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Microbiological profile of artisanal coalho cheese produced in the Seridó region of Rio Grande do Norte, Brazil

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Abstract

The production of artisanal cheeses is generally done empirically, without strict controls on the hygiene and final quality of these products. The aim of this study was to investigate the microbiological profile of artisanal *coalho* cheese produced in the Seridó region of Rio Grande do Norte. Forty-five samples were collected from eleven municipalities. The results for *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* sp. were in line with current legislation. When analyzing coliforms at 35 and 45°C, more than 40% of the samples were contaminated to above legal limits. The fat content of the cheeses was below the regulated level, but the moisture content was in line with the category standards. In conclusion, the microbiological profile of artisanal *coalho* cheese produced in the Seridó region of Rio Grande do Norte does not yet meet the standards set by the Brazilian Ministry of Agriculture and Livestock decree 146, since the standards stipulated for total and thermotolerant coliforms still need to be better monitored, as contamination by these microorganisms is indicative of hygiene failures in the manufacturing processes. Standardizing artisanal products remains a challenge, highlighting the need for enhanced monitoring of product quality and health standards.

Keywords: safe food; pathogenic bacteria; good manufacturing practices; regional product.

Practical application: Monitoring hygiene-health standards of artisanal cheeses guarantees public health.

1 INTRODUCTION

Cheese is defined as the fresh or matured product obtained through the partial separation of whey or milk serum, coagulated by the physical action of rennet, specific enzymes, specific bacteria, or organic acids, alone or in combination, all of which yeld a product of suitable quality for consumption (Brasil, 1996).

According to the specifications of the Technical Regulations for the Identity and Quality of Cheese (Brasil, 2001), *coalho* cheese is cheese that is obtained by coagulating milk using rennet or other appropriate coagulating enzymes, whether or not complemented by the action of selected lactic acid bacteria and is normally marketed up to 10 (ten) days after manufacture.

Brazil boasts over 30 types of artisanal or typical cheeses produced across its regions, including the renowned *coalho* cheese from the Northeast (Kothe et al., 2022). Historically, artisanal cheese production has been empirical, without standardization and strict hygiene and final quality control (Neves et al., 2021). However, the commercial valorization of artisanal products and the consequent inclusion of these products in formal markets has prompted the need to comply with certain sanitary and legal standards (Bezerra, 2022).

Food safety of artisanal cheeses has been the subject of much discussion in Brazil due to their high susceptibility to contamination by a wide range of pathogens such as: *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* (Gérard et al., 2020). In addition, incidences of foodborne infections linked to cheese consumption have been reported globally, including in Brazil (Carvalho et al., 2019).

The milk obtained or handled under unsatisfactory hygienic conditions may harbor a high load of contaminating microorganisms, jeopardizing the quality of milk and dairy products, especially those produced with raw milk (Camargo et al., 2021). Milk quality is influenced by numerous factors, including nutritional and non-nutritional elements, originating from milk processing, fermentation, genetics, diet, lactation period, among others. Given the variation in type and level of metabolites present in milk, studying milk quality becomes essential for ensuring the nutritional value, authenticity, and food safety of dairy products (Suh, 2022).

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Cheese contamination often results from failures in hygienic and sanitary practices during milking, the health conditions of animals and workers, equipment cleanliness, inadequate handling and storage of the milk, as well as the absence of good manufacturing practices (GMP) during cheese production (Penna et al., 2021). In order to obtain high-quality products, complying with good agricultural practices (GAP) and GMP procedures is imperative. GMP are general (essential) hygiene requirements and good preparation practices for food intended for human consumption, including the stages of manufacturing, fractioning, storage, transportation, and marketing (Brasil, 1997).

This pursuit for quality is intricately linked to production standards and effective management of production activities across the entire production chain, including government entities, professionals, producers, retailers, and, finally, consumers (Bezerra, 2022).

Ordinance No. 146 of the Ministry of Agriculture, Livestock, and Supply (*Ministério da Agricultura e Pecuária* — MAPA), which regulates the industrial and sanitary inspection of products of animal origin, outlines the microbiological requirements for cheese marketing (Brasil, 1996). Hygiene and food safety criteria are carried out through the detection of certain microorganisms, considered indicators (coliforms) or pathogens.

The aim of this research was to investigate the microbiological profile related to hygiene and health safety indicators, as well as the physical-chemical characteristics such as pH, fat content, and humidity of *coalho* cheese produced in the Seridó region of Rio Grande do Norte (RN).

2 MATERIAL AND METHODS

2.1 Sample collection

Between November 2019 and December 2020, forty-five samples of artisanal *coalho* cheese were randomly collected from cheese makers, street markets, and local shops, ready for sale in the following municipalities: Caicó, São João do Sabugi, Jucurutu, Currais Novos, Jardim do Seridó, São Fernando, Jardim de Piranhas, Timbaúba dos Batistas, Lagoa Nova, Tenente Laurentino, and Cruzeta, all located in the Seridó Region of RN. The samples were packed in an isothermal box containing ice and transported to the laboratory, where they were kept at a temperature of 2-8°C. The analyses were carried out gradually according to the samples and the data tabulated for later assessment. Microbiological analysis was carried out at the Milk Quality Laboratory (LABOLEITE) of *Universidade Federal do Rio Grande do Norte/Escola Agrícola de Jundiaí* (UFRN/EAJ) in Macaíba, RN.

2.2 Microbiological analysis

To prepare the microbiological analyses, each of the packages containing the cheese samples underwent thorough external cleaning, followed by aseptic removal of the cheese. Microbiological quality assessment of the cheese samples was conducted in accordance with the protocols outlined by the Association of Officiating Analytical Chemists (AOAC, 2005). This method facilitates the rapid determination of bacterial loads by counting on Compact Dry plates, which are pre-sterilized and contain nutrients supplemented with selective substances, chromogenic enzyme substrate, and a gelling agent. The plates, with a surface area of 20 cm^2, were incubated and the microorganisms detected and counted, according to their characteristics. Results were expressed in colony-forming units per milliliter (CFU/mL) or per gram (CFU/g).

The samples were prepared using 25g of the sample in 225 mL of peptone water for the first homogenization (10^{-1}) and then subjected to serial dilutions, 10^{-2} and 10^{-3}. Subsequently, 1 mL of sample from each dilution was added and incubated in automatic diffusion plates specifically developed for the analysis. *Listeria* spp.*, Staphylococcus aureus, Salmonella* spp., coliforms at 35°C, and coliforms at 45°C were incubated according to the specifications described in Table 1 (AOAC, 2005).

2.3 Analysis of physico-chemical characteristics

Analyses of pH, humidity, and fat in the total solids of the cheeses were carried out in triplicate at the Animal Nutrition Laboratory of UFRN/EAJ, Macaíba, RN, according to the methodologies described below.

2.3.1 pH

The pH analysis was based on measuring the concentration of hydrogen ions in the sample. The pH meter was calibrated with pH 4 and 7 buffer solutions. To measure pH, the samples were prepared in accordance with the Manual of Physical-Chemical Food Analysis of the Adolfo Lutz Institute (2008). 20 g of a homogeneous and representative cheese sample were added to 20 mL of distilled water. After homogenization, pH was directly measured using a calibrated pH meter.

2.3.2 Humidity

Humidity content was analyzed using a Humidity Analyzer (Ohaus MB23) using 3 g portions of a uniform and representative cheese sample. This parameter is determined through the weight loss of the sample influenced by heat.

2.3.3 Fat

For fat content analysis, 3 g of each homogeneous and representative cheese sample was weighed directly in a butyrometer. A beaker was attached to the bottom so that it was well sealed. Then, 5 mL of water, 10 mL of sulfuric acid solution, and 1 mL

Table 1. Inoculation time and temperature for the development of the different species of bacteria on Compact Dry ® plates.

Species	Inoculation time (hours)	Temperature
Coliforms 35°C and *E. coli*	24	35 ± 2°C
Salmonella sp.	20–24	41–43°C
Coliforms 45°C	18–24	40–42°C
Listeria spp.	24	35–37°C
Staphylococcus aureus	24	35–37°C

Source: AOAC (2005).

of isoamyl alcohol were added and the butyrometer was transferred to a water bath at 65°C to facilitate sample dissolution. The butyrometer was closed and shaken until a homogeneous liquid was obtained. Once dissolved, the top cap was removed, and water was added up to the last mark on the butyrometer. The cap was replaced, and the sample was centrifuged for 10 minutes at 1,200 rpm. Finally, the percentage of fat was read directly on the butyrometer scale.

3 RESULTS AND DISCUSSION

The production of *coalho* cheese is widespread in Rio Grande do Norte and holds significant socio-economic and cultural importance for the region. Therefore, ensuring the hygienic and sanitary quality of the product is paramount, as well as the production of a safe and consumable food item.

Bacteria from the Enterobacteriaceae family serve as crucial indicators of hygiene and food safety. Although cheese is considered a healthy and nutritious food, some food-borne diseases (FBD) are associated with its consumption. *Listeria monocytogenes*, *Salmonella* sp, *Staphylococcus aureu*s, and *Escherichia coli* are among the main indicator pathogens associated with cheese contamination (Choi et al., 2016).

The findings of this study were analyzed in accordance with Ordinance No. 146 (Brasil, 1996). The frequency count of the microorganisms investigated was calculated using a descriptive statistical method. Table 2 shows the results of the frequency of microbiological legal acceptability for the presence of *Staphylococcus aureus*, *Listeria* spp., *Salmonella* sp., and Coliforms at 35 and 45°C. MAPA Ordinance No. 146 establishes: absence of *Salmonella* and *Listeria monocytogenes* in a 25 g sample, and tolerance limits of 1 x 10^3 most probable number (MPN)/g for *Staphylococcus*, 5.0 x 10^3 CFU/g for Coliforms at 35°C, and 5.0 x 10^2 CFU/g for Coliforms at 45°C.

In 10 (22.2%) of the samples, the presence of *Staphylococcus aureus* was detected, with counts ranging from 2 to 50 CFU/g. These counts fall within the acceptable limit prescribed by current legislation for cheese identity and quality.

The *Staphylococcus aureus* count observed in the analyzed samples can be deemed satisfactory, with the highest count recorded at 50 CFU/g. This fining contrasts with the results reported by Bomfim et al. (2020), who found that 91.66% of *coalho* cheese samples sold in street markets in Bahia were contaminated by *Staphylococcus*, of which 87.5% were above the limit tolerated by law.

The low count of this microorganism can be attributed to the training provided by Sebrae, RN, which has raised awareness among producers regarding the adoption of GMP. According to Oliveira et al. (2019), contamination by *Staphylococcus aureus* is classified as the most important in the manufacture of *coalho* cheese, as it may indicate lapses in personal hygiene or the acquisition of contaminated raw materials. In the case of artisanal cheeses, contamination through milk due to bovine mastitis is a plausible scenario (Johler et al., 2018).

Of the 45 samples analyzed, 42 (93%) had different species of *Listeria* spp. (Figure 1). However, no *Listeria monocytogenes* was identified.

For the Listeria analysis, there is great concern about the presence of *L. monocytogenes*, which was not detected in the samples analyzed. This bacterium thrive in ready-to-eat foods, surviving in extreme environmental conditions such as: acidity, high salinity, low nutrient concentrations, and with resistance to a wide temperature range, surviving variations from 0 to 45°C (Oswaldi et al., 2022). Thus, GAP and GMP are important tools to prevent this agent from spreading in the environment and gaining access to food via milk through bovine mastitis, contaminated water, poorly sanitized equipment and utensils, and other sources. Torres et al. (2021), analyzing samples of *coalho* cheese and butter cheese in the state of Alagoas, found some samples positive for *Listeria* spp and 3.3% (2/60) of the samples positive for *L. monocytogenes*. Although the count may be considered low, it underscores health authorities' concerns regarding artisanal cheeses, which can pose serious risks to public health, since they are capable of causing serious illnesses when contaminated, especially for vulnerable groups such as pregnant women, newborns, the aged and immunocompromised (McLauchlin et al., 2004).

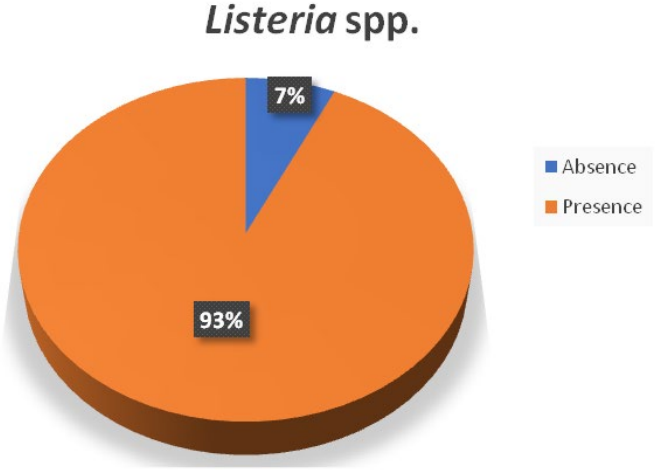

Figure 1. Percentage of samples contaminated by *Listeria* spp.

Table 2. Frequency of microbiological acceptability according to Ordinance No. 146 of MAPA for the presence of *Staphylococcus aureus*, *Listeria spp.*, *Salmonella* sp., and Coliforms at 35 and 45°C.

Microbiological Legal Standard	Samples				
	***Staphylococcus aureus* (%)**	***Listeria* spp. (%)**	***Salmonella* sp. (%)**	***Coliforms 35°C* (%)**	***Coliforms 45°C* (%)**
Acceptable	100	93	100	60	58
Unacceptable	-	-	-	40	42

Salmonella sp. was not detected in the samples studied. The absence of *Salmonella* spp. in the cheese samples analyzed may be related to improved control measures during the cheese-making and storage processes. Almeida et al. (2021) analyzed published data on the microbiological quality of handmade *coalho* cheeses and found that 90% of the samples analyzed had bacterial counts above those permitted by law. The presence of total Coliforms, *Salmonella* spp., and *Listeria monocytogenes* was identified.

According to the sampling plans developed by the International Commission on Microbiological Specifications for Food (ICMSF) for the assessment of microbiological risks related to food consumption, *Salmonella* sp. is classified at level 10, on a scale ranging from 1 to 15. *Salmonella* is one of the main microorganisms causing food poisoning in Brazil. Santos et al. (2020), analyzing the microbiological quality of artisanal *coalho* cheeses sold in street markets, detected the presence of *Salmonella* sp. in 20% of the samples analyzed. This figure may be related to the absence of local sanitation control in street markets, where the samples were collected, potentially leading to environmental contamination.

Eighteen (40%) of the coliform counts at 35°C were found to be above the acceptable limits, with counts reaching 2.84 x 10^5 MPN/g. In the 45°C coliform count, 19 (42.2%) samples surpassed legal thresholds, with the highest count being 1.56 x 10^5 MPN/g. These data highlight the imperative to enhance production practices in order to comply with the legislation and underscores the importance of monitoring these products to uphold legal standards. Similar figures were observed by Bomfim et al. (2020), where the total coliform count was above 1.1 x 10^3 MPN/g, 62.5% of which were thermotolerant coliforms.

Silva et al. (2012) assessed the microbiological quality of artisanal *coalho* cheese produced in the state of Pernambuco and found total and thermotolerant coliforms and the presence of *Escherichia coli* in 100% of the samples studied. These data prove the importance of monitoring the adequacy of these products in relation to the standards stipulated by laws and regulatory bodies. Brazilian regulation 368/97 of MAPA (Brasil, 1997) includes instructions regarding the origin of raw materials, the hygienic and sanitary conditions of establishments that produce/industrialize food for human consumption, the hygiene requirements of establishments, the personal hygiene of employees and their state of health, hygiene requirements during production, the use of drinking water, packaging, and laboratory control. Adhering to these measures is imperative to prevent food contamination and safeguard public health.

The implementation of food safety programs by small producers involves some difficulties: lack of information and knowledge on the part of employees, financial limitations, personal attitudes, or even the burden of excessive documentation requirements (Carrascosa et al., 2016). Corroborating Carrascosa et al. (2016), Barros et al. (2019) found a degree of reluctance on the part of artisanal cheese producers to adopt hygienic-sanitary practices in their production environment, rendering much of the cheese susceptible to microbiological contamination. Barros et al. (2019) also highlights the importance of raising awareness, knowledge, and training for cheese factory owners and their employees to enforce GMP in dairy operations, ensuring the production of healthy, nutritious, and innocuous food.

In addition to microbiological criteria, the legal parameters for cheese evaluation encompass physicochemical aspects, as recommended by the Technical Regulation for the Identity and Quality of *Coalho* Cheese (Brasil, 2001). This regulation outlines the identity and minimum quality requirements that *coalho* cheese intended for human consumption must meet (Supplementary Figure).

The average values of the physicochemical characteristics of the cheeses analyzed are shown in Table 3.

The humidity levels of the cheeses studied ranged from 35.47 to 48.48%, but the average humidity of the samples was 42.46%. According to the specifications of the Technical Regulations for the Identity and Quality of Cheese (Brasil, 2001), *coalho* cheese is a medium to high humidity cheese (humidity between 36.0 and 45.9%). Thus, it may be concluded that the humidity content of the cheeses analyzed varied within the limit established for the category.

The fat content varied between 18 and 34%, with an average of 26.9%. The Technical Regulations for the Identity and Quality of Cheese (Brasil, 2001) state that the fat content in the total solids of *coalho* cheese should vary between 35 and 60%, so the cheeses analyzed were not in accordance with the regulations in force for this parameter. Producers of *coalho* cheese generally subject the milk to a skimming process, carried out in an entirely artisanal manner, which may compromise the fat percentage of the resulting cheese. In addition, they also use the fat removed from the milk to make a typical regional butter-from-the-land, also known as *sertão* butter or bottle butter, serving as an additional source of income for the producers. The fat content of *coalho* cheese can undergo significant changes depending on factors such as the quality of the raw material, the skimming process and the formation and handling of the curd (Silva et al., 2020).

The pH values of the samples varied between 5.34 and 6.68, with an average value of 6.11. This parameter is important to determine as it influences the chemical reactions catalyzed by enzymes from the microbiota and rennet. Cheeses with a very high pH provide favorable conditions for the development of bacteria (Sousa et al., 2014). This variation in pH may be due to various factors, including the lack of standardization of production, distinct milk characteristics and preparation techniques, as well as environmental influences.

Table 3. Average values of the physicochemical characteristics of the handmade *coalho* cheese samples produced in the Seridó region of Rio Grande do Norte.

Parameters (%)	Mean ± SD	CV
Fat	26.9 ± 3.5	13.0
Humidity	42.46 ± 3.1	7.3
pH	6.11 ± 0.3	5.4

SD: Standard Deviation; CV: Coefficient of Variation.

4 CONCLUSION

Despite the results for *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes* being compliant with official regulations, the microbiological profile of artisanal *coalho* cheese from the state of Rio Grande do Norte still needs to be improved. Embracing good manufacturing practices is imperative. Further research can provide data on the current microbiological quality situation of local artisanal *coalho* cheese, enabling the adoption of corrective measures to improve hygiene and food safety indicators.

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