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Pasteurised sugarcane juice supplemented with *Lactobacillus casei* and prebiotics: physicochemical stability, sensory acceptance and probiotic survival

[1]Santos, M. A., [2]Costa, G. M., [2]Dias, S. S., [2]Klososki, S. J., [2]Barão, C. E., [1]Gomes, R. G. and [1,2*]Pimentel, T. C

[1]*Universidade Estadual de Maringá, Pós-Graduação em Engenharia de Alimentos, 87020-900, Maringá, Paraná, Brazil*
[2]*Instituto Federal do Paraná (IFPR), Campus Paranavaí, 87703-536, Paranavaí, Paraná, Brazil*

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Abstract

The objective of the present work was to evaluate the effect of the direct addition of the freeze-dried probiotic culture (*Lactobacillus casei*, 2 g/L) and prebiotics (oligofructose or polydextrose, 20 g/L) on the physicochemical characteristics, probiotic survival and on the acceptance of pasteurised sugarcane juice during refrigerated storage (7°C / 28 days). The incorporation of oligofructose or polydextrose resulted in products with physicochemical characteristics, texture, acceptance and storage stability similar to the pure product. *L. casei* remained viable in the product (> 10^9 CFU/mL), but its addition increased the acidity, turbidity, luminosity and green colour of the products, and decreased the acceptance during the storage period. The present work demonstrated that it is possible to directly add the freeze-dried probiotic culture (*L. casei*) to the sugarcane juice, replacing the traditional methods of activation and propagation of the culture in Man Rogosa Sharp broth or juice, resulting in an easier and faster manufacturing process and high probiotic counts (> 10^9 CFU/mL). However, the probiotic inoculum should be further optimised in order to minimise the physicochemical and sensory alterations.

Introduction

Sugarcane (*Saccharum officinarum* L.) juice is a sucrose-rich beverage obtained by crushing mature sugarcane and extracting the juice (Chauhan *et al.*, 2017). It is a non-alcoholic beverage, characterised as a viscous and opaque liquid, with colour that ranges from light grey to dark green (Manikantan *et al.*, 2017). Sugarcane juice has significant values of carbohydrates, minerals (potassium, calcium, phosphorus, magnesium and iron) and vitamins (complex B and C), being an energetic product (80-100 kcal/100 mL) with pH ranging from 4 to 5 (Suganthi *et al.*, 2018). The presence of high quantities of sugars, traces of polyphenols and organic acids and the high polyphenol oxidase activity cause quick fermentation and dark brownish appearance to the product, resulting in unmarketable juice only several hours after extraction (Mattos *et al.*, 2017; Nishad *et al.*, 2017). Furthermore, quality degradation due to microbial spoilage is also a factor of concern (Sreedevi *et al.*, 2017). Pasteurisation at suitable conditions could overcome these problems; but, despite the large potential for the beverage industry, commercialisation of sugarcane juice is restricted to the immediate consumption of the raw juice shortly after extraction (Mattos *et al.*, 2017; Nishad *et al.*, 2017).

Dairy products, such as cheeses (Silva *et al.*, 2017; 2018; Sperry *et al.*, 2018), ice creams (Balthazar *et al.*, 2018a) and fermented milks (Batista *et al.*, 2017) are the main carriers of probiotic cultures. However, in recent years, there has been a great demand for non-dairy probiotic products due to the increase in lactose intolerant, allergic to milk protein or strictly vegetarian individuals, and people who do not appreciate the milk flavour (Alves Filho *et al.*, 2017; Patel *et al.*, 2017; Min *et al.*, 2018). Fruit and vegetable beverages can be good carriers of probiotic cultures, as they are rich in essential nutrients (minerals, vitamins, fibres and antioxidants), consumed by people of all age groups and on a regular basis, do

*Corresponding author.
Email: tatiana.pimentel@ifpr.edu.br

not contain other microorganisms that can compete with probiotic cultures, and do not have allergenic components (Panghal *et al.*, 2018). The probiotic culture *Lactobacillus casei* has high survival rates in fruit juices (Sperry *et al.*, 2018) and its addition did not impact on the consumer acceptance of the products (Pimentel *et al.*, 2015; da Costa *et al.*, 2017).

Probiotic cultures are added to fruit and vegetable beverages in an activated form and after propagation of the probiotic in Man Rogosa Sharp (MRS) broth (Sheehan *et al.*, 2007; Ding and Shah, 2008; Pimentel *et al.*, 2015; Valero-Cases and Frutos, 2017); or in juices (da Costa *et al.*, 2017). However, this methodology requires long time to obtain the activated culture (24-54 h), requires a refrigerated centrifuge for the separation of the biomass, and increases the risk of contamination because of the various steps required. These factors limit the industrial interest and preclude the production of probiotic juices by small and medium industries (da Costa *et al.*, 2017). The direct addition of the freeze-dried probiotic culture would be time consuming, does not need specific equipment and has lower microbiological safety concern. However, the sensory characteristics could be impaired, as well as the viability of the probiotic culture, because the microorganisms would not be in an active form. Presently, studies that evaluated the direct addition of the freeze-dried probiotic cultures to non-fermented fruit and vegetable beverages are scarce (Saarela *et al.*, 2006).

Prebiotics are substrates selectively used by the host microorganisms to confer a health benefit (Gibson *et al.*, 2017). Dairy products are the most important carriers of prebiotic components (Balthazar *et al.*, 2016; 2017; Belsito *et al.*, 2017). In the products, prebiotics could assist in increasing the survival of the probiotic cultures during storage (da Costa *et al.*, 2017). Oligofructose has already been used as a prebiotic in apple juice (Pimentel *et al.*, 2015) and orange juice (da Costa *et al.*, 2017), and its protective effect on probiotic cultures was dependent on the food matrix. To the best of the authors' knowledge, there are no studies that applied polydextrose as a prebiotic in fruit and vegetable beverages thus far. The prebiotic effect of the oligofructose is established (Gibson *et al.*, 2017), while the consumption of polydextrose was associated to preservation of bone tissue (Franco *et al.*, 2018), stimulation of colonic microbiota (Lamichhane *et al.*, 2018), hypolipidemic effect (Raza *et al.*, 2017), among others. A product containing both probiotics and prebiotics is called synbiotic (Zoghi *et al.*, 2017).

Therefore, the purpose of the present work was to evaluate the effect of the direct addition of the freeze-dried probiotic culture (*L. casei*) and prebiotics (oligofructose or polydextrose) on the physicochemical characteristics, probiotic survival and on the acceptance of pasteurised sugarcane juice during refrigerated storage (7°C / 28 days).

Materials and methods

Material

Sugarcane stems, oligofructose (P95, Orafti®), polydextrose (Tate and Lyle®), probiotic culture of *L. casei* (*L. casei*-01, Christian Hansen®) and glass flasks (Farma®, 50 mL) were used.

Formulations

Six formulations of sugarcane juice were prepared: PUR (pure juice), PRO (juice added with 2 g/L of probiotic culture), OLIGO (juice added with 20 g/L of oligofructose), POLY (juice added with 20 g/L of polydextrose), SYNB-O (juice added with 20 g/L of oligofructose and 2 g/L of probiotic culture) and SYNB-P (juice added with 20 g/L of polydextrose and 2 g/L of probiotic culture).

Sugarcane juice processing

The sugarcane stems were sanitised (6 mL/L Pury Vitta® fruit disinfectant with 0.96 g / 100 mL active chlorine), peeled, and the juice extracted by crushing them in a cane grinder (Botini, São Paulo, Brazil).

Formulations containing oligofructose (OLIGO and SYNB-O) or polydextrose (POLY and SYNB-P) were added with 20 g/L of the prebiotic component. This concentration was based on Brazilian regulation in order to use the functional property claim (minimum of 2.5 g per serving of 200 mL) (ANVISA, 2016) and in previous studies that indicated a minimum daily consumption of 2-4 g of oligofructose (Closa-Monasterolo *et al.*, 2017) or polydextrose (Forssten *et al.*, 2015) to trigger the beneficial health effects of these components.

The formulations were placed in glass flasks, pasteurised at 80°C for 20 min in a thermostatic bath (Marconi®, Piracicaba, Brazil) and cooled in an ice bath until reaching 37°C. The formulations added with probiotic cultures (PRO, SYNB-O and SYNB-P) were directly supplemented with 2 g/L of the freeze-dried probiotic culture in an aseptic environment. The concentration of the probiotic culture was defined in preliminary tests in order to obtain suitable probiotic counts (> 10^8 CFU/mL) in the products.

The formulations were stored at 7°C for 28 days, which is the shelf life of pasteurised commercial fruit juices. The storage temperature (7°C) was selected to test the conventional commercialisation conditions of the products, as it is close to the temperature of domestic and commercial refrigerators. Previous studies have evaluated the survival of the probiotic culture only at 4°C (Ding and Shah, 2008; Pimentel *et al.*, 2015; da Costa *et al.*, 2017).

Physicochemical evaluation and probiotic survival

Measurements of moisture, protein, lipid, ash and carbohydrate were performed following the methodologies proposed by the Association of Official Analytical Chemists (AOAC, 2004). The pH was determined using a digital potentiometer (MS Tecnopon Instrumentation, mPA210, Piracicaba, Brazil) and the titratable acidity was determined based on AOAC (2004) and expressed as a percentage (%) of citric acid.

The total soluble solids (TSS) content was determined in a digital refractometer (Instruterm®, São Paulo, Brazil) and the results were expressed in °Brix. For colour instrumental evaluation (L^*, a^* and b^*), a colorimeter (Konica Minolta, CR-400, Osaka, Japan) was used. The coordinates L^*, a^* and b^* were obtained using the CIE system, where L^* is a measure of the lightness, a^* varies from green (−) to red (+) and b^* varies from blue (−) to yellow (+) (Nishad *et al.*, 2017). The turbidity was analysed in a spectrophotometer (T80, UV/VIS Spectrometer, PG Instruments Ltd., United Kingdom) at 600 nm wavelength (Shah *et al.*, 2010).

The texture parameters (firmness, consistency, cohesiveness and viscosity) were determined by a single compression test using TA-TX Express Texturometer (Stable Micro Systems, London, England). The formulations (40 mL), in a plastic container of 55 mm diameter and 60 mm height, were compressed by a cylindrical probe of 36 mm diameter (P36 R) to a depth of 10 mm, using pre-test and test speeds of 1 mm/s, post-test speed of 10 mm/s and trigger of 1 g (da Costa *et al.*, 2017). The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with some modifications (Wang *et al.*, 2017). The results were expressed in mg of gallic acid equivalents per litre (mg GAE/L).

The *L. casei* survival on the sugarcane juice was performed on MRS agar (Himedia, Mumbai, India) and anaerobic incubation (Anaerobac, Probac®) at 37°C for 72 h (Tharmaraj and Shah, 2003).

Sensory evaluation

There were two sensory panels, one on the 1st day and the other on the 28th day of storage of the products. The panels were composed of untrained 94 consumers (51 women, 41 men and 2 not specified), ranging from 15 to over 50 of age, with majority (84 individuals) being 15-25 of age. The individuals were students, professors and staff of the same university and majority (65 individuals) participated in both sensory evaluations.

The acceptance test (appearance, aroma, flavour, texture and overall impression) was carried out using a 9-point hedonic scale (1 - disliked very much and 9 - liked very much); and the purchase intention test with a 5-point scale (1 = certainly would not buy, 5 = certainly would buy) (Dantas *et al.*, 2016; Batista *et al.*, 2017; Balthazar *et al.*, 2018b). The consumers evaluated the six formulations in monadic form and in random order. The formulations (30 mL) were served in plastic cups (50 mL), coded with random three-digit numbers, at approximately 7°C. Potable water (25°C) and gluten-free rice crackers were available for palate cleansing.

Design of the experiment and statistical analysis

The experiment was repeated twice. The physicochemical characterisation and the probiotic survival in the juice were conducted in triplicate on each storage day (1, 7, 14, 21 and 28 days). The proximate composition was evaluated in triplicate only on the 1st and 28th days of storage. Acceptance and purchase intention were performed on the 1st and 28th days of storage. The experiment followed a completely randomised design and a split plot design was used to analyse the data (main treatment was the formulation and the secondary treatment was the storage time). Acceptance and purchase intention were performed using a completely randomised block design (treatments were the formulations and time and the blocks the judges). The results were submitted to Analysis of Variance (ANOVA) and Tukey test (p = 5%) to determine significant differences between treatments. Statistical analyses were performed using ASSISTAT 7.7 and Statistical Analysis System (SAS) software.

Results and discussion

Proximate composition

The proximate composition of the sugarcane juice formulations is shown in Table 1. The proximate composition (g/100 mL) fell in the following range: moisture (75.70-80.10), carbohydrate (19.68-22.88), protein (0.28-0.44), lipid (0.12-0.17) and ash (0.21-0.27), corroborating the results of a previous study (Ramachandran *et al.*, 2017). Therefore, the sugarcane juice presents high water and carbohydrate contents and low concentrations of protein and lipid.

The addition of *L. casei* (PRO) and the refrigerated storage (7°C) did not influence the proximate

Table 1. Proximate composition of the sugarcane juice formulations (g/100 mL) during refrigerated storage (7°C).

Parameter	Storage time (days)	Formulations					
		PUR	PRO	OLIGO	POLY	SYNB-O	SYNB-P
Moisture	1	79.23 ± 0.14^{aA}	79.47 ± 0.70^{aA}	76.75 ± 0.63^{aBC}	77.47 ± 0.20^{aB}	76.25 ± 0.35^{aC}	77.82 ± 0.16^{aB}
	28	79.50 ± 0.20^{aA}	80.10 ± 0.30^{aA}	77.50 ± 0.70^{aBC}	78.02 ± 0.30^{aB}	75.70 ± 0.20^{aC}	78.10 ± 0.20^{aB}
Protein	1	0.34 ± 0.06^{aA}	0.44 ± 0.11^{aA}	0.40 ± 0.11^{aA}	0.36 ± 0.06^{aA}	0.39 ± 0.06^{aA}	0.44 ± 0.08^{aA}
	28	0.28 ± 0.06^{aA}	0.39 ± 0.10^{aA}	0.32 ± 0.08^{aA}	0.34 ± 0.09^{aA}	0.40 ± 0.05^{aA}	0.42 ± 0.06^{aA}
Lipid	1	0.15 ± 0.08^{aA}	0.14 ± 0.06^{aA}	0.13 ± 0.05^{aA}	0.17 ± 0.04^{aA}	0.13 ± 0.12^{aA}	0.15 ± 0.07^{aA}
	28	0.13 ± 0.07^{aA}	0.13 ± 0.10^{aA}	0.14 ± 0.08^{aA}	0.16 ± 0.10^{aA}	0.12 ± 0.07^{aA}	0.16 ± 0.11^{aA}
Ash	1	0.23 ± 0.04^{aA}	0.22 ± 0.11^{aA}	0.25 ± 0.09^{aA}	0.22 ± 0.02^{aA}	0.21 ± 0.04^{aA}	0.24 ± 0.04^{aA}
	28	0.23 ± 0.05^{aA}	0.23 ± 0.08^{aA}	0.27 ± 0.10^{aA}	0.23 ± 0.05^{aA}	0.22 ± 0.09^{aA}	0.25 ± 0.05^{aA}
Carbohydrate	1	20.04 ± 0.07^{aC}	19.68 ± 0.73^{aC}	22.32 ± 0.66^{aAB}	21.74 ± 0.20^{aAB}	22.88 ± 0.42^{aA}	21.30 ± 0.17^{aB}
	28	19.85 ± 0.10^{aC}	20.03 ± 0.10^{aC}	22.48 ± 0.75^{aAB}	22.09 ± 0.52^{aAB}	22.68 ± 0.50^{aA}	21.40 ± 0.75^{aB}

Means ± standard deviation in the same row followed by different uppercase letters indicate statistically significant differences at $p \leq 0.05$ between formulations of sugarcane juice for the same storage day ($n = 6$). Means ± standard deviation in the same column followed by different lowercase letters indicate statistically significant differences at $p \leq 0.05$ for each formulation affected by storage time. PUR: pure, PRO: with probiotic, OLIGO: with oligofructose, POLY: with polydextrose, SYNB-O: with oligofructose and probiotic, SYNB-P: with polydextrose and probiotic.

composition of the sugarcane juice ($p > 0.05$), while the addition of oligofructose (OLIG and SYNB-O) or polydextrose (POLY and SYNB-P) caused a decrease in the moisture content and an increase in the carbohydrate content ($p \leq 0.05$). Oligofructose and polydextrose are soluble oligosaccharides (Mussatto and Mancilha, 2007). Their addition in foods causes an increase in the total solids content and carbohydrates and, consequently, a decrease in the moisture content (da Costa *et al.*, 2017).

Physicochemical characteristics

The physicochemical characteristics of the sugarcane juice formulations are presented in Table 2. The products had a pH of 3.3-5.4, titratable acidity of 0.07-0.35% citric acid, TSS of 18.9-21.6 °Brix and TPC of 364.30-891.59 mGAE/L. The addition of oligofructose (OLIGO) or polydextrose (POLY) did not cause changes in pH and titratable acidity ($p > 0.05$) nor influence the stability of the sugarcane juices during refrigerated storage (7°C), with maintenance of these parameters similar to the pure product (PUR). Only an increase in TSS was observed ($p \leq 0.05$) in prebiotic formulations, which could be related to the chemical composition of oligofructose and polydextrose as soluble oligosaccharides (da Costa *et al.*, 2017).

The formulations added with probiotics (PRO, SYNB-O and SYNB-P) were more acidic than the pure product (PUR) (lower pH values and higher titratable acidity values) ($p \leq 0.05$), with similar TSS ($p > 0.05$). During storage, the acidity of the probiotic products was further enhanced ($p \leq 0.05$), while the TSS was maintained ($p > 0.05$). Probiotic cultures might have metabolised some sugars present in the sugarcane juice, resulting in the production of acids and consequent increases in the acidity (Pimentel *et al.*, 2015). The maintenance of the TSS might be related to sucrose hydrolysis, resulting in fructose and glucose, the latter being consumed by the probiotic culture but not altering the final TSS. The highest acidity of the probiotic products may be advantageous from the microbiological point of view, as it may lead to an increase in the shelf life of the product. However, it may result in decreased viability of the probiotic culture and consumer acceptance of the products (da Costa *et al.*, 2017).

There was no effect of adding oligofructose (OLIGO) or polydextrose (POLY) ($p > 0.05$) on the TPC, and the formulations maintained the initial levels ($p > 0.05$) during the storage time. Probiotic addition (PRO, SYNB-O and SYNB-P) resulted in higher concentrations of TPC in some of the evaluated storage time. The higher TPC content could be related to the higher acidity of the probiotic products, as the phenolic compounds are more stable at this condition (de Souza *et al.*, 2017). The results indicate good processing and storage conditions, as the phenolic compounds could suffer from enzymatic and/or chemical oxidation when exposed to improper processing conditions and prolonged periods of storage (Abreu *et al.*, 2011). Phenolic compounds act as antioxidant agents and may contribute to the reduction of the risk of chronic non-communicable diseases. In foods, they are responsible for colour, astringency, aroma and oxidative stability (Balasundram *et al.*, 2006; Klangpetch, 2017). The content of TPC of the sugarcane juices (364.30 to 891.59 mGAE/L) was lower than the observed in red grape juices (1728 mGAE/L), but similar to

Table 2. Physicochemical characteristics of the sugarcane juice formulations during refrigerated storage (7°C).

Parameter***	Storage time (days)	Formulations					
		PUR	PRO	OLIGO	POLY	SYNB-O	SYNB-P
pH	1	5.24 ± 0.42^{aA}	4.15 ± 0.30^{aB}	5.14 ± 0.18^{aA}	5.21 ± 0.11^{aA}	4.10 ± 0.24^{aB}	3.99 ± 0.34^{aB}
	7	5.34 ± 0.19^{aA}	3.87 ± 0.07^{abB}	5.21 ± 016^{aA}	5.23 ± 0.14^{aA}	3.90 ± 0.11^{abB}	3.66 ± 0.11^{abB}
	14	5.27 ± 0.05^{aA}	3.77 ± 0.25^{bB}	5.31 ± 0.42^{aA}	5.19 ± 0.15^{aA}	3.72 ± 0.22^{bcB}	3.53 ± 0.25^{bcB}
	21	5.12 ± 0.03^{aA}	3.77 ± 0.61^{bB}	5.09 ± 0.08^{aA}	5.17 ± 0.01^{aA}	3.46 ± 0.12^{cC}	3.30 ± 0.15^{cC}
	28	5.14 ± 0.15^{aA}	3.56 ± 0.09^{bB}	5.09 ± 0.17^{aA}	5.10 ± 0.14^{aA}	3.46 ± 0.06^{cB}	3.31 ± 0.05^{cB}
Titratable acidity (% citric acid)	1	0.08 ± 0.01^{aB}	0.17 ± 0.01^{cA}	0.09 ± 0.02^{aB}	0.08 ± 0.00^{aB}	0.17 ± 0.01^{dA}	0.18 ± 0.01^{eA}
	7	0.07 ± 0.00^{aC}	0.20 ± 0.03^{cB}	0.09 ± 0.02^{aC}	0.08 ± 0.02^{aC}	0.20 ± 0.01^{cAB}	0.23 ± 0.03^{dA}
	14	0.08 ± 0.01^{aC}	0.23 ± 0.02^{bB}	0.09 ± 0.02^{aC}	0.07 ± 0.01^{aC}	0.24 ± 0.00^{bB}	0.28 ± 0.03^{cA}
	21	0.07 ± 0.02^{aC}	0.27 ± 0.00^{aB}	0.01 ± 0.01^{aC}	0.08 ± 0.02^{aC}	0.26 ± 0.02^{bB}	0.31 ± 0.03^{bA}
	28	0.07 ± 0.02^{aC}	0.26 ± 0.01^{aB}	0.09 ± 0.03^{aC}	0.07 ± 0.01^{aC}	0.29 ± 0.01^{aB}	0.35 ± 0.04^{aA}
TSS (°Brix)	1	19.57 ± 0.92^{aC}	19.57 ± 1.34^{aC}	21.03 ± 0.63^{aB}	20.87 ± 0.88^{aB}	21.63 ± 0.54^{aA}	21.13 ± 0.85^{aB}
	7	19.58 ± 1.59^{aC}	19.15 ± 2.01^{aC}	21.12 ± 1.45^{aB}	21.20 ± 1.46^{aB}	21.62 ± 1.02^{aA}	20.98 ± 1.23^{aB}
	14	19.73 ± 1.21^{aC}	19.32 ± 1.85^{aC}	20.82 ± 0.82^{aB}	20.87 ± 1.61^{aB}	21.15 ± 1.19^{aA}	20.53 ± 1.59^{aB}
	21	19.20 ± 1.29^{aC}	18.90 ± 1.72^{aC}	20.73 ± 1.00^{aB}	20.30 ± 0.65^{aB}	20.92 ± 0.66^{aA}	20.42 ± 1.16^{aB}
	28	19.92 ± 1.01^{aC}	19.98 ± 2.07^{aC}	21.47 ± 0.97^{aB}	21.65 ± 1.28^{aB}	21.88 ± 0.31^{aA}	21.45 ± 1.09^{aB}
Phenolic compounds (mg equivalents of gallic acid/L of sugarcane juice)	1	387.3 ±84.3^{aC}	710.1 ± 200.8^{aAB}	537.2 ± 125.8^{aBC}	436.3 ± 1889.7^{aC}	833.96 ± 142.14^{aA}	891.59 ± 265.73^{aA}
	7	488.2 ± 287.2^{aAB}	571.8 ± 159.7^{aA}	364.3 ± 154.0^{aB}	401.8 ± 137.0^{aAB}	496.84 ± 244.18^{bAB}	571.76 ± 123.42^{bA}
	14	421.9 ± 212.4^{aA}	534.3 ± 118.1^{aA}	378.7 ± 234.9^{aA}	381.6 ± 122.0^{aA}	496.84 ± 150.22^{bA}	571.01 ± 109.16^{bA}
	21	381.6 ± 107.9^{aB}	502.6 ±72.9^{aAB}	442.1 ± 78.6^{aAB}	430.6 ± 89.9^{aAB}	623.62 ± 164.89^{abA}	589.05 ± 159.36^{bA}
	28	499.73 ± 89.27^{aBC}	675.49 ± 87.07^{aAB}	459.39 ± 47.24^{aC}	522.78 ± 92.7 ^{aABC}	715.83 ± 113.85^{abA}	643.79 ± 4.06^{bABC}
*L**	1	27.30 ± 0.71^{aA}	27.93 ± 0.70^{aA}	26.34 ± 1.00^{aA}	26.90 ± 1.20^{aA}	27.81 ± 0.62^{cA}	28.01 ± 0.73^{aA}
	7	27.12 ± 1.10^{aBC}	29.02 ± 0.46^{aAB}	26.32 ± 1.22^{aC}	26.94 ± 1.51^{aBC}	29.88 ± 3.01^{abcA}	28.85 ± 0.25^{aAB}
	14	26.28 ± 0.54^{aB}	29.89 ± 0.91^{aA}	26.15 ± 1.12^{aB}	26.12 ± 0.54^{aB}	30.79 ± 4.08^{abA}	29.38 ± 0.66^{aA}
	21	26.04 ± 0.21^{aB}	29.86 ± 0.60^{aA}	26.32 ± 0.32^{aB}	25.55 ± 0.57^{aB}	29.06 ± 1.17^{bcA}	29.13 ± 1.22^{aA}
	28	25.63 ± 0.37^{aC}	29.95 ± 1.02^{aAB}	25.20 ± 0.25^{aC}	25.26 ± 0.48^{aC}	31.61 ± 3.97^{aA}	29.25 ± 1.00^{aB}
*a**	1	0.47 ± 0.68^{aB}	-0.08 ± 0.67^{aC}	0.53 ± 1.04^{aB}	1.03 ± 0.80^{aA}	-0.5 ± 0.54^{aC}	-0.05 ± 0.47^{aC}
	7	0.81 ± 0.89^{aB}	-0.34 ± 0.86^{aC}	0.94 ± 1.06^{aB}	1.08 ± 0.75^{aA}	0.01 ± 0.96^{aC}	0.14 ± 0.76^{aC}
	14	0.70 ± 0.52^{aB}	-0.46 ± 1.09^{aC}	0.62 ± 0.93^{aB}	0.95 ± 0.56^{aA}	-0.54 ± 0.94^{aC}	-0.36 ± 1.14^{aC}
	21	0.55 ± 0.25^{aB}	-0.83 ± 0.60^{aC}	0.43 ± 0.65^{aB}	0.84 ± 0.45^{aA}	-0.90 ± 0.72^{aC}	-0.66 ± 0.69^{aC}
	28	0.34 ± 0.42^{aB}	-0.89 ± 0.61^{aC}	0.28 ± 0.52^{aB}	0.61 ± 0.24^{aA}	-0.83 ± 0.72^{aC}	-0.72 ± 0.85^{aC}
*b**	1	5.34 ± 1.19^{aAB}	5.01 ± 0.95^{aB}	5.28 ± 1.75^{aAB}	6.68 ± 2.20^{aA}	4.59 ± 0.34^{bB}	5.19 ± 0.34^{aB}
	7	6.18 ± 1.77^{aA}	5.72 ± 1.10^{aA}	6.01 ± 2.21^{aA}	5.96 ± 1.35^{abA}	6.94 ± 3.66^{aA}	6.01 ± 1.51^{aA}
	14	5.44 ± 0.63^{aA}	6.23 ± 1.26^{aA}	5.27 ± 1.76^{aA}	5.53 ± 1.15^{abA}	6.21 ± 0.74^{abA}	6.52 ± 1.04^{aA}
	21	4.63 ± 0.39^{aA}	5.59 ± 0.18^{aA}	4.31 ± 0.54^{aA}	4.90 ± 0.54^{abA}	5.58 ± 0.19^{abA}	5.59 ± 0.36^{aA}
	28	4.52 ± 0.28^{aBCD}	5.77 ± 0.30^{aAB}	4.01 ± 0.56^{aD}	4.34 ± 0.18^{bCD}	6.03 ± 0.52^{abA}	5.65 ± 0.25^{aABC}
Turbidity at 600nm	1	1.79 ± 0.02^{aB}	2.05 ± 0.02^{aA}	1.79 ± 0.10^{aB}	1.69 ± 0.03^{abB}	2.04 ± 0.09^{aA}	2.00 ± 0.04^{aA}
	7	1.56 ± 0.02^{bB}	1.95 ± 0.01^{aA}	1.56 ± 0.01^{bB}	1.48 ± 0.01^{bB}	1.97 ± 0.01^{aA}	1.91 ± 0.18^{aA}
	14	1.85 ± 0.10^{aB}	2.13 ± 0.06^{aA}	1.86 ± 0.04^{aB}	1.74 ± 0.07^{aB}	2.18 ± 0.02^{aA}	2.09 ± 0.03^{aA}
	21	1.78 ± 0.06^{aB}	2.11 ± 0.07^{aA}	1.82 ± 0.09^{aB}	1.86 ± 0.25^{aB}	2.02 ± 0.22^{aA}	2.08 ± 0.03^{aA}
	28	1.85 ± 0.05^{aB}	2.09 ± 0.09^{aA}	1.84 ± 0.11^{aB}	1.73 ± 0.03^{aB}	2.15 ± 0.07^{aA}	2.07 ± 0.09^{aA}

Means ± standard deviation in the same row followed by different uppercase letters indicate statistically significant differences at $p \leq 0.05$ between formulations of sugarcane juice for the same storage day (n = 6). Means ± standard deviation in the same column followed by different lowercase letters indicate statistically significant differences at $p \leq 0.05$ for each formulation affected by storage time. PUR: pure, PRO: with probiotic, OLIGO: with oligofructose, POLY: with polydextrose, SYNB-O: with oligofructose and probiotic, SYNB-P: with polydextrose and probiotic.

those of other juices, such as apple (339 mGAE/L), grapefruit (535 mGAE/L), orange (755 mGAE/L), pineapple (358 mGAE/L) and prune (441 mGAE/L) (Balasundram *et al.*, 2006).

The sugarcane juice had a dark yellow-green coloration and was cloudy, with values in the following ranges: L^* = 25.20-31.61, a^* = -0.89-1.08, b^* = 4.01-6.68 and turbidity at 600 nm = 1.48-2.18. The addition of probiotic culture (PRO) caused an increase in L^* (luminosity) (from the 14th day of storage), decrease in a^* (greener) and increase in turbidity ($p \leq 0.05$), indicating that the addition of *L. casei* resulted in products with a lighter colour, greener and more turbid. The addition of polydextrose (POLY) resulted in more reddish products (higher values of a^*) ($p \leq 0.05$), while the addition of oligofructose (OLIGO) did not alter the colour parameters (L^*, a^* and b^*) and the turbidity ($p > 0.05$). The changes in colour and turbidity are related to the addition of probiotic culture and prebiotics in lyophilised and/or powder form consisting of small white or yellowish lumps.

The formulations presented similar colour parameters (L^*, a^* and b^*) and turbidity at the 1st and 28th days of storage ($p > 0.05$, except polydextrose). The maintenance of the colour and turbidity parameters of sugarcane juice added with probiotics is an indication that there was no marked death of the probiotic cultures. According to da Costa *et al.* (2017) and Shah *et al.* (2010), compromised probiotic cells (lysed or killed) would result in colour change and increased turbidity in fruit beverages.

The texture characteristics (firmness, consistency, cohesiveness and viscosity index) of the sugarcane juices are presented in Table 3. Regardless of the

Table 3. Texture parameters of the sugarcane juice formulations during refrigerated storage (7°C).

Parameter	Storage time (days)	Formulations					
		PUR	PRO	OLIGO	POLY	SYNB-O	SYNB-P
Firmness (g)	1	27.59 ± 0.58^{aABC}	27.26 ± 0.34^{aBC}	27.57 ± 0.47^{aAB}	27.79 ± 0.55^{aA}	27.10 ± 0.63^{aABC}	27.17 ± 1.07^{aC}
	7	27.61 ± 0.47^{aABC}	27.32 ± 0.72^{aBC}	27.92 ± 0.91^{aAB}	27.61 ± 1.21^{aA}	27.32 ± 1.06^{aABC}	27.22 ± 0.65^{aC}
	14	30.85 ± 4.36^{aABC}	31.18 ± 4.19^{aBC}	31.18 ± 4.44^{aAB}	31.45 ± 3.99^{aA}	31.34 ± 3.72^{aABC}	30.96 ± 3.74^{aC}
	21	31.42 ± 4.69^{aABC}	30.96 ± 4.41^{aBC}	31.84 ± 4.34^{aAB}	31.69 ± 4.71^{aA}	31.20 ± 5.14^{aABC}	30.94 ± 5.00^{aC}
	28	31.91 ± 3.58^{aABC}	31.32 ± 3.93^{aBC}	31.53 ± 3.95^{aAB}	32.10 ± 4.12^{aA}	31.69 ± 3.74^{aABC}	31.17 ± 3.30^{aC}
Consistency (g.s)	1	159.01 ± 3.97^{aA}	156.22 ± 1.91^{aA}	158.26 ± 2.30^{aA}	158.50 ± 3.20^{aA}	171.89 ± 43.08^{aA}	155.18 ± 7.36^{aA}
	7	159.45 ± 3.84^{aA}	157.58 ± 4.62^{aA}	161.22 ± 6.00^{aA}	159.31 ± 8.01^{aA}	157.58 ± 6.33^{aA}	157.57 ± 3.73^{aA}
	14	200.65 ± 52.97^{aA}	202.10 ± 51.35^{aA}	203.06 ± 54.06^{aA}	205.12 ± 49.06^{aA}	203.09 ± 48.02^{aA}	199.99 ± 48.16^{aA}
	21	204.89 ± 53.79^{aA}	200.85 ± 51.31^{aA}	206.69 ± 51.61^{aA}	206.59 ± 53.62^{aA}	202.27 ± 56.23^{aA}	245.87 ± 143.52^{aA}
	28	206.04 ± 48.24^{aA}	203.67 ± 47.47^{aA}	204.19 ± 49.41^{aA}	206.71 ± 50.03^{aA}	204.37 ± 45.15^{aA}	201.22 ± 45.37^{aA}
Cohesiveness (g)	1	5.88 ± 0.19^{aA}	6.07 ± 0.14^{aA}	6.16 ± 0.16^{aA}	6.12 ± 0.21^{aA}	5.92 ± 0.21^{aA}	6.10 ± 0.24^{aA}
	7	5.96 ± 0.22^{aA}	6.12 ± 0.25^{aA}	5.96 ± 0.18^{aA}	6.18 ± 0.16^{aA}	6.14 ± 0.34^{aA}	5.90 ± 0.19^{aA}
	14	5.94 ± 0.41^{aA}	5.94 ± 0.18^{aA}	6.08 ± 0.21^{aA}	6.18 ± 0.23^{aA}	6.12 ± 0.09^{aA}	5.94 ± 0.13^{aA}
	21	5.99 ± 0.19^{aA}	6.14 ± 0.18^{aA}	6.08 ± 0.15^{aA}	6.16 ± 0.24^{aA}	6.20 ± 0.39^{aA}	6.07 ± 0.32^{aA}
	28	6.08 ± 0.15^{aA}	6.14 ± 0.23^{aA}	6.12 ± 0.24^{aA}	6.05 ± 0.15^{aA}	6.10 ± 0.29^{aA}	6.20 ± 0.38^{aA}
Viscosity index (g.s)	1	0.75 ± 0.10^{aA}	0.78 ± 0.08^{aA}	0.82 ± 0.06^{aA}	0.83 ± 0.11^{aA}	0.78 ± 0.07^{aA}	0.80 ± 0.12^{aA}
	7	0.78 ± 0.14^{aA}	0.82 ± 0.13^{aA}	0.72 ± 0.11^{aA}	0.83 ± 0.08^{aA}	0.84 ± 0.14^{aA}	0.73 ± 0.09^{aA}
	14	0.85 ± 0.06^{aA}	0.89 ± 0.11^{aA}	0.89 ± 0.07^{aA}	0.87 ± 0.13^{aA}	0.74 ± 0.04^{aA}	0.83 ± 0.14^{aA}
	21	0.80 ± 0.14^{aA}	0.87 ± 0.08^{aA}	0.76 ± 0.08^{aA}	0.88 ± 0.09^{aA}	0.79 ± 0.09^{aA}	0.80 ± 0.11^{aA}
	28	0.79 ± 0.04^{aA}	0.77 ± 0.10^{aA}	0.76 ± 0.10^{aA}	0.78 ± 0.10^{aA}	0.79 ± 0.12^{aA}	0.75 ± 0.15^{aA}

Means ± standard deviation in the same row followed by different uppercase letters indicate statistically significant differences at $p \leq 0.05$ between formulations of sugarcane juice for the same storage day ($n = 6$). Means ± standard deviation in the same column followed by different lowercase letters indicate statistically significant differences at $p \leq 0.05$ for each formulation affected by storage time. PUR: pure, PRO: with probiotic, OLIGO: with oligofructose, POLY: with polydextrose, SYNB-O: with oligofructose and probiotic, SYNB-P: with polydextrose and probiotic.

added component (probiotic, oligofructose or polydextrose), all texture attributes remained similar to those observed in the pure product ($p > 0.05$). The formulations showed no differences in the texture parameters ($p > 0.05$) during storage. The maintenance of the texture parameters during refrigerated storage (7°C) is important, since it indicates that the addition of the probiotic and prebiotic components does not modify the product and that after 28 days of storage, the sugarcane juices are similar to the newly manufactured products (da Costa *et al.*, 2017).

The results of the physicochemical characteristics and texture parameters indicate that the addition of prebiotics (oligofructose or polydextrose) had no negative impact on the characteristics of the sugarcane juices, while the addition of the probiotic culture resulted in more acidic, cloudier, lighter and greener coloured products.

Probiotic culture viability

The *L. casei* survival during storage is shown in Figure 1. On the first day of storage, the sugarcane juice formulations showed similar counts of *L. casei* ($p > 0.05$), demonstrating that the microorganisms were added in the same concentration in all products. During the storage period, the probiotic count decreased in all formulations ($p \leq 0.05$), however, the viability remained higher than 10^9 CFU/mL, despite the acidic conditions of the probiotic products (pH 3.46-4.15) and the high carbohydrate concentration (19.68-22.88 g/100 mL). Minimum counts of 10^7 CFU/mL are suggested for probiotic foods (Ding and Shah, 2008; Pimentel *et al.*, 2015; da Costa *et al.*, 2017).

The results indicate that the *L. casei* probiotic culture was resistant, sugarcane juice was a suitable medium for probiotic supplementation, and the products could be stored for 28 days at 7°C, which is the conventional condition of commercialisation of refrigerated juices. In addition, it proves that it is possible to add the probiotic culture in the freeze-dried form, resulting in products with sufficient counts, without needing to propagate the probiotic in MRS broth or juice and separate the biomass in refrigerated centrifuge. This result is extremely important for the beverage industry, since it makes the production easier, consumes shorter time and reduces the chances of contamination.

The addition of prebiotic components (SYNB-O and SYNB-P) had no influence on probiotic culture viability ($p > 0.05$) (PRO). The protective effect of prebiotics on probiotic cultures seems to be related to the physicochemical characteristics of the fruit and vegetable beverages. The sugarcane juice did not have an extremely low pH (3.3-5.4), presented nitrogenous compounds in sufficient amounts (protein content > 0.3%, Nualkaekul *et al.*, 2012), high total sugars (18 g/100 mL, Manikantan *et al.*, 2017) and adequate vitamin C content (2.98 mg/100 g, Ramachandran *et al.*, 2017), which helped to maintain the viability of *L. casei*.

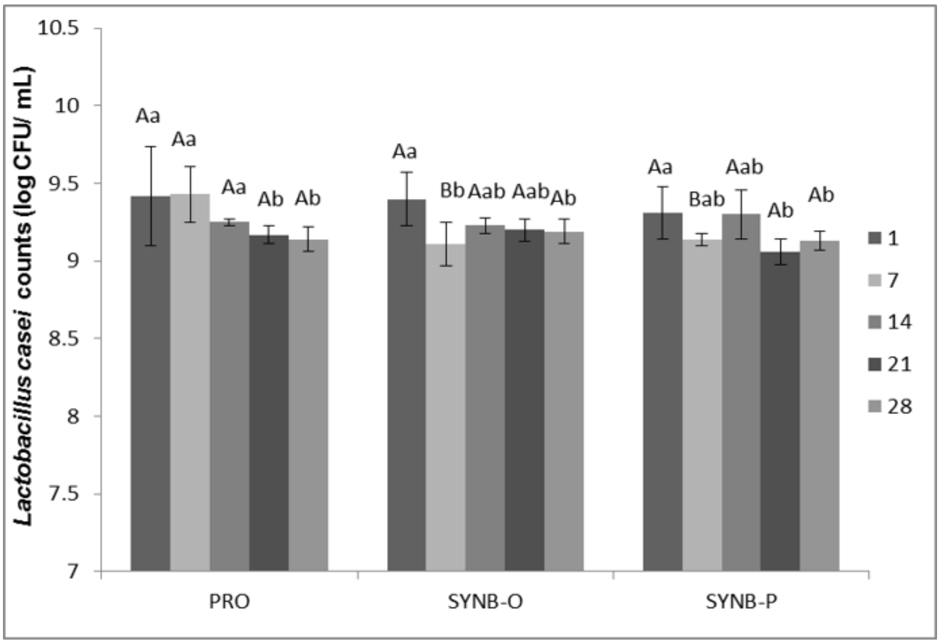

Figure 1. Viability (log CFU/mL) of the Lactobacillus casei in formulations of sugarcane juices (PRO, SYNB-O and SYNB-P) during cold storage (7ºC). The error bars represent the standard deviation of the mean ($n = 6$). Different uppercase letters indicate statistically significant differences at $p \leq 0.05$ between formulations for the same storage day, and different lowercase letters indicate statistically significant differences at $p \leq 0.05$ for each formulation affected by storage time. Days of storage: 1, 7, 14, 21 and 28. Formulation: PRO (probiotic), SYNB-O (probiotic + oligofructose) and SYNB-P (probiotic + polydextrose).

Sensory analysis

Table 4 shows the average acceptance scores (appearance, aroma, flavour, texture and overall impression) and the purchase intention of the sugarcane juice formulations. The products presented scores from 4.93 to 7.12 on a 9-point scale, indicating that the consumers were indifferent to some formulations and liked slightly to moderate the others. The low acceptance scores in the pure products (5.93-6.78) is related to the use of a water bath in the pasteurisation of the products, and to the fact that the consumers are used to the immediate consumption of the raw juice shortly after extraction. da Costa *et al.* (2017) reported that these technological problems could be solved easily on an industrial scale, as the industries have heat exchangers for the thermal treatment, which use higher temperatures and lower retention time (seconds) than the batch method, thus maintaining the sensory properties of the juices.

The addition of oligofructose (OLIGO) or polydextrose (POLY) did not alter the acceptance of sugarcane juice in any of the evaluated attributes (appearance, aroma, flavour, texture, overall impression and purchase intention) ($p > 0.05$). Furthermore, the colour changes provided by polydextrose increased the acceptance on the appearance (day 28) ($p \leq 0.05$). The results indicate that both oligofructose and polydextrose could be used as prebiotic components without negative impact on the sensory acceptance of the products.

The addition of the probiotic culture resulted in decreased acceptance in most of the attributes, and the negative impact was accentuated during storage ($p \leq 0.05$). These results could be related to the increased acidity, turbidity and luminosity of the probiotic products when compared to the pure products (Table 2). Previous studies that used the propagation of the probiotic cultures before addition to fruit juices (Pimentel *et al.*, 2015; da Costa *et al.*, 2017) did not observe a negative impact of the cultures on the acceptance of the products. Therefore, the addition of the freeze-dried culture resulted in a higher metabolic activity of the probiotic cultures and, consequently, in more changes on the physicochemical characteristics and reduced acceptance.

The sugarcane juices added with *L. casei* could improve the immunological system (Mirzaei *et al.*, 2008), present antioxidant and anticarcinogenic properties (Liu *et al.*, 2011), among other benefits. The formulations with prebiotics (oligofructose or polydextrose) could increase the faecal mass, improve the absorption of minerals (calcium, magnesium and iron) from the diet, decrease intestinal inflammatory disorders, and reduce the risk of colon cancer, among others (Gibson *et al.*, 2017).

The results of the present work are of relevance considering the easiness of the sugar cane juice consumption and the possible probiotic effect with the ingestion of just one cup per day. In addition, the ingestion of probiotic cultures in processed foods is recommended instead of using supplements (Champagne *et al.*, 2018). Future studies should characterise the sensory properties of the products by using a trained panel (Janiaski *et al.*, 2016) and innovative methods based on consumer perception (Torres *et al.*, 2017; Oliveira *et al.*, 2017; Esmerino *et al.*, 2017a). In addition, temporal (Esmerino *et al.*, 2017b) and projective methods (Soares *et al.*, 2017; Pinto *et al.*, 2018; Pacheco *et al.*, 2018) should also be conducted.

Table 4. Sensory acceptance of the formulations of sugarcane juices during refrigerated storage (7°C).

Attribute	Storage time (days)	Formulation					
		PUR	PRO	OLIGO	POLY	SYNB-O	SYNB-P
Appearance	1	6.78 ± 2.06^{Aa}	6.14 ± 2.06^{ABa}	7.03 ± 1.85^{Aa}	6.64 ± 1.95^{Aa}	5.90 ± 2.25^{ABa}	5.53 ± 2.39^{Ba}
	28	5.29 ± 2.10^{Bb}	5.74 ± 2.19^{ABa}	5.01 ± 2.17^{Bb}	6.41 ± 8.77^{Aa}	5.88 ± 2.14^{ABa}	5.89 ± 2.02^{ABa}
Aroma	1	6.33 ± 2.11^{Aa}	6.22 ± 1.82^{Aa}	6.67 ± 1.87^{Aa}	6.34 ± 2.06^{Aa}	6.18 ± 2.11^{Aa}	6.00 ± 2.14^{Aa}
	28	5.93 ± 1.86^{Aa}	5.10 ± 2.23^{Bb}	6.04 ± 1.91^{Ab}	5.86 ± 2.08^{Aa}	5.13 ± 2.31^{Bb}	5.47 ± 2.12^{ABa}
Flavour	1	6.65 ± 2.28^{Aa}	5.90 ± 2.04^{Ba}	7.11 ± 1.78^{Aa}	7.07 ± 2.03^{Aa}	5.72 ± 2.41^{Ba}	5.60 ± 2.41^{Ba}
	28	6.70 ± 2.02^{Aa}	4.93 ± 2.51^{Bb}	6.63 ± 1.91^{Aa}	6.15 ± 2.39^{Ab}	4.56 ± 2.53^{Bb}	5.31 ± 2.64^{Ba}
Texture	1	6.74 ± 2.13^{Aa}	6.31 ± 1.92^{ABa}	7.12 ± 1.73^{Aa}	7.12 ± 1.96^{Aa}	$5.84 \pm 2,26^{Ba}$	5.86 ± 2.14^{Ba}
	28	6.66 ± 1.69^{Aa}	5.72 ± 2.35^{BCa}	6.81 ± 1.54^{Aa}	6.37 ± 2.09^{ABb}	5.32 ± 2.51^{Ca}	5.87 ± 2.25^{BCa}
Overall impression	1	6.73 ± 2.12^{Aa}	6.17 ± 1.85^{ABa}	7.17 ± 1.60^{Aa}	6.99 ± 1.95^{Aa}	5.87 ± 2.11^{Ba}	5.65 ± 2.23^{Ba}
	28	6.32 ± 1.79^{Aa}	5.22 ± 2.47^{Bb}	6.23 ± 1.77^{Ab}	6.18 ± 2.09^{Ab}	4.90 ± 2.41^{Bb}	5.53 ± 2.42^{Ba}
Purchase intention	1	3.46 ± 1.30^{Aa}	2.97 ± 1.26^{Ba}	3.80 ± 1.02^{Aa}	3.81 ± 1.19^{Aa}	2.84 ± 1.33^{Ba}	2.87 ± 1.33^{Ba}
	28	3.38 ± 1.12^{Aa}	2.67 ± 1.21^{Ba}	3.28 ± 1.10^{Ab}	3.15 ± 1.20^{Ab}	2.48 ± 1.22^{Bb}	2.82 ± 1.35^{Ba}

Means ± standard deviation in the same row followed by different uppercase letters indicate statistically significant differences at $p \leq 0.05$ between formulations of sugarcane juice for the same storage day ($n = 6$). Means ± standard deviation in the same column followed by different lowercase letters indicate statistically significant differences at $p \leq 0.05$ for each formulation affected by storage time. PUR: pure, PRO: with probiotic, OLIGO: with oligofructose, POLY: with polydextrose, SYNB-O: with oligofructose and probiotic, SYNB-P: with polydextrose and probiotic.

Conclusion

The pasteurised sugarcane juice has been demonstrated as a suitable matrix for the incorporation of oligofructose or polydextrose as prebiotics, resulting in products with physicochemical characteristics, texture, acceptance and storage stability similar to the pure product. This is the first time that polydextrose was used as a prebiotic component in fruit juices. Based on the results; it had similar behaviour to the oligofructose. The *L. casei* remained viable in the product (> 10^9 CFU/mL) for 28 days of refrigerated storage (7°C) and its addition resulted in products more acidic, turbid, with lighter and greener colour, and less accepted than the pure product.

The present work showed that it is possible to directly add the freeze-dried probiotic culture (*L. casei*) to the sugarcane juice, replacing the traditional methods of activation and propagation of the culture in MRS broth or juice, thus resulting in easier and faster manufacturing process and high counts (> 10^9 CFU/mL), which is the most important property when applying probiotic. However, the probiotic inoculum should be optimised in order to minimise the physicochemical and sensory alterations of the products. The results of the present work could be used by medium and small beverage industries to manufacture high value-added items, considering that the pasteurised sugarcane juice could be stored for 28 days, thus facilitating its commercialisation. Furthermore, it could be added with two prebiotic components without a negative impact, and could also be a probiotic product. Nevertheless, some adjustments to reduce the negative impact on the sensory properties are warranted.

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