Development of a frozen drink based on prebiotic lactic beverage: physicochemical, microbiological and sensory aspects

Desenvolvimento de "frozen" de bebida láctea prebiótica: aspectos físico-químicos, microbiológicos e sensoriais

Desarrollo de "frozen" sobre la base de bebida láctica prebiótica: aspectos fisicoquímicos, microbiológicos y sensoriales

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Elisangela de Andrade Castro
ORCID: https://orcid.org/0000-0002-1618-927X
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: elisangelaandrade@ifce.edu.br
Poliana Brito de Sousa
ORCID: https://orcid.org/0000-0003-1037-8747
Instituto Federal de Educação, Ciência e Tecnologia do Piauí, Brazil
E-mail: pollysousa100@gmail.com
Elizabeth Mariano Batista
ORCID: https://orcid.org/0000-0001-5250-4110
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: elisabethmariano@hotmail.com
Everlândia Silva Moura Miranda
ORCID: https://orcid.org/0000-0002-2671-7005
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: everlandiamoura7@yahoo.com
Sandra Maria Lopes dos Santos
ORCID: https://orcid.org/0000-0001-9342-3931
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: anisulivan@gmail.com
Luana Guabiraba Mendes
ORCID: https://orcid.org/0000-0001-8184-8588
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: luangmendes@gmail.com

Antônio Belfort Dantas Cavalcante[1]
ORCID: https://orcid.org/0000-0001-8440-2979
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: belfort.dantas@ifce.edu.br
Elda Fontinele Tahim
ORCID: https://orcid.org/0000-0002-4135-7714
Instituto Centro de Ensino Tecnológico, Brazil
E-mail: fontineletahim@gmail.com
Marlene Nunes Damaceno
ORCID: https://orcid.org/0000-0002-3553-6740
Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Brazil
E-mail: marlene@ifce.edu.br

Abstract

The improper disposal of residual whey from dairy production represents a serious environmental problem in Brazil, causing a lot of effort applied in the development of dairy products with partial replacement of milk by whey. In this study, three formulations of a frozen dairy drink containing whey and different amounts of fructooligosaccharides, in replacing saccharose, were developed and evaluated in relation to physicochemical, microbiological and sensory properties. All physicochemical and microbiological parameters were within Brazilian regulations for frozen edibles. Evaluated by 120 volunteers, the Acceptability Index was above 80% for all formulations and attributes and the purchase intention favourable the acquisition of the product. The tested formulations represent a nutritious and safe alternative for the use of residual whey from dairy farms and an innovation on the market of functional frozen edibles.

Keywords: Functional food; Fructooligosaccharides; Edible ice cream.

Resumo

O descarte inadequado de soro residual da produção leiteira representa um sério problema ambiental no Brasil ocasionando muito esforço aplicado no desenvolvimento de produtos lácteos com substituição parcial do leite pelo soro de leite. Neste estudo, três formulações de “frozen” de bebida láctea contendo soro de leite e diferentes concentrações de

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frutooligossacarídeos, em substituição à sacarose, foram desenvolvidas e avaliadas em relação às propriedades físico-químicas, microbiológicas e sensoriais. Todos os parâmetros físico-químicos e microbiológicos estavam de acordo com os regulamentos brasileiros para produtos comestíveis congelados. Avaliadas por 120 voluntários, o índice de aceitabilidade foi superior a 80% para todas as formulações e atributos e a intenção de compra favorável à aquisição do produto. As formulações testadas representam uma alternativa nutritiva e segura para o uso de soro de leite residual de fazendas leiteiras e uma inovação no mercado de comestíveis congelados funcionais.

Palavras-chave: Alimento funcional; Frutooligossacarídeo; Gelados comestíveis.

Resumen

La eliminación inadecuada del suero residual de la producción de leche representa un grave problema ambiental en Brasil provocando mucho esfuerzo aplicado en desarrollar productos lácteos, reemplazando en parte la leche con suero. En este estudio, tres formulaciones de “frozen” sobre la base de bebida láctica que contiene suero y diferentes concentraciones de fructooligosacáridos, reemplazando sacarosa, se ensayaron con respecto a las propiedades fisicoquímicas, microbiológicas y sensoriales. Todos los parámetros fisicoquímicos y microbiológicos estaban de acuerdo con las regulaciones brasileñas para comestibles congelados. Evaluadas por 120 voluntarios, el índice de aceptabilidad estaba por encima de 80% para todas las formulaciones y atributos y la intención de compra favoreció la adquisición del producto. Las formulaciones ensayadas representan una alternativa nutritiva y segura para el uso de suero residual de granjas lecheras, y una innovación en el mercado de comestibles congelados funcionales.

Palabras clave: Alimentos funcionales; Fructooligosacáridos; Helados comestibles.

1. Introduction

Foods not only satiate the appetite but provide energy and nutrients for a vast array of processes essential for growth, survival and the proper functioning of the organism. Greater life expectancy and growing incidences of chronic diseases have spurred interest in healthy dietary habits, including functional foods. In addition to ensuring adequate nutrition, functional foods help modulate important organic functions, improve health and well-being, and reduce the risk of diseases (Lomovskiy, Bychkova, Slavikovskaya & Lomovsky, 2020). The prospects offered by such foods are drawing increasing attention from consumers, manufacturers and researchers alike, as shown by the current boom in publications on prebiotic, probiotic and symbiotic products (Hussein, Awad, El-Sayed & Ibrahim, 2020).

Prebiotics may be defined as indigestive dietary ingredients that act beneficially in the bowel by selectively stimulating the development and/or activity of one or more bacterial species in the colon. Probiotics are living microorganisms which benefit the host's health when administered in proper amounts (Li et al., 2020; Raddatz et al., 2020). Foods containing both prebiotics and probiotics are classified as symbiotic (Raddatz et al., 2020).

Many dairy industries now produce functional foods, such as frozen yogurt, containing probiotic cultures and/or prebiotic substances. Yogurt is essentially produced from milk submitted to lactic fermentation with *Lactobacillus bulgaricus* and *Streptococcus thermopilus* and enriched with other dietary substances, after which it may be aerated or frozen (Brasil, 2005; El-Kholy, Aamer & Ali, 2020).

Whey is one of the residues of dairy production which is often improperly disposed of, generating negative environmental impacts (Cotrim, Cotrim & Coimbra, 2019). The frozen drink produced in the present study used whey as an essential ingredient. The processing followed the basic principles of yogurt preparation but differed with regard to the lactic beverage.

The prebiotics most widely used in food processing include fructooligosaccharides (FOS), inulin, isomalto-oligosaccharides, glucooligosaccharides, xylooligosaccharides and trans-galactooligosaccharides. Inulin and FOS have received the most attention from researchers (Markowiak & Slizewska, 2017; Silva et al., 2019) and, in Brazilian legislation, are the only prebiotics with documented effects on the composition of the intestinal microbiota (Brasil, 2008).

The purpose of the present study was to develop and evaluate the physicochemical, microbiological and sensory properties of a frozen dairy drink containing whey and different concentrations of FOS.

2. Methodology

This work corresponds to a basic research of experimental nature and quantitative approach. This research can be classified as explanatory in terms of objectives, as it seeks to verify the cause and effect relationships of data collected in the laboratory (Cervo, Bervian & Silva, 2007; Pereira, Shitsuka, Parreira & Shitsuka, 2018).

2.1 Materials

Skimmed milk UHT, whole dry milk, whey powder, lactic cultures (*L. bulgaricus* and *S. thermophilus*), mombin pulp (cajá-umbu), refined sugar, stabilizer, emulsifier, food dye.

2.2 Sample preparation

Following the preliminary tests to adjust fermentation time, fermentation temperature and beating time, the frozen drink was developed in two stages: the preparation of a lactic beverage, and the preparation of a syrup containing two thirds mombin pulp (cajá-umbu). Three formulations were tested: F1=control formulation containing 14.58% sucrose and 0% FOS, F2=formulation containing 11.58% sucrose and 3% FOS, and F3=formulation containing 8.58% sucrose and 6% FOS. The other ingredients remained unchanged (Table 1).

Table 1 - Formulations of frozen drink based on fermented lactic beverage enriched with prebiotic fiber (FOS) and mombin pulp (cajá-umbu).

Ingredients	Frozen drink formulations (%)*		
	F1	F2	F3
FOS	-	3.00	6.00
Fermented lactic beverage	51.00	51.00	51.00
Refined sugar	14.58	11.58	8.58
Whole dry milk	2.40	2.40	2.40
Mombin pulp	30.02	30.02	30.02
Stabilizer (neutral admixture)	1.00	1.00	1.00
Emulsifier/stabilizer (Emustab)	1.00	1.00	1.00

*Abbreviations are: F1, Frozen drink from fermented beverage (0% of FOS -control formulation); F2, Frozen drink from fermented beverage containing 3% FOS; F3, Frozen drink from fermented beverage containing 6% FOS.
Source: Authors.

2.3 Lactic beverage

Skimmed milk UHT (54%), reconstituted whey powder (36%) and fat-free milk powder (7%) were mixed and incubated for 5 min at 90 ºC, followed by cooling to 43 ºC and direct inoculation of lactic cultures (*L. bulgaricus* and *S. thermophilus*) (3%). The mix was incubated for approximately 5 hours and sampled in duplicate at hourly intervals for pH analysis and fermentation time determination until pH 4.6. This level of acidity was chosen for optimal sensory qualities and storage life. Following fermentation, the lactic beverage was matured for 12 hours at 4 ± 1 ºC. The resulting beverage was used to produce the frozen drink.

2.4 Mombin-flavored frozen drink

The frozen drink was prepared by adding emulsifier/stabilizer, whole dry milk, neutral admixture and mombin pulp to the previously prepared lactic beverage. FOS was added to two of the three formulations (F2 and F3).

Prepared 24 hours prior to usage, the syrup consisted of frozen fruit pulp (67%), white sugar (29.98%), starch (3%) and food dye (0.02%). After homogenization of the ingredients in an industrial blender for 3 minutes, the syrup was allowed to rest in a polyethylene container for 12 hours at -18 ºC. Then emulsifier/stabilizer was added, and the syrup was homogenized in a mixer for 5 minutes in order to incorporate air, followed by storage at -18 °C until use.

2.5 Physicochemical properties of raw materials and formulations

The raw materials (lactic beverage, syrup and dry whey) and the three formulations were submitted to physicochemical analysis to determine pH, titratable acidity, soluble solids, total solids, total and reducing sugars, and vitamin C content.

The pH value was measured with a digital pH meter (Hanna Instruments®, model HI 7004L) method 981.12 (AOAC, 2002). Titratable acidity was quantified by titration using phenolphthalein as indicator, according to method 947.05 (AOAC, 2002). The results were expressed as percentage of citric acid (mombin pulp) or percentage of lactic acid (formulations).

The soluble solids content was measured with a bench refractometer (Optronics Abbe Refractometer) and expressed as degrees Brix. Total and reducing sugars content was determined with the Lane-Eynon method (Lane & Eynon, 1934). Total solids were quantified by gravimetric analysis, according to method 925.23 (AOAC, 2002).

Vitamin C content was determined by titrimetry using a DFI (2,6-dichlorophenolindophenol) until reaching a permanent pink color. The results were expressed as mg ascorbic acid per 100 mL of sample, according to method 985.33 (AOAC, 2002). All the formulations were prepared and tested in triplicate.

2.6 Centesimal composition and total energy of formulations

Fat content was determined with the Gerber method (Brasil, 1987) while moisture rates were determined by incubating the samples at 105 ºC until reaching a constant weight (method 935.29; AOAC, 2002). Protein analyses were carried out based on nitrogen content, as determined by the micro-Kjeldahl method. The protein content was estimated by multiplying the nitrogen value by 6.38 (method 991.20; AOAC, 2002). The ash content was determined by incinerating the sample in a muffle furnace at 550 ºC until reaching a constant weight (method 930.22; AOAC, 2002). Finally, the carbohydrate content was calculated using the equation: 100 - (moisture + fat + protein + ash).

The total energy value of the formulations was calculated using a conversion factor of 4 kcal g^{-1} for carbohydrates and proteins and 9 kcal g^{-1} for lipids. The results were given in kcal per 100 g of product (Brasil, 2003).

2.7 FOS determination

The total FOS content was measured by spectrophotometry (method 999.03; AOAC, 2002) using the Megazyme-Fructan HK kit (Megazyme International, Wicklow, Ireland). The process consisted of hydrolyzing sucrose and low-polymerization maltosaccharides into fructose and glucose with a specific enzyme (sucrase/maltase). After pH adjustment, the amount of glucose and fructose released at this step (A) was quantified by absorbance at 340 nm. In the next step (B), part of the sample was treated with purified fructanase in order to hydrolyze fructan into fructose and glucose. The glucose and fructose in the aliquot were then treated with hexokinase/phosphate-glucose isomerase/glucose 6-phosphate and quantified by absorbance at 340 nm. The FOS content of the sample (expressed in percentage) was obtained by subtracting A from B.

2.8 Microbiological assessment

The 25 g aliquot was diluted in 225 mL sterile 0.85% salt solution (1:10). The sample was adjusted to a pH value between 6.6 and 7.2 by adding 1 N NaOH.

The evaluation of thermotolerant coliforms (*Escherichia coli*) was done by inoculating 1.0 mL of the diluted sample on a 3M Petrifilm™ E. coli/Coliform Count Plate™ using a pipette positioned perpendicularly. Following the manufacturer's instructions, the lower film was carefully covered with the upper film to avoid bubble formation. The plates were incubated for 24-48 hours at 35 ± 1 ºC. Expressed as UFC g^{-1}, the results were interpreted in accordance with the criteria of method 991.14 (AOAC, 2002).

The evaluation of *Staphylococcus aureus* was done by incubating inoculate on 3M Petrifilm Staph Express Count plate for 24 ± 2 hours at 35 ± 1 ºC. Red-violet colonies surrounded by a pink zone were identified as *S. aureus* and quantified according to method 2003.07 (AOAC, 2012).

Testing for *Salmonella* sp. was done with method 967.25 (AOAC, 2002). The results were expressed as absence/presence of *Salmonella* sp. in 25 g of sample.

2.9 Assessment of quality and acceptance

The research project was submitted to analysis by the Committee of Ethics in Research in Humans - CEP, IFCE, under decree 667.712, aiming at complying with the items in Resolution No. 466, 12/12/2012 of the National Health Council (CNS), which provides on ethics in research involving human beings (Brasil, 2013).

The formulations were submitted to sensory evaluations of acceptance and purchase intention by 120 non-trained volunteers. Each participant was given approximately 30 g of each sample at -9 ± 1 ºC, served in disposable cups labeled with 3-digit numbers.

Acceptance (appearance, aroma, flavor, texture and general impression) was scored on a hedonic scale from 1 (dislike extremely) to 9 (like extremely) (Meilgaard, Carr & Civille, 2006). Purchase intention was graded on a structured scale from 1 (would certainly not buy) to 5 (would certainly buy). Based on the obtained scores, the Acceptability Index (AI) was calculated using the equation described by Dutcosky (2013), where AI = (Average score obtained for the product / Highest score given to the product) x 100.

2.10 Statistical analysis

The collected data were submitted to analysis of variance, and differences between mean values were compared with the Tukey test. The level of statistical significance was set at 5% ($p<0.05$). All analyses were performed with the software SISVAR® (Ferreira, 2008).

3. Results and Discussion

3.1 Physicochemical properties of raw materials and formulations

The results of the physicochemical analyses and centesimal composition (protein, moisture, ashes, fat, carbohydrates, water activity, titratable acidity, pH, soluble solids and vitamin C) of the raw materials (lactic beverage, syrup and dry whey) are displayed in Table 2. The high protein (4.10 g 100 g^{-1}) and moisture (86.10 g 100 g^{-1}) content found in the lactic beverage is due to the addition of reconstituted dry whey, while the low carbohydrate content (8.42%) may be due to the conversion of lactose into lactic acid during fermentation (Lech, 2020).

Table 2 - Physicochemical properties of lactic beverage (LB), syrup (SY) and dry whey (DW).

Component	Raw materials (mean ± SD)*		
	LB	SY	DW
Protein (g 100 g^{-1})	4.10 ± 0.40	0.47 ± 0.07	11.33 ± 0.83
Moisture (g 100 g^{-1})	86.10 ± 0.17	60.51 ± 0.52	3.40 ± 0.16
Ashes (g 100 g^{-1})	1.36 ± 0.05	0.37 ± 0.02	5.03 ± 0.03
Fat (g 100 g^{-1})	0.00 ± 0.00	n/a	0.28 ± 0.04
Carbohydrates (g 100 g^{-1})	8.42 ± 0.17	n/a	79.99 ± 0.70
Water activity	n/a	0.72 ± 0.01	n/a
Titratable acidity (% lactic acid)	0.97 ± 0.01	0.58 ± 0.58	1.21 ± 0.01
pH	4.60 ± 0.01	2.77 ± 0.03	6.52 ± 0.01
Soluble solids (ºBrix)	n/a	42.17 ± 1.17	n/a
Vitamin C (mg 100 g^{-1})	n/a	14.73 ± 1.10	n/a

*Abbreviations are: LB, lactic beverage; SY, syrup and DW, dry whey; n/a, not analysed. Values are the mean ± SD (n = 3).
Source: Authors.

Brazilian standards of identity and quality for whey (Brasil, 2020) specify a recommendable lactose content of 61.0 g 100 g^{-1}, a minimum protein content of 10.0 g 100 g^{-1}, a maximum moisture value of 5.0 g 100 g^{-1}, an ash content of 9.5-15.0 g 100 g^{-1}, and acidity of < 0.35 g 100 g^{-1}. The total carbohydrate content (which is not covered by regulations) was 79.99%. The protein and moisture contents found in the analysed whey powder were within the recommended limits. The low content of total lipids enables it to be classified as skimmed fermented milk. The ash content was 5.03 g 100 g^{-1}, which is lower than the reference values for whey. The acid content (1.21 g 100 g^{-1}) was well above the maximum value specified in the legislation, but this had no influence on the other properties of the formulations. The average pH value was 6.52 according to the established in the legislation. Westergaard (2001) recommends a minimum pH value of 6.30 for whey. The pH ranges close to those described in this study were also observed by Wherry, Barbano and Drake (2019), which were 6.49 and 6.52. The average fat content was 0.28 g 100 g^{-1}. The regulations make no mention of fat, however, according to Westergaard (2001), whey rarely exceeds 0.05 g 100 g^{-1}. Similar results were observed in a study by Wherry et al. (2019), that obtained a content of 0.04 g 100 g^{-1} of fat.

The syrup analysis yielded a mean protein content of 0.47 g 100 g^{-1}, which is lower than that found by most other authors (Bramont et al., 2018; Lima, Lima, Oliveira & Fernandes Neto, 2012). The relatively low moisture rate observed was due to the addition of solids (starch and sugar) and to the moisture loss during the heating process. The mean vitamin C content of our syrup was 14.73 mg 100 g^{-1}, which is within the range (3.8-16.4 mg 100 mL^{-1}) reported by Carvalho, Ritzinger, Soares Filho and Ledo (2008). Titratable acidity and pH were 0.58% and 2.77, respectively. Low pH values benefit the preservation of processed fruit by inhibiting microorganism growth. The high content of soluble solids in the syrup was due to the addition of sugar and starch and the subsequent concentration.

The results of the physicochemical analyses of the three formulations (F1, F2, F3) are shown in Table 3. Brazilian law provides no guidelines for acidity (lactic acid) in fermented milk-based ice cream, but a level of 0.6-1.5% is recommended for fermented milk (e.g., yogurt) (Brasil, 2007). The samples tested in this study fell within this range.

Table 3 - Physicochemical parameters of formulations of frozen drink based on fermented lactic beverage enriched with prebiotic fiber (FOS) and mombin pulp (cajá-umbu).

Parameter	Frozen drink formulations (mean ± SD)*		
	F1	F2	F3
pH	4.45 ± 0.03^{ab}	4.44 ± 0.03^{a}	4.48 ± 0.02^{b}
Titratable acidity (% lactic acid)	0.80 ± 0.01^{a}	0.80 ± 0.01^{a}	0.79 ± 0.01^{a}
Soluble solids (ºBrix)	36.00 ± 0.75^{a}	35.30 ± 0.79^{a}	37.00 ± 1.06^{b}
Total solids (g 100 g^{-1})	37.41 ± 0.39^{a}	37.25 ± 0.26^{a}	37.10 ± 0.19^{a}
Vitamin C (mg 100 g^{-1})	14.33 ± 0.87^{a}	15.22 ± 0.81^{a}	17.04 ± 0.85^{b}
FOS (%)	n/a	2.42 ± 0.040^{a}	4.69 ± 0.06^{b}

*Abbreviations are: F1, Frozen drink from fermented beverage (0% of FOS -control formulation); F2, Frozen drink from fermented beverage containing 3% FOS; F3, Frozen drink from fermented beverage containing 6% FOS; n/a, not analysed. Values are the mean ± SD (n = 9), different superscript letters (a, b) show significant differences (p<0.05) in a row.
Source: Authors.

All three formulations had relatively low pH values. This was expected due to fermentation during processing, just as with any milk-based frozen edible. The pH value was further lowered by the addition of acidic syrup.

Brazilian regulations specify a minimum of 28% total solids for milk-based ice creams (Brasil, 2005). These are adequate values if soluble solids are important, as they interfere with product viscosity (Marques, Antunes & Gama, 2017). The mean amounts of solids in our formulations (F1=37.41 g 100 g^{-1}; F2=35.25 g 100 g^{-1}; F3=37.10 g 100 g^{-1}) were within the range recommended by law and by several authors (Barcelos et al., 2019; Gonçalves and Eberle, 2008; Akalin & Erisir, 2008), and are higher values than those found in the literature (Carlos et al., 2019; Marques et al., 2017). F2 and F3 did not differ significantly ($p \geq 0.05$) from the control formulation (F1) in this respect.

As opposed to F2 and F3, Vitamin C content was lower in F1 (0% FOS) than syrup (14.73 mg 100 g^{-1}). F3 differed significantly ($p<0.05$) from F1 and F2. Because ascorbic acid is highly degradable during processing and storage, retention is used as a nutritional quality maintenance index. Since the syrup was the only source of ascorbic acid in the formulations, the vitamin C content in F2 and F3 was expected to be somewhat lower than that of the syrup. However, some ingredient in the chemically more complex formulations may have interfered with the titration process or even changed the endpoint.

The mean final FOS content was slight smaller than the amount added to the

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formulations, with a significant difference between F2 (2.42%) and F3 (4.60%) ($p<0.05$). Ideally, prebiotics should remain chemically stable during food processing (including heating, pH reduction, Maillard's reactions) in order to preserve their functional properties in the finished product Bersaneti, Garcia, Mali and Celligoi (2019). According to Tian et al. (2019) the addition of FOS to foods increases satiety, thereby reducing the consumption of calories and preventing weight gain (usually in the form of fat).

The remaining parameters there was no significant difference ($p\geq0.05$). This was expected since the only difference between the formulations was with regard to sucrose content, part of which was replaced by soluble fiber.

The nutrient content of each formulation is listed in Table 4. As shown, the addition of FOS did not interfere significantly ($p\geq0.05$) with ash, fat, and moisture contents. According to Clarke (2004), 100 g of ice cream should contain 60-72 g of water. The moisture contents of our formulations fell within this range.

Table 4 - Nutrient content in formulations of frozen drink based on fermented lactic beverage enriched with prebiotic fiber (FOS) and mombin pulp (cajá-umbu).

Component	Frozen drink formulations (mean ± SD)[*]		
	F1	F2	F3
Moisture (g 100 g^{-1})	62.56 ± 0.34^a	62.74 ± 0.27^a	62.90 ± 0.19^a
Ashes (g 100 g^{-1})	0.89 ± 0.03^a	0.88 ± 0.03^a	0.87 ± 0.03^a
Fat (g 100 g^{-1})	0.79 ± 0.02^a	0.80 ± 0.01^a	0.80 ± 0.01^a
Protein (g 100 g^{-1})	3.18 ± 0.33^{ab}	3.04 ± 0.26^a	3.47 ± 0.34^b
Reducing sugars (%)	14.86 ± 0.27^c	12.85 ± 0.20^b	9.69 ± 0.34^a
Total sugar (%)	20.89 ± 0.38^c	18.69 ± 0.26^b	15.47 ± 0.35^a
Calorie count (kcal 100 g^{-1})	103.51 ± 1.34^c	94.68 ± 0.40^b	83.48 ± 2.57^a

[*]Abbreviations are: F1, Frozen drink from fermented beverage (0% of FOS -control formulation); F2, Frozen drink from fermented beverage containing 3% FOS; F3, Frozen drink from fermented beverage containing 6% FOS; Values are the mean ± SD (n = 9), different superscript letters (a, b) show significant differences ($p<0.05$) in a row.
Source: Authors.

Brazilian regulations provide no guidelines for the composition and quality of frozen drinks made from fermented lactic beverage, but a minimum of 2.5% protein is specified for milk-based ice cream in a directive regulating frozen edibles. Thus, with regard to protein content, the three formulations tested met the legal requirements (Brasil, 2005).

The three formulations did not differ significantly ($p \geq 0.05$) with regard to ash content (0.87-0.89 g 100 g^{-1}). Currently, ash content is not regulated by Brazilian law (Brasil, 2005).

Fat helps improve the texture, creaminess, taste and elasticity of foods, in addition to providing 9 kcal g^{-1}. Conventional ice cream formulations have high concentrations of sucrose and fat related to texture, consistency and taste, but the growing demand for healthy foods has spurred the development of alternative products which retain desirable sensory features while employing non-fattening ingredients, such as soluble fibers (inulin and FOS) used for texture in ice cream (Bersaneti et al., 2019; Carlos et al., 2019). The lipid content in our formulations was 0.79 g 100 g^{-1} (F1) and 0.80 g 100 g^{-1} (F2 and F3) ($p \geq 0.05$). The lipid content in the formulations was 0.79 g.100 g^{-1} (F1) and 0.80 g.100 g^{-1} (F2 and F3) and did not differ from each other ($p \geq 0.05$), making it possible to classify the frozen drink as a "soft" or "low-fat" (Brasil, 1978). This is further reinforced by the reduced amount of sucrose and low percentage of overrun (data not shown).

The amount of total and reducing sugars in sucrose varied significantly ($p<0.05$) between F1 (20.89%), F2 (18.69%) and F3 (15.47%). Adding FOS as a partial replacement for sucrose a widely adopted technique to control sweetness in processed foods (Yun, 1996) reduced the calorie count of the formulations from 103.51 kcal 100 g^{-1} (F1) to 94.69 kcal 100 g^{-1} (F2) and 83.48 kcal 100 g^{-1} (F3).

3.2 Microbiological assessment

Current Brazilian regulations for the microbiological quality of frozen edibles (Brasil, 2001) specify limits for thermotolerant coliforms (5 x 10 UFC g^{-1}), coagulase-positive staphylococci (5 x 10^2 UFC g^{-1}) and *Salmonella* sp. in 25 g (none). The ingredients of ice cream (fat, milk, sugars, stabilizers, emulsifiers, flavoring, dyes) provide a favorable milieu for the growth of microorganisms; nevertheless, contamination is usually the result of negligence in production procedures.

The tests yielded excellent results regarding thermotolerant coliforms (< 1 x 10 UFC g^{-1}), coagulase-positive staphylococci (< 1 x 10^2 UFC g^{-1}), and *Salmonella* sp. in 25 g (absent), regardless of formulation. In other words, the frozen drink complied with regulations and was safe to consume.

3.3 Quality and acceptance evaluation

The mean scores of the three formulations were between 7 (like moderately) and 8 (like very much). F1 received slightly higher scores for flavor and general acceptance (Table 5), but the three formulations did not differ significantly ($p \geq 0.05$) despite the replacement of sucrose by FOS.

Table 5 - Sensory properties of frozen drink based on fermented lactic beverage enriched with prebiotic fiber (FOS) and mombin pulp (cajá-umbu) evaluated on a 9-point hedonic scale by 120 volunteers.

Attributes	Frozen drink formulations (mean ± SD)*		
	F1	F2	F3
Appearance	7.80 ± 1.10^a	7.71 ± 1.50^a	7.80 ± 1.28^a
Aroma	7.38 ± 1.30^a	7.31 ± 1.42^a	7.50 ± 1.26^a
Flavor	7.65 ± 1.33^a	7.39 ± 1.37^a	7.36 ± 1.51^a
Texture	7.48 ± 1.43^a	7.40 ± 1.54^a	7.50 ± 1.45^a
General acceptance	7.62 ± 1.18^a	7.43 ± 1.36^a	7.53 ± 1.34^a

*Abbreviations are: F1, Frozen drink from fermented beverage (0% of FOS -control formulation); F2, Frozen drink from fermented beverage containing 3% FOS; F3, Frozen drink from fermented beverage containing 6% FOS; SD=standard deviation. Superscripts (a, b) indicate significant differences ($p<0.05$) within the same row.
Nine-point hedonic scale from 9 = like extremely to 1 = dislike extremely.
Source: Authors.

Table 6 shows the Aceeptability Index of the three formulations according to attribute. Regardless of attribute, our AR values were better than those of Dutcosky (2013), who adopted 70% as cut-off.

Table 6 - Acceptance rates (%) for sensory properties of frozen drink based on fermented lactic beverage enriched with prebiotic fiber (FOS) and mombin pulp (cajá-umbu), based on a sample of 120 volunteers.

Attributes	Frozen drink formulations*		
	F1	F2	F3
Appearance	86.67	85.67	86.67
Aroma	82.00	81.22	83.33
Flavor	85.00	82.11	81.78
Texture	83.11	82.22	83.33
General acceptance	84.67	82.55	83.67

*Abbreviations are: F1, Frozen drink from fermented beverage (0% of FOS -control formulation); F2, Frozen drink from fermented beverage containing 3% FOS; F3, Frozen drink from fermented beverage containing 6% FOS.
Source: Authors.

The average purchase intention scores were similar ($p \geq 0.05$) for the three formulations (F1=3.90, F2=3.80, F3=3.91), suggesting the participants would probably buy the product. In short, all three formulations were deemed attractive and marketable. The addition of up to 6% FOS had no measurable negative impact on acceptance.

4. Conclusion

The replacement of sucrose by FOS significantly reduced the content of total sugar and non-reducing sugars without affecting the remaining physicochemical parameters. Acceptance was not impacted by the addition of FOS.

From the technological and nutritional perspective, the use of 3-6% FOS in the processing of a frozen drink based on fermented lactic beverage produced good results, contributing to the expansion of the Brazilian market of functional foods.

The tested formulations represent a nutritious and safe alternative for the use of residual whey from dairy farms, and an innovation on the market of functional frozen edibles.

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Percentage of contribution of each author in the manuscript

Elisangela de Andrade Castro – 30%

Poliana Brito de Sousa – 5%

Elisabeth Mariano Batista – 5%

Everlândia Silva Moura Miranda – 5%

Sandra Maria Lopes dos Santos – 15%

Luana Guabiraba Mendes – 5%

Antônio Belfort Dantas Cavalcante – 15%

Elda Fontinele Tahim – 5%

Marlene Nunes Damaceno – 15%