




Article

Alternative Fermented Soy-Based Beverage: Impact of Inulin on the Growth of Probiotic Strains and Starter Culture

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Abstract: The number of people with dietary restrictions on dairy products has increased significantly due to lactose intolerance/allergy or adoption of vegan diets. Organic acid-producing probiotics have been used in fermented beverages, such as those based on soy, with good results. Such molecules have in fact been described for their role in sensory analyses and benefits to human health. Therefore, this study suggested the evaluation of an alternative soy extract-based beverage that could act as a functional food. For this purpose, products and biomass concentrations were monitored throughout soy extract fermentation through acidification kinetics and cell count. The effect of inulin on the growth of the probiotic strains *Bifidobacterium longum* and *Lactobacillus acidophilus* in co-culture with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was evaluated (technical replicates). It was observed that the addition of inulin reduced the time of fermentation by *L. acidophilus*, while no statistically significant effect was observed in the post-acidification period. In *B. longum* fermentation, the process did not change in the presence of inulin, but there was a significant increase in viability and survival in the post-acidification period. Therefore, it can be concluded that the strains studied can be used in the formulation of soy-based drinks and that inulin positively influenced the viability of both probiotics in fermented drinks tested.

Keywords: organic acids; inulin; fermented soy-based beverages; co-culture; *Bifidobacterium longum*; *Lactobacillus acidophilus*; *Streptococcus thermophilus*



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1. Introduction

The current context exhibits a strong trend in the demand for functional foods. The consumer market is becoming increasingly selective, prioritizing foods and ingredients that not only offer healthiness and nutrition but also deliver concrete health benefits. It is widely recognized that maintaining a nutritionally balanced diet can lower the incidence of specific diseases such as cancer, hypertension, and diabetes [1] by modulating metabolism [2]. Consequently, the scientific community holds substantial interest in comprehending and validating the clinical impacts instigated by certain foods, aiming to define dietary patterns that can contribute to promoting health [3,4].

In the 1980s, Japan's health authorities illuminated the connection between the dietary choices of the population and the progressively rising life expectancy rates among the elderly. This led to the start of the concept of functional foods [3]. In Brazil, the legal definition of functional foods was established by Resolution n° 18 of 04/30/1999 from the Health Surveillance Secretariat of the Ministry of Health. According to this definition,

functional foods encompass “all food or ingredient that, in addition to the basic nutritional functions, when consumed as part of the usual diet, produces metabolic and/or physiological effects and/or beneficial health effects and must be safe for consumption without medical supervision” [5]. The demand for such products is robust, propelling the expansion of the ingredients and functional foods market. The segment predicted to be the most lucrative is the one centered around products designed to enhance intestinal functionality [6].

A prototype of these functional foods is the fermented beverage derived from soy extract, which boasts several characteristics that contribute to maintaining regular intestinal function. Moreover, it serves as an alternative for individuals suffering from lactose intolerance or milk protein allergies. Soy extract is in fact a protein-rich plant-based source with a minimal fat content [7]. Soy-based products have been linked to the reduction in various diseases, including breast and prostate cancer, osteoporosis, and coronary heart disease. Additionally, they play a role in diminishing oxidative stress and enhancing renal function [8,9].

Among the prominent functional ingredients that have gained significant attention within the food industry in recent years, prebiotics have taken center stage. It was reported that as of 2015, a substantial investment of USD 2.90 billion was allocated towards incorporating these compounds into foods across various production chains [10]. Prebiotics refer to non-digestible carbohydrates that exert their primary effects within the gastrointestinal tract. The central mechanism of their action revolves around the modulation of the intestinal microbiota, promoting the proliferation of beneficial bacteria within the colon and triggering the release of microbial metabolites [10,11].

One of the most extensively researched and widely recognized prebiotics in the realm of scientific literature is inulin [11], a fructo-oligosaccharide sourced from the roots of chicory, dahlia, and Jerusalem artichoke (*Helianthus tuberosus*) that remains resistant to digestion by α -amylase and other hydrolytic enzymes present in the upper gastrointestinal tract [2,12]. It is established that inulin fosters the growth of beneficial bifid bacteria within the colon, inducing effects similar to those resulting from breastfeeding. Additional clinical effects attributed to prebiotics in general encompass a diminished risk of colon cancer, enhancements in the immune system, increased resistance to specific pathogens, decreased blood lipid levels, and improved mineral absorption [12]. Notably, inulin is a prebiotic that can be seamlessly integrated into food products due to its low-calorie nature, lack of influence on taste or aroma, and capacity to serve as a texture-modifying fat substitute [12].

Research has demonstrated that the inclusion of inulin can accelerate the rate of milk acidification by starter cultures and probiotics [11,13], the latter being live microorganisms that, when administered in suitable amounts, confer a range of advantages to host health [14]. Moreover, when combined with probiotics, inulin has the potential to enhance the consistency of infant feces [2]. Predominantly, lactic acid bacteria (LABs) belonging to the genera *Bifidobacterium* and *Lactobacillus* are commonly employed probiotics. Through the synthesis of organic acids, they are capable of lowering intestinal pH, rendering the colonization of certain pathogens unviable, and augmenting mineral absorption capacity [11]. Recent investigations [15,16] have also suggested that these probiotics possess anti-allergic properties as well as the ability to mitigate symptoms related to lactose intolerance due to their secretion of β -galactosidases [17].

Lactobacillus acidophilus stands as one of the most extensively studied lactobacilli species, primarily due to its wide-ranging applications within the food industry [16]. This Gram-positive, microaerophilic, rod-shaped microorganism [18] is found in both the human gastrointestinal tract and the oral cavity [16]. Within the human gastrointestinal tract, the most prevalent species of *Bifidobacterium* is *Bifidobacterium longum* [11]. This Gram-positive, non-sporulating, non-motile bacterium establishes a presence in the gut from early childhood and is even present in breast milk [16]. While it holds immense potential as a probiotic for utilization in the food industry, much like other bifid species, *B. longum* has a slow growth rate that can affect product taste and texture [15,19].

In light of this, several studies [11,19] have demonstrated that the incorporation of starter cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* in co-culture with *Streptococcus thermophilus* can effectively expedite the fermentation process and mitigate excess acetic acid production, thereby preventing undesirable flavors [11]. This approach is considered a viable strategy to expand the applicability of *B. longum* in dairy products [11,20]. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* initiate the fermentation process and, through mutual cooperation, exchange metabolites. Particularly, *L. delbrueckii* subsp. *bulgaricus* displays robust proteolytic activity, yielding free peptides and amino acids that stimulate the growth of *S. thermophilus*. In turn, *S. thermophilus* supplies formic, folic, and pyruvic acid, alongside carbon dioxide, to *L. delbrueckii* subsp. *bulgaricus*. Furthermore, lactic acid generated by *S. thermophilus* lowers milk pH to an optimal level for *L. delbrueckii* subsp. *bulgaricus*, thus stimulating its growth [21,22].

Thus, the objective of this study was to prepare a fermented beverage derived from soy with properties enhanced by the inclusion of the probiotics *B. longum* and *L. acidophilus* cultivated in conjunction with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. The experiments aimed to ascertain the feasibility of growth and the viability of bacterial strains in the selected fermentation substrate. Additionally, the impact of inulin as a prebiotic on both the fermentation process and the subsequent post-acidification stages was evaluated.

2. Materials and Methods

2.1. Microbial Cultures

This study was carried out using the following bacterial strains in lyophilized form: *Bifidobacterium longum* BL-D5, *Lactobacillus acidophilus* LYO40, *Streptococcus thermophilus* T040, and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB340; all were provided by Danisco-Dupont, Madison, MA, USA.

2.2. Experimental Design

Triplicate runs were performed (technical replicates), according to the experimental design outlined in Table 1, on soy base either supplemented with inulin (3 g/100 mL) or not (0 g/100 mL) using a probiotic strain (*B. longum* or *L. acidophilus*) in co-culture with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (StLb).

Table 1. Experimental design adopted to study the influence of inulin (0 and 3 g/100 mL) on the fermentation of soy extract and the viability of microorganisms. The fermentations were performed in triplicate.

Co-Culture	Inulin Concentration (g/100 mL)	
<i>B. longum</i> + StLb	0	3
<i>L. acidophilus</i> + StLb	0	3

2.3. Inoculum Preparation

Each probiotic strain was weighed using an analytical precision balance and rehydrated in 50 mL of powdered soy milk (Soymilke, Olvebra, Eldorado do Sul, RS, Brazil) at 42 °C for 15 min before being utilized. The initial average microbial count for each inoculum ranged from approximately 10^6 to 10^8 CFU mL⁻¹. Soybean commercial base was used for the preparation of the beverages. For this purpose, 12 g of commercial soybean base was added to 100 mL of filtered water, and then the mixture was pasteurized. Finally, beverages with a total solids content of 12% (in samples without inulin) and 15% (in those that received 3% inulin) were obtained. The mixtures were heat-treated at 90 °C for 5 min under constant mixing using a Thermomix (Vorwerk & Co. KG, TM31, Wuppertal, Germany). Furthermore, the soy base was cooled to 10 °C using an ice bath and left overnight while awaiting the fermentation process. After that, the probiotic strains were inoculated into 500 mL of the soy-based preparation.

2.4. Acidification Kinetics

Each fermentation described in Table 1 was conducted in triplicate at a temperature of 42 °C and was stopped upon reaching a pH of 4.7. The progress of fermentation was closely monitored using the Cinac system (Ysebaert, Frépillon, France) as described by Spinnler and Corrieu [23]. This system enabled continuous real-time pH measurement and subsequent calculation of the acidification rate throughout the fermentation period. The following kinetic parameters were taken into consideration (Table 2): (a) inulin concentration (0 to 3 g/100 mL), (b) initial pH, (c), the times required to obtain a pH of 5.5 ($t_{pH5.5}$), 5.0 ($t_{pH5.0}$) and 4.7 ($t_{pH4.7}$) expressed in hours, (d) the maximum acidification rate (V_{max}) measured in pH units per minute, (e) the time to reach V_{max} (t_{max}) expressed in hours, and (f) the pH at t_{max} ($pH_{V_{max}}$). The fermentation process was stopped by ceasing operations in the Cinac System, followed by immersing the mixture in an ice bath for 10 min. Subsequently, the coagulated mixture was agitated using a stainless steel rod equipped with a perforated disc. The fermented soy milk was then dispensed into 50 mL containers and hermetically sealed using Selopar equipment (BrasHolanda, Pinhais, PR, Brazil). All batches of fermented soy milk were stored at a temperature of 4 °C.

Table 2. Kinetic parameters of acidification by *Streptococcus thermophilus* (St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) in co-culture with *Lactobacillus acidophilus* (La) or *Bifidobacterium longum* (Bl) in soy-based medium at 42 °C until reaching pH 5.5 ($t_{pH5.5}$), 5.0 ($t_{pH5.0}$), and 4.7 ($t_{pH4.7}$).

Co-Culture	Inulin Concentration (%)	Initial pH	$t_{pH5.5}$	$t_{pH5.0}$	$t_{pH4.7}$	V_{max}^a	t_{max}^b	$pH_{V_{max}}^c$
			(h)	(h)	(h)	(upH.min ⁻¹ · 10 ³)	(h)	
St/Lb/La	0	6.64	4.25	5.25	6.33	8.52	4.58	5.33
St/Lb/La	3	6.63	4.00	4.92	5.92	9.32	4.58	5.19
St/Lb/Bl	0	6.64	4.58	5.67	6.67	9.68	4.17	5.71
St/Lb/Bl	3	6.63	4.54	5.67	6.92	10.69	4.50	5.52

^a V_{max} = maximum acidification rate, upH = pH units. ^b t_{max} = time to reach V_{max} . ^c $pH_{V_{max}}$ = pH at t_{max} .

2.5. Post-Acidification

Post-acidification of the fermented soy milk samples was assessed at specific time intervals (0, 1, 14, and 28 days) during storage at 4 °C by measuring the pH with a pH meter (model Q-400M1, Quimis, São Paulo, SP, Brazil).

2.6. Microbial Count

To quantify the viable cells of the selected microorganisms, predetermined time points (0, 1, 14, and 28 days) of storage at 4 °C were selected. The microorganisms were cultured on selective media considered to be appropriate for each species. *B. longum* was plated on BSM agar (Fluka, Sigma-Aldrich, St Louis, MO, USA), *L. acidophilus* on MRS agar (Difco, Detroit, MI, USA), *S. thermophilus* on M17 agar (Difco) supplemented with 10% (*w/v*) lactose, and *L. delbrueckii* subsp. *bulgaricus* on MRS agar (Difco) acidified to pH 5.4 with acetic acid [13].

3. Results and Discussion

3.1. Production of Organic Acids by Probiotics Grown in Soy-Based Beverage

When observing Table 2, it is evident that the initial pH values of the media both without and with inulin were practically coincident, which ensures consistent conditions across all the fermentations. The fermentation by the *Lactobacillus acidophilus*-containing co-culture with 3% inulin was faster than without inulin (0%). For instance, the medium with inulin took 5.92 h to achieve pH 4.7, while that without inulin took 6.33 h (41 min more). On the contrary, inulin addition did not have any significant effect on the *Bifidobacterium longum*-containing co-culture. It is worth noting that the time spent and the fermentation rate achieved were similar among the evaluated groups, and the presence of inulin did not

directly influence the fermentation rate; therefore, the rate of acidification depended solely on the microorganism used.

Figure 1 illustrates the difference in the fermentation time of *L. acidophilus* co-cultures with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in the presence of inulin or not. The sample containing inulin exhibited a shorter fermentation time, as inulin accelerated the process. The curves, which started from the same point and ran in parallel for approximately one hour, then diverged, making the lag between them more noticeable. Towards the end of the fermentation, such a lag became more pronounced, given that the sample with 3% inulin reached the desired pH (4.7) before the one without inulin. Therefore, inulin may have enhanced the fermentation by this microorganism, acting as a prebiotic capable of stimulating its metabolism. Among the various benefits expected from this action, we can mention the reduction in costs in the industrial production of the drink [11].

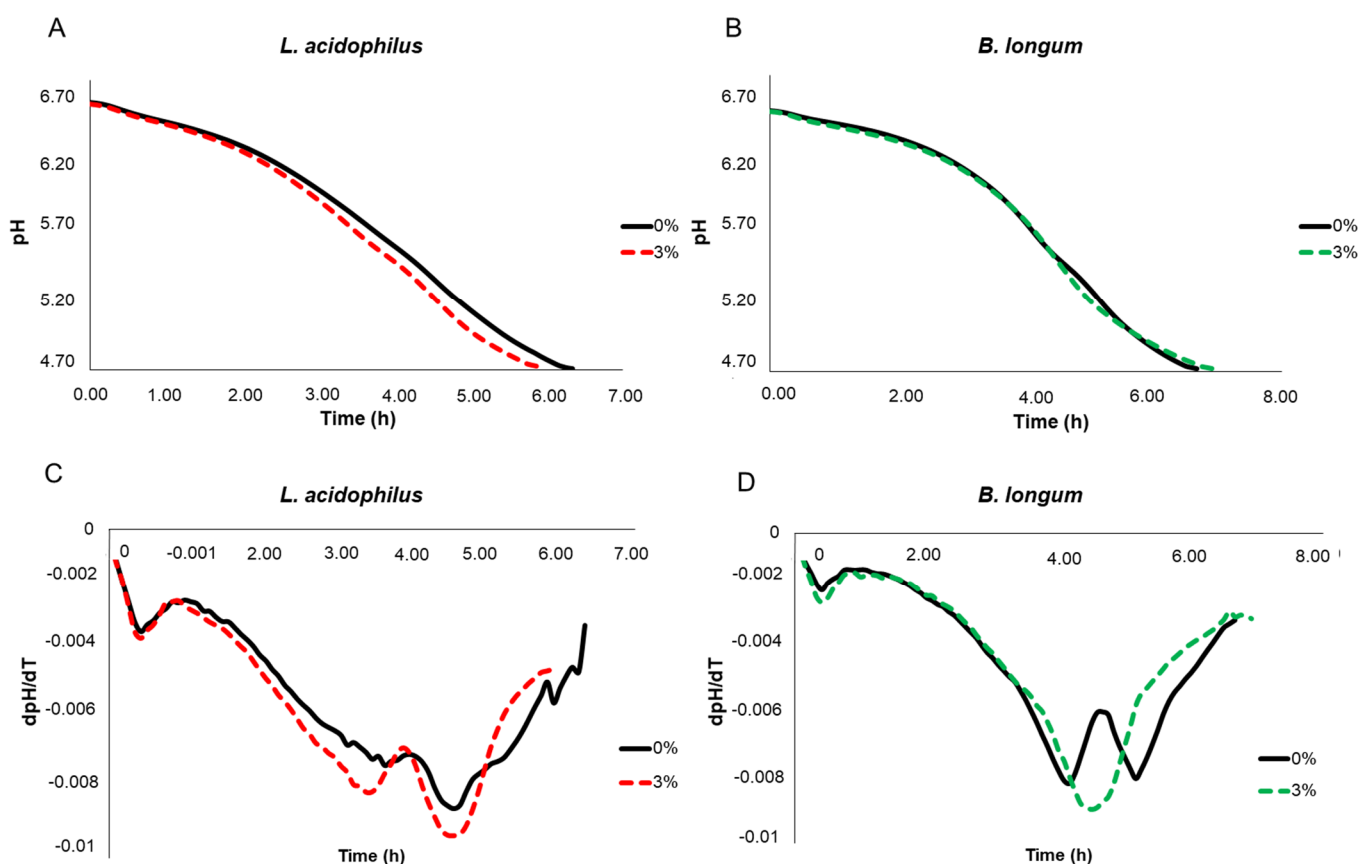


Figure 1. Acidification kinetics. pH vs. time (h) curve depicting the acidification by *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus acidophilus* (A) or *Bifidobacterium longum* (B) in soy-based fermented beverage at 42 °C until reaching pH 4.7. Behavior of the derivative of pH with respect to time during fermentation by *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* (C) or *B. longum* (D) at 42 °C.

Based on the results of the fermentation carried out by *L. acidophilus* in coculture with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Figure 1), the presence of inulin appeared to induce some changes in the fermentation process. The two curves started similarly and remained so for a while. After approximately 1 h, they diverged, and the acidification of the sample containing inulin accelerated, achieving a pH of 4.7 before the one without inulin. Moreover, after about 4 h, the peak was higher in the inulin-containing sample, followed by a more pronounced decline. The rate of acidification of this sample increased rapidly,

highlighting a slowdown in the process, which then stabilized. This is in contrast with the sample without inulin, which continued to show a decline.

The results illustrated in Figure 1 confirm that inulin did not have a discernible impact on the fermentation by the *B. longum*-containing co-culture, as the time taken for the process was nearly coincident for both samples. This observation is reflected in the overlapping of curves starting at pH 6.63 until reaching pH 4.7. The curves started at the same point and exhibited minimal variations over time, regardless of the presence or absence of inulin. This phenomenon arose due to the distinct response of each microorganism to this prebiotic compound. Although inulin provided the system with dietary fibers, the fermentation process did not exploit them for acceleration. While inulin did not affect the fermentation time, it played a significant role in other important aspects, such as the viability and survival of *B. longum*. Analyzing Figure 1, which depicts the acidification profile of this bacterium in co-culture with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, it is evident that the presence of inulin did not exert any appreciable effect at the 4 h mark. Both curves started similarly and progressed in tandem until this point, where the inulin-free sample exhibited a slower acidification rate when compared to the inulin-enriched one. The acidification rate of the latter then began to slow down, maintaining this pace until reaching pH 4.7, while that of the former increased, but only briefly, before gradually approaching the desired pH. It is noteworthy that even though the presence of inulin did not exert any significant influence on the fermentation rate, it accelerated the subsequent acidification phase.

The use of soy milk-based beverages as a growth medium for probiotic bacteria has been proposed in various studies because it offers significant advantages [24–26]. The primary advantage is that soy milk serves as a safe alternative for individuals with lactose intolerance or cow's milk allergy. Its use provides consumers with an option to consume probiotics without triggering gastrointestinal discomfort. Moreover, soy milk is naturally rich in essential nutrients, such as high-quality proteins, fibers, and isoflavones, which can serve as substrates for the growth and activity of probiotic bacteria within the intestinal environment [27,28]. Recent studies have demonstrated that soy milk can support the development and viability of several probiotic species, mainly belonging to the *Lactobacillus* and *Bifidobacterium* genera [27,29,30], thus allowing for the production of fermented products enriched with beneficial microorganisms able to enhance consumers' digestive and immune health. The influence of soy milk as a cultivation medium for probiotic bacteria has been explored in the literature [27,29,30]. The findings reveal that soy milk not only enhanced the growth of the tested probiotic bacteria but also promoted the production of beneficial metabolites, such as organic acids. This suggests that soy milk not only serves as a nutritious vehicle for probiotics but also has the potential to amplify their beneficial effects. Therefore, the utilization of soy milk as a base for cultivating probiotic bacteria emerges as a promising option, supported by recent scientific evidence that underscores its advantages for gastrointestinal and overall health [27,29,30].

3.2. Effect of Inulin on Cell Viability

The microbial count carried out over 28 days revealed some important information regarding the possible effect of inulin on cell viability. The results in Tables 3 and 4 show that the starting number of bacterial cells in the inulin-containing sample was higher than without inulin, which suggests that the presence of inulin stimulated the fermentation by *L. acidophilus* throughout the entire process. Concerning *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*, there were minimal differences between their growths. However, the final count of *B. longum* in the inulin-free sample (after 28 days) (Table 4) was 1.6 log units lower than in that enriched with inulin; therefore, it can be inferred that the addition of inulin may be related to the maintenance of cell viability throughout the fermentation or may influence growth.

Table 3. Mean viable cell counts (log) and standard deviations of the co-culture of *Streptococcus thermophilus* (St), *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb), and *Lactobacillus acidophilus* (La) in soy-based beverages before fermentation (d0), after 24 h (d1) of fermentation, and after 14 days (d14) and 28 days (d28) of storage at 4 °C. Matrix without inulin addition and with 3% inulin addition.

Run	d0	d1	d14	d28
Without inulin				
La	6.75 ± 0.03	8.86 ± 0.03	7.51 ± 0.09	6.45 ± 0.03
St	6.08 ± 0.01	9.01 ± 0.02	8.08 ± 0.03	7.43 ± 0.07
Lb	6.85 ± 0.05	8.76 ± 0.03	7.32 ± 0.03	6.35 ± 0.01
With 3% inulin				
La	7.78 ± 0.07	9.15 ± 0.01	7.13 ± 0.07	6.63 ± 0.04
St	7.17 ± 0.00	9.91 ± 0.01	7.97 ± 0.01	7.60 ± 0.06
Lb	7.70 ± 0.03	9.75 ± 0.01	7.38 ± 0.03	6.54 ± 0.07

Table 4. Mean viable cell counts (log) and standard deviations of the co-culture of *Streptococcus thermophilus* (St), *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb), and *Bifidobacterium longum* (Bl) in soy-based beverages before fermentation (d0), after 24 h (d1) of fermentation, and after 14 days (d14) and 28 days (d28) of storage at 4 °C. Matrix without inulin addition and with 3% inulin addition.

Test	d0	d1	d14	d28
Without inulin				
Bl	5.46 ± 0.06	8.82 ± 0.00	4.96 ± 0.01	3.54 ± 0.09
St	6.00 ± 0.01	8.70 ± 0.06	8.25 ± 0.01	7.83 ± 0.02
Lb	5.17 ± 0.08	9.56 ± 0.00	8.12 ± 0.01	7.99 ± 0.01
With 3% inulin				
Bl	5.73 ± 0.05	8.71 ± 0.00	5.25 ± 0.07	5.14 ± 0.03
St	6.04 ± 0.02	9.16 ± 0.02	8.17 ± 0.01	8.37 ± 0.16
Lb	5.32 ± 0.00	9.53 ± 0.14	8.25 ± 0.00	7.89 ± 0.08

Inulin is a carbohydrate that has gained significant attention for its impact on fermentation and its promotion of bacterial growth. In studies using it as a substrate in fermentative processes, inulin has demonstrated the ability to provide energy to various microorganisms, particularly beneficial bacteria. Its structure is complex, consisting of fructose units linked by β (2→1) glycosidic bonds, which makes it a useful substrate for inulinase-producing bacteria. This enzyme breaks the glycosidic bonds, releasing fructose molecules that can be easily metabolized by these microorganisms [31–33]. This process not only produces fermentation byproducts such as short-chain fatty acids, contributing to the distinctive flavors and textures of fermented foods, but also creates an environment conducive to the growth and proliferation of probiotic bacteria. Thus, inulin supplementation can have an important impact on fermentation dynamics, improving the nutritional and sensory quality of food products. Furthermore, the prebiotic nature of inulin further accentuates its role in promoting bacterial growth. As a non-digestible fiber, inulin reaches the colon virtually intact, where it becomes a substrate for specific groups of bacteria possessing the necessary enzymes to break it down [31,32,34]. This process promotes the growth of beneficial bacteria, such as Bifidobacteria and Lactobacilli, while inhibiting the proliferation of potentially harmful microorganisms. The resulting shift in the gut's microbial composition can lead to improved gut health, increased nutrient absorption, and even potential modulation of the immune system. Overall, the dual effects of inulin on fermentation and bacterial growth make it a valuable component in both food processing and promotion of intestinal microbiota balance [31,35].

3.3. Post-Acidification

From the results gathered in Table 5, it can be observed that inulin did not exert any statistically significant influence on the post-acidification profile of the *L. acidophilus*-containing co-culture. This can be observed by comparing the pH values at d1, d14, and d28 in both samples. On the other hand, it is also evident that this prebiotic managed to maintain, during storage, the pH of the *B. longum*-containing co-culture at almost its starting value after the fermentation.

Table 5. Mean pH values of *Streptococcus thermophilus* (St), *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb), and *Lactobacillus acidophilus* (La) or *Bifidobacterium longum* (Bl) co-cultures after 24 h (d1) of fermentation, and after 14 days (d14) and 28 days (d28) of storage at 4 °C.

Co-Culture	Inulin Concentration (%)	d1	d14	d28
St/Lb/La	0	4.78 ± 0.02	4.62 ± 0.01	4.58 ± 0.01
St/Lb/La	3	4.73 ± 0.02	4.60 ± 0.01	4.59 ± 0.01
St/Lb/Bl	0	4.75 ± 0.02	4.58 ± 0.01	4.48 ± 0.01
St/Lb/Bl	3	4.73 ± 0.02	4.68 ± 0.01	4.65 ± 0.01

The acidity of a fermented beverage can influence several aspects of a food product, such as taste, texture, and general quality. Therefore, studies related to post-acidification of fermented beverages play a key role in the food industry. Adjustments to the sensory attributes of a food product can ensure that it will meet consumer and market demands; in addition, post-acidification studies can generate useful information for the development of food products with a balance of flavors, odors, and increased shelf life. This is an area of great relevance to the food sciences, as its findings have a direct impact on the nutritional value and health implications of fermented beverages [36–39]. By adjusting acidity levels after fermentation, for instance, scientists can enhance the availability of nutrients (minerals and vitamins), as well as modulate the growth and increase the survival rate of beneficial probiotics present in the beverage. With the growing demand for functional beverages that promote health, research in the field of post-acidification may offer ways to ensure that these products provide advantages both in terms of sensory attributes (flavor) and health-related gains (nutritional benefits). In addition to the nutritional benefits, post-acidification can also yield economic advantages for industries and contribute to environmental damage reduction. Techniques for adjusting acidity after fermentation can in fact optimize industrial processes, resulting in a significant reduction in waste and environmental impacts [39].

4. Conclusions

The development of soy-based dairy beverages and the use of probiotics and prebiotics can play an important role in sustainability, promoting health and increasing the dietary options available to people with dietary restrictions. Furthermore, adding probiotics and prebiotics to these products can improve their nutritional value and increase the digestibility of soybeans. In this study, the effect of inulin on the growth and viability of microorganisms in a fermented drink based on soy extract was evaluated. For this purpose, commercial lactic acid bacteria with proven beneficial effects were used, namely *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The acidification profile observed after the acidification kinetic tests indicates that the strains tested adapted well to the soy-based drink. These results suggest that probiotics could be used in the development of functional foods based on soy milk, and that the product can be a viable and quality option for people with dietary restrictions involving lactose. In turn, tests with inulin revealed that it was not able to influence the time of fermentation by *B. longum*; the pH reached by the product during 28 day-storage at 4 °C showed that inulin did not influence the post-acidification process. However, this prebiotic played a significant role in other aspects of the product fermented

by *B. longum*, such as viability and survival, especially in post-acidification, which may give the product greater durability.

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