EVALUATION OF MANGO JUICE AS A SUBSTRATE FOR THE PRODUCTION OF FERMENTED SYMBIOTIC BEVERAGES: AN ALTERNATIVE FUNCTIONAL FOOD

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ABSTRACT

Diets based on ultra-processed foods, coupled with a sedentary lifestyle, can trigger chronic diseases. Probiotic products present a promising alternative for addressing these diseases. However, many of them are of dairy origin, limiting access for some individuals. Therefore, this study aimed to develop a non-dairy fermented symbiotic mango juice, assessing the effects of adding inulin and xylitol on the survival of *Lacticaseibacillus rhamnosus* ATCC 7469 under refrigeration. The study involved mango juice production with pH adjustment to 6.0, 24-hour fermentation, and refrigerated storage with varying additions of inulin and xylitol at different concentrations, using a factorial design (2^2). In the analysis of fermented juices with varying concentrations of inulin and xylitol, survival only decreased when xylitol was at the lowest level (5 g/L), regardless of the inulin concentration. Intermediate concentrations of inulin and xylitol, 3.5 and 7.5 g/L, respectively, were considered optimal for obtaining a lower-calorie symbiotic mango juice while maintaining survival above 100% during refrigerated storage. The non-dairy fermented mango juice is a promising substrate for *L. rhamnosus* ATCC 7469, and the addition of inulin and xylitol increased both survival and nutritional quality.

Keywords: Fermentation. Fruit juices. Functional food. Prebiotic. Sweetener.

1 INTRODUCTION

Diets rich in ultra-processed foods and a sedentary lifestyle can cause chronic diseases. However, consuming functional foods, such as fermented probiotics and symbiotic drinks, can be a healthy alternative for improving health. Symbiotic foods contain a mixture of probiotics and prebiotics and are known as symbiotic mixtures[1]. These foods perform metabolic, physiological, and/or nutritional functions[2,3]. Fermented probiotic products have been part of food culture since ancient peoples[4]. *Lacticaseibacillus* species, such as *L. rhamnosus* ATCC 7469, are common in the fermentation of probiotic products, favoring their application in foods due to their resistance to low pH and tolerance to high acidity and bile salts [5,6]. On the other hand, prebiotics, such as inulin, are not digestible by the human gastrointestinal tract, but are metabolized by the gut microbiota[7], and are widely used in food preparations with probiotics [8,9]. However, many probiotic foods on the market are dairy-based[10], excluding consumers with lactose intolerance, allergies, or milk-related dietary restrictions. The use of non-dairy sources, such as fruit juices, is a promising alternative.

Fruits, rich food in bioactive compounds, are a healthy choice for adding probiotics, and mango, due to its health benefits, is a valuable option[11,12]. In addition to probiotics, supplements such as xylitol, a low-calorie sweetener, are present in foods to add nutritional value[13]. Xylitol, like inulin, has prebiotic properties, promoting healthy gut function. Currently, few studies have explored the potential of mango juice as a vehicle for probiotics, and even fewer have evaluated the effects of adding functional ingredients, such as xylitol, on the survival of probiotic microorganisms in fruit juices [9,11–13]. This study seeks to develop a juice of mango and evaluate the effects of inulin and xylitol on the survival of *L. rhamnosus* ATCC 7469 during cold storage.

2 MATERIAL & METHODS

Microorganism: The pure culture of *Lacticaseibacillus rhamnosus* ATCC 7469 was obtained from Plast Labor (Rio de Janeiro, Brazil) and preserved at -20°C in 10% glycerol (v/v)[14]. For inoculum development, 2 mL of the microbial suspension were transferred to 50 mL of selective *Lactobacillus* medium (MRS – MAN, ROGOSA, and SHARPE) in a 125 mL flask, which was incubated at 37°C for 24 hours in a bacteriological incubator (LABOR, SP-101).

Probiotic Mango Juice Preparation: The pulp concentration used was 55% (v/v). After dilution in distilled water, the pH was adjusted to 6.0 with NaOH (2M). Pasteurization occurred at 67°C ± 2°C for 35 minutes, followed by thermal shock in an ice bath for 5 minutes[15,16]. Fermentation, in 125 mL Erlenmeyer flasks with 45 mL pasteurized juice, involved inoculation with 5 mL (10% v/v) of *L. rhamnosus* ATCC 7469. Juices were incubated at 37°C for 24 hours in a bacteriological incubator.

Production of Fermented Symbiotic Juices: After fermentation, all juices were enriched with inulin and xylitol obtained from ROVAL, and stored at 4ºC. The study on the influence of these additives on probiotic survival involved applying a 2^2 factorial design with replicates at the central point to evaluate the effect of inulin and xylitol. The concentrations used for inulin were 2, 3.5, and 5 g/L, while the concentrations for xylitol were 5, 7.5, and 10 g/L, for the lower (-1), central (0), and upper (+1) levels, respectively. All assays were performed in triplicate, except for central point assays in the second stage, which were done in duplicate.

Refrigerated Storage: After adding functional ingredients (inulin and xylitol), symbiotic fermented juices were stored for 28 days under refrigeration (4°C). All juices were analyzed for pH, cell viability and probiotics survival. Cell viability determination for *L. rhamnosus* cell viability was determined at 0 and 28 days of refrigerated storage. Viability results were expressed in Log CFU/mL (Colony Forming Units per milliliter), determined in triplicate through serial dilutions and spread plate technique on Petri dishes containing MRS Agar. Petri dishes were incubated in a bacteriological incubator (LABOR, SP-101) at 37°C for 48 hours. Bacterial survival was determined using initial (V_i) and final (V_f) microorganism viabilities in probiotic or symbiotic fermented mango juices under refrigeration. The determination of the pH during refrigerated storage was performed using a digital potentiometer (JENWAY 3510, Meter).

Data Analysis: Averages, standard deviations, and coefficients of variation were determined in Excel. Excel and Origin Pro 2023b software were used for table and graph construction, respectively. Statistical analyses were performed using Statistica 7.0 software.

3 RESULTS & DISCUSSION

The values of cell viability and survival in the factorial design assays of fermented symbiotic juices, at the beginning and after 28 days of refrigerated storage, are presented in Figures 1a and 1b.

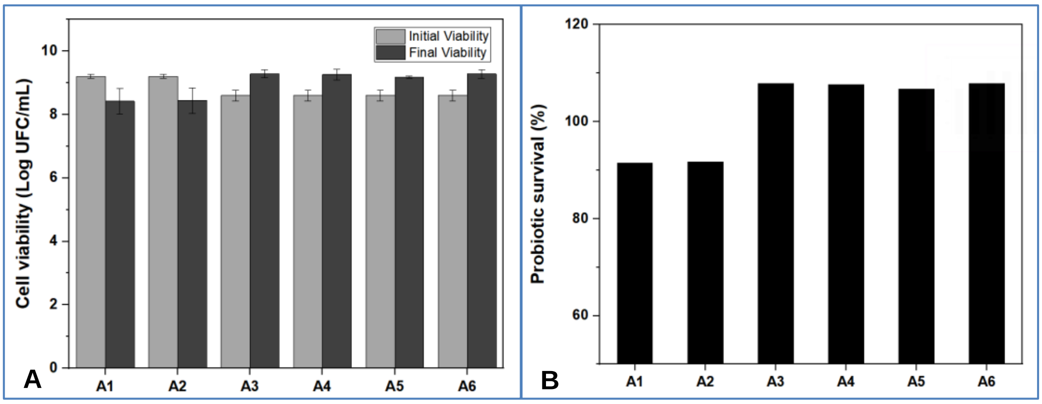

Figure 1. (A) Cell viability of *L. rhamnosus* ATCC 7469 in fermented symbiotic mango juices, at 0 and 28 days of refrigerated storage; (B) Probiotic survival of *L. rhamnosus* ATCC 7469 in fermented symbiotic juices, at 28 days of refrigerated storage.

The assessment of the final viability of *L. rhamnosus* ATCC 7469 in probiotic mango juices revealed variations between 8 and 9 Log CFU/mL after 28 days of storage. Figure 1a shows that both the survival and viability of the microorganism were influenced by the concentration of additives in the formulation of probiotic mango juices. With the application of a factorial design, the average viability of *L. rhamnosus* was 8.9 ± 0.34 Log CFU/mL (Figure 1a). In assays 1 and 2, with the lowest xylitol concentration (5 g/L), there was a decrease in viability, regardless of inulin concentration (2 g/L or 5 g/L). However, in assays 3 and 4, with higher xylitol (10 g/L) and inulin concentrations, there was a significant increase. In central point assays (5 and 6), with intermediate concentrations of inulin (3.5 g/L) and xylitol (7.5 g/L), an increase in viability was also observed after 28 days of storage. Overall, the microorganism's viability remained within expectations for probiotic/symbiotic foods stored at low temperatures. Promising results were achieved due to the increase in xylitol concentration in assays 3 and 4 compared to assays 1 and 2. This increase favored the growth and stability of microbial viability during storage. As indicated by previous studies, xylitol acts as a prebiotic, stimulating the growth and metabolism of probiotic bacteria, especially of the *Lacticaseibacillus* genus [17,18].

Despite this, it is important to note that the use of xylitol in probiotic fruit juices still lacks further exploration, requiring additional studies to assess its influence on food preparations. Figure 1b presents the probiotic survival values of *L. rhamnosus* ATCC 7469 in fermented symbiotic juices after 28 days of refrigerated storage. The results obtained after 28 days of storage indicate that all survival values were above 90%, regardless of the additive concentration, as evidenced in figure above. Assays 1 and 2 showed survival of 91.5% and 91.7%, respectively, while in the other assays, survivals were approximately 107%. This survival trend reflects the same pattern observed in the cell viability of *L. rhamnosus* ATCC 7469. Considering that increasing the concentration of inulin or xylitol did not have a significant effect on survival, the use of concentrations of 2 to 5 g/L and 5 to 10 g/L for inulin and xylitol, respectively, is suggested. However, it is important to note that high concentrations of both additives can be potentially toxic or harmful to both probiotic microorganisms and consumers. Research indicates that inulin doses exceeding 5 g/L may reduce the adhesion of *L. rhamnosus* to intestinal epithelial cells[19]. In the case of xylitol, high concentrations (> 12%) have shown inhibitory effects on the viability of *L. acidophilus*[18]. Additionally, it is important to observe that it is not recommended for humans to consume more than 30 g/day of inulin or xylitol, as excessive consumption of these additives may pose health risks to consumers, according to regulatory guidelines [20].

Regarding the pH profile of the assays during refrigerated storage, it was possible to observe that assays 1 and 2 exhibited the smallest pH variations, while in the other assays, an apparent relationship was observed between increased viability (Figure 1a) and higher pH variation. On average, the pH of fermented symbiotic juices ranged from 3.6 to 3.9, with an average variation of 0.6% after 28 days of refrigerated storage. It is important to note that, even with fluctuations, all pH values at the end of storage remained between 3 and 4, aligning with observations in fermented probiotic fruit juices [8,9,21]. Low pH is often associated with low probiotic survival in fruit juices[22]. In this study, even with pH variations, *L. rhamnosus* ATCC 7469 demonstrated survival capability in all juices, regardless of the factorial design application, during refrigerated storage. Cell viability and survival for both juices remained above 8 Log CFU/mL and 100%, respectively, after approximately 30 days of refrigerated storage. The increase in viability in assays 3 and 4 coincided with the decline in juice pH, both showing a similar range of variation. These results suggest that a higher concentration of xylitol likely favored the growth of *L. rhamnosus* in the juices. In addition to assays 3 and 4, two other assays (T5 and T6) showed similar results to each other. Regardless of the concentration of inulin and xylitol used, these four assays demonstrated analogous behavior, resulting in close values among them.

These findings are particularly relevant for the large-scale production of fermented probiotic juices, as they indicate that an intermediate concentration of xylitol (7.5 g/L) provided similar positive effects on the viability and survival of *L. rhamnosus*, comparable to the maximum concentration (10 g/L). Statistical analysis of the factorial design revealed non-significant effects of the factors. The negative effect of inulin suggests that an increase in its concentration reduces the survival of the probiotic microorganism, while the positive effect of xylitol indicates that an increase in its concentration is associated with higher survival. In summary, this study contributes to understanding the influence of inulin and xylitol on the viability of *L. rhamnosus* ATCC 7469 in mango probiotic juices, highlighting the effectiveness of intermediate concentrations of xylitol to optimize probiotic survival. These results provide valuable insights for the production and formulation of fermented probiotic juices, indicating strategies to improve the quality and stability of these products.

4 CONCLUSION

The results suggest that mango juice can be effectively used as a base for fermented probiotic and symbiotic beverages, expanding options for consumers with dietary restrictions. Formulation optimization, with an emphasis on intermediate concentrations of inulin and xylitol, proved more suitable for producing fermented symbiotic mango juices, avoiding the need for excessive additive amounts to achieve survival exceeding 100% during refrigerated storage. Furthermore, this study provides an effective strategy to enhance the survival and quality of probiotic microorganisms during refrigerated storage through the addition of functional ingredients.

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ACKNOWLEDGEMENTS

The authors express their gratitude to the UFPE (Federal University of Pernambuco), the PROPESQI (Pro-Rectorate for Research and Innovation), and CNPQ (National Council for Scientific and Technological Development).