EFFECT OF CLARIFICATION OF FERMENTED PROBIOTIC JUICES FROM GUAVA, MANGO, AND PASSION FRUIT ON THE VIABILITY OF *Lacticaseibacillus rhamnosus* ATCC 7469

Thiago R. Silva, Ester Ribeiro*

[*] *Department of Antibiotics – Federal University of Pernambuco, Cidade Universitária, Recife, Brazil.*
[2] *UFPE, Recife, Brazil.*
** Corresponding author's phone number and email address: +5581997045404/ ester.ribeiro@ufpe.br*

ABSTRACT

The study developed and analyzed fermented juices and probiotics from guava, mango, and passion fruit, with the goal of offering affordable options for consumers with lactose intolerance or milk allergy. *Lacticaseibacillus rhamnosus* ATCC 7469 was used as a probiotic microorganism and kinetic studies of microbial growth were performed during fermentation of clarified juices. Methods to measure absorbance, cell viability, pH, lactic acid, glucose, and fructose concentration were applied to monitor the fermentation process. Fruit pulp was diluted at 50% v/v and clarified with agar at 1g/L, varying the size of the inoculum (5%, 7.5% and 10% v/v). The results showed that all fermented juices reached acceptable concentrations of viable microorganisms (8 log), with pH reduction throughout fermentation, especially in clarified juices. Regarding glucose and fructose intake, unclarified mango juices with 5% inoculum had higher glucose intake, while clarified guava juices stood out in fructose intake. It was concluded that clarified juices fermented with *L. rhamnosus* ATCC 7469 have potential as probiotic products, and the size of the inoculum influences the quality and efficiency of fermentation, contributing to the production of inclusive and affordable probiotic beverages.

Keywords: Juices; Fruits; Lactic Acid; Fermentation.

1 INTRODUCTION

The consumption of functional foods is on the rise due to health awareness, reflected by the global market of 183,22 billion USD in 2023. In Brazil, ANVISA recognizes "functional foods", highlighting their potential to improve health. Probiotics are the most widely consumed dietary supplements globally, and the search for new raw materials to deliver them is an area of interest. The market is growing in alternative probiotic products due to the demand for non-dairy options, as underscored by studies [(1-2)]. Probiotics, classified in 2002[3] as "health-beneficial live microorganisms," including genera such as *Lactaseibacillus, Bifidobacterium*, and *Saccharomyces*, have been associated with improvements in immunity and potentially in the treatment of several diseases, as highlighted by recent studies[7].

The genus *Lacticaseibacillus* is composed of lactic acid-producing bacteria, found in substrates such as decomposing vegetables and dairy products[8], playing a crucial role in the production of fermented and preserved foods, and the species *L. rhamnosus* is recognized for its versatility and benefits to gastrointestinal health [(2, 4, 5, 6)].

There is a growing interest in non-thermal techniques, such as juice clarification with enzymes such as pectinases, which reduce turbidity and improve sensory quality. These enzymes, derived from microorganisms, are low-cost, renewable and effective in the juice industry, while clarifying agents based on polysaccharides, such as agar-agar and xanthan gum, have also been effective in reducing turbidity and improving the antioxidant properties of juices [(6,7,8)].

The objective of this study was to produce probiotic juices using fruit pulps that are widely accepted by Brazilian consumers like guava, mango, and passion fruit, which were fermented by *L. rhamnosus* ATCC 7469, varying the size of the inoculum. The research investigated microbial viability, pH, biomass, glucose, fructose, and lactic acid concentrations to investigate the fermentation process and to ensure high-quality products and food safety.

2 MATERIAL & METHODS

Lacticaseibacillus rhamnosus ATCC 7469 used in this work, was maintained in 10% glycerol (v/v), at -20 °C, according to the methodology proposed by Chang and Liew [9]. Mango pulp (*Mangifera indica L.*), yellow passion fruit (*Passiflora edulis*) and paloma guava (*Psidium guajava L.*) were stored at -20ºC (FE 22, Eletrolux). The clarification was based on the method proposed which uses agar-agar (1g/L)[7]. Then, pasteurization was performed in a water bath (TE-084, Pyrotec) for 35 minutes at 67 ± 2 °C, with subsequent cooling in an ice bath[5].

The microorganism was reactivated and 5%, 7.5% or 10% v/v inoculum were transferred to 125 mL Erlenmeyer flasks, containing the culture medium (clarified and unclarified juices). After the inoculum was added, the total volume was 50 mL. The flasks were placed in an oven (SP-101/216, LABOR) at 37ºC for fermentation, during 12 hours. Absorbance was used to determine the maximum specific growth rate (μ_{max}) in the clarified juices, during the development of the inoculum.

A pH meter (P62000, Gehaka) was used to determine the pH. Cell viability, expressed in Colony Forming Units per Milliliter (CFU/mL), was determined after spread plate plating in MRS agar medium[10]. The samples were incubated in an incubator (SP-101/216, LABOR) at 37ºC for 48 hours for subsequent CFU counting.

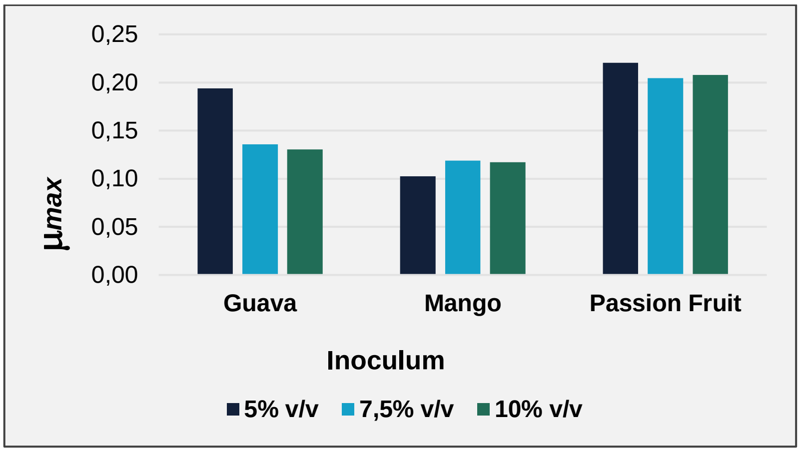
The biomass of the fermented clarified juices was determined using the dry weight methodology and the results were expressed as ΔX, in g/L. The determination of lactic acid, glucose and fructose during fermentation was performed through high-performance liquid chromatography[5]

3 RESULTS & DISCUSSION

It was observed that the increase in absorbance in fermentation with the 5 % v/v inoculum was more pronounced in the first 3 hours in the clarified guava juice, which was confirmed with the value of the maximum specific growth velocity, μ_{max}. The 10% v/v inoculum showed the highest absorbances, as in the studies [10,11,12]. However, in relation to the value of the maximum specific growth velocity, μ_{max}, the 7.5% v/v inoculum presented the best fit 0.9973 (R2) when compared to the 5% v/v (0.9963) and 10% v/v (0.9606) inoculum. The results come from the interference of the medium used, since the availability of nutrients and acidity of the passion fruit juice medium was favorable for the adaptation of the microorganism and consequently the speed of its growth. In the comparison of the results of the three juices, based on the maximum cell growth speeds, the 5% v/v inoculum stood out as effective in terms of growth speed for guava and passion fruit juices.

Figure 1. Values of the maximum specific growth velocity,

μ_{max} of clarified guava, mango and passion fruit juices with different percentages of the inoculum (5% v/v, 7.5% v/v and 10% v/v).



Source: Own authorship (2024)

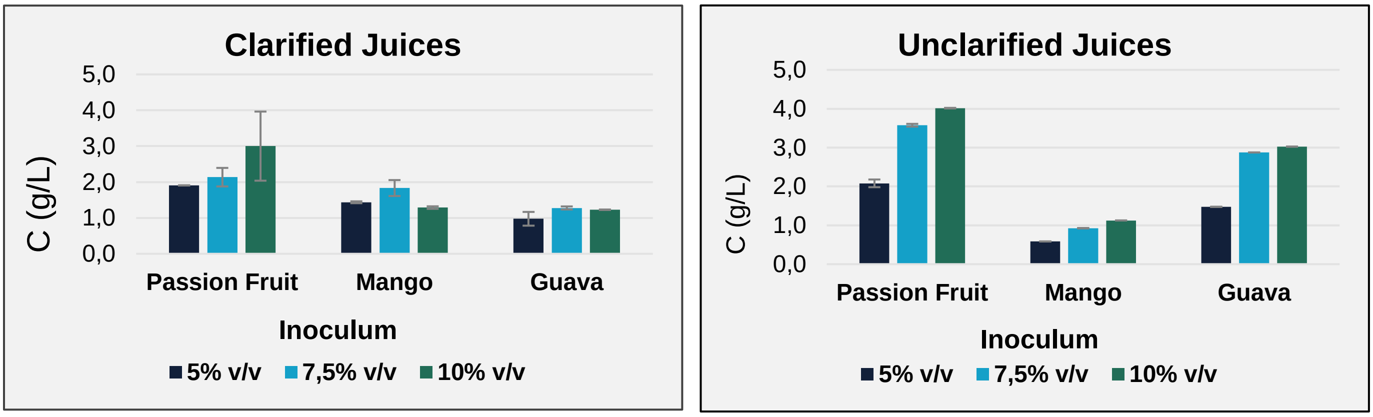
In guava juice, the 10% v/v inoculum showed a mean higher than 8 log (CFU/mL) after 12 hours. All fermentations showed an average above 7 log (CFU/mL) after 12 hours. The clarified mango juice showed all averages above 8 log (CFU/mL) for all inoculums after 12 hours of fermentation when compared to the clarified guava and passion fruit juices. Regarding microbial biomass, an increase (ΔX) was observed for all inoculum concentrations. Evident growths were obtained in the fermentations of mango and guava juices in all inoculums used. In passion fruit juice, similarities were observed in the biomass produced in relation to the different inoculums (5% v/v, 7.5% v/v and 10% v/v). For the passion fruit juice samples, the 5% v/v inoculum resulted in a biomass of 0.5 g/L (ΔX) after 12 hours of fermentation, as well as in the mango juice. In the clarified guava and mango juices, the 5% and 7% v/v inoculum resulted in a higher amount of biomass in 12 hours, with the exception of the clarified passion fruit juice, where the 7.5% and 10% v/v inoculum presented a more expressive amount of biomass.

The pH drop was observed in all juices, but in the mango juice with 5% v/v inoculum it was more significant, while the guava and mango juices showed pronounced reductions in the final pH in relation to the passion fruit juice. Regarding glucose and fructose intake, mango showed better consumption of unclarified juices (95.3% and 92.5%, respectively), while guava stood out in clarified juices (75.7% glucose and 98.6% fructose), suggesting that clarification may increase carbohydrate intake. The reduction of pH during fermentation, especially in clarified juices, indicated high acidification in these media, associated with the consumption of glucose and fructose, favoring the growth of fermentative microorganisms. The type of juice and the degree of clarification influenced glucose and fructose intake, with variable results for the 10% v/v inoculum, depending on the type of juice.

In the clarified juices of passion fruit, mango and guava, there was an increase in the concentration of lactic acid with the increase of the inoculum, with the inoculum of 10% v/v resulting in the highest concentrations: 3.00 g/L for passion fruit, 1.83 g/L for mango and 1.28 g/L for guava (Figure 2). The unclarified juices showed even higher concentrations, such as 4.0 g/L for passion fruit with 10% v/v inoculum, indicating that clarification reduces lactic acid production. Additionally, clarified juices tend to maintain a more stable pH over time, suggesting less acidification due to the removal of solids during clarification.

Both types of juice, however, showed a reduction in pH over time, indicating the possibility of lactic acid production in both cases. Regarding lactic acid production and pH variation, unclarified juices can be considered more viable in terms of efficiency in lactic acid production, generally presenting higher concentrations of this compound, which can be advantageous in applications that demand high concentrations of lactic acid (Figure 2). On the other hand, clarified juices may be preferable in terms of stability and quality, as they tend to maintain a more stable pH over time, providing a base for products that seek this characteristic.

Figure 2. Comparative graphs of lactic acid production (g/L) in clarified and unclarified juices using different inoculum sizes (5% v/v, 7.5% v/v and 10% v/v).



Source: Own authorship (2023).

4 CONCLUSION

The study emphasized the importance of precise inoculum choice for different fruit juices, due to variations in cell viability, biomass production, carbohydrate intake, pH, and lactic acid. All juices and inoculum tested showed satisfactory cell viability after 12 hours of fermentation, but there was variation in biomass production, especially among the different inoculums and juices. Carbohydrate consumption correlated with microbial growth, with notable variations, especially in passion fruit juice. pH and lactic acid analyses throughout the process emphasized the need for an individualized selection of the appropriate inoculum, as it was not possible to determine an ideal inoculum for all the juices evaluated. The 5% v/v inoculum has been shown to be effective in some cases, but its suitability may vary, highlighting the importance of considering the particularities of each juice and the objectives of fermentation.

REFERENCES

1 ALVES, Niédila Nascimento; MESSAOUD, Ghazi Ben; DESOBRY, Stéphane; COSTA, José Maria Correia; RODRIGUES, Sueli. Effect of drying technique and feed flow rate on bacterial survival and physicochemical properties of a non-dairy fermented probiotic juice powder. Journal Of Food Engineering, [S.L.], v. 189, p. 45-54, nov. 2016. Elsevier BV.

2 NEMATOLLAHI, Amene; SOHRABVANDI, Sara; MORTAZAVIAN, Amir Mohammad; JAZAERI, Sahar. Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. Electronic Journal Of Biotechnology, [S.L.], v. 21, p. 49-53, maio 2016. Elsevier BV.

3 FAO/WHO. Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. Londres, Ontario, Canada. 30 Abril e 1 Maio, 2002.

4 MISHRA, Birendra Kumar; HATI, Subrota; DAS, Sujit; PRAJAPATI, Jashbhai B. Biofunctional Attributes and Storage Study of Soy Milk Fermented by Lactobacillus rhamnosus and Lactobacillus helveticus. Food Technology And Biotechnology, [S.L.], v. 57, n. 3, p. 399-407, 2019. Faculty of Food Technology and Biotechnology - University of Zagreb.

5 PIMENTEL, Tatiana Colombo; KLOSOSKI, Suellen Jensen; ROSSET, Michele; BARÃO, Carlos Eduardo; MARCOLINO, Vanessa Aparecida. Fruit Juices as Probiotic Foods. Sports And Energy Drinks, [S.L.], p. 483-513, 2019. Elsevier.

6 SANTI, Lucélia; BEYS-DA-SILVA, Walter O.; BERGER, Markus; YATES, John R.; BRANDELLI, Adriano; VAINSTEIN, Marilene H.. Penicillium oxalicum secretomic analysis identify plant cell wall degrading enzymes important for fruit juice extraction. Journal Of Food Science And Technology, [S.L.], v. 58, n. 5, p. 1764-1775, 6 ago. 2020. Springer Science and Business Media LLC.

7 LACHOWICZ, Sabina; OSZMIAńSKI, Jan; KALISZ, Stanisław. Effects of various polysaccharide clarification agents and reaction time on content of polyphenolic compound, antioxidant activity, turbidity and colour of chokeberry juice. Lwt, [S.L.], v. 92, p. 347-360, jun. 2018. Elsevier BV.

8 TRINDADE, Lucas Vinícius; DESAGIACOMO, Carla; POLIZELI, Maria de Lourdes Teixeira de Moraes; DAMASIO, André Ricardo de Lima; LIMA, Aline Margarete Furuyama; GOMES, Eleni; BONILLA-RODRIGUEZ, Gustavo Orlando. Biochemical Characterization, Thermal Stability, and Partial Sequence of a Novel Exo-Polygalacturonase from the Thermophilic Fungus Rhizomucor pusillus A13.36 Obtained by Submerged Cultivation. Biomed Research International, [S.L.], v. 2016, p. 1-10, 2016. Hindawi Limited.

9 CHANG, C. P.; LIEW, S. L.. Growth Medium Optimization for Biomass Production of a Probiotic Bacterium, L actobacillus rhamnosus ATCC 7469. Journal Of Food Biochemistry, [S.L.], v. 37, n. 5, p. 536-543, 12 nov. 2012. Hindawi Limited.

10 FARIAS, N., SOARES, M., & GOUVEIA, E. Enhancement of the viabi¬lity of Lactobacillus rhamnosus ATCC 7469 in passion fruit juice: Appli¬cation of a central composite rotatable design. LWT - Food Science and Technology. 2016.

11 HOJSAK, I., ABDOVIĆ, S., SZAJEWSKA H., MILOSEVIĆ, M., KRZ¬NARIĆ, Z., & KOLACEK S. Lactobacillus GG in the Prevention of No¬socomial gastrointestinal and Respiratory Tract Infections. Pediatrics, 125, 1171 – 1177, 2010.

[12] SANTOS, Eloyza; ANDRADE, Raissa; GOUVEIA, Ester. Utilization of the pectin and pulp of the passion fruit from Caatinga as probiotic food carriers. Food Bioscience, [S.L.], v. 20, p. 56-61, dez. 2017. Elsevier BV. http://dx.doi.org/10.1016/j.fbio.2017.08.005.