms and enterobacteria found above the acceptability levels in the national standards corroborated the deficiencies in the conditions of milk production, the infrastructure of cheese factories, and the processing and storage of cheeses, which favor the growth of FTD-causing pathogens, coinciding with Rodríguez *et al.* 2016, and Martínez *et al.* (2020), who claimed that the presence of enterobacteria gains relevance considering that they are indicators of the possible presence of biological hazards associated with FTDs.

Fungi and yeasts are frequent in the environment of cheese factories, and are generally present in the milk and in the manufacturing facility. The values of fungi and yeasts observed in artisan cheeses evidenced a homogeneous behavior. These results coincide with the reports of Martínez *et al.*, 2020 in cheese; Ruíz *et al.* (2017), and Merchán *et al.* (2019), who found values between 2.0 and 5.0 \log_{10} UFC/g of filamentous fungi and viable yeasts in the artisan cheese in the coastal regions, respectively. It is important to highlight the risk posed by the presence of fungi due to their association with mycotoxins (Daou *et al.*, 2020).

The results of *S.aureus* showed significant differences between the raw milk and the artisan cheese, evidencing that apart from milk quality, the flaws in the process of making, storage, and transportation may have influenced it too. Accordingly, Flores *et al.* (2020) stressed on the fact that because of the ubiquity of this microorganism it can be directly transmitted through the milk from udders infected with subclinical mastitis, or other ways, such as the outer surfaces of animals, the milking environment, and the staff.

Martínez *et al.* (2016) said that the raw material to make fresh artisan cheese is one of the main sources of contamination. Moreover, González and Franco (2015); Vásquez *et al.* (2018); Martínez *et al.* (2019) said that the quality of artisan cheese is influenced by factors like *S.aureus* which has been reported as a contaminating microorganism in cheese factories. It constitutes a biological hazard since it produces toxins and extracellular enzymes capable of causing several clinical forms of food poisoning, depending on the amounts of food ingested.

González and Franco (2015), and Mendoza *et al.* (2020) noted that *S.aureus* creates a risk for the consumer of artisan cheese from non-pasteurized milk, considering that the pathogenic traits increase their resistance and survival.

The association effect obtained between milk and cheese contamination by microorganisms in all the cantons studied evidenced the biggest correlation of *S.aureus* (Table 5). The other microorganisms showed values above 0.6.

Table 5. Results of the effect of association effect obtained between milk and cheese contamination by microorganisms in all the cantos studied

Statistics	TC Milk/ Cheese	MA Milk/ Cheese	Enterob. Milk/ Cheese	<i>S.aureus</i> Milk/ Cheese	FY Milk/ Cheese
Correlation coefficient	0.74	0.70	0.71	0.83	0.61
R ² (%)	54.32	49.00	50.70	70.45	37.06
R ² (adjusted for g.l.)	54.03	48.67	50.38	70.25	36.65
Durbin-Watson	0.983206	0.937945	1.50004	0.957135	0.541478
P=	0.0000	0.0000	0.0008	0.0000	0.0000
Regression equation	TC Cheese = 2.49812 + 0.880941* TC Milk	MA Cheese = 1.75263 + 1.06687* MA Milk	Enterob. Cheese = 2.91386 + 0.801063* Enterob. Milk	<i>S.aureus</i> Cheese = - 0.398347 + 1,4732* <i>S.aureus</i> Milk	FY Cheese = 0.249447 + 1.39641* FY Milk

MA: Mesophile aerobic microorganisms; TC Total coliforms; Enterob. Enterobacteria; *S. aureus*: *Staphylococcus aureus*; FY: Fungi and yeasts

The correlation values between milk and cheese contaminations for all the microorganisms may have been influenced by the chemical and microbiological composition of the source milk and the technological process of manufacture; the flaws in the hygienic-sanitary conditions of cheese manufacturing facilities due to malpractice in milking, collection, and transportation of milk, as well as inappropriate cooling conditions, or the absence of them linked to milk and cheese. Similar results were reported in Peru (Guzmán *et al.*, 2015) and in Cuba (Flores *et al.*, 2020; Armenteros *et al.*, 2018). Furthermore, Herrera and Santos (2015), Molina (2018) found counts between 10³ – 10⁶ UFC/g of all the microorganisms studied in fresh cheese made from raw milk, the later associated with FTD outbreaks, with a higher frequency than the ones manufactured using pasteurized milk.

In general, these results evidence the need of improving the diagnostic of microbiological hazards, and the conception of a strategy of participatory strategy designed with the farmers, which facilitates a discussion with a value chain approach, and risk analysis, since the consumption of artisan cheese in the province of Manabi increases the chances of the presence of FTD-causing microorganisms in the people. The pertinence of this strategy would be given by the fact that the artisan cheese made in this area, as a product seeking competitiveness, can be introduced with the advances demanded by the food safety of an artisan product, in compliance with the legal standards, traceability, costumes, and the cultural traditions.

CONCLUSIONS

The fresh milk and artisan cheese from the four cantons of the north of Manabi province included in the study do not meet the sanitary quality requirements, exceeding the microbiological limits by the national standards, with a high relevance in canton El Carmen, and a moderately strong relation between these two items for each indicator microorganism.

RECOMMENDATIONS

The authors recommend further research to establish the correlation between the lack of good manufacturing practices and their direct effect on the high levels of microorganisms observed. Based on these results, a strategy of participatory strategy designed with the farmers should be designed, which includes a value chain approach, and risk analysis, since the consumption of artisan cheese in the province of Manabi increases the chances of the presence of FTD-causing microorganisms in the people.

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AUTHOR CONTRIBUTION

Conception and design of research: RAAS, MAA, MCC, MPR, RFD; analysis and interpretation of data: RAAS, MAA, MCC, MPR, RFD; redaction of the manuscript: RAAS, MAA, MCC, MPR, RFD.

CONFLICT OF INTERESTS

The authors declare the existence of no conflicts of interests.