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EVALUATION OF *Limosilactobacillus fermentum* ATCC 23271 SURVIVAL IN OSMOTICALLY DEHYDRATED SAPODILLA (*Achras zapota* L.) SLICES

AVALIAÇÃO DA SOBREVIVÊNCIA DE Limosilactobacillus fermentum ATCC 23271 EM FATIAS DE SAPOTI (Achras zapota L.) DESIDRATADAS OSMOTICAMENTE

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ABSTRACT

Sapodilla (*Achras zapota* L.) is a very perishable fruit rich in carbohydrates, vitamins, and minerals. The process of osmotic dehydration (OD) was applied to reduce the initial moisture of sapodilla and increase its shelf life. Dehydrated fruits have been pointed as interesting matrices to incorporate probiotics. The present study aims to evaluate the survival of *Limosilactobacillus fermentum* ATCC 23271, a strain with probiotic potential, in osmotically dehydrated sapodilla (*Achras zapota* L.) during storage and under gastrointestinal stress. The incorporation of *L. fermentum* ATCC 23271 was carried out during the process of osmotic dehydration (OD) of sapodilla slices under predetermined temperature, time, and sucrose solution concentration (40 °C, 165 min, 50 °Brix). After the DO, the survival of the microorganism during storage (0, 7, 14 and 28 days) at 4 °C was analyzed and a simulation of rapid digestion in the gastrointestinal tract (2 h) of the stored samples was carried out. *L. fermentum* ATCC 23271 resisted the DO process with a population of 10^9 CFU/g even after 14 days of storage. In the following weeks, the population reached 10^8 CFU/g ($p<0.01$). Furthermore, *L. fermentum* ATCC 23271 survived the simulated passage through the gastrointestinal tract, with values greater than 10^8 CFU/g for samples stored for up to 21 days. Taken together, the results demonstrate that *L. fermentum* ATCC 23271 is a strain capable of resisting the DO process, making osmotically dehydrated sapodilla slices a potential probiotic product.

Keywords: probiotic; storage; gastrointestinal tract.

RESUMO

O sapoti (*Achras zapota* L.) é uma fruta muito perecível, rica em carboidratos, vitaminas e minerais. O processo de desidratação osmótica (DO) foi aplicado para reduzir a umidade inicial do sapoti e aumentar sua vida útil. Frutas desidratadas têm sido apontadas como matrizes interessantes para incorporação de probióticos. O presente estudo tem como objetivo avaliar a sobrevivência de *Limosilactobacillus fermentum* ATCC 23271, cepa com potencial probiótico, em sapoti (*Achras zapota* L.) osmoticamente desidratado durante armazenamento e sob estresse gastrointestinal. A incorporação de *L. fermentum* ATCC 23271 foi realizada durante o processo de desidratação osmótica (DO) de fatias de sapoti sob temperatura, tempo e concentração de solução de sacarose pré-determinados (40 °C, 165 min, 50 °Brix). Após a DO, foi analisada a sobrevivência do microrganismo durante o armazenamento (0, 7, 14 e 28 dias) a 4 °C e realizada uma simulação de digestão rápida no trato gastrointestinal (2 h) das amostras armazenadas. *L. fermentum* ATCC 23271 resistiu ao processo de DO com população de 10^9 UFC/g mesmo após 14 dias de armazenamento. Nas semanas seguintes, a população atingiu 10^8 UFC/g ($p<0,01$). Além disso, *L. fermentum* ATCC 23271 sobreviveu à passagem simulada pelo trato gastrointestinal, com valores superiores a 10^8 UFC/g para amostras armazenadas por até 21 dias. Em conjunto, os resultados demonstram que *L. fermentum* ATCC 23271 é uma cepa capaz de resistir ao processo de DO, tornando as fatias de sapoti osmoticamente desidratadas um potencial produto probiótico.

Palavras-chave: probiótico; armazenar; trato gastrointestinal.

INTRODUCTION

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host and have been associated with improved digestive health and a strengthened immune system (BODKE; JOGDAND, 2022; PRAMANIK; VENKATRAMAN; KARTHIK; VAIDYANATHAN, 2023; VERA-SANTANDER; HERNÁNDEZ-FIGUEROA; JIMÉNEZ-MUNGUÍA; MANI-LÓPEZ; LÓPEZ-MALO, 2023). They are widely found as dietary supplements and functional foods, especially in dairy food products (GAO *et al.*, 2021; GREEN; ARORA; PRAKASH, 2020; WANG; WEN; TANG; QU; RAO, 2023).

Non-dairy probiotic food products represent an emerging group of functional foods due to the growing number of individuals with dairy food restrictions (due to lactose intolerance or milk protein allergy), vegans, and vegetarians (ASPRI; PAPADEMAS; TSALTAS, 2020; KUMAR, D. *et al.*, 2022; KUMAR, S. *et al.*, 2022; MIN; BUNT; MASON; HUSSAIN, 2019). Fruits are reservoirs of many nutritionally important compounds, such as vitamins, minerals, dietary fiber, and sugars (mainly fructose, glucose, and sucrose), in addition to their antioxidant capacity and other functional properties. Taken together, these characteristics make fruits suitable matrices for incorporating and stabilizing the growth of probiotics (BUSTOS; FONT; TARANTO, 2023; GUIMARÃES *et al.*, 2020).

Nevertheless, several challenges are found in the development of non-dairy probiotics products, including low pH or high-water activity (KUMAR, S. *et al.*, 2022; MIN; BUNT; MASON; HUSSAIN, 2019). In this context, osmotic dehydration (OD) has been pointed as an interesting alternative to reduce the initial moisture of the fruit, allowing the efficient incorporation of probiotics into the fruit matrix (BUSTOS; FONT; TARANTO, 2023; RASCÓN *et al.*, 2018; VIJAY; VIKRAMAN; MARY; CHAUHAN; KAPOOR, 2021). OD is a process of immersing food in a concentrated solution (e.g., sugar, salt, or an active physiological component) for a specified time and temperature, allowing spontaneous migration of water from the food tissues into the osmotic solution while the solids migrate from the solution towards the surface and interior of the biological material (KAUR; SINGH; ZALPOURI; SINGH, 2022; PANDISELVAM *et al.*, 2022; RASCÓN *et al.*, 2018).

The OD process using sucrose was effectively applied in Sapodilla (*Achras zapota* L.), a very perishable fruit that is rich in carbohydrates, vitamins, and minerals (COIMBRA *et al.*, 2017, 2022). The OD process was optimized for Sapodilla evaluating the effects of different temperature values (30–50 °C), sucrose concentration (40–60% °Brix) and immersion time (90–240 min). The sample with highest scores of acceptability and purchase intention (sample 9) was obtained using sucrose solution at 50 °Brix, under an operation temperature of 40 °C and immersion for 165 min (COIMBRA *et al.*, 2022).

The development of sapodilla as a probiotic carrier by osmotic dehydration using sucrose, along with studies on the stability of the infused probiotic under gastrointestinal stress and during storage have yet to be explored. The present study aimed to evaluate the incorporation of *Limosilactobacillus fermentum* ATCC 23271 in osmotically dehydrated sapodilla and its viability during storage and under simulation of digestion in the gastrointestinal tract.

MATERIAL AND METHODS

Material

Sapodilla fruits were randomly purchased from a local supermarket in São Luís, MA, Brazil. The fruits were visually selected by color (completely brown), size (average diameter of 4.6 cm), ovoid shape and absence of physical damage. Commercial sucrose was used as an osmotic agent. The culture of *L. fermentum* ATCC 23271 is kept under refrigeration (-80°C) and was reactivated in Man, Rogosa and Sharpe Agar (MRS) (Bio Labor) at 37 °C for 24 h.

Sapodilla sample preparation

The general aspects of the raw material were visually inspected, and the samples were weighed and selected according to the degree of maturation (12 to 16°Brix). Then, the material was washed in running water and sanitized with sodium hypochlorite solution (2.5% w/v) for 15 min. Subsequently, the fruits were manually peeled with a stainless steel knife. The fruits were cut into longitudinal slices (5 × 1 × 1 cm) and the seeds were removed. The samples were subjected to a bleaching process using a steam flow (100°C for 5 min on each side) and immediately cooled in ice water for 5 min. After blanching, the slices were drained on paper towels.

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Osmotic dehydration with L. fermentum ATCC 23271

The impregnation of the probiotic occurred during the OD (EMSER; BARBOSA; TEIXEIRA; BERNARDO DE MORAIS, 2017). *L. fermentum* ATCC 23271 was cultivated in MRS broth at 37 °C. After 24h, samples (0.1 mL) were transferred to MRS broth (1:100) and incubated at 37 °C for 24h to reach the stationary phase. The probiotic culture was then centrifuged, the supernatant was discarded, and the cells were washed twice in sterile PBS (phosphate buffered saline). Cells were resuspended in 10 mL of sterile osmotic solution (50 °Brix) to obtain an optical density of 1.0 at 600 nm. Sapodilla slices were immersed in a sucrose solution (sample/solution ratio of 1:20) containing *L. fermentum* ATCC 23271 at 110 rpm in a shaker (Marconi, MA-410) at 40 °C during 165 min (COIMBRA *et al.*, 2022). After, a sapodilla slice (about 1 g) was washed in sterile deionized water, gently dried and added to PBS (9 mL). These sample was serially diluted (1:10), plated on MRS agar and cultivated at 37°C for 24 h. The results were expressed as colony-forming units per gram (CFU/g).

Viability under storage

Sapodilla samples (6 g) were stored in individual Petri dishes sealed with sealed with parafilm at 4°C for 28 days. The bacterial load was determined after 0, 7, 14 and 28 days, as described in 2.3.

Survival under gastrointestinal tract conditions

Each stored sample was submitted to simulated gastric and enteric conditions. For this, a sapodilla slice (approximately 1 g) from each sample was triturated in a stomacher for 120 seconds. In the simulated stomach assay, the samples were added to 49 mL of buffered peptone water (Merck) adjusted to pH 3.0 with hydrochloric acid (1 M HCl, Merck). In the simulated enteric assay, a sterile solution of bile salts was added (final concentration of 0.3% (w/v), Pronadisa), after increasing the pH from 3.0 to 7.0 with a sterile solution of sodium hydroxide (1 M NaOH, Pronalab) (BARBOSA; BORGES; TEIXEIRA, 2015). In both assays, samples were taken at time 0 (inoculation time) and after 30 min, 60 min and 120 min. Bacterial viability was performed as described above.

Statistical analysis

The data obtained were analyzed using GraphPad Prism software version 8.0. A p-value < 0.05 was considered statistically significant.

RESULTS

Viability of L. fermentum ATCC 23271 in osmotically dehydrated sapodilla

The first step of this work was to evaluate the viability of *L. fermentum* ATCC 23271 incorporated into sapodilla during the osmotic dehydration process. *L. fermentum* ATCC 23271 resisted the OD process presenting $4.17 \pm 0.29 \times 10^9$ CFU/g. This population remained stable in the first week ($3.97 \pm 0.55 \times 10^9$ CFU/g; $p>0.05$) with a slightly decreased in the second week ($1.85 \pm 0.44 \times 10^9$ CFU/g; $p<0.05$) (Figure 1). In the following weeks, the population reached 10^8 CFU/g ($p<0.01$).

Figure 1. Survival of *L. fermentum* ATCC 23271 in dehydrated sapodilla slices during storage at 4 °C. Statistical differences in relation to the first day of storage: *p<0.05; *p<0.01.

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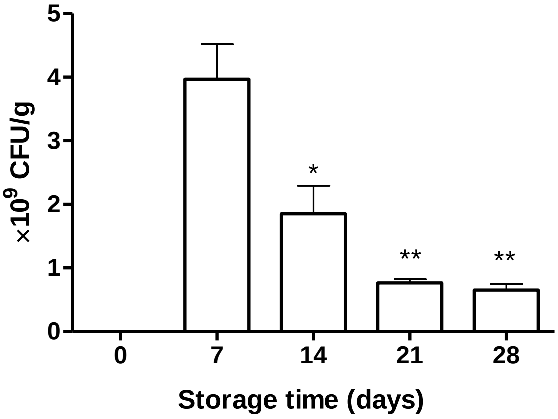

Viability of L. fermentum ATCC 23271 in osmotically dehydrated sapodilla under simulated gastrointestinal conditions

Following, the viability of *L. fermentum* ATCC 23271 in stored samples was evaluated after simulated gastrointestinal conditions (Figures 2 and 3). In the sample stored for 7 days, *L. fermentum* ATCC 23271 remained stable for up to 120 min in stomach conditions with 10^9 CFU/g (Figure 2A), while in the intestinal conditions there was a significant reduction ($p<0.05$) in the number of colonies, however the population was kept at 10^9 CFU/g up to 60 minutes (Figure 3A). For the sample stored for 14 days, no statistical differences were observed regarding the incubation times in both conditions ($p>0.05$) (Figures 2B and 3B). Regarding storage for 21 days (Figures 2C and 3C), significant reductions were detected after 60 minutes and 120 minutes in stomach conditions, and 120 minutes in intestinal conditions. However, the count in all periods was 10^8 CFU/g. Finally, in the sample stored for 28 days, no growth was detected after 120 minutes in stomach or intestinal conditions (Figures 2D and 3D).

Figure 2. Survival of *L. fermentum* ATCC 23271 in osmotically dehydrated sapodilla slices after simulated passage in the stomach. (A) Evaluation of the sample stored for 7 days; (B) Evaluation of the sample stored for 14 days; (C) Evaluation of the sample stored for 21 days; (D) Evaluation of the sample stored for 28 days. ***p<0.001; ****p<0.0001; ND= Not detected.

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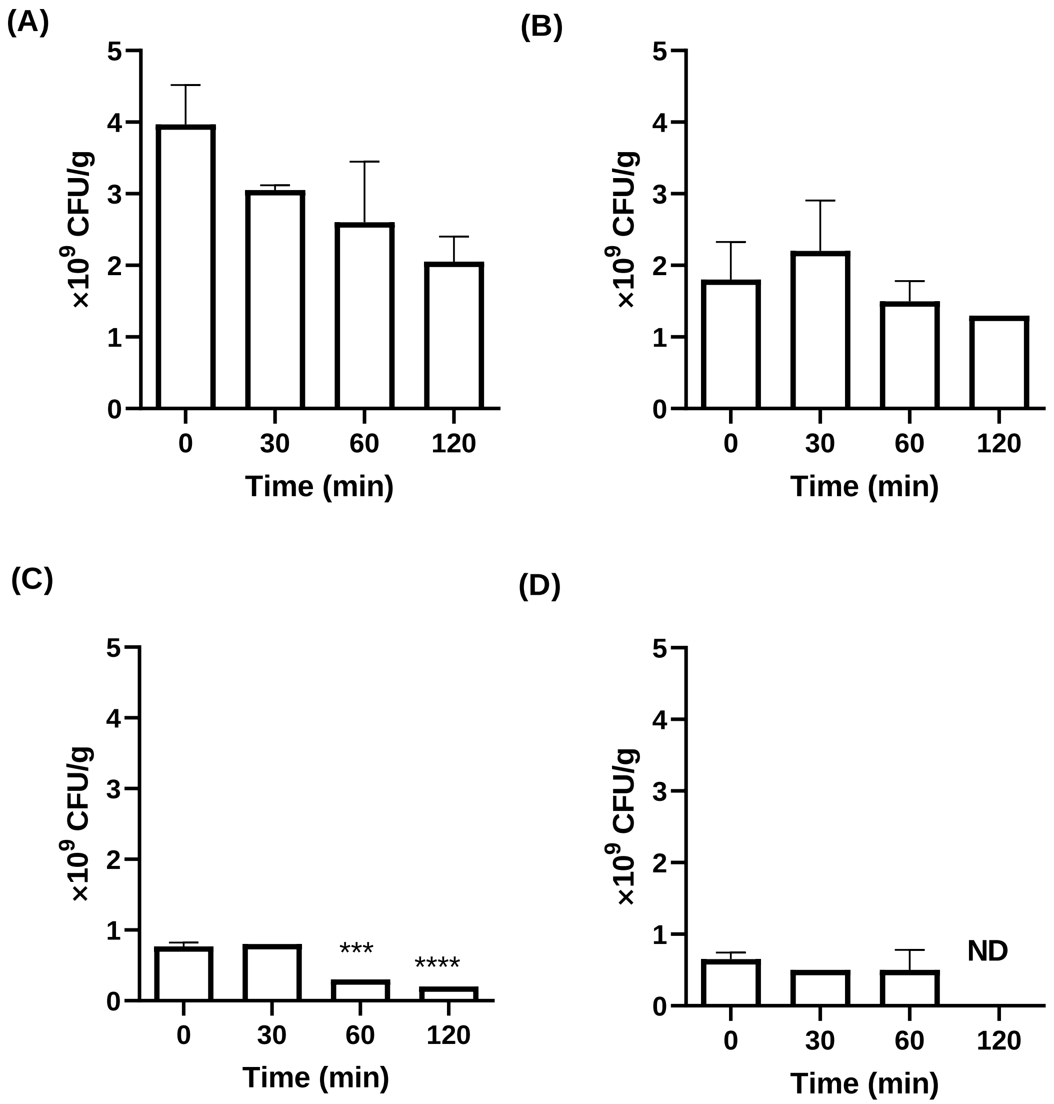

Figure 3. Survival of *L. fermentum* ATCC 23271 in osmotically dehydrated sapodilla slices after simulated passage in the intestine. (A) Evaluation of the sample stored for 7 days; (B) Evaluation of the sample stored for 14 days; (C) Evaluation of the sample stored for 21 days; (D) Evaluation of the sample stored for 28 days. **p<0.01; ***p<0.001; ND= Not detected.

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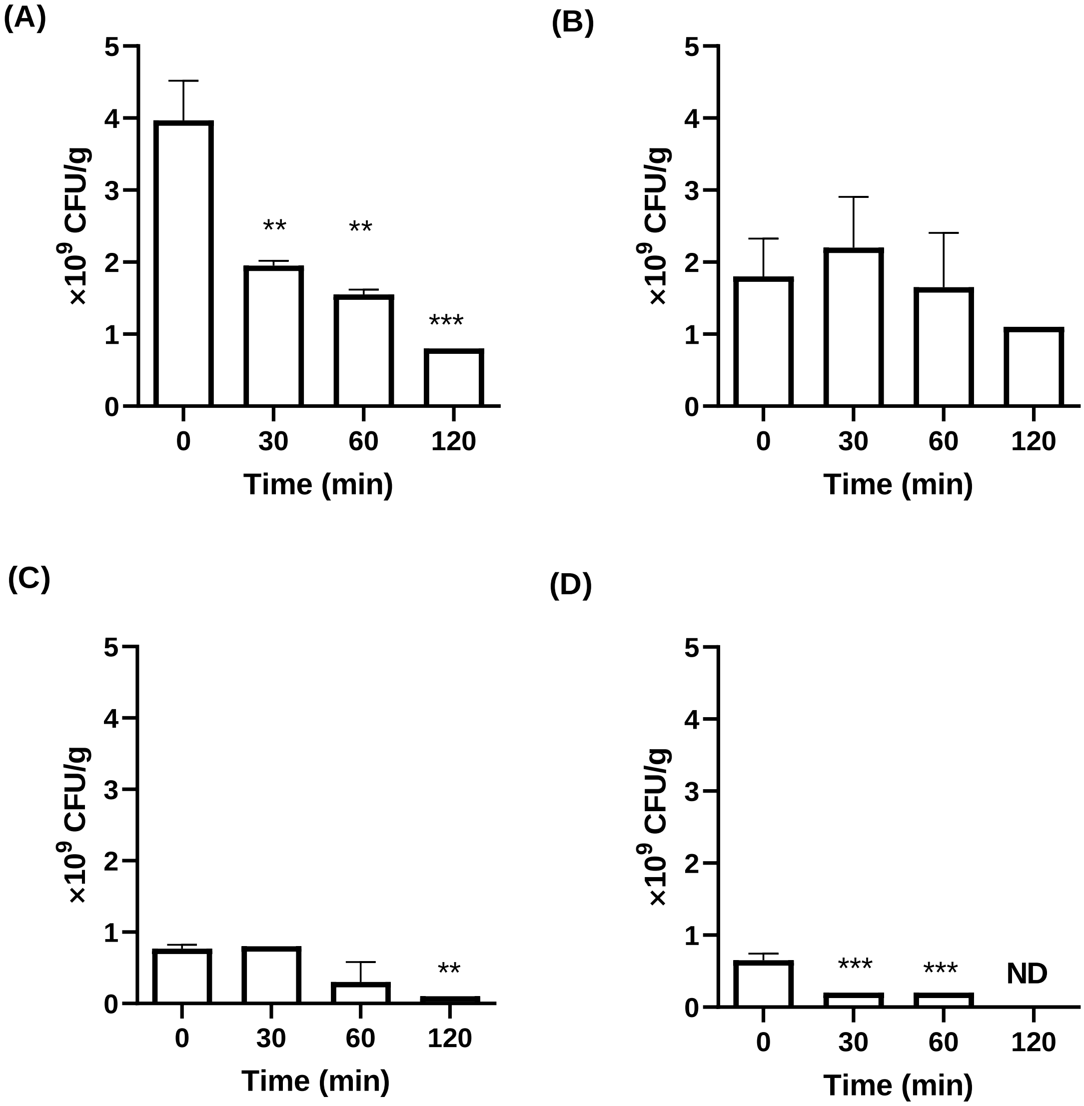

DISCUSSION

Dehydrated fruits have been indicated as an interesting alternative for probiotic delivery (BUSTOS; FONT; TARANTO, 2023; EMSER; BARBOSA; TEIXEIRA; BERNARDO DE MORAIS, 2017; RASCÓN *et al.*, 2018). In this study, *L. fermentum* ATCC 23271 was successfully incorporated in osmotically dehydrated sapodilla. *L. fermentum* ATCC 23271 has been described as a strain with probiotic properties and with antimicrobial effects (CARMO, DO *et al.*, 2016; SANTOS *et al.*, 2021). In addition, this strain has been used for development of new food products (DEMISSIE; HUMBLOT; BAXTER; NEALON; RYA., 2020; MENDES *et al.*, 2021).

The viability of probiotics in the food matrix depends on factors such as pH, temperature and storage time, oxygen levels and the presence of competing and inhibitor microorganisms, fruit structure and food manufacturing conditions (MENDONÇA *et al.*, 2022; PRISCO, DE; MAURIELLO, 2016). The results show that *L. fermentum* ATCC 23271 showed high viability in dehydrated sapodilla during the storage at 4 °C, with only a 1 log reduction after 21 days of storage. This small reduction in the viability of L. fermentum ATCC 23271 can be explained by the presence of sugars that have the ability to confer a protective effect on probiotic strains under simulated conditions of gastric stress (SIANG; WAI; LIN; PHING, 2019). In addition, the storage temperature of 4 °C has been shown to be the best temperature to maintain probiotic viability in a fruit matrix over time (EMSER; BARBOSA; TEIXEIRA; BERNARDO DE MORAIS, 2017).

In order to induce a beneficial effect in human beings, the probiotic population of must be within the range of 10^8 to 10^9 CFU in the daily product recommendation or present populations from 10^6 to 10^7 CFU/g or mL in the final product (BRASIL, 2002; MENDONÇA *et al.*, 2022). To achieve this goal, strains must be selected considering their natural ability to survive during the stages of production, storage and distribution of functional foods, as well as their resistance to passing through the gastrointestinal tract (FERRANDO; QUIBERONI; REINHEMER; SUÁREZ, 2015; MENDONÇA *et al.*, 2022). Therefore, the viability of probiotic cultures during transit in the gastrointestinal tract is of fundamental importance.

Importantly, *L. fermentum* ATCC 23271 survived the adverse conditions of gastrointestinal tract in the samples stored up to 21 days (> 10^8 CFU/g). These results can be explained by the presence of sugars that have the ability to confer a protective effect on probiotic strains (SIANG; WAI; LIN; PHING, 2019). The tolerance of probiotic strains to biliary stress is important for the successful colonization of intestinal epithelium (VIJAY; VIKRAMAN; MARY; CHAUHAN; KAPOOR, 2021). The results obtained with *L. fermentum* ATCC 23271 in dehydrated sapodilla were better than those reported for *Lactiplantibacillus plantarum* 299v in osmotically dehydrated cut apple (EMSER; BARBOSA; TEIXEIRA; BERNARDO DE MORAIS, 2017) and *Lactiplantibacillus plantarum* NCIM 2372 and *Lacticaseibacillus casei* NCIM 2126 in osmotically dehydrated pineapple (VIJAY; VIKRAMAN; MARY; CHAUHAN; KAPOOR, 2021).

CONCLUSION

Osmotic dehydration proved to be a good method for probiotic incorporation into the sapodilla matrix. *L. fementum* ATCC 23271 was successfully incorporated into osmotically dehydrated sapodilla slices and maintained viability throughout the storage period (28 days at 4°C. In addition, *L. fermentum* ATCC 23271 survived fast fast simulation of passage through the gastrointestinal tract (2 h), with values greater than 10^8 CFU/g, even in samples stored for up to 21 days. Therefore, the probiotic food generated can be considered a functional food, with sufficient probiotics amount to exert benefits to the health of the host. The product is applicable in different food products and would be suitable for consumers who do not eat dairy products.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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