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Probiotic fermented beverages processed with water-soluble rice extract and added with curdlan oligosaccharides and oligofructose: physicochemical characteristics, rheological parameters, and storage stability

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Abstract

The effect of oligofructose and/or curdlan oligosaccharides (CO) addition on the characteristics of probiotic fermented beverages processed with water-soluble rice extract (WSRE) was evaluated. The products were assessed for the physicochemical characteristics, rheological parameters, and probiotic survival (*L. casei)* during storage (28 days, 7 °C). Probiotic counts higher than 10^8 CFU mL^{-1} and 10^6 CFU mL^{-1} were observed during storage and after simulated gastrointestinal conditions (SGIC). Oligofructose addition increased the red color of the products (increase in a* values) and acted as a prebiotic component during storage and SGIC, increasing probiotic survival. CO addition resulted in higher acidity (lower pH and higher titratable acidity values) and lower consistency (lower *k* and higher *n* values). It acted as a prebiotic component during fermentation, storage, and SGIC, promoting the highest increase in probiotic survival. Furthermore, CO improved the storage stability, reducing the post-acidification and the alterations in the rheological parameters.

Keywords: *L. casei*; *Oryza sativa*; prebiotic; nondairy products.

Practical Application: It demonstrates that curdlan oligosaccharides can be used as prebiotic components in developing synbiotic fermented beverages of water-soluble rice extract.

1 Introduction

Probiotics or true probiotics are living microorganisms that confer beneficial effects on the individual when administered in adequate amounts (Hill et al., 2014; Zendeboodi et al., 2020). *L. casei*-01 consumption has been associated with several health effects, such as reduction of postprandial glycemia (Grom et al., 2020), decrease in oxidative stress (Vasconcelos et al., 2019), improvements in the hematological and lipid profiles (Sperry et al., 2018), and reduction of the risk of colon cancer (Balthazar et al., 2021). Other *L. casei* species may also improve bone health (Lee et al., 2020) and modulate the gut-bone axis (Eor et al., 2020). Nowadays, most of the probiotic products available on the market are dairy products (yogurts and fermented milks) (Mantovani et al., 2020; Camelo-Silva et al., 2021; Al-Sulbi & Shori, 2022).

There is a demand for nondairy probiotic products, mainly because of the increased number of lactose intolerant, casein allergic, and vegan individuals (Savedboworn et al., 2017; Araujo-Rodrigues et al., 2021). Rice is a highly energetic product, as it contains starch (90%), proteins (7-8%), minerals (iron, phosphorous, and calcium), and B vitamins (Silva et al., 2020). Furthermore, rice protein has a high nutritive value because of its high lysine content (Rabo & Dewidar, 2017). In this way, the water-soluble rice extract (WSRE) could be an alternative to substitute milk in nondairy products, as rice is present in the population's diet, has a low cost of acquisition, and has mild flavor (Prasad et al., 2018). In addition, it has been recognized as the most hypoallergenic of the plant water-soluble extracts and recommended as the best alternative to cow milk for people with milk, soy, or nut allergies and lactose intolerance (Xiong et al., 2022). Finally, the production of rice-based beverages is associated with only 22-38% of the greenhouse gas emissions compared to dairy products' production (Craig & Fresán, 2021).

However, studies concerning probiotic fermented beverages processed with WSRE are still scarce (Costa et al., 2017; Freire et al., 2017; El-Sayed & Ramadan, 2020), and they used other cereals (soy, cassava or maize) together with rice. Probiotic addition to fermented beverages processed only with WSRE could be more complicated since the drinks can have insufficient peptides and free amino acids concentrations. In addition, the probiotic cultures are generally more sensitive to the environmental conditions in these matrices (Pimentel et al., 2015). Therefore, including prebiotic components can be a suitable alternative to increase the probiotic survival in the products.

Prebiotics are non-viable food components that confer health benefits to the host associated with the modulation of the microbiota (Gibson et al., 2017). Inulin-type fructans (oligofructose and inulin) are the most used prebiotic components, and they are commercially obtained, mainly from chicory roots (Fonteles & Rodrigues, 2018). The utilization of prebiotic

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ingredients synthesized by microorganisms has emerged, as it is possible to control the growth conditions and produce prebiotic components with unique properties (Mangolim et al., 2017). Curdlan is a microbial extracellular homopolysaccharide of β-1,3-glucan (Verma et al., 2020). The hydrolysis of curdlan into oligosaccharides with lower molecular weight can increase its solubility, improve the health effects, and increase probiotic survival (Shi et al., 2018; Tang et al., 2019). However, the impact of the addition of curdlan oligosaccharides (CO) on probiotic survival in nondairy products has not been previously studied.

Synbiotics are a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms, which confers a health benefit. Thus, a 'synergistic synbiotic' is a synbiotic in which the substrate is designed to be selectively utilized by the co-administered microorganism(s). At the same time, a 'complementary synbiotic' is a synbiotic composed of a probiotic combined with a prebiotic, which is designed to target autochthonous microorganisms (Swanson et al., 2020). In this way, in a synergistic synbiotic, it is expected that the prebiotic compound could increase the viability of the probiotic culture in food and after gastrointestinal digestion (Santos et al., 2019). Therefore, the objective of the present study was to evaluate the impact of the addition of oligofructose and/or CO as prebiotic components on the physicochemical characteristics, rheological parameters, and probiotic survival of probiotics (*L. casei*) fermented beverages processed with WSRE.

2 Materials and methods

2.1 Material

Rice (Migra, Meleiro, Brazil), refined sugar (Alto Alegre, Colorado, Brazil), concentrated pasteurized grape juice (Maguary, Araguari, Brazil), oligofructose (P95, Orafti®, DP = 4-5, Tienen, Belgium), and curdlan (*Fujifilm Wako Chemicals*®) were used in the experiment. *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (YF-L812, Christian Hansen®, Valinhos, Brazil) were used as starter cultures, and *L. casei* (*L. casei*-01, Christian Hansen®) was used as a probiotic culture.

2.2 Curdlan oligosaccharides processing

For obtaining the CO, a curdlan solution (1.5 g L^{-1}) was prepared in 0.024 M sodium acetate buffer with pH adjusted to 5.0 using 1M HCl and stirred for 48 h at 25 °C. Subsequently, a volume of 0.38 mL L^{-1} of Viscozyme (Rosset et al., 2012) was added to the fermenter vessel (Bioflo® Celligen® 115 - Eppendorf) containing 5 L of the solution and kept at 45 °C and 150 rpm for 7 h. Next, the enzyme inactivation was performed by heating the aliquots of the reaction mixture at 95 °C for 10 min. Then, the degree of polymerization (DP) was determined according to Mangolim 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 was then lyophilized.

2.3 Processing of the water-soluble rice extract (WSRE)

The rice was weighed, washed under potable water, and added with distilled water (30 g of rice L $water^{-1}$). According to the manufacturer's recommendation, the mixture was processed in a commercial extractor (QLink®) for 35 min. Then, the WSRE was homogenized and filtered through a sieve (Costa et al., 2017).

2.4 Probiotic fermented beverage processed with WSRE

Four probiotic fermented beverage formulations were prepared: CONTR (control), OLIGO (with oligofructose), CURDLAN (with CO), and MIX (with oligofructose + CO). First, the WSRE was added with 100 g L^{-1} of sucrose and the prebiotic component (35 g L^{-1} of oligofructose for OLIGO, 35 g L^{-1} of CO for CURDLAN 17.5 gL^{-1} of oligofructose, and 17.5 g L^{-1} of CO for MIX). Next, the mixture was pasteurized at 85 °C for 20 min in a water bath (Marconi®, Piracicaba, Brazil) and cooled to 38 °C. Then, 0.2 g L^{-1} of the probiotic culture and 30 mLL^{-1} of the starter culture were added, and the mixture was incubated at 38 °C for 5 h. After fermentation, the probiotic fermented beverage was added with 75 g L^{-1} of grape juice, packaged in polypropylene plastic packages (80 mL capacity), and stored at 7 °C for 28 days.

2.5 Physicochemical characteristics and rheological parameters of the probiotic fermented beverage processed with WSRE

The pH was determined using a digital potentiometer (MS Technopon®, Piracicaba, Brazil). The titratable acidity was determined according to the methodology proposed by Association of Official Analytical Chemists (2000) and expressed in g 100 mL^{-1} of NaOH. The total soluble solids (TSS) were determined in a digital refractometer (Instruterm®, São Paulo, Brazil) and expressed as °Brix.

The chemical composition (moisture, protein, ash, lipids, and carbohydrates) was determined according to Association of Official Analytical Chemists (2000). The color parameters (L*, a*, and b*) were determined using a colorimeter (Konica Minolta®, model CR-410, Tokyo, Japan).

For rheological parameters, 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).

2.6 Probiotic survival

Probiotic survival was determined 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 °C 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).

2.7 Design of the experiment and statistical analysis

The experiment was repeated four times following a completely randomized design, and the analyzes were performed in triplicates. The chemical composition was evaluated on the 1st and 28th days, while physicochemical characteristics, rheological parameters,

and probiotic survival were determined every seven days for 28 days. In the SGIC test, probiotic survival was determined after each digestion step (gastric, 1st enteric phase, and 2nd enteric phase). A split-plot design was used to analyze the data (the primary treatment was the formulation, and the secondary treatment was the storage time). The results were submitted to analysis of variance (ANOVA) and Tukey test (p = 5%) using ASSISTAT 7.7 (UFCG, Campina Grande, Brazil) and Statistical Analysis System (SAS, Institute Inc., Cary, NC, USA) software.

3 Results and discussion

3.1 Curdlan oligosaccharides degree of polymerization

The curdlan used in the present study had a DP of 548.15. The initial DP of the mixture was 248.58, and the DP had a progressive decrease until 7 h of hydrolysis, reaching a DP of 6.32 (Table 1). After that, the DP was maintained in the range of 6.39-6.95. Therefore, aiming for a process with lower cost and time saving, the time of curdlan hydrolysis selected was 7 h, and the CO presented an average DP of 6.32.

3.2 Physicochemical characteristics and rheological parameters of the probiotic fermented beverage processed with WSRE

As no differences were observed for chemical composition during storage ($p > 0.05$), only the results of 1st day are showed. The fermented beverages presented chemical composition in the following ranges (g 100 g^{-1}, Table 2): moisture (85.7-88.5), ash (0.55-0.56), proteins (0.36-0.38), and carbohydrates (10.55-13.30). The products did not have significant concentrations of fat. The addition of the prebiotic components did not alter ($p > 0.05$) the beverages' ash, protein, and fat contents. The addition of oligofructose did not change ($p > 0.05$) the moisture and carbohydrate contents. In contrast, a decrease in the moisture content and an increase in the carbohydrate content were observed when CO (CURDLAN) and oligofructose + CO (MIX) were added ($p < 0.05$). The addition of soluble oligosaccharides can increase the total solids of the products, and, consequently, a decrease in the moisture content and an increase in the carbohydrate content are observed (Santos et al., 2019). Maintaining the moisture and carbohydrate contents after the addition of oligofructose can be associated with this prebiotic component's higher water retention capacity (Apolinário et al., 2014). This fact can also justify the differences between CURDLAN and MIX formulations in moisture and carbohydrate contents. The MIX formulation presents higher moisture and lowers carbohydrate contents than the CURDLAN formulation ($p < 0.05$). The present study results demonstrate that the addition of the prebiotic components has no negative impact on the nutritional value of the probiotic fermented beverages.

Table 1. Curdlan degree of polymerization (DP) during hydrolysis.

Sample	Time (h)	mol de term g $sample^{-1}$	DPn
Curdlan, brand Wako, lot: STE7258	0	2.6764E-05	248.5807
	0.5	0.00014833	44.85192
	1	0.00034233	19.43442
	2	0.00059327	11.214
	3	0.00072197	9.214933
	4	0.00079386	8.380451
	5	0.00084475	7.875605
	6	0.00092404	7.199779
	7	0.00105221	6.322813
	8	0.0010409	6.391506
	9	0.00095703	6.951644
	10	0.00099095	6.713651

The probiotic fermented beverages presented a pH of 2.95-4.18, titratable acidity (TA) of 0.14-0.56 g 100 mL^{-1} of NaOH, and TSS of 10.31-12.53 °Brix (Table 3). The addition of oligofructose made no impact on the pH and TA values ($p > 0.05$, day 1), while the addition of CO (CURDLAN) or both prebiotics (MIX) resulted in a decrease in the pH values and increased in the TA ($p < 0.05$). The starter and/or probiotic cultures probably used part of the CO during fermentation, resulting in small organic acids (Batista et al., 2017). The higher initial acidity of the fermented beverages added with CO can impact the probiotic survival and sensory acceptance (Januário et al., 2018).

All fermented beverage formulations behaved similarly during the storage period, with decreases in the pH values and increases in the TA values ($p < 0.05$). The decline in pH and the increase in the acidity are associated with the post-acidification of the products, as the starter and/or probiotic cultures continue the fermentative process during the storage period, with the production of small amounts of organic acids (Costa et al., 2019). It could be observed that the post-acidification process was less pronounced in the formulations added with CO, as CURDLAN and MIX formulations presented a lower decrease in pH (0.46-0.55 units) and a lower increase in TA (0.29-0.32 g 100 mL^{-1} of NaOH) than the CONTR formulation (1.2 units and 0.36 g 100 mL^{-1} of NaOH, respectively, $p < 0.05$). The results

Table 2. Chemical composition of the probiotic fermented beverage processed with water-soluble rice extract.

Parameter (g 100 g^{-1})	Formulations*			
	CONTR	OLIGO	CURDLAN	MIX
Moisture	88.16 ± 0.46[a]	88.52 ± 0.09[a]	85.77 ± 0.65[c]	86.66 ± 0.99[b]
Ash	0.57 ± 0.03[a]	0.56 ± 0.02[a]	0.56 ± 0.02[a]	0.55 ± 0.02[a]
Protein	0.39 ± 0.06[a]	0.35 ± 0.037[a]	0.36 ± 0.01[a]	0.36 ± 0.01[a]
Fat	Not identified	Not identified	Not identified	Not identified
Carbohydrate	10.88 ± 0.55[a]	10.55 ± 0.14[a]	13.30 ± 0.68[c]	12.42 ± 1.03[b]

Means ± standard deviation on the same line with different letters are significantly different by the Tukey test ($p < 0.05$, n = 12). *Formulations: CONTR (control sample); OLIGO (with oligofructose); CURDLAN (with curdlan oligosaccharides), MIX (with oligofructose + curdlan).

Table 3. Physicochemical characteristics of the probiotic fermented beverage processed with water-soluble rice extract.

Parameters	Storage time (days)	Formulations*			
		CONTR	OLIGO	CURDLAN	MIX
pH	1	4.15 ± 0.02^{Aa}	4.18 ± 0.01^{Aa}	3.69 ± 0.01^{Ba}	3.53 ± 0.01^{Ca}
	7	3.84 ± 0.01^{Ab}	3.81 ± 0.01^{Bb}	3.41 ± 0.01^{Cb}	3.24 ± 0.01^{Db}
	14	3.14 ± 0.01^{Cc}	3.14 ± 0.01^{Cc}	3.21 ± 0.02^{Bc}	3.25 ± 0.03^{Ab}
	21	3.06 ± 0.01^{Dd}	3.10 ± 0.02^{Cd}	3.23 ± 0.03^{Ac}	3.14 ± 0.01^{Bc}
	28	2.95 ± 0.01^{Ce}	2.95 ± 0.02^{Ce}	3.14 ± 0.02^{Ad}	3.07 ± 0.01^{Bd}
Titratable acidity (g 100 mL^{-1} of NaOH)	1	0.14 ± 0.02^{Be}	0.15 ± 0.01^{Be}	0.24 ± 0.01^{Ae}	0.23 ± 0.01^{Ad}
	7	0.28 ± 0.02^{Bd}	0.26 ± 0.01^{Bd}	0.33 ± 0.01^{Ad}	0.32 ± 0.01^{Ac}
	14	0.36 ± 0.01^{Cc}	0.34 ± 0.02^{Cc}	0.41 ± 0.01^{Ac}	0.38 ± 0.01^{Bb}
	21	0.38 ± 0.01^{Cb}	0.41 ± 0.01^{Bb}	0.45 ± 0.01^{Ab}	0.39 ± 0.01^{Cb}
	28	0.50 ± 0.01^{Ba}	0.43 ± 0.01^{Ca}	0.56 ± 0.01^{Aa}	0.52 ± 0.01^{Ba}
TSS (°Brix)	1	11.56 ± 0.27^{Ab}	10.50 ± 0.13^{Bb}	11.73 ± 0.14^{Aa}	11.71 ± 0.11^{Aa}
	7	11.53 ± 0.27^{Ab}	10.38 ± 0.11^{Bb}	11.36 ± 0.26^{Ab}	11.51 ± 0.13^{Aa}
	14	11.51 ± 0.18^{Ab}	10.65 ± 0.46^{Bb}	11.38 ± 0.07^{Ab}	11.55 ± 0.05^{Aa}
	21	12.53 ± 0.11^{Aa}	10.31 ± 0.16^{Cb}	11.60 ± 0.18^{Bab}	11.43 ± 0.11^{Ba}
	28	12.71 ± 0.22^{Aa}	12.26 ± 0.17^{Ba}	11.26 ± 0.05^{Db}	11.60 ± 0.01^{Ca}
L*	1	48.41 ± 0.91^{Aa}	43.3 ± 1.66^{Ba}	40.63 ± 0.69^{Cb}	43.49 ± 0.57^{Ba}
	7	38.11 ± 0.42^{Db}	39.79 ± 0.87^{Cb}	45.27 ± 1.00^{Aa}	43.34 ± 0.71^{Ba}
	14	36.12 ± 0.21^{Bc}	33.80 ± 0.59^{Ccd}	37.60 ± 0.13^{Ac}	36.43 ± 0.29^{ABbc}
	21	34.33 ± 0.69^{Bd}	34.95 ± 0.85^{Abc}	35.74 ± 0.28^{Ad}	35.18 ± 0.52^{Abc}
	28	34.68 ± 0.56^{Bd}	33.44 ± 0.11^{Cd}	35.70 ± 0.45^{Bd}	37.32 ± 0.58^{Ab}
a*	1	6.44 ± 0.21^{Bc}	7.49 ± 0.39^{Ac}	6.15 ± 0.57^{Bb}	6.36 ± 0.33^{Bbc}
	7	8.41 ± 0.10^{Ab}	8.67 ± 0.01^{Ab}	6.24 ± 0.10^{Cc}	6.68 ± 0.22^{Cab}
	14	8.76 ± 2.65^{Bb}	8.72 ± 0.18^{Bb}	6.61 ± 0.06^{Ca}	6.32 ± 0.07^{Cc}
	21	9.44 ± 0.24^{Aa}	9.51 ± 0.02^{Aa}	6.51 ± 0.03^{Ba}	6.42 ± 0.08^{Cbc}
	28	9.60 ± 0.17^{Aa}	9.42 ± 0.28^{Aa}	6.25 ± 0.059^{Cb}	6.85 ± 0.04^{Ba}
b*	1	6.17 ± 0.20^{Aa}	5.61 ± 0.20^{Ba}	4.84 ± 0.06^{Ca}	5.69 ± 0.03^{Ba}
	7	3.57 ± 0.03^{Cb}	3.53 ± 0.22^{Cb}	4.93 ± 0.21^{Aa}	4.32 ± 0.52^{Bb}
	14	2.74 ± 0.33^{Bc}	2.78 ± 0.08^{Bc}	3.16 ± 0.08^{Ab}	2.11 ± 0.04^{Cc}
	21	2.21 ± 0.21^{Ad}	1.74 ± 0.40^{Bd}	1.43 ± 0.03^{BCc}	1.26 ± 0.02^{Cd}
	28	1.52 ± 0.07^{Ae}	1.15 ± 0.03^{Be}	1.41 ± 0.06^{ABc}	1.52 ± 0.09^{Ad}

Means ± standard deviation on the same line with different capital letters means that formulations are significantly different for the same storage time by the Tukey test ($p < 0.05$, $n = 12$). Means ± standard deviation on the columns with different small letters means significant differences during storage for the same formulation by the Tukey test ($p < 0.05$, $n = 12$). L* ranging from 0 (black) to 100 (white), a* ranging from red (+a*) to green (−a*), and b* ranging from yellow (+b*) to blue (−b*). *Formulations: CONTR (control sample); OLIGO (with oligofructose); CURDLAN (with curdlan oligosaccharides), MIX (with oligofructose + curdlan).

suggest that the presence of oligofructose did not impact the pH and TA values. At the same time, the addition of CO promoted an initial higher acidity but decreased the post-acidification process.

The addition of oligofructose resulted in a decrease in the TSS values of the products ($p < 0.05$), while the addition of CO (CURDLAN) or both prebiotics (MIX) resulted in maintenance of this parameter ($p > 0.05$) at day 1. At the end of the storage time (day 28), all prebiotic formulations (OLIGO, CURDLAN, and MIX) presented lower TSS values than the CONTR formulation ($p < 0.05$), but the products with CO (CURDLAN and MIX) showed the lowest values ($p < 0.05$). The results of the TSS values suggest that the prebiotic compounds were probably partially consumed by the starter and/or probiotic cultures, mainly during the storage period. The consumption was more pronounced for the products added with CO.

The fermented beverages presented L* from 33.44-48.41, a* from 6.15-9.60, and b* from 1.15-6.17 (Table 3). Therefore, the products had a dark reddish color, which is associated with the grape juice used. The addition of the prebiotic components resulted in fermented beverages with less yellow color (decrease in b* values) and darker coloration (reduction in L* values) ($p < 0.05$). Furthermore, the addition of oligofructose increased the red color (a* values) of the products ($p < 0.05$). The color changes are related to the acquisition of prebiotics in lyophilized and/or powder form consisting of small white, yellowish, or slightly red lumps (Santos et al., 2019).

All fermented beverage formulations behaved similarly during the storage period, with decreases in the L* and b* values and increases in the a* values ($p < 0.05$), suggesting that the dark reddish color was accentuated. The rise in the acidity of the products during storage (Table 3) can be associated with the more reddish color, as acidification of the medium causes protonation of anthocyanins, shifting the balance towards the formation of the flavilium cation (red coloration) (Lopes et al., 2007). Furthermore, proteolysis could have occurred during the storage period due to the continuous activity of the proteases

from the probiotic and starter cultures, resulting in a darkening of the products (Costa et al., 2017). The similar behavior of all formulations upon the color parameters is an indication that there was no marked death of the probiotic cultures (Santos et al., 2019), as lysed or killed cells result in more pronounced color changes (Costa et al., 2019).

The rheological parameters of the fermented beverage formulations are shown in Table 4. The flow curves presented reasonable adjustment (R^2= 0.96-0.99), and the products could be characterized as pseudoplastic fluids (n < 1). According to Miranda et al. (2019), pseudoplastic fluids are characterized by a decrease in the apparent viscosity as a function of the deformation rate. The addition of oligofructose did not alter the *n* and k values of the fermented beverages ($p > 0.05$). However, the addition of CO (CURDLAN and MIX formulations) caused a reduction in k values and increases in *n* values ($p < 0.05$), which indicates that the fermented beverages became less consistent. CO can interact with other CO or proteins, forming a compact and continuous network with a gel-like structure. However, this gel presents low stability (Mangolim et al., 2017). Therefore, the low strength of the curdlan gel can be associated with the reduced consistency of the products. The impact of the reduction in consistency provided by CO should be evaluated from the consumer's point of view, considering their expectations about the product. If the fermented beverage is compared to fermented milks, which are liquid and homogeneous products, the reduced consistency could positively impact the sensory acceptance.

All fermented beverage formulations behaved similarly during the storage period, with decreases in the k values and increases/maintenance in the *n* values ($p < 0.05$), suggesting a loss of consistency during storage. The fermented beverages had a low concentration of protein (Table 2), and the rice protein has low water absorption capacity, which can cause a reduction in the water holding capacity of the products during storage and, consequently, loss of consistency (Akin & Ozcan, 2017). However, it could be observed that the alterations in the rheological parameters were less pronounced in the formulations added with CO (decrease of 8.05 mPa.s in k and increase of 0.02 in *n*) compared to the control (17.27 mpa.s reduction in k and increase of 0.03 in *n,* $p < 0.05$). The results suggest that oligofructose did not impact the rheological parameters, while the addition of CO promoted an initial lower consistency but improved the rheological stability during storage.

3.3 Probiotic survival

All the fermented beverages formulations had *L. casei* counts higher than 10^8 cfu/mL during the entire storage (28 days, Table 4), higher than the minimum count needed to consider a product as a probiotic food (10^6 CFU mL^{-1}, Savedboworn et al., 2017). Thus, all formulated fermented beverages could be considered probiotic products for 28 days of storage under refrigeration at 7 °C. Therefore, although the fermented beverage had a low protein content (0.35-0.39 g 100 g^{-1}) and high acidity (pH of 2.95-4.18), it was suitable for probiotic maintenance.

The addition of oligofructose (OLIGO) did not impact ($p > 0.05$) on the probiotic counts at day 1 but increased the probiotic survival during storage ($p < 0.05$). The addition of CO (CURDLAN and MIX) increased the probiotic counts at day 1 and during storage ($p < 0.05$), with a more pronounced effect for the CURDLAN formulation ($p < 0.05$). Therefore, oligofructose acted as a prebiotic component during the storage of the products, increasing probiotic survival. On the other hand, CO acted as a prebiotic component both during fermentation and storage of the products, increasing the probiotic survival. It was observed that CO presented a higher prebiotic potential than oligofructose, resulting in higher probiotic counts ($p < 0.05$).

Table 4. Rheological parameters of the probiotic fermented beverage processed with water-soluble rice extract.

Parameters	Storage time (days)	Formulations*			
		CONTR	OLIGO	CURDLAN	MIX
Consistency Index (k)	1	76.62 ± 3.07^{Aa}	82.07 ± 6.46^{Aa}	54.12 ± 5.32^{Ca}	62.95 ± 4.70^{Ba}
	7	75.50 ± 4.17^{Aa}	79.07 ± 4.47^{Aa}	$51.37 \pm 3.73C^{ab}$	62.95 ± 4.70^{Ba}
	14	72.27 ± 1.02^{Aa}	50.42 ± 1.07^{BCb}	46.27 ± 1.96^{Bb}	56.17 ± 1.19^{Aab}
	21	63.52 ± 3.54^{Ab}	43.55 ± 2.76^{Cbc}	46.50 ± 2.90^{Cb}	55.12 ± 0.44^{Bb}
	28	59.35 ± 0.49^{Ab}	42.30 ± 1.50^{Bc}	46.07 ± 2.06^{Cb}	53.87 ± 1.44^{Bb}
Flow Behavior Index (n)	1	0.72 ± 0.01^{Bb}	0.71 ± 0.01^{Bb}	0.76 ± 0.01^{Aa}	0.75 ± 0.01^{Aa}
	7	0.71 ± 0.01^{Bb}	0.69 ± 0.02^{Cb}	0.74 ± 0.02^{Ab}	0.75 ± 0.01^{Aa}
	14	0.75 ± 0.01^{Ca}	0.77 ± 0.01^{Aa}	0.77 ± 0.01^{ABa}	0.75 ± 0.01^{BCa}
	21	0.75 ± 0.01^{Ca}	0.77 ± 0.01^{Aa}	0.77 ± 0.01^{ABa}	0.75 ± 0.01^{BCa}
	28	0.75 ± 0.01^{Ba}	0.77 ± 0.01^{Aa}	0.78 ± 0.01^{Aa}	0.76 ± 0.01^{Aba}
R^2	1	0.99	0.97	0.99	0.97
	7	0.98	0.96	0.98	0.97
	14	0.99	0.98	0.99	0.99
	21	0.99	0.98	0.98	0.98
	28	0.99	0.99	0.98	0.98

Means ± standard deviation on the same line with different capital letters means that formulations are significantly different for the same storage time by the Tukey test ($p < 0.05$, n = 12). Means ± standard deviation on the columns with different small letters means significant differences during storage for the same formulation by the Tukey test ($p < 0.05$, n = 12). Results of rheological parameters obtained by Power Law at 11 °C. Consistency Index in mPa.s. Flow behavior index is dimensionless. R^2 = coefficient determination. *Formulations: CONTR (control sample); OLIGO (with oligofructose); CURDLAN (with curdlan oligosaccharides), MIX (with oligofructose + curdlan).

These results indicate that the probiotic culture probably used CO during fermentation and storage of the products (Batista et al., 2017). *L. casei* could have genes encoding carbohydrate-active enzymes specific for degradation of CO, helping break down the chains and utilization by probiotic cultures (Shi et al., 2018). The higher initial acidity of the products added with CO (Table 2) did not negatively impact the probiotic survival.

All formulations behaved similarly during the storage period when comparing the products on the 1st and 28th days of storage, maintaining the viability of the probiotic culture ($p > 0.05$). However, some fluctuations occurred in the intermediate storage times, increasing and decreasing probiotic counts ($p < 0.05$). The loss of viability of probiotic cultures in some weeks may be related to the reduction in pH during storage (Table 3) due to the accumulation of organic acids (Costa et al., 2019). On the other hand, viability recovery may be related to amino acids released during storage and the adaptation of cultures to the environment (Januário et al., 2018).

The probiotic culture survived to SGIC in all evaluated fermented beverages, with counts greater than 10^6 CFU mL^{-1} in all considered steps (gastric and enteric phases, Figures 1B-D), which is higher than the minimum suggested counts (10^6 CFU mL^{-1}, Millette et al., 2013). The addition of oligofructose (OLIGO) did not impact ($p > 0.05$) on the probiotic survival in the 2nd enteric phase (Figure 1D) but increased the probiotic survival in the gastric and 1st enteric phase ($p < 0.05$, Figures 1B and 1C). The addition of CO (CURDLAN and MIX) increased the probiotic survival in all the SGIC steps ($p < 0.05$), with a more pronounced effect for the CURDLAN formulation ($p < 0.05$). Oligofructose and CO are substrates available for probiotic culture utilization. They could be used as a carbon source for its maintenance and prevent the probiotics from injuries caused by acidity (Costa et al., 2019).

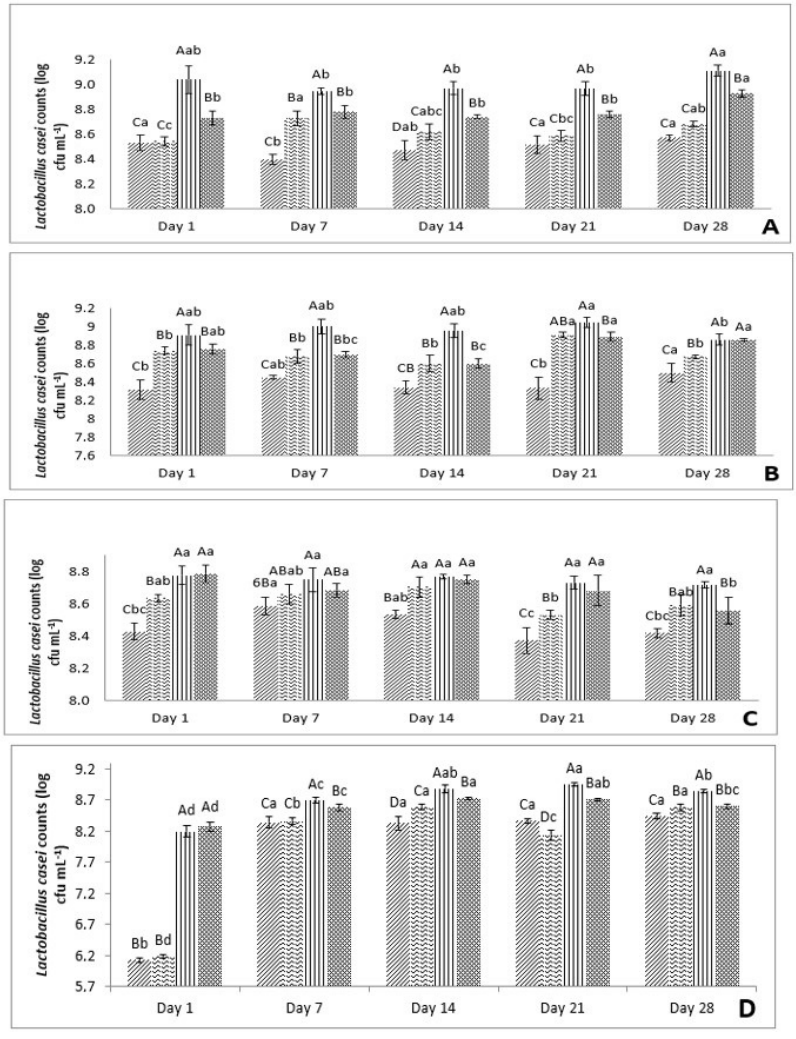

Figure 1. Viability (log CFU mL^{-1}) of the *Lactobacillus casei* in formulations of probiotic fermented beverage processed with water-soluble rice extract during refrigerated storage (7 °C) in the product (A) and simulated gastrointestinal conditions (B= gastric phase, C= first enteric phase, D = second enteric phase). Formulations: (▨) CONTR (control sample), (▒) OLIGO (with oligofructose), (▥) CURDLAN (with curdlan), (▩) MIX (with oligofructose and curlan). The error bars represent the standard deviation (n = 12). Different capital letters mean that formulations are significantly different for the same storage time by the Tukey test ($p < 0.05$). Different lowercase letters mean significant differences during storage for the same formulation by the Tukey test ($p < 0.05$).

4 Conclusion

This study is the first to demonstrate that CO can be used as a prebiotic component in the development of synbiotic fermented beverages of WSRE, with better results than those provided by oligofructose, an established prebiotic component. CO addition resulted in higher acidity (lower pH and higher titratable acidity values) and less consistency (lower *k* and higher *n* values). Furthermore, it acted as a prebiotic component during fermentation, storage, and SGIC, promoting the highest increase in probiotic survival. Moreover, CO improved the storage stability, reducing the post-acidification and the alterations in the rheological parameters. The results are significant from the industry point of view, as CO can be obtained using microbial processes.

Conflict of interest

No known competing financial interests or personal relationships could have appeared to influence the work reported in this paper.

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