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Evaluation of the Effects of Thermal Treatment on the Microbiological Quality of *Passiflora cincinnata* (Wild Passion Fruit) Flour

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Abstract

The aim of this study was to evaluate the effectiveness of thermal treatment in reducing the microbial load of wild passion fruit (*Passiflora cincinnata*) peel flour, aiming for its safe use as a food ingredient. Peels were sanitized with a 200 ppm chlorine solution for 15 min, dried at 55 °C, and ground; however, microbial counts remained above acceptable limits. A preliminary test at 65 °C confirmed the absence of contamination exceeding legal thresholds, and the flour was subsequently thermally treated at 65 °C for 24 h. Microbiological analyses were conducted for molds, yeasts, *Escherichia coli*, *Bacillus cereus*, and *Salmonella*. Fungal counts were $\log_{10}$-transformed and statistically analyzed using analysis of variance and Tukey's test ($p < .05$). Results showed a significant reduction in fungal load after thermal treatment, with *Bacillus*, *E. coli*, and *Salmonella* absent or below detectable limits. *Enterobacter/Klebsiella* group microorganisms were detected in raw peels and 55 °C dried flour, indicating contamination of the raw material. Thermal treatment at 65 °C reduced microbial loads to levels compliant with current Brazilian regulatory legislation. The study emphasizes the importance of validating thermal processes to ensure the microbiological safety of flours and recommends further research on the preservation of nutritional and bioactive compounds under these conditions.

Keywords: agro-industrial residues; drying; food safety; microbial reduction; thermal processing.

Practical Application: Thermal treatment ensures microbiological safety of *Passiflora cincinnata* peel flour, enabling its use as a functional food ingredient.

1 INTRODUCTION

Passion fruit of the genus *Passiflora* is industrially processed to obtain pulp and juice, generating large amounts of peel as waste. With appropriate treatment, this by-product can be transformed into an ingredient with functional or nutraceutical properties, offering health benefits to consumers (Bentacur-Ancona et al., 2025). *Passiflora cincinnata* is a Passifloraceae species native to the Caatinga, a biome unique to Brazil. It presents several pharmacological properties associated with its high flavonoid content. Vitexin, isovitexin, orientin, isoorientin, and their derivatives are the main chemical and pharmacological markers of this plant (Oliveira Júnior et al., 2024). Its fruit consists of 34% pulp, 26% seeds, and 40% peel, meaning that processing can generate up to 66% waste (Reis et al., 2023).

During transport, foods are exposed to multiple environments and, when crossing countries, are subject to various regulations and quality standards. Handling, transport, and processing increase the risk of contamination throughout the production chain. Consequently, residues may contaminate foods from farm to table, and technical issues can compromise quality, potentially leading to outbreaks affecting thousands of people (Libert et al., 2025).

If not properly controlled, industrial environments can facilitate contamination by pathogenic and spoilage microorganisms, making constant monitoring essential to ensure food safety (Crandall et al., 2024). The World Health Organization (WHO) established the Global Antimicrobial Resistance and Use Surveillance System to address antimicrobial resistance and monitor resistance in common bacteria and invasive fungi, among other factors (WHO, 2022). Monitoring contamination sources is therefore crucial, as foods can serve as vectors of diseases and foodborne intoxications if good practices are not followed.

Thermal treatment, or heat disinfection, is one of the simplest and most widely used disinfection methods in water, sanitation, and food sectors, particularly in resource-limited settings. Pathogen reduction during thermal treatment depends on the combination of temperature and exposure time (Espinosa et al., 2020).

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Agro-industries produce vast amounts of effluents annually, which, if released untreated into the environment, can negatively impact human and animal health as well as ecosystems (Vučurović et al., 2024). Therefore, industrial by-products can be repurposed into food ingredients and products, reinserting materials that would otherwise be discarded into the market, in line with the circular bioeconomy concept (Gómez-García et al., 2021).

This study aimed to evaluate the efficacy of thermal treatment in reducing the microbial load of *P. cincinnata* peel flour, ensuring its safe use as a food ingredient. Microbiological analyses were conducted before and after thermal treatment to verify whether the process could guarantee the safety of the by-product for subsequent food formulation.

1.1 Relevance of the work

This study highlights the potential use of *Passiflora cincinnata* peel flour, an underutilized agro-industrial residue, as a safe food ingredient after thermal treatment. The results demonstrate that drying at 65 °C effectively reduces microbial contamination to levels compatible with food safety standards, supporting its application in food formulations. Beyond promoting food safety, this approach contributes to waste valorization and aligns with the principles of circular bioeconomy, reinforcing the importance of integrating sustainable practices into agro-industrial processes.

2 MATERIAL AND METHODS

2.1 Raw material

The *P. cincinnata* peels used in this study were donated by the Cooperativa Grande Sertão, located in Montes Claros—MG, Brazil. The samples were initially sanitized with a 200 ppm chlorine solution for 10 min and then dried at 55 °C.

Before applying the thermal treatment at 65 °C to the flour, a preliminary test was conducted to assess the presence of microbial contamination in the peels after 24 h of drying. As the results indicated no contamination exceeding the limits established by current legislation, the thermal treatment of the flour proceeded.

2.2 Heat treatment

The thermal treatment consisted of exposing the peels to approximately 65 °C for 24 h in an air circulation oven, model SL-102, SOLAB. After treatment, the samples were cooled to room temperature, packed in plastic bags, and stored at room temperature until microbiological analyses were performed (Figure 1).

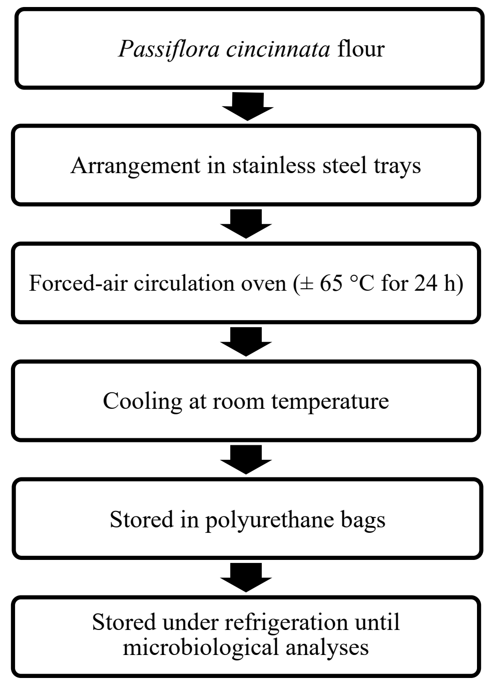


Figure 1. Flowchart of thermal treatment.

2.3 Microbiological analyses

2.3.1 Molds and yeasts

The total count of molds and yeasts was performed according to method 7.2—Total Count of Molds and Yeasts on Plates, as described in Chapter 7 of the *Manual of Methods for Microbiological Analysis of Foods and Water* (Silva et al., 2010). The analyses were conducted using successive dilutions up to 10^{-5}, with two sample replicates and two plating replicates per dilution.

2.3.2 Escherichia coli

The APHA Method 9.3—*VRB Plating Method* (total coliforms in food) was used, as described in Chapter 9—Enumeration of total coliforms, thermotolerant coliforms, and *E. coli*, from the *Manual of Methods for Microbiological Analysis of Foods and Water* (Silva et al., 2010). Analyses were performed with serial dilutions up to 10-^{3}, using two sample repetitions and two plating replicates per dilution.

2.3.3 Bacillus cereus

The Direct Plating Count Method 11.2, described in Chapter 11—*B. cereus*, from the *Manual of Methods for Microbiological Analysis of Foods and Water* (Silva et al., 2010), was used. Analyses were performed with serial dilutions up to 10^{-3}, using two sample repetitions and two plating replicates per dilution. However, for the 10^{-1} dilution, six replicates with 300 µL and two replicates with 100 µL of the inoculated sample were prepared.

2.3.4 Salmonella

Method 19.2, according to International Organization for Stand1ardization (ISO) 6579:2007(E) from the ISO, was used for the detection of *Salmonella* spp., as described in Chapter 19—*Salmonella*, of the *Manual of Methods for Microbiological Analysis of Foods and Water* (Silva et al., 2010). Analyses were performed in two repetitions of the flour sample.

2.3.5 Rugai qualitative test

For the presumptive identification of the isolated bacteria, the Rugai and Araújo test was used. The isolated colonies were inoculated into tubes containing Rugai medium and incubated at 37 °C for 24 h. After this period, the reactions were observed, and the results were compared with the profiles described by Rugai and Araújo (1968).

2.3.6 Statistical analysis

The data obtained from the microbiological analyses (counts of filamentous fungi and yeasts) were converted to decimal logarithms (log_{10} CFU/g) in order to meet the assumptions of normality and homogeneity of variances required for parametric tests. The transformed means were subjected to analysis of variance, and when significant differences were detected ($p < .05$), Tukey's test was applied for mean comparison at a 5% significance level. All statistical analyses were performed using R software (R version 4.5.1).

3 RESULTS

According to the analyses conducted, the results indicated the presence of fungi in samples of fresh wild passion fruit, wild passion fruit flour dried at 55 °C, and wild passion fruit flour treated at 65 °C (Table 1).

Table 2 summarizes the qualitative results obtained from microbiological tests carried out in different samples of *maracujá* products. The analyses include fresh *maracujá*, *maracujá-do-mato* flour dried at 55 °C, *maracujá* peel dried at 65 °C, and *maracujá-do-mato* flour treated at 65 °C. These results allow the evaluation of the hygienic-sanitary quality of the raw material and processed products, as well as the potential impact of drying conditions on microbiological safety.

Table 1. Filamentous fungi and yeast counts in samples of fresh passion fruit peels, dried *Passiflora cincinnata* at 65 °C, and *P. cincinnata* flour treated at 65 °C.

Treatment	Averages
FM65	2.340a ± 0.12
FM55	3.152a ± 0.10
FMN	3.165b ± 0.35
p-value	$p < .01$
CV	*7.59*

FM55: *P. cincinnata* flour dried at 55 °C; FM65: *P. cincinnata* flour treated at 65 °C; FMN: Fresh passion fruit *(P. cincinnata)*; CV: coefficient of variation. Values are expressed as log_{10} colony-forming units per gram (CFU/g). Means were calculated from two replicates per treatment, with standard deviation.

In Figure 2, the stage of the *Salmonella* test can be observed, showing contamination on plates containing XLD (Xylose Lysine Deoxycholate Agar) and HE (Hektoen Agar). Contamination was also detected in the Rugai medium, suggesting that the isolated microorganism possibly belongs to the genus *Enterobacter* or *Klebsiella* (Figure 3).

Figure 3 illustrates the results obtained with the Rugai test. In photo "a," contamination is evidenced by the alteration of the culture medium, while in photo "b," the medium remains unchanged, indicating the absence of contamination. This visual comparison highlights the contrast between positive and negative results in the applied microbiological analysis.

4 DISCUSSION

Based on the results, a decrease in the fungal microbial load was observed from the fresh wild passion fruit to the flour dried at 55 °C and the flour subjected to heat treatment at 65 °C. This reduction is attributed to the application of heat and temperature adjustment, as the thermal treatment at 65 °C reduced the number of dilutions expressing the minimum detectable count range for microorganisms. In a study conducted by Wang et al. (2022), thermal treatment at 60 and 70 °C significantly mitigated the microbial loads of *E. coli*.

As shown in Table 2, the analyzed samples revealed that microorganisms of the genus *Bacillus* either did not grow or presented counts below the minimum detection limit established by the applied method. This finding suggests the effectiveness of the processing in inhibiting or eliminating these microorganisms, which may pose a risk to food quality and microbiological safety. In addition, the tests for *E. coli* showed no bacterial growth in any of the samples, and the results for *Salmonella* were negative, further supporting the product's safety with regard to these pathogens of public health importance.

Through the *Salmonella* test, it was possible to detect the presence of microorganisms of the genus *Enterobacter*, or possibly *Klebsiella*, in the samples, both in the wild passion fruit flour dried at 55 °C and in the fresh wild passion fruit peel. These results indicate that the contamination originated from the raw material, which had not undergone any prior processing. It is noteworthy that the peels were sanitized with a 200 ppm solution for 10 min; however, this procedure was not sufficient to eliminate the microorganisms present.

In a study by Hartantyo et al. (2020), the presence of *Klebsiella pneumoniae* in raw foods was reported, highlighting that this bacterium can be multidrug-resistant and carry virulence

Table 2. Qualitative results of microbiological tests in samples of fresh maracujá, maracujá-do-mato flour dried at 55 °C, maracujá peel dried at 65 °C, and maracujá-do-mato flour treated at 65 °C.

Sample Type	*E. coli*	*Salmonella*	*Enterobacter/Klebsiella*	*Bacillus spp.*	General Microbiological Situation
Fresh passion fruit	ND	Negative	Positive	ND	Not compliant*
Passion fruit dried at 55 °C	ND	Negative	Positive	ND	Not compliant*
Passion fruit dried at 65 °C (bark)	ND	Negative	ND	ND	Compliant
Passion fruit flour treated at 65 °C	ND	Negative	ND	ND	Compliant

*Non-compliant: results outside the limits established by legislation. ND: not detected (below the limit of detection of the method).

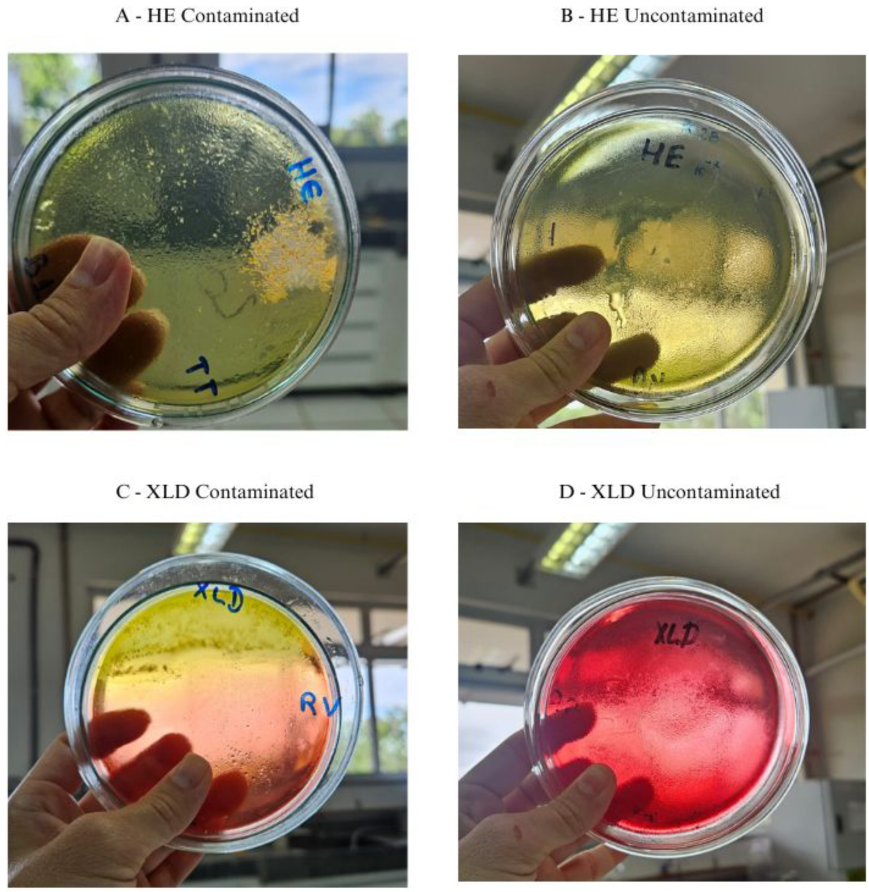
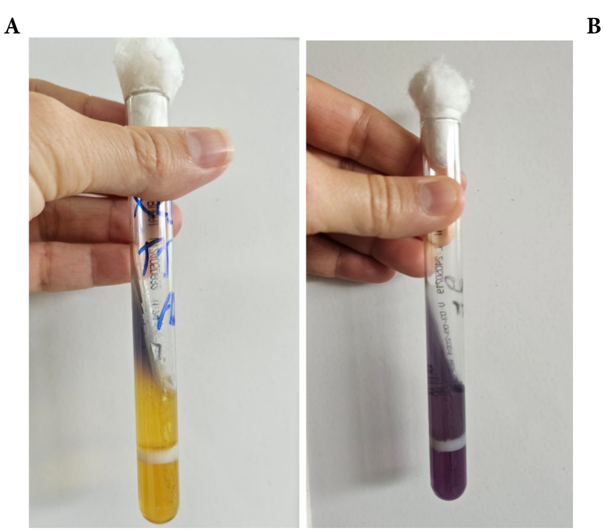


Figure 2. Salmonella test plates.



In Figure 3A the Rugai test tube shows contamination indicated by medium alteration, whereas Figure 3B presents the medium without alteration.

Figure 3. Qualitative test on Rugai medium: (**A**) Contaminated Rugai and (**B**) Uncontaminated Rugai.

genes. The author also noted that the detection of this microorganism in ready-to-eat foods may be related to its thermotolerant nature, as the growth of *K. pneumoniae* is significant at 60 °C, and complete inactivation may not occur at this temperature. Based on this information, a thermal treatment at 65 °C was chosen for the maracujá-do-mato flour.

In the study by Sousa et al. (2023), different time/temperature combinations were tested to reduce the microbial load in olive pomace, showing that thermal treatments were effective in significantly decreasing the native microbiota. This result reinforces the applicability of thermal treatment as a viable alternative to ensure the microbiological safety of food by-products, which aligns with the effect observed in the present study with the application of 65 °C for 24 h.

According to Agência Nacional de Vigilância Sanitária (Anvisa) Resolution RDC No. 724/2022 (Brasil, 2022), flours intended for human consumption must be free of *Salmonella* spp. in 25 g of sample and present a maximum of 10^2 CFU/g of thermotolerant coliforms. In this study, the thermal treatment at 65 °C for 24 h effectively reduced the microbial load, resulting in levels compatible with the limits established by legislation.

The thermal resistance of microorganisms in low-moisture food matrices, such as flours, has been widely studied due to outbreaks associated with dry products. Lin et al. (2020) demonstrated that the physical structure of the food and moisture content significantly influence the effectiveness of thermal inactivation of microorganisms, using *Enterococcus faecium* as a surrogate for *Salmonella* spp. In their study, in matrices with reduced water activity (aw ≈ 0.65), high-temperature treatments, such as 85 °C for over 20 min, were required to achieve significant microbial reductions, although the efficiency was lower than in higher-moisture foods. These findings can be correlated with the results obtained in the present study, in which the application of 65 °C was sufficient to eliminate microorganisms suspected to belong to the *Klebsiella/Enterobacter* group, suggesting that these microorganisms in the analyzed matrix exhibit lower thermal resistance or that the experimental conditions favored their elimination. This highlights the importance of validating thermal processes to ensure the microbiological safety of flours, especially considering the complexity of the matrix and the profile of contaminants.

In the present study, drying at 65 °C was chosen to inactivate microorganisms, which is consistent with protocols for reducing microbial load in plant-based matrices. Although temperatures above 60 °C may cause losses of heat-sensitive bioactive compounds, such as vitamin C and volatile compounds, studies indicate that flavonoids and certain polyphenols may remain relatively stable within this thermal range, particularly when exposure time is controlled (Elgamal et al., 2023). Therefore, 65 °C represents a balance between sanitization efficacy and the potential preservation of bioactive compounds of functional interest. However, further studies are necessary to determine the optimal treatment time and to validate the presence and stability of these functional compounds.

5 CONCLUSIONS

According to this study, the observed contamination originated from the raw material supplier. Applying thermal treatment to flour previously dried at 55 °C reduced the microbial load, but the results indicate that drying at 65 °C is more effective for ensuring microbiological safety. However, further studies are needed to evaluate whether the nutritional properties of *P. cincinnata* peel are preserved under this drying condition.

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