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IMPACT OF ROASTED *CANEPHORA* COFFEE EXTRACT ON VIABILITY OF STARTER AND PROBIOTIC CULTURES IN YOGURT

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ABSTRACT

Yogurt is renowned for its health benefits due to live bacterial cultures such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. However, these cultures do not survive the gastrointestinal tract, necessitating the addition of probiotics. Additionally, roasted canephora coffee extract can enhance the microbiological quality of yogurt due to its antimicrobial properties. Thus, this study aimed to assess the impact of roasted canephora coffee extract on the development of starter and probiotic cultures in yogurt during 42 days of storage. Four yogurt formulations were prepared: P (standard), PEC (standard + coffee extract), PLa (standard + *Lactobacillus acidophilus*), and PLaEC (standard + *Lactobacillus acidophilus* + coffee extract). The addition of 5% coffee extract was incorporated into the PEC and PLaEC formulations. Microbial growth was monitored at weekly intervals. Results showed that the addition of coffee extract did not significantly affect the growth of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus acidophilus*, maintaining counts above 7 Log CFU/g. In conclusion, coffee extract can be a viable addition to yogurt, preserving bacterial cultures and providing functional benefits to consumers.

Keywords: Fermented dairy products. *Lactobacillus acidophilus*. Antimicrobial agents. Food preservation. Functional foods.

1 INTRODUCTION

Yogurt, a fermented dairy product, is widely recognized for its health benefits due to the presence of live bacterial cultures, primarily *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These products serve as an efficient matrix for delivering probiotics, live microorganisms that confer health benefits to the consumer when consumed in adequate amounts. [1,2] However, the starter cultures in traditional yogurt typically do not survive gastrointestinal conditions, necessitating the addition of probiotics to ensure these benefits. [3,4] Using plant extracts with antimicrobial properties, such as coffee, may enhance the microbiological quality of dairy products without compromising their safety or flavor. Coffee is known for its unique flavor characteristics, roast levels, presence or absence of caffeine, chlorogenic acids, and melanoidins, making it one of the most consumed beverages worldwide.[5] Among the main benefits of coffee are its antioxidant effects and preventive properties against cancer, cardiovascular diseases, obesity, and diabetes. [6] Additionally, coffee extract can control microbial growth in food without posing risks to consumers. [7]

Recent studies demonstrate that extracts from *Coffea canephora (known commercially as* robusta) and *Coffea arabica* (arabica) inhibit pathogenic microorganisms such as *Salmonella enterica*, *Escherichia coli*, and *Staphylococcus aureus*, without affecting the growth of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*. This suggests that coffee extract can be used as a preservative in functional foods, promoting consumer health and extending product shelf life. [8] Therefore, the aim of this study is to evaluate the impact of roasted robusta coffee extract on the development of starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and the probiotic (*Lactobacillus acidophilus*) in yogurt during 42 days of refrigerated storage. It also aims to contribute insights into the feasibility of adding coffee as a natural flavoring and preservative in dairy products, enhancing their functional and sensory value.

2 MATERIAL & METHODS

The yogurt production was conducted at the Research and Teaching Laboratories of UTFPR (Federal Technological University of Paraná) – Campus Medianeira. The process began with preparing the yogurt formulation ([9]) using standardized pasteurized whole milk to 12% (w/v) total solids with the addition of skimmed milk powder and granulated sugar. After thermal treatment at 95 ± 1 °C for 5 minutes and cooling to 42 ± 1 °C, the starter and probiotic cultures were added according to the manufacturers' recommendations. The formulations were fermented at 42 ± 1 °C until reaching a pH close to 4.60 ± 0.05 and a minimum acidity of 0.60 g of lactic acid/100g. After fermentation, the yogurts were cooled to 10 °C, manually stirred, divided into portions, and placed in containers. In the formulations corresponding to the PEC and PLaEC assays, coffee extract 5% (5 g/100 g) was added (Table 1). Subsequently, the yogurts were stored under refrigeration (5 ± 1 °C).

Microbial growth of starter and probiotic cultures was assessed by plate counting using selective media: M17 agar for *Streptococcus thermophilus* [10]; De Man, Rogosa, and Sharpe (MRS) agar adjusted to pH 4.58 for *Lactobacillus delbrueckii* subsp. *bulgaricus* [11, 12]; MRS agar with D-sorbitol solution for *Lactobacillus acidophilus* [12,13]. Additionally, following the Normative Instruction No. 161, dated July 1, 2022 [14], monitoring was conducted for the growth of *Escherichia coli* [15], *Salmonella* sp. [16], and molds and yeasts [17]. Plate counts were performed in triplicate every 7 days over 42 days of refrigerated storage (T1 = 1 day, T2 = 7 days, T3 = 14 days, T4 = 21 days, T5 = 28 days, T6 = 35 days, T7 = 42 days), and results were expressed as log CFU/g.

Table 1 Formulations of assays for probiotic yogurt production and incorporation of coffee extracts.

Experiment	Composition
P	Standard (cultura *starter* de *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus)*
PEC	Standard + robusta coffee extract (5,0%) [8]
PLa	Standard + *Lactobacillus acidophilus*
PLaEC	Standard + *Lactobacillus acidophilus* + robusta coffee extract (5,0%) [8]

3 RESULTS & DISCUSSION

The results of the microbial counts for the starter culture are presented in Figure 1. It was found that the formulated assays met the microbiological requirements for total lactic acid bacteria in yogurts [18], with counts exceeding 7 log CFU/g throughout the seven weeks of storage. For *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, no significant differences in bacterial growth were observed between assays with and without adding coffee extract. These results indicate that the coffee extract had no influence on the viability of these microorganisms in the studied formulations.

Some research emphasizes [19, 20] that the viability of starter bacteria during yogurt shelf life is a critical quality parameter. Therefore, when researching new ingredients such as coffee, evaluating their impact on these microorganisms is essential. [19] Another author [21] highlights the importance of maintaining adequate counts of these microorganisms, as excessively low or high values of bacteria in the starter culture, which were not detected in the present study, can result in flavor defects in yogurt. In a study on the shelf life of coffee-flavored yogurt, another researcher [20] found that yogurt containing 0.2% soluble coffee had 7.46 log CFU/g of viable lactic acid bacteria at the beginning of storage and 7.30 log CFU/g after 28 days of storage, demonstrating that the addition of soluble coffee did not compromise microbial viability, a result consistent with the present study.

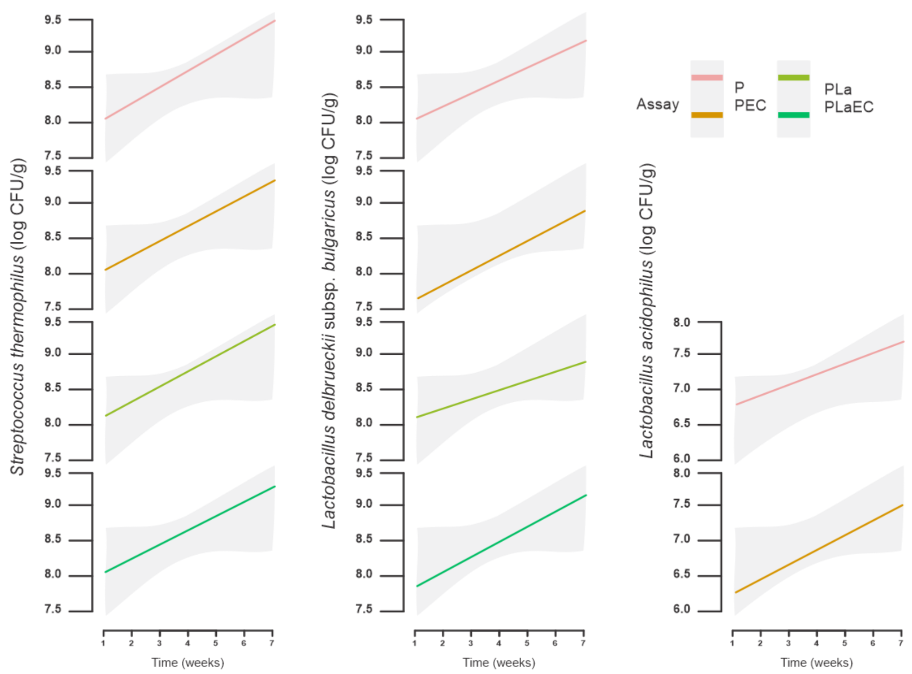

Figure 1 Microorganism counts of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* e *Lactobacillus acidophilus* (log CFU/g) across different assays over time in weeks.
*The smoothed line represents the linear relationship of the data (averages) between time and microorganism counts (log CFU/g), and the shaded area around the line represents the confidence interval.

The results of *Lactobacillus acidophilus* growth in the assays can also be seen in Figure 1. Brazilian legislation does not establish limits for *Lactobacillus acidophilus* counts; however, for the use of bifidobacteria (another probiotic microorganism), the count must be at least 6 log CFU/g or mL. [18] Additionally, probiotic strains must remain viable throughout the yogurt's shelf life. Thus, based on the aforementioned count, it can be stated that the assays complied with the recommended guidelines for probiotic microorganisms. Another important factor regarding *Lactobacillus acidophilus* growth is that no significant influence of coffee extract addition on its population growth was identified. These results are crucial to ensure that adding coffee does not compromise the viability of probiotic strains during storage. As already discussed by some authors [22, 23], maintaining the viability of probiotic strains is crucial to ensure the health benefits for consumers, with the recommended minimum daily intake of probiotics being 6 - 9 log CFU/g or mL, or at least 8 log CFU per serving. [23, 24] Therefore, it can be concluded that the yogurts produced in this research maintained adequate concentrations of probiotic microorganisms. Previous studies highlight the importance of probiotic bacteria survival and viability throughout the product's shelf life. In this context, the present research is aligned with these findings [25], which investigated the in vitro effect of coffee species, roast degree, and decaffeination on probiotic bacteria and *Escherichia coli*. They found that aqueous extracts of medium roast coffee from *Coffea arabica* and *Coffea canephora* species (0.5 to 1.5% soluble coffee) promoted the growth of all tested probiotic bacteria (*Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *lactis*, and *Bifidobacterium animalis*), but not the growth of pathogens.

Thus, the results obtained in this study indicate that the addition of coffee extract in probiotic yogurt formulations did not affect the growth of starter and probiotic cultures. This suggests that coffee-enhanced yogurts can be a viable option by maintaining desirable microbiological characteristics of the product while offering health benefits to consumers aligned with minimum daily probiotic intake recommendations, coupled with the health benefits of coffee consumption.

4 CONCLUSION

The results of this study indicate that the addition of roasted Robusta coffee extract to yogurt formulations did not significantly affect the growth of starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) or the probiotic (*Lactobacillus acidophilus*) during 42 days of refrigerated storage. Maintaining adequate counts of probiotic microorganisms throughout the storage period suggests that coffee extract could be a beneficial addition to yogurts, not only as a flavoring agent but also as a potential natural preservative. This corroborates with previous studies emphasizing the importance of bacterial viability and the health benefits of probiotic strains in dairy products. Therefore, adding coffee extract to probiotic yogurts may be considered a viable strategy to enhance these products' microbiological and sensory quality, while promoting functional benefits for consumers. Future studies could further explore how bioactive compounds from coffee interact with bacterial cultures and expand the use of plant extracts in different food matrices.

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