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AVALIAÇÃO BACTERIOLÓGICA DE SUCOS DE POLPAS DE FRUTAS COMERCIALIZADOS EM PRAÇAS PÚBLICAS DE SÃO RAIMUNDO DAS MANGABEIRAS E BALSAS, MARANHÃO, BRASIL

BACTERIOLOGICAL EVALUATION OF FRUIT PULP JUICES MARKETED IN PUBLIC SQUARES OF SÃO RAIMUNDO DAS MANGABEIRAS AND BALSAS, MARANHÃO, BRAZIL

EVALUACIÓN BACTERIOLÓGICA DE JUGOS DE PULPA DE FRUTA COMERCIALIZADOS EN PLAZAS PÚBLICAS DE SÃO RAIMUNDO DAS MANGABEIRAS Y BALSAS, MARANHÃO, BRASIL

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RESUMO

O consumo de sucos de polpas de frutas vem aumentando a cada ano devido à praticidade em sua preparação e à presença de nutrientes, o que contribui para uma vida saudável. Entretanto, devido ao processamento inadequado desse alimento, algumas bactérias patogênicas podem se proliferar e causar infecções nos consumidores. Dessa forma, o objetivo deste trabalho foi realizar uma avaliação bacteriológica de amostras de sucos de pontos comerciais localizados em São Raimundo das Mangabeiras (SRM) e Balsas (BS), Maranhão. Foram coletadas duas amostras de sucos variados em cinco pontos de venda aleatórios situados em praças públicas de cada município, totalizando 20 amostras. No laboratório, foram realizadas a detecção e a contagem de colônias de bactérias mesófilas, coliformes totais e termotolerantes, *Staphylococcus* coagulase-positivo, *Escherichia coli* e *Salmonella*. Todas as amostras apresentaram crescimento de colônias de bactérias mesófilas, mas em quantidade viável ao consumo humano. Cerca de 86% das amostras

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apresentaram coliformes totais e 100% apresentaram coliformes termotolerantes; entretanto, os níveis permaneceram dentro dos limites estabelecidos pela legislação brasileira vigente. Não houve a presença de *Staphylococcus* e *Salmonella*; porém, em uma das amostras de suco de cajá coletadas em SRM e nas amostras de caju, abacaxi e cupuaçu coletadas em BS, houve a presença da bactéria *E. coli*. Portanto, nossos dados indicam que a maioria dos sucos avaliados estão adequados para o consumo; no entanto, a detecção de *E. coli* em algumas amostras indica a necessidade de adoção de boas práticas de higiene na manipulação desses alimentos.

Palavras-chave: polpas de frutas; Coliformes; *Escherichia coli*; bactérias patogênicas.

ABSTRACT

The consumption of fruit pulp-based juices has been increasing annually owing to their convenience of preparation and high nutrient content, which contribute to a healthy lifestyle. However, owing to inadequate handling, processing, and storage, pathogenic bacteria may proliferate and cause severe infections in consumers. Thus, the objective of this study was to conduct a bacteriological evaluation of juice samples from commercial establishments located in São Raimundo das Mangabeiras (SRM) and Balsas (BS), Maranhão. Two juice samples were collected from five randomly selected vending sites located in public squares in each municipality, yielding a total of 20 samples. Microbiological analyses were performed in the laboratory to detect and enumerate mesophilic bacteria, total coliforms, thermotolerant coliforms, coagulase-positive *Staphylococcus*, *Escherichia coli*, and *Salmonella*. All samples exhibited growth of mesophilic bacteria, although at levels acceptable for human consumption. Approximately 86% of the samples presented total coliforms, and 100% presented thermotolerant coliforms; however, the levels remained within the limits established by current Brazilian legislation. *Staphylococcus* and *Salmonella* were not detected. Nevertheless, *E. coli* was identified in one sample of hog plum juice from SRM and in samples of cashew, pineapple, and cupuaçu from BS. Therefore, our data indicate that most of the evaluated juices are suitable for consumption; however, the detection of *E. coli* in some samples highlights the need for continuous monitoring and the implementation of good hygiene practices during the handling of these foods.

Key words: fruit pulp; Coliforms; *Escherichia coli;* pathogenic bacteria.

RESUMEN

El consumo de jugos a base de pulpa de fruta aumenta cada año debido a la practicidad de su preparación y a su contenido de nutrientes, lo que contribuye a una vida saludable. Sin embargo, debido a un procesamiento inadecuado, pueden proliferar bacterias patógenas y causar infecciones en los consumidores. Por ello, el objetivo de este trabajo fue realizar una evaluación bacteriológica de muestras de jugos provenientes de establecimientos comerciales en São Raimundo das Mangabeiras (SRM) y Balsas (BS), Maranhão. Se recolectaron dos muestras de diversos jugos en cinco puntos de venta seleccionados al azar en plazas públicas de cada municipio, sumando un total de 20 muestras. En el laboratorio, se llevó a cabo

la detección y el recuento de colonias de bacterias mesófilas, coliformes totales y termotolerantes, *Staphylococcus* coagulasa-positivo, *Escherichia coli* y *Salmonella*. Todas las muestras presentaron crecimiento de colonias de bacterias mesófilas, pero en cantidades aptas para el consumo humano. Alrededor del 86% de las muestras presentaron coliformes totales y el 100% presentó coliformes termotolerantes; no obstante, los niveles se mantuvieron dentro de los límites establecidos por la legislación brasileña vigente. No se detectó presencia de *Staphylococcus* ni de *Salmonella*; sin embargo, se detectó la bacteria *E. coli* en una muestra de jugo de *cajá* recolectada en SRM y en las muestras de jugos de *caju* (marañón), piña y *cupuaçu* recolectadas en BS. Por lo tanto, nuestros datos indican que la mayoría de los jugos evaluados son aptos para el consumo; sin embargo, la detección de *E. coli* en algunas muestras señala la necesidad de adoptar buenas prácticas de higiene en la manipulación de estos alimentos.

Palabras clave: pulpas de fruta; coliformes; *Escherichia coli*; bacterias patógenas.

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INTRODUCTION

Brazil is one of the world's leading fruit producers, a position resulting from its vast floristic diversity, favorable climate, and extensive territorial reach[1]. According to data from the Food and Agriculture Organization, the country ranks third in global fruit production, with a yield of approximately 58 million tons in 2023[2]. This growth is associated, among other factors, with the population's adoption of healthier dietary habits, such as increased consumption of fruit juices[3].

Natural juices are significant sources of vitamins, minerals, and bioactive compounds that contribute to metabolic function and health maintenance. Furthermore, their ease of preparation and broad sensory acceptance make these products an accessible and practical option for daily consumption[4,5]. However, inadequate handling and improper storage may facilitate the proliferation of pathogenic microorganisms that can cause foodborne infections and intoxications[5,6]. Such contamination is frequently linked to poor hygiene during the preparation, handling, and commercialization stages. Consequently, the microbiological quality of these products can be compromised at various points in the production chain, from fruit harvesting to final consumption[5,7].

Among the bacteria frequently monitored in food quality analysis studies, mesophilic bacteria, coliforms (including *Escherichia coli*), *Salmonella* spp., and *Staphylococcus aureus* are of particular prominence[8]. The genus *Salmonella* is widely recognized for its capacity to infect both humans and animals, causing gastroenteritis and typhoid fevers that may progress to severe food poisoning or even death[9]. In turn,

E. coli is naturally present in the intestinal microbiota of humans and warm-blooded animals and is disseminated into the environment through feces[10]. The presence of this species in food indicates fecal contamination and can cause symptoms such as diarrhea, vomiting, abdominal pain, and nausea[4]. Finally, *S. aureus* (coagulase-positive) is a gram-positive microorganism that can colonize the nasal passages and skin of approximately half of the healthy population. This bacterium exhibits high adaptability and can proliferate in various foods, producing enterotoxins that cause food poisoning even after the food has been heated[11,12].

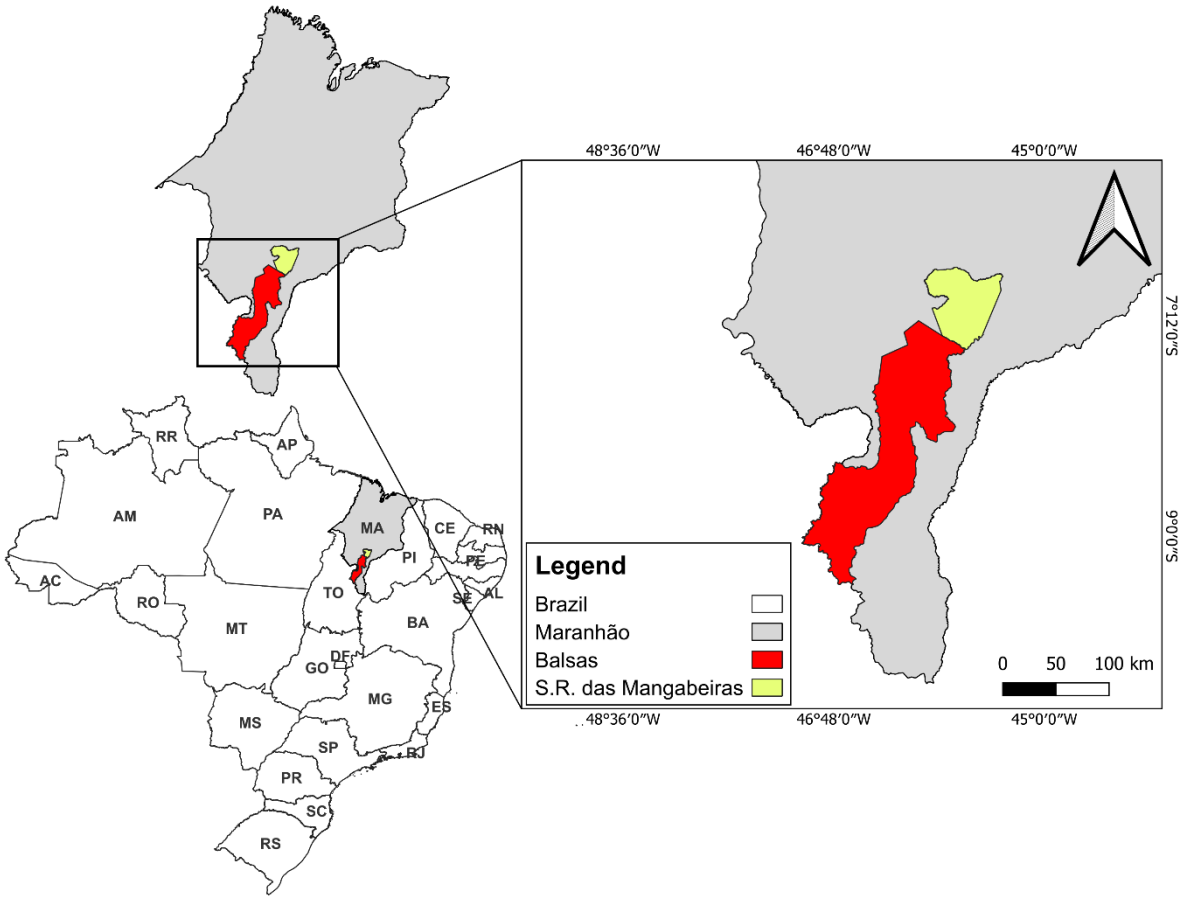
Given these risks, there is an evident need for constant monitoring of the bacteriological quality of fruit juices sold in public spaces, as consumption of products contaminated with pathogenic bacteria is directly related to public health. However, in many cities in the state of Maranhão, scientific information regarding the microbiological safety of juices sold in public squares is scarce. Therefore, this study aimed to evaluate and quantify the presence of pathogenic bacteria in fruit juices commercialized in public squares in the cities of São Raimundo das Mangabeiras and Balsas, Maranhão, Brazil.

MATERIALS AND METHODS

Study area

The study was conducted in the municipalities of São Raimundo das Mangabeiras, with an area of 3,521.5 km² and a population of 18,672 inhabitants, and Balsas, covering approximately 13,141 km² and with approximately 100,000 inhabitants[13], both located in the Southern Mesoregion of Maranhão, Brazil **(Figure 1)**. The predominant biome is the Cerrado, and its climate is characterized as tropical and sub-humid dry, with two well-defined periods: a rainy season (November–April) and a dry season (May–October).

Figure 1 – Location map of the municipalities of São Raimundo das Mangabeiras and Balsas, Southern Maranhão, Brazil.



Sample collection

In each municipality, five sales points located in public squares were randomly selected, totaling ten collection sites. We commercially obtained two samples from each of the five sales points, totaling 20 natural juice samples, with 10 from each municipality (Balsas and São Raimundo das Mangabeiras). The flavors of the juices varied according to availability at the time of field collection and did not constitute an experimental variable, as the aim of the study was not to compare different fruits. For ethical reasons, the specific identities and locations of the sellers were kept confidential.

All juice samples evaluated in this study were prepared at the time of collection. Each 200-mL sample was placed in sterile glass containers, stored in a thermal box, and transported to the Laboratory of Microbiology and Biotechnology (LAMBIO) at the Federal Institute of Maranhão, São Raimundo das Mangabeiras, MA, Brazil. All glassware and culture media used in this study were previously autoclaved at 121°C for 20 minutes to ensure complete sterilization. Furthermore, all procedures were conducted within a laminar flow hood to prevent external contamination[5].

The bacteriological evaluations followed the methodologies recommended by the American Public Health Association (APHA)[14] and the Environmental Company of the State of São Paulo (CETESB)[15], with modifications.

Microbiological assessments

All glassware sterilization procedures, culture media preparation, and sample processing for bacteriological analyses were performed according to previously established protocols[5]. A 25-mL aliquot of juices, in triplicate, at concentrations of 1:1, 1:10, and 1:100 was used for each bacteriological assay. Mesophilic bacteria were enumerated using plate count agar in triplicate, with results expressed in CFU/25 mL. Total and thermotolerant coliforms were quantified by the multiple tube technique and expressed as MPN/25 mL[5,16]. To confirm the presence of *E. coli*, samples from TTC-positive tubes were streaked onto Eosin Methylene Blue (EMB) agar plates and incubated at 35°C for 24 hours[5,16]. *E. coli* presence was demonstrated based on the growth of characteristic colonies featuring a black center and metallic sheen. To detect *Salmonella* spp., 25 mL of sample was pre-enriched in Lactose Broth (10^{-1} to 10^{-3} dilutions) at 35°C for 24 hours, followed by selective enrichment in Rappaport-Vassiliadis broth at 42°C for 24 hours. The culture was then streaked onto XLD and HE agar plates and incubated at 35°C for 24 hours. *Salmonella* presence was confirmed by characteristic black-centered colonies with red borders on XLD agar and green borders on HE agar[5,16]. The presence of coagulase-positive *Staphylococcus* was verified using Baird-Parker agar, followed by coagulase, catalase, and Gram staining[16]. Positive controls, using American Type Culture Collection strains, and negative controls (culture media only) were included in all bacteriological assays to ensure the reliability and quality control of the results. The results obtained were compared with the standards established by the Brazilian Health Regulatory Agency (ANVISA) in Normative Instruction No. 161, of July 1, 2022 (Brasil, 2022)[17].

RESULTS

São Raimundo das Mangabeiras

All samples evaluated from São Raimundo das Mangabeiras exhibited growth of mesophilic bacteria. The highest colony-forming unit (CFU) count was recorded in sample A (hog plum), reaching 2.5 x 10^5 CFU/25 mL, while the lowest values were observed in sample H (acerola/*Malpighia* spp.), with 6.4 x 10^2 CFU/25 mL (Table 1). Notwithstanding, none of the samples exceeded the limits established by current Brazilian legislation[17].

Table 1 – Mean number of colony-forming units (CFU/25 mL) of mesophilic bacteria found in fruit pulp juice samples collected in São Raimundo das Mangabeiras, Maranhão, Brazil.

Samples	Mesophilic bacteria (CFU/25 mL)
A	2.5×10^5
B	7.4×10^2
C	6.3×10^4
D	3.3×10^4
E	1.6×10^4
F	7.2×10^3
G	8.5×10^4
H	6.4×10^2
I	1.1×10^3
J	1.0×10^4

Legend: A = hog plum; B = passion fruit; C = acerola; D = cupuaçu; E = guava; F = orange; G = pineapple; H = acerola; I = acerola; J = hog plum. Maximum value permitted[17]: 1×10^6 CFU/25 mL.

Regarding coliform analysis, sample A (hog plum) showed no growth of total coliforms (TC). However, this same sample yielded a most probable number (MPN) of 3 for thermotolerant coliforms (TTC)/25 mL. Conversely, sample H (acerola) exhibited the highest concentrations of both TC and TTC, at 43 and 29 MPN/25 mL, respectively. Nevertheless, all TC and TTC values found remain within the acceptable standards defined by Brazilian legislation[17] (Table 2).

None of the evaluated juice samples showed colony growth of *Salmonella* or *Staphylococcus*. However, in sample J (hog plum), the presence of *E. coli* colonies was observed, indicating that this specific sample is unfit for human consumption (Table 2).

Table 2 – Quantification of the most probable number (MPN) of total (TC) and thermotolerant (TTC) coliforms found in fruit pulp juice samples collected in São Raimundo das Mangabeiras, Maranhão, Brazil.

Samples	**TC (MPN/25 mL)**	**TTC (MPN/25 mL)**
A	0	3 (0.15–9.6)
B	9.2 (1.4–38)	3 (0.15–9.6)
C	9.2 (1.4–38)	3.6 (0.17–18)
D	3 (0.15–9.6)	3 (0.15–9.6)
E	14 (3.6–42)	3 (0.15–9.6)
F	6.2 (1.2–18)	3 (0.15–9.6)
G	3 (0.15–9.6)	3 (0.15–9.6)
H	43 (9–180)	29 (8.7–94)
I	16 (4.5–42)	7.4 (1.3–18)
J	11 (3.6–38)	3.6 (0.17–18) *

Legend: A = hog plum; B = passion fruit; C = acerola (*Malpighia* spp); D = cupuaçu; E = guava; F = orange; G = pineapple; H = acerola; I = acerola; J = hog plum. (*) presence of *Escherichia coli.* Maximum value permitted for Enterobacteriaceae[17]: 1×10^2.

Balsas

In Balsas, all evaluated samples exhibited growth of mesophilic bacteria. The highest CFU count was recorded in sample H (cashew), reaching 9.0 x 10^4 CFU/25 mL, whereas the lowest values were observed in sample I (pineapple), with 4.0 x 10^1 CFU/25 mL (Table 3). Nevertheless, none of the samples exceeded the limits established by current Brazilian legislation for this parameter[17].

Table *3* – Mean number of colony-forming units (CFU/25 mL) of mesophilic bacteria found in fruit pulp juice samples collected in Balsas, Maranhão, Brazil.

Samples	Mesophilic bacteria (CFU/25 mL)
A	3.8 x 10^3
B	7.0 x 10^2
C	5.0 x 10^2
D	3.4 x 10^4
E	1.7 x 10^2
F	4.5 x 10^2
G	7.0 x 10^2
H	9.0 x 10^4
I	4.0 x 10^1
J	2.7 x 10^3

Legend: A = cupuaçu (*Theobroma* spp.); B = passion fruit; C = acerola (*Malpighia* spp.); D = guava; E = hog plum; F = acerola; G = soursop; H = cashew; I = pineapple; J = cupuaçu. Maximum value permitted[17]: 1 x 10^6 CFU/25 mL.

Regarding coliforms, sample B (passion fruit) exhibited the lowest concentrations of TC and TTC, recorded at 3.0 MPN/25 mL. Conversely, sample F (acerola) showed the highest TC concentration, reaching 210 MPN/25 mL. The highest values for TTC were detected in samples H (cashew), I (pineapple), and J (cupuaçu/*Theobroma* spp.), each yielding 23 MPN/25 mL. All evaluated samples showed no growth of bacteria belonging to the genera *Salmonella* and *Staphylococcus*. Therefore, based on these specific parameters, the samples were considered fit for consumption; however, characteristic growth of *E. coli* colonies was observed in samples H (cashew), I (pineapple), and J (cupuaçu). The presence of this pathogen demonstrates a health risk to the population associated with the consumption of these specific samples.

Table 4 – Quantification of the most probable number (MPN) of total (TC) and thermotolerant (TTC) coliforms found in fruit pulp juice samples collected in Balsas, Maranhão, Brazil.

Samples	TC (MPN/25 mL)	TTC (MPN/25 mL)
A	9.2 (1.4–38)	9.2 (1.4–38)
B	3 (0.15 – 9.6)	3 (0.15–11)
C	3.6 (0.17–18)	7.4 (1.3–20)
D	3.6 (0.17–18)	3.6 (0.17–18)
E	3.6 (0.17–18)	9.2 (0.14–38)
F	210 (40–430)	7.2 (1.3–18)
G	93 (18–420)	15 (3.7–42)
H	23 (4.6–94)	23 (4.6–94) *
I	23 (4.6–94)	23 (4.6–94) *
J	3.6 (0.17–18)	23 (4.6–94) *

Legend: A = cupuaçu (*Theobroma* spp.); B = passion fruit; C = acerola (*Malpighia* spp.); D = guava; E = hog plum; F = acerola; G = soursop; H = cashew; I = pineapple; J = cupuaçu. (*) presence of *Escherichia coli*. Maximum value permitted for Enterobacteriaceae[17]: 1 x 10^2.

DISCUSSION

This study evaluated, for the first time, the bacteriological quality of fruit pulp juices commercialized in public squares across two municipalities in Southern Maranhão: São Raimundo das Mangabeiras and Balsas. We observed that most samples exhibited growth of mesophilic bacteria and coliforms within the limits established by current legislation[17]. However, some samples tested positive for *E. coli*, indicating fecal contamination and a potential health risk to consumers.

Mesophilic bacterial counts remained within acceptable limits in the evaluated juice samples collected in São Raimundo das Mangabeiras and Balsas. Although all values comply with Brazilian legislation, counts nearing the permitted thresholds suggest failures in hygiene and preservation practices during juice preparation and handling. In a study conducted on orange juices sold in Uberlândia, Minas Gerais, Brazil[18], two samples were found with too numerous to count mesophilic bacteria, demonstrating that this parameter can serve as a critical indicator for evaluating the sanitary quality of food.

Coliform analysis also revealed that in all positive samples, the levels of this bacterial group complied with current Brazilian legislation. However, thermotolerant coliform-positive samples indicate the presence of fecal contamination, possibly associated with the use of contaminated water, inadequate handling, and/or insufficient sanitization of the utensils used in juice preparation. Bandeira et al.[5], when analyzing fruit pulps sold in supermarkets in São Raimundo das Mangabeiras, also identified high coliform levels even under freezing conditions, reinforcing that hygiene failures in the production chain remain recurrent**.** Furthermore, Silva et al.[19] have demonstrated that "detox" juices sold in Bahia contained coliforms above the acceptable limit, in addition to yeasts, fungi, and parasites, showing that microbial contamination is a frequent issue in natural beverages sold outside controlled environments.

The most significant finding in our study was the presence of *E. coli* in four juice samples. This bacterium was present in one hog plum juice sample collected in São Raimundo das Mangabeiras and in one sample each of cashew, pineapple, and

cupuaçu collected in Balsas. Therefore, even with low coliform levels, the occurrence of *E. coli* is still possible. The detection of this bacterium is a major indicator of fecal contamination and reveals deficiencies in good manufacturing practices. Pathogenic strains of *E. coli* can cause gastroenteritis, diarrhea, vomiting, and, in severe cases, hemolytic uremic syndrome[20]**.** Pilo et al.[21] have reported similar results in Belo Horizonte (MG), where over half of the evaluated juices were contaminated with *E. coli*. In Mexico, Hernández-Anguiano et al.[22] have found that 91% of fresh juice samples were contaminated with this bacterium, evidencing that this is a recurrent problem in various regions of the world.

In contrast, the absence of *Salmonella* spp. and coagulase-positive *Staphylococcus* in all juice samples evaluated in this study represents a positive outcome, given that these bacteria are among the primary etiological agents of foodborne outbreaks[9,23]**.** *Salmonella* is frequently associated with gastroenteritis and enteric fevers, while *S. aureus* can produce thermostable enterotoxins that resist heating and cause acute food poisoning[10,11,24]. Ribeiro et al.[25] have highlighted that *Salmonella* remains one of the leading bacteria causing foodborne outbreaks in Brazil, accounting for 646 cases between 2000 and 2023, which underscores the importance of periodic inspections. Despite this, studies conducted in São Paulo[26] and Mato Grosso[27] also did not detect these microorganisms in juices or coconut water sold *in natura*, suggesting that factors such as acidity and low exposure time may limit their growth.

However, Araújo et al.[28] have proved that molecular methods (qPCR) are more sensitive for detecting *Salmonella* spp., showing that the absence of microorganisms using conventional microbiological methods does not necessarily imply the actual absence of contamination. Additionally, while the natural acidity of juices, especially those from fruits like acerola and pineapple, may partially inhibit microbial growth, it is insufficient to eliminate pathogens[29]. This condition reinforces the need for proper handling and storage practices to prevent recontamination.

Although it was not possible in this study to determine the exact origin of the contamination in the evaluated juices, the primary causes are generally inadequate handling, the use of non-potable water, and incorrect storage. Insufficient sanitary inspections and a lack of knowledge of good practices among vendors exacerbate this scenario, especially in inland municipalities where infrastructure and access to technical training are more limited.

Thus, it is essential to strengthen public policies and educational initiatives to train food handlers, alongside awareness campaigns on hygiene and preservation. The implementation of simple measures, such as the use of treated water, proper sanitization of utensils, and constant refrigeration of juices, can significantly reduce contamination risks. Therefore, while the results indicate that most evaluated samples fall within legal standards, the detection of *E. coli* in some of them reinforces the need for continuous monitoring and the improvement of Good Manufacturing Practices. Strengthening sanitary education and surveillance is imperative to ensure food safety for the population and to reduce the risk of bacterial disease outbreaks.

CONCLUSION

The bacteriological analysis of natural juices commercialized in public squares in São Raimundo das Mangabeiras and Balsas revealed that, overall, the evaluated samples complied with the microbiological standards established by current legislation. However, the detection of *E. coli* in samples of hog plum, pineapple, cupuaçu, and cashew juices evidences hygienic-sanitary failures during handling, preparation, and/or storage stages, indicating fecal contamination and representing a potential health risk to consumers. Thus, these findings reinforce the critical importance of adopting Good Manufacturing Practices, including the use of treated water, sanitized utensils, and adequate storage conditions.

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