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Bacteriological assessment of fruit pulp sold in supermarkets in São Raimundo das Mangabeiras, Maranhão State, Brazil

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ABSTRACT. Natural fruit pulp is highly appreciated because it retains the flavor and nutrients of the fresh fruit. However, fruit pulp is susceptible to contamination by pathogenic bacteria, mainly because of improper handling and storage. Therefore, this study evaluated and quantified the presence of pathogenic bacteria in fruit pulp sold in São Raimundo das Mangabeiras, Maranhão, Brazil. Two fruit pulp samples each of açaí (A and B), acerola (C and D), cajá (E and F), cashew (G and H), and passion fruit (I and J) were obtained from supermarkets and subjected to the following microbiological analyses: enumeration of mesophilic bacteria using plate count agar; quantification of total (TC) and thermotolerant (TTC) coliforms using the multiple tube technique; qualitative detection of *Escherichia coli* on eosin methylene blue agar and that of *Salmonella* on xylose lysine base agar and enteric agar. Mesophilic bacteria were found to grow in all the samples, with the highest value obtained for the acerola pulp (sample D; average: 1.08×10^5 colony-forming units g^{-1}). However, all samples were in accordance with the Brazilian legislation for this parameter. The most probable number of colonies (MPN g^{-1}) of TC and TTC were ⩾1,100 in açaí (sample A), cajá (sample E), and cashew (sample G) pulps, which is in disagreement with the maximum value allowed. *E. coli* and *Salmonella* were not found to grow in the samples evaluated in this study. Collectively, the data indicate inadequate handling and storage in the production chain of fruit pulp sold in the municipality of São Raimundo das Mangabeiras, suggesting that the population in the region is exposed to a probable risk of food contamination.

Keywords: pathogenic bacteria; coliforms; *Escherichia coli*; *Salmonella*.

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Introduction

Brazil is one of the main fruit producers worldwide owing to its diverse flora and climate and extensive territorial area (Silva & Abud, 2017; Costa, Gomes, Erazo, Carvalho, & Alencar, 2020). According to the Food and Agriculture Organization [FAO] (Food and Agriculture Organization Corporate Statistical Database [FAOSTAT], 2021), Brazil is the third largest producer worldwide, having produced 59 million tons of fruits in 2021. Production at a large scale may be attributed to the adoption of healthier habits by the population, such as consumption of fruit juices (Brasil, 2014). Moreover, consumption of fruit juices has increased since the advent of the coronavirus disease (COVID-19) owing to the possibility that the chemical compounds present in fruits might strengthen the immune system and help eliminate the virus (SARS-CoV-2) (Sousa et al., 2021a). Indeed, fruits have high nutritional content and are mainly composed of vitamins, minerals, fibers, proteins, lipids, carbohydrates, and natural antioxidants, which help reduce oxidative stress (Santos et al., 2019).

However, some fruits are available only during specific seasons and get spoiled more easily, which makes their consumption and transport unfeasible (Morais, Araújo, & Machado, 2010). Thus, fruit pulp production is considered a suitable alternative that guarantees the availability of fruits during the entire year, in addition to minimizing waste (Silva & Abud, 2017). Fruit pulp production is important for the agroindustry of Brazil and has a relatively low cost and ease of storage and packaging (Jesus et al., 2018; Neri-Numa, Sancho, Pereira, & Pastore, 2018).

In addition to the increase in fruit pulp consumption in Brazil, the informal market associated with the processing and sale of this type of foods has also increased (Santos, Figueiredo Neto, & Donzeli, 2016). However, many establishments do not strictly follow the practice guidelines for fruit pulp production, which may lead to contamination by pathogenic microorganisms (Oliveira & Machado, 2021). The microbiological quality of fruit pulp declines due to poor hygiene (cleaning and sanitizing) and irregularities at freezing stages (Silva, Coutinho, & Soares, 2016). Thus, fruit pulp quality must be constantly monitored through microbiological tests to evaluate the presence of pathogenic organisms of public health relevance (Silva & Abud, 2017).

Therefore, in this study, a bacteriological evaluation of the different types of fruit pulps sold in the supermarkets of São Raimundo das Mangabeiras was performed to assess the viability of this product for consumption by the local population.

Material and methods

Samples and microbiological procedures

Samples were collected from the supermarkets located in the city of São Raimundo das Mangabeiras, Maranhão, Brazil between August, 2022 and November, 2022. Ten fruit pulp samples from different supermarkets and brands were obtained and analyzed. All the evaluated samples were within their expiration date as informed by the manufacturer. Two samples of each of the five different types of fruits [açaí (A and B), acerola (C and D), cashew (E and F), cajá (G and H), and passion fruit (I and J)] were selected from the five largest supermarkets in the city. The supermarkets and suppliers (small and micro producers) were randomly sampled, and the two samples of each fruit were obtained from different supermarkets. The collection site and identity of the supplier of the fruit pulps were kept anonymous. The samples were then packed in sterile bags, placed inside a thermal box with ice, and transported to the Laboratory of Microbiology and Biotechnology (LAMBIO) from the *Instituto Federal do Maranhão*, São Raimundo das Mangabeiras for bacteriological evaluation.

All glassware and culture media used in this study were autoclaved at 120°C for 30 min. to completely sterilize the material. All procedures were performed in a laminar flow hood (Veco, São Paulo, Brazil) to avoid external contamination. The bacteriological evaluations were based on procedures recommended by the American Public Health Association [APHA] (2013) and Companhia Ambiental do Estado de São Paulo [CETESB] (2018), with some modifications. The results obtained were compared with those established by Agência Nacional de Vigilância Sanitária [Anvisa], *Resolução de Diretoria Colegiada* - RDC n. 12 (Brasil, 2001), and *Instrução Normativa* n. 60 (Brasil, 2019) from the Brazilian legislation.

Evaluation of mesophilic bacteria

Each sample (25 g) was diluted in 225 mL of peptone water (GranuCult, Darmstadt, Germany) to obtain a 10^{-1} dilution, which was used to prepare two solutions with dilutions of 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) (Himedia, India), according to the pour plate technique. These plates were inverted and incubated in a bacteriological oven (Quimis, São Paulo, Brazil) for 48 hours at 35ºC to evaluate the growth and count the bacterial colony-forming units (CFU). The colonies were counted using an automatic counter (Quimis). To calculate CFU g^{-1}, the most homogeneous counts of triplicates of one of the three dilutions were used. The average value was obtained and multiplied by the dilution factor of the selected sample.

Evaluation of total and thermotolerant coliforms

To evaluate the presence of total (TC) and thermotolerant (TTC) coliforms, a presumptive test was initially performed, followed by a confirmatory test. For the presumptive test, the sample (25 g) was diluted in 225 mL of lactose broth (LB) (GranuCult, Germany) to prepare three solutions with different dilutions (10^{-1}, 10^{-2}, and 10^{-3}), with a final volume of 10 mL in tubes containing lids and inverted Durham tubes. Samples were then incubated in a bacteriological oven (Quimis) at 35ºC for 48 hours. Thereafter, the presumptive test was considered positive for the tubes containing gas (verified by opening the lid and Durham's tube evaluation).

Samples positive in the presumptive test were used in the confirmation test to determine the presence of TC and TTC. For TC, 100 µL of each of the positive samples was added to new tubes with lids containing 10 mL

of 2% brilliant green bile broth (GranuCult, Germany). Samples were processed in triplicates for each dilution (10^{-1}, 10^{-2}, and 10^{-3}), according to the multiple-tube technique. The tubes were then incubated at 35°C for 24 hours. Positive samples were evaluated as described previously for the presumptive test. Positive tubes were counted according to the sample dilution, and the results were recorded as most probable number of colonies per gram (MPN g^{-1}).

For TTC, 100 µL of each of the positive samples (presumptive test) was added to new tubes with lids containing 10 mL of *E. coli* broth (Sigma Aldrich, Missouri, USA); the test was performed in triplicates for each dilution (10-1, 10-2, and 10-3). The samples were incubated in a water bath (Quimis) at 44.5°C for 24 hours. The test was considered positive for the tubes showing gas formation, and the results were recorded as MPN g^{-1} as described above.

Detection of *Escherichia coli*

Samples positive in the confirmatory test for TTC were used to determine the presence of *E. coli*. A sample of each positive tube was seeded on Petri dishes containing methylene blue eosin agar (GranuCult, Germany) using a sterile swab (Olen, China) according to the streaking technique. The plates were then incubated at 35°C for 24 hours. The presence of *E. coli* was confirmed by observing the growth of typical colonies with a black center and metallic coloration.

Detection of *Salmonella* spp.

To detect *Salmonella* spp., the sample (25 g) was diluted in 225 mL of LB (Kasvi) (pre-enrichment medium) to prepare concentrations of 10^{-1}, 10^{-2}, and 10^{-3}, which were incubated at 35°C for 24 hours. Then, 1 mL aliquot of each sample was added to tubes with lids containing Rapport Vassiliadis broth (Kasvi, Spain) for enrichment, followed by incubation at 42°C for 24 hours. Thereafter, a sterile swab (Olen) was used to seed each sample on Petri dishes containing xylose lysine deoxycholate (XLD) agar (GranuCult, Germany) and Hektoen enteric (HE) agar (GranuCult) using the streaking method, followed by incubation at 35°C for 24 hours. The presence of *Salmonella* was confirmed by the observation of colonies with a black center and a red border on XLD agar and green border on HE agar.

Results

As shown in Table 1, mesophilic bacteria grew in all the evaluated samples and dilutions (Figure 1 A and B). In general, passion fruit pulp samples (I and J) showed the lowest average number of CFUs for mesophilic bacteria, with values of 4.5×10^2 and 2.5×10^2 CFU g^{-1}, respectively (Table 1). Sample D (acerola) had the highest number of CFUs (1.08×10^5 CFU g^{-1}) of mesophilic bacteria (Table 1). However, the numbers of CFUs of mesophilic bacteria in all the samples were within the standard established by the Brazilian legislation RDC Nº 12 (01/02/2001) (Brasil, 2001).

Sample B (açaí) had the lowest amount [9.2 (1.4–38) MPN g^{-1}] of TC. The TC values for samples A (açaí) and E (cajá), with an estimate of >1,100 MPN g^{-1} (Table 2), were in disagreement with the Brazilian legislation. Similarly, sample B (açaí) had the lowest TTC values [21 (4.5–42) MPN g^{-1}]. TTC values for sample E (cajá) (>1,100 MPN g^{-1}) and sample G (cashew) [1,100 (180–4,100) MPN g^{-1}] were in disagreement with the legislation (Table 2).

Table 1. Colony forming units (CFU) of mesophilic bacteria present in the samples of fruit pulp sold in supermarkets of São Raimundo das Mangabeiras, Maranhão, Brazil.

Samples	Mesophilic bacteria (CFU g^{-1})	Maximum value allowed *
A	1.4×10^2	
B	7.7×10^4	
C	4.7×10^4	
D	1.08×10^5	
E	1.4×10^5	10^6
F	1.2×10^5	
G	3.9×10^2	
H	8.8×10^2	
I	4.5×10^2	
J	2.5×10^2	

A, B = açaí; C, D = acerola; E, F = cajá; G, H = cashew; I, J = passion fruit. * Standard established by the Resolução de Diretoria Colegiada - RDC No. 12 (January 2, 2001).

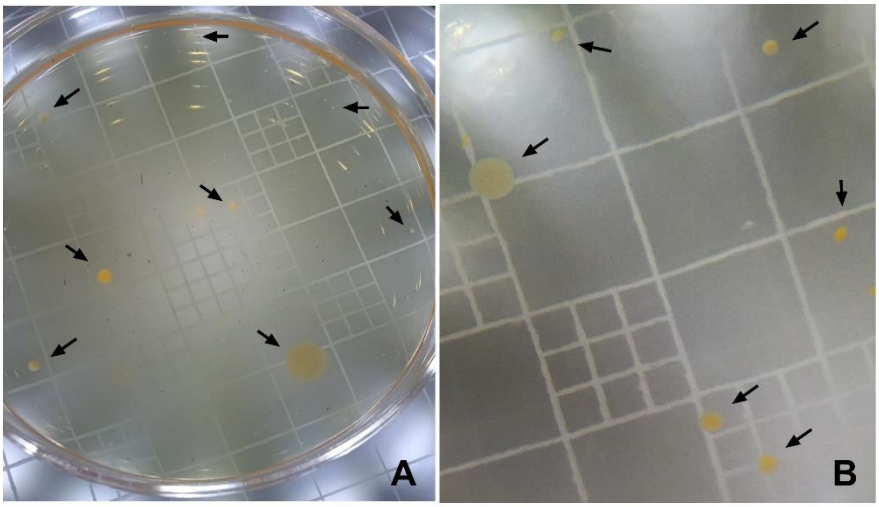

Figure 1. Representative photographs (A and B) of plate count agar (PCA) with distinct (color and shape) colonies of mesophilic bacteria (arrows) found in fruit pulp samples obtained in supermarkets of São Raimundo das Mangabeiras, Maranhão, Brazil.

Table 2. Total (TC) and thermotolerant coliforms (TTC) present in fruit pulp samples sold in supermarkets of São Raimundo das Mangabeiras, Maranhão, Brazil.

Samples	TC/35°C (MPN g^{-1})	TTC/44.5°C (MPN g^{-1})	Maximum value allowed*
A	>1,100 (420) #	43 (9–180)	
B	9.2 (1.4–38)	21 (4.5–42)	
C	93 (18–420)	23 (4.6–94)	
D	23 (4.6–94)	240 (42–1,000)	
E	>1,100 (420) #	>1,100 (420) #	10^2
F	93 (18–420)	150 (37–420)	
G	150 (37–420)	1,100 (180–4,100) #	
H	210 (40–430)	240 (42–1,000)	
I	460 (90–2,000)	28 (8.7–94)	
J	210 (40–430)	23 (4.6–94)	

A, B = açaí; C, D = acerola; E, F = cajá; G, H = cashew; I, J = passion fruit. * Standard established by the Instrução Normativa n. 60, de 23 de dezembro de 2019 (Brasil, 2019). # Values in disagreement with the established maximum standards.

The evaluated samples showed no characteristic growth of *E. coli* and *Salmonella* colonies in the selective culture media used in this study, indicating that all evaluated fruit pulps were in agreement with the legislation for this parameter.

Discussion

To the best of our knowledge, this study investigated the food safety of fruit pulp sold in the supermarkets in São Raimundo das Mangabeiras for the first time by assessing the presence and quantification of potentially pathogenic bacteria. Most samples showed bacterial growth within the maximum permitted limit by the current Brazilian legislation; however, some samples showed higher numbers of TC and TTC than those permitted, indicating poor handling/storage of these fruit pulps and a potential source of contamination for the population of São Raimundo das Mangabeiras.

The highest number of mesophilic bacteria was found in acerola (sample D) fruit pulp (1.08×10^5 CFU g^{-1}); however, all analyzed samples agreed with the current Brazilian legislation for this microbiological parameter. The number of mesophilic bacteria obtained in our sample of acerola was higher than that reported by Lanz, Nachtigal, and Severo (2019) and Sousa et al. (2022), who evaluated the microbiological quality of fruit pulp, including that of acerola, sold in Rio Grande do Sul and Maranhão (municipality of Porto Franco), respectively. Values that disagree with the legislation have already been found for fruit pulps sold in the states of Paraíba (Santos & Vieira, 2020) and Rondônia (Barcelos, Vallati, Almeida, & Prazeres, 2017). The presence of mesophilic bacteria in food is associated with poor handling, and may contribute to outbreaks of food- and water-borne diseases (FWBDs) (Pinheiro, Wada, & Pereira, 2010).

The numbers of TC and TTC found in açaí (sample A), cajá (sample E), and cashew (sample G) pulps did not agree with the Brazilian norms established for this type of food. Previous studies conducted in the states

of Amazonas (Costa et al. 2020), Rondônia (Santos & Romão, 2018), Amapá (Jesus et al., 2018), Bahia (Ferreira et al., 2019), and Maranhão (Arcanjo et al., 2014; Marinho, Moura, Rabelo, Silva, & Furtado, 2018) also reported high numbers of coliforms in açaí pulp. Moreover, high coliform levels have also been found in cashew samples collected from the states of Minas Gerais (Alvarenga, Vieira, Santos, & Fernandes, 2017) and Ceará (Guimarães et al., 2021), demonstrating that sanitation problems associated with the production chain of fruit pulp exist in different regions of Brazil. These fruits have great socioeconomic importance for Brazil (Santana, 2004; D'Arace et al., 2019; Sousa et al., 2021b); therefore, it is necessary to focus on the microbiological parameters of their sub-products, such as fruit pulp.

Coliform bacteria are among the main pathogenic microorganisms associated FWBDs (Nicholson, Neumann, Dowling, & Sharma, 2017). High levels of TC are directly associated with poor hygienic conditions of food (De Bruin, Otto, & Korsten, 2016). Additionally, high levels of TTC (a group that includes *E. coli*) strongly indicate fecal contamination (Montezani, Giuffrida, Andrade, & Silva, 2017). Therefore, our data demonstrate that precarious sanitary conditions exist during the handling, production, and/or storage of fruit pulp sold in São Raimundo das Mangabeiras.

Escherichia coli is one of the main pathogenic bacteria of the coliform group and is naturally present in the intestinal microbiota of humans and warm-blooded animals, thus being constantly eliminated into the environment and contaminating soil, water, and food (Silva et al., 2010). The main symptoms associated with *E. coli* infection are diarrhea, vomiting, abdominal pain, and nausea (Gurgel, Silva, & Silva, 2020). In addition to this species, bacteria of the genus *Salmonella* are also of great relevance within the Enterobacteriaceae family (Chlebicz & Śliżewska, 2018). This bacterium is one of the main causes of FWBDs in humans worldwide, including Brazil (Connor & Schwartz, 2005; Brasil, 2011; Montezani et al., 2017). In this study, these bacteria were not detected in the analyzed fruit pulp samples. However, the presence of *Salmonella* has already been detected in fruit pulps from several Brazilian states (Cohen, Matta, Furtado, Medeiros, & Chisté, 2011; Souza, Macedo, Leandro, Almeida, & Fonseca, 2016; Coutinho et al., 2017; Santos & Vieira 2020), suggesting that the microbiological quality of fruit pulp needs be constantly monitored.

Fruit pulp can be contaminated post-harvest generally due to poor hygiene of the handlers and non-sterile equipment, utensils, and transport vehicles used during their production and distribution. In addition, the use of contaminated water and storage irregularities (supplier and supermarket) are also important sources of fruit pulp contamination (Alegbeleye, Singleton, & Sant'ana, 2018; Sant'anna, Franco, & Maffei, 2020). As Brazil is one of the largest fruit producers worldwide, it is essential to guarantee the safety of fruit pulp to ensure consumer health and credibility in the international market. Thus, evaluation of different fruit pulps for the detection of pathogenic microorganisms is crucial.

The data of this current study are concerning because they indicate a favorable environment for the growth of pathogenic bacteria in fruit pulp that is sold in supermarkets in São Raimundo das Mangabeiras, which may cause serious infections in the population. However, we suggest that more samples be evaluated, and a wide variety of microorganisms be investigated for a better diagnosis of the food safety of fruit pulp consumed in this municipality.

Conclusion

Bacteriological evaluation of the various fruit pulps sold in São Raimundo das Mangabeiras revealed that most samples had acceptable levels of bacterial growth. However, the amounts of TC and TTC found in some samples of açaí, cajá, and cashew were in disagreement with the amounts permitted by the current Brazilian legislation, thus strongly indicating negligence during the handling and storage of these fruit pulps and making them unfeasible for consumption by the population.

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