Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00058

Effect of commercially available probiotics on quality parameters of plain yogurts

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Abstract

This study evaluated the effect of two commercially available probiotics (*Bifidobacterium animalis* BB-12®, Chr. Hansen, and *Lacticaseibacillus rhamnosus* + *Lacticaseibacillus paracasei* culture FreshQ®9, Chr. Hansen) on quality parameters of plain yogurts and the development of *Aspergillus* sp. and *Escherichia coli* inoculated in the products. Sensory evaluations were performed on the second day after the manufacture of yogurts, while microbiological analysis and pH measurements were carried out at 5-day intervals during 30 days of storage at 4°C. Yogurt containing FreshQ®9 had lower pH values and reduced fermentation time than control or BB-12® yogurts, also presenting higher sensory grades. *Aspergillus* sp. and *E. coli* counts decreased in all types of yogurts, although the probiotic cultures did not affect these microbial adulterants. Data indicate that *L. rhamnosus* in combination with *L. paracasei* improved the overall quality of yogurt. Further studies are needed to explore the potential bioprotective effect of the evaluated probiotics against other spoilage microorganisms in yogurt.

Keywords: probiotics; *Lacticaseibacillus rhamnosus*; *Lacticaseibacillus paracasei*; *Bifidobacterium animalis*; spoilage; yogurt.

Practical Application: Commercially available probiotic containing *Lacticaseibacillus rhamnosus* + *Lacticaseibacillus paracasei* has the potential to improve some quality parameters of yogurts, despite the absence of a bioprotective effect against microbial adulterants in the product.

1 INTRODUCTION

Yogurt and other fermented milks are important milk products largely consumed worldwide. In terms of nutrient composition, these products are considered complete foods that provide essential nutrients containing carbohydrates, lipids, proteins, vitamins, and minerals (Souza et al., 2021). Milk fermentation is based on the addition of bacterial strains that mainly decrease the pH, with or without coagulation of casein fractions through isoelectric precipitation (Aktaş et al., 2022). In the manufacture of yogurts, the fermentation process is accomplished by the symbiotic microorganisms *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which remain viable, active, and abundant in the product during shelf-life (Aktaş et al., 2022). Despite the acidic nature of yogurt, some microorganisms including fungi species and pathogens can grow in the product and negatively affect its quality and nutritional characteristics (Kumar Puniya, 2015; Marth & Steele, 2001).

Probiotics are defined as live microorganisms that, when consumed in adequate amounts, lead to health benefits for the consumer (Hill et al., 2014). Several fermented food products and beverages contain probiotic microbes as starter cultures. The consumption of yogurt with probiotics can promote a healthier cardiovascular system, maintain body weight, and strengthen the immune system (Roobab et al., 2020). In addition to health-promoting properties, depending on the organic substrates, some probiotics are capable of producing many small organic molecules or metabolites, including flavoring and aromatic organic acids, esters, and carbonyl compounds, as well as further bioactive chemical contents in fermented products (Zendeboodi et al., 2020).

Among the most relevant probiotics, *Lacticaseibacillus rhamnosus*, *L. paracasei,* and *Bifidobacterium animalis* have been widely used in the food industry to manufacture dairy products and other fermented foods due to their probiotic and antimicrobial activities (Oliveira et al., 2020). Both species exhibit several desirable features generally required for a good probiotic strain, including the ability to survive in the human gastrointestinal tract after ingestion, especially colonization of the ileum and colon (Zendeboodi et al., 2020). The ingestion of *L. rhamnosus* is also associated with positive effects on human health, mainly the prevention and treatment of acute diarrhea in children, allergies, and the colonization of harmful organisms, among others (Sun et al., 2019). Moreover, *L. rhamnosus* strains contribute to the stability and increased shelf-life of food products by acting as bio-preservative agents (Singh, 2018), i.e., by inhibiting the *in vitro* growth of various foodborne pathogens, such as *Escherichia coli* and *Salmonella typhimurium* (Calderón et al., 2007), *Pseudomonas* sp. (Alcântara et al., 2019), *Staphylococcus aureus,* and *Listeria monocytogenes* (Prezzi et al., 2020). In addition to bio-preservation, it has been speculated that food-protecting microbial cultures may also improve growth

Received 12 June, 2023.
Accepted 11 Nov., 2023.
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Conflict of interest: nothing to declare.
Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant #2022/03952-1).

and fermentation, thus enhancing the stability and quality of yogurts (Olivares-Tenorio & Klotz-Ceberio, 2020). Therefore, the aim of this study was to evaluate the bioprotective effect of two commercially available probiotics, one containing *L. rhamnosus* and *L. paracasei*, and another prepared with *B. animalis*, on the pH, sensory attributes, and the development of *Aspergillus* sp. and *E. coli* as microbial contaminants in plain yogurts during 30 days of storage.

2 MATERIALS AND METHODS

2.1 Bacterial strains

The commercially available probiotics used in the study were *B. animalis* BB-12® culture and FreshQ®9 culture, which contained *L. rhamnosus* + *L. paracasei*, both manufactured by Chr. Hansen (Hørsholm, Denmark). *Aspergillus* sp. ANYP01, previously isolated from a native strain of yerba mate by the Cemit Research Laboratories of the National University of Asunción, Paraguay, and *E. coli* ATCC® 2592 were reconstituted in standard plate count agar (PCA). Then, one colony was taken with a sterile inoculation loop and transferred to test tubes containing 10 mL of peptone broth. The suspensions of *Aspergillus* sp. and *E. coli* ATCC® 2592 were vortexed and incubated at 25°C for 3 days and at 36°C for 24 h, respectively. After incubation, the concentrations of *Aspergillus* sp. and *E. coli* ATCC® 2592 in the inoculum suspensions were determined by using Petrifilm™ Yest and Mold Count plates (3M, Saint Paul, MN) and Petrifilm™ REC (3M, Saint Paul, MN), respectively. The suspensions were diluted with peptone broth until reaching approximately 4.0×10^3 cells/mL of *Aspergillus* sp. and 1.0×10^2 cells/mL of *E. coli* ATCC® 2592.

2.2 Manufacture of yogurts

The plain solid yogurts (16 L) used in the experiment were produced in the laboratories of the Faculty of Chemical Sciences of the National University of Asunción, based on the procedures described by Fernandes et al. (2007). Commercially available sterilized (ultra-high temperature) whole milks containing 12.3% of total solids were placed in a fermentation vat and heated to 42°C. Subsequently, the milk was inoculated (2% inoculum) with a starter culture (SC) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Yo-Flex®, YF-L904, Chr. Hansen, Hørsholm, Denmark) to reach a concentration of 1.0×10^9 colony-forming units (CFU)/mL of the SC in the final yogurt. Then, the mixture was split into three equal parts of 4 L each, one without any probiotic addition (control), one further inoculated with *B. animalis* (BA) BB-12® culture (Chr. Hansen, Hørsholm, Denmark), and one inoculated with FreshQ®9 culture (Chr. Hansen, Hørsholm, Denmark) containing *L. rhamnosus* + *L. paracasei* (LR+LP). After mixing, the inoculated milks were packaged in 100-mL polypropylene flasks and incubated at 42°C for nearly 6 h. The fermentation time was controlled during the production of the yogurts, with pH measures taken every hour until reaching a pH of 4.6–4.7. The concentration of probiotic bacteria in the final yogurts (BA and LR+LP) was 1.0×10^9 CFU/mL. The prepared yogurts were cooled immediately after incubation and stored at 4°C for 30 days.

2.3 Inoculation of microbial suspensions into yogurts

Duplicate sub-samples (1 L) of each experimental yogurt (control, LR+LP, and BA) were placed in a laminar hood and inoculated with 5 mL of *Aspergillus* sp. and 6 mL of *E. coli* ATCC® 2592 suspensions, prepared as described in Section 2.1, to obtain counts of approximately 2.0×10^4 and 6.0×10^2 CFU/mL for these microorganisms in the final yogurt samples, respectively. All inoculated sub-samples of yogurt were stored at 4°C for 30 days.

2.4 Sensory analysis of yogurts

Sensory analysis of the three types of experimental yogurts prepared without *Aspergillus* sp. or *E. coli* ATCC® 2592 inoculation was conducted after 2 days of storage at 4°C. For this purpose, 25 untrained tasters and regular consumers of yogurt were selected and invited to score the acceptance order regarding the consistency, acidity, taste, and final preference of the products, based on a scale of 1–3, where 1 = like less and 3 = like more, according to Norma Técnica Colombiana (ICOTEC, 1996) (NTC 3930).

2.5 Microbiological and pH analysis of inoculated yogurts

Microbiological and pH analyses were carried out in the sub-samples from the three types of experimental yogurts inoculated with *Aspergillus* sp. and *E. coli* ATCC® 2592 suspensions at days 1, 5, 10, 15, 20, 25, and 30 of storage. Yogurt samples were prepared using dilutions from 10^{-1} to 10^{-5}. For *Aspergillus* sp. counts, Petrifilm™ Yest and Mold Count plates (3M, Saint Paul, MN) were incubated at 20–25°C for 5 days (Association of Official Agricultural Chemists, 2000) (AOAC method 997.02). *E. coli* ATCC® 2592 counts were determined using Petrifilm™ REC (3M, Saint Paul, MN), which were incubated at 36°C for 24 h (Association of Official Agricultural Chemists, 1994) (AOAC method 991.14). Analysis of pH in yogurts was carried out using a pH meter (Oakton®, Vernon Hills, IL), according to the Association of Official Agricultural Chemists (1982) (AOAC method 981.12).

2.6 Statistical analysis

Sensory evaluation results were submitted to ANOVA, followed by the application of the Friedman test to check for differences among sensory attributes of the experimental yogurts. The CFU values were transformed into log10, and the data were subjected to the nonparametric Kruskal–Wallis test. Statistical analyses were conducted using the procedures established in the SPSS software version 20 (SPSS Inc, Chicago, IL, USA). All statements of significance were based on 5% probability.

3 RESULTS AND DISCUSSION

This study significantly contributes to the probiotic field by investigating the impact of commercially available probiotics on the quality parameters of plain yogurts and the development of spoilage agents within the products, aiming at developing healthier and safer food options. In this study, for three types of yogurts, the mean count of SC was approximately 2.0×10^7 CFU/mL. The fermentation times of experimental yogurts

as a function of pH reduction are represented in Figure 1. Interestingly, the LR+LP probiotic yogurts containing *L. rhamnosus* + *L. paracasei* reached the isoelectric point of caseins approximately 1 h earlier than the other two yogurts (control and BA), thus providing a faster and more pronounced acidification in LR+LP yogurt. This finding supports the potential application of the studied probiotics at the industrial scale of yogurt productions, to reduce processing costs and, consequently, provide economic benefits in the dairy industry.

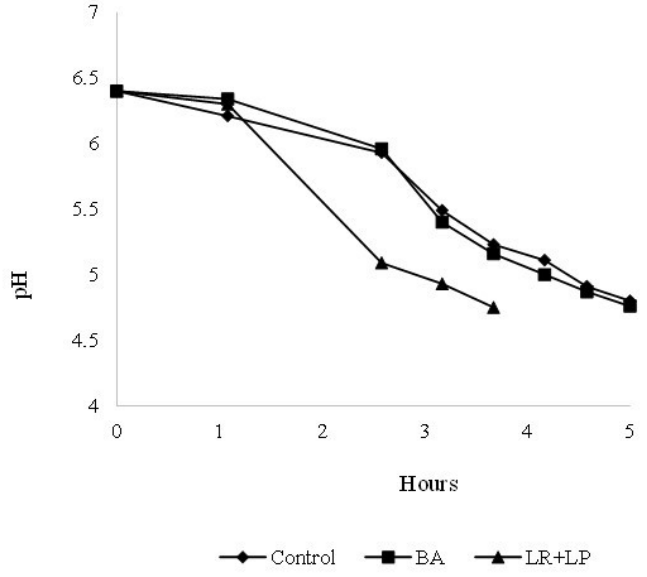

Figure 1. Fermentation time (mean of duplicate samples) of control and probiotic yogurts, according to their pH changes. BA: yogurt inoculated with *B. animalis* BB-12® culture (Chr. Hansen, Hørsholm, Denmark); LR+LP: yogurt inoculated with FreshQ®9 culture (Chr. Hansen, Hørsholm, Denmark) containing *Lacticaseibacillus rhamnosus* + *L. paracasei*.

The pH values of the three types of yogurts during 30-day storage are presented in Figure 2. The values were similar ($p > 0.05$) and remained unchanged within the operational pH ranges of yogurts (Fernandes et al., 2007). It should be noted that pH is not an inhibitory factor for the growth of molds such as *Aspergillus* sp. and *E. coli*, as both microorganisms are viable in a wide pH range.

The sensory grades for consistency, acidity, taste, and final preference of yogurts manufactured with or without probiotic addition are presented in Figure 3. Except for acidity, all the evaluated attributes differed ($p < 0.05$) among the three types of yogurts. Compared with the control and BA yogurts, the product prepared with the addition of LR+LP probiotic culture had higher grades ($p < 0.05$) for consistency, taste, and final preference, thus indicating its potential application to improve the general acceptance of yogurts containing probiotics.

Ramos et al. (2019) carried out a sensorial analysis of a probiotic yogurt by applying consumer tests. According to the results, it was possible to elaborate probiotic yogurts with pleasant sensory qualities using *Lactiplantibacillus plantarum* strains, where the products achieved good consistency, a moderate level of acidity, and a low cheese flavor. A study carried out by Kamal et al. (2018) showed that the sensory acceptability of probiotic yogurt with *L. rhamnosus* was comparable to that of normal yogurt. So, applying *L. rhamnosus* as a bio-preservative in yogurts constitutes technological and economic advantages.

Figure 4 shows the counts of *Aspergillus* sp. (Figure 4A) and *E. coli* (Figure 4B) in the experimental yogurts (control, BA,

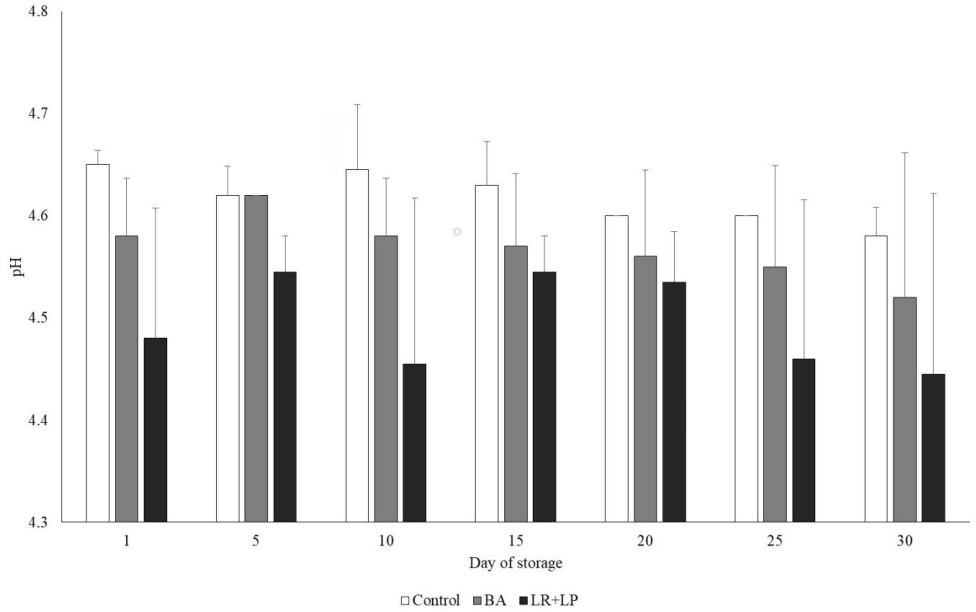

Figure 2. pH values during 30-day storage of yogurts manufactured without any probiotic addition (control: □), inoculated with *B. animalis* (BA: ▒) BB-12® culture (Chr. Hansen, Hørsholm, Denmark), and inoculated with FreshQ®9 culture (Chr. Hansen, Hørsholm, Denmark) containing *Lacticaseibacillus rhamnosus* + *L. paracasei* (LR+LP: ■). Results are expressed as mean ± SD (in bars) of duplicate samples. No significant differences were found at $p < 0.05$.

and LR+LP) during 30 days of storage at 4°C. In all yogurts, the microbial load decreased during storage, especially from days 10 to 15. Although *Aspergillus* sp. counts (Figure 4A) in the BA yogurts (inoculated with *B. animalis* BB-12® culture) on days 10, 15, and 20 were lower than the control or LR+LP products, no differences ($p > 0.05$) were found between the mean values of the yogurts along the storage period.

E. coli counts (Figure 4B) decreased to zero in the probiotic yogurts (inoculated with *B. animalis* BB-12® and FreshQ®9 culture containing *L. rhamnosus* + *L. paracasei*) on day 10 of storage, while non-detection of this contaminant in control yogurts was achieved only on day 25. However, there was no difference ($p > 0.05$) between the *E. coli* counts among the three types of yogurts within the overall 30 days of storage.

Although the acidic pH of yogurt is considered a natural barrier to the growth of several microorganisms, both *Aspergillus* sp. and *E. coli* as inoculated in the experimental yogurts can develop in the products during storage and affect their safety and shelf-life. Probiotic cultures are currently of great relevance worldwide, not only for the benefits to human health but also for the effect of competition against pathogenic microorganisms. Regarding this last point, the production of organic acids, carbanilic compounds, and bacteriocins could explain the mechanism by which probiotics exert their antagonistic effect on other bacteria (Calderón et al., 2007).

Similarly, Fernández et al. (2017) studied the efficacy of using microorganisms as an inhibitor for mold proliferation in cheese. In this regard, seven strains of bioprotective cultures, alone or in pairs, were chosen and tested for their abilities to prevent the growth of *Penicillium chrysogenum* in solidified milk matrix and cheese. *L. rhamnosus* A238 alone or in combination with *B. animalis* subsp. *lactis* A026 inhibited mold growth for at least 21 days at 6°C, probably due to the production of secondary metabolites and/or nutrient competition. Thus, the evaluated bacteria have the potential to be used as bio-preservatives in fresh milk products, including cheese.

The efficacy of three commercial protective cultures called PC1 (*Lactobacillus* spp.), PC2 (*L. rhamnosus*), and PC3 (*L. rhamnosus*) as bio-preservatives in fresh cheese was tested against nine yeast and 11 mold strains that affect the quality of cheese (Makki et al., 2020). The mold strains were inoculated on a cheese surface, with approximately 20 CFU/g, and stored at 6 ± 2°C for 21 days. Significant inhibition was detected for individual yeast strains, and mold growth was observed every week for up to 70 days post-inoculation. The results showed that

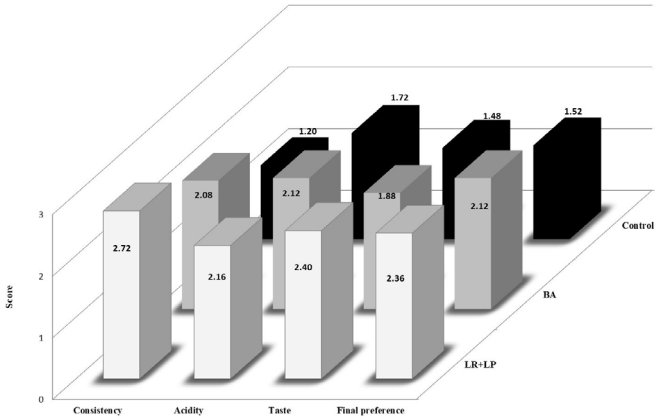

Figure 3. Sensory grades for attributes of yogurts manufactured without any probiotic addition (control: □), inoculated with *B. animalis* (BA: ▒) BB-12® culture (Chr. Hansen, Hørsholm, Denmark), and inoculated with FreshQ®9 culture (Chr. Hansen, Hørsholm, Denmark) containing *Lacticaseibacillus rhamnosus* + *L. paracasei* (LR+LP: ■).

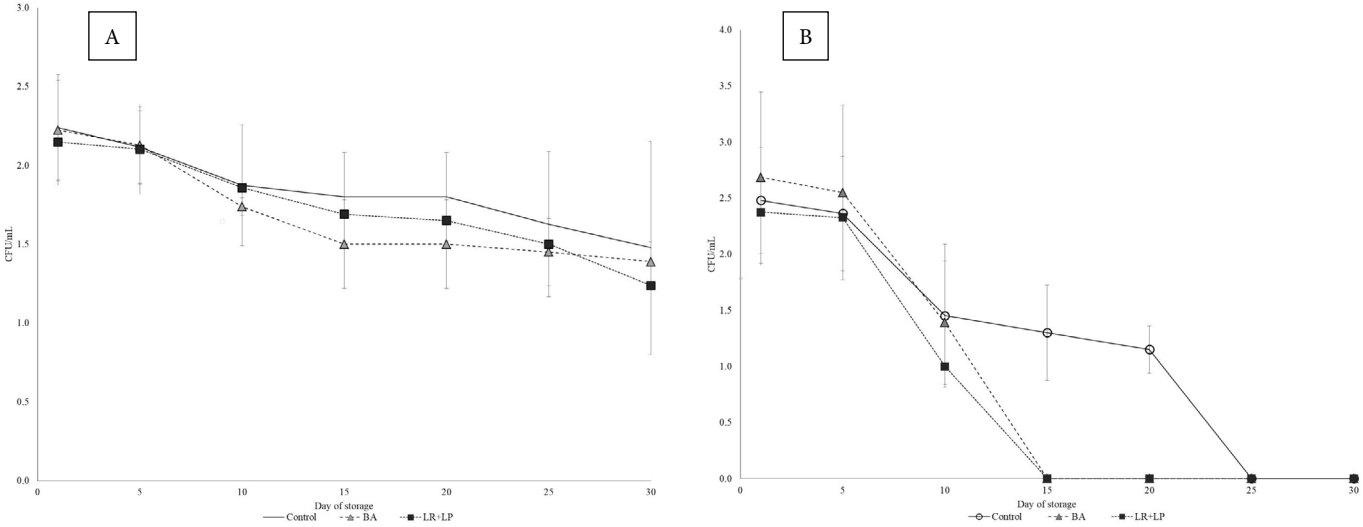

Figure 4. (A) *Aspergillus* sp. and (B) *Escherichia coli* counts during 30-day storage of yogurts manufactured without any probiotic addition (control), inoculated with *B. animalis* (BA) BB-12® culture (Chr. Hansen, Hørsholm, Denmark), and inoculated with FreshQ®9 culture (Chr. Hansen, Hørsholm, Denmark) containing *Lacticaseibacillus rhamnosus* + *L. paracasei* (LR+LP). Results are expressed as mean ± standard deviation (in bars) of Log10 of CFU per mL of duplicate samples. On each day of storage, no differences were found between the mean values of yogurts at the 0.05 level of probability.

commercial probiotic cultures vary in efficacy against yeast and mold strains in cheeses. The cultures analyzed varied in genus, species, and inoculum level. Therefore, each bioprotective culture can be ideal against a specific strain, concerning the food matrix and other factors, affecting fungal growth. Although commercial probiotic cultures are widely marketed due to their natural antimicrobial action, the specific antifungal efficacy is highly variable in different types of foods.

Martínez and Trujillo (2017) studied the bioconservation of fresh cheeses, using the probiotic *L. rhamnosus* HOWARU™ in contradiction to *Listeria monocytogenes* ATCC 19118. It was observed that the probiotic allowed the organoleptic characteristics to be maintained constantly while decreasing the growth of the pathogenic bacteria by improving the conservation of the tested samples. The concentration of 1.1×10^6 CFU/mL for *L. rhamnosus* HOWARU™ was perceived as efficient and inhibited *L. monocytogenes* ATCC 19118 at a concentration of 7.6×10^3 CFU/mL. Consequently, it was revealed that the bio-preservative kept the cheeses fresh for up to 8 days, maintaining the pH within the normal range (pH 5.6–6.4).

L. rhamnosus significantly inhibited *E. coli* O157:H7, *S. aureus*, *Yersinia enterocolitica*, and *Salmonella enterica* in different types of yogurts (Kamal et al., 2018). This antimicrobial effect was associated with inhibitory compounds rather than acids resulting from fermentation. In brief, the addition of *L. rhamnosus* to yogurt appears to be a beneficial and applicable biocontrol strategy. This supports the antimicrobial properties of *L. rhamnosus* and its possible application in the bio-preservation of different fermented foods (Kamal et al., 2018). Another study analyzing Minas Frescal cheese and the bioprotective effect of *L. rhamnosus* on the growth of *S. aureus* and *L. monocytogenes* showed an inhibitory effect only for *L. monocytogenes* (1.1–1.6 Log CFU/g decrease) (Prezzi et al., 2020).

In the case of yogurts, it is known that the lactic bacteria responsible for the acid fermentation of milk are good competitors with the contaminating or spoiling microorganisms. Subsequently, the use of the probiotic culture *L. rhamnosus* in yogurts has a bactericidal effect similar to other probiotics and lactic bacteria used in yogurt and fermented milk. So, it does not exhibit a significant additional bactericidal effect.

4 CONCLUSION

In the three types of processed yogurts, the fermentation time was compared, where the pH curves showed that the probiotic yogurts with *L. rhamnosus* reached the isoelectric point of caseins in less time than the other two yogurts, which could be a favorable aspect in the production and costs on an industrial scale. The microbiological results revealed a decrease in the molds and *E. coli* in the yogurts stored at a temperature below 4°C for 30 days. Comparatively, no significant differences were examined, demonstrating that the probiotic culture of *L. rhamnosus* did not provide any additional bioprotective effect against the count of molds and *E. coli*. Sensory results indicated that the yogurt with the probiotic culture of *L. rhamnosus* was preferred by the tasters compared to the other yogurts. The results of this study suggest that more research is needed on the use of *L. rhamnosus* in yogurt and fermented milk to confirm if it exerts an important bio-preservative effect.

REFERENCES

Aktaş, H., Yıldız, S., & Çetin, B. (2022). Probiotic and technological properties of isolates from homemade and industrial yoghurts. *Journal of Food Processing and Preservation*, e17135. https://doi.org/10.1111/jfpp.17135

Alcântara, A. L. D., Bruzaroski, S. R., Luiz, L. L., de Souza, C. H. B., Poli-Frederico, R. C., Fagnani, R., & de Santana, E. H. W. (2019). Antimicrobial activity of *Lactobacillus rhamnosus* against *Pseudomonas fluorescens* and *Pseudomonas putida* from raw milk. *Journal of Food Processing and Preservation*, *43*(9), 1-6. https://doi.org/10.1111/jfpp.14082

Association of Official Agricultural Chemists (AOAC) (1982). *pH of Acidified Foods (Official Method 981.12)*. AOAC. Retrieved from http://www.aoacofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=1159

Association of Official Agricultural Chemists (AOAC) (1994). *Coliforms and* Escherichia coli *Counts in Foods (Official Method 991.14)*. AOAC. Retrieved from http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=8

Association of Official Agricultural Chemists (AOAC) (2000). *Yeast and Mold Counts in Foods (Official Method 997.02)*. AOAC. Retrieved from https://docslib.org/doc/10931992/aoac-997-02-yeast-and-mold-count-in-foods

Calderón, O., Padilla, C., Chaves, C., Villalobos, L., & Arias, M. L. (2007). Evaluación del efecto del cultivo probiótico Lactobacillus rhamnosus adicionado a yogurt natural y con probióticos comerciales sobre poblaciones de *Staphylococcus aureus, Escherichia coli O157:H7*, *Listeria monocytogenes* y *Salmonella enteritidis*. *Archivos Latinoamericanos de Nutrición*, *57*(1), 51-56. Retrieved from https://www.researchgate.net/publication/239531005

Fernandes, A. M., Oliveira, C. A. F., & Lima, C. G. (2007). Effects of somatic cell counts in milk on physical and chemical characteristics of yoghurt. *International Dairy Journal*, *17*(2), 111-115. https://doi.org/10.1016/j.idairyj.2006.02.005

Fernández, B., Vimont, A., Desfossés-Foucault, É., Daga, M., Arora, G., & Fliss, I. (2017). Antifungal activity of lactic and propionic acid bacteria and their potential as protective culture in cottage cheese. *Food Control*, *78*, 350-356. https://doi.org/10.1016/j.foodcont.2017.03.007

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*, 506-514. https://doi.org/10.1038/nrgastro.2014.66

Instituto Colombiano de Normas Técnicas y Certificación (ICOTEC) (1996). *NTC 3930: Análisis Sensorial. Metodología. Ordenación de acuerdo con un criterio específico (Ranking)*. ICOTEC. Retrieved from https://tienda.icontec.org/gp-analisis-sensorial-metodologia-ordenamiento-de-acuerdo-con-un-criterio-especifico-ranking-ntc3930-2015.html

Kamal, R. M., Alnakip, M. E., Abd El Aal, S. F., & Bayoumi, M. A. (2018). Biocontrolling capability of probiotic strain *Lactobacillus rhamnosus* against some common foodborne pathogens in yoghurt. *International Dairy Journal*, *85*, 1-7. https://doi.org/10.1016/j.idairyj.2018.04.007

Kumar Puniya, A. (Ed.). (2015). *Fermented Milk and Dairy Products*. CRC Press. https://doi.org/10.1201/b18987

Makki, G. M., Kozak, S. M., Jencarelli, K. G., & Alcaine, S. D. (2020). Evaluation of the efficacy of commercial protective cultures against mold and yeast in queso fresco. *Journal of Dairy Science*, *103*(11), 9946-9957. https://doi.org/10.3168/jds.2020-18769

Marth, E. H., & Steele, J. L. (Eds.). (2001). *Applied Dairy Microbiology* (2nd Ed.). https://doi.org/10.1201/9781482294606

Martínez, J. C., & Trujillo, M. F. (2017). *Determinación de la bioconservación de quesos frescos de Metapan, utilizando una cepa probiótica* Lactobacillus rhamnosus *Howaru™ frente a* Listeria monocytogenes *ATCC 19118* [Tesis de grado]. Universidad de El Salvador. Retrieved from http://ri.ues.edu.sv/id/eprint/15057/1/1610'3714.pdf

Olivares-Tenorio, M. L., & Klotz-Ceberio, B. (2020). Evaluación del efecto antifúngico de metabolitos de cultivos bioprotectores: aplicación en derivados lácteos. *Biotecnología en el Sector Agropecuario y Agroindustrial*, *18*(2), 15-25. https://doi.org/10.18684/BSAA(18)15-25

Oliveira, C. A. F., Muaz, K., Møller, C. A. O., Corassin, C. H., & Rattray, F. P. (2020). Probiotics and mycotoxins. In: Cruz, A. G., Ranadheera, C. S., Nazzaro, F., & Mortazavian, A. *Probiotics and Prebiotics in Foods: Challenges, Innovations, and Advances* (p. 309-328). Academic Press. https://doi.org/10.1016/B978-0-12-819662-5.00005-7

Prezzi, L. E., Lee, S. H. I., Nunes, V. M. R., Corassin, C. H., Pimentel, T. C., Rocha, R. S., Ramos, G. L. P. A., Guimarães, J. T., Balthazar, C. F., Duarte, M. C. K. H., Freitas, M. Q., Esmerino, E. A., Silva, M. C., Cruz, A. G., & Oliveira, C. A. F. (2020). Effect of *Lactobacillus rhamnosus* on growth of *Listeria monocytogenes* and *Staphylococcus aureus* in a probiotic Minas Frescal cheese. *Food Microbiology*, *92*, 103557. https://doi.org/10.1016/j.fm.2020.103557

Ramos, J., Barazarte, H., & Pernalete, M. (2019). Evaluación sensorial de un yogurt probiótico aplicando pruebas de consumidores. *Revista Científica A.S.A.*, 48-67. Retrieved from http://bdigital2.ula.ve:8080/xmlui/bitstream/handle/654321/4776/artículo2.pdf?sequence=1&isAllowed=y

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., & Aadil, R. M. (2020). Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science*, *32*, 17-28. https://doi.org/10.1016/j.cofs.2020.01.003

Singh, V. P. (2018). Recent approaches in food bio-preservation-a review. *Open Veterinary Journal*, *8*(1), 104-111. https://doi.org/10.4314/ovj.v8i1.16

Souza, C., Mousavi-Khaneghah, A., & Oliveira, C. A. F. (2021). The occurrence of aflatoxin M_1 in industrial and traditional fermented milk: A systematic review study. *Italian Journal of Food Science*, *33*(1), 53-64. https://doi.org/10.15586/ijfs.v33iSP1.1982

Sun, J., Chen, H., Qiao, Y., Liu, G., Leng, C., Zhang, Y., Lv, X., & Feng, Z. (2019). The nutrient requirements of *Lactobacillus rhamnosus GG* and their application to fermented milk. *Journal of Dairy Science*, *102*(7), 5971-5978. https://doi.org/10.3168/jds.2018-15834

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, *32*, 103-123. https://doi.org/10.1016/j.cofs.2020.03.009