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Physico-chemical and microbiological evaluation of artisanal cheeses made from cow's milk in the agreste region of Paraíba state

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Abstract

The aim of this study was to evaluate the physicochemical and microbiological characteristics of rennet cheese and butter cheese produced by hand from cow's milk in Paraiba. The study was carried out using 24 cheese samples, six samples per city (three rennet cheeses and three butter cheeses), in which physicochemical analyses were carried out to determine the nutritional composition of the samples and microbiological analyses to quantify enterobacteria and *Escherichia coli*. In this study, only samples without inspection records (federal, state, and/or municipal) were used. Around 80% of the premises had hygiene problems, such as a lack of proper cleaning and poor facilities. Only 20% maintained hygienic handling, while all the sites took care of personal protective equipment such as uniforms, masks, and caps. The values of the physicochemical parameters varied between the cities, with moisture contents ranging from 47.12 to 61.27% for rennet cheeses and from 38.13 to 57.44% for butter cheeses between the cities evaluated. The detection of *E. coli* at around 17% highlighted the importance of adopting strict quality control and hygiene measures at all stages of the production of dairy products in order to guarantee safe, high-quality end products. Based on these results, it can be concluded that the curd and butter cheeses produced by hand in four cities in Paraíba face challenges related to hygiene and sanitation, infrastructure, and inadequate handling in the shops.

Keywords: artisanal cheeses; food safety; enterobacteria; *Escherichia coli.*

Practical Application: Encourage farmers to produce their products better, as the material has high microbiological indices.

1 INTRODUCTION

Milk is considered one of the most nutritionally complete foods and is an important part of the human diet. However, it is worth noting that the composition of milk is an excellent medium for the development of a wide range of microorganisms, including pathogenic ones (Silva et al., 2008). Dairy production is an economic pillar in many regions, with a significant proportion going to the dairy industry, resulting in a wide variety of products. However, a specific portion of the milk produced goes to artisanal producers, boosting the production of cheeses that carry invaluable cultural and regional value (Chaves et al., 2021).

The main types of cheese produced in the Northeast are rennet and butter, which are traditional products and play a fundamental role in the local culture and economy. It is estimated that around 50% or more of cow's milk production in the Northeast is used to make artisanal rennet cheese. The artisanal production of these cheeses is a tradition that spans generations and represents a source of income for many families (Cavalcante, 2023).

However, the artisanal cheese trade is often characterized by informality, which translates into a lack of regulation and proper inspection. Lack of supervision in dairy production can result in products contaminated by bacteria, antibiotics, or pathogens, raising serious concerns about food safety (Dalla Colletta, 2007).

This lack of control not only compromises nutritional quality but also puts consumers' health at risk, potentially exacerbating food safety problems and increasing the spread of food-borne diseases (Marins et al., 2014). This highlights the need for policies and regulations to guarantee the quality and safety of dairy products.

Eating contaminated food, such as low-quality cheese, can pose serious risks to public health, triggering foodborne illnesses with significant consequences. Despite an increased understanding of the microorganisms responsible for these illnesses, they remain a significant problem, affecting economic productivity (Forsythe, 2013). It is crucial to standardize cheese-making processes, strengthen inspection, and especially educate producers and sellers about good hygiene and conservation practices for animal products intended for sale. The aim of this study was to evaluate the physicochemical and microbiological characteristics of rennet cheese and butter cheese produced by hand from cow's milk in Paraiba.

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2 MATERIALS AND METHODS

2.1 Research site and sample collection

The research was carried out in the city of Areia and the surrounding towns of Remígio, Alagoa Grande, and Arara, all located in Agreste Paraibano. Uninspected curd cheeses and butter were bought from fairs and markets near the fairs. For each town, six cheese samples were purchased (three rennet cheeses and three butter cheeses), totaling 24 cheese samples.

Pieces of cheese weighing 250 g each (rennet and butter) were bought and packed in a cooler with ice for transport to the Dairy School located in the Department of Animal Science, Agricultural Sciences Centre (Campus II) at UFPB, and then refrigerated until they were analyzed.

When purchasing the cheeses, a form was filled out for each location, taking into account the following aspects observed at the time of purchase: sanitary conditions and structure of the location, equipment and utensils, hygiene of the handler, method of handling, use of uniform, mask, and cap, and place of display and storage of the cheeses.

2.2 Physicochemical analysis

pH: pH measurements were obtained using a portable pH meter, previously calibrated, by inserting the electrode directly into the analyzed cheese solution (Brasil, 2019).

Total solids (TS): Moisture was determined using the gravimetric method in a drying oven (105°C), following the technique described by Brasil (2019). Moisture was calculated by the difference (100 − TS = moisture).

Fat: The curd cheese and butter samples were analyzed in duplicate according to the methodology of Normative Instruction 30, June 26, 2018, to determine the fat content, using the Gerber Lacto-butyrometer.

Fat in the dry extract (FDE) was obtained using the following ratio (Equation 1):

$$FDE = \frac{\% \ Fat \times 100}{\% \ TS} \quad (1)$$

Protein: Protein was determined according to the methodology laid down in Normative Instruction 30, June 26, 2018, by determining total nitrogen using the Kjeldahl method.

Ash: Ash was obtained by incinerating the sample in a muffle furnace at a temperature of 550°C until it reached a constant weight, in accordance with Normative Instruction 30, June 26, 2018.

2.3 Microbiological analysis

Initially, the samples were prepared by taking aliquots of approximately 10 g of the cheese sample. The 10 g of cheese sample was added to 90 mL of distilled water and homogenized for 1 min, obtaining dilutions of 10^{-1} and 10^{-2}. After homogenization, 1 mL was removed from this bottle and added to another bottle with 99 mL of distilled water and homogenized for 1 min, obtaining a dilution of 10^{-3}. The culture was carried out in sterile disposable petri dishes in duplicate.

A volume of 1 mL for the 10^{-1} and 10^{-3} dilutions and 0.1 mL for the 10^{-2} dilution of each sample was poured into sterile Petri dishes, followed by approximately 15 mL of Violet Red Bile agar medium for the analysis of enterobacteria. The inoculum was mixed into the culture medium using gentle circular movements in the shape of a figure of eight. After the medium had completely solidified, the plates were inverted and incubated at 32°C for 24 h for the enterobacteria counts.

The same procedure was used to count *Escherichia coli*, except that the medium was eosin methylene blue agar. A total of 10 mL of the culture medium was added to each plate. The plates were incubated at 32°C for 23 h, and those with values between 30 and 300 colony-forming units were considered eligible for counting. The counts were made using a magnifying glass attached to a colony counter.

2.4 Statistical analysis

The results of the analyses were tabulated and organized in tables using the Microsoft Excel® software. The data were analyzed using descriptive statistics to calculate the means according to the variables studied.

3 RESULTS

The display of cheeses in the places analyzed revealed significant problems in terms of hygiene and structure. Most of the establishments had issues such as a lack of adequate cleaning and poor facilities. The way in which the cheeses were handled also raised concerns, as seen in Graph 1, with only 20% of the premises maintaining hygienic handling, while 80% had hygiene problems. In addition, the absence of personal protective equipment (PPE), such as uniforms, masks, and caps, was observed at all sites, posing a serious threat to the prevention of food contamination (Table 1).

As far as storing the cheeses is concerned, approximately 67% (Graph 1) of the establishments did not have an appropriate storage area, opting instead to display the cheeses on counters. This can increase the risk of cheese deterioration due to inadequate temperatures and environmental impurities. In order to guarantee the quality and safety of the cheeses, it is essential that establishments adopt hygienic practices, implement the use of PPE, and establish storage that is compatible with what is required for the products.

Each city showed different characteristics in terms of moisture, protein, fat, ash, and pH in their products (Table 2). These variations may be due to factors such as the ingredients used, production practices, and environmental conditions. It is therefore important to consider these factors when evaluating and comparing the quality of these products.

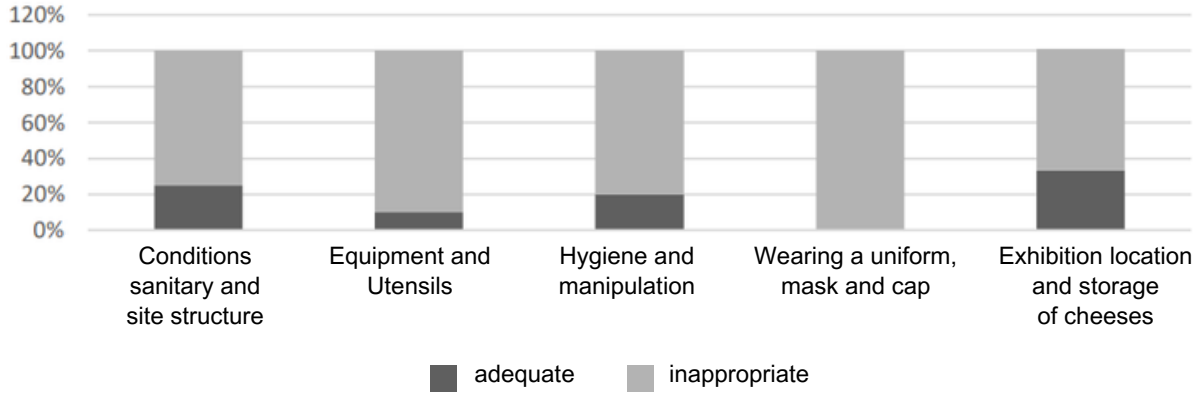
The moisture content varied from 47.12 to 61.27% for rennet cheeses and 38.13 to 57.44% for butter cheeses, indicating variations in the way they were made. The samples from the cities of Areia, Arara, and Alagoa Grande for both types were within the limit set by the recommendation of Normative Instruction No. 30 of June 26, 2001, while the average of the samples from Remígio showed a value of 61.27% for rennet and 57.44% for butter, exceeding the recommended 55% humidity.

Despite the absence of microbiological standards for the genus *Enterobacteriaceae*, Table 3 provides quantitative data on the count of enterobacteria and *E. coli* in different samples of curd and butter cheeses. *Enterobacteriaceae* were found in 100% of the curd cheese samples and in 36% of the butter cheese samples (Table 3).

Of the 23 cheeses analyzed, the results revealed that four of them (approximately 17%) contained the bacterium *E. coli*, while the other 19 (around 83%) showed no traces of this bacterium. It is important to note that, of the four cases of *E. coli* (17%), three samples (13%) complied with the standards established by legislation, as defined in Normative Instruction No. 161, of July 1, 2022. According to this regulation, the maximum limit allowed for the presence of *E. coli* in cheese can vary, depending on the moisture content of the cheese, ranging from 102 to 103 CFU/g.

Table 1. Form applied in the shopping areas.

Aspect evaluated	Evaluation
Sanitary conditions and site structure	
Equipment and utensils	
Handler hygiene	
Way of handling	
Use of uniform, mask, and cap	
Display and storage location	



Graph 1. Hygienic and sanitary conditions in artisanal cheese shops in the cities of Arara, Areia, Alagoa Grande, and Remigio.

Table 2. Physico-chemical composition of curd cheese and butter cheese samples produced by hand in the cities of Arara, Areia, Alagoa Grande, and Remígio, PB.

Variables (%)	Cities							
Cud cheese	Arara	Areia	A. Grande	Remígio	**Average**	SD	Max	Min
Total solid	52.82	50.88	52.88	38.73	48.83	6.79	52.88	38.73
Moisture	47.18	49.12	47.12	61.27	51.17	6.79	61.27	47.12
Crude protein	22.58	28.06	20.26	23.06	23.49	3.29	28.06	20.26
Fat	25.40	20.68	22.76	24.17	23.25	2.03	25.40	20.68
FDE	48.41	40.58	42.94	63.32	48.81	10.21	63.32	40.58
Ash	3.37	3.41	3.49	3.29	3.39	0.09	3.49	3.29
pH	5.87	5.35	5.50	5.31	5.51	0.26	5.87	5.31
Butter cheese	Arara	Areia	A. Grande	Remígio	Average	SD	Max	Min
ST (%)	61.87	53.69	42.56	54.94	53.27	5.35	61.87	42.56
Moisture	38.13	46.31	57.44	45.06	46.73	5.35	57.44	38.13
Crude protein	18.84	22.00	23.08	21.55	21.37	1.26	23.08	18.84
Fat	41.03	27.57	31.85	26.43	31.72	4.72	41.03	26.43
FDE	66.92	51.41	60.34	47.98	56.66	6.97	66.92	47.98
Ash	1.85	1.42	1.87	2.16	1.82	0.20	2.16	1.42
pH	5.77	5.00	5.28	5.37	5.36	0.22	5.77	5.00

FDE: fat dry extract.

Table 3. *Enterobacteriaceae* (ENT) and *Escherichia coli* (*E.coli)* counts using the colony forming units (CFU/G) method.

Cities	Cud cheese	ENT (UFC/g)	*E. coli* (UFC/g)	Butter cheese	ENT (UFC/g)	*E. coli* (UFC/g)
Aara	**1**	3×10^3	$< 1 \times 10^1$	**1**	3×10^3	5
	2	3×10^3	$< 1 \times 10^1$	**2**	5	$< 1 \times 10^1$
	3	3×10^3	$< 1 \times 10^1$	**3**	$< 1 \times 10^1$	3×10^3
Areia	**1**	3×10^3	2.3×10^2	**1**	3×10^3	$< 1 \times 10^1$
	2	3×10^3	$< 1 \times 10^1$	**2**	2.4×10^2	$< 1 \times 10^1$
	3	1.6×10^3	$< 1 \times 10^1$	**3**	1.5×10^1	$< 1 \times 10^1$
Alagoa Grande	**1**	3×10^3	5×10^2	**1**	3×10^3	$< 1 \times 10^1$
	2	3×10^3	8.5×10^1	**2**	5	$< 1 \times 10^1$
	3	3×10^3	$< 1 \times 10^1$	**3**	3×10^3	$< 1 \times 10^1$
Remígio	**1**	3×10^3	$< 1 \times 10^1$	**1**	$< 1 \times 10^1$	$< 1 \times 10^1$
	2	3×10^3	$< 1 \times 10^1$	**2**	5.1×10^2	$< 1 \times 10^1$
	3	3×10^3	$< 1 \times 10^1$	**3**	----	----

4 DISCUSSION

The moisture content in cheese plays a fundamental role in influencing water activity (Aw) and the metabolic actions of microorganisms during ripening. This has direct implications for the pH, texture, flavor, and aroma of the cheese, as observed by Bansal and Veena (2024). Inadequate humidity can negatively affect these characteristics, impacting the final quality of the product. Therefore, precise control of moisture content is essential to guarantee the quality and safety of cheeses.

The pH values for rennet cheese obtained in this study are in line with other studies. As observed by Freitas Filho et al. (2012), who analyzed artisanal rennet cheeses in Calçados (PE), the pH values found ranged from 5.2 to 6.5, demonstrating a range similar to that observed in this study. Although the specific pH values for butter cheese are not recommended by the technical regulations, Leite (2018) and Nassu et al. (2009) present pH values between 5.10 and 6.06 for the butter cheeses analyzed. Determining the pH of cheeses is of great importance due to its implications for the product's characteristics. As recommended by Sousa et al. (2014), pH influences texture, microbial activity, and ripening reactions in cheeses. This is because variations in pH affect the activity of enzymes from rennet and the microbiota present in the cheese, which plays a critical role in shaping flavor and texture during ripening.

The fat content found in the curd cheeses analyzed showed general compliance with the classification required by MAPA in IN No. 30 of 2001, which defines curd cheese as having a fat content in TS of between 35.0% and 60.0%. However, it is important to note that the city of Remígio had a fat content in dry extract above the upper limit, reaching 63.32%. This difference may have implications for the sensory characteristics of the cheese, such as texture and flavor, and also for the other physicochemical parameters evaluated, as observed by Freitas Filho et al. (2020). On the contrary, the regulations recommend a percentage of FDE for butter cheese of between 25 and 55%; however, the samples from the cities of Arara and Remígio exceeded the maximum, and the others were within the recommended range.

However, it should also be borne in mind that the FDE content is calculated on the basis of the TS, and therefore variations in the latter also influence the former. On the contrary, the amount of butter added to the manufacturing process will also influence the fat and FDE results.

There were also variations in protein content between the cities, reflecting the different production conditions and ingredients used. However, it is interesting to note that the values found for the rennet cheese samples in this study are in line with the results of other research, such as the study by Silva et al. (2020), who assessed the nutritional composition of artisanal rennet cheeses in São Luís, Maranhão, and found similar values. The same is true of the values found for butter cheeses by Cavalcante (2023), who analyzed the standardization of butter cheese manufacturing technology. Furthermore, a reference to previous studies, such as Freitas Filho et al. (2012), highlights that the protein content in cooked cheeses can vary significantly from 20% to 30%, depending on the manufacturing system.

The higher presence of enterobacteria in rennet cheese compared to butter cheese can be explained by the lack of milk pasteurization in some cases, less rigorous hygiene conditions, higher pH, lower acidity, and shorter ripening time in rennet cheese (Cavalcante, 2023). Furthermore, additional ingredients and beneficial bacterial cultures in butter cheese can inhibit the growth of enterobacteria. These factors combined contribute to the difference in the presence of enterobacteria between the two types of cheese.

In the context of food, the presence of enterobacteria in products of animal origin can indicate impurities and hygiene conditions, becoming a concern for food safety (Dalmina et al., 2020). Therefore, the monitoring and control of enterobacteria are crucial in various areas of microbiology and public health.

Similar results were found by Barbosa et al. (2017) when analyzing the survival of *E. coli* in rennet cheese manufactured

and kept under refrigeration from experimentally contaminated milk. This highlights that in situations where there are low counts of *E. coli*, these bacteria can remain viable, creating the possibility that they multiply and reach unacceptable levels, which can represent a risk to product safety.

Previous studies have corroborated the association between the presence of enterobacteria and shortcomings in hygiene practices, especially during the milking and handling of raw milk (Tavares, 2021; Zegarra et al., 2009). The detection of enterobacteria, such as *E. coli* and *Salmonella*, in cheeses is often related to the presence of fecal waste and inadequate sanitization measures. These findings highlight the importance of adopting strict quality control and hygiene measures at all stages of dairy product production in order to guarantee safe, high-quality end products. Furthermore, they highlight the ongoing need for monitoring and regulation in the dairy industry, with a view to protecting public health and reducing the risks of bacterial contamination in cheeses.

5 CONCLUSION

Variations in moisture content, pH, fat, protein, and the presence of bacteria between cities highlight the different production practices. The presence of bacteria, such as *E. coli*, points to the need for improvements in hygiene during manufacturing.

It is essential to implement practical measures, such as training producers on hygiene, establishing proper storage standards, and clearer regulations. Such adjustments can improve the quality of cheeses, guarantee food safety, and strengthen the dairy sector, ensuring more reliable products for consumers.

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