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## BACTERIOLOGICAL AND CHEMICAL ASSESSMENT OF RAW MILK SOLD IN SÃO RAIMUNDO DAS MANGABEIRAS, MARANHÃO, BRAZIL

AVALIAÇÃO BACTERIOLÓGICA E QUÍMICA DO LEITE CRU COMERCIALIZADO EM SÃO RAIMUNDO DAS MANGABEIRAS, MARANHÃO, BRASIL

EVALUACIÓN BACTERIOLÓGICA Y QUÍMICA DE LECHE CRUDA VENDIDA EN SÃO RAIMUNDO DAS MANGABEIRAS, MARANHÃO, BRASIL

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### ABSTRACT

Owing to its nutrient content, milk is among the most consumed foods; however, milk can be easily contaminated or adulterated because of inadequate handling and hygiene practices during its production chain. Thus, in this study, we aimed to evaluate the bacteriological, and chemical compositions of raw milk sold in São Raimundo das Mangabeiras in Northeast Brazil. Ten samples were obtained between November 2022 and June 2023. Mesophilic bacteria were enumerated, total (TC) and thermotolerant (TTC) coliforms were quantified, and the presence of *Escherichia coli* and *Salmonella* spp. were evaluated. Lactose (%), protein (%), fat (%), and added water (%) contents were also determined. Results showed mesophilic bacterial growth in 80,0% of our samples, with the highest value being  $1 \times 10^3$  colony-forming units (CFU)/mL. In contrast, 100% of our samples contained TC and TTC, with the highest values ranging from 150–210 most probable number (MPN)/mL. None of the samples contained *Salmonella* spp.; however, one sample (10,0%) contained *E. coli*. Moreover, 80,0% of our samples had low fat, and 30,0% low lactose. In addition, 1,0% added water was detected in one sample (10,0%). Therefore, some raw milk sold in São Raimundo das Mangabeiras are not suitable for consumption, indicating inadequate handling of this food product.

### KEYWORDS

*Escherichia coli*; food quality; Microbiology.

## RESUMO

O leite é um dos alimentos mais consumidos no mundo, pois é rico em nutrientes; entretanto, esse alimento pode ser facilmente contaminado ou adulterado, devido as práticas de manejo e higiene inadequadas. Assim, o objetivo desse trabalho foi avaliar a composição química e bacteriológica do leite cru comercializado em São Raimundo das Mangabeiras, Nordeste do Brasil. Um total de 10 amostras foram obtidas entre novembro de 2022 a junho de 2023, e processadas para: enumeração de bactérias mesófilas; quantificação de coliformes totais (CT) e termotolerantes (CTT); e avaliação da presença de *Escherichia coli* e *Salmonella* sp. Além disso, também avaliamos o teor de lactose (%), proteína (%), gordura (%) e água adicionada (%). Um total de 80,0% das nossas amostras apresentou crescimento de colônias de bactérias mesófilas, com o maior valor de  $1 \times 10^3$  unidades formadoras de colônias (UFC)/mL. Por outro lado, um total de 100% das nossas amostras apresentou CT e CTT. Os maiores valores variaram entre 150 – 210 número mais provável (NMP)/mL. Nenhuma amostra teve a presença de *Salmonella* sp; entretanto, uma (10,0%) apontou a presença de *E. coli*. Além disso, um total de 80,0% das nossas amostras apresentou baixo teor de gordura e 30,0% baixa taxa de lactose. Também verificamos a quantidade de 1,0% de água adicionada em uma de nossas amostras (10,0%). Portanto, algumas amostras de leite cru comercializado em São Raimundo das Mangabeiras não são apropriadas para o consumo da população, indicando uma manipulação inadequada desse alimento.

## PALAVRAS-CHAVE

*Escherichia Coli*. Qualidade de Alimentos. Microbiologia.

## RESUMÉN

La leche es uno de los alimentos más consumidos en el mundo, pues es rica en nutrientes; sin embargo, este alimento puede contaminarse o adulterarse fácilmente debido a prácticas inadecuadas de manipulación e higiene. Por lo tanto, el objetivo de este trabajo fue evaluar la composición química y bacteriológica de la leche cruda comercializada en São Raimundo das Mangabeiras, Nordeste de Brasil. Se obtuvieron un total de 10 muestras entre noviembre de 2022 y junio de 2023, las cuales se procesaron para: recuento de bacterias mesófilas; cuantificación de coliformes totales (CT) y termotolerantes (CTT); y evaluación de la presencia de *Escherichia coli* y *Salmonella* sp. Además, también evaluamos el contenido de lactosa (%), proteína (%), grasa (%) y agua añadida (%). Un total del 80,0% de nuestras muestras mostraron crecimiento de colonias de bacterias mesófilas, siendo el mayor valor de  $1 \times 10^3$  unidades formadoras de colônias (UFC)/mL. Por otro lado, un total del 100% de nuestras muestras mostraron CT y CTT. Los valores más altos oscilaron entre 150 – 210 número más

probable (NMP)/mL. Ninguna muestra tuvo presencia de *Salmonella* sp; sin embargo, uno (10,0%) indicó la presencia de *E. coli*. Además, un total del 80,0% de nuestras muestras tenían un bajo contenido en grasas y 30,0% bajo en lactosa. También comprobamos la cantidad de 1,0% de agua añadida en una de nuestras muestras (10,0%). Por lo tanto, algunas muestras de leche cruda vendidas en São Raimundo das Mangabeiras no son aptas para el consumo de la población, lo que indica un manejo inadecuado de este alimento.

## PALABRAS CLAVE

*Escherichia coli*; Calidad de los alimentos; Microbiología.

## 1 INTRODUCTION

Milk is one of the most complete foods because of its nutritional properties that satisfy physiological demands and increase immunity in developing animals, including humans (GIVENS, 2020; HUANG *et al.*, 2023). Additionally, the protein content of milk aids the formation of tissues, muscles, and bones (NIELSEN *et al.*, 2023).

However, owing to its abundant nutrients, milk is highly susceptible to the proliferation of pathogenic microorganisms (ALBUQUERQUE *et al.*, 2017; FUSCO *et al.*, 2020), mainly coliforms, *Escherichia coli*, and *Salmonella* (TEBALDI *et al.*, 2008; GUTH *et al.*, 2022), which can cause serious illnesses (CASTRO-ROSAS *et al.*, 2012). In milk derived from production animals, contamination may come from the udder, which may have contact with contaminated land (e.g., with feces) (BEKUMA; GALMESSA, 2018), hands of producers, and dirty instruments (NYOKABI *et al.*, 2021). Therefore, the entire milk production chain must be monitored to ensure food safety (GRIFFITHS, 2010; BORREANI *et al.*, 2019).

In addition to microbiological parameters, raw milk that is intended for consumption must satisfy chemical standards, such as fat, protein, and lactose contents (BRASIL, 2018). These parameters can be affected when inappropriate management is applied during milk production, including the addition of prohibited substances such as water. In Brazil, most fresh milk producers are from small farms that do not receive financial support from the government and are unaware of the correct measures for collecting, processing, and storing milk. Consequently, fresh milk derived from these small producers may contain pathogenic microorganisms or may have altered chemical parameters, making it unsuitable for consumption (MARTINELLI *et al.*, 2014).

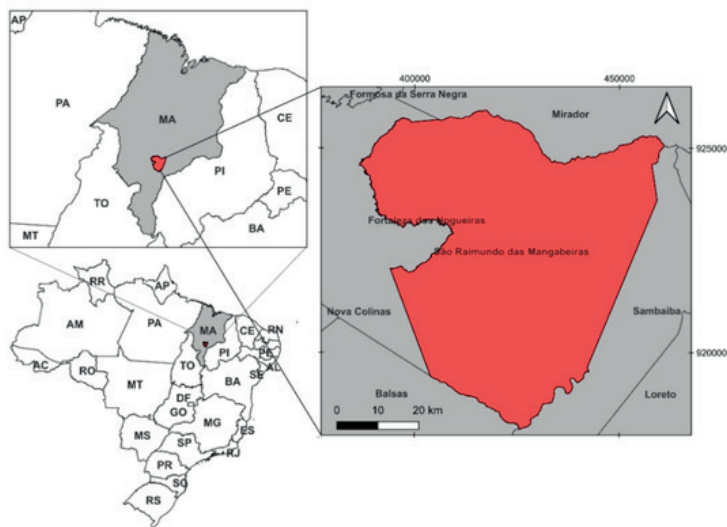
Therefore, in this study, we aimed to perform bacteriological and chemical analyses of raw milk sold in the municipality of São Raimundo das Mangabeiras, Northeast Brazil.

## 2 METHODS

### 2.1 SAMPLES COLLECTION

Ten raw milk samples (1000 mL) were obtained from different commercial establishments in the municipality of São Raimundo das Mangabeiras (Figure 1) between November 2022 and June 2023. Each commercial establishment had different milk supplier, according to the sellers. The samples were stored in sterile bags inside a thermal box and transported to the Laboratory of Microbiology and Biotechnology (LAMBIO) of the Federal Institute of Maranhão, São Raimundo das Mangabeiras, for bacteriological and chemical analyses.

**Figure 1** – Geographical location of São Raimundo das Mangabeiras, South of Maranhão, Brazil



Source: Prepared by the authors

### 2.2 BACTERIOLOGICAL ASSESSMENT

All glassware sterilization procedures, culture media preparation, and sample processing for bacteriological analyses were performed according to a previously established protocol (BANDEIRA *et al.*, 2023). In summary, mesophilic bacteria were enumerated in milk samples (25mL) diluted in peptone water 0,1% ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ). From solution of each dilution, a 1-mL aliquot (in triplicate) was taken and added to Petri dishes containing 15 mL of plate count agar (PCA), according to the pour plate technique. These plates were inverted and incubated in a bacteriological oven for 48 hours at 35°C (BANDEIRA *et al.*, 2023).

The total (TC) and thermotolerant (TTC) coliforms were quantified using the multiple tube technique. Briefly, for the presumptive test, the samples (25mL) were diluted in 225 mL of lactose broth (LB) to prepare three dilutions ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ). Samples were then incubated in a bacteriological oven at 35°C for 48 hours, and the positive ones were used in the confirmation test to determine the presence of TC and TTC. A total of 100  $\mu$ L of each of the positive samples was added to new tubes with lids containing 2% brilliant green bile broth (BGBB) for detection of TC or *E. coli* broth (ECB) for detection of TTC, using three dilutions ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ) in triplicate for both procedures.

The tubes containing BGBB were incubated at 35°C for 24 hours (bacteriological oven), and the tubes containing ECB were incubated in a water bath at 44.5°C for 24 hours. Positive tubes were counted according to the sample dilution, and the results were recorded as most probable number (MPN) of colonies/mL (BANDEIRA *et al.*, 2023).

Additionally, we investigate the presence of *E. coli* in the positive samples for TTC by seeding an aliquot of these samples on Petri dishes containing methylene blue eosin agar (EMB). For *Salmonella* detection, we diluted the milk samples (25mL) in tubes containing LB ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ), at 35°C for 24 hours (pre-enrichment test), followed by an enrichment procedure in tubes containing Rapport Vassiliadis broth (42°C for 24 hours). Afterward, the samples were seed on Petri dishes containing xylose lysine deoxycholate (XLD) agar and Hektoen enteric (HE) agar using the streaking method, followed by incubation at 35°C for 24 hours (BANDEIRA *et al.*, 2023).

The results were compared with those established by the Brazilian Normative Instruction from the Secretariat of Agricultural Defense (SDA) n°62, 26/08/2003 (BRASIL, 2003) and the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA)-Normative Instruction 76, 26/11/2018.

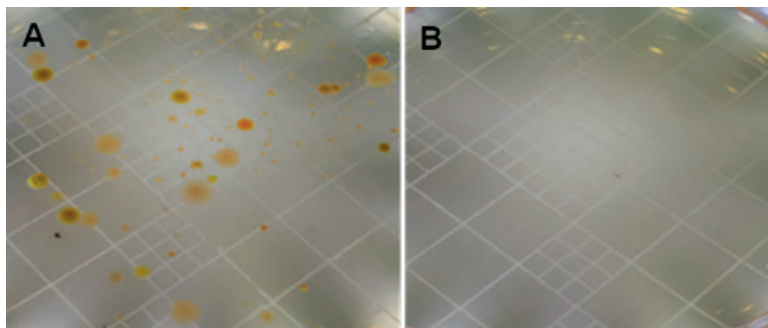
## 2.3 CHEMICAL COMPOSITION

Fat (%), lactose (%), protein (%), and water (%) contents were evaluated automatically using the Ultrasonic Milk Analyzer equipment (Master Complete, Akso®), following the manufacturer's recommendations. Each sample was assessed in duplicate.

## 3 RESULTS

A total of 80% of our milk samples showed growth of mesophilic bacterial colonies (Figure 2. A), and for the other 20%, we did not detect colony-forming units (CFUs) growth (Figure 2. B). In general, the sample 5 showed the lowest number of CFUs ( $1.1 \times 10^1$ ), and the sample 3 demonstrated the highest number of CFUs ( $1.0 \times 10^3$ ) (Table 1). However, none of the milk samples presented values in disagreement with the Brazilian legislation for this parameter.

**Figure 2** – Presence (A) and absence (B) of mesophilic bacterial growth on Plate Count Agar in raw milk samples sold in São Raimundo das Mangabeiras, Maranhão, Brazil



Source: Research data

**Table 1** – Enumeration of mesophilic bacteria in raw milk samples sold in São Raimundo das Mangabeiras, Maranhão, Brazil

Samples	Mesophilic bacteria (CFU/mL)	Maximum value allowed <sup>#</sup>
1	0	
2	0	
3	$1.0 \times 10^3$	
4	$5.3 \times 10^2$	
5	$1.1 \times 10^1$	$10^5$
6	$7.0 \times 10^2$	
7	$8.3 \times 10^2$	
8	$6.4 \times 10^2$	
9	$5.1 \times 10^2$	
10	$3.4 \times 10^2$	

CFU, colony-forming units. <sup>#</sup>According to Brazilian Normative Instruction from Secretariat of Agricultural Defense (SDA) n°62, of 26/08/2003

Source: Research data

All 10 samples (100%) showed the growth of total and thermotolerant coliforms (Table 2). Samples 5 and 1 had the lowest (15 [4.5–42] MPN/mL) and highest (150 [37–420] MPN/mL) amount of TC, respectively. Sample 5 also had the lowest amount of TTC (15 [3.7–42] MPN/mL), and sample 2 had the highest amount of this group of bacteria (210 [40–430] MPN/mL) (Table 2). All samples presented

coliform values according to Brazilian legislation. Additionally, *Salmonella* was not observed in milk samples. However, we showed a suggestive growth of *E. coli* in sample 5, which was verified by the formation of black colonies with a metallic green shine on EMB agar.

**Table 2** – Quantification of total (TC) and thermotolerant (TTC) coliforms in raw milk samples sold in the municipality of São Raimundo das Mangabeiras, Maranhão, Brazil

Samples	TC at 35°C (MPN/mL)	TTC at 44.5°C (MPN/mL)	Maximum value allowed <sup>#</sup>
1	150 (37–420)	150 (37–420)	
2	28 (8.7–94)	210 (40–430)	
3	28 (8.7–94)	28 (8.7–94)	
4	36 (8.7–94)	29 (8.7–94)	
5	15 (4.5–42)	11 (3.6–42)	10 <sup>4</sup>
6	93 (18–420)	21 (4.5–42)	
7	20 (4.5–42)	15 (3.7–42)	
8	24 (4.6–94)	28 (8.7–94)	
9	34 (8.7–94)	43 (9–180)	
10	44 (17–200)	35 (8.7–94)	

**TC, total coliforms; TTC, thermotolerant coliforms, MPN, most probable number.** <sup>#</sup>According to Brazilian Normative Instruction from Secretariat of Agricultural Defense (SDA) n°62, of 26/08/2003. Source: Research data

Chemical analyses of the milk samples revealed that 80,0% had fat content below the normal value (3.0g/100g) (Table 3). Most samples (70,0%) had normal lactose content (min. 4.3 g/100 g), except for samples 6, 7, and 8, which had below-normal values (Table 3). All the samples had a normal protein content (min. 2.9 g/100 g). However, 10,0% (sample 4) had added water (1,0%), which is not allowed for commercialization.

**Table 3** – Chemical properties of raw milk samples sold in São Raimundo das Mangabeiras, Maranhão, Brazil

Samples	Fat (%)	Lactose (%)	Protein (%)	Added water (%)
1	4.0	4.8	3.2	0.0
2	3.0	6.4	4.3	0.0
3	0.4*	4.9	3.3	0.0

Samples	Fat (%)	Lactose (%)	Protein (%)	Added water (%)
4	0.6*	4.4	2.9	1.0*
5	0.7*	4.7	3.1	0.0
6	0.5*	3.9*	3.7	0.0
7	0.3*	4.2*	3.4	0.0
8	0.7*	3.7*	3.5	0.0
9	0.9*	4.8	3.9	0.0
10	0.5*	4.3	3.7	0.0

\*values in disagreement with the Brazilian Ministry of Agriculture and Livestock (MAPA) -Normative Instruction 76, de 26/11/2018.

Source: Research data

## 4 DISCUSSION

To the best of our knowledge, this is the first study known to evaluate the bacteriological and chemical properties of raw milk sold in São Raimundo das Mangabeiras, southern Maranhão, Brazil. Some milk samples showed the growth of potentially pathogenic bacteria and alterations in the chemical properties, which could be harmful to consumer health and reduce the commercial value of this product.

Mesophilic bacterial growth was observed in most samples; however, it was within the limits established by Brazilian legislation for this type of food. High levels of these bacteria were detected in raw milk samples from the states of Rio Grande do Norte (OLIVEIRA *et al.*, 2020), Bahia (ALVES *et al.*, 2023), Pará (SILVA *et al.*, 2023) e Rondônia (ROCHA *et al.*, 2023). This group of bacteria is routinely investigated in studies that evaluate milk quality, because their high abundance may indicate a lack of adequate hygiene during milking (SILVA *et al.*, 2023).

In addition to enumerating mesophilic bacteria, we evaluated the most probable number of total and thermotolerant coliforms in raw milk samples. Although all samples showed the presence of coliforms, none showed values in disagreement with the Brazilian legislation. However, previous studies performed in the states of Pará (LIMA *et al.*, 2016; SILVA *et al.*, 2023), Rondônia (ROCHA *et al.*, 2023), Minas Gerais (SÁ, 2011), and Rio Grande do Sul (SILVA *et al.*, 2010) demonstrated high levels of coliforms in raw milk. The growth of this bacterial group in milk samples, even at low levels, indicates possible fecal contamination of the udders of animals and hands of producers, as well as of water and/or utensils (SABEDOT *et al.*, 2011; FURLAN; VALEJO, 2017).

Although the milk samples showed low levels of total and thermotolerant coliform growth, we detected the suggestive presence of *E. coli* in one sample. This bacterium was also detected in milk samples from Bahia (SOUSA *et al.*, 2021), Minas Gerais (BATISTA *et al.*, 2014), and São Paulo (RIBEIRO *et*



*al.*, 2019). According to milk quality standards, *E. coli* must be absent to avoid possible infections in consumers (BRASIL, 2018). The main source of contamination is mammalian feces, including those of humans (FREITAS-GUIMARÃES; LANGONI, 2009). Most strains of *E. coli* are harmless; however, some are pathogenic such as the enterohemorrhagic strain (EHEC), which produces verotoxins that induce severe diarrhea and can lead to kidney failure, and hemolytic anemia (BIBBAL *et al.*, 2022).

Furthermore, we evaluated the chemical components of the raw milk samples. Main component changes were associated with a low-fat content. In previous studies, low fat content was found in milk samples from different regions of Brazil (OLIVEIRA *et al.*, 2012; SANTOS; FOGAÇA, 2019). This low-fat content may be associated with the consumption of low-quality food, making digestion more difficult and reducing nutrient conversion (JOBIM *et al.*, 2008). The fat content of milk indicates the health of the herd, and its concentration can vary depending on the time of year, breed, dilution due to increased production, and physiological state of the animals (GEBERT; BASSANI, 2022). A reduction in the fat concentration of milk reduces its nutritional value (ROSA *et al.*, 2017), requiring a greater volume to compensate for the reduction in its quality.

Regarding lactose content, only three samples had values outside the required standard. Brasil *et al.* (2013) also showed a reduction in the lactose concentration in milk samples produced using a mechanized system. The lactose content in milk is dependent on glucose production in the liver due to propionate production in the rumen, especially in diets with a greater inclusion of concentrate (PEREIRA *et al.*, 2012). In addition, lactose concentration is directly linked to osmotic pressure in the mammary glands; thus, high levels of lactose production determine the volume of milk produced and excreted by the mammary glands (HETTINGA, 2019). However, as mentioned above, the chemical composition of the milk may also alter according to diet, health, milking management, season, lactation stage, genetics and breed of the herd (GUERIOS; SANTOS, 2020).

Additionally, one of our samples contained 1% added water, which is not allowed for sale and consumption because it causes changes in the physical and chemical composition of milk (ABRANTES *et al.*, 2014). The presence of added water in milk samples was also verified in a previous study performed in the state of Paraíba (PAIVA *et al.*, 2018) and Bahia (ALVES *et al.*, 2023). Adding water to milk is a fraudulent and illegal practice that is widely performed by low-scale businesses, compromising food quality (PANCIERE; RIBEIRO, 2021).

Finally, although informal commercialization of raw milk is prohibited by Brazilian legislation (Decree-Law No. 66,183 of November 5, 1970), due to the absence of an official health inspection, this food is normally sold in São Raimundo das Mangabeiras. The risk of ingesting raw milk is due to the possible proliferation of pathogenic microorganisms (ROCHA *et al.*, 2023), as demonstrated in our study. Furthermore, fraudulent practices (e.g., addition of water) were detected in raw milk sold in São Raimundo das Mangabeiras. This practice reduces the quality of the milk and compromises its shelf-life, as it dilutes its basic components. Additionally, this added water can vehicle pathogenic bacteria, which further contributes to the contamination of raw milk. As previously mentioned, all these aspects can directly impact the consumer's health.

## 5 CONCLUSION

Most of the bacteriological and chemical characteristics of raw milk sold in São Raimundo das Mangabeiras present values according to Brazilian legislation. However, *E. coli* was detected in 10% of our samples, and some had low fat and lactose contents, as well as added water. These characteristics compromise the quality of the raw milk sold in this city and may negatively impact consumer health.

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