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Microbiological analysis of brigadeiros sold in Barretos, São Paulo, Brazil

Análise microbiológica de brigadeiros comercializados em Barretos, São Paulo, Brasil

Nilson Carvalho Cristino Junior[1,2], Nathalia Garlich[1], Amir Horiquini[1]

[1] Barretos Educational Foundation University Center – UNIFEB.
[2] Bachelor's student in Biomedicine, scholarship holder PIBIC

Abstract

The habit of consuming food and sweets is a global reality, and Brazil is no exception. Chocolates, lollipops, candies, and their derivatives are routinely consumed by individuals of all age groups, including children, adolescents, and adults. According to the Brazilian Association of the Chocolate, Peanut, and Confectionery Industry, approximately 693,000 tons of sweets were consumed in Brazil in 2021, representing 75% of the population as consumers of these products. This study aimed to detect possible hygiene deficiencies in the production and handling of artisanal sweets. Three samples of *brigadeiros* (Brazilian chocolate truffles) were collected at different points of sale for microbiological analysis. The analysis revealed significant variations in contamination levels among the samples. One sample showed alarming levels of contamination, indicating serious flaws in hygiene and handling practices. Cross-contamination, often associated with poor hand hygiene among food handlers, was identified as a critical risk. The results highlight the importance of hygiene protocols and the implementation of good manufacturing practices to prevent contamination. Regulatory measures, such as RDC 331/2019 and IN 60/2019 by Anvisa, were highlighted as key for maintaining food safety standards. Implementing these practices is essential to reduce risks to public health and ensure the quality of food products.

Keywords: Microbiological analysis, Food safety, *Escherichia coli,* Hygiene practices, Contamination control.

Resumo

O hábito de consumir alimentos e doces é uma realidade global, e o Brasil não é exceção. Chocolates, pirulitos, balas e seus derivados são consumidos rotineiramente por indivíduos de todas as faixas etárias, incluindo crianças, adolescentes e adultos. De acordo com a Associação Brasileira da Indústria de Chocolate, Amendoim e Confeitaria, aproximadamente 693 mil toneladas de doces foram consumidas no Brasil em 2021, representando 75% da população como consumidores desses produtos. O objetivo da pesquisa foi identificar possíveis deficiências de higiene na produção e manuseio de doces artesanais. Três amostras de brigadeiros foram coletadas em diferentes pontos de venda para análise microbiológica. A análise revelou variações significativas nos níveis de contaminação entre as amostras. Uma amostra apresentou níveis alarmantes de contaminação, indicando falhas graves nas práticas de higiene e manuseio. A contaminação cruzada, frequentemente associada à má higiene das mãos dos manipuladores de alimentos, foi identificada como um risco crítico. Os resultados ressaltam a importância de protocolos de higiene e da implementação de boas práticas de fabricação para prevenir a contaminação. Medidas regulatórias, como a RDC 331/2019 e a IN 60/2019 da Anvisa, foram destacadas como essenciais para manter os padrões de segurança alimentar. A implementação dessas práticas é essencial para reduzir os riscos à saúde pública e garantir a qualidade dos produtos alimentícios.

Palavras-chave: Análise microbiológica, Segurança alimentar, *Escherichia coli*, Práticas de higiene, Controle de contaminação.

Autor para correspondência: Amir Horiquini – E-mail: horikini@icloud.com
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Introduction

The habit of consuming sweet foods and treats is a global reality, and Brazil is no exception. Chocolates, lollipops, candies, and their derivatives are routinely consumed by individuals of various age groups, including children, adolescents, adults, and the elderly. According to the Brazilian Association of the Chocolate, Peanut, and Candy Industry (Abicab), in 2021, approximately 693 thousand tons of sweets were consumed in Brazil (Cardo, 2022), representing 75% of the Brazilian population as consumers of these products (Associação Brasileira da Indústria de Chocolates, Cacau, Amendoim, Balas e Derivados, 2020)

Currently, sweets are linked to foodborne diseases (FBD), which affect millions of Brazilians, largely due to the ingestion of food contaminated with biological, chemical, or physical agents, including viruses, fungi, bacteria, parasites, toxins, heavy metals, and others, causing symptoms such as fever, nausea, and vomiting (Brasil, 2025; Klein et al., 2017; Levy et al., 2022; Nepomuceno et al., 2023; Vieira et al., 2022; Wittler, 2023).

The pathogens most frequently associated with FBD in Brazil include *Salmonella, Escherichia coli, Staphylococcus aureus, coliforms, Bacillus cereus, Shigella, rotavirus, Clostridium perfringens*, norovirus, and hepatitis A (Melo et al., 2018; Brasil, 2020).

Microbial growth in foods is related to various factors, mainly extrinsic ones such as air temperature and humidity, and intrinsic ones like pH. The presence of coliforms, such as *Escherichia coli*, in food can pose a health risk, leading to intoxications and infections caused by microorganisms (Volcão et al., 2016).

Some sweets, like brigadeiros, which are typically made with condensed milk, sugar, and cocoa powder, can become contaminated with microorganisms, as their composition, combined with favorable temperature, supports microbial growth. Furthermore, preparation conditions can exacerbate contamination risks, such as improper hygiene practices and the use of unsuitable ingredients (Boakye et al., 2023; Liao et al., 2023).

Moreover, preparation conditions play a crucial role in the microbiological safety of these foods. Poor hygiene practices and failures in temperature control during processing can significantly increase contamination risks (Alam et al., 2019; Evans & Redmond, 2019; Gautam & Curtis, 2021; Lompo et al., 2024; Novák et al., 2020; Resende & Fernandes, 2017; Rutaro et al., 2024). A frequently underestimated but critical factor is the direct handling of food by producers. The hands of food handlers can serve as efficient vectors for the transfer of microorganisms to the final product, especially when proper hygiene practices are not strictly followed (Berhanu et al., 2021; Costa et al., 2020; Labović et al., 2023; Teferi et al., 2021).

Cross-contamination through handlers' hands is particularly relevant in brigadeiro production, as these sweets are often manually shaped. Studies have shown that even small lapses in hand hygiene can lead to the introduction of pathogens such as *Escherichia coli* and other enterobacteria into the food (Ponath et al., 2018). This contamination route is especially concerning in informal or small-scale production settings, where strict hygiene controls may be more challenging (Abreu et al., 2011; Amin et al., 2019; Augustin et al., 2020; Barreiro et al., 2022; Bhandari et al., 2022; D'Mello-Guyett et al., 2020; Ferreira et al., 2022; Gutema et al., 2021; Neuhaus et al., 2023; Tuyet Hanh & Hanh, 2020)

The presence of coliforms in food is an important indicator of poor hygiene conditions. Total coliforms are Gram-negative, rod-shaped bacteria that can ferment lactose, producing acid and gas at 35°C. They can be found in aquatic environments, soil, food, and animals and are considered indicators of fecal contamination and poor hygiene during food processing (Atwill & Jeamsripong, 2021; Bonso et al., 2023; Botha et al., 2023; Britton et al., 2021; Caggiano et al., 2020; Dankwa et al., 2020; Kim & Cheigh, 2022; Liao et al., 2023; Milton et al., 2020; Nunes-Carvalho et al., 2020; Santos et al., 2020; Yan et al., 2022; Zhang et al., 2021). On the other hand, thermotolerant coliforms are a subcategory of coliform bacteria capable of surviving and multiplying at higher temperatures, with the ability to ferment lactose while producing gas. These organisms are frequently found in foods, which may indicate possible fecal contamination (Andrade et al., 2017; Porto et al., 2011). Therefore, both groups of bacteria are important indicators of the presence of other pathogenic microorganisms, such as bacteria, viruses, and parasites (Sales et al., 2015).

To control the presence of fecal coliforms, the National Health Surveillance Agency (Anvisa) is responsible for regulating, controlling, and

supervising foods, beverages, and other products, ensuring the quality and safety of consumables to prevent diseases and promote the health of the Brazilian population (Agência Nacional de Vigilância Sanitária, 2021). It establishes microbiological standards for foods, which are measured based on the presence or absence of microorganisms according to Normative Instruction (IN) 60/2019 and Resolution of the Collegiate Board (RDC) 331/2019.

RDC 331/2019 regulates all aspects related to food, from production to commercialization, including storage, transport, and distribution. Additionally, complementing RDC 331/2019, IN 60/2019 establishes a list of microbiological standards defining the allowable amount of pathogenic microorganisms, toxins, or metabolites in food. Thus, the National Health Surveillance Agency (Anvisa) plays a fundamental role in protecting public health, ensuring food quality, and preventing diseases related to the consumption of food contaminated with microorganisms harmful to human health (Brasil, 2019a, 2019b).

The objective of this study was to evaluate the microbiological contamination of brigadeiros sold in the Barretos-SP region, specifically focusing on the presence of total coliforms and *Escherichia coli*. Through this analysis, the study aims to identify potential sanitary and hygiene deficiencies during the production, handling, and commercialization of these artisanal sweets. Additionally, the study seeks to highlight the importance of implementing good manufacturing practices and stringent quality control measures to ensure food safety and minimize public health risks associated with microbial contamination.

Materials and Methods

Brigadeiros sold in the Barretos-SP region were selected, following the parameters established by Codex Alimentarius, the International Commission on Microbiological Specifications for Foods (ICMSF), and the Bacteriological Analytical Manual (Food and Drug Administration) for sample collection, transport, and storage for analysis. The microorganisms analyzed were total coliforms and *Escherichia coli*, common indicators of fecal contamination and food sanitation quality (Agência Nacional de Vigilância Sanitária, 2019; Food and Agriculture Organization, 2023; Food and Drug Administration, 2022).

Brigadeiro samples were collected in duplicate from three different sales points in the Barretos-SP region. Vendor selection was done randomly and without identifying establishments or sellers, aiming to represent the conditions of brigadeiro sales and production in the region. Samples were collected under sterile conditions and immediately transported to the laboratory in an insulated thermal box (styrofoam) for microbiological analysis.

Each brigadeiro sample was submerged in a sterile 0.3% peptone water solution to facilitate microbiological sampling from the surface of the brigadeiro. For the simultaneous detection of *Escherichia coli* and total coliforms, the Colipaper Petri microbiological kit (Alfakit) was used, which is a multifunctional analytical tool designed for the detection, quantification, and differentiation of microorganisms in various matrices, including water, effluents, aquatic environments, and food and beverage industry products. Based on membrane filtration associated with chromogenic and fluorogenic media, the hydration of the medium followed by inoculation of the sample and incubation promotes microbial growth directly on the Colipaper Petri paper, facilitating the visual identification of colonies and allowing the visual identification of Escherichia coli and total coliforms with high sensitivity and specificity. This chromatic differentiation simplifies reading and ensures greater diagnostic reliability, in addition to enabling quantification at levels higher than conventional methods. Samples were inoculated and incubated at 36°C as recommended by the kit manufacturer, monitoring the growth of total coliforms and *Escherichia coli*. After incubation periods of 24 and 48 hours, developed colonies were visually analyzed using a digital colony counter, differentiating between dark blue to violet colonies as *E. coli* and pink to red colonies, along with the blue to violet ones, as total coliforms (Alfakit, 2024).

Results and Discussion

The microbiological analysis of the brigadeiro samples sold in the Barretos-SP region, using the Colipaper kit, revealed significant variations in contamination levels by total coliforms and *Escherichia coli* across different samples (Figure 1). In sample A (Figure 1A), no visible colonies were observed for either total coliforms or *E. coli* in both replicates (Figures 1A and 1B). This result

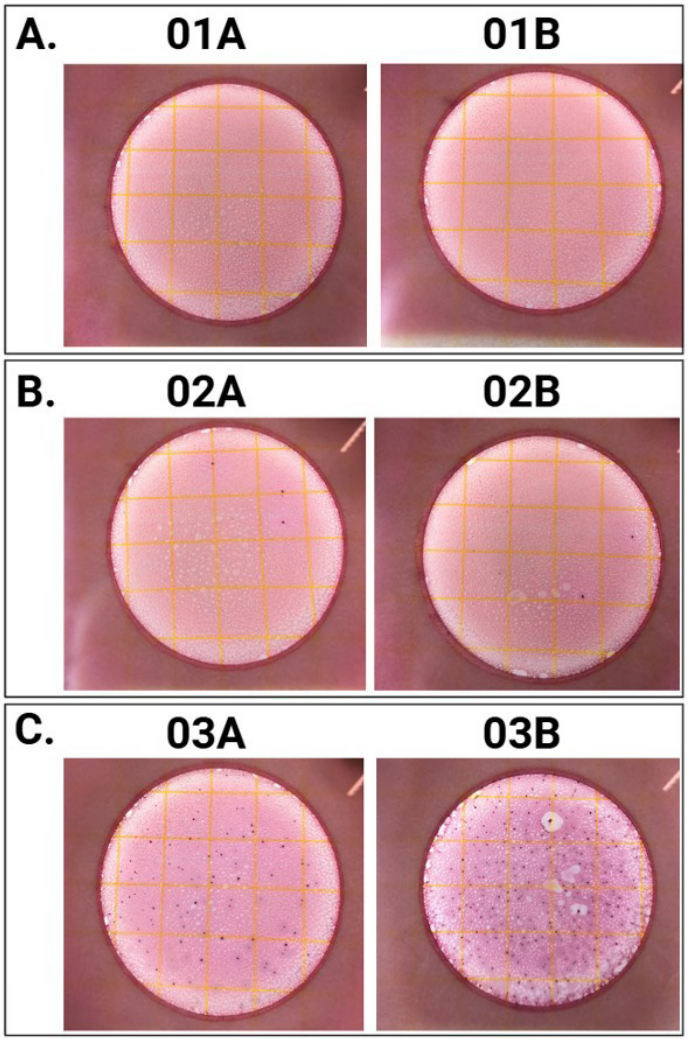

Figure 1: Photo of the Colipaper kit plates showing the duplicates of each brigadeiro sample**.** A. Brigadeiro sample 1 showed no detectable results. B. Brigadeiro sample 2 presented some evidence of total coliforms and *E. coli*. C. Brigadeiro sample 3 showed high levels of total coliforms and *E. coli*. Source: the author (2024).

suggests that the product was prepared under appropriate hygienic and sanitary conditions, indicating good handling and storage practices. Sample B (Figure 1B) showed a low level of contamination, with some visible colonies in both replicates (Figures 2A and 2B). The presence of colonies indicates that contamination occurred during the production or handling process of the food. In contrast, sample C (Figure 1C) displayed an alarming level of contamination, especially in replicate 03B, where a high density of colonies of both total coliforms and *E. coli* was observed. This result indicates serious failures in food hygiene and handling practices, representing a potential health risk for consumers.

The presence of *E. coli*, evidenced by dark blue to violet colonies, is particularly concerning as it indicates recent fecal contamination and the possible presence of enteric pathogens. Total coliforms, represented by pink to red colonies in addition to blue to violet colonies, are general indicators of food hygiene and sanitary quality. The variability observed among the samples highlights the importance of implementing and

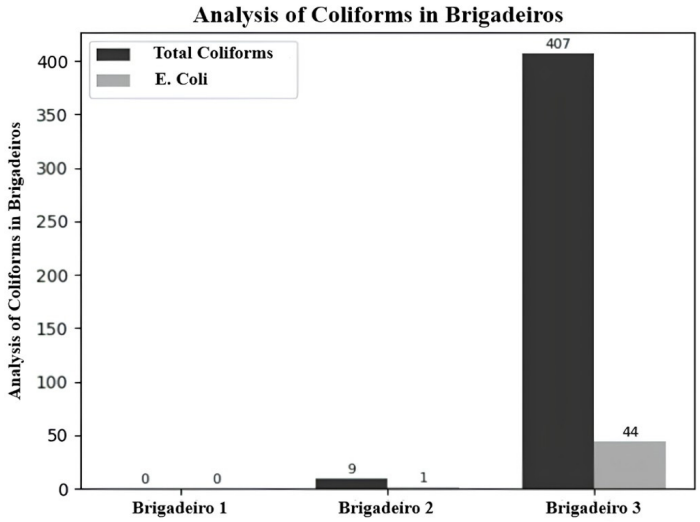

Figure 2: Bar graph illustrating the amount of Total Coliforms and *E. coli* in brigadeiros from three different vendors. Source: the author (2024).

strictly adhering to good manufacturing practices in artisanal food production.

Figure 2 presents the analysis of total coliforms and *E. coli* in three different brigadeiro samples from various vendors. Significant variability is observed among the samples, indicating differences in the hygiene and sanitary conditions of the production and sales locations.

In the Brigadeiro 1 sample, both total coliforms and *E. coli* showed undetectable results (0 units). This result suggests that the sample was produced and handled under appropriate hygienic conditions, preventing the presence of bacteria indicative of fecal contamination.

The Brigadeiro 2 sample showed 9 units of total coliforms and 1 unit of *E. coli*. The presence of total coliforms indicates contamination due to inadequate hygiene conditions during production, handling, or storage. The detection of *E. coli* is concerning as it directly indicates fecal contamination. The presence of *E. coli* emphasizes the need for improvements in hygiene and food safety procedures.

Finally, the Brigadeiro 3 sample exhibited the highest values, with 407 units of total coliforms and 44 units of *E. coli*. These results are alarming and point to a severe sanitary failure at the production site. The high count of total coliforms suggests significant contamination, potentially due to inadequate cleaning, storage, or handling practices. The elevated presence of *E. coli* reinforces evidence of fecal contamination, representing a serious public health risk as this bacterium may be pathogenic.

The results presented in Figure 2 demonstrate considerable variability in the microbiological quality of brigadeiros sold in the Barretos-SP region. While one sample showed no detectable contamination, the other two samples displayed concerning levels of total coliforms and E. coli. These findings underscore the importance of implementing and monitoring strict hygiene and quality control practices at all stages of food production and commercialization.

The high contamination observed in some samples highlights the urgent need for corrective actions, such as training in good manufacturing practices for food handlers, facility upgrades, and the implementation of sanitary control procedures. Additionally, regular inspections by competent authorities are essential to ensure the safety of the food offered to consumers.

The distribution of total coliforms, as shown in Figure 3, reveals significant variability among the samples. The median of total coliforms is approximately 100 units, with an interquartile range from around 25 to 200 units. The maximum observed value is 407 units.

This wide dispersion indicates that some samples are subject to inadequate conditions, resulting in

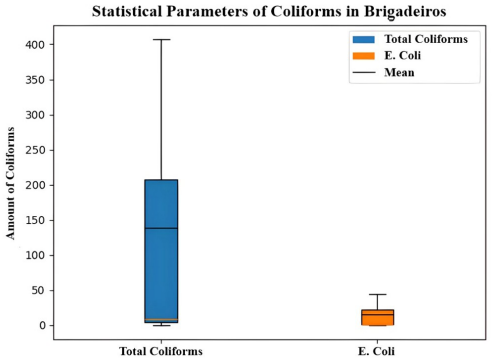

Figure 3: Box plot illustrating the total, minimum, maximum, and average amounts of Total Coliforms and *E. coli* in brigadeiros. Source: the author (2024).

high coliform counts. The presence of outliers, such as the Brigadeiro 3 sample, which showed an exceptionally high count, suggests critical control points that require special attention. The observed variation highlights the importance of consistent hygiene practices to reduce microbiological contamination.

The distribution of *E. coli* is more restricted, ranging from 0 to 10 units. The maximum observed value is 44 units, again in the Brigadeiro 3 sample. Any detection of *E. coli* is concerning due to its pathogenic potential and indication of fecal contamination.

Subsequently, the Pearson correlation coefficient was calculated, resulting in r = 0.992, indicating a very strong positive correlation between the two variables. The associated p-value was 0.000086, demonstrating that the observed correlation is statistically significant, with an extremely low probability that this result is due to chance. Despite these results indicating a clear trend of increasing *E. coli* as Total Coliforms increase, it is important to consider that obtaining a larger number of samples could provide a more robust analysis.

The microbiological analyses of brigadeiros sold in Barretos-SP show a significant correlation with studies that examined the hygiene of food handlers' hands. As observed by Silva et al. (2020), inadequate hand hygiene among handlers is a critical factor in food contamination, with the presence of total coliforms commonly detected. This finding aligns with the high contamination of total coliforms and *E. coli* identified in the Brigadeiro 3 sample in the present study, suggesting that failures in handlers' hygiene practices contributed to the high levels of contamination observed (Silva et al., 2020).

Additionally, Soares (2011) and Vitoria (2017) highlights that food handlers' hands are an important route of cross-contamination, with results indicating high coliform levels on hands before any hygiene training. This reinforces the need for educational interventions to improve handling practices. These parallels point to the urgency of continuous training and supervision to ensure the microbiological safety of food, thus preventing risks to public health (Soares, 2011; Vitoria, 2017).

The implementation of education and training programs for food handlers can be an effective measure to reduce contamination. Furthermore, adopting strict quality control procedures, including regular microbiological analysis of products, is essential to ensure consumer safety.

Conclusion

This study evaluated the microbiological contamination of brigadeiros sold in Barretos, São Paulo, focusing on total coliforms and Escherichia coli. The results revealed marked differences between samples: while one showed no contamination, another presented low levels, and a third displayed high contamination, including E. coli, indicating serious failures in hygiene and handling practices. These findings confirm the existence of sanitary deficiencies in part of artisanal production and highlight the associated risks to public health. Therefore, the study concludes that the microbiological quality of brigadeiros in the region is variable and directly dependent on the adoption of good manufacturing and handling practices, reinforcing the importance of implementing strict hygiene measures. Moreover, further studies with larger sample sizes are needed to expand understanding of the direct impacts of microbial contamination on food safety and public health.

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