Inulin addition improved probiotic survival in soy-based fermented beverage

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Abstract
Currently, the growing demand for non-dairy functional foods leads to the constant development of new products. The objective of the present work was to obtain a soy-based fermented beverage employing the strains Lactiplantibacillus plantarum CIDCA 8327 or Lactocaseibacillus paracasei BGP1 and to analyze the effect of post-fermentation addition of inulin of low or high average polymerization degree on the bacterial resistance. Also, the antimicrobial and antioxidant activity of the fermented soy-based beverages were analyzed. The soy-based matrix was shown to be a suitable substrate for the growth of both lactic acid bacteria, and the fermented beverages obtained presented bioactive properties such as antioxidant activity and bactericidal effect against pathogen microorganisms. The addition of inulin after the fermentation process avoid the hydrolysis and so, preserve its polymerization degree and thus the potential prebiotic effect. The incorporation of inulin to the soy-based fermented beverages increased the bacterial count after 30 days of refrigerated storage up to 8.71 ± 0.15 and 8.41 ± 0.10 log CFU/mL for L. paracasei and L. plantarum respectively. The resistance to the gastrointestinal conditions of the strain L. paracasei BGP1 in the fermented beverage was improved up to 70% when inulin of high polymerization degree was added. Meanwhile the strain L. plantarum CIDCA 8327 showed a survival of 97 and 94% in the fermented beverage added with inulin of low or high polymerization degree, respectively. These results contribute to the development of non-dairy products containing inulin and probiotics and the diversification agri-based functional foods.

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**Introduction**

Although the primary sector involved in the production of functional foods is represented nowadays by the dairy industry, other sectors that employ alternative matrices as carriers of bioactive compounds such as probiotics and prebiotics are emerging. This fact is in line with the worldwide trend of consumption of “lactose-free” beverages and foods, especially with the increase in the diagnosis of lactose intolerance and allergies and the increase in the number of vegetarians, among others (Granato et al. 2010).

Probiotics, generally including species of Lactobacilli and Bifidobacteria, are defined as live microorganisms that, when administered in adequate amounts, are thought to confer health benefits on the host (Hill et al. 2014). In order to provide these effects, *Lactobacillus* strains have to be delivered in a food system with a minimum level of $10^6$ CFU/mL at the time of consumption. They also must resist physical and chemical barriers along the gastrointestinal tract, and have to confer specific beneficial health effects (Bao et al. 2012).

The so-called vegetable milk refers to a water vegetable extract and represents an attractive option for probiotic delivery because of its intrinsic nutritional value and health benefits that could replace cow milk when its inclusion in the diet is restrained (Bernat et al. 2014). In this sense, ‘soymilk’ refers to the traditional oriental beverage that consists of a water extract of soy beans. This product provides a rich supply of nutrients and functional components, including proteins, peptides, isoflavones, saponins, oligosaccharides, dietary fiber, plant sterols, protease inhibitors, lectins, phytic acid, and polyamines (Fukuda et al. 2017). Moreover, soy-based formula for infant containing soy protein isolate and carbohydrates are found in the market as an alternative for cow-milk based infant formula. Also, products obtained by the fermentation of soymilk with lactic acid bacteria (LAB) have emerged as a viable alternative to traditional yoghurts, with a particular interest in the enhanced health-promoting effects of these products (Bao et al. 2012; Marazza et al. 2012; Wang et al. 2006; Zhao and Shah, 2014). Interest in soy-based beverage production has led to developing strategies to improve its nutritional value and the health benefits that they confer. So, it has been shown that the soymilk fermentation by LAB contributes to increasing the nutritional value while reducing the off-flavor associated with soy products, though improving the general acceptability of the beverages (Bergsveinson et al. 2017; Rekha and Vijayalakshmi 2011). In addition, several studies...
have evidenced that soymilk represents a suitable matrix to be employed as a driver for probiotic delivery (Bedani and Rossi 2013; Donkor et al. 2007a, b). In addition to the health benefits, the incorporation of prebiotics, like inulin, has been reported to provide also technological advantages on physicochemical properties in addition to exerting a protective effect on probiotics survival during processing and storage (de Souza et al. 2009). Inulin is a component of the water-soluble fiber fraction that can preferentially stimulate the growth and activity of a limited number of desirable bacteria in the colon, which improves host health (Kolida et al. 2002). Moreover, it was demonstrated that incorporating inulin in combination with probiotics to soy yoghurt enhanced its textural and rheological characteristics (Mishra and Mishra 2013).

The strain Lactiplantibacillus plantarum CIDCA8327 is a facultative heterofermentative lactic acid bacteria isolated from kefir grains (Garrote et al. 2001). This strain showed inhibitory activity against some pathogens (Golowczyc et al. 2008) and promisory probiotic potential for the development of functional foods (Gangoiti et al. 2017; Londero et al. 2015). The commercial strain Lactcaseibacillus paracasei BGP1 is a recognized probiotic microorganisms employed for functional food processing (Céspedes et al. 2013). This work aimed to obtain a functional soy-based beverage by fermentation with Lactiplantibacillus plantarum CIDCA 8327 or Lactcaseibacillus paracasei BGP1 and to analyze the effect of different polymerization degrees inulin addition to the fermented beverages, on the bacterial viability during storage as well as the bacterial tolerance to gastrointestinal conditions. In addition, the antimicrobial and antioxidant activity of the fermented soy-based beverages were analyzed.

**Materials and methods**

**Bacterial strains and culture media**

For soy-based formula fermentation, the commercial probiotic strain Lactcaseibacillus paracasei BGP1 (Clericci, SACCO, Italy) and the strain Lactiplantibacillus plantarum CIDCA 8327 isolated from kefir grains (Garrote et al. 2001; Golowczyc et al. 2008) were used. For LAB culture, MRS broth (Britania, Argentina) was used. For pathogens, the media Mac Conkey Agar (Britania, Argentina), Salmonella-Shigella Agar (Britania, Argentina), and Salted Mannitol (Brizuela, Argentina) were employed for the enumeration of viable E. coli, Salmonella sp., and Staphylococcus aureus. Nutrient broth (NB) (Britania, Argentina) was used for all pathogen growth.

LAB strains were stored at – 20 °C in MRS with skim milk, and pathogen strains were kept in NB with glycerol 20% w/v. To activate microbial cultures, incubations in MRS (for LAB) or NB (for pathogens) at 37 °C for 24–48 h were conducted.

**Bioactive propeties**

**Antimicrobial activity of the fermented soy-based beverages**

Fermented soy-based beverages with L. paracasei or L. plantarum, unfermented acidified soy-based formula (ASF) adjusted to pH 4 with lactic acid or unfermented soy-based formula (Control), were inoculated at 1% v/v with the pathogen strain grown in NB at 37 °C for 24 h. The plate count viable microorganism analysis was performed immediately after the inoculation (initial) and after 24 h of incubation at 37 °C (final) in selective agar plates for the pathogen microorganisms studied. The results were expressed as CFU/mL. The growth degree was calculated as the difference between the final and the initial log CFU/mL. Three independents experiments were done.

**Antioxidant activity of the fermented soy-based beverages**

Antioxidant activity of fermented soy-based beverage was evaluated by DPPH radical scavenging activity measured according to Brand-Williams et al. (1995). Aliquots (100 μL) of fermented beverage supernatant (obtained by centrifugation 10 min, 10,000 xg) were added to 1.9 mL of a methanolic solution of DPPH radical (100 μM). After agitation, the mixture was left in the dark for 30 min, and the absorbance was measured at 517 nm employing a spectrophotometer (UV-1800PC, Mapda Instruments, China). The antioxidant activity was expressed as a percentage of
DPPH radical scavenging calculated according to the following formula:

\[
\% \text{DPPH radical scavenging} = 100 \times \frac{(AC - AS)}{AC}
\]

where AC corresponds to the absorbance of DPPH solution, and AS is the absorbance of DPPH solution after 30 min of reaction with the supernatants of the fermented soy-based beverages. Three independents experiments were done.

**Effect of the inulin addition to the fermented soy-based beverages**

**Formulation of the beverage with inulin addition**

A commercial infant formula powder, based on isolated soy protein and glucose syrup (Nutrilon Soya, Nutricia Bago, Argentina) was used. The powder was reconstituted with distilled water according to manufacture instructions and then sterilized. The strains *L. plantarum* CIDCA 8327 or *L. paracasei* BGP1 were grown in MRS at 37 °C for 24 h, harvested by centrifugation (10 min, 7000 × g), washed twice, and resuspended in PBS buffer (pH 7). Soy-based formula was inoculated at 1% v/v with bacterial suspension in PBS and incubated at 37 °C for 8 h. Then, the pH of the fermented soy-based beverage was adjusted to 4 with lactic acid, and its final lactic acid concentration was measured employing a commercial kit (Lactate, Wiener Lab., Argentina). After the fermentation process, inulin with different average polymerization degrees: Low, DPn ≥ 10 (GR, Beneo® Orafti, Belgium) or high, DPn ≥ 23 (HP, Beneo® Orafti, Belgium) was added at 3% w/v. Finally, samples (with and without inulin) were placed in hermetically sealed plastic containers and stored at 4 °C for 30 days.

**Bacterial viability in fermented soy-based beverage during the storage**

The bacterial viability (CFU/mL) in the fermented soy-based beverages samples after 0, 10, 20, and 30 days of storage at 4 °C was assayed by plate count in MRS agar. The effect of post-fermentation addition of inulin on the bacterial viability during refrigerated storage of the fermented products was also evaluated, by triplicate.

**Bacterial tolerance to simulated gastrointestinal conditions**

In order to evaluate the resistance of the LAB strains present in the fermented soy-based beverages to simulated gastrointestinal conditions, two serial incubations were used according to Grimoud et al. (2010). The first was in a gastric solution containing 125 mM NaCl, 7 mM KCl, 45 mM NaHCO₃, and 0.3% w/v pepsin (SIGMA-Aldrich, USA), and the final pH was adjusted to 2.5 with concentrated HCl. The second was in an intestinal solution containing 22 mM NaCl, 3.2 mM KCl, 7.6 mM NaHCO₃, 0.1% w/v pancreatin (SIGMA-Aldrich, USA), and 0.15% w/v bile sales (SIGMA-Aldrich, USA) and the final pH adjusted to 8 with 4 N NaOH. For the tolerance test, 2.5 mL of fermented beverage with or without inulin, were mixed with 2.5 mL of gastric solution (2×), the pH adjusted to 2.5 with HCl, and the final mixture incubated for 90 min at 37 °C. Then, 5 mL of intestinal solution (2×) was added, the pH adjusted to 8 with NaOH, and the final mixture was incubated for 180 min at 37 °C. Bacterial viability was assessed by plate count in MRS agar (37 °C, 48 h), and the percentage of survival was calculated as follows:

\[
\% \text{Survival} = 100 \times \frac{(\log \text{CFU/mL})_{\text{after}}}{(\log \text{CFU/mL})_{\text{before}}}
\]

where log CFU/mL represents cell viability at the beginning (before) and after incubation (after) in the simulated gastrointestinal conditions, as described above. Three independent assay were performed.

**Statistical analysis**

Results were expressed as mean ± standard deviation of three independent experiments with duplicates on each assay. One-way analysis of variance was performed. Mean differences were statistically tested using the LSD Fisher multiple comparison test conducted by the InfoStat® Software (Version 2008, Argentina). A p value ≤ 0.05 indicated significant differences.

**Results**

**Fermentation of soy-based formula by LAB strains**

The ability of the probiotic strains *Lactobacillus paracasei* BGP1 and *Lactiplantibacillus plantarum* CIDCA 8327 to grow and to ferment the soy-based formula, was evaluated. It was observed that both LAB grew in the soy-based formula at the two temperatures evaluated (Fig. 1). Besides, the growth rate in the soy-based formula was strain dependent. It was observed that the temperature had no significant effect on the growth of *L. plantarum* in the soy-based formula; meanwhile the growth of *L. paracasei* at 37 °C was significantly higher concerning the growth observed at 42 °C.

The reconstituted soy-based formula (unfermented) presented a pH value of 6.80 ± 0.27. Fig. 2a shows the pH values during fermentation of soy-based formula at 37 and
42 °C for 24 h with L. paracasei BGP 1 and L. plantarum CIDCA 8327. It was observed that the pH remained constant during soy-based formula fermentation at 42 °C with both strains throughout the assay period. Instead, at 37 °C, the fermentation with L. paracasei produced a significant decrease in the pH reaching a minimum (4.22 ± 0.22) after 24 h, while L. plantarum reached the minimum pH (4.12 ± 0.07) after 12 h. During the soy-based formula fermentation with both LAB at 37 °C, the titratable acidity increased (Fig. 2b) as the pH decreased.

The titratable acidity of the soy-based formula, expressed as percentage of lactic acid during the first 8 h of fermentation at 37 °C, was in the range between 0.07 and 0.15%, and after 24 h the amount of lactic acid produced by L. plantarum was higher compared with L. paracasei (0.64 and 0.25%, respectively).

Finally, the fermentation process for producing the soy-based fermented beverages with both LAB was conducted at 37 °C during 8 h, then the beverages were bottled and stored at 4 °C for further analysis. The Table 1 shows the results about bacterial concentration (log CFU/mL), pH and titrable acidity (lactic acid %w/v) obtained in these conditions.

### Soy-based fermented beverages: antimicrobial and antioxidant activities

The antimicrobial activity of the soy-based fermented beverages obtained with the strains L. paracasei BGP1 and L.
The artificially acidified soy-based formula presented a bacteriostatic effect against the pathogens; moreover the concentrations obtained for *Salmonella* and *S. aureus* after 24 h were significantly lower than those obtained in the unfermented control soy-based formula. The soy-based fermented beverages with each LAB strain presented bactericidal effect against the three pathogens. In the case of soy-based beverage fermented with *L. plantarum*, no growth of *E. coli* and *Salmonella* sp. was detected (< 10² CFU/mL), while *S. aureus* presented a decrease of 4.47 log CFU/mL cycles. The concentrations found after 24 h of incubation in soy-based beverage fermented with *L. paracasei* were significantly lower than the initial values showing a decrease of 2.90; 2.83 and 0.54 log CFU/mL cycles for *E. coli*, *Salmonella* sp., and *S. aureus*, respectively.

The percentage of inhibition of the DPPH radical found in the soy-based beverage fermented with *L. plantarum* was 25.3 ± 1.8% and did not show significant differences from the unfermented control (27.3 ± 1.0%). However, the percentage of inhibition values found for the soy-based beverage fermented with *L. paracasei* were 24.3 ± 1.8% and resulted significantly lower than the unfermented control. In addition, no significant differences were found between the fermentation with both strains.

At this point the antimicrobial and antioxidant activities of the fermented beverages were described, and this product was employed as basis for the final product formulations.

**Storage of soy-based fermented beverage with addition of inulin**

After 30 days of storage at 4 °C the soy-based fermented beverages presented viable counts of 2 × 10⁷ and 6 × 10⁷ CFU/mL for *L. paracasei* and *L. plantarum*, respectively. Also, the viability of LAB strains in the fermented soy-based beverage with inulin (3% w/v) of low (DPn ≥ 10) or high (DPn ≥ 23) average polymerization degree was evaluated (Fig. 4). For both LAB strains, the soy-based beverage with post-fermentation addition of inulin presented significantly higher viable counts than in the soy-based beverage without inulin (control) after storage. Moreover, the post-fermentation addition of low or high DPn inulin to the beverages contributed to maintaining the viability of both LAB strains during storage, since the final counts (30 days) did not present significant...
differences with respect to the initial counts, regardless of the polymerization degree of the inulin added.

The variation of the pH values of the fermented beverage is another factor that should be taken into account as an indicative parameter of the product performance during refrigerated storage. After 30 days of storage at 4 °C, the fermented soy-based beverage without inulin presented pH values of 4.02 ± 0.10 and 3.44 ± 0.3 for L. paracasei and L. plantarum, respectively. It is worth to mention that no significant differences between the pH values of the fermented beverage with or without inulin stored were obtained for each strain. The pH values of the fermented soy-based beverages with inulin of low and high polymerization degree were 3.74 ± 0.2 and 3.79 ± 0.3 for L. paracasei, or 3.25 ± 0.3 and 3.31 ± 0.3 for L. plantarum, respectively.

Survival of LAB in soy-based fermented beverages with inulin after gastrointestinal simulation

The soy-based beverages fermented with L. paracasei and L. plantarum without or with inulin addition, were subjected to simulated gastrointestinal conditions, and the results concerning the bacterial survival are shown in Table 2. The survival of L. plantarum in fermented soy-based beverage submitted to in vitro gastrointestinal conditions was higher than the survival of L. paracasei, in the respective fermented soy-based beverage, suggesting that this characteristic is microorganism dependent. Moreover, it was observed that the post-fermentation addition of inulin (either of low or high DPn) contributed to increase the percentage of survival of L. plantarum after simulated gastrointestinal treatment. Meanwhile, the post-fermentation addition of inulin with high DPn produced a higher increase of the survival of L. paracasei in fermented soy-based beverage than when inulin of the lower DPn was added.

Table 2 Percentage of survival of LAB strains in fermented soy-based beverages with addition of inulin (3% w/v) after simulated gastrointestinal treatment

| Strain          | Percentage of survival (%) | Control | Inulin low DPn | Inulin high DPn |
|-----------------|----------------------------|--------|----------------|-----------------|
| L. paracasei BGP1 | 52.4 ± 3.1<sup>a</sup> | 48.1 ± 2.2<sup>a</sup> | 70.6 ± 8.6<sup>b</sup> |
| L. plantarum CIDCA8327 | 87.2 ± 3.2<sup>b</sup> | 97.9 ± 1.2<sup>b</sup> | 94.6 ± 2.0<sup>b</sup> |

DPn: average polymerization degree (Low: ≥ 10; High: ≥ 23). Values in each row with the same superscript are not significantly different (p > 0.05). ANOVA followed by LSD Fisher Test

Discussion

Many challenges arise when attempting to incorporate probiotic bacteria in foods matrices, mainly related to their growth, survival, viability, stability and functionality in food processing, storage and consumption as well as changes of sensory characteristics of probiotic foods (Min et al. 2018). It is known that many LAB strains possess the metabolic tools to degrade oligosaccharides through α-galactosidase activity (Silvestroni et al. 2002) and are capable of adapting their metabolism and growing in soy-based products (Aguirre et al. 2014; Farnworth et al. 2007; Granato et al. 2012). The LABs might present different metabolic activities, evidencing differences in their nucleotide sequence described by technologies that have the power to rapidly reveal important genetic detail for the LAB involved in these fermentations, as reported by Bergsvinsson et al. (2017). In this sense, in this work, it was observed a satisfactory growth of L. paracasei BGP1 and L. plantarum CIDCA 8327 in the soy-based formula. For both strains, a low pH and high titratable acidity was found in the soy-based formula fermented at 37 °C, similar to the results reported by Wang et al. (2002) in soymilk fermented with combinations of bifidobacteria and lactic acid bacteria and dos Santos et al. (2019) for soymilk fermented with kefir grains. Other work showed higher lactic acid concentrations for soy beverages containing cane juice fermented with L. johnsonii NCC533 and L. rhamnosus ATCC 53103 (Farnworth et al. 2007). The variability in the lactic acid content as a metabolic product detected in the fermented beverages is dependent on the metabolic and adaptive characteristics of each particular strain or starter and also the fermentation conditions.

Many studies have reported that soy fermented beverages containing probiotics present good general acceptability by potential consumers since the fermentation improves the product’s flavor and texture (Donkor et al. 2007a, b; Granato et al. 2012; Shimakama et al. 2003). The preliminary promissory results obtained in the present work leads to complete characterization of this new functional non-dairy beverage considering the sensory analysis for the future studies. Among other parameters, the pH influences the stability, aroma, flavor, texture, and shelf-life of the fermented soymilk products.

Both bacteria and also their spent culture supernatants employed in the present work, showed inhibitory effect against E. coli, Salmonella sp. and S. aureus. In general, the antimicrobial activity of Lactobacillus strains is well documented; for instance, in coincidence with the results obtained in this work, Bao et al. (2012) demonstrated that the strain Lactobacillus plantarum IMAU70004 presented antagonistic activity against the foodborne pathogens.
Salmonella typhimurium S50333, Escherichia coli O157 882364, and Staphylococcus aureus AC1.2465; meanwhile other studies show different degrees of antagonism against S. aureus and E. coli exerted by Lactobacillus strains (Ebhodaghe et al. 2012; Shokryazdan et al. 2014). The spent culture supernatant contains bacterial metabolic products that might exert inhibitory effects, as suggested by Bian et al. (2011) for L. reuteri DPC16 spent culture against food-borne pathogens, due to acidic effect in a pH-dependent way. Other research concluded that the inhibitory effect of Lactobacillus supernatants against pathogens was due to their organic acid productions (Shokryazdan et al. 2014).

The fermented beverages obtained also exhibited antimicrobial activity against the three pathogens analyzed in this work. In line with these results, Kumari and Vij (2015) reported antimicrobial activity of soymilk fermented with L. rhamnosus C6 against S. aureus and E. coli 0157:H7 ATCC 35150. Moreover, soymilk fermented with L. helveticus V3 inhibited the growth of S. aureus and S. typhi, while fermentation with S. thermophilus MD2 inhibited the growth E. coli (Hati et al. 2018). The antimicrobial activity against pathogens exhibited by the fermented beverages could be attributed either to the presence of organic acid produced by the strains (Ebhodaghe et al. 2012) and also to bioactive peptides produced during soymilk fermentation (Singh et al. 2015).

The antioxidant capacity of the soy-based formula and the fermented products obtained in this work was moderate and no increase of this activity was obtained after fermentation. In contrast, Marazza et al. (2012) and Rani and Pradeep (2015) informed that the antioxidant activity increase after fermentation of soymilk with L. rhamnosus and L. paracasei KUMBB005. Also, other works reported higher antioxidant activity of soymilk after the fermentation with other LAB and bifidobacteria (Wang et al. 2006). Meanwhile the antioxidant activity obtained in the present work is in accordance to the results reported for soy protein extract fermented with L. acidophilus CSCC 24, L. paracasei CSCC 279, L. zeae ASCCC 15820, and L. rhamnosus WQ2 (Zhao and Shah 2014). The antioxidant activity seems to have a positive correlation either with fermentation time and the bacterial proteolysis capacity (Monajjemi et al. 2012; Singh et al. 2015), and so it is possible that 8 h of fermentation were not enough to evidence an increase in the DPPH radical scavenging activity of the beverage developed in the present work. However, the antioxidant activity of the fermented soymilks could increase by the gastrointestinal conditions due to larger hydrolysis of soy proteins and the release of bioactive peptides (Capriotti et al. 2015). Finally, given that the fermentation time and proteolysis degree affect the organoleptic and bioactive characteristics of the product, a compromise relationship is generated between these parameters.

The soy-based fermented beverages were characterized and employed as a basis for the development of the functional beverage with addition of inulin. In most of the studies inulin is mainly added before the soymilk fermentation, so it is available to be hydrolyzed and employed as fermentable substrate by bacteria (Battistini et al. 2018; Bedani et al. 2013; Saarela et al. 2006). In the present work, to avoid inulin from being hydrolyzed during the fermentation and to analyze the effect that inulin polymerization degree has on the bacterial survival, this compound was added after the bacterial soy-based formula fermentation. During the storage of the beverages, the probiotics are exposed to a low pH values that may damage both the cell wall and the cell membrane, thus influencing ΔpH and the membrane potential, acidifying of the cytosol and affecting in consequence the bacterial viability (Papadimitriou et al. 2016). The soy-based beverage with inulin presented significantly higher viable counts than in the soy-based beverage without inulin after storage, regardless of the polymerization degree. These results can be attributed to the better environment and the direct interactions between inulin polymer structure with bacterial proteins and lipids from the membranes, contributing to their survival during storage, as was also described for different food applications (Khorasany and Shahdadi 2021; Schaw et al. 2007). The protective effect of inulin during during freeze-drying is attributed to direct interactions with proteins and membranes. Other authors showed that the addition of inulin in fermented soymilk exerted a protective effect on LAB viability and activity during fermentation (Donkor et al. 2007a, b; Oliveira et al. 2011; Valero-Cases and Frutos 2017). One of the advantages of the post-fermentation addition of inulin can be related to keeping the chemical structure of this ingredient, since it is not hydrolyzed nor consumed by bacteria during the storage at 4 °C, as was evidenced by thin layer chromatography analysis (data not shown).

The metabolic activity of the LAB cultures employed for soymilk fermentation is reduced during refrigerated storage, however some variation of the pH of the final products may take place. The difference between the pH values after 30 days of storage of each fermented soy-based beverage obtained, could be related to the metabolic activity intrinsic of each strain. This was evidenced in the present work since in one hand, no significant differences were obtained between the initial and final pH of the soy-based beverage fermented with L. paracasei with or without inulin. On the other hand, the beverage fermented with L. plantarum showed a significant decrease in the pH values after the storage, and no significant differences were obtained in the final pH values between the samples. As suggested by other authors, the acidity could be maintained or decreased
during storage in fermented soy-products (Capriotti et al. 2015; dos Santos et al. 2019; Saito et al. 2014). In addition, inulin-supplementation of fermented soy milk did not produce changes in pH values of after 28 days of storage at 4 °C, as reported by Mishra and Mishra (2018). It should be taken in account that there is a negative correlation between the titable acidity and pH with the consumers acceptability (Raja et al. 2009; Souza et al. 1991). However, fermented beverages with a pH under 4 showed higher stability against spoilage and resulted a refreshing product with a mild acidic taste (Simova et al. 2002).

Probiotic foods must demonstrate the ability to preserve the viability of the strains after passage through the gastrointestinal tract (Buriti et al. 2010). It was determined that the soy matrix contributed to improve the survival of both strains when compared with their survival as free cells, as described by Iraporda et al. (2019). In accordance other authors reported that the survival rate of different LAB after simulated gastrointestinal conditions was higher in a fermented soy beverage than in culture media (Bedani et al. 2013; Guo et al. 2009). Moreover, it was observed that the post-fermentation addition of inulin increase the percentage of survival of *L. plantarum* after simulated gastrointestinal treatment. Meanwhile the post-fermentation addition of inulin with high DPn increased the survival of *L. paracasei* in fermented soy-based beverage. Therefore, the mean molecular weight, and hence the DPn of the inulin, could be correlated with the degree of protection that this polysaccharide exerts on bacterial tolerance to gastrointestinal conditions, as suggested by Valero-Cases and Frutos (2017). This result leads to highlighting a differential action with regards of the inulin structure; thus, post-fermentation inulin incorporation could be advantageous.

**Conclusion**

In this work, it was shown that the soy-based formula is a suitable matrix for the growth of both *Lactocaseibacillus paracasei* BGP1 and *Lactiplantibacillus plantarum* CIDCA 8327, obtaining a fermented product that has a bactericidal effect against pathogen microorganisms, while the antioxidant activity of the products was maintained. Moreover, the beverages’ appearances were homogeneous and remained stable during storage. In particular, the strain *L. plantarum* CIDCA 8327 exhibited better performance with respect to *L. paracasei* BGP1. In order to avoid inulin from being hydrolyzed during the fermentation process, and though preserving its chemical structure, this bioactive ingredient was incorporated after the bacterial fermentation. As a relevant result of this work, the post-fermentation addition of inulin to the fermented soy-based beverages contributed to increasing the bacterial viability during refrigerated storage, and improved the bacterial tolerance to simulated gastrointestinal conditions. Finally, this study demonstrate that the fermented soy-based beverages with inulin represents a good matrix for probiotic delivery, as alternative to the traditional dairy probiotic beverages also contributing to diversify the type of agri-based food products.

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**Declarations**

**Conflict of interest** The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

**References**

Aguirre L, Hebert EM, Garro MS, de Giori GS (2014) Proteolytic activity of *Lactobacillus* strains on soybean proteins. LWT-Food Sci Technol 59(2):780–785. https://doi.org/10.1016/j.lwt.2014.06.061

Bao Y, Zhang Y, Li H, Liu Y, Wang S, Dong X, Xu F, Yao G, Sun T, Zhang H (2012) In vitro screen of *Lactobacillus plantarum* as probiotic bacteria and their fermented characteristics in soymilk. Ann Microbiol 62(3):1311–1320. https://doi.org/10.1007/s13213-011-0377-4

Battistini C, Gullón B, Ichimura ES, Gomes AMP, Ribeiro EP, Kunigk L et al (2018) Development and characterization of an innovative synbiotic fermented beverage based on vegetable soybean. Braz J Microbiol 49(2):303–309. https://doi.org/10.1016/j.bjm.2017.08.006

Bedani R, Rossi EA, Isay Saad SM (2013) Impact of inulin and okara on *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. Food Microbiol 34(2):382–389. https://doi.org/10.1016/j.fm.2013.01.012

Bergsveinson J, Kajala I, Ziola B (2017) Next-generation sequencing approaches for improvement of lactic acid bacteria-fermented plant-based beverages. AMS Microbiol 3(1):8. https://doi.org/10.3934/microbiol.2017.1.8

Bernat N, Cháfer M, Chiralt A, González-Martínez C (2014) Hazelnut milk fermentation using probiotic *Lactobacillus rhamnosus*
GG and inulin. Int J Food Sci Technol 49(12):2553–2562. https://doi.org/10.1111/ijfs.12585

Bian L, Molan AL, Maddox I, Shu Q (2011) Antimicrobial activity of Lactobacillus reuteri DPC16 supernatants against selected food borne pathogens. World J Microbiol Biotechnol 27(4):991–998. https://doi.org/10.1007/s11274-010-0543-z

Brand-Williams W, Cuvelier ME, Berest CLWT (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28(1):25–30. https://doi.org/10.1016/0022-0267(95)80008-5

Buriti FC, Castro IA, Saad SM (2010) Effects of refrigeration, freezing and replacement of milk fat by inulin and whey protein concentrate on texture profile and sensory acceptance of symbiotic guava mousse. Food Chem 123(4):1190–1197. https://doi.org/10.1016/j.foodchem.2010.05.085

Capriotti AL, Caruso G, Cavaliere C, Sampieri R, Ventura S, Chi ozzi RZ, Lagana A (2015) Identification of potential bioactive peptides generated by simulated gastrointestinal digestion of soybean seeds and soy milk proteins. J Food Comp Anal Aci 44:205–213. https://doi.org/10.1016/j.jfca.2015.08.007

Céspedes M, Cárdenas P, Staffolani M, Ciappini M, Vinderola G (2013) Performance in nondairy drinks of probiotic L. casei strains usually employed in dairy products. J Food Sci 78(5):756–762
de Souza Oliveira RP, Perego P, Converti A, De Oliveira MN (2009) The effect of inulin as a prebiotic on the production of probiotic fibre-enriched fermented milk. Int J Dairy Tech 62(2):195–203. https://doi.org/10.1111/j.1471-0307.2009.00471.x

Donkor ON, Henriksson A, Vasiljevic T, Shah NP (2007a) Rheological properties and sensory characteristics of set-type soy yogurt. J Agric Food Chem 55(24):9868–9876. https://doi.org/10.1021/jf071050r

Donkor ON, Henriksson A, Vasiljevic T, Shah NP (2007b) α-Galactosidase and proteolytic activities of selected probiotic and dairy cultures in fermented soymilk. Food Chem 104(1):10–20. https://doi.org/10.1016/j.foodchem.2006.10.065
dos Santos DC, de Oliveira Filho JG, Santana ACA, de Freitas BSM, Silva FG, Takeuchi KP, Egea MB (2019) Optimization of soy milk fermentation with kefir and the addition of inulin: physicochemical, sensory and technological characteristics. LWT-Food Sci Technol 104:30–37. https://doi.org/10.1016/j.lwt.2019.01.030

Ebbodaghe SO, Abiose SH, Adeniran HA (2012) Assessment of physico-chemical characteristics, viability and inhibitory effect of Bifidobacteria in soymilk. J Food Res 1(2):159. https://doi.org/10.5539/jfr.v1n2p159

Farnworth ER, Mainville I, Desjardins MP, Gardner N, Fliss I, Chambon L, Abraham AG, Semorile L, De Antoni G (2008) Characterization of homofermentative Lactobacillus plantarum strain usually employed in dairy products. J Food Comp Anal Aci 21(6):514. https://doi.org/10.1016/j.jfca.2008.05.025

Guo Z, Wang J, Yan L, Chen W, Liu XM, Zhang HP (2009) In vitro comparison of probiotic properties of Lactobacillus casei Zhang, a potential new probiotic, with selected probiotic strains. LWT-Food Sci Technol 42(10):1640–1646. https://doi.org/10.1016/j.lwt.2009.05.025

Hatt S, Patel N, Mandal S (2018) Comparative growth behaviour and biofunctionalilty of lactic acid bacteria during fermentation of soy milk and bovine milk. Prob Antimicrob 10(2):277–283. https://doi.org/10.5897/s12602-017-9279-5

Khill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Berni Canani R, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11(8):506–514. https://doi.org/10.1038/nrgastro.2014.66

Iraporda C, Rabea IA, Manrique GD, Abraham AG (2019) Influence of inulin rich carbohydrates from Jerusalem artichoke (Helianthus tuberosus L.) tubers on probiotic properties of Lactobacillus strains. LWT-Food Sci Technol 101:738–746. https://doi.org/10.1016/j.lwt.2018.11.074

Khorasany S, Shahdadi F (2021) Improvements in survival of probiotic bacteria, rheology and sensory characteristics of yogurts during storage. Nutr Food Sci Res 8(1):35–43

Kolida S, Tuohy K, Gibson GR (2002) Prebiotic effects of inulin and oligofructose. Br J Nutr 87(2):193–197. https://doi.org/10.1079/BJN2002537

Kumari P, Vij S (2015) Growth and antimicrobial activity of proteolytic probiotic Lactobacillus rhamnosus C6 in soymilk and whey. Indian J Dairy Sci 68(3):229–238

Londero A, Iraporda C, Garrote GL, Abraham AG (2015) Cheese whey fermented with kefir micro-organisms: antagonism against Salmonella and immunomodulatory capacity. Int J Dairy Technol 68(1):118–126

Marazza JA, Nazareno MA, de Giori GS, Garro MS (2012) Enhancement of the antioxidant capacity of soymilk by fermentation with Lactobacillus rhamnosus. J Funct Foods 4(3):594–601. https://doi.org/10.1016/j.jff.2012.03.005

Min M, Bunt CR, Mason SL, Hussain MA (2018) Non-dairy probiotic food products: an emerging group of functional foods. Crit Rev Food Sci Nutr 59(16):2626–2641

Mishra S, Mishra BN (2013) Effect of synbiotic interaction of fructooligosaccharide and probiotics on the acidification profile, textural and rheological characteristics of fermented soy milk. Food Bioproc Tech 6(11):3166–3176. https://doi.org/10.1007/s11947-012-1021-4

Mishra S, Mishra BN (2018) Comparative study of the synbiotic effect of inulin and fructooligosaccharide with probiotics with regard to the various properties of fermented soy milk. Food Sci Technol Int 24(7):564–575. https://doi.org/10.1177/10282013218776529

Monajjemi M, Aminin AN, Ilkhani AR, Mollaamin F (2012) Nanoemulsion of inulin rich carbohydrates from Jerusalem artichoke (Helianthus tuberosus L.) tubers on probiotic properties of Lactobacillus strains. LWT-Food Sci Technol 101:738–746. https://doi.org/10.1016/j.lwt.2018.11.074

Oliveira RPDS, Perego P, De Oliveira MN, Converti A (2011) Effect of inulin as a prebiotic to improve growth and counts of a...
probiotic cocktail in fermented skim milk. LWT-Food Sci Technol 44(2):520–523. https://doi.org/10.1016/j.lwt.2010.08.024

Papadimitriou K, Alegria Á, Bron PA, De Angelis M, Gobbetti M, Kleerebezem M, Kok J (2016) Stress physiology of lactic acid bacteria. Microbiol Mol Biol Rev 80(3):837–890. https://doi.org/10.1128/MMBR.00076-15

Raja A, Gajalaksshmi P, Raja MMM, Imran MM (2009) Effect of Lactobacillus lactis cremorii isolated from kefir against food spoilage bacteria. Am J Food Technol 4(5):201–209. https://doi.org/10.3923/ajft.2009.201.209

Rani VU, Pradeep BV (2015) Antioxidant properties of soy milk fermented with Lactobacillus paracasei B005. Int J Pharm Sci Res 30(1):39–42

Rekha CR, Vijayalakshmi G (2011) Isoflavone phytoestrogens in soymilk fermented with β-glucosidase producing probiotic lactic acid bacteria. Int J Food Sci Nutr 62(2):111–120. https://doi.org/10.3109/09637486.2010.513680

Saarela M, Virkajarvi I, Nohynek L, Vaari A, Matto J (2006) Fibres as carriers for Lactobacillus rhamnosus during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. Int J Food Microbiol 112(2):171–178. https://doi.org/10.1016/j.ijfoodmicro.2006.05.019

Saito VST, Dos Santos TF, Vinderola CG, Romano C, Nicoli JR, Araújo LS et al (2014) Viability and resistance of lactobacilli isolated from cocoa fermentation to simulated gastrointestinal digestive steps in soy yoghurt. J Food Sci 79(2):M208–M213. https://doi.org/10.1111/1750-3841.12326

Schwab C, Vogel R, Gänzle MG (2007) Influence of oligosaccharides on the viability and membrane properties of Lactobacillus reuteri TMW1.106 during freeze-drying. Cryobiology 55(2):108–114

Shimakama Y, Matsubara S, Yuki N, Ikeda M, Ishikawa F (2003) Evaluation of Bifidobacterium brevis strain Yakult fermented soymilk as a probiotic food. Int J Food Microbiol 81(2):131–136. https://doi.org/10.1016/S0168-1605(02)00224-6

Shokryazdan P, Sioe CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M, Ho YW (2014) Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. BioMed Res Int. https://doi.org/10.1155/2014/927268

Silvestroni A, Connes C, Sesma F, De Giori GS, Piard JC (2002) Characterization of the melA locus for α-galactosidase in Lactobacillus plantarum. Appl Environ Microbiol 68(11):5464–5471. https://doi.org/10.1128/AEM.68.11.5464-5471.2002

Simova E, Beshkova D, Angelov A, Hristozova T, Frengová G, Spasov Z (2002) Lactic acid bacteria and yeasts in kefir grains and kefir made from them. J Ind Microbiol Biotechnol 28(1):1–6. https://doi.org/10.1023/A:1009835700131

Singh BP, Vij S, Hati S, Singh D, Kumari P, Minj J (2015) Antimicrobial activity of bioactive peptides derived from fermentation of soymilk by Lactobacillus plantarum C2 against common food-borne pathogens. Int J Ferm Foods 4(1):91–99

Souza G, de OL, Vetra AJ (1991) Acceptability of the flavour of soy bean milk yoghurt with added cow milk. Dairy Sci Abst 13(9):729

Valero-Cases E, Frutos MJ (2017) Effect of inulin on the viability of L. plantarum during storage and in vitro digestion and on composition parameters of vegetable fermented juices. Plant Foods Hum Nutr 72(2):161–167. https://doi.org/10.1007/s11130-017-0601-x

Wang YC, Yu RC, Chou CC (2002) Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiol 19(5):501–508. https://doi.org/10.1006/fmic.2002.0506

Wang YC, Yu RC, Chou CC (2006) Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. Food Microbiol 23(2):128–135. https://doi.org/10.1016/j.fm.2005.01.020

Zhao D, Shah NP (2014) Changes in antioxidant capacity, isoflavone profile, phenolic and vitamin contents in soymilk during extended fermentation. LWT-Food Sci Technol 58(2):454–462. https://doi.org/10.1016/j.lwt.2014.03.029

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