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Fermented beverage obtained from soy and rice incorporated with inulin and oligosaccharides derived from succinoglycan

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Abstract

The aim of this study was to evaluate the effect of incorporating inulin and oligosaccharides resulted from the enzymatic hydrolysis of the commercial succinoglycan, regarding the physicochemical properties, rheology, syneresis and probiotic viability of soy and rice-based fermented beverages added with *Lactobacillus paracasei*. Four formulations were prepared and supplemented with 3.5% of inulin; 0.5% of succinoglycan oligosaccharides; 3.0% of inulin and 0.5% of succinoglycan oligosaccharides, and evaluated for 28 days of storage under refrigeration at 5 ºC. The succinoglycan oligosaccharides presented an average degree of polymerization (DPn) of 8. All the formulations were characterized as pseudoplastic fluids. Regarding syneresis, the formulations with succinoglycan oligosaccharides and Mix exhibited a reduction of it and this was not observed in the formulation with inulin. Regarding the probiotic viability, all the formulations maintained the subsistence of *Lactobacillus paracasei* with counts above 10^8 CFU mL^{-1} up to the 28th storage day, however, only the incorporated prebiotic formulations presented sufficient numbers of viable cells after exposure to simulated gastrointestinal conditions. Therefore, this fermented beverage proved to be an innovative alternative of non-dairy products interesting for the food industry to attend the population's aspirations for functional products.

Keywords: fermented beverage, succinoglycan oligosaccharides, prebiotics, inulin, probiotics.

Practical Application: This study demonstrates the effectiveness of the use of succinoglucan oligosaccharides as prebiotic ingredients in the preparation of fermented soy and rice beverage.

1 Introduction

The most popular functional foods are those with added probiotics and prebiotics (Guimarães et al., 2018). Probiotics are defined as living microorganisms that, when administered in proper amounts, provide benefits to the host's health (Gurpilhares et al., 2019; Turkmen et al., 2019). Among these benefits are: the inhibition of undesirable bacteria, reduction of symptoms caused by lactose intolerance, the improvement of the immunological potential, anti-tumorigenic action and reduces the risk of developing cancer (Handa & Sharma, 2016; Fazilah et al., 2018; Vitola et al., 2018). For these microorganisms to perform these functions, probiotic products must have at least 10^6 – 10^7 CFU mL^{-1} viable cells at the time of consumption (Fazilah et al., 2018). These microorganisms, however, must survive not only the shelf life of the product but, also, the passage through the gastrointestinal tract (Soares et al., 2019). Different resources have been used to increase the survival rate of probiotics, among them, the simultaneous administration of prebiotics (Bedani et al., 2013).

To be considered a prebiotic component, some criteria must be attended, such as, resistance to the digestive process, be fermentable by beneficial intestinal microorganisms and, also, selectively stimulate the growth and/or activity of a restricted quantity of bacteria in the gastrointestinal system (Gibson, 2004; Nawaz et al., 2018).

Inulin is a prebiotic ingredient that can be found as a reserve carbohydrate in a wide variety of plants, such as chicory, dahlia and the Jerusalem artichoke (Shoaib et al., 2016). Inulin may have a protective effect on probiotic bacteria, as it increases their subsistence and activity during the storage and passage through the gastrointestinal trait (Bedani et al., 2013).

The β-glucans are soluble fibers usually located in algae, plants, yeasts, fungi, and bacteria. Among the β-glucans, succinoglycans are characterized as acid extracellular heteropolysaccharides, produced by Sinorhizobium, Agrobacterium and other bacteria from the soil. Succinoglycans are constituted by galactose and glucose monomers present in the ratio of 1-7, which are

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connected by β-glucosidic, β-(1→4), β-(1→3), and β-(1→6) bonds. Its structure, also, comprises succinate, acetate, and pyruvate, as non-carbohydrate substituents (Jofré et al., 2018; Ruiz et al., 2015; Simsek et al., 2009).

The enzymatic hydrolysis of β-glucans performed through β-glucanases consists of a route to obtain oligosaccharides with oligomers of higher molecular mass (DP ≥ 4), which exhibit biological properties, such as prebiotics (Bauermeister et al., 2010).

The main food items with added probiotics are fermented milk and yogurts (Costa et al., 2017; Olivares et al., 2019). However, due to the large number of people with lactose intolerance and allergic to milk proteins, there was an increase in demand for vegetable-based beverages (Costa et al., 2017; Savedboworn et al., 2017). The soybean has proteins, dietary fibers, minerals, vitamins, and polyunsaturated fatty acids. Besides that, it is a functional seed that helps to reduce the risk of cardiovascular diseases, type 2 diabetes and cancer (Bedani et al., 2013; Bedani et al., 2014). Rice is a good source of nutrition and energy (He et al., 2018) and, also, it provides low fat content (Mandial et al., 2018). For this reason, the mixture of soybean and rice may be beneficial.

Therefore, the objective of this research was to prepare a soy and rice-based fermented beverage added with *Lactobacillus paracasei* and evaluate in four formulations the effect of adding inulin and oligosaccharides resulting from the enzymatic hydrolysis of the commercial succinoglycan. This analysis assessed the physical-chemical properties, rheology, syneresis, and probiotic viability of the formulations during storage.

2 Materials and methods

2.1 Materials

In this research the following products were used: non-transgenic bulk soybean obtained from local trade in Maringá (Brazil), refined sugar (Alto Alegre, Colorado, Brazil), polished rice (Grão de Ouro, Maringá, Brazil), inulin with a degree of polymerization (DPn) equal to 10 (SM Empreendimentos Farmacêuticos Ltda, São Paulo, Brazil), commercial succinoglycan (Rheozan, donated by Rhodia Solvay São Paulo, Brazil), lactic culture constituted by *Streptococcus thermophillus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Chr-Hansen, Valinhos, Brazil), *Lactobacillus paracasei* probiotics (donated by the company Coana, Florianópolis, Brazil), and Viscozyme commercial enzyme (given by the company Novozymes, Araucária, Brazil). All the chemicals used in this study were of analytical grade.

2.2 The enzymatic hydrolysis of the commercial succinoglycan to obtain oligosaccharides

For the enzymatic hydrolysis of the commercial succinoglycan 5 L of a solution containing 1.5 g L^{-1} of succinoglycan was prepared in 0.025 M sodium acetate buffer, pH adjusted to 5.0 with the addition of HCL 1M. This solution was maintained under agitation for 48 h at room temperature and, posteriorly, a volume of 0.38 ml L^{-1} of Viscozyme enzyme was added to the fermenter container Bioflo Celligen 115 (Eppendorf, Hamburg, Germany). The reaction was conducted at 45 °C and 150 rpm for 6 h. Aliquots from the reaction mixture were periodically removed to verify the DPn of the hydrolyzed succinoglycan and boiled at 95° C for 10 minutes to inactivate the reaction. Then, the degree of polymerization (DP) was determined according to Mangolin et al. (2017) and Zhang & Lynd (2005). Finally, the solution was centrifuged in a refrigerated centrifuge at 4 °C and 9000 rpm for 10 min, and the supernatant was discarded. The product (succinoglycan oligosaccharides) was frozen, lyophilized, and maintained in a freezer for posterior use.

2.3 Obtention of water-soluble soy and rice extract

For the manufacturing of the water-soluble soy and rice extract, 50 g of grains were used in a 70:30 ratio of soy and rice, respectively, which were added to 1 L of water and processed in the equipment Vegan Milk Machine (Polishop, Jundiaí, Brazil). After the end of the process, the final product was filtered and cooled (8 °C to 10 °C). During processing, the grains were crushed and also subjected to heat and this resulted in the inactivation of the enzyme lipoxygenase present in soybean.

2.4 Preparation of lactic culture

To the previously obtained water-soluble soy and rice extract was added a 50 U envelope of lactic culture that was composed of *Streptococcus thermophillus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The lactic culture was homogenized for 10 to 15 minutes and distributed in 50 mL containers that were frosted for posterior utilization.

2.5 Preparation of fermented beverages

Four formulations of fermented beverages were produced and they were named Control (no prebiotic addition), Inulin (with 35 g L^{-1} of inulin prebiotic), Succinoglycan (with 5 g L^{-1} of succinoglycan oligosaccharides), and Mix (with 30 g L^{-1} of inulin and 5 g L^{-1} of succinoglycan oligosaccharides).

To produce the four formulations of fermented beverages the following substances were added to 1 L of water-soluble soy and rice extract: 100 g L^{-1} of sucrose for the Control formulation, 95 g L^{-1} of sucrose for the Succinoglycan formulation, and 65 g L^{-1} of sucrose for the other formulations that were added with prebiotics. Posteriorly, the beverages were homogenized and pasteurized at 85 ± 1 °C for 20 minutes. Following this, they were cooled to a temperature of 40 ± 1 °C, added with 30 g L^{-1} of lactic culture which was previously prepared, and 2 g L^{-1} of lyophilized *Lactobacillus paracasei*. Then, the formulations were incubated in the greenhouse for 5 h at 38 ± 1 °C. They were stored on previously autoclaved glass flasks and kept at 5 ± 1 °C for 28 days.

2.6 Determination of pH, total titratable acidity, total soluble solids (TSS) and water activity (Aw)

The pH of the four formulations was measured using the MS TECNOPON mPA210 potentiometer (Piracicaba, Brazil). The total titratable acidity was determined by titrimetry according to the Ministry of Agriculture, Livestock and Food Supply - MAPA methodology with results expressed in g 100 mL^{-1} (Brasil, 2006).

The total soluble solids (TSS) content, given in ºBrix, was read in the 2WAJ refractometer (Biobrix, Curitiba, Brazil). And the water activity (Aw) measures were conducted in the Dew Point Water Activity Meter 4 TE Aqualab equipment (Pullman, The United States of America). All the analyses were carried out in sextuplicate.

2.7 Determination of rheology

The rheological parameters measurements were performed in the four formulations of fermented beverages on the 1st, 14th and 28th days of storage under refrigeration. The steady-state flow curve tests were performed on a DV2T viscometer model (Brookfield, USA) using an SC4-18 spindle and constant temperature of 11 °C, according to the methodology described by Miranda et al. (2019).

2.8 Determination of syneresis

The syneresis measurements of the four formulations were determined in triplicate, in accordance with Debon et al. (2012), with some modifications. The samples (10 g) from the four formulations were centrifuged at 350 g in a refrigerated centrifuge Rotanta 460R (Hettich Lab Technology, Massachusetts, The United States of America) to 5 ºC for 10 minutes. The supernatant was collected and weighted, and the syneresis was calculated through Equation 1:

$$\text{Syneresis (\%)} = (\text{Supernatant (g)} / \text{Fermented beverage (g)}) * 100 \quad (1)$$

2.9 Determination of the probiotic viability

Probiotic survival was determined in the four formulations during storage and in the simulated gastrointestinal conditions (SGIC). *L. casei* counts were determined on Man Rogosa and Sharp (MRS, Himedia®, Mumbai, India) agar supplemented with 2 mL L^{-1} of a 0.05 g 100 mL^{-1} vancomycin solution and anaerobic incubation at 37 ºC for 72 h (Tharmaraj & Shah, 2003). The survival of the probiotic culture to gastric and enteric conditions was carried out according to the methodology described by Costa et al. (2019). The analyses were performed in duplicated on the 1st, 14th and 28th days of storage under refrigeration.

Table 1. Average degree of polymerization (DPn) of succinoglycan oligosaccharides.

Time (min)	Mol $C_nH_{2n}On\ g_{sample}^{-1}$	Mol reducing end g_{sample}^{-1}	DP
1	0.004676	0.000450856	10.37137
15	0.004676	0.000507786	9.208607
30	0.004676	0.000516775	9.048431
60	0.004676	0.000519771	8.996270
180	0.004676	0.000537149	8.705213
240	0.004676	0.000555726	8.414213
300	0.004676	0.000576700	8.108197
360	0.004676	0.000584491	8.000128
480	0.004676	0.000603667	7.745994
540	0.004676	0.000619847	7.543800
600	0.004676	0.000609659	7.669856

DPn was estimated as the glucosyl monomer concentration ratio (mol $C_nH_{2n}On\ g_{sample}^{-1}$), defined by the phenol-sulfuric acid technique, divided by the concentration of the reducing end (mol reducing end g_{sample}^{-1}), stipulated by the modified 2,2'-bicinchoninate (BCA) method.

2.10 Analysis of the results

The results obtained in this research were submitted to analysis of variance (ANOVA) and the means compared by Tukey's test, considering the significance level of 5% ($p \leq 0,05$), using the Sisvar 5.6 program.

3 Results and discussion

3.1 Enzymatic hydrolysis of commercial succinoglycan for obtaining oligosaccharides

Table 1 presents the results of the average degree of polymerization (DPn) of succinoglycan oligosaccharides obtained at different time intervals after the enzymatic hydrolysis of commercial succinoglycan.

The commercial succinoglycan used in this research presented an average initial DP of 114.89. At the beginning of the process, there was a significant reduction in the DP, which showed slight oscillations during 10 hours of testing. Thus, in order to optimize the process time and also to avoid energy costs, it was established as standard the reaction time of 6 h, in which it was possible to obtain succinoglycan oligosaccharides with an average DP of 8. Shi et al. (2018) also performed the enzymatic hydrolysis of β-glucan, more specifically of curdlan. In this process, an enzyme from the GH 64 family was used β- (1 → 3) – glucans, and the enzymatic reaction was conducted for 16 h at 42 ºC. The authors obtained products with DP varying between 2 and 5 units.

3.2 Determination of pH, total titratable acidity and total soluble solids (TSS) and water activity (Aw)

Table 2 presents the results of pH, total titratable acidity and total soluble solids of the soy and rice-based fermented beverage formulations on the 1st, 14th and 28th day of storage at 5 ºC.

On the first day of storage, it was observed that the addition of prebiotics to the fermented beverage formulations caused a reduction in pH values ($p < 0.05$) when compared to the Control, except for the Succinoglycan formulation (Table 2). This reduction in the pH of the Inulin and Mix formulations can be attributed to the presence of inulin which stimulated the production of organic acids. According to a study by Silva Sabo et al. (2015), when *L. plantarum* cultures were supplemented with 1% of inulin, the released fructose monomers were assimilated via Embden-Meyerhof-Parnas, which led to higher lactic acid production.

During storage time, as expected, the pH values decreased with a proportional increase in the total titratable acidity (TTA) ($p < 0.05$). A similar situation was observed by Santos et al. (2019) for a soy-based fermented beverage with kefir using inulin as a probiotic with a pH reduction from 5.19 to 3.79 and TTA from 0.64 to 2.66% in 28 days of storage at 7 ± 0, 1 ºC. The reduction in pH values and the elevation of acidity during

storage are consequences of post-acidification of the product and are related to the production of organic acids resulting from the fermentation of carbohydrates by starter culture microorganisms and probiotics (Bedani et al., 2013; Costa et al., 2019).

Regarding the total soluble solids (TSS) content, on the first day of storage, the formulations with the addition of prebiotics showed lower values when compared to the Control. Such occurrence is the result of partial replacement of sucrose by inulin and/or succinoglycan oligosaccharides, since these ingredients have larger and therefore less soluble chains.

During storage, the soluble solids content of the formulation with inulin showed an increase in values ($p < 0.05$), while the Succinoglycan and Mix formulation remained practically stable during 28 days of storage. The results for the inulin formulation are probably related to the ability of the *Lactobacillus* strain to use oligofructoses as a substrate for its metabolism and organic acid production.

A water activity (Aw) study was also carried out and, the addition of prebiotics to the formulations did not result in a sufficient increase of solutes to reduce Aw and thus decrease the metabolism of microorganisms.

3.3 Determination of rheology

The rheological parameters of the four fermented beverage formulations are shown in Table 3.

The values of flow behavior index (*n*) and consistency index (K) were adjusted by the Power Law model. All formulations showed *n* less than 1, which indicates that such formulations are characterized as non-Newtonian fluids and have pseudoplastic behavior. For this type of fluid, the apparent viscosity of the material decreases with the increase in the deformation rate (Costa et al., 2016; Miranda et al., 2019).

The addition of inulin (Inulin formulation) resulted in a slight reduction in the consistency index (K) on the 1st and 14th day of storage. According to De Castro et al. (2009), this behavior can be attributed to a possible plasticizing effect of oligofructose, which results in less humectation and reduction of the hydrodynamic volume of the protein and, thus, lower viscosity.

In a study by Guimarães et al. (2018) it was observed that, in general, the addition of 6% of inulin and 0.5% and 0.05% of acacia and gellan gums, respectively, significantly interfered in the rheological parameters of whey fermented beverage formulations. The changes were: inulin crystallization, gel formation and protein interactions. The results found in the cited study were very different from the results observed in the present research, in which a higher amount of inulin was added. Probably, the authors reached this conclusion through the use of gums that are thickening agents.

The addition of succinoglycan oligosaccharides to the formulations caused a reduction of *n* and an increase of K. This

Table 2. Analysis of pH, total titratable acidity and total soluble solids of the four beverage formulations.

Parameters	Time (days)	Formulation			
		Control	Inulin	Succinoglycan	Mix
pH	1	4.30 ± 0.00^{Ba}	4.07 ± 0.01^{Ca}	4.36 ± 0.04^{Aa}	4.06 ± 0.02^{Ca}
	14	3.61 ± 0.06^{Ab}	3.41 ± 0.07^{Cb}	3.55 ± 0.07^{Bb}	3.42 ± 0.01^{Cb}
	28	3.35 ± 0.02^{Ac}	3.22 ± 0.02^{Bc}	3.35 ± 0.01^{Ac}	3.20 ± 0.01^{Bc}
Titratable acidity (% citric acid)	1	0.18 ± 0.01^{Bc}	0.23 ± 0.01^{Ac}	0.16 ± 0.01^{Bc}	0.22 ± 0.01^{Ac}
	14	0.36 ± 0.01^{Cb}	0.45 ± 0.01^{Ab}	0.30 ± 0.01^{Db}	0.43 ± 0.01^{Bb}
	28	0.50 ± 0.00^{Ba}	0.50 ± 0.01^{Ba}	0.44 ± 0.01^{Ca}	0.61 ± 0.02^{Aa}
TSS (°Brix)	1	10.70 ± 0.16^{Aa}	10.20 ± 0.13^{Cc}	10.37 ± 0.07^{Ba}	10.37 ± 0.07^{Ba}
	14	10.53 ± 0.05^{Ab}	10.50 ± 0.06^{Ab}	10.15 ± 0.05^{Bb}	10.25 ± 0.08^{Ba}
	28	10.18 ± 0.04^{Bc}	10.90 ± 0.12^{Aa}	10.23 ± 0.09^{Bab}	10.28 ± 0.09^{Ba}

Mean values ± standard deviation; Different capital letters in the same row indicate that there was a statistical difference ($p < 0.05$, $n = 6$) between formulations, different lowercase letters in the column indicate that there was a statistical difference ($p < 0.05$, $n = 6$) in the storage days for each formulation.

Table 3. Rheological parameters of fermented beverage formulations.

Parameters	Time(Days)	Control	Inulin	Succinoglycan	Mix
K (mPa)	1	127.80 ± 1.40	83.77 ± 1.37	1571.10 ± 56.10	976.50 ± 10.60
	14	99.96 ± 3.10	82.50 ± 0.80	1804.20 ± 19.20	797.20 ± 7.00
	28	77.34 ± 0.51	78.13 ± 2.10	887.80 ± 18.80	225.80 ± 1.40
n	1	0.72 ± 0.00	0.69 ± 0.00	0.33 ± 0.01	0.31 ± 0.00
	14	0.66 ± 0.00	0.69 ± 0.00	0.14 ± 0.00	0.33 ± 0.00
	28	0.68 ± 0.00	0.70 ± 0.00	0.31 ± 0.00	0.55 ± 0.00
η_{ap} (100 s^{-1}) ($mPa.s^{-1}$)	1	35.69 ± 0.00	20.00 ± 0.00	72.81 ± 0.00	40.90 ± 0.00
	14	21.18 ± 0.00	19.34 ± 0.00	34.86 ± 0.00	36.77 ± 0.00
	28	17.80 ± 0.00	19.27 ± 0.00	36.67 ± 0.00	27.91 ± 0.00

The abbreviations are: K, consistency index; *n*, flow behavior index and η_{ap}, apparent viscosity. Mean values ± standard deviation.

indicates that the formulations have become more consistent. According to Ruiz et al. (2015), the rheological behavior of succinoglycans can be influenced by several factors, such as the substitutes acetate, pyruvate and succinate. This fact was confirmed in a study carried out by Simsek et al. (2009), in which it was found that a small amount of the succinyl group in the succinoglycan molecule significantly increased the consistency index. The increase in consistency of the Succinoglycan and Mix formulations, resulting from the addition of succinoglycan oligosaccharides, can become an appealing organoleptic feature because it will meet the consumer's preference when they opt for more consistent formulations.

3.4 Determination of syneresis

Figure 1 presents the results of syneresis for the four fermented beverage formulations evaluated in this research.

Except for the first day of storage, the Inulin formulation did not present a significant difference when compared to the Control ($p > 0.05$), which indicates that the addition of the inulin prebiotic did not affect the syneresis, due to its low viscosity and low degree of polymerization. Similar behavior was observed by Guimarães et al. (2018) when adding 6% of inulin with the degree of polymerization DP ≤ 10 to the whey drink with graviola flavor, however, when they used inulin with the degree of polymerization DP ≥ 23, lower values for syneresis were obtained.

On the other hand, the addition of succinoglycan oligosaccharides to the Succinoglycan and Mix formulations provided lower syneresis values throughout the evaluated storage period in relation to the Control and Inulin formulations. The higher viscosity observed in these formulations is an indication that they presented more hydration potential of the molecules, which contributed to the lower syneresis.

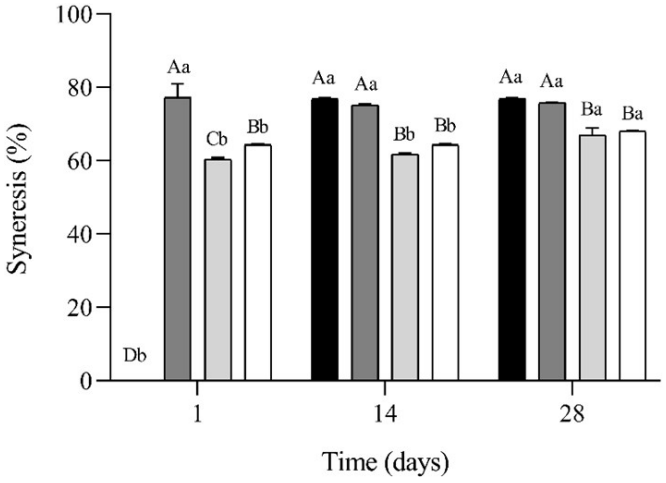

Figure 1. Syneresis (%) of the four fermented beverage formulations: Control (■); Inulin (■); Succinoglycan (■); Mix (■). The error bars represent the standard deviation. Different capital letters indicate statistical difference ($p < 0.05$, $n = 3$) between formulations. Different lowercase letters indicate a statistical difference between storage periods ($p < 0.05$, $n = 3$).

With storage, there was a slight increase in syneresis for both formulations with succinoglycan oligosaccharides, which may have occurred due to a drop in pH. Factors such as molecular weight, temperature, pH, ionic strength of the solutions, electrical load of the hydrocolloids and concentrations of polymers are some of the aspects that can interfere with the quality of protein-polysaccharide interactions (Yousefi & Jafari 2019).

In a research, Barbosa et al. (2020) made fermented beverages from different percentages of water-soluble soy and Brazil nut extracts. The authors obtained syneresis values ranging from 50% to 70%. These values were relatively close to the ones achieved in this research, in general.

3.5 Determination of probiotic viability during storage and exposure to simulated gastrointestinal conditions (SGIC)

Figure 2 shows the results of the probiotic viability of *Lactobacillus paracasei* in fermented beverages during refrigerated storage and exposed to simulated gastrointestinal conditions.

All formulations presented counts greater than 10^8 mL^{-1} CFU (Figure 2A) during the storage period. These values are in accordance with Brazilian legislation, which recommends a minimum population of cells ranging from 10^8 to 10^9 CFU mL^{-1} per daily portion of product to provide beneficial health effects (Pimentel et al., 2015). Soy-based products are promising in terms of maintaining probiotic viability since the grain is a great substrate for the development of these bacteria (Bedani et al., 2013; Mishra & Mishra, 2018). The veracity of this statement was confirmed in this research, since the viable cell counts obtained for the Control formulation, without the addition of prebiotics, remained above 10^8 CFU mL^{-1} during the entire period evaluated. Therefore, any of the formulations evaluated could be considered probiotic during the 28 days of refrigerated storage.

The viability of *L. paracasei* for the formulations of fermented beverages submitted to simulated gastrointestinal conditions (SGIC) is presented in Figures 2B, 2C and 2D. The probiotic microorganisms survived the SGIC in all the evaluated formulations, showing counts over 10^4 CFU mL^{-1}. Thus, reductions of 3.79, 2.67, 2.63 and 2.27 log cycles are observed for the Control, Inulin, Succinoglycan and Mix formulations, respectively. The reduced counts of *L. paracasei* were close to the results obtained in the study conducted by Costa et al. (2019), that is, 2 and 3 log cycles. In this study, the probiotic viability of *L. casei* was evaluated in yogurts sweetened with natural ingredients (stevia, erythritol and xylitol) and prebiotics (oligofructose or polydextrose).

In the gastric phase (Figure 2B) there was a reduction in the viability of the culture of *L. paracasei* for all formulations in the period evaluated. Thus, the Control formulation presented the highest average reduction, 2.39 log cycles, followed by the Succinoglycan, Inulin and Mix formulations, with 1.95, 1.37 and 1.33, respectively. These reductions in probiotic viability indicate that the strain is highly sensitive to simulated gastric juice. It is also noted that the presence of the prebiotic inulin was essential to improve the survival of the microorganism. In the same way, Buriti et al. (2010), noted that the incorporation of inulin to guava mousses improved the tolerance of *L. acidophillus* La-5 to gastrointestinal conditions at the beginning of storage.

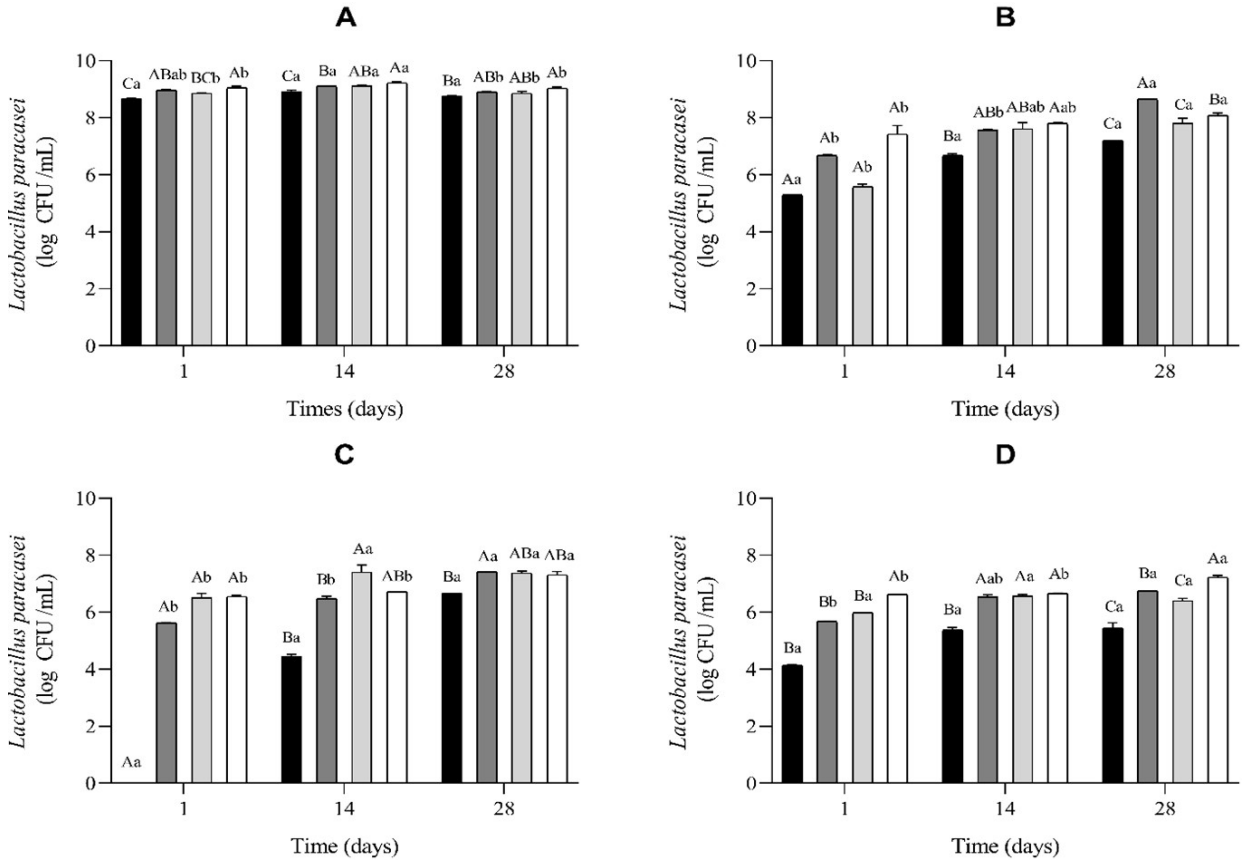

Figure 2. Probiotic viability (CFU mL^{-1} log) of the four fermented beverage formulations: Control (■), Inulin (■), Succinoglycan (■) and Mix (■), during storage (A) and SGIC: gastric phase (B), enteric phase I (C) and enteric phase II (D). The error bars represent the standard deviation. Different capital letters indicate statistical difference ($p < 0.05$, $n = 2$) between formulations. Different lowercase letters indicate a statistical difference between storage periods ($p < 0.05$, $n = 2$).

The authors associated the protective effect of inulin with its ability to bind to the water available in the food matrix and form gel, which is composed of a three-dimensional network of microcrystals that interact and form small aggregates that conglomerate considerable amounts of water. This structure may have involved the bacterial cells within the food matrix and thus contributed to physical protection.

The probiotic microorganism was also exposed to enteric phases I and II (Figures 2C and 2D), which are added bile and have the ability to affect phospholipids and proteins in cell membranes, and interrupt cell homeostasis (Begley et al., 2005). In the enteric phase I (Figure 2C) the formulations with prebiotics presented higher counts than the Control on all days evaluated, but with a significant difference only on the 14th and 28th day, for the Succinoglycan and Inulin formulations, respectively. The storage did not affect the survival of the microorganism, as the counts were, in general, maintained or increased. Probiotic survival during exposure to the gastrointestinal tract is influenced by both the matrix and food ingredients, which can bind to bile acids and prevent them from exerting their toxicity on probiotics (Bedani et al., 2013).

According to Millette et al. (2013), for a probiotic culture to provide benefits to the individual it is necessary that it survives and reaches the gastrointestinal tract with counts of at least 10^6 and 10^7 CFU g^{-1}. Therefore, at the end of the SGIC exposure (Figure 2D - enteric phase II), the Control formulation would not be able to provide probiotic health benefits on any day evaluated. Inulin, Succinoglycan and Mix formulations, on the other hand, proved to be suitable until the end of the storage period. A similar research was conducted by Bedani et al. (2013), who investigated the survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 in different soy milk treatments which were supplemented with inulin and okara and submitted to gastrointestinal conditions in vitro during 28 days of storage at 4 °C. These authors observed that the protective effect of okara flour and/or inulin on probiotics was not significant, while the food matrix improved the survival of *Bifidobacterium animalis* Bb-12 and therefore could be considered a good vehicle for the delivery of this microorganism, and also an important protective agent against gastrointestinal juices.

Therefore, it was observed in this research that both food matrix components and prebiotic supplementation were essential to protect *L. paracasei* cells during exposure to simulated gastrointestinal conditions, and the combination of inulin and succinoglycan oligosaccharides provided even greater resistance to probiotic cells.

4 Conclusion

The fermented beverage developed in this research proved to be a bold food alternative for people who have lactose intolerance, allergy to milk proteins or have chosen a different lifestyle. This beverage is a functional food that combines the nutritional properties present in soybean and rice with the benefits of adding microorganisms with probiotic functions, and also the positive effects of inulin and succinoglycan oligosaccharides, which favored increased probiotic resistance during exposure to simulated gastrointestinal conditions, in addition reducing syneresis, positively interfere in rheological parameters and influence less in the variation of physical-chemical parameters such as acidity and soluble solids of drinks.

In this way, the elaborated product can collaborate with the food industry in meeting the wishes of the current population regarding functional foods and thus help in the cure or prevention of diseases such as cardiovascular, certain types of cancer, allergies, intestinal problems, among others. Moreover, this is an innovative product that can meet the needs of the market in creating and launching new products for companies that intend to maintain or establish their leadership in the functional food market.

References

Barbosa, M. L. S., Alencar, E. R., Leandro, E. S., Borges, R. M., Mendonça, M. A., & Ferreira, W. F. S. (2020). Characterization of fermented beverages made with soybean and Brazil nut hydrosoluble extracts. *International Journal of Gastronomy and Food Science*, 21, 100228. http://dx.doi.org/10.1016/j.ijgfs.2020.100228.

Bauermeister, A., Rezende, M. I., Giese, E. C., Dekker, R. F. H., & Barbosa, A. M. (2010). Fungal β-1,3-Glucanases: production and biotechnological applications. *Semina. Ciências Exatas e Tecnológicas*, 31, 75-86. http://dx.doi.org/10.5433/1679-0375.2010v31n2p75.

Bedani, R., Rossi, E. A., & Saad, S. M. I. (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 Microbiology*, 34(2), 382-389. http://dx.doi.org/10.1016/j.fm.2013.01.012. PMid:23541206.

Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *Lebensmittel-Wissenschaft + Technologie*, 55(2), 436-443. http://dx.doi.org/10.1016/j.lwt.2013.10.015.

Begley, M., Gahan, C. G. M., & Hill, C. (2005). The interaction between bacteria and bile. *FEMS Microbiology Reviews*, 29(4), 625-651. http://dx.doi.org/10.1016/j.femsre.2004.09.003. PMid:16102595.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. (2006, December 12). Instrução Normativa nº 68, de 12 de dezembro de 2006. Oficializa os métodos analíticos oficiais físico-químicos, para controle de leite e produtos lácteos, em conformidade com o anexo desta Instrução Normativa, determinando que sejam utilizados nos Laboratórios Nacionais Agropecuários. *Diário Oficial [da] República Federativa do Brasil*.

Buriti, F. C. A., Castro, I. A., & Saad, S. M. I. (2010). Viability of *Lactobacillus acidophilus* insynbiotic guava mousses and its survival under in vitro simulated gastrointestinalconditions. *International Journal of Food Microbiology*, 137(2-3), 121-129. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.11.030. PMid:20056289.

Castro, F. P., Cunha, T. M., Barreto, P. L. M., Amboni, R. D. C., & Prudêncio, E. (2009). Effect of oligofructose incorporation on the properties of fermented probiotic lactic beverages. *International Journal of Dairy Technology*, 62(1), 68-74. http://dx.doi.org/10.1111/j.1471-0307.2008.00447.x.

Costa, G. M., Paula, M. M., Barão, C. E., Klososki, S. J., Bonafé, E. G., Visentainer, J. V., Cruz, A. G., & Pimentel, T. C. (2019). Yoghurt added with *Lactobacillus casei* and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. *International Dairy Journal*, 97, 139-148. http://dx.doi.org/10.1016/j.idairyj.2019.05.007.

Costa, K. K. F. D., Garcia, M. C., Ribeiro, K. D. O., Soares, M. S. Jr, & Caliari, M. (2016). Rheological properties of fermented rice extract with probiotic bacteria and different concentrations of waxy maize starch. *LWT - Food Science and Technology*, 72, 71-77. http://dx.doi.org/10.1016/j.lwt.2016.04.014.

Costa, K. K. F. D., Soares, M. S. Jr., Rosa, S. I. R., Caliari, M., & Pimentel, T. C. (2017). Changes of probiotic fermented drink obtained from soy and rice byproducts during cold storage. *Lebensmittel-Wissenschaft + Technologie*, 78, 23-30. http://dx.doi.org/10.1016/j.lwt.2016.12.017.

Debon, J., Prudêncio, E. S., Petrus, J. C. C., Fritzen-Freire, C. B., Müller, C. M. O., Amboni, R. D. de M., & Vieira, C. R. W. (2012). Storage stability of prebiotic fermented milk obtained from permeate resulting of the microfiltration process. *LWT - Food Science and Technology*, 47(1), 96-102. http://dx.doi.org/10.1016/j.lwt.2011.12.029.

Fazilah, N. F., Ariff, A. B., Khayat, M. E., Rios-Solis, L., & Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods*, 48, 387-399. http://dx.doi.org/10.1016/j.jff.2018.07.039.

Gibson, G. R. (2004). Fibre and effects on probiotics (the prebiotic concept). *Clinical Nutrition Supplements*, 1(2), 25-31. http://dx.doi.org/10.1016/j.clnu.2004.09.005.

Guimarães, J. T., Silva, E. K., Costa, A. L. R., Cunha, R. L., Freitas, M. Q., Meireles, M. A. A., & Cruz, A. G. (2018). Manufacturing a prebiotic whey beverage exploring the influence of degree of inulin polymerization. *Food Hydrocolloids*, 77, 787-795. http://dx.doi.org/10.1016/j.foodhyd.2017.11.021.

Gurpilhares, D. B., Cinelli, L. P., Simas, N. K., Pessoa, A. Jr., & Sette, L. D. (2019). Marine prebiotics: polysaccharides and oligosaccharides obtained by using microbial enzymes. *Food Chemistry*, 280, 175-186. http://dx.doi.org/10.1016/j.foodchem.2018.12.023. PMid:30642484.

Handa, S., & Sharma, N. (2016). *In vitro* study of probiotic properties of *Lactobacillus plantarum* F22 isolated from chang – a traditional fermented beverage of Himachal Pradesh, India. *Journal of Genetic Engineering and Biotechnology*, 14(1), 91-97. http://dx.doi.org/10.1016/j.jgeb.2016.08.001. PMid:30647602.

He, M., Qiu, C., Liao, Z., Sui, Z., & Corke, H. (2018). Impact of cooking conditions on the properties of rice: combined temperature and cooking time. *International Journal of Biological Macromolecules*, 117, 87-94. http://dx.doi.org/10.1016/j.ijbiomac.2018.05.139. PMid:29792958.

Jofré, E., Liaudat, J. P., Medeot, D., & Becker, A. (2018). Monitoring succinoglycan production in single Sinorhizobium meliloti cells by Calcofluor white M2R staining and time-lapse microscopy. *Carbohydrate Polymers*, 181, 918-922. http://dx.doi.org/10.1016/j.carbpol.2017.11.059. PMid:29254054.

Mandial, D., Khullar, P., Mahal, A., Kumar, H., Singh, N., Ahluwalia, G. K., & Bakshi, M. S. (2018). Applications of rice protein in nanomaterials synthesis, nanocolloids of rice protein, and bioapplicability. *International*

Journal of Biological Macromolecules, 120(Pt A), 394-404. http://dx.doi.org/10.1016/j.ijbiomac.2018.08.101. PMid:30144545.

Mangolim, C. S., Silva, T. T., Fenelon, V. C., Nascimento, A., Sato, F., & Matioli, G. (2017). Use of FT-IR, FT-Raman and thermal analysis to evaluate the gel formation of curdlan produced by Agrobacterium sp. IFO 13140 and determination of its rheological properties with food applicability. *Food Chemistry*, 232, 369-378.

Millette, M., Nguyen, A., Amine, K. M., & Lacroix, M. (2013). Gastrointestinal survival of bacteria in commercial probiotic products. *International Journal of Probiotics & Prebiotics*, 8, 149-156.

Miranda, R. F., Paula, M. M., Costa, G. M., Barão, C. E., Silva, A. C. R., Raices, R. S. L., Gomes, R. G., & Pimentel, L. T. C. (2019). Orange juice added with *L. casei*: is there an impact of the probiotic addition methodology on the quality parameters? *Lebensmittel-Wissenschaft + Technologie*, 106, 186-193. http://dx.doi.org/10.1016/j.lwt.2019.02.047.

Mishra, S., & Mishra, H. N. (2018). Comparative study of the synbiotic effect of inulin and fructooligosaccharide with probiotics with regard to the various properties of fermented soy milk. *Food Science & Technology International*, 24(7), 564-575. http://dx.doi.org/10.1177/1082013218776529. PMid:29764202.

Nawaz, A., Javaid, A. B., Irshad, S., Hoseinifar, S. H., & Xiong, H. (2018). The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. *Fish & Shellfish Immunology*, 76, 272-278. http://dx.doi.org/10.1016/j.fsi.2018.03.004. PMid:29510254.

Olivares, A., Soto, C., Caballero, E., & Altamirano, C. (2019). Survival of *microencapsulated Lactobacillus casei* (prepared by vibration technology) in fruit juice during cold storage. *Electronic Journal of Biotechnology*, 42, 42-48. http://dx.doi.org/10.1016/j.ejbt.2019.10.002.

Pimentel, T. C., Madrona, G. S., Garcia, S., & Prudêncio, S. H. (2015). Probiotic viability, physicochemical characteristics and acceptability during refrigerated storage of clarified apple juice supplemented with Lactobacillus paracasei ssp. paracasei and oligofructose in different package type. *LWT - Food Science and Technology*, 63, 415-422.

Ruiz, S. P., Martinez, C. O., Noce, A. S., Sampaio, A. R., Baesso, M. L., & Matioli, G. (2015). Biosynthesis of succinoglycan by Agrobacterium radiobacter NBRC12665 immobilized on loofa sponge and cultivated in sugar canemolasses. Structural and rheological characterization of biopolymer. *Journal of Molecular Catalysis. B, Enzymatic*, 122, 15-28. http://dx.doi.org/10.1016/j.molcatb.2015.08.016.

Santos, D. C., Oliveira, J. G. Fo., Santana, A. C. A., Freitas, B. S. M., Silva, F. G., Takeuchi, K. P., & Egea, M. B. (2019). Optimization of soymilk fermentation with kefir and the addition of inulin: physicochemical, sensory and technological characteristics. *Lebensmittel-Wissenschaft + Technologie*, 104, 30-37. http://dx.doi.org/10.1016/j.lwt.2019.01.030.

Savedboworn, W., Niyomrat, S., Naknovn, J., & Phattayakorn, K. (2017). Impact of inulin on viability and storage stability of probiotic *Lactobacillus plantarum* TISTR 2075 in fermented rice extract. *Agriculture and Natural Resources*, 51(6), 463-469. http://dx.doi.org/10.1016/j.anres.2018.03.008.

Shi, Y., Liu, J., Yan, Q., You, X., Yang, S., & Jiang, Z. (2018). In vitro digestibility and prebiotic potential of curdlan (1→3)-β-D-glucan oligosaccharides in *Lactobacillus* species. *Carbohydrate Polymers*, 188, 17-26. http://dx.doi.org/10.1016/j.carbpol.2018.01.085. PMid:29525154.

Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H. R., Shakeel, A., Ansari, A., & Niazi, S. (2016). Inulin: properties, health benefits and food applications. *Carbohydrate Polymers*, 147, 444-454. http://dx.doi.org/10.1016/j.carbpol.2016.04.020. PMid:27178951.

Silva Sabo, S., Converti, A., Todorov, S. D., Domínguez, J. M., & Souza Oliveira, R. P. (2015). Effect of inulin on growth and bacteriocin production by *Lactobacillus plantarum* in stationary and shaken cultures. *International Journal of Food Science & Technology*, 50(4), 864-870. http://dx.doi.org/10.1111/ijfs.12711.

Simsek, S., Mert, B., Campanella, O. H., & Reuhs, B. (2009). Chemical and rheological properties of bacterial succinoglycan with distinct structural characteristics. *Carbohydrate Polymers*, 76(2), 320-324. http://dx.doi.org/10.1016/j.carbpol.2008.10.033.

Soares, M. B., Martinez, R. C. R., Pereira, E. P. R., Balthazar, C. F., Cruz, A. G., Ranadheera, C. S., & Sant'Ana, A. S. (2019). The resistance of *Bacillus, Bifidobacterium*, and *Lactobacillus* strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Research International*, 125, 108542. http://dx.doi.org/10.1016/j.foodres.2019.108542. PMid:31554104.

Tharmaraj, N., & Shah, N. P. (2003). Selective enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*, bifidobacteria, *Lactobacillus casei, Lactobacillus rhamnosus*, and propionibacteria. *Journal of Dairy Science*, 86(7), 2288-2296. http://dx.doi.org/10.3168/jds.S0022-0302(03)73821-1. PMid:12906045.

Turkmen, N., Akal, C., & Özer, B. (2019). Probiotic dairy-based beverages: a review. *Journal of Functional Foods*, 53, 62-75. http://dx.doi.org/10.1016/j.jff.2018.12.004.

Vitola, H. R. S., Dannenberg, G. S., Marques, J. L., Lopes, G. V., Silva, W. P., & Fiorentini, A. M. (2018). Probiotic potential of *Lactobacillus casei* CSL3 isolated from bonive colostrum silage and its viability capacity immobilized in soybean. *Process Biochemistry*, 75, 22-30. http://dx.doi.org/10.1016/j.procbio.2018.09.011.

Yousefi, M., & Jafari, S. M. (2019). Recent advances in application of different hydrocolloids in dairy products to improve their techno-functional properties. *Trends in Food Science & Technology*, 88, 468-483. http://dx.doi.org/10.1016/j.tifs.2019.04.015.

Zhang, Y. H., & Lynd, L. R. (2005). Determination of the number-average degree of polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis. *Biomacromolecules*, 6(3), 1510-1515. http://dx.doi.org/10.1021/bm049235j. PMid:15877372.