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Original Article

Microbiological evaluation of homemade mayonnaise and self-serve acai sold in Araguaína, Tocantins

Avaliação microbiológica de maionese caseira e açaí self-service comercializados em Araguaína, Tocantins

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Abstract

Foodborne diseases are common illnesses caused by the consumption of food contaminated with microorganisms, such as viruses, fungi, bacteria, and protozoa. Every year, 600 million people become ill and 420,000 people die as a result of consuming contaminated food. Therefore, food safety is an important issue. In this study, samples of homemade spiced mayonnaise and self-serve acai sold in the city of Araguaína, Tocantins, Brazil were analyzed for microbiological contaminants. Acai was collected from 10 stores, one sample from each store, and tested for mold, yeast, and coliforms, as well as coliform identification and total and thermotolerant coliform counts. Mayonnaise was collected from 20 snack bars, one sample from each. These samples were inoculated on MacConkey and *Salmonella Shigella* agar plates, and the plates were analyzed for growth. *Salmonella* spp. were detected in some Mayonnaise samples, and coliforms were detected in all acai samples; 60% of samples had thermotolerant coliforms, and only 40% were within the limits established by ANVISA. The collected samples of mayonnaise and acai were contaminated with molds and yeasts above the established limit of 10^3 CFU/g. Thus, the analyzed mayonnaise and acai samples were contaminated and unfit for consumption, demonstrating the importance of hygienic-sanitary measures in food handling.

Keywords: thermotolerant coliforms, food contamination, *Salmonella*, food quality, good handling practices.

Resumo

As doenças transmitidas por alimentos são doenças comuns causadas pelo consumo de alimentos contaminados com microrganismos, como vírus, fungos, bactérias e protozoários. Todos os anos, 600 milhões de pessoas ficam doentes e 420.000 pessoas morrem como resultado do consumo de alimentos contaminados. Portanto, a segurança alimentar é uma questão importante. Neste estudo, amostras de maionese temperada caseira e açaí self-service vendidas na cidade de Araguaína, Tocantins, Brasil foram analisadas quanto a contaminantes microbiológicos. O açaí foi coletado em 10 lojas, uma amostra de cada loja, e testado para mofo, levedura e coliformes, bem como identificação de coliformes e contagem de coliformes totais e termotolerantes. A maionese foi coletada de 20 lanchonetes, sendo uma amostra de cada. Essas amostras foram inoculadas em placas de ágar MacConkey e Salmonella Shigella, e as placas foram analisadas quanto ao crescimento. Salmonela spp. foram detectados em algumas amostras de maionese e coliformes foram detectados em todas as amostras de açaí; 60% das amostras apresentaram coliformes termotolerantes, e apenas 40% estavam dentro dos limites estabelecidos pela ANVISA. As amostras coletadas de maionese e açaí estavam contaminadas com bolores e leveduras acima do limite estabelecido de 103 UFC/g. Assim, as amostras de maionese e açaí analisadas estavam contaminadas e impróprias para o consumo, demonstrando a importância das medidas higiênico-sanitárias na manipulação de alimentos.

Palavras-chave: coliformes termotolerantes, contaminação de alimentos, *Salmonella*, qualidade de alimentos, boas práticas de manipulação.

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1. Introduction

Food safety is key to promoting health and ensuring quality of life, as unsafe food can become contaminated with bacteria, viruses, parasites, or harmful chemicals that can cause more than 200 diseases (World Health Organization, 2022).

Foodborne diseases (FBDs) are caused consumption of food products contaminated with infectious or toxic agents, i.e., biological or chemical agents, and are an important public health problem worldwide (Oliveira et al., 2010; Melo et al., 2018; Andrade Júnior et al., 2019; World Health Organization, 2022). Inadequate food sanitization or preparation practices can lead to microbial contamination with viruses, bacteria, fungi, and parasites, which can cause outbreaks of FBDs (Garcia et al., 2020; Oliveira et al., 2022; Rodrigues and Moraes Filho, 2020). The most common cause of FBDs is bacterial contamination, by organisms such as *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes, Bacillus cereus,* and *Staphylococcus aureus* (Fröder et al., 2021; Oliveira et al., 2010). Each year, an estimated 600 million people become ill and 420,000 people die as a result of consuming contaminated food (World Health Organization, 2022).

Foods contaminated with pathogens may look normal, but ingestion of such tainted foods can cause stomach pain, nausea, vomiting, diarrhea, and fever (Oliveira et al., 2010). Therefore, good sanitation practices are important for preparing foods from fruits, since pathogenic microorganisms may be on the outside of these fruits; the final product may become contaminated due to inadequate washing, poor hygiene of the food handlers, or unhygienic equipment (Santos et al., 2008). Thus, microbiological analysis of foods is an important food safety measure. In this study, we microbiologically analyzed homemade spiced mayonnaise and self-serve acai produced and sold in the city of Araguaína, Tocantins, Brazil.

2. Material and Methods

2.1. Samples

This study was conducted using commercialized spiced mayonnaise and self-serve acai samples collected from stores in Araguaína, Tocantins, Brazil. For the microbiological analysis of the homemade mayonnaise, one sample was collected from 20 snack bars between November–December 2019 and February–May 2020. For the microbiological analysis of acai, one sample was collected from 10 stores between December 2019 and March 2020.

2.2. Sample preparation and plating

For the microbiological analysis of homemade mayonnaise, the samples were prepared and inoculated on the same day. A 2.5 g portion of each sample was placed a 50 ml beaker and diluted (1:10, w/v) with sterile saline and then homogenized by stirring. Under sterile conditions, 10 µl of the dilution was inoculated into MacConkey agar by depositing the material near the edge of the plate and streaking it out with a platinum loop. Colonies that appeared on these plates were collected and streaked on Salmonella Shigella (SS) agar plates. Both plates were incubated at 35 °C (± 1 °C) for 24 hours and were analyzed for microorganism growth.

Acai was microbiologically analyzed to count molds and yeasts and to detect, identify, and count total and thermotolerant coliforms according to the Manual of Official Food Analysis Methods (Brasil, 2018). Following the APHA 21:2015 method, 25 g of each acai sample was weighed out separately and then diluted in 225 mL of 0.1% peptone water. The diluted samples were incubated at 35–37 °C for 24 hours to simulate microbial growth for pre-enrichment. After incubation, serial dilutions were prepared by adding 25 mL of the pre-enrichment to 225 mL of 0.1% peptone water (1:10 dilution), and then mixing 1 mL of this dilution with 9 mL of 0.1% peptone water to generate a 1:100 dilution; this was repeated to obtain a 1:1000 dilution. To determine the mold and yeast counts, three appropriate sample dilutions were selected, and 0.1 mL of each dilution was spread onto acidified dextrose potato agar plates with a Drigalski loop and dried until all liquid was completely absorbed. After drying, the plates were incubated at 25 °C for 5 days in the dark, without inversion. After incubation, colonies were counted while avoiding moving the plates to limit secondary growth, which would invalidate the count (Salfinger and Tortorello, 2015).

2.3. Assessments of total and thermotolerant coliforms in acai

2.3.1. Presumptive test

Three appropriate dilutions of each acai sample were inoculated into a series of three tubes with Lauryl Sulfate Tryptose (LST) broth, adding 1 ml of dilution to 9 ml of LST. The tubes were incubated at 35 °C (± 0.5 °C) for 24 h (± 2 h) in a microbial growth oven. Both growth and gas production were detected. If a sample was positive for growth and gas production, total and thermotolerant coliforms were counted. If a sample was negative, i.e., no growth or growth or gas production was detected, the samples were incubated until 48 h (± 2 h), and the assessments were repeated.

2.3.2. Total coliform detection and counting

A loop of each positive LST culture was transferred to a tube containing 2% Brilliant Green Bile (BGB) broth. The BGB tubes were incubated at 35 °C (± 0.5 °C) for 24 h (± 2 h) and then observed for growth and gas production. If no growth or growth without gas production was detected, the samples were incubated until 48 h (± 2 h), and the assessments were repeated. If gas production and growth, indicative of the presence of total coliforms, was confirmed, the number of BGB tubes were noted, and the Most Probable Number (MPN)/g or ml was determined using an MPN table.

2.3.3. Thermotolerant coliform confirmation and counting

A loop of each positive LST culture (with growth and gas production) was transferred to an *E. coli* (EC) broth tube.

The tubes were incubated for 24 h (± 2 h) in a water bath at 45.5 °C (± 0.2 °C) and then observed for growth and gas production. Once growth and gas production were confirmed, indicating the presence of total coliforms, the number of positive BGB tubes was noted, and the MPN/g or ml was determined using an MPN table.

3. Results and Discussion

All 20 of the analyzed mayonnaise samples showed bacterial growth on MacConkey agar and high positivity for gram-negative bacteria (Figure 1).

Of the samples inoculated on SS agar, only one showed no *Salmonella* spp. or *Shigella* spp. growth. The colonies showed morphological and dye characteristics indicative of *Salmonella* spp. (Figure 2). Based on the results of this analysis, the collected homemade mayonnaise samples contained microbiological counts beyond current limits, according to RDC resolution no. 331 (December 23, 2019), and IN Normative Instruction no. 60 (December 23, 2019), which state that mayonnaise must be absent of *Salmonella* (Brasil, 2019a, b). Other studies of homemade mayonnaise found similar results; most samples showed high contamination by pathogenic microorganisms (Santos et al., 2021; Felinto et al., 2021). Santos et al. (2021) observed contamination in properly refrigerated samples of homemade mayonnaise, indicating that it had been prepared under poor sanitary standards. In the current study, proper refrigeration was not assessed.

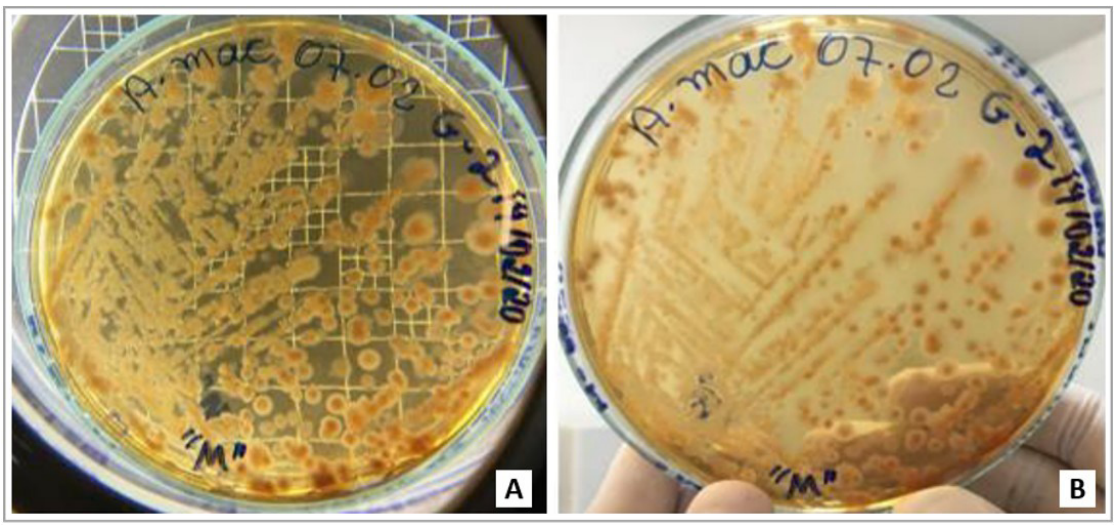

Figure 1. A homemade spiced mayonnaise sample inoculated onto a MacConkey agar plate. A. Plate on a colony counter. B. Bacterial growth on the MacConkey agar plate.

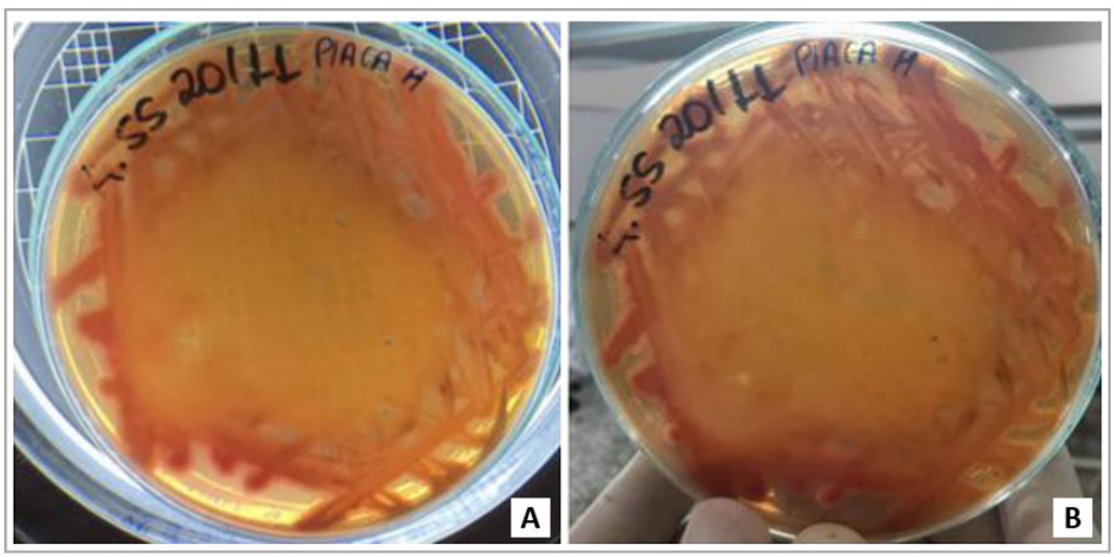

Figure 2. A homemade spiced mayonnaise sample inoculated onto Salmonella Shigella (SS) medium agar. A. SS agar plate on a colony counter. B. Bacterial growth on the SS agar plate.

Table 1. Analysis of the total coliforms, thermotolerant coliforms, and molds, and yeasts in self-serve acai samples collected from 10 supermarkets in Araguaína, Tocantins, Brazil.

Sample	Total coliforms (col/ml)	Thermotolerant coliforms (col/ml)	Molds and yeasts (CFU/g)
1	2	Not detected	1.62×10^5
2	0.91	Not detected	1.44×10^5
3	>110	>110	5.8×10^3
4	9.3	Not detected	Uncountable
5	>110	110	1.6×10^4
6	>110	dd15.0	1.34×10^4
7	>110	>110	1.76×10^4
8	>110	>110	Uncountable
9	21.0	Not detected	2.3×10^4
10	>110	>110	9.6×10^4

Microbiological analyses of the acai samples showed that total coliforms were detected in 100% of the collected samples (Table 1). The National Health Surveillance Agency, ANVISA, established microbiological standards for foods ready for consumer consumption in Normative Instruction No. 60 (December 23, 2019), setting a maximum limit of 100 col/ml. Of the samples evaluated, four (40%) were below the limit established by ANVISA, and the other six (60%) were above the limit and were positive for thermotolerant coliforms.

Analysis of the acai samples for molds and yeasts showed counts above the limit established by the Brazilian legislation (RDC no. 12/2001/ANVISA) of 10^3 CFU/g (Brasil, 2001). Thus, all the acai samples analyzed in this study were unfit for consumption (Table 1).

Few studies have performed microbiological analyses of self-serve acai. In Minas Gerais, a study evaluating acai vitamins found a high percentage of samples with unsatisfactory microbiological levels and identified inadequate hygiene in the stores, including by the food handlers (Mello and Resende, 2018). A study conducted by Souza et al. (2015) of 10 self-serve ice cream samples in a city in the state of Mato Grosso showed that all samples had high total coliform counts due to unsatisfactory hygiene. Nine samples contained *Salmonella* sp. Consumption of food contaminated by *Salmonella* carries a risk of salmonellosis, a disease causing gastrointestinal symptoms, such as abdominal pain, diarrhea, low-grade fever, and vomiting (Shinohara et al., 2008). Improper food handling can increase contamination and the transmission of FBDs. FBDs are caused by different microorganisms, and some can lead to death (Ferreira, 2021). Previous results and our findings demonstrate that proper hygienic-sanitary measures are necessary to ensure the safety of self-serve food products, since they are constantly handled by different people.

4. Conclusion

Microbiological analysis of homemade spiced mayonnaise and self-serve acai sold in different stores in the city of Araguaína, Tocantins, Brazil showed high levels of contamination by pathogenic microorganisms, with values above the established limits. Therefore, human consumption of these foods represents a risk, as it is considered a potential source of infection and FBD outbreaks. These study results indicate the need to implement good hygiene practices during food handling and effective supervision of these measures by sanitary surveillance agencies and authorization to process and market these foods within the specifications provided by law.

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