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Hygienic-sanitary quality of peanuts commercialized in Campinas, São Paulo, Brazil, and the toxigenic potential of fungi isolated from peanuts

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Abstract

Food safety is a topic of high relevance, with *Salmonella* and *Escherichia coli* being pathogenic microorganisms associated with foodborne outbreaks worldwide, and *Aspergillus flavus* is a contaminant able to produce aflatoxins, a highly toxic mycotoxin that may be present in various commodities. Food contamination can occur at various stages along the production and processing pathways. Peanuts, despite being low-cost products with numerous nutritional advantages, are susceptible to microbiological contamination by foodborne bacteria and aflatoxins. Thus, to assess the food safety of commercially available peanuts in Campinas, two sample groups were collected and analyzed: bulk peanuts and packaged peanuts. The analysis involved the presence or absence of *Salmonella*, enumerating the populations of *E. coli*, Enterobacteria, yeast, and molds, particularly *Aspergillus* spp., and evaluating the toxigenic potential of the isolated strains. Among the main findings, it was observed that the samples were not contaminated with *Salmonella* or *E. coli*; however, Enterobacteria, yeast, and molds and toxicogenic potential were detected, particularly in bulk peanut samples. This demonstrates the importance of washing or treating fresh foods before consumption and highlights the need for companies handling these products to implement more rigorous quality control and monitoring measures to ensure the safety of the commercialized products.

Keywords: *Aspergillus; Salmonella;* foodborne; mycotoxins.

Practical Application: This information enables us to gain a comprehensive understanding of the rigor of phytosanitary control measures in the region of Campinas, São Paulo, Brazil, informing us about the importance of good manufacturing practices and providing insights into the actual quality of peanuts being commercialized.

1 INTRODUCTION

Peanut (*Arachis hypogaea L.*) is one of the most consumed oilseeds around the world and provides nutritional and medicinal benefits due to its bioactive components, such as phenolics, flavonoids, polyphenols, and resveratrol; therefore, developing countries can benefit local communities by cultivating peanuts (Akram et al., 2018; Costa et al., 2020; Souza & Ferrarezi Junior, 2022). Several studies have found a positive correlation between peanut consumption and decreased risks of life-threatening diseases, attributed to its bioactive components that may enhance overall health and wellness (Akram et al., 2018; Syed et al., 2021; Toomer, 2018).

Brazil is among the largest producers and exporters, having exported 297,000 tons in 2023, with São Paulo being the main contributor (Companhia Nacional de Abastecimento [CONAB], 2025; Sampaio et al., 2024). However, peanuts have recently emerged as potential sources of infection with foodborne bacteria, fungi, and high levels of aflatoxins (Costa et al., 2020; Uçkun & Var, 2018).

For acceptable food quality, the presence of *Salmonella* is unacceptable due to its ability to cause salmonellosis, being considered as one of the most important agents involved in food disease outbreaks, in addition to its endemic characteristics and high morbidity (Shinohara et al., 2008). Nascimento et al. (2018) found that peanuts may become contaminated by *Salmonella* in the supply chain, especially at the post-harvest process. It was previously understood that *Salmonella* does not proliferate at water activity (a_w) levels below 0.94; however, currently this is not a limiting factor, since *Salmonella* has been detected in samples with a_w levels below 0.91.

Enterobacteriaceae have the capacity to acquire multiple resistance mechanisms to a wide range of antimicrobials and are now classified as emerging contaminants. In addition, they can survive and replicate under stress or in hostile environments since they are tolerant to adverse factors, such as temperature, humidity, and pH variations (Menezes et al., 2024). The Enterobacteriaceae family is ubiquitous and includes *Escherichia coli* and over 210 other species, some of which are plant pathogens,

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while others are part of the normal flora of animals. Most of them are frequently associated with intestinal and extraintestinal infections (Jenkins et al., 2017). *E. coli* and *Enterobacteria* could be related to the contamination of peanuts, mainly of products that have not been properly sanitized, whose production site has been exposed to organic fertilizer from animal waste, that were produced in an area close to animal production, or whose sanitization and irrigation water was contaminated (Nascimento et al., 2018; Ramirez & Giron, 2025).

Furthermore, other microorganisms and toxins might affect peanut quality and safety. Mycotoxins are toxins produced by the secondary metabolism of filamentous fungi that could contaminate food throughout the supply chain, from the field to processing (Moss, 1996). Aflatoxins are mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Brazil has favorable environmental conditions, such as high temperatures and high relative humidity, which are typical of tropical regions; such conditions promote the growth of aflatoxin-producing fungi (Calori-Domingues & Fonseca, 1995). Contamination with aflatoxins can lead to economic losses as well as health concerns.

Certain aflatoxins are of particular relevance: B series (AFB1 and AFB2), G series (AFG1 and AFG2), and M series (AFM1 and AFM2). *A. flavus* and *A. parasiticus* generate the B series and B and G series, respectively. According to the International Agency for Research on Cancer, AFB1 is classified as a human carcinogen due to evidence linking it to cancer development in humans (Dhanasekaran et al., 2011).

To avoid various outbreaks and contaminations, there are some microbiological analysis standards determined by Normative Instruction No. 161 of July 1, 2022 (Agência Nacional de Vigilância Sanitária [ANVISA], 2022b). For nuts, almonds, peanuts, and edible seeds, samples must present an absence of *Salmonella*; for *E. coli*, the acceptable number of samples is 2 in 5 random sampling units from the same batch, in a microbiological limit that ranges from 10 (m) to 10^2 (M) (ANVISA, 2022b).

Therefore, evaluating the hygienic-sanitary quality of peanuts by assessing the bacterial presence and toxigenic potential of isolated fungi provides an overview of the quality of products sold in the Campinas region. This assessment serves as a warning of the importance of adhering to good manufacturing practices.

The objectives of the present study were to evaluate the hygienic and sanitary quality of peanuts sold in establishments in the city of Campinas, São Paulo, Brazil, through the isolation and enumeration of *E. coli*, Enterobacteria, molds, and yeast; the presence of *Salmonella* sp.; and the isolation and verification of the toxigenic potential of *Aspergillus* sp.

1.1 Relevance of the work

Ensuring the safety and quality of peanut products is essential for maintaining market access and competitiveness. Research findings can inform trade policies and market regulations, supporting the economic viability of the peanut industry; research into safety and quality can inform industry practices and standards, leading to improvements in production, processing, storage, and transportation methods; and identifying potential hazards and developing strategies to mitigate risks, thus safeguarding public health and consequently ensuring that peanuts reach consumers without compromising safety or quality. Governments and regulatory bodies often rely on scientific research to establish safety standards and regulations; research findings contribute to the evidence base upon which these regulations are built, ensuring that peanut products meet required safety standards. Consumers expect the food they purchase to be safe and of high quality, and this work is expected to inform them about the importance of washing or treating food before consuming it and the monitoring of the quality of peanuts sold in the region.

2 MATERIALS AND METHODS

Peanut samples were collected from random commercial establishments in Campinas, São Paulo, Brazil. One sample was collected from each location, resulting in a total of 100 samples. Of these 100 samples, 54 were obtained in bulk and 46 were packaged.

2.1 Sample preparation and initial dilution

In a homogenizer (SMASHER®, Biomérieux), 25 g of sample was added to 225 mL of peptone water (Oxoid) to obtain the initial sample solution (ISS or 10^{-1} dilution). The ISS was used in the *Salmonella*, *E. coli,* Enterobacteria, yeast, and molds count or detection.

For all analyses, except *Salmonella*, two dilutions of the ISS were prepared using 1 mL of ISS (10^{-1} dilution) in 9 mL of 0.85% NaCl solution (dilution 10^{-2}) and 1 mL of 10^{-2} in 9 mL of 0.85% NaCl solution (dilution 10^{-3}) for the analyses.

2.2 Analyses of Salmonella

The ISS was incubated at 37°C for 24 h. After incubation, 1 mL was added to tubes with 10 mL of tetrathionate broth (KASVI) with 0.2 mL of potassium iodide and incubated at 37°C for 24 h. Also, 100 μL of ISS was transferred to 10 mL tubes of Rappaport-Vassiliadis broth (Oxoid) and incubated for 24 h at 42°C.

After the incubation period, a loopful of both broths was streaked on plates of xylose lysine deoxycholate Agar (Oxoid), bismuth sulfite agar (Oxoid), and Hektoen Enteric Agar (Merck). The plates were divided into two sides, one for the Rappaport-Vassiliadis broth loopful (R) and the other for the tetrathionate broth loopful (T), and incubated at 37°C for 24 h. From the growth colonies isolated from each media, three with suspected *Salmonella* characteristics were further analyzed by biochemical screening in tubes with triple sugar iron Agar (Oxoid) (Andrews et al., 2001).

2.3 Analyses of E. coli

From ISS (10^{-1}), 10^{-2}, and 10^{-3} dilutions, 100 μL were added into plates of MacConkey Agar (KASVI), spread with a

Drigalsky loop, and incubated at 37°C for 18–24 h in aerobic conditions. Then, the colonies were counted, and three of them with *E. coli* characteristics were further analyzed by biochemical screening (methyl red, Voges-Proskauer, indole, and citrate tests) (Andrews et al., 2001).

2.4 Enterobacteria count

According to the Pour Plate method, 1 mL of ISS (10^{-1}), 10^{-2}, and 10^{-3} dilutions was added to sterilized plates. Molten cooled violet red bile glucose agar was poured over it and mixed gently. The media was allowed to solidify and set. Plates were inverted and incubated for 18–24 h at 35°C. After incubation, the colonies were counted (Silva et al., 2018).

2.5 Yeast and mold count, and Aspergillus sp. identification

From ISS (10^{-1}), 10^{-2}, and 10^{-3} dilutions, 100 μL were added into dichloran-glycerol (DG18) agar (Oxoid) plates, spread with a Drigalsky loop, and incubated at 25°C for 5 days in darkness. After incubation, colonies were counted, and those with *Aspergillus* sp. characteristics were isolated (Ryu & Wolf-Hall, 2015).

The morphologic identification was conducted according to the protocol of Pitt & Hocking (2009). For molecular identification, the DNA from the strains was extracted following the instructions of the DNA easy kit (Invitrogen), and a polymerase chain reaction according to White et al. (1990) for the internal transcribed spacer (ITS) locus. After sequencing, similarity analysis was performed by Basic Local Alignment Search Tool on the NCBI (National Center for Biotechnology Information) website, and a phylogeny was inferred using the maximum parsimony method (Peterson, 2008).

2.6 Toxigenic potential of Aspergillus sp.

The samples identified by microscopy were inoculated in yeast extract with supplements broth (BD) for 10 days at 25°C.

From the central region of the colony, a *plug* was collected, five drops of chloroform were dispensed on it, and then the plug was pressed into a silica gel plate (G60) of 20 cm for thin layer chromatography (TLC) with 5 µL of aflatoxin B1 standard solution. For TLC, 100 mL of mobile phase with chloroform and acetone in a 9:1 ratio was used (Lin & Dianese, 1976). After inserting the plate into the vat, we waited until the mobile phase reached 12 cm above the application of samples and aflatoxin standard. Finally, toxigenic potential was observed under ultraviolet light by comparing the stains obtained from the extract with that of the standard toxin according to the simple screening method reported by Filtenborg et al. (1983).

3 RESULTS

3.1 E. coli and Salmonella sp.

From the total of 100 samples of peanuts, both in bulk and packaged, 100% were found to be free of *E. coli* and *Salmonella* contamination.

3.2 Enterobacteria count

For bulk peanut samples, 81.48% (44 samples) showed colony count greater than 10^2 CFU/g, including sample 20, which was uncountable. The remaining percentage of samples had counts lower than 10^1 CFU/g (Figure 1). However, the packaged peanut samples showed 65.22% (30 samples) with a count greater than 10^2 CFU/g, while the other 34.78% exhibited values lower than 10^1 CFU/g (Figure 2).

It is pertinent to note that regardless of whether the samples were collected in bulk or packaged, certain locations exhibited a higher susceptibility to Enterobacteria contamination. This susceptibility was notably higher in bulk peanut samples.

3.3 Yeast and mold count

Yeast and mold colonies were observed in all samples. Among the bulk peanut samples, 75.92% (41 samples) showed a lower count, less than or equal to 5×10^2 CFU/g; 18.52% (10 samples) exhibited a count higher than 5×10^2 CFU/g but lower than 10^4 CFU/g, while 5.56% (three samples) showed counts exceeding 10^4 CFU/g (Figure 3). Notably, in the last samples, sample 2 had a count of 4.2×10^5 CFU/g, which is out of the ratio of Figure 3.

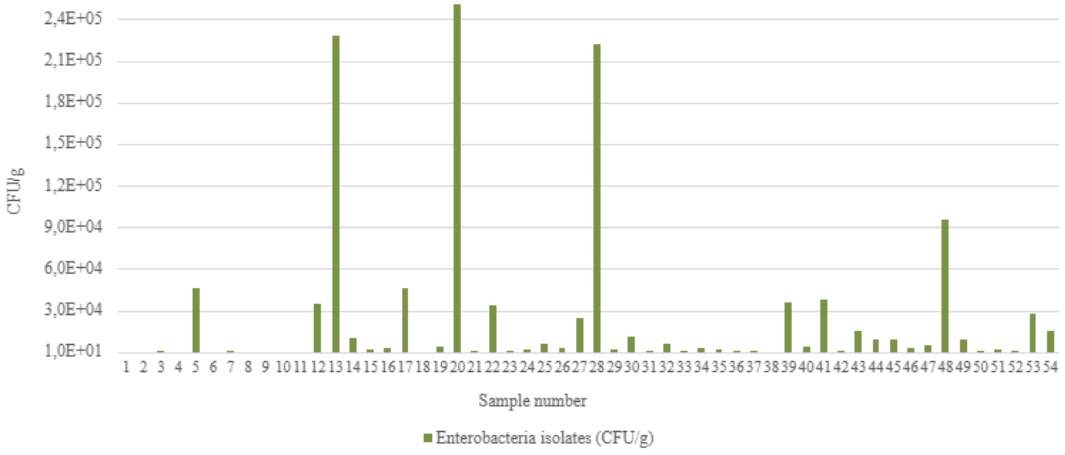


Figure 1. Enterobacteria count of bulk peanut samples.

For the packaged peanut samples, 76.09% (35 samples) showed values lower than or equal to 5×10^2 CFU/g, 21.74% (10 samples) showed a count higher than 5×10^2 CFU/g but lower than 10^4 CFU/g, while 2.17% (one sample) showed counts exceeding 10^4 CFU/g (Figure 4).

3.4 Aspergillus sp. identification and its toxigenic potential

Characteristic colonies of *Aspergillus* sp. were identified on malt extract agar (Figure 5). Among the total samples, only 14% showed colonies with characteristic *Aspergillus* sp. morphology, which were submitted to microscopy to confirm the

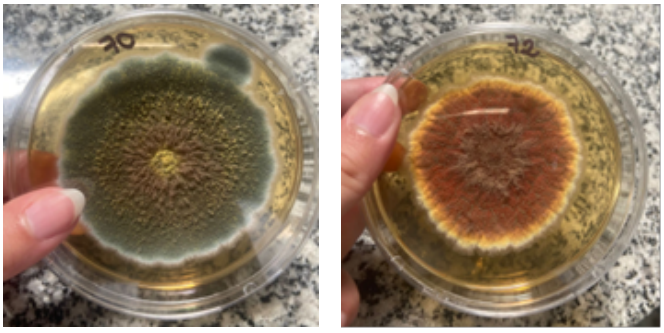


Figure 5. Characteristic colonies of *Aspergillus* sp. on malt extract agar.

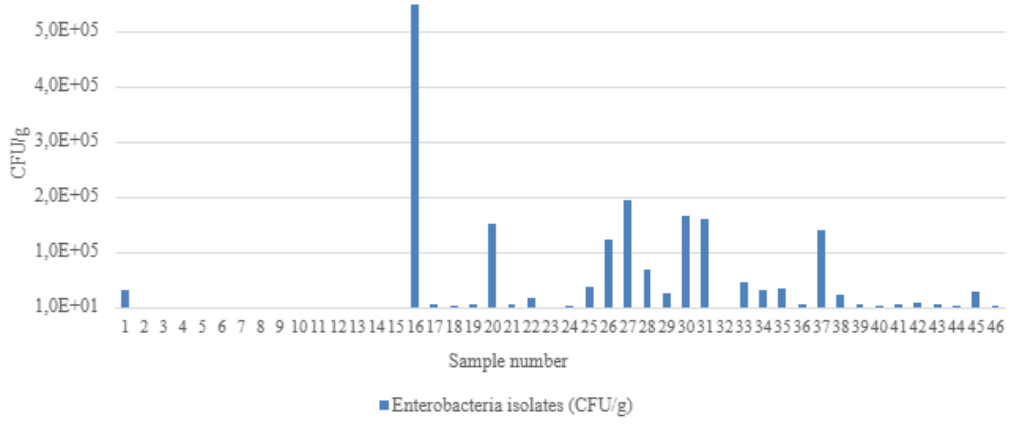


Figure 2. Enterobacteria count of packaged peanut samples.

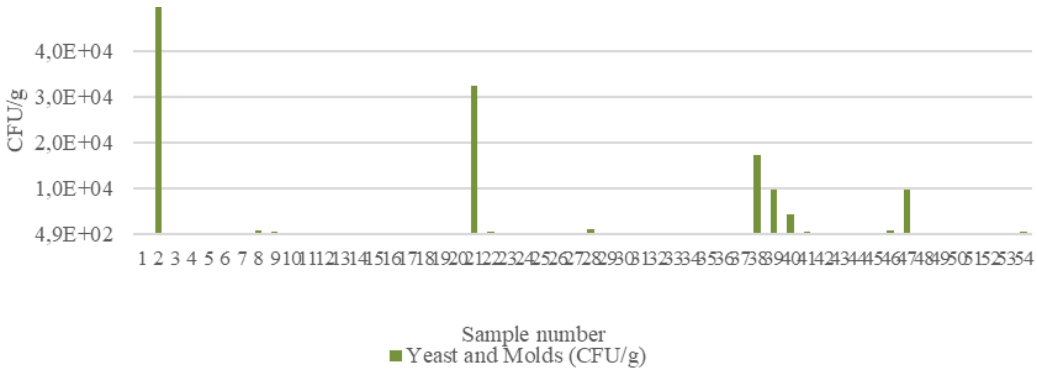


Figure 3. Yeast and mold count of bulk peanut samples.

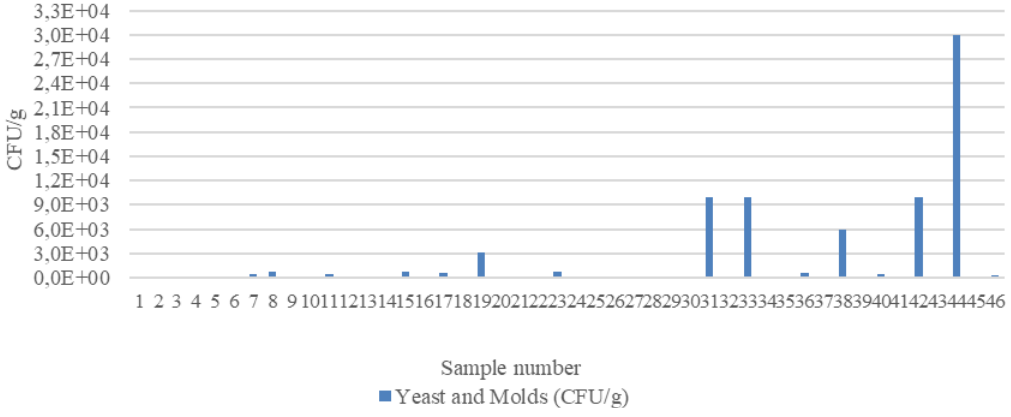


Figure 4. Yeast and mold count of packaged peanut samples.

identification. Figure 6 shows a conidiophore characteristic of *Aspergillus* sp.

The 14 samples that were morphologically identified were subjected to molecular identification through sequencing of the ITS region. The phylogeny presented in Figure 7 displays bootstrap values indicated on the branches. Branches supported by bootstrap presented values higher than 60%, and the outgroup is represented by the species *Aspergillus tamarii*. The alignment consisted of 39 taxons, 398 nucleotides, and 36 PICs (parsimony-informative characters).

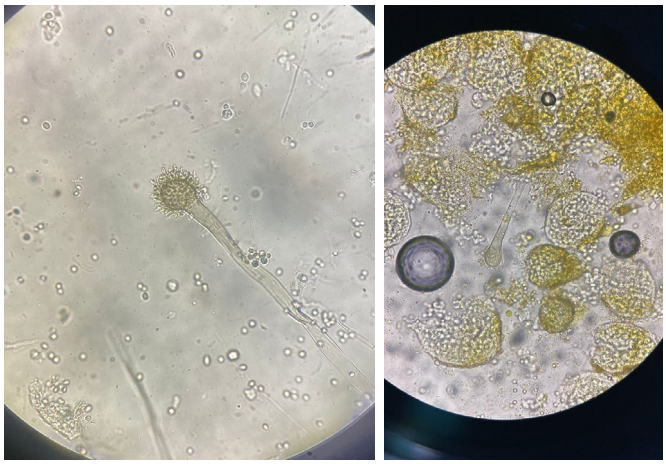


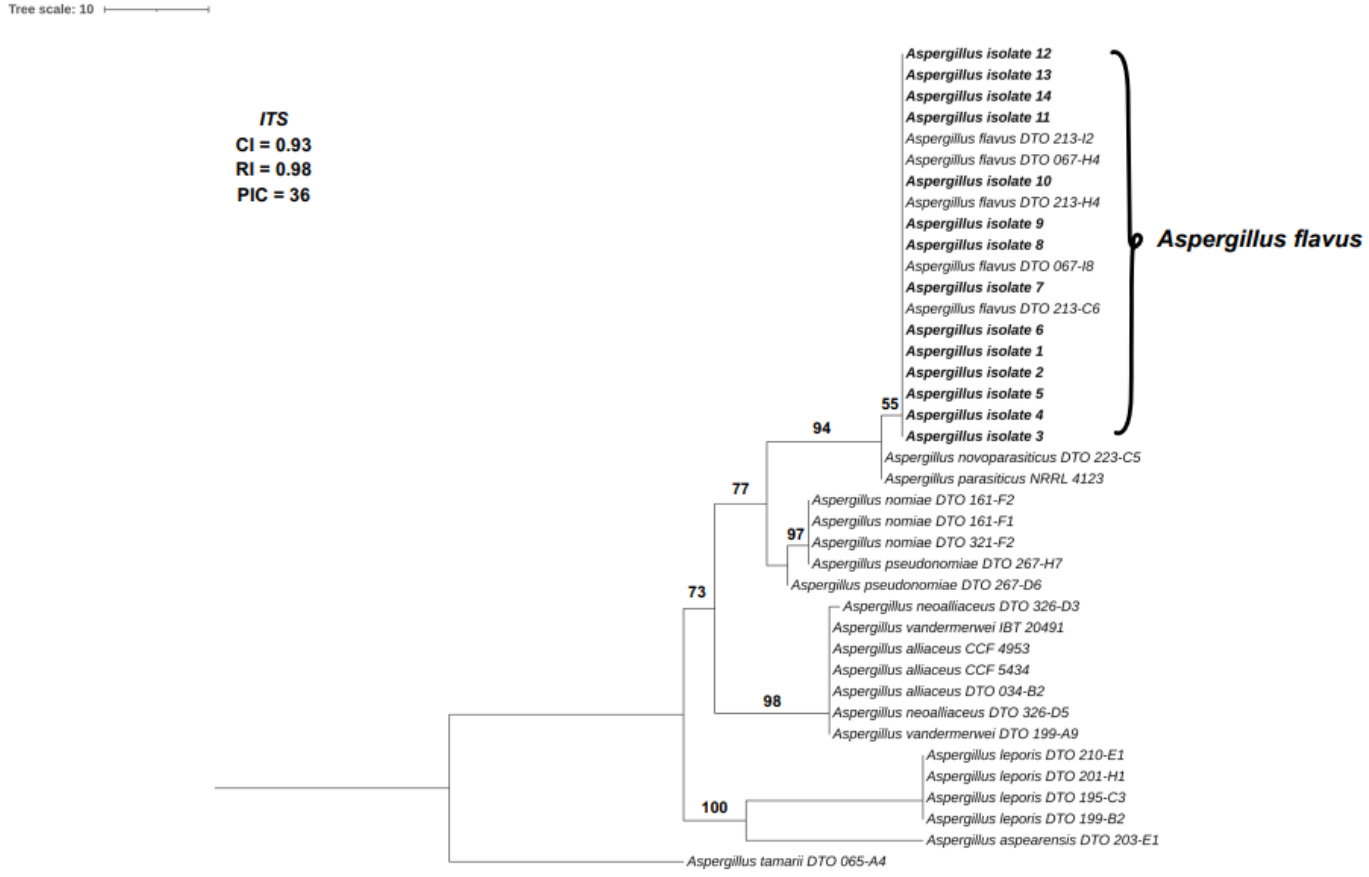
Figure 6. Microscopy of characteristic conidiophore of *Aspergillus* sp. obtained from peanut samples (20x zoom).

The analysis resulted in a tree of 89 most parsimonious trees. Statistical support was indicated by bootstrap analysis, indicated at each node of the phylogeny. Therefore, the 14 samples were confirmed to be *Aspergillus* sp., and the isolates were grouped into the clade containing *A. flavus* reference sequences.

Then, from 100 samples, 14% were positive for *Aspergillus* by morphology and molecular sequencing, and according to the simple screening method, 7% of the total samples showed toxigenic potential by the confirmation of the comparison of the stains with that of the standard toxin (aflatoxin B1). There was no evidence of the production of other toxins such as aflatoxin B2, G1, and G2, and, by the identification of the isolates, it is possible to affirm that there were no species potentially producers of G series aflatoxins such as *A. parasiticus*.

Specifically, for the bulk peanut samples, 16.67% (nine samples) showed positive colonies of *Aspergillus*; however, not all of those produced mycotoxins, and just four samples were able to produce aflatoxin B1, which means that, of the bulk peanut samples, 7.41% showed toxigenic potential.

On the other hand, 10.87% (five samples) of the packaged peanuts were positive for *Aspergillus*, and of them, three samples produced aflatoxin B1; therefore, 6.52% of the packaged peanut samples showed toxigenic potential.



PIC: number of parsimony-informative characters; CI: consistency index; RI: retention index; Aspergillus flavus isolates.

Figure 7. Phylogeny obtained by the parsimony method inferred by the internal transcribed spacer locus of peanut isolates.

4 DISCUSSION

The discussion on the safety and quality of food products is paramount for ensuring public health. In this context, the absence of pathogens such as *E. coli* and *Salmonella* in the samples analyzed in our study holds significant implications for food safety standards and the prevention of foodborne diseases. These results complied with the Brazilian maximum limits of *Salmonella* absence and not exceeding 10^2 CFU/g of *E. coli* (ANVISA, 2022b). Similar results were reported by Uçkun & Var (2018) for shelled and unshelled peanut samples from storage, with salmonella presence being negative. Costa et al. (2020) reported that all peanut samples were negative for coliforms, but showed a concerning quantification of mesophiles, which was attributed to the handling of the samples not complying with good manufacturing standards, as several cooperatives lack technical regulations such as standard operational procedures. Therefore, prevention and monitoring measures are important to avoid the risk of foodborne infections in the human population for countries where these outbreaks occur, since they could cost billions per year due to medical expenses, absence from work, and drops in productivity (Shinohara et al., 2008).

For peanuts, there are no specific regulatory parameters for Enterobacteria, but for those products for which there is a limit, the regulation stipulates that, out of five samples analyzed, only two samples can present an intermediate result, between 10 and 10^2 CFU/g, with no sample showing a result above 10^2 CFU/g; therefore, considering that parameter, both sample groups exceeded the allowed limits, since there were samples above 10^2 CFU/g (ANVISA, 2022b). According to Chang et al. (2013), few foodborne disease outbreaks have been attributed to peanuts, and almost all were attributed to poor handling practices after roasting. To guarantee a product that is safe for consumption, it is necessary to ensure proper peanut processing and handling after harvest (Chang et al., 2013). Therefore, the count of Enterobacteria on the peanut samples of this study was concerning, as it is an indicator of poor hygienic quality and because the Enterobacteriaceae family could cause gastrointestinal infections (International Commission on Microbiological Specifications for Foods [ICMSF], 2011). Consequently, it is important to raise the awareness of the companies as to the need to review and improve their peanut handling processes to prevent contamination by Enterobacteriaceae from becoming a serious issue. This concern was also found in a research study on the peanut supply chain, which found high levels of Enterobacteriaceae counts in post-harvest samples. At the end of the secondary process, 16% of the samples remained contaminated. Six samples of the primary production and one sample of peanut butter tested positive for *E. coli*, while *Salmonella* was detected in nine samples. However, there was a high prevalence of Enterobacteriaceae and a low prevalence of *E. coli* throughout the peanut supply chain (Nascimento et al., 2018).

Peanuts can be stored for extended periods, requiring precautions to avoid humidity, as foods with high fat content and low water content, such as peanuts, are prone to fungal contamination in humid environments (Jay, 2005). The current regulatory instruction IN No. 161 of 2022 in Brazil, does not specify microbiological standards for molds and yeasts in peanuts for direct consumption. However, for peanut bars, the regulation stipulates that, out of five samples analyzed, only one sample can present an intermediate result, between 5×10^2 CFU/g and 10^4 CFU/g, with no sample exceeding 10^4 CFU/g (ANVISA, 2022b). Considering this standard, bulk peanut samples represent the greatest risk for consumers, as, out of 54 samples, 11 showed intermediate values for mold and yeast growth, whereas, for the sample size (54), 10.8 is the number of samples allowed to have intermediate results, and had three samples with results greater than 10^4 CFU/g. On the other hand, for the packaged peanut samples, of 46 samples, 10 showed intermediate values, and one sample showed a count greater than 10^4 CFU/g; such results highlight a certain concern regarding hygienic standards and good practices for manufacturing, storing, and growing peanuts in the region. These results differ from those reported in a microbiological analysis of peanut samples from the public market of Porto Alegre, RS, Brazil, in which mesophiles and total coliforms were quantified, but there was an absence of mold and yeast, which was attributed to the roasting process, in which the harvest and storage contamination could have been removed (Spinelli et al., 2018).

Otherwise, since the risk of foodborne contamination seems to be low for the peanut samples, it could be considered that the main concerns should also focus on contamination by aflatoxin and cross-contamination that could occur after processing, as reported by Chang et al. (2013). Considering dry fruits and grains, storage conditions with high humidity and temperature and the rich nutritional composition of the products produce an environment conducive to the development of microorganisms such as *Aspergillus* and *Penicillium*, which can damage, discolor, and rot the products, negatively affecting their nutritional and commercial quality (Santos et al., 2016). Furthermore, the development of those microorganisms could favor the contamination by mycotoxins. In a study of aflatoxin and cyclopiazonic acid production, *A. flavus* strains were isolated from peanut samples: out of 47 isolated strains, 91.5% were able to produce aflatoxins, highlighting the contamination risk posed by these toxins due to the conditions being favorable for the growth of *A. flavus* (Gonçalez et al., 2013). In Brazil, the main fungi responsible for mycotoxins harmful to human and animal health include fungi of the genera *Aspergillus* sp., *Fusarium* sp., *Paecilomyces* sp., and *Byssochlamys* sp. (Gonçalves et al., 2018), which were confirmed by this study, whose results showed that 14% of the samples analyzed showed *Aspergillus* sp. growth, with only a few presenting toxicogenic potential, totaling 7% of samples with toxicogenic potential to produce aflatoxin B1.

The presence of aflatoxins in peanuts was studied by other authors, such as Liu et al. (2022), who analyzed the aflatoxin levels of peanuts, peanut oil, and peanut meal, since aflatoxin contamination could pass from the raw material to the processed product, and found that raw material had 13.31 µg/kg of AFB_1, 2 µg/kg of AFB_2, 43.68 µg/kg of AFG_1, and 13.65 µg/kg of AFG_2. The work showed that such values decreased after processing and were enhanced by the fumigation of the raw material. On the other hand, according to Qu et al. (2020), peanuts were susceptibly contaminated by AFB_1 along the production chain, and the optimal conditions for aflatoxin

were 33°C temperature and 0.9933 water activity. Therefore, the AFB_1 levels were monitored after drying and showed that all samples were below 2 µg/kg.

Most positive samples for *Aspergillus* sp. in this study were from the bulk peanut group. This underscores the importance of submitting foods to some form of processing, such as drying treatments, to mitigate the risk of foodborne contamination. The choice of treatment method will vary depending on the type of food and the specific microorganisms involved.

In the case of aflatoxins, they are thermally stable and difficult to eliminate once formed, and food processing is also ineffective in reducing toxin levels. Techniques for decontaminating large batches are generally economically unfeasible. There is a possibility of limited use of chemicals to control fungal growth during storage in the industry; however, this must be conducted with extreme caution by strictly following application instructions and adhering to usage guidelines as well as regulatory standards (Brito et al., 2021; Liu et al., 2022). In Brazil, there is a maximum tolerated limit ranging from 20 µg/kg of aflatoxin for peanuts and derivatives, and, to ensure compliance with the standard, health surveillance agencies across the country analyze products such as peanuts, peanut sweets, and other derivatives (ANVISA, 2022a).

In any case, good agricultural practices, adequate post-harvest processes, and storage conditions with low temperatures and low relative humidity are essential to ensure that the commercialized peanuts are safe for consumption and of high quality. As concluded by Chang et al. (2013), preventing contamination is the best method to prevent foodborne disease outbreaks. However, according to the findings of this study, it is possible that these conditions are not being correctly complied with throughout the production chain. It is recommended that stakeholders review processes and storage conditions to avoid future complications.

5 CONCLUSION

For both *E. coli* and *Salmonella*, all peanut samples showed compliance with safe microbiological standards. Considering the broader context, the presence of Enterobacteria, molds, and yeast serves as a vital indicator of the hygienic and sanitary quality of the products. Poor microbiological quality may result from mishandling during processing or inappropriate storage conditions. Notably, bulk peanut samples, requiring less handling and occasionally prone to contamination from the field, may exhibit a higher susceptibility to compromised sanitary hygiene quality. This observation underlines the necessity of refining sanitation practices during processing and storage, particularly for bulk products. Implementing effective treatments to sanitize peanuts in their natural state before consumption could significantly enhance overall food safety. Although at a lower frequency, packaged peanut samples also displayed indications of contamination and toxicogenic risk. This underscores the imperative for companies in the region to intensify monitoring and control measures to uphold the hygienic and sanitary quality of their products, thus mitigating the risk of foodborne disease outbreaks.

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