



## Yoghurt added with *Lactobacillus casei* and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival

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### ABSTRACT

The effect of addition of natural sugar substitutes (stevia, erythritol or xylitol) and prebiotics (oligo-fructose or polydextrose) on physicochemical characteristics, probiotic survival (*Lactobacillus casei*) and sensory acceptance of yoghurts during storage (7 °C, 28 days) was evaluated. Yoghurts with sucrose or sucralose were also prepared. Xylitol and sucralose added yoghurts had physicochemical characteristics and sensory acceptance similar to those of the product with sucrose. Xylitol was more efficient in maintenance of textural characteristics and probiotic survival in simulated gastrointestinal conditions (SGIC) than sucralose. Addition of erythritol and stevia changed the textural parameters, reduced acceptance and decreased probiotic survival in SGIC. Addition of oligofructose or polydextrose improved texture and increased probiotic survival but decreased flavour acceptance. All formulations could be considered probiotic products during shelf life (7 °C, 28 days) with counts higher than 10<sup>7</sup> cfu mL<sup>-1</sup> in the product and 10<sup>4</sup> cfu mL<sup>-1</sup> after SGIC.

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### 1. Introduction

Sucrose is the main ingredient used in the food industry for promoting sweet taste, it can improve the consistency of products, and does not present bitter or residual tastes (Carocho, Morales, & Ferreira, 2017). However, due to an increase in consumption of foods with low or no sugars, the food industries are trying to include sugar substitutes in their formulations (Furlan & Campderrós, 2017; Gao, Brennan, Mason, & Brennan, 2017). The sucrose substitutes most used are artificial sweeteners, such as saccharin, acesulfame K, aspartame and sucralose (Miele et al., 2017), the latter being considered the best substitute for sugar, because it has slight residual bitter taste, stability to high temperature and acidity and causes few sensorial alterations in products

(Furlan & Campderrós, 2017). However, consumption of artificial sweeteners is associated to increased risk of type 2 diabetes (Fagherazzi et al., 2017), higher body weight (Bian et al., 2017), increased cardiometabolic risk (Azad et al., 2017), and cytotoxic and mutagenic activities (Oliveira et al., 2017), among others.

Thus, there is an increase in demand for natural and healthy products, increasing development of dairy products with natural sweeteners (Miele et al., 2017) and/or added prebiotic components. In the category of natural sweeteners are erythritol (produced by the fermentation of glucose using osmophilic yeasts; Carly et al., 2017), xylitol (obtained by microbial processes involving bacteria, fungi or yeasts and having as a substrate xylose or hemicellulose hydrolysates; Pappu & Gummadi, 2017), and stevia (extracted from leaves of *Stevia rebaudiana* Bertoni; Gao et al., 2017). Among the prebiotic components are oligofructose and polydextrose, which can also be used as sugar substitutes, but it is recommended they be used with sweeteners, because of their low sweetener power (Apolinário et al., 2014). Prebiotic components can promote health

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benefits to consumers associated with modulation of their microbiota (Das, Choudhary, & Thompson-Witrick, 2019; Gibson et al., 2017).

Probiotics are living microorganisms, that confer beneficial effects on the individual, when administered in adequate amounts (Hill et al., 2014). To exert a beneficial impact on consumer health, probiotic cultures must be viable in the product throughout the shelf-life ( $>10^6$  cfu mL<sup>-1</sup>) (Sangami & Sri, 2017; Savedboworn et al., 2017). Survival depends on the conditions of the medium and ingredients used. Only one study evaluated the effect of different sweeteners addition on the viability of probiotic cultures in yoghurts (Vinderola, Costa, Regenhardt, & Reinheimer, 2002); however, it used only traditional artificial sweeteners (aspartame and acesulfame K). In addition, probiotics should be able to survive under the conditions of the gastrointestinal tract, i.e., be resistant to stomach acidity and bile salts (Champagne, Gomes da Cruz, & Daga, 2018). However, few studies take this latter aspect into account when evaluating probiotic survival in dairy products.

The objective of the present study was to evaluate the effect of addition of natural sugar substitutes (stevia, erythritol or xylitol) and prebiotics (oligofructose or polydextrose) on the physico-chemical characteristics, probiotic culture survival (*Lactobacillus casei*) and sensory acceptance of yoghurts during refrigerated storage (7 °C for 28 days). Sucrose and sucralose added products were used as controls.

## 2. Material and methods

### 2.1. Material

Oligofructose (P95, Orafiti®, Tienen, Belgium), polydextrose (Tate & Lyle®, London, UK), xylitol (La Pianezza®, Santa Barbara do Oeste, Brazil), erythritol (La Pianezza®), stevia A (90% of rebaudioside, Stevita®, Maringá, Brazil), stevia B (20% of rebaudioside, Stevita®) and sucralose (Splenda, Tate & Lyle®) were used as sweeteners or prebiotic components in the experiment. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (YF-L812, Christian Hansen®, Valinhos, Brazil) were used as starter cultures and *L. casei* (*L. casei*-01, Christian Hansen®) as probiotic culture.

### 2.2. Starter culture preparation

The starter culture was prepared according to the recommendations of the manufacturer. The content of the package (25 g) was poured into 1 L of sterilised milk, mixed, and stored frozen (50-mL aliquots). For every yoghurt batch, it was thawed and inoculated.

### 2.3. Yoghurt preparation

Eight formulations of yoghurt were prepared (Table 1): SUC (sucrose), SUCRA (sucralose), XYL (xylitol), ST-A (stevia A), ST-B

(stevia B), ERY (erythritol), ERY-O (erythritol + oligofructose) and ERY-P (erythritol + polydextrose). The concentration of sweeteners was determined in preliminary tests using pairwise comparison tests (Stone, 2012) to evaluate the sweet taste intensity. Thus, all formulations had the same sweet taste intensity when compared with the sucrose added product. The stevia sweeteners differed in rebaudioside content and were tested to observe if concentration of this component resulted in higher acceptance of products by consumers and better-quality products, as it is stated that stevia with higher rebaudioside content has lower bitter taste (Poele et al., 2018). As the prebiotic components have low sweetening capacity (Apolinário et al., 2014), oligofructose and polydextrose were used in association with erythritol, which was the natural sweetener with the lowest sweetener capacity (70% of the sucrose sweetener power) (Carocho et al., 2017), aiming for synergism and cost reduction. Sucralose was used as control, because it is considered the artificial sweetener that best substitutes for sucrose (Furlan & Campderrós, 2017).

For yoghurt production, sucrose or the sweeteners/prebiotics and 35 g L<sup>-1</sup> of skimmed milk powder (SMP) was added to full-fat milk. The SMP was used to adjust the total solids content and produce products with desirable texture characteristics. The mixture was pasteurised at 85 °C for 30 min in a water bath (Marconi®, Piracicaba, Brazil) and cooled to 42 °C (Akalin, Fenderya, & Akbulut, 2004). Then, 30 mL L<sup>-1</sup> starter culture and 0.2 g L<sup>-1</sup> probiotic culture (approximately 7.5 log cfu mL<sup>-1</sup>) were added; and the mix was incubated at 42 °C for 5 h. After fermentation, the yoghurt was beaten, packed in translucent polypropylene plastic (52 mm high and 52 mm diameter) containers with 80 mL of capacity and stored at 7 °C for 28 days.

### 2.4. Chemical composition and physicochemical analysis

The chemical composition of the yoghurt formulations was determined on the 1st day of storage, according to the methodologies proposed by AOAC (2004).

The fatty acid profile was performed on the 1st day of storage. Fatty acid methyl esters (FAME) were prepared by methylation of total lipids, according to Florence et al. (2012). Methyl esters were separated by gas chromatography using a Thermo model Trace Ultra 3300 (Thermo Scientific, Waltham, MA, USA) equipped with a flame ionisation and cyanopropyl capillary column (100 m × 0.25 i.d., 0.25-µm film thickness, CP-7420). Gas flow rates were 1.2 mL min<sup>-1</sup> carrier gas (H<sub>2</sub>), 30 mL min<sup>-1</sup> make-up gas (N<sub>2</sub>) and 35 and 350 mL min<sup>-1</sup> for the flame gases (H<sub>2</sub> and synthetic air, respectively). The sample splitting rate was 1:80, and samples (2 µL) were injected in duplicate. Operational parameters were as follows: detector and injection port temperatures of 240 °C, column temperature of 185 °C for 7.5 min, programmed to increase at 4 °C min<sup>-1</sup> to 235 °C and maintenance at this temperature for 1.5 min. Peak areas were determined using ChromQuest 5.0 software (Thermo Scientific, Waltham, MA, USA). For fatty acid

**Table 1**  
Experimental design of sweeteners used in yoghurt formulations.

Formulation	Sweetener (g L <sup>-1</sup> )							
	Sucrose	Sucralose	Xylitol	Stevia A	Stevia B	Erythritol	Oligofructose	Polydextrose
SUC	120	–	–	–	–	–	–	–
SUCRA	–	0.2	–	–	–	–	–	–
XYL	–	–	120	–	–	–	–	–
ST-A	–	–	–	0.6	–	–	–	–
ST-B	–	–	–	–	0.6	–	–	–
ERY	–	–	–	–	–	240	–	–
ERY-O	–	–	–	–	–	120	60	–
ERY-P	–	–	–	–	–	120	–	60

identification, the retention times were compared with those of standard methyl esters.

Quantification was performed using tricosanoic acid methyl ester as an internal standard (23:0), and theoretical flame ionisation detector (FID) correction factors were applied to obtain concentrations. Fatty acid contents were calculated in mg g<sup>-1</sup> of total lipids using equation (1).

$$FA = \frac{A_X W_{IS} C_{F_X}}{A_{IS} W_X C_{F_{AE}}} \times 100 \quad (1)$$

where FA is the mg of fatty acids per g of total lipids; AX is the peak area (fatty acids); AIS is the peak area of the internal standard (IS) methyl ester of tricosanoic acid (23:0); WIS is the IS weight (mg) added to the sample (in mg); WX is the sample weight (in mg); CFX is the theoretical correction factor; CFAE is the conversion factor required to express results in mass of fatty acids rather than mass of methyl esters.

The atherogenic index (AI; Batista et al., 2017), thrombogenic index (TI, Batista et al., 2017), desired fatty acids (DFA; Barłowska et al., 2018) and hypercholesterolemic saturated fatty acids (HSFA; Barłowska et al., 2018) were calculated according to equations (2)–(5).

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\sum MUFA + \sum PUFA(n-6) (n-3)] \quad (2)$$

$$TI = (C14:0 + C16:0 + C18:0) / [0.5 \times \sum MUFA + 0.5 \times \sum PUFA(n-6) + 3 \times \sum PUFA(n-3) + (n-3)/(n-6)] \quad (3)$$

$$DFA = MUFA + PUFA + C18:0 \quad (4)$$

$$HSFA = C12:0 + C14:0 + C16:0 \quad (5)$$

The physicochemical characteristics were evaluated on days 1, 7, 14, 21 and 28 of storage. The pH of products was determined using a pre-calibrated digital potentiometer (MS Tecnoyon Instrumentation, mPA210, Piracicaba, Brazil). The titratable acidity was determined according to AOAC (2004). The total soluble solids (TSS) content was determined in a digital refractometer (Instruterm®, São Paulo, Brazil) and expressed as °Brix. For colour instrumental evaluation, a colorimeter (Minolta®, model CR400, Osaka, Japan) was used. The equipment provided the parameters L\* (luminosity), a\* (red-green component) and b\* (yellow-blue component).

Texture parameters (firmness, consistency, cohesiveness and viscosity index) were determined in a TAXT Plus texturometer (Stable Micro Systems, London, UK) by simple compression. The formulations, in their original containers, were compressed with a cylindrical probe with a 36 mm diameter (P 36R) and penetration of 10 mm at pre-test and test speeds of 1 mm s<sup>-1</sup>, post-test speed of 10 mm s<sup>-1</sup> and 1 g of trigger (Januário et al., 2017).

## 2.5. Sensory analysis

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 58 untrained individuals (34 women, 24 men), ranging in age from 15 to over 50 years, with majority (49 individuals) aged 15–25 years. Individuals were students, professors and staff of the same university and participated in both sensory evaluations.

The acceptance test (appearance, aroma, flavour, texture and overall impression) used a hedonic scale of 9 points (1, disliked very much; 9, liked very much) (Cruz et al., 2010); and the purchase intention test used a 5-point scale (1, certainly would not buy; 5,

would certainly buy). This project was approved by the Ethics Committee involving human (CAAE: 54928016.4.0000.5688, Opinion number: 2.037.951). The judges evaluated the eight formulations in two evaluation sessions, at a temperature of 7 °C, one at a time, in a randomised order. Drinking water at room temperature and cream cracker biscuits were available for mouth cleaning before and between evaluations of the formulations.

## 2.6. Probiotic survival

Probiotic survival was determined in the yoghurt formulations during storage and in the simulated gastrointestinal conditions (SGIC). Survival in the product was evaluated on days 1, 7, 14, 21 and 28 of storage, while survival in the SGIC was performed on days 1 and 28 of storage.

After homogenisation of the samples, 1 mL of each yoghurt formulation was diluted with 9 mL of sterile 0.85% saline solution, mixed and serially diluted. *L. casei* counts were determined on Man Rogosa and Sharp (MRS, Himedia, Mumbai, India) agar supplemented with 2 mL L<sup>-1</sup> of a 0.05% (w/v) vancomycin solution and anaerobic incubation at 37 °C for 72 h (Tharmaraj & Shah, 2003).

The survival of the probiotic to gastric and enteric conditions was carried out according to the methods of Bedani, Vieira, Rossi, and Saad (2014) and Burity, Castro, and Saad (2010), with some modifications. The probiotic yoghurt (50 mL) was transferred to sterile shot tubes (100 mL) and diluted with 0.85% NaCl solution. The pH was adjusted to 2.3–2.6 with 1 M HCl and pepsin and lipase were added until a concentration of 3 g L<sup>-1</sup> and 0.9 mg L<sup>-1</sup>, respectively. Samples were, then, incubated at 37 °C, with shaking at approximately 150 rpm, for 2 h, simulating the gastric phase (GF). After incubation, using an alkaline solution (150 mL 1 M NaOH + 14 g NaH<sub>2</sub>PO<sub>4</sub> in 1 L), the pH was raised to 5.4–5.7 and bile and pancreatin were added reaching concentrations of 10 g L<sup>-1</sup> and 1 g L<sup>-1</sup>, respectively. Samples were incubated again at 37 °C for 2 h, with shaking, simulating the first enteric phase (1EF). In the last step, the pH was raised to 6.8–7.2 using the same alkaline solution, and the bile and pancreatic concentrations were adjusted to 10 g L<sup>-1</sup> and 1 g L<sup>-1</sup>, respectively. Samples were incubated again at 37 °C for 2 h, with shaking, simulating the second enteric phase (2EF). The total assay last 6 h. The *L. casei* count was performed initially and after the full GT simulation (6 h).

## 2.7. Design of the experiment and statistical analysis

The experiment was repeated twice and followed a completely randomised design. All analyses for chemical composition, physicochemical characteristics, texture parameters and probiotic survival were done in triplicates, except fatty acid profile, which was done in duplicates. 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/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 (UFCEG, Campina Grande, Brazil) and Statistical Analysis System (SAS, Institute Inc., Cary, NC, USA) software.

## 3. Results and discussion

### 3.1. Chemical composition

The chemical composition of the yoghurt formulations is presented in Table 2. The chemical composition fell within the

**Table 2**  
Chemical composition of yoghurt formulations containing various sweetening systems.<sup>a</sup>

Parameter	Formulations							
	SUC	SUCRA	XYL	ST-A	ST-B	ERY	ERY-O	ERY-P
Moisture (g 100 g <sup>-1</sup> )	73.68 ± 0.10 <sup>b</sup>	86.40 ± 0.39 <sup>a</sup>	76.11 ± 0.49 <sup>b</sup>	85.10 ± 1.52 <sup>a</sup>	84.82 ± 1.43 <sup>a</sup>	68.78 ± 1.57 <sup>c</sup>	68.39 ± 1.8 <sup>c</sup>	67.91 ± 1.39 <sup>c</sup>
Ash (g 100 g <sup>-1</sup> )	2.29 ± 0.04 <sup>ab</sup>	1.78 ± 0.01 <sup>ab</sup>	1.58 ± 0.01 <sup>b</sup>	1.93 ± 0.01 <sup>ab</sup>	2.25 ± 0.01 <sup>ab</sup>	1.78 ± 0.01 <sup>ab</sup>	2.50 ± 0.01 <sup>a</sup>	1.83 ± 0.01 <sup>ab</sup>
Protein (g 100 g <sup>-1</sup> )	3.18 ± 0.14 <sup>abc</sup>	3.50 ± 0.24 <sup>a</sup>	2.89 ± 0.22 <sup>bc</sup>	2.87 ± 0.21 <sup>c</sup>	3.16 ± 0.07 <sup>abc</sup>	3.14 ± 0.16 <sup>abc</sup>	3.34 ± 0.10 <sup>ab</sup>	3.41 ± 0.16 <sup>a</sup>
Fat (g 100 g <sup>-1</sup> )	3.37 ± 0.23 <sup>a</sup>	3.76 ± 0.10 <sup>a</sup>	3.67 ± 0.42 <sup>a</sup>	3.72 ± 0.48 <sup>a</sup>	3.62 ± 0.20 <sup>a</sup>	3.60 ± 0.43 <sup>a</sup>	3.27 ± 0.22 <sup>a</sup>	3.29 ± 0.23 <sup>a</sup>
Carbohydrates (g 100 g <sup>-1</sup> )	17.50 ± 0.95 <sup>b</sup>	4.56 ± 0.38 <sup>d</sup>	15.90 ± 0.97 <sup>b</sup>	6.38 ± 0.42 <sup>c</sup>	6.15 ± 0.75 <sup>c</sup>	22.70 ± 0.89 <sup>a</sup>	22.50 ± 0.94 <sup>a</sup>	23.57 ± 0.74 <sup>a</sup>

<sup>a</sup> Means ± standard deviation in the same row followed by different lower-case superscript letters indicate statistically significant differences at  $p \leq 0.05$  between formulations of yogurt ( $n = 6$ ). Abbreviations are: SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebaudioside; ST-B, stevia, 20% rebaudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.

following ranges (g 100 g<sup>-1</sup>): moisture (67.9–86.4), ash (1.58–2.50), proteins (2.87–3.50), fat (3.27–3.76) and carbohydrates (4.56–23.57), similar to the chemical composition observed in previous studies (Janiaski, Pimentel, Cruz, & Prudencio, 2016; Januário et al., 2017; Pimentel, Garcia, & Prudêncio, 2012). Substitution of sucrose by SUCRA, natural sweeteners (XYL, ST-A, ST-B, ERY) and prebiotics (ERY-O and ERY-P) did not influence ( $p > 0.05$ ) the protein, fat and ash contents of the yoghurts when compared with the sucrose added product.

The effect of sweeteners on moisture and carbohydrate contents was dependent on the sweetening power and, consequently, on the amount of the sweetener added. Sweeteners with high sweetener capacity (sucralose and stevia A and B) were added in small concentrations (0.2 and 0.6 g L<sup>-1</sup>, respectively) and resulted in products (SUCRA, ST-A and ST-B) with higher moisture and lower carbohydrate contents than sucrose added product (120 g L<sup>-1</sup>) ( $p < 0.05$ ). Addition of XYL resulted in products with similar moisture and carbohydrate contents ( $p > 0.05$ ) when compared with the product with SUC, because both components were added in similar

concentrations (120 g L<sup>-1</sup>). Finally, addition of ERY and prebiotics (ERY-O and ERY-P) resulted in products with lower moisture and higher carbohydrate contents than sucrose added product, as erythritol alone or in combination with prebiotics was added in higher concentrations (180–240 g L<sup>-1</sup>) than the sucrose (120 g L<sup>-1</sup>).

Table 3 shows the fatty acids profile of the yoghurts and the AI, TI, DFA, and HSFA indices. The yoghurts were characterised by the presence of saturated fatty acids (SFA; 572–588 mg g<sup>-1</sup>), followed by monounsaturated fatty acids (MUFA; 272–286 mg g<sup>-1</sup>) and polyunsaturated fatty acids (PUFA; 30–33 mg g<sup>-1</sup>). The substitution of sucrose by different sweeteners resulted in a different fatty acid profile in the yoghurts. In general, higher levels of SFA were observed in the products with sweeteners addition ( $p \leq 0.05$ ), mainly associated to the increased levels of 6:0, 16:0 and 18:0. At the same time, higher levels of 16:1 n-9, 18:2 n-6, and 14:1 n-7, were observed ( $p \leq 0.05$ ), with no significant impact in the PUFA values ( $p > 0.05$ ).

These differences could be the result of possible differences in the fermentation process by starter cultures in the presence of the

**Table 3**  
Fatty acid profile of yogurt formulations containing various sweetening systems.<sup>a</sup>

Parameter	Formulations							
	SUC	SUCRA	XYL	ST-A	ST-B	ERY	ERY-O	ERY-P
4:0	11.4 ± 0.3 <sup>a</sup>	10.9 ± 0.2 <sup>a</sup>	11.2 ± 0.1 <sup>a</sup>	11.3 ± 0.1 <sup>a</sup>	10.8 ± 0.4 <sup>a</sup>	10.9 ± 0.2 <sup>a</sup>	11.7 ± 0.3 <sup>a</sup>	11.2 ± 0.2 <sup>a</sup>
6:0	10.2 ± 1.8 <sup>c</sup>	13.7 ± 1.1 <sup>ab</sup>	11.3 ± 0.2 <sup>bc</sup>	15.7 ± 0.3 <sup>a</sup>	14.1 ± 0.1 <sup>ab</sup>	14.5 ± 0.3 <sup>ab</sup>	15.2 ± 0.8 <sup>a</sup>	14.4 ± 0.2 <sup>ab</sup>
8:0	23.1 ± 0.1 <sup>a</sup>	23.1 ± 0.1 <sup>a</sup>	21.9 ± 1.2 <sup>a</sup>	23.0 ± 0.4 <sup>a</sup>	23.6 ± 1.1 <sup>a</sup>	26.3 ± 0.7 <sup>a</sup>	25.8 ± 2.9 <sup>a</sup>	26.1 ± 0.4 <sup>a</sup>
10:0	33.5 ± 0.6 <sup>ab</sup>	31.8 ± 0.1 <sup>b</sup>	32.3 ± 0.1 <sup>ab</sup>	34.2 ± 0.4 <sup>a</sup>	33.7 ± 0.4 <sup>ab</sup>	33.5 ± 0.6 <sup>ab</sup>	33.5 ± 0.8 <sup>ab</sup>	33.4 ± 0.6 <sup>ab</sup>
12:0	1.7 ± 0.1 <sup>b</sup>	1.7 ± 0.1 <sup>b</sup>	1.7 ± 0.1 <sup>b</sup>	2.9 ± 0.1 <sup>a</sup>	1.7 ± 0.1 <sup>b</sup>	1.7 ± 0.1 <sup>b</sup>	1.7 ± 0.3 <sup>b</sup>	1.7 ± 0.1 <sup>b</sup>
14:0	105.7 ± 0.4 <sup>b</sup>	106.5 ± 0.6 <sup>b</sup>	106.4 ± 0.4 <sup>b</sup>	109.3 ± 0.7 <sup>a</sup>	107.6 ± 0.1 <sup>ab</sup>	105.8 ± 0.5 <sup>b</sup>	107.1 ± 0.5 <sup>b</sup>	105.9 ± 0.7 <sup>b</sup>
14:1 n-11	8.4 ± 0.1 <sup>a</sup>	9.3 ± 0.1 <sup>a</sup>	9.2 ± 0.1 <sup>a</sup>	9.3 ± 0.1 <sup>a</sup>	9.5 ± 0.1 <sup>a</sup>	9.3 ± 0.1 <sup>a</sup>	9.5 ± 1.4 <sup>a</sup>	9.5 ± 0.1 <sup>a</sup>
14:1 n-9	5.9 ± 0.1 <sup>a</sup>	4.3 ± 0.7 <sup>b</sup>	6.0 ± 0.1 <sup>a</sup>	4.4 ± 0.1 <sup>b</sup>	4.1 ± 0.1 <sup>b</sup>	4.2 ± 0.1 <sup>b</sup>	4.3 ± 0.1 <sup>b</sup>	4.3 ± 0.1 <sup>b</sup>
14:1 n-7	5.8 ± 0.1 <sup>b</sup>	5.5 ± 0.8 <sup>b</sup>	9.0 ± 1.5 <sup>a</sup>	6.3 ± 0.1 <sup>b</sup>	6.0 ± 0.1 <sup>b</sup>	6.4 ± 0.1 <sup>b</sup>	6.5 ± 0.1 <sup>b</sup>	6.5 ± 0.1 <sup>b</sup>
16:0	278.7 ± 0.7 <sup>bc</sup>	284.7 ± 2.2 <sup>a</sup>	285.5 ± 1.0 <sup>a</sup>	276.5 ± 0.9 <sup>c</sup>	281.2 ± 0.9 <sup>ab</sup>	281.7 ± 0.2 <sup>ab</sup>	282.1 ± 0.8 <sup>ab</sup>	281.6 ± 1.1 <sup>ab</sup>
16:1 n-9	2.2 ± 0.1 <sup>cd</sup>	2.4 ± 0.1 <sup>ab</sup>	2.6 ± 0.1 <sup>a</sup>	2.1 ± 0.1 <sup>d</sup>	2.6 ± 0.1 <sup>a</sup>	2.3 ± 0.1 <sup>bc</sup>	2.3 ± 0.1 <sup>bc</sup>	2.3 ± 0.1 <sup>bc</sup>
16:1 n-7	11.5 ± 0.5 <sup>ab</sup>	10.3 ± 0.1 <sup>abc</sup>	9.8 ± 0.2 <sup>bc</sup>	12.0 ± 0.1 <sup>a</sup>	11.8 ± 0.8 <sup>ab</sup>	10.4 ± 1.1 <sup>abc</sup>	10.2 ± 0.2 <sup>abc</sup>	8.7 ± 0.4 <sup>c</sup>
18:0	108.1 ± 0.8 <sup>b</sup>	111.6 ± 1.1 <sup>a</sup>	111.6 ± 0.2 <sup>a</sup>	109.9 ± 0.1 <sup>ab</sup>	110.3 ± 0.7 <sup>ab</sup>	111.3 ± 0.2 <sup>a</sup>	111.0 ± 0.6 <sup>a</sup>	110.9 ± 1.0 <sup>a</sup>
18:1t	30.3 ± 0.4 <sup>a</sup>	30.9 ± 0.3 <sup>a</sup>	31.2 ± 0.2 <sup>a</sup>	30.6 ± 0.1 <sup>a</sup>	30.6 ± 1.4 <sup>a</sup>	30.5 ± 0.3 <sup>a</sup>	29.3 ± 0.2 <sup>a</sup>	30.4 ± 0.1 <sup>a</sup>
18:1 n-9	219.2 ± 0.6 <sup>ab</sup>	220.6 ± 1.1 <sup>a</sup>	218.7 ± 0.9 <sup>abc</sup>	215.5 ± 0.8 <sup>bcd</sup>	215.5 ± 0.3 <sup>bcd</sup>	214.9 ± 0.4 <sup>d</sup>	209.9 ± 1.1 <sup>e</sup>	215.0 ± 1.7 <sup>cd</sup>
18:2t	1.8 ± 0.1 <sup>b</sup>	1.8 ± 0.1 <sup>b</sup>	1.8 ± 0.1 <sup>b</sup>	1.56 ± 0.1 <sup>b</sup>	1.65 ± 0.4 <sup>b</sup>	1.6 ± 0.1 <sup>b</sup>	2.5 ± 0.1 <sup>a</sup>	1.6 ± 0.1 <sup>b</sup>
18:2 n-6	15.3 ± 0.1 <sup>c</sup>	16.4 ± 0.1 <sup>a</sup>	15.9 ± 0.1 <sup>ab</sup>	15.9 ± 0.2 <sup>b</sup>	16.0 ± 0.2 <sup>ab</sup>	15.9 ± 0.1 <sup>b</sup>	16.0 ± 0.1 <sup>ab</sup>	16.1 ± 0.2 <sup>ab</sup>
18:3 n-3	4.2 ± 0.7 <sup>a</sup>	5.8 ± 2.3 <sup>a</sup>	4.3 ± 0.3 <sup>a</sup>	3.8 ± 0.1 <sup>a</sup>	6.6 ± 0.7 <sup>a</sup>	4.7 ± 1.4 <sup>a</sup>	3.8 ± 0.1 <sup>a</sup>	4.1 ± 0.2 <sup>a</sup>
18:2 c9t11	8.9 ± 1.1 <sup>a</sup>	9.2 ± 0.1 <sup>a</sup>	8.5 ± 0.1 <sup>a</sup>	9.2 ± 0.2 <sup>a</sup>	9.3 ± 0.3 <sup>a</sup>	9.3 ± 0.1 <sup>a</sup>	9.3 ± 0.1 <sup>a</sup>	9.4 ± 0.1 <sup>a</sup>
SFA	572.0 ± 3.2 <sup>b</sup>	584.0 ± 3.1 <sup>a</sup>	581.9 ± 0.9 <sup>a</sup>	582.8 ± 2.9 <sup>a</sup>	583.0 ± 0.4 <sup>a</sup>	584.9 ± 1.3 <sup>a</sup>	588.0 ± 3.7 <sup>a</sup>	585.1 ± 0.1 <sup>a</sup>
MUFA	283.3 ± 0.5 <sup>ab</sup>	283.2 ± 2.9 <sup>ab</sup>	286.6 ± 2.9 <sup>a</sup>	280.3 ± 1.1 <sup>abc</sup>	280.1 ± 2.6 <sup>abc</sup>	278.0 ± 1.6 <sup>bc</sup>	272.0 ± 2.7 <sup>c</sup>	276.7 ± 1.1 <sup>bc</sup>
PUFA	30.1 ± 1.7 <sup>a</sup>	33.1 ± 2.5 <sup>a</sup>	30.5 ± 0.3 <sup>a</sup>	30.4 ± 0.1 <sup>a</sup>	33.5 ± 1.1 <sup>a</sup>	31.6 ± 0.1 <sup>a</sup>	31.5 ± 0.5 <sup>a</sup>	31.5 ± 0.5 <sup>a</sup>
AI	2.3 ± 0.1 <sup>b</sup>	2.3 ± 0.1 <sup>b</sup>	2.3 ± 0.1 <sup>b</sup>	2.4 ± 0.1 <sup>ab</sup>	2.4 ± 0.1 <sup>ab</sup>	2.4 ± 0.1 <sup>ab</sup>	2.5 ± 0.1 <sup>a</sup>	2.4 ± 0.1 <sup>ab</sup>
TI	3.0 ± 0.1 <sup>ab</sup>	3.0 ± 0.1 <sup>ab</sup>	3.1 ± 0.1 <sup>ab</sup>	3.1 ± 0.1 <sup>ab</sup>	3.0 ± 0.1 <sup>ab</sup>	3.1 ± 0.1 <sup>ab</sup>	3.2 ± 0.1 <sup>a</sup>	3.1 ± 0.1 <sup>ab</sup>
DFA	421.5 ± 0.4 <sup>abcd</sup>	428.0 ± 1.5 <sup>ab</sup>	428.7 ± 2.9 <sup>a</sup>	420.5 ± 1.2 <sup>bcd</sup>	423.8 ± 2.0 <sup>abc</sup>	420.9 ± 0.3 <sup>abcd</sup>	414.4 ± 3.4 <sup>d</sup>	418.8 ± 2.5 <sup>cd</sup>
HSFA	386.1 ± 0.3 <sup>b</sup>	392.9 ± 2.7 <sup>a</sup>	393.6 ± 1.4 <sup>a</sup>	388.7 ± 1.6 <sup>ab</sup>	390.5 ± 0.8 <sup>ab</sup>	389.2 ± 0.7 <sup>ab</sup>	391.0 ± 0.1 <sup>ab</sup>	389.2 ± 0.4 <sup>ab</sup>

<sup>a</sup> Values (mg fatty acid g<sup>-1</sup> total lipids) are expressed as mean ± standard deviation. Analysis performed in duplicate. Means with different lowercase superscripts in the same row indicate presence of statistical difference ( $p < 0.05$ ) among treatments by Tukey Test. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. AI = (C12:0 + 4 C14:0 + C16:0)/[∑MUFA + ∑PUFA(n-6) and (n-3)]; TI = (C14:0 + C16:0 + C18:0)/(0.5 × ∑MUFA + 0.5 × ∑PUFA(n-6) + 3 × ∑PUFA(n-3) + (n-3)/(n-6)); DFA = MUFA + PUFA + C18:0; HSFA = C12:0 + C14:0 + C16:0 (n = 4). Abbreviations are: SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebaudioside; ST-B, stevia, 20% rebaudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.

different sweeteners (Florence et al., 2012). Furthermore, it could be associated with inhibition of production of fatty acids by starter and probiotic cultures in the presence of sucrose (Lin, 2000). Batista et al. (2015) reported that modification in the ingredients used or process parameters during yoghurt preparation can provide a variable effect on the lipolytic activity of starter and probiotic cultures, by favouring certain reactions pathways and resulting in different fatty acid profile. The increase in unsaturation has already been reported for several microorganisms (starter or probiotic cultures) and it was suggested to be a universally conserved adaptation response (Espírito Santo et al., 2012). Results of the present study suggest that there was a possible inhibition of the starter cultures in the presence of sucrose, which was not observed when the other sweeteners were added. Therefore, it contributed to higher lipolytic activity in the sweetener-added yoghurts, resulting in higher contents of SFA and some MUFA and/or PUFA.

Considering health indices (AI and TI), all yoghurts presented low values (Sperry et al., 2018). Addition of XYL, SUCRA, ST-A, ST-B, ERY and ERY-P did not result in a significant effect ( $p > 0.05$ ) when compared with the product with sucrose. The addition of ERY-O resulted in a product with higher AI ( $p \leq 0.05$ ). Thus, although addition of sweeteners resulted in a different fatty acid profile, the health effect of the yoghurts with sucrose would be maintained with the sweeteners, except for ERY-O products, which had slightly higher AI. The atherogenic index is associated to the blockage of arteries, and a high atherogenic index can lead to cell adhesion in the immune and circulatory systems. In contrast, a lower atherogenic index prevents occurrence of micro and macro-coronary disease and is related to healthy lipid indices (Balthazar et al., 2016).

### 3.2. Physicochemical characteristics

The physicochemical characteristics of the yoghurt formulations are presented in Table 4. Yoghurts presented pH of 3.95–4.43 and titratable acidity of 0.70–1% of lactic acid during the storage period, similar to those observed in previous studies (Januário et al., 2017; Pimentel et al., 2012). Substitution of sucrose with natural and prebiotic sweeteners did not result in alteration of the pH and titratable acidity of the yoghurts (XYL, SUCRA, ERY, ERY-O, ERY-P, ST-A and ST-B) when compared with the control product (SUC) ( $p > 0.05$ ), except for the ERY product on day 1, that had lower pH ( $p \leq 0.05$ ).

During the storage period, yoghurts with SUCRA, XYL, ST-A and ST-B behaved in a similar way to the product with sucrose (SAC), with decreases in the pH and increases in the titratable acidity ( $p \leq 0.05$ ). Erythritol added yoghurts (ERY, ERY-O and ERY-P) showed a decrease in pH ( $p \leq 0.05$ ), but maintenance of the titratable acidity ( $p > 0.05$ ). A previous study reported antagonistic activity of erythritol on some LAB (Klewicki & Klewicka, 2004), which could have resulted in less production of lactic acid during the storage of the yoghurt with erythritol compared with the other products. Products with erythritol presented a lower decrease in pH values (0.15–0.28) than the product with sucrose (0.3), suggesting a lower post-acidification. Decline in pH and increase in acidity are the result of post-acidification of products and related to the continuity of the fermentative process by starter and probiotic cultures during the storage period, with small production of lactic acid (Gengatharan, Dykes, & Choo, 2017).

Similarly, as discussed for carbohydrate content, the TSS values of the products added with high intensity sweeteners (SUCRA, ST-A and ST-B) were lower than the values observed for sucrose added

**Table 4**  
Physicochemical characteristics of yogurt formulations containing various sweetening systems.<sup>a</sup>

Parameter	Storage day	Formulations							
		SUC	SUCRA	XYL	ST-A	ST-B	ERY	ERY-O	ERY-P
pH	1	4.30 ± 0.11 <sup>aA</sup>	4.30 ± 0.11 <sup>aA</sup>	4.30 ± 0.11 <sup>aA</sup>	4.40 ± 0.11 <sup>aA</sup>	4.40 ± 0.11 <sup>abA</sup>	4.10 ± 0.11 <sup>abB</sup>	4.43 ± 0.15 <sup>aA</sup>	4.40 ± 0.11 <sup>aA</sup>
	7	4.20 ± 0.11 <sup>abABC</sup>	4.20 ± 0.11 <sup>abABC</sup>	4.16 ± 0.11 <sup>bcB</sup>	4.23 ± 0.11 <sup>abABC</sup>	4.26 ± 0.11 <sup>bcABC</sup>	4.10 ± 0.11 <sup>abc</sup>	4.30 ± 0.11 <sup>baB</sup>	4.36 ± 0.11 <sup>aA</sup>
	14	4.26 ± 0.12 <sup>abAB</sup>	4.29 ± 0.17 <sup>aA</sup>	4.22 ± 0.19 <sup>abAB</sup>	4.17 ± 0.17 <sup>abAB</sup>	4.18 ± 0.12 <sup>caB</sup>	4.06 ± 0.11 <sup>abB</sup>	4.30 ± 0.13 <sup>abA</sup>	4.34 ± 0.10 <sup>aA</sup>
	21	4.22 ± 0.13 <sup>abA</sup>	4.19 ± 0.130 <sup>abA</sup>	4.14 ± 0.12 <sup>ba</sup>	4.14 ± 0.11 <sup>ba</sup>	4.20 ± 0.16 <sup>ca</sup>	4.21 ± 0.15 <sup>aA</sup>	4.24 ± 0.11 <sup>ba</sup>	4.24 ± 0.15 <sup>abA</sup>
	28	4.00 ± 0.11 <sup>baB</sup>	4.08 ± 0.11 <sup>baB</sup>	4.14 ± 0.17 <sup>baB</sup>	4.08 ± 0.12 <sup>baB</sup>	4.16 ± 0.16 <sup>caB</sup>	3.95 ± 0.12 <sup>cb</sup>	4.26 ± 0.13 <sup>ba</sup>	4.12 ± 0.11 <sup>baB</sup>
Titratable acidity (% lactic acid)	1	0.82 ± 0.12 <sup>ba</sup>	0.89 ± 0.11 <sup>ba</sup>	0.81 ± 0.13 <sup>ba</sup>	0.87 ± 0.11 <sup>ba</sup>	0.85 ± 0.11 <sup>ba</sup>	0.80 ± 0.11 <sup>aA</sup>	0.77 ± 0.13 <sup>aA</sup>	0.75 ± 0.12 <sup>aA</sup>
	7	0.82 ± 0.45 <sup>ba</sup>	0.98 ± 0.12 <sup>aA</sup>	0.83 ± 0.11 <sup>abA</sup>	0.95 ± 0.15 <sup>abA</sup>	0.95 ± 0.11 <sup>abA</sup>	0.82 ± 0.11 <sup>aA</sup>	0.75 ± 0.11 <sup>aA</sup>	0.82 ± 0.12 <sup>aA</sup>
	14	0.80 ± 0.14 <sup>ba</sup>	0.94 ± 0.14 <sup>abA</sup>	0.86 ± 0.13 <sup>abA</sup>	0.97 ± 0.13 <sup>aA</sup>	0.92 ± 0.12 <sup>abA</sup>	0.84 ± 0.11 <sup>aA</sup>	0.78 ± 0.14 <sup>aA</sup>	0.75 ± 0.11 <sup>aA</sup>
	21	0.80 ± 0.13 <sup>ba</sup>	0.99 ± 0.16 <sup>aA</sup>	0.78 ± 0.19 <sup>ba</sup>	0.99 ± 0.12 <sup>aA</sup>	0.95 ± 0.15 <sup>abA</sup>	0.81 ± 0.12 <sup>aA</sup>	0.77 ± 0.13 <sup>aA</sup>	0.83 ± 0.14 <sup>aA</sup>
	28	0.90 ± 0.46 <sup>aA</sup>	1.00 ± 0.11 <sup>aA</sup>	0.87 ± 0.16 <sup>aA</sup>	1.00 ± 0.12 <sup>aA</sup>	1.00 ± 0.12 <sup>aA</sup>	0.80 ± 0.11 <sup>aA</sup>	0.70 ± 0.12 <sup>aA</sup>	0.80 ± 0.11 <sup>aA</sup>
TSS (°Brix)	1	18.13 ± 0.11 <sup>aCD</sup>	9.60 ± 0.26 <sup>aE</sup>	17.96 ± 0.47 <sup>aD</sup>	9.33 ± 0.35 <sup>aE</sup>	8.33 ± 0.28 <sup>aE</sup>	23.46 ± 0.77 <sup>aA</sup>	20.56 ± 0.15 <sup>aB</sup>	21.26 ± 0.23 <sup>abC</sup>
	7	18.46 ± 0.28 <sup>aCD</sup>	9.63 ± 0.577 <sup>aE</sup>	17.70 ± 0.30 <sup>aD</sup>	9.70 ± 0.10 <sup>aE</sup>	9.06 ± 0.53 <sup>aE</sup>	24.16 ± 1.03 <sup>aA</sup>	21.13 ± 0.58 <sup>aB</sup>	21.70 ± 0.16 <sup>abC</sup>
	14	17.80 ± 0.35 <sup>aCD</sup>	8.43 ± 4.969 <sup>aE</sup>	17.33 ± 0.10 <sup>aD</sup>	9.43 ± 0.58 <sup>aE</sup>	9.20 ± 0.10 <sup>aE</sup>	25.46 ± 2.90 <sup>aA</sup>	20.93 ± 0.15 <sup>aB</sup>	20.86 ± 0.68 <sup>abC</sup>
	21	17.80 ± 0.35 <sup>aCD</sup>	8.43 ± 0.832 <sup>aE</sup>	17.33 ± 0.55 <sup>aD</sup>	9.43 ± 0.58 <sup>aE</sup>	9.20 ± 0.10 <sup>aE</sup>	15.46 ± 2.89 <sup>aA</sup>	20.93 ± 0.15 <sup>aB</sup>	20.86 ± 0.68 <sup>abC</sup>
	28	18.27 ± 0.54 <sup>aCD</sup>	8.33 ± 1.05 <sup>aE</sup>	15.33 ± 4.79 <sup>aD</sup>	8.90 ± 0.34 <sup>aE</sup>	8.91 ± 0.17 <sup>aE</sup>	23.00 ± 1.47 <sup>aA</sup>	21.50 ± 0.26 <sup>aB</sup>	17.10 ± 1.44 <sup>bc</sup>
L*	1	84.68 ± 0.12 <sup>aA</sup>	86.70 ± 0.17 <sup>aA</sup>	85.34 ± 0.12 <sup>aA</sup>	85.82 ± 0.25 <sup>aA</sup>	85.93 ± 0.28 <sup>aA</sup>	83.03 ± 0.35 <sup>aA</sup>	84.03 ± 0.19 <sup>aA</sup>	83.65 ± 0.13 <sup>aA</sup>
	7	84.17 ± 0.18 <sup>aA</sup>	86.42 ± 0.12 <sup>aA</sup>	84.65 ± 0.29 <sup>aA</sup>	86.37 ± 0.18 <sup>aA</sup>	86.15 ± 0.25 <sup>aA</sup>	82.97 ± 0.14 <sup>aA</sup>	83.77 ± 0.17 <sup>aA</sup>	83.52 ± 0.26 <sup>aA</sup>
	14	84.88 ± 0.56 <sup>aA</sup>	86.72 ± 0.44 <sup>aA</sup>	85.00 ± 1.2 <sup>aA</sup>	86.22 ± 0.19 <sup>aA</sup>	86.11 ± 0.35 <sup>A</sup>	82.99 ± 0.17 <sup>aA</sup>	84.02 ± 0.24 <sup>aA</sup>	85.97 ± 2.29 <sup>aA</sup>
	21	76.39 ± 6.90 <sup>bb</sup>	86.47 ± 0.28 <sup>aA</sup>	85.07 ± 0.19 <sup>aA</sup>	84.56 ± 1.52 <sup>A</sup>	74.49 ± 0.64 <sup>bb</sup>	77.67 ± 5.92 <sup>bb</sup>	83.46 ± 0.90 <sup>aA</sup>	83.36 ± 0.29 <sup>aA</sup>
	28	73.21 ± 2.64 <sup>ba</sup>	73.22 ± 2.26 <sup>ba</sup>	73.44 ± 1.75 <sup>ba</sup>	70.10 ± 4.87 <sup>ba</sup>	73.39 ± 1.10 <sup>ba</sup>	71.95 ± 4.32 <sup>ca</sup>	71.83 ± 1.93 <sup>ba</sup>	72.55 ± 1.44 <sup>ba</sup>
a*	1	3.45 ± 0.12 <sup>aA</sup>	3.33 ± 0.15 <sup>aA</sup>	3.55 ± 0.13 <sup>aA</sup>	3.51 ± 0.26 <sup>aA</sup>	3.39 ± 0.20 <sup>aA</sup>	3.57 ± 0.16 <sup>aA</sup>	3.23 ± 0.17 <sup>aA</sup>	3.1 ± 0.13 <sup>aA</sup>
	7	3.93 ± 0.13 <sup>aA</sup>	3.39 ± 0.17 <sup>aA</sup>	3.63 ± 0.19 <sup>aA</sup>	3.57 ± 0.14 <sup>aA</sup>	3.41 ± 0.31 <sup>aA</sup>	3.6 ± 0.15 <sup>aA</sup>	3.31 ± 0.14 <sup>aA</sup>	3.24 ± 0.16 <sup>aA</sup>
	14	3.46 ± 0.16 <sup>aA</sup>	3.16 ± 0.96 <sup>aA</sup>	3.7 ± 0.71 <sup>aA</sup>	3.46 ± 0.12 <sup>aA</sup>	3.49 ± 0.16 <sup>aA</sup>	3.59 ± 0.12 <sup>aA</sup>	3.36 ± 0.14 <sup>aA</sup>	3.19 ± 0.15 <sup>aA</sup>
	21	2.85 ± 1.22 <sup>aA</sup>	3.37 ± 0.17 <sup>aA</sup>	3.58 ± 0.15 <sup>aA</sup>	3.13 ± 1.08 <sup>aA</sup>	2.82 ± 0.11 <sup>aA</sup>	2.89 ± 0.35 <sup>aA</sup>	3.24 ± 0.32 <sup>aA</sup>	3.23 ± 0.51 <sup>aA</sup>
	28	2.73 ± 0.22 <sup>aA</sup>	2.51 ± 0.18 <sup>aA</sup>	2.68 ± 0.13 <sup>aA</sup>	2.4 ± 0.78 <sup>aA</sup>	2.74 ± 0.18 <sup>aA</sup>	2.41 ± 0.24 <sup>aA</sup>	2.57 ± 0.33 <sup>aA</sup>	2.47 ± 0.33 <sup>aA</sup>
b*	1	10.39 ± 0.14 <sup>aA</sup>	9.97 ± 0.13 <sup>aA</sup>	10.11 ± 0.12 <sup>aA</sup>	10.15 ± 0.18 <sup>aA</sup>	10.15 ± 0.18 <sup>aA</sup>	10.15 ± 0.19 <sup>aA</sup>	10.19 ± 0.15 <sup>aA</sup>	10.81 ± 0.13 <sup>abA</sup>
	7	10.38 ± 0.17 <sup>aA</sup>	10.12 ± 0.15 <sup>ab</sup>	10.11 ± 0.17 <sup>ab</sup>	9.99 ± 0.12 <sup>ab</sup>	10.10 ± 0.12 <sup>ab</sup>	10.18 ± 0.13 <sup>ab</sup>	10.20 ± 0.19 <sup>ab</sup>	11.19 ± 0.16 <sup>aA</sup>
	14	10.40 ± 0.10 <sup>aA</sup>	10.27 ± 0.50 <sup>ab</sup>	9.91 ± 0.66 <sup>ab</sup>	10.12 ± 0.19 <sup>ab</sup>	10.11 ± 0.19 <sup>ab</sup>	10.25 ± 0.13 <sup>ab</sup>	10.15 ± 0.12 <sup>ab</sup>	11.28 ± 0.19 <sup>aA</sup>
	21	9.24 ± 0.51 <sup>bc</sup>	10.14 ± 0.16 <sup>aAB</sup>	10.11 ± 0.11 <sup>aAB</sup>	10.64 ± 0.20 <sup>aA</sup>	8.35 ± 0.59 <sup>bc</sup>	9.47 ± 0.262 <sup>ab</sup>	9.52 ± 0.13 <sup>abB</sup>	10.79 ± 0.13 <sup>abA</sup>
	28	8.43 ± 0.17 <sup>bb</sup>	8.50 ± 0.22 <sup>bb</sup>	8.51 ± 0.14 <sup>bb</sup>	8.21 ± 0.19 <sup>bb</sup>	8.62 ± 0.12 <sup>bb</sup>	8.23 ± 0.13 <sup>bb</sup>	9.03 ± 0.19 <sup>baB</sup>	9.98 ± 0.10 <sup>ba</sup>

<sup>a</sup> Means ± standard deviation in the same row followed by different upper-case letters indicate statistically significant differences at  $p \leq 0.05$  between formulations of yoghurts for the same storage day ( $n = 6$ ); means ± standard deviation in the same column followed by different lower-case letters indicate statistically significant differences at  $p \leq 0.05$  for each formulation affected by storage time ( $n = 6$ ). Abbreviations are: SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebudioside; ST-B, stevia, 20% rebudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.

product ( $p \leq 0.05$ ). The XYL yoghurt presented similar TSS to the SUC yoghurt ( $p > 0.05$ ). Finally, the yoghurts with added erythritol (ERY, ERY-O and ERY-P) presented higher TSS than the control (SUC). During storage, the TSS values were maintained in all yoghurt formulations ( $p > 0.05$ ).

Colour parameters of the yoghurt formulations are presented in Table 4. All the yoghurt formulations presented slightly yellow colour ( $L^* = 70\text{--}86$ ,  $a^* = 2.4\text{--}3.9$  and  $b^* = 8.2\text{--}11.2$ ). Addition of sugar substitutes did not have an impact on the colour parameters of the products, as the sweetener added products (XYL, SUCRA, ERY, ERY-O, ERY-P, ST-A and ST-B) had similar  $L^*$ ,  $a^*$  and  $b^*$  values to the sucrose added yoghurt during storage ( $p > 0.05$ ). The only exception was for ST-A and ERY-P on day 21. Maintenance of the yoghurt colour is important, because the consumers expect a white or light-yellow product when buying non-flavoured yoghurts. During storage, all formulations darkened and became less reddish, as decreases in  $L^*$  and  $b^*$  parameters were observed ( $p \leq 0.05$ ). The opaque white colour of milk is related to the presence of protein, mainly casein (Aryana, Barnes, Emmick, McGrew, & Moser, 2006). During storage, proteolysis occurs, mainly because of the activity of the proteases from starter and probiotic cultures. Therefore, darkening could be related to some proteolysis that might have occurred (Costa, Junior, Rosa, Caliani, & Pimentel, 2017).

The physicochemical results indicate that addition of *L. casei* to all yoghurt formulations and substitution of sucrose by various sweeteners did not result in excessive acidification during storage and/or technological problems during the fermentation of the product, resulting in yoghurts with suitable pH and titratable acidity at 5 h of fermentation, which is the time commonly used by the dairy industries. Substitution of sucrose with sweeteners and prebiotics maintained colour and physicochemical stability of the yoghurts during storage. The addition of erythritol caused an initial lower pH, and this should be evaluated, as it can be related to decreased probiotic viability and consumer acceptance (Januário et al., 2017).

### 3.3. Texture characteristics

Texture characteristics of the yoghurt formulations are presented in Table 5. Addition of XYL resulted in a yoghurt with similar

texture characteristics (firmness, cohesiveness, consistency and viscosity index) to the SUC yoghurt during refrigerated storage ( $p > 0.05$ ). Addition of SUCRA resulted in a product with lower firmness, consistency, cohesiveness and viscosity index on the 1st day of storage when compared with the product with sucrose ( $p \leq 0.05$ ). This difference could be attributed to the fact that sucralose was added in a low amount ( $0.6 \text{ g L}^{-1}$ ) and did not contribute to the texture characteristics of the yoghurts as sucrose does (Carocho et al., 2017). During storage, the sucralose added yoghurt behaved similarly to the sucrose yoghurt, with decrease in texture parameters (firmness, cohesiveness, consistency and viscosity index) until day 14 and then increased until day 28 ( $p \leq 0.05$ ), with final values similar to initial values ( $p > 0.05$ ).

Addition of stevia (ST-A or ST-B) resulted in a product with initial higher values for the texture parameters (firmness, consistency, and viscosity index) ( $p \leq 0.05$ ) than the sucrose yoghurt (SUC, day 1). Considering the cohesiveness, only ST-A formulation presented higher values than the control (SUC,  $p \leq 0.05$ ). The increase in the texture parameters provided by stevia could be related to the formation of stronger networks due to hydrophobic interactions and interactions with milk proteins. Hernández-Rodríguez, Lobato-Calleros, Ramírez-Santiago, Rodríguez-Huezo, and Meraz (2017) observed that the substitution of sucrose by stevia resulted in yoghurts with a more compact gel network, in which the presence of non-micellar material was observed between casein clusters. The impact of stevia addition on the increase of firmness was also reported by Gao et al. (2017) when replacing sucrose by stevia in muffins. Products with stevia (ST-A or ST-B) presented lower texture parameters values at the end of storage (day 28) ( $p \leq 0.05$ ). Thus, interactions provided by stevia addition weakened during the storage period, resulting in decreases in the texture parameters values.

Addition of erythritol increased firmness and consistency ( $p \leq 0.05$ ), with no significant impact on cohesiveness and viscosity index ( $p > 0.05$ , day 1). The erythritol was added in a higher concentration ( $240 \text{ g L}^{-1}$ ) than the sucrose ( $120 \text{ g L}^{-1}$ ) and this product had lower pH on the 1st day of storage (Table 4), resulting in a firmer and more consistent product. During storage, the ERY yoghurt behaved similarly to the SUC yoghurt. Addition of

**Table 5**  
Texture characteristics of yoghurt formulations containing various sweetening systems.<sup>a</sup>

Parameter	Storage day	Formulations							
		SUC	SUCRA	XYL	ST-A	ST-B	ERY	ERY-O	ERY-P
Firmness (g)	1	69.0 ± 2.1 <sup>aCD</sup>	55.7 ± 1.2 <sup>bE</sup>	70.1 ± 2.4 <sup>aC</sup>	95.4 ± 3.2 <sup>aA</sup>	85.5 ± 2.9 <sup>aB</sup>	89.5 ± 1.3 <sup>aAB</sup>	62.7 ± 1.8 <sup>cD</sup>	73.7 ± 2.9 <sup>aC</sup>
	7	69.3 ± 0.9 <sup>aABC</sup>	58.9 ± 6.8 <sup>bD</sup>	75.9 ± 5.5 <sup>aA</sup>	67.1 ± 1.9 <sup>bBC</sup>	63.4 ± 4.4 <sup>bCD</sup>	75.3 ± 3.2 <sup>bA</sup>	71.9 ± 3.5 <sup>abAB</sup>	74.4 ± 4.9 <sup>aA</sup>
	14	53.9 ± 1.8 <sup>bCD</sup>	52.5 ± 0.6 <sup>cD</sup>	60.1 ± 4.3 <sup>bBC</sup>	62.7 ± 1.8 <sup>bAB</sup>	55.5 ± 3.0 <sup>cCD</sup>	57.9 ± 3.9 <sup>cBCD</sup>	67.8 ± 1.9 <sup>bcA</sup>	66.7 ± 5.6 <sup>bA</sup>
	21	67.9 ± 8.2 <sup>aBC</sup>	68.0 ± 2.6 <sup>aBC</sup>	72.9 ± 1.9 <sup>abAB</sup>	65.5 ± 5.4 <sup>bCD</sup>	60.6 ± 3.0 <sup>bcD</sup>	75.6 ± 7.8 <sup>bA</sup>	76.6 ± 1.7 <sup>aA</sup>	72.2 ± 1.6 <sup>abAB</sup>
	28	69.2 ± 1.0 <sup>aB</sup>	63.2 ± 2.4 <sup>abcd</sup>	68.5 ± 1.5 <sup>bBC</sup>	61.8 ± 9.8 <sup>bDE</sup>	55.6 ± 1.1 <sup>bcE</sup>	76.9 ± 5.5 <sup>bA</sup>	71.5 ± 0.6 <sup>abAB</sup>	74.9 ± 5.6 <sup>aAB</sup>
Consistency (g s)	1	408.7 ± 9.6 <sup>aD</sup>	342.7 ± 13.0 <sup>bE</sup>	443.9 ± 11.6 <sup>abCD</sup>	634.9 ± 23.6 <sup>aA</sup>	556.2 ± 31.1 <sup>aB</sup>	573.5 ± 10.1 <sup>aB</sup>	388.3 ± 11.8 <sup>cDE</sup>	474.5 ± 29.4 <sup>abC</sup>
	7	441.4 ± 2.7 <sup>aBC</sup>	365.2 ± 36.7 <sup>bD</sup>	499.5 ± 52.6 <sup>aAB</sup>	395.6 ± 18.3 <sup>bCD</sup>	383.4 ± 33.9 <sup>bD</sup>	484.9 ± 30.8 <sup>aB</sup>	474.3 ± 25.7 <sup>abAB</sup>	477.9 ± 66.9 <sup>abAB</sup>
	14	314.4 ± 1.2 <sup>bC</sup>	319.5 ± 7.5 <sup>bc</sup>	365.1 ± 33.9 <sup>bBC</sup>	383.6 ± 9.0 <sup>bAB</sup>	321.6 ± 18.2 <sup>cdC</sup>	314.7 ± 62.6 <sup>cc</sup>	430.8 ± 26.3 <sup>bcA</sup>	421.1 ± 52.3 <sup>bAB</sup>
	21	427.6 ± 61.4 <sup>aBC</sup>	435.5 ± 37.7 <sup>aBC</sup>	461.3 ± 36.2 <sup>abAB</sup>	393.1 ± 46.7 <sup>bCD</sup>	365.8 ± 30.8 <sup>bcD</sup>	470.2 ± 75.8 <sup>aB</sup>	512.7 ± 16.6 <sup>aA</sup>	473.2 ± 26.5 <sup>abAB</sup>
	28	436.6 ± 24.3 <sup>aA</sup>	371.6 ± 16.9 <sup>bB</sup>	437.9 ± 13.7 <sup>bA</sup>	351.6 ± 59.8 <sup>bBC</sup>	307.3 ± 1.5 <sup>dC</sup>	469.1 ± 48.7 <sup>bA</sup>	456.9 ± 32.1 <sup>abA</sup>	488.5 ± 38.8 <sup>aA</sup>
Cohesiveness (g)	1	42.9 ± 3.6 <sup>aB</sup>	25.9 ± 2.6 <sup>abd</sup>	42.6 ± 3.0 <sup>abB</sup>	64.0 ± 3.2 <sup>aA</sup>	50.6 ± 1.7 <sup>aB</sup>	47.7 ± 1.9 <sup>aB</sup>	30.1 ± 3.1 <sup>aCD</sup>	41.3 ± 4.2 <sup>aBC</sup>
	7	40.2 ± 1.1 <sup>aA</sup>	27.1 ± 6.1 <sup>aB</sup>	47.1 ± 5.6 <sup>aA</sup>	36.18 ± 2.5 <sup>bAB</sup>	35.5 ± 6.2 <sup>bAB</sup>	39.1 ± 4.0 <sup>abA</sup>	40.3 ± 3.6 <sup>aA</sup>	43.1 ± 4.2 <sup>aA</sup>
	14	22.3 ± 1.3 <sup>bAB</sup>	16.1 ± 8.4 <sup>bb</sup>	26.8 ± 1.3 <sup>cAB</sup>	32.8 ± 2.9 <sup>bCA</sup>	22.5 ± 2.7 <sup>cAB</sup>	23.5 ± 1.2 <sup>cAB</sup>	33.3 ± 2.8 <sup>aA</sup>	30.6 ± 2.4 <sup>bA</sup>
	21	34.6 ± 7.9 <sup>aA</sup>	36.1 ± 6.5 <sup>aA</sup>	37.5 ± 2.5 <sup>abA</sup>	31.3 ± 3.5 <sup>abAB</sup>	22.5 ± 3.5 <sup>cB</sup>	37.6 ± 2.4 <sup>abA</sup>	40.2 ± 3.9 <sup>aA</sup>	34.9 ± 3.5 <sup>abA</sup>
	28	37.0 ± 1.3 <sup>aA</sup>	30.3 ± 6.3 <sup>aABC</sup>	34.5 ± 2.2 <sup>bcAB</sup>	24.9 ± 9.0 <sup>cBC</sup>	20.1 ± 1.5 <sup>cc</sup>	36.5 ± 6.9 <sup>bA</sup>	34.2 ± 1.0 <sup>aAB</sup>	38.1 ± 5.9 <sup>abA</sup>
Viscosity index (g s)	1	36.7 ± 4.6 <sup>aC</sup>	21.8 ± 2.6 <sup>bcD</sup>	36.6 ± 2.3 <sup>abC</sup>	66.4 ± 1.6 <sup>aA</sup>	51.2 ± 8.2 <sup>aB</sup>	43.6 ± 1.4 <sup>aBC</sup>	26.6 ± 2.5 <sup>bD</sup>	36.5 ± 2.9 <sup>aC</sup>
	7	33.9 ± 0.2 <sup>aAB</sup>	18.4 ± 8.9 <sup>bCC</sup>	39.9 ± 8.9 <sup>aA</sup>	30.1 ± 2.8 <sup>aB</sup>	29.3 ± 5.2 <sup>bB</sup>	36.3 ± 3.9 <sup>aB</sup>	35.16 ± 3.0 <sup>abAB</sup>	38.16 ± 5.7 <sup>aA</sup>
	14	17.4 ± 2.0 <sup>bB</sup>	14.5 ± 1.3 <sup>cb</sup>	23.3 ± 4.9 <sup>cAB</sup>	27.9 ± 1.9 <sup>bA</sup>	16.1 ± 4.9 <sup>cb</sup>	17.9 ± 2.8 <sup>cb</sup>	30.13 ± 1.5 <sup>bA</sup>	33.2 ± 4.3 <sup>bA</sup>
	21	31.7 ± 8.0 <sup>aAB</sup>	35.3 ± 3.3 <sup>aA</sup>	35.4 ± 3.2 <sup>abA</sup>	26.3 ± 7.2 <sup>bB</sup>	15.6 ± 3.0 <sup>cc</sup>	33.1 ± 9.9 <sup>abAB</sup>	38.2 ± 0.9 <sup>aA</sup>	29.5 ± 1.9 <sup>abAB</sup>
	28	33.5 ± 1.1 <sup>aA</sup>	23.2 ± 3.3 <sup>bb</sup>	31.6 ± 1.5 <sup>bAB</sup>	15.3 ± 5.5 <sup>cc</sup>	17.6 ± 1.5 <sup>cc</sup>	34.7 ± 6.7 <sup>bA</sup>	29.5 ± 5.2 <sup>bb</sup>	35.0 ± 5.2 <sup>abA</sup>

<sup>a</sup> Means ± standard deviation in the same row followed by different upper-case letters indicate statistically significant differences at  $p \leq 0.05$  between formulations of yoghurts for the same storage day ( $n = 6$ ); means ± standard deviation in the same column followed by different lower-case letters indicate statistically significant differences at  $p \leq 0.05$  for each formulation affected by storage time ( $n = 6$ ). Abbreviations are: SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebaudioside; ST-B, stevia, 20% rebaudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.

prebiotics to the products with erythritol improved the texture characteristics (firmness), resulting in products (ERY-O and ERY-P) similar to the control (SUC) ( $p > 0.05$ ).

The results of the texture parameters, in a general view, demonstrated that the substitution of the sucrose with different types of sweeteners had impact on the texture characteristics of the products. It is important to observe if these alterations result in a negative impact on the consumer acceptance of the products. Xylitol was the only sweetener that did not have impact on the texture characteristics compared with the product with sucrose.

### 3.4. Sensory acceptance

Results of the sensory acceptance of the yoghurts formulations are presented on Table 6. Consumers evaluated the yoghurt formulations with scores between 4.6 and 8.1 on a 9-point scale, indicating that they disliked slightly some formulations and liked very much other formulations. Only the formulation with stevia B (ST-B) presented scores on the dislike part of the scale (<5).

Sucralose and xylitol effectively acted as sugar substitutes, as the formulations showed similar acceptance to the product with sucrose in all evaluated attributes (appearance, aroma, flavour, texture and overall impression) on 1st and 28th day of storage ( $p > 0.05$ ). These results are compatible with those observed for the physicochemical (Table 4) and texture (Table 5) characteristics of yoghurts, with no negative impact of the addition of these components on pH, titratable acidity, TSS and colour parameters. Lower firmness, cohesiveness, consistency and viscosity index observed in products with sucralose on the 1st day of storage did not have a negative impact on acceptance of the product by consumers. In fact, sucralose is recognised as the most suitable artificial sweetener to replace sucrose (Furlan & Campderrós, 2017). The present study demonstrates that this artificial sweetener can be replaced by a natural sweetener (xylitol) without impairment to the physicochemical and sensorial characteristics of products.

Addition of ERY resulted in a product with decreased acceptance (appearance, flavour, texture and overall impression) when compared with sucrose added product (SUC) only on the 1st day of storage ( $p \leq 0.05$ ). On the 28th day, both products presented similar acceptance in all evaluated attributes ( $p > 0.05$ ). Reduced acceptance may be related to lower pH (Table 4) and higher values in texture parameters (firmness and consistency) (Table 5) of this product on the 1st day of storage. One solution to this problem would be to stop the fermentation process at a higher pH and, thus, keep the texture parameters closer to the product with sucrose. It is

important to mention, however, that the ERY product had scores in the overall impression higher than 6, demonstrating that the consumers slightly liked this yoghurt.

The products with addition of prebiotics (ERY-O and ERY-P) showed lower acceptance in flavour and overall impression (ERY-O only on day 1, and ERY-P on days 1 and 28) ( $p < 0.05$ ) when compared with the product with sucrose. Also, product with oligofructose (ERY-O) presented lower acceptance on texture on day 1 ( $p < 0.05$ ). As there were no differences in pH and titratable acidity of yoghurts (Table 4), prebiotics may have altered the taste of the formulated yoghurts in some way, which was already mentioned in previous studies (Heydari et al., 2018; Pimentel, Prudêncio, & Rodrigues, 2011). Addition of prebiotics to the product with erythritol (ERY-O and ERY-P) resulted in improved texture acceptance, corroborating the results observed instrumentally (Table 5). The suggestion is to reduce the concentration of the prebiotics in the products (that was 6%) to reduce their negative impact on the flavour of the products, provided that their efficiency as a functional component is guaranteed. It is important to mention that oligofructose performed better than polydextrose, with the ERY-O product presenting score in the overall impression higher than 6 (slightly liked).

Addition of stevia (ST-A and ST-B) resulted in decreased acceptance in flavour, overall impression, purchase intention and texture (day 1) compared with the sucrose product ( $p < 0.05$ ). The results of texture acceptance corroborate those observed instrumentally, as products with stevia showed higher values in texture parameters on the 1st day of storage (Table 5). Lower acceptance in flavour is possibly related to the residual bitter taste of this sweetener (Oleson & Murphy, 2017). The ST-B had lower scores than the ST-A in the attributes evaluated in the 28th of storage ( $p \leq 0.05$ ), indicating that an increase in rebaudioside content has an influence on acceptance of the yoghurts by the consumer mainly in those already stored. Therefore, due to the results obtained, it is suggested to use preferentially stevia with a higher rebaudioside content. Subsequent studies should evaluate alternative ways of reducing the residual bitter taste of this sweetener, such as use of fruit pulps in yoghurt flavouring. Furthermore, descriptive tests with trained panel should be conducted to better elucidate the differences among the yoghurt formulations.

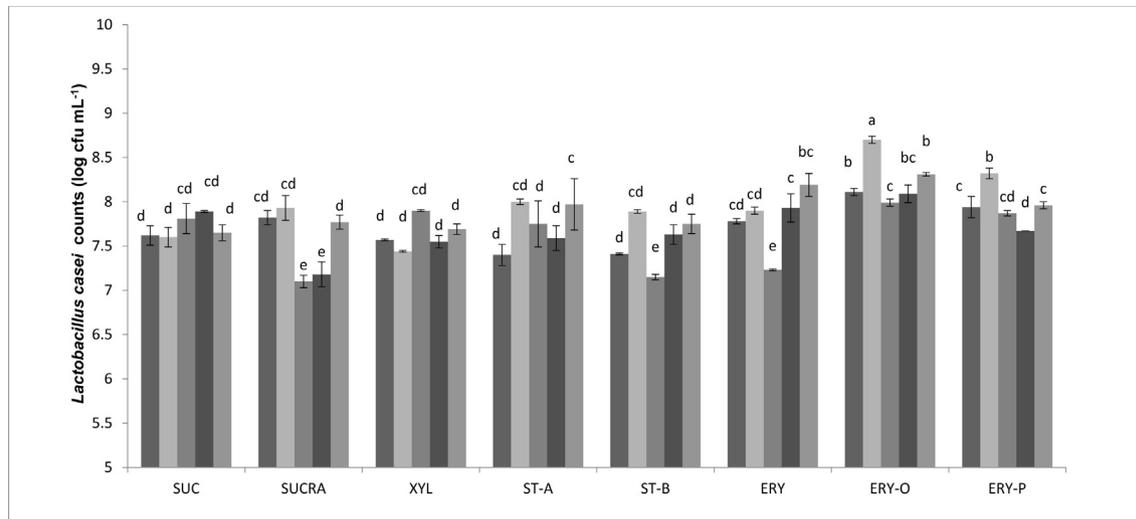
### 3.5. Probiotic viability

Probiotic survival in the formulated yoghurts is presented in Fig. 1. All formulations had *L. casei* counts higher than  $10^7$  cfu mL<sup>-1</sup>

**Table 6**  
Sensory acceptance of yoghurt formulations containing various sweetening systems.<sup>a</sup>

Parameter	Storage day	Formulations							
		SUC	SUCRA	XYL	ST-A	ST-B	ERY	ERY-O	ERY-P
Appearance	1	7.9 ± 1.9 <sup>Aa</sup>	7.8 ± 2.0 <sup>Aa</sup>	7.9 ± 1.8 <sup>Aa</sup>	7.4 ± 1.9 <sup>Aa</sup>	7.5 ± 2.5 <sup>Aa</sup>	6.5 ± 1.6 <sup>Ba</sup>	7.4 ± 2.0 <sup>ABa</sup>	7.2 ± 2.4 <sup>ABa</sup>
	28	7.6 ± 1.8 <sup>Aa</sup>	7.5 ± 1.9 <sup>Aa</sup>	7.3 ± 2.1 <sup>ABa</sup>	7.4 ± 2.0 <sup>ABa</sup>	6.6 ± 2.3 <sup>Bb</sup>	7.1 ± 1.8 <sup>ABa</sup>	7.1 ± 1.8 <sup>ABa</sup>	7.0 ± 2.2 <sup>ABa</sup>
Aroma	1	7.7 ± 1.8 <sup>Aa</sup>	7.6 ± 2.0 <sup>Aa</sup>	7.6 ± 2.0 <sup>Aa</sup>	6.8 ± 1.9 <sup>Aa</sup>	6.8 ± 2.3 <sup>Aa</sup>	6.9 ± 2.4 <sup>Aa</sup>	6.9 ± 1.9 <sup>Aa</sup>	7.1 ± 2.3 <sup>Aa</sup>
	28	6.6 ± 1.8 <sup>Ab</sup>	6.2 ± 2.3 <sup>Ab</sup>	6.5 ± 2.0 <sup>Ab</sup>	6.3 ± 2.2 <sup>Aa</sup>	5.8 ± 2.2 <sup>Ab</sup>	6.2 ± 2.0 <sup>Aa</sup>	6.5 ± 1.8 <sup>Aa</sup>	6.2 ± 2.4 <sup>Aa</sup>
Flavour	1	8.1 ± 1.9 <sup>Aa</sup>	7.6 ± 2.0 <sup>Aa</sup>	7.7 ± 2.0 <sup>Aa</sup>	5.3 ± 2.4 <sup>BCa</sup>	5.0 ± 2.4 <sup>Ca</sup>	6.2 ± 2.3 <sup>BCa</sup>	6.4 ± 2.3 <sup>Ba</sup>	6.3 ± 2.6 <sup>Ba</sup>
	28	6.9 ± 2.0 <sup>Ab</sup>	7.0 ± 2.2 <sup>Aa</sup>	6.7 ± 2.2 <sup>Aa</sup>	5.7 ± 2.2 <sup>Ba</sup>	4.6 ± 2.4 <sup>Ca</sup>	6.2 ± 2.4 <sup>Aa</sup>	5.9 ± 2.4 <sup>Ba</sup>	5.5 ± 2.2 <sup>BCa</sup>
Texture	1	7.9 ± 1.7 <sup>Aa</sup>	6.8 ± 1.9 <sup>Aa</sup>	7.3 ± 2.0 <sup>ABa</sup>	6.6 ± 2.1 <sup>Ba</sup>	6.6 ± 2.3 <sup>Ba</sup>	5.8 ± 2.2 <sup>Ca</sup>	6.5 ± 1.9 <sup>Ba</sup>	7.0 ± 1.8 <sup>Aa</sup>
	28	7.1 ± 2.0 <sup>Aa</sup>	7.0 ± 1.8 <sup>Aa</sup>	7.0 ± 2.2 <sup>Aa</sup>	6.7 ± 2.0 <sup>Aa</sup>	5.7 ± 2.4 <sup>Ba</sup>	6.63 ± 2.4 <sup>Aa</sup>	6.1 ± 2.0 <sup>Aa</sup>	6.1 ± 2.3 <sup>Aa</sup>
Overall impression	1	7.8 ± 1.8 <sup>Aa</sup>	7.3 ± 2.0 <sup>ABa</sup>	7.8 ± 2.0 <sup>Aa</sup>	6.0 ± 2.2 <sup>Ca</sup>	5.6 ± 2.4 <sup>Ca</sup>	6.2 ± 2.4 <sup>Ca</sup>	6.7 ± 1.9 <sup>BCa</sup>	6.6 ± 1.8 <sup>BCa</sup>
	28	6.9 ± 1.8 <sup>ABb</sup>	7.1 ± 2.1 <sup>Aa</sup>	6.9 ± 2.0 <sup>ABa</sup>	6.0 ± 2.1 <sup>Ba</sup>	5.0 ± 2.5 <sup>Ca</sup>	6.1 ± 2.0 <sup>Ba</sup>	6.4 ± 2.0 <sup>Ba</sup>	5.8 ± 2.3 <sup>Ca</sup>
Purchase intention	1	4.2 ± 1.2 <sup>Aa</sup>	4.0 ± 1.2 <sup>ABa</sup>	4.2 ± 1.5 <sup>ABa</sup>	2.7 ± 1.2 <sup>Ca</sup>	2.9 ± 1.2 <sup>Ca</sup>	3.5 ± 1.2 <sup>BCa</sup>	3.3 ± 1.4 <sup>BCa</sup>	3.2 ± 1.3 <sup>BCa</sup>
	28	3.5 ± 1.3 <sup>Ab</sup>	3.7 ± 1.3 <sup>Aa</sup>	3.6 ± 1.5 <sup>Ab</sup>	2.9 ± 1.5 <sup>BCa</sup>	2.5 ± 1.2 <sup>Ca</sup>	3.1 ± 1.3 <sup>ABa</sup>	3.0 ± 1.2 <sup>BCa</sup>	2.8 ± 1.5 <sup>BCa</sup>

<sup>a</sup> Means ± standard deviation in the same row followed by different upper-case letters indicate statistically significant differences at  $p \leq 0.05$  between formulations of yoghurts for the same storage day ( $n = 58$ ); means ± standard deviation in the same column followed by different lower-case letters indicate statistically significant differences at  $p \leq 0.05$  for each formulation affected by storage time ( $n = 58$ ). Abbreviations are: SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebaudioside; ST-B, stevia, 20% rebaudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.



**Fig. 1.** Viability ( $\log \text{cfu mL}^{-1}$ ) of the *Lactobacillus casei* in formulations of yoghurts during cold storage ( $7\text{ }^{\circ}\text{C}$ ). The error bars represent the standard deviation ( $n = 6$ ). Different lowercase letters indicate statistically significant differences at  $p \leq 0.05$ . Days of storage: ■, 1; ▨, 7; ▩, 14; ▪, 21; ▫, 28. SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebaudioside; ST-B, stevia, 20% rebaudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.

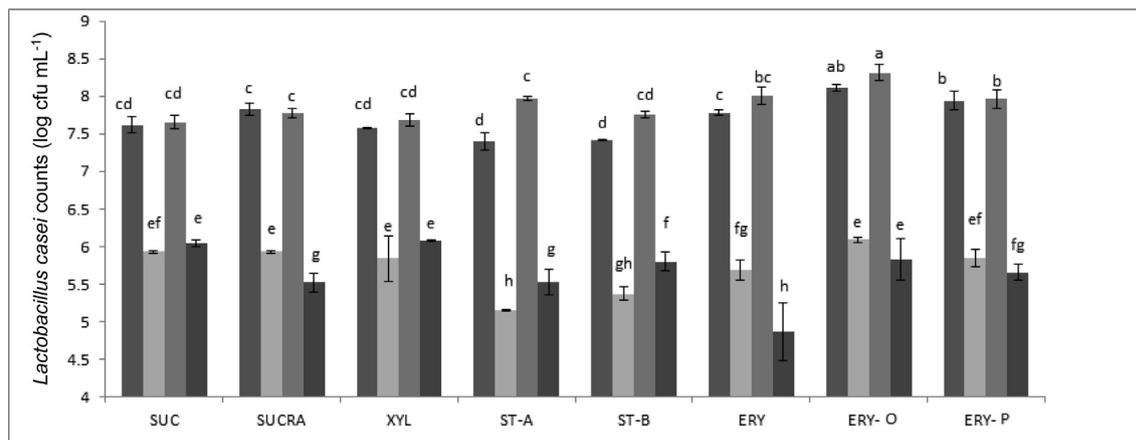
during storage, which is high than the minimum count necessary to consider a product as probiotic ( $10^6 \text{ cfu mL}^{-1}$ ; [Savedbown et al., 2017](#); [Sangami & Sri, 2017](#)). Thus, regardless of addition of sucrose, sweeteners or prebiotics, all formulated yoghurts could be considered probiotic after 28 days of refrigerated storage ( $7\text{ }^{\circ}\text{C}$ ).

Addition of the sweeteners SUCRA, XYL, ST-A, ST-B, and ERY did not impact on the probiotic survival during processing, as these formulations presented similar probiotic viability ( $p > 0.05$ ) to the sucrose added product on the 1st day of storage. The results indicate that the lower pH observed for the ERY formulation had no influence on the probiotic viability ( $p > 0.05$ ). These formulations behaved similar to the control (SUC) during the storage period, with maintenance of the probiotic culture viability (SUCRA, XYL, ERY, ST-B) ( $p > 0.05$ ) or slight increase of their counts (ST-A) ( $p \leq 0.05$ ) when comparing the products on the 1st and 28th day of storage. In a general, there was a decrease in the probiotic counts in the middle of the storage time and an increase until the end of storage ( $p \leq 0.05$ ). Similar behaviour was observed in previous studies ([Januário et al., 2017](#); [Pimentel et al., 2012](#)). The initial loss of viability of probiotic cultures may be related to the decrease in pH

during storage ([Table 4](#)), due to the accumulation of organic acids. Further recovery of the viability can be related to the free amino acids released during storage and to the adaptation of the cultures to the environment ([Januário et al., 2017](#)).

Addition of oligofructose (ERY-O) resulted in higher counts of the probiotic cultures in the initial weeks of storage (days 1, 7, 14 and 21) when compared with the product without oligofructose (ERY). The addition of polydextrose (ERY-P) resulted in higher counts on days 7, 14 and 21. The probiotic counts at 28th day of storage in ERY, ERY-P and ERY-O yoghurts were similar ( $p > 0.05$ ), but higher than those found in the control (SUC) ( $p \leq 0.05$ ). Therefore, oligofructose and polydextrose acted as prebiotic components in the yoghurts. It could be observed that the effect of oligofructose on the protection of the viability of the probiotic culture was higher than polydextrose. [Marinaki, Kandyliis, Dimitrellou, Zakyntinos, and Varzakas \(2016\)](#) also observed a protective effect of the prebiotic raffinose on the viability of *L. casei* ATCC 393 in yoghurts during 4 weeks of storage at  $4\text{ }^{\circ}\text{C}$ .

Probiotic survival in the formulated yoghurts during SGIC is presented in [Fig. 2](#). The *L. casei* probiotic culture survived through the



**Fig. 2.** Viability ( $\log \text{cfu mL}^{-1}$ ) of the *Lactobacillus casei* in formulations of yoghurts during simulated gastrointestinal conditions on (left to right) days 1 (■, initial and ▨, after SGIC) and 28 (▩, initial and ▪, after SGIC). The error bars represent the standard deviation ( $n = 6$ ). Different lowercase letters indicate statistically significant differences at  $p \leq 0.05$ . SUC, sucrose; SUCRA, sucralose; XYL, xylitol; ST-A, stevia, 90% rebaudioside; ST-B, stevia, 20% rebaudioside; ERY, erythritol; ERY-O, erythritol + oligofructose; ERY-P, erythritol + polydextrose.

gastrointestinal conditions in all evaluated yoghurts, presenting counts higher than  $10^4$  cfu mL<sup>-1</sup>. Thus, a 2–3 log reduction of probiotic cultures was observed during passage through stomach and intestine simulated conditions. Survival of the probiotic culture in the present study was slightly higher than observed in previous studies, with reductions of 3.1–6.7 log cfu mL<sup>-1</sup> in the counts of *L. casei* and *Lactobacillus acidophilus* (Krasaekoopt & Watcharapoka, 2014) and 3.2–4.7 log cfu mL<sup>-1</sup> for *L. acidophilus* (Bedani, Rossi, & Saad, 2013) in yoghurts after SGIC. Millete, Nguyen, Amine, and Lacroix (2013) suggested that, for the probiotic culture to exert its beneficial effects on the host, it must be able to survive and reach the GI tract in sufficient numbers, at least  $10^6$ – $10^7$  cfu g<sup>-1</sup>. The products with sucrose and xylitol presented these counts even after the passage throughout the entire gastrointestinal tract. The authors also note that products with probiotic survivals between 1 and 5 log cfu g<sup>-1</sup> are considered of limited gastrointestinal resistance (Millete et al., 2013). Therefore, all the other formulations of this study presented limited gastrointestinal resistance. However, it is important to mention that in vitro and in vivo studies should be performed to finally conclude the optimum probiotic survival necessary to observe the expected health effects (Klingberg & Budde, 2006).

Addition of the sweetener XYL did not impact probiotic survival during simulated gastrointestinal conditions when compared with the conventional product (SUC) ( $p > 0.05$ ). The addition of SUCRA, ERY, ST-A and ST-B resulted in decreased counts of probiotic cultures after SGIC ( $p \leq 0.05$ ). However, the decrease after SGIC was only in the samples stored for 28 days with the SUCRA and ERY formulations, while for formulations with stevia the diminution was observed in samples stored for 1 and 28 days. Probably, stevia had an inhibitory activity on the probiotic survival, which was not observed in the product but in the SGIC. Deniņa, Semjonovs, Fomina, Treimane, & Linde (2014) reported that the growth of *Lactobacillus reuteri* strains was inhibited in the presence of stevia sweeteners stevioside and rebaudioside A. The observation of the inhibitory effect only in SGIC can be related to the harsh environment and to the lower protection exerted by the dairy matrix in this condition.

Furthermore, the addition of the prebiotics (ERY-O and ERY-P) protected the probiotic cultures during SGIC when compared with product without prebiotics (ERY), with probiotic counts similar ( $p > 0.05$ ) to those observed for the conventional product (SUC, except ERY-P on day 28). Oligofructose and polydextrose are available substrates for probiotic culture metabolism, serving as a carbon source for the maintenance of the cell. Furthermore, they can prevent injuries caused by acidity or provide a physical protection from damage caused by the environment (Delgado-Fernández, Corzo, Olano, Hernández-Hernández, & Moreno, 2019; Pimentel, Madrona, García, & Prudencio, 2015).

From the results observed in the present study, none of the sweeteners have a negative impact on probiotic survival during storage of yoghurts. During SGIC, stevia (A and B) and erythritol had a slightly negative impact, with decreases of 0.5–1 log in the probiotic counts in comparison with control formulation (SUC). However, suitable probiotic counts were observed in the yoghurts during storage ( $>10^7$  cfu mL<sup>-1</sup>) and after SGIC ( $>10^4$  cfu mL<sup>-1</sup>). This is an interesting result, since dairy industries are trying to develop probiotic products without added sugar, focussing on the diabetic public and on people with health habits.

#### 4. Conclusions

Xylitol and sucralose could be considered sucrose substitutes in yoghurt formulations, as their addition resulted in products with physicochemical characteristics (pH, titratable acidity, TSS, colour

parameters L\*, a\* and b\*), sensory acceptance and probiotic survival in the product similar to that in the yoghurt with sucrose. Xylitol was more efficient than sucralose in the maintenance of the texture characteristics of the yoghurts and probiotic survival during SGIC. Therefore, this study proved that sucralose can be replaced by a natural sweetener (xylitol).

Addition of erythritol caused an initial low pH and increased texture parameters (firmness and consistency), which resulted in a decreased acceptance of the products on the 1st day of storage and lower probiotic survival in SGIC on the 28th day of storage. Therefore, it is advisable to stop the fermentation of erythritol added products at higher pH values. Addition of stevia resulted in initial increased texture parameters, decreased acceptance by consumers (mainly on the flavour) and lower probiotic survival in SGIC. Stevia with a higher rebaudioside content performed better, therefore, it is recommended to select this type of stevia to use in yoghurt formulations. Addition of oligofructose or polydextrose as prebiotics resulted in improved texture and probiotic survival, however, these components had a negative impact on the flavour of the products. Future studies should evaluate decreasing the prebiotic concentration in the products and study more deeply the impact of addition on flavour. Independently of the sucrose, sweetener or prebiotic addition, all formulations could be considered probiotic products during the entire shelf life (28 days at 7 °C) with counts higher than  $10^7$  cfu mL<sup>-1</sup> in the product and higher than  $10^4$  cfu mL<sup>-1</sup> after SGIC.

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#### References

- Akalin, A. S., Fenderya, S., & Akbulut, N. (2004). Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage. *International Journal of Food Science and Technology*, 39, 613–621.
- AOAC. (2004). *Official methods of analysis of the AOAC International* (19th ed.). Washington, DC, USA: AOAC.
- Apolinário, A. C., de Lima Damasceno, B. P. G., de Macêdo Beltrão, N. E., Pessoa, A., Converti, A., & da Silva, J. A. (2014). Inulin-type fructans: A review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers*, 101, 368–378.
- Aryana, K. J., Barnes, H. T., Emmick, T. K., McGrew, P., & Moser, B. (2006). Lutein is stable in strawberry yogurt and does not affect its characteristics. *Journal of Food Science*, 71, 467–472.
- Azad, M. B., Abou-Setta, A. M., Chauhan, B. F., Rabbani, R., Lys, J., Copstein, L., et al. (2017). Nonnutritive sweeteners and cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. *Canadian Medical Association Journal*, 189, 929–939.
- Balthazar, C. F., Júnior, C. C., Moraes, J., Costa, M. P., Raices, R. S. L., Franco, R. M., et al. (2016). Physicochemical evaluation of sheep milk yogurts containing different levels of inulin. *Journal of Dairy Science*, 99, 4160–4168.
- Barłowska, J., Pastuszka, R., Rysiak, A., Król, J., Brodziak, A., Kedzińska-Matysek, M., et al. (2018). Physicochemical and sensory properties of goat cheeses and their fatty acid profile in relation to the geographic region of production. *International Journal of Dairy Technology*, 70, 1–10.
- Batista, A. L. D., Silva, R., Cappato, L. P., Almada, C. N., Garcia, R. K. A., Silva, M. C., et al. (2015). Quality parameters of probiotic yogurt added to glucose oxidase compared to commercial products through microbiological, physical–chemical and metabolic activity analyses. *Food Research International*, 77, 627–635.
- Batista, A. L. D., Silva, R., Cappato, L. P., Ferreira, M. V. S., Nascimento, K. O., Schmiele, M., et al. (2017). Developing a synbiotic fermented milk using probiotic bacteria and organic green banana flour. *Journal of Functional Foods*, 38, 242–250.

- 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, 382–389.
- 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. *LWT Food Science Technology*, 55, 436–443.
- Bian, X., Chi, L., Gao, B., Tu, P., Ru, H., & Lu, K. (2017). The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PLoS One*, 12, 0178426.
- Burití, F. C. A., Castro, I. A., & Saad, S. M. I. (2010). Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and its survival under in vitro simulated gastrointestinal conditions. *International Journal of Food Microbiology*, 137, 121–129.
- Carly, F., Vandermies, M., Telek, S., Steels, S., Thomas, S., Nicaud, J. M., et al. (2017). Enhancing erythritol productivity in *Yarrowia lipolytica* using metabolic engineering. *Metabolic Engineering*, 42, 19–24.
- Carocho, M., Morales, P., & Ferreira, I. C. F. R. (2017). Sweeteners as food additives in the XXI century: A review of what is known, and what is to come. *Food and Chemical Toxicology*, 107, 302–317.
- Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, 22, 160–166.
- Costa, K. K. F. D., Junior, M. S. S., Rosa, S. I. R., Caliani, M., & Pimentel, T. C. (2017). Changes of probiotic fermented drink obtained from soy and rice byproducts during cold storage. *LWT - Food Science and Technology*, 78, 23–30.
- Cruz, A. G., Cadena, R. S., Walter, E. H. M., Mortazavian, A. M., Granato, D., Faria, J. A. F., et al. (2010). Sensory analysis: Relevance for prebiotic, probiotic, and synbiotic product development. *Comprehensive Reviews in Food Science and Food Safety*, 9, 358–373.
- Das, K., Choudhary, R., & Thompson-Witrick, K. A. (2019). Effects of new technology on the current manufacturing process of yogurt to increase the overall marketability of yogurt. *LWT Food Science and Technology*, 108, 69–80.
- Delgado-Fernández, P., Corzo, N., Olano, A., Hernández-Hernández, O., & Moreno, F. J. (2019). Effect of selected prebiotics on the growth of lactic acid bacteria and physicochemical properties of yoghurts. *International Dairy Journal*, 89, 77–85.
- Denipa, I., Semjonovs, P., Fomina, A., Treimane, R., & Linde, R. (2014). The influence of stevia glycosides on the growth of *Lactobacillus reuteri* strains. *Letters in Applied Microbiology*, 58, 278–284.
- Espirito Santo, A. P., Cartolano, N. S., Silva, T. F., Soares, F. A. S. M., Gioielli, L. A., Perego, P., et al. (2012). Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts. *International Journal of Food Microbiology*, 154, 135–144.
- Fagherazzi, G., Gusto, G., Affret, A., Mancini, F. R., Dow, C., Balkau, B., et al. (2017). Chronic consumption of artificial sweetener in packets or tablets and type 2 diabetes risk: Evidence from the E3N-European prospective investigation into cancer and nutrition study. *Annals of Nutrition Metabolism*, 70, 51–58.
- Florence, A. C. R., Béal, C., Silva, R. C., Bogsan, C. S., Pilleggi, A. L. O., Gioielli, L. A., et al. (2012). Fatty acid profile, trans-octadecenoic,  $\alpha$ -linolenic and conjugated linoleic acid contents differing in certified organic and conventional probiotic fermented milks. *Food Chemistry*, 135, 2207–2214.
- Furlan, L. T. R., & Campderros, M. E. (2017). The combined effects of Stevia and sucralose as sugar substitute and inulin as fat mimetic on the physicochemical properties of sugar-free reduced-fat dairy dessert. *International Journal of Gastronomy and Food Science*, 10, 16–23.
- Gao, J., Brennan, M. A., Mason, S. L., & Brennan, C. (2017). Effects of sugar substitution with “steviana” on the sensory characteristics of muffins. *Journal of Food Quality*, 1, 1–11.
- Gengatharan, A., Dykes, G. A., & Choo, W.-S. (2017). The effect of pH treatment and refrigerated storage on natural colourant preparations (betacyanins) from red pitahaya and their potential application in yoghurt. *LWT - Food Science and Technology*, 80, 437–445.
- Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14, 491–502.
- Hernández-Rodríguez, L., Lobato-Calleros, C., Ramírez-Santiago, C., Rodríguez-Huezo, M. E., & Meraz, M. (2017). Microstructure and rheology of yogurt added with protein-*L. plantarum*-polysaccharide coacervate and stevia in substitution of milk-Fat and sucrose. *Revista Mexicana de Ingeniería Química*, 16, 77–89.
- Heydari, S., Amiri-Rigi, A., Ehsani, M. R., Mohammadifar, M. A., Khorshidian, N., Koushki, M. R., et al. (2018). Rheological behaviour, sensory properties and syneresis of probiotic yoghurt supplemented with various prebiotics. *International Journal of Dairy Technology*, 71, 175–184.
- Hill, C., Garner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11, 506–514.
- Janiaski, D. R., Pimentel, T. C., Cruz, A. G., & Prudencio, S. H. (2016). Strawberry-flavored yogurts and whey beverages: What is the sensory profile of the ideal product? *Journal of Dairy Science*, 99, 5273–5283.
- Januário, J. G. B., da Silva, I. C. F., de Oliveira, A. S., de Oliveira, J. F., Dionísio, J. N., Klososki, S. J., et al. (2017). Probiotic yoghurt flavored with organic beet with carrot, cassava, sweet potato or corn juice: Physicochemical and texture evaluation, probiotic viability and acceptance. *International Food Research Journal*, 24, 359–366.
- Klewicki, R., & Klewicka, E. (2004). Antagonistic activity of lactic acid bacteria as probiotics against selected bacteria of the Enterobacteriaceae family in the presence of polyols and their galactosyl derivatives. *Biotechnology Letters*, 26, 317–320.
- Klingberg, T. D., & Budde, B. B. (2006). The survival and persistence in the human gastrointestinal tract of five potential probiotic lactobacilli consumed as freeze-dried cultures or as probiotic sausage. *International Journal of Food Microbiology*, 109, 157–159.
- Krasaekoopt, W., & Watcharapoka, S. (2014). Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT Food Science and Technology*, 57, 761–766.
- Lin, T. Y. (2000). Conjugated linoleic acid concentrations as affected by lactic cultures and additives. *Food Chemistry*, 69, 27–31.
- Marinaki, E., Kandylis, P., Dimitrellou, D., Zakyntinos, G., & Varzakas, T. (2016). Probiotic yogurt production with *Lactobacillus casei* and prebiotics. *Current Research in Nutrition and Food Science Journal*, 4, 48–53.
- Miele, N. A., Cabisidan, E. K., Plaza, A. G., Masi, P., Cavella, S., & Di Monaco, R. (2017). Carbohydrate sweetener reduction in beverages through the use of high potency sweeteners: Trends and new perspectives from a sensory point of view. *Trends in Food Science & Technology*, 64, 87–93.
- Millette, M., Nguyen, A., Amine, K. M., & Lacroix, M. (2013). Gastrointestinal survival of bacteria in commercial probiotic products. *International Journal of Probiotics and Prebiotics*, 8, 149–156.
- Oleson, S., & Murphy, C. (2017). Prediction of stevia liking by sucrose liking: Effects of beverage background. *Chemosensory Perception*, 10, 49–59.
- Oliveira, V. A., Oliveira, V. M. A., Oliveira, T. W. N., Damasceno, A. N. C., Silva, C. E. O., Medeiros, S. R. A., et al. (2017). Evaluation of cytotoxic and mutagenic effects of two artificial sweeteners by using eukaryotic test systems. *African Journal of Biotechnology*, 16, 547–551.
- Pappu, S. M., & Gummadi, S. N. (2017). Artificial neural network and regression coupled genetic algorithm to optimize parameters for enhanced xylitol production by *Debaryomyces nepalensis* in bioreactor. *Biochemical Engineering Journal*, 120, 136–145.
- Pimentel, T. C., Garcia, S., & Prudêncio, S. H. (2012). Effect of long-chain inulin on the texture profile and survival of *Lactobacillus paracasei* ssp. *paracasei* in set yoghurts during refrigerated storage. *International Journal of Dairy Technology*, 65, 104–110.
- Pimentel, T. C., Madrona, G. S., Garcia, S., & Prudencio, 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.
- Pimentel, T. C., Prudêncio, S. H., & Rodrigues, R. S. (2011). Néctar de pêssego potencialmente simbiótico. *Alimentos e Nutrição*, 22, 455–464.
- Poele, E. M., Devlamynck, T., Jäger, M., Gerwig, G. J., van de Walle, D., Dewettinck, K., et al. (2018). Glucansucrase (mutant) enzymes from *Lactobacillus reuteri* L80 efficiently transglucosylate Stevia component rebaudioside A, resulting in a superior taste. *Scientific Reports*, 8, 1516.
- Sangami, R., & Sri, R. S. (2017). Emerging trends in improving viability, advanced stability techniques and health claims of healthy microbiome – the probiotics. *International Journal of Current Microbiology and Applied Sciences*, 6, 194–200.
- Savedbowen, W., Kerdwan, N., Sakorn, A., Charoen, R., Tipkanon, S., & Pattayakorn, K. (2017). Role of protective agents on the viability of probiotic *Lactobacillus plantarum* during freeze drying and subsequent storage. *International Food Research Journal*, 24, 787–794.
- Sperry, M. F., Silva, H. L., Balthazar, C. F., Esmerino, E. A., Verruck, S., Prudencio, E. S., et al. (2018). Probiotic Minas Frescal cheese added with *L. casei* 01: Physicochemical and bioactivity characterization and effects on hematological/biochemical parameters of hypertensive overweight women—A randomized double-blind pilot trial. *Journal of Functional Foods*, 45, 435–443.
- Stone, H. (2012). *Sensory evaluation practices*. London, UK: Academic Press.
- 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, 2288–2296.
- Vinderola, C. G., Costa, G. A., Regenhart, S., & Reinheimer, J. A. (2002). Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. *International Dairy Journal*, 12, 579–589.