Ciência Animal Brasileira
Ciência Animal Brasileira
Brazilian Animal Science
e-ISSN 1809-6891

DOI: 10.1590/1809-6891v23e-72395E

Section: Veterinary medicine
Research article

Dairy drink fermented with chia seed and acerola syrup: physicochemical, microbiological, and sensory characterization

Bebida láctea fermentada com semente de chia e xarope de acerola: caracterização físico-química, microbiológica e sensorial

Juliana de Abreu Costa[1*], Julliet Teixeira de Oliveira Santos[1], Rafael Gomes Abreu Bacelar[1], Rosana Martins Carneiro[1], Darlisson Slag Neri Silva[1], Maria Marlúcia Gomes Pereira Nóbrega[1], Maria Christina Sanches Muratori[1]

[1]Universidade Federal do Piauí (UFPI), Teresina, Piauí, Brazil
[*]Correspondent: juliana.abreu04@hotmail.com

Abstract
This research aimed to develop a fermented dairy beverage using whey as a dairy base with chia seed (*Salvia hispanica* L.) and acerola syrup (*Malpighia emarginata*) and to evaluate physicochemical parameters (pH, water activity, total soluble solids, acidity, syneresis), proximate composition (moisture, ash, proteins, milk fat, carbohydrates, and energy value), β-carotene, ascorbic acid, microbiological and sensory parameters. The acidity results ranged from 1% to 1.27%; pH from 3.86 to 4.11; soluble solids from 15.67 to 21.6; water activity from 0.93 to 0.99; syneresis from 46.67 to 68.08; they presented satisfactory conditions for thermotolerant coliforms. *Salmonella* spp. showed cell viability of *L. acidophilus* : E4 (2.9 x 10^7 to 9.7 x 10^7) and E8 (1.3 x 10^7 to 8.6 x 10^7) , however *Bifidobacterium* spp. did not show any viability. The drinks had a good acceptance rate by the tasters (7.0). The humidity results were from 74.21% to 74.34%; ash from 0.42% to 0.55%; proteins from 2.93% to 2.99%, milk fat from 1.47% to 0.93%; carbohydrate from 20.97% to 21.19%; energy value from 108.83% to 105.09% and β-carotene from 12.33% to 8.19%; and ascorbic acid ranged from 222.23 (mg/100g) to 418.10 (mg/100g). It is concluded that formulated dairy drinks are considered a viable alternative for the food industry, with the differential of including chia seed and acerola pulp, due to their good sensory acceptance, physical-chemical stability, satisfactory sensory analysis, microbiological standards suitable in the 21-day storage period.
Keywords: Industry; quality; whey; *Salvia hispanica* L.; *Malpighia emarginata*.

Resumo
O objetivo desta pesquisa foi desenvolver uma bebida láctea fermentada utilizando o soro de leite como base láctea com semente de chia (*Salvia hispanica* L.) e xarope de acerola (*Malpighia emarginata*), avaliar parâmetros físico-químicos (pH, atividade de água, sólidos solúveis totais, acidez, sinérese), composição centesimal (umidade, cinzas, proteínas, matéria gorda láctea, carboidratos e valor energético), β-caroteno, ácido ascórbico, parâmetros microbiológicos e sensorial. Os resultados de acidez variaram de 1% a 1,27%; pH de 3,86 a 4,11; sólidos solúveis de 15,67 a 21,6; atividade de água de 0,93 a 0,99; sinérese de 46,67 a 68,08. Apresentaram condições satisfatórias para Coliformes termotolerantes e *Salmonella* spp., apresentou viabilidade celular de *L. acidophilus*: E4 (2,9 x 10^7 a 9,7 x 10^7) e E8 (1,3 x 10^7 a 8,6 x 10^7), porém *Bifidobacterium* spp. não apresentou viabilidade. As bebidas tiveram bom índice de aceitação pelos provadores (7,0). Os resultados de umidade foram de 74,21% a 74,34%; cinzas de 0,42% a 0,55%; proteínas de 2,93% a 2,99%, matéria gorda láctea de 1,47% a 0,93%; carboidrato de 20,97% a 21,19%; valor energético de 108,83% a 105, 09% e β-caroteno de 12,33% a 8,19%; e o ácido ascórbico variou de 222,23 (mg/100g) a 418,10 (mg/100g). Conclui-se que as bebidas lácteas formuladas são consideradas uma alternativa viável para indústria de alimentos, com o diferencial da inclusão da semente de chia e polpa de acerola, devido a sua boa aceitação sensorial, estabilidade físico-química, análise sensorial satisfatória, padrões microbiológicos adequados durante o período de estocagem de 21 dias.
Palavras-chaves: Indústria; qualidade; soro de leite; *Salvia hispanica* L.; *Malpighia emarginata*.

Received: March 29, 2022. Accepted: June 13, 2022. Published: July 22, 2022.

Introduction

The term "dairy beverage" broadly encompasses different products made from milk and whey. Whey is widely used to make dairy drinks in Brazil. This practice was regulated by the Ministry of Agriculture, Livestock, and Supply, with the publication of Normative Instruction Nº 16, on August 23rd, 2005, which approved the Milk Beverage Identity and Quality Technician [1].

In the cheese manufacturing process, whey is generated, which has a significant polluting action; that is, if destined for disposal, it can cause severe environmental damage when released into the environment in an untreated way, given its significant rate of organic matter represented, mainly by lactose and proteins [2]. Whey protein has been drawing a lot of attention due to its excellent biological value, as it has a high content of essential amino acids in its composition, which can increase the nutritional value of foods used in the human diet and can release a variety of bioactive peptides, which have antioxidant, antibacterial, immunomodulatory activities, as well as the inhibition of the converting enzyme of angiotensin-I [3,4]. The use of this dairy co-product in the preparation of food for human consumption reduces the environmental impact. Furthermore, it economically favors the industries by excluding expenses with effluent treatment and the economic return with the sale of these foods [5].

The search for healthy foods has increased the preference for the intake of fruits and fruit pulps. Currently, the use of fruits in the production of dairy drinks is related to marketing strategies focused on these products, which aim to improve sensory characteristics and offer new healthy foods [6]. Acerola is a tropical fruit with significant economic and nutritional potential, a high concentration of vitamin C in its composition, carotenoids, anthocyanins, iron, and calcium. Its consumption is made *in natura* and processed in juices, jellies, ice creams, syrups, and liqueurs, among other products [7]. The addition of chia seed in dairy drinks is an alternative for nutritional increment due to its protein content and polyunsaturated fatty acids, promoting benefits for individuals suffering from cardiovascular diseases, diabetes, and immune response disorders [8,9].

Based on this context, this research was conducted to develop a fermented dairy drink, added with chia seed (*Salvia hispanica* L.) and flavored with acerola syrup (*Malpighia emarginata*) and to evaluate its physicochemical, microbiological, and sensory characteristics.

Material and methods

The fermented dairy drinks were formulated at the Laboratory of the Dairy Sector of the Center for Studies, Research and Food Processing (NUEPPA) of the Federal University of Piauí (UFPI). The experiment was divided into 2 stages; the first stage was characterized by the definition of the best formulations based on the physical-chemical analyses of the developed dairy drinks. In the second stage, the optimized formulations were characterized based on proximate analyses, ascorbic acid, β-carotene, microbiological, the viability of probiotic bacteria, and sensory.

We used 1500mL of sweet whey from curd cheese manufactured by a dairy industry in Teresina, Piauí, Brazil, to prepare the fermented dairy drinks. The other ingredients were purchased from the local market in their conventional packaging: a) frozen acerola pulp (NutriVita ®, Porto Alegre/RS, Brazil) individually packaged in plastic bags (100g) packed in 500g packages; b) chia seed (Produza Foods ®) in 250g sachets; c) Itambé ® whole milk powder in 200g sachets; d) Longá ® whole pasteurized milk in 1000mL plastic packages and e) Olho D'água ® crystal sugar in 1000g plastic packages. Before purchase, packaging integrity and expiration date were checked. The lyophilized mixed culture DVS ABT-4 (Chr Hansen®) for direct use was adopted for the fermentation of beverage, according to the manufacturer's recommendations, containing *Lactobacillus acidophilus, Bifidobacterium animalis* Bb-12 and *Streptococcus thermophilus.*

Experimental planning

The formulations started from a minimum value of dairy base (51% of the total ingredients) that the legislation determines for the drink to be classified as a dairy drink (BRASIL, 2005a) and a minimum amount of acerola pulp so that the drink presented an ascorbic acid content to supply the daily intake index recommended by the National Health Surveillance Agency through ordinance No. 33, 1998 [10], at 60 mg/100g (Adults). After preliminary evaluation, the experimental design consisted of 8 trials (E1, E2, E3, E4, E5, E6, E7, and E8), considering the variables: dairy base (X1), syrup (X2) and chia seed (X3), whose proportions are shown in Table 1, as well as the formulations obtained. The dairy base (X1) comprised 70% pasteurized whole milk, 30% whey, and 3% whole milk powder. The syrup (X2) contained 80% acerola pulp and 20% crystal sugar. Chia (X3) was composed of 100% of the seeds.

Table 1. Experimental design for fermented dairy beverage added with chia seeds (*Salvia hispanica* L.) and acerola syrup (*Malpighia emarginata*)

Assay	Dairy base* (X1)		Syrup** (X2)		Chia (X3)	
	g	%	g	%	g	%
E1	700	70	290	29	10	1
E2	700	70	280	28	20	2
E3	650	65	340	34	10	1
E4	650	65	330	33	20	2
E5	600	60	390	39	10	1
E6	600	60	380	38	20	2
E7	550	55	440	44	10	1
E8	550	55	430	43	20	2

*Dairy base (X1) composed of 70% pasteurized whole milk, 30% whey, and 3.0% whole milk powder. **Syrup (X) composed of 80% acerola pulp and 20% crystal sugar.

Fermented milk beverage processing

Initially, the milk base was formulated from pasteurized whole milk, whey, and whole milk powder. The whey was submitted to slow pasteurization under constant agitation until reaching the temperature between 63 and 65°C, remaining for 30 minutes under this condition. At the same time, the powdered milk was dissolved in the pasteurized whole milk, and heating was carried out until reaching a temperature of 50°C. After these procedures, the two parts were mixed and cooled to the inoculation temperature (42°C - 45°C). Then, the lyophilized mixed culture DVS ABT-4 (Chr Hansen ®) for direct use was added, containing *Lactobacillus acidophilus, Bifidobacterium animalis* Bb-12 and *Streptococcus thermophilus*. Fermentation was carried out in an oven at 42°C and ended after reaching an average pH of 4.5, approximately four hours after the start of the process. After this step, the product was cooled to a refrigeration temperature of approximately 10°C. After cooling, it could be noticed that the clot was broken.

After the production of the milk base, the acerola syrup was formulated by homogenizing the acerola pulp with crystal sugar. Then, the mixture was boiled, under constant stirring, for approximately 30 minutes, for the evaporation of residual water from the acerola pulp. After cooling the acerola syrup to room temperature, the milk base, syrup, and chia seed were mixed to prepare the formulations according to the experimental plan (Table 1).

The fermented milk beverage formulations were homogenized, stored in glass jars with a metal lid with a capacity of 1,200 mL, previously identified, sanitized with a chlorinated solution (100ppm/20 minutes), and subjected to UV lamp irradiation for 20 minutes. The contents of each glass jar were divided into previously identified plastic flasks with a capacity of 250 mL. Subsequently, the vials of each treatment were separated for analysis at the time of immediate analysis (control), 7, 14, and 21 days of storage, under refrigeration at ± 4°C.

Physico-chemical analyzes of ingredients and formulations of dairy drinks

The dairy base (pasteurized whole milk, whey, and whole milk powder), chia seeds, and syrup (acerola pulp and sugar) were subjected to analysis of moisture (%), proteins (%), lipids (%), ash (%), and carbohydrates. In addition, the whey, chia seeds, acerola pulp, sugar, and syrup were submitted for analysis of titratable acidity quantification (% lactic acid), pH analysis, and total soluble solids readings (ºBrix) and water activity. The samples of fermented dairy drinks were submitted to the titratable acidity analysis, pH, total soluble solids, water activity, and syneresis, where all parameters were evaluated in triplicate [11].

The optimized formulations (E4 and E8) presented the best pH values (averages 4.01 and 3.91, respectively). Thus, they were chosen to continue the study, characterized based on the proximate analysis (moisture, ash, proteins, lipids, carbohydrates, caloric value), ascorbic acid, β-carotene, microbiological, the viability of probiotic bacteria, and sensory analysis.

For moisture analysis, a moisture meter (OHAUS®, Parsippany, New Jersey, USA) was used, following the manufacturer's instructions. In the ash analysis, the sample was incinerated in a muffle furnace at 550 ºC for 5 hours [11]. Protein analysis was performed using the Kjeldahl method with the process of digestion, distillation, and titration of the samples. The result obtained after the titration was calculated using a formula to obtain the total nitrogen value [12]. The lipid content was determined by the Gerber method [11]. Carbohydrate content was defined as the difference between 100 and the sum of protein, fat, moisture, and ash. Finally, the energy value was calculated considering carbohydrates and proteins with 4 kcal/g and lipids with 9 kcal/g [13].

The analysis of water activity was performed with the device previously calibrated, as determined by the manufacturer (PawKit Decagon ®, São Paulo/SP, Brazil). In the pH analysis, 10g of the sample was weighed in a 100mL beaker and diluted in 100mL distilled water. The measurement was carried out using a previously calibrated benchtop device, model K39-2014B, from Kasvi ®. The total soluble solids readings (ºBrix) were made by refractometry, using the Abbé refractometer, Biobrix ®, scale from 0% to 95% corrected to 20ºC, as

determined by the manufacturer. Titratable acidity was determined using sodium hydroxide solution (NaOH) at 0.1 mol/L (correction factor=1) and 1% phenolphthalein as an indicator solution, expressed in g lactic acid/100g [11].

The percentage of syneresis was calculated by the mass of whey separated from the gel network during centrifugation, divided by the mass of milky beverage, multiplied by 100 [14]. Ascorbic acid analysis was performed during 21 days of storage using the titrimetric method until the color turned pink, obtaining the ascorbic acid content in milligrams from the volume spent in the titration [15].

For β-carotene analysis, the fermented milk beverage was freeze-dried for 48h in a freeze-dryer (LIOTOP ®, L101, São Paulo, Brazil). The solvents and reagents used to quantify carotenoids were standard β-carotene and acetone. The total concentration of carotenoids was spectrophotometrically determined as described by [16,17], with some adaptations. Analyzes were performed in duplicate and under dim light.

Microbiological analysis

We performed the Microbiological analyzes of *Salmonella* spp. and most likely number (MPN/g) of thermotolerant Coliforms, according to recommended standards [18]. Initially, 25g of the sample was weighed and diluted in 225mL of peptone water (Ion ®) at 0.1%, forming the initial dilution (10^{-1}). From this, serial decimal dilutions up to 10^{-3} were prepared. Next, samples in flasks containing peptone water were incubated at 37°C for 24 hours for pre-enrichment to search for *Salmonella* spp. Finally, 1.0 mL of the sample was inoculated in three series of three tubes containing Sodium Lauryl Sulfate Broth (Tmmedia ®, Delhi, Republic of India) [13] to search for thermotolerant Coliforms.

Probiotic viability

To count *Lactobacillus acidophilus* and *Bifidobacterium animalis,* MRS Agar (Kasvi ®, São José do Pinhais/PR, Brazil) was used with modifications by adding maltose solution (Dinâmica ®, São Paulo/SP, Brazil), inhibitory compounds of chloride lithium (Dinâmica ® São Paulo/SP, Brazil) and sodium propionate (Sigma ®, Missouri, USA). The procedure started with weighing 25g of the sample and diluting in 225mL of peptone water (Ion ®) at 0.1%, forming an initial dilution (10^{-1}). From this, serial decimal dilutions were prepared up to 10^{-6}. The analyses used dilutions from 10^{-4} to 10^{-6}.

For the counting of *L. acidophilus,* the MRS (Kasvi ®) was sterilized at 121ºC for 15 minutes and modified by adding a maltose solution. The maltose solution (Dinâmica ®) was prepared using 25g of maltose dissolved in 50mL of distilled water, and sterilized in a 25µm membrane filter. Next, 4mL of the solution was added to 100ml of agar base at a temperature of approximately 50ºC. Finally, the mixture was carefully homogenized to avoid incorporating air [19].

For the quantification of *B. animalis,* MRS (Kasvi®) was used as a base medium and added with inhibitory compounds: 0.002g/mL of lithium chloride (Dinâmica®) and 0.003 g/mL of sodium propionate (Sigma®)[20]. The plates were evaluated in duplicates with deep inoculation for both microorganisms, incubated in anaerobic conditions at 37°C for 72 h. Counts were expressed in colony-forming units per milliliters (CFU/mL in log10).

Sensory analysis

The Ethics Committee of the Federal University of Piauí (UFPI) approved this research under the Consubstantiated Opinion number 2,640,867. The acceptance test was carried out with a nine-point hedonic scale (ranging from 1- "I disliked it very much" to 9- "I liked it very much"), in which the parameters of texture, flavor, aroma, and global impression were evaluated, and 5-point product purchase intention (ranging from 1 - "I would certainly not buy it" to 5 - "I would certainly buy it") [11,21]. Previously, each participant was asked to consent to the study by signing the Free and Informed Consent Term (ICF), which informed about the project's objectives and methodology. In addition, 120 untrained tasters were considered to participate in the sensory panel, members of IFPI, aged between 18 and 55 years old and of both sexes, who received specific guidance on the tests before being submitted. Each taster received the two formulations of the milk drink in disposable cups containing 20 mL, coded with random three-digit numbers, and served under controlled conditions.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) and Tukey's test to compare the means, establishing statistical significance at 5% probability ($p<0.05$). For the analysis of ascorbic acid, the t-Student test was used ($p<0.05$). The calculations were performed using the Statistica® and Excel® 2010 (Windows) programs.

Results and discussion

Characterization of ingredients

The proximate and physicochemical characterization of the ingredients used in the formulations of dairy drinks is shown in Table 2.

Table 2. Centesimal and physicochemical characterization of ingredients used in dairy beverage formulations

Parameters	Ingredients					
	Syrup*	Chia	Dairy base**	Acerola pulp	Sugar	Whey
Moisture (%)	63.11	6.62	85.62	-	-	-
Lipids (%)	0.27	25.49	2.27	-	-	-
Protein (%)	0.54	19.71	3.13	-	-	
Ash (%)	0.25	10.07	0.06	-	-	-
Carbohydrates (%)	35.83	38.11	8.92	-	-	-
Energy value (Kcal)	147.91	460.69	68.63	-	-	-
Acidity	-	-	-	-	-	0.12
pH	3.76	6.87	-	3.67	7.73	6.5
ºBrix	44.1	2.2	-	6.3	-	6.4
Aw	0.83	0.61	-	0.75	0.45	0.99

* Syrup prepared with acerola pulp and sugar. ** Dairy base composed of pasteurized whole milk, whey, and whole milk powder. - Unrealized.

The moisture content observed in the syrup can be explained by the water in the acerola pulp. The ash and carbohydrate values may have been influenced by adding sugar to the acerola pulp, given the number of solids added to the product. Chia presented the highest lipids, proteins, ash, carbohydrates, and energy values among the ingredients analyzed. The lipid content presented by chia was 25.49%. The protein portion of the seed also showed a significant value, 19.71%, superior to other traditional foods such as wheat, rice, corn, and barley [22]; consequently, the ash content also presented a high value, 10.07%. The high concentration of ash indicates its importance as a source of minerals.

The dairy base had a moisture content of 85.62% due to pasteurized whole milk and whey. The food's moisture is related to its stability, quality, composition, and shelf life [23]. The pulp presented 6.3 ºBrix and a pH of 3.67, meeting the values established by the legislation [24]. The whey presented values within the Whey Identity and Quality Standard, which determines a pH value between 6.0 and 6.8; titratable acidity in lactic acid (g/100g) of at least 0.08 to 0.14 and total solids content (g/100mL) of at least 5.0 [25]. The composition of the whey varies due to several factors, such as the type of cheese produced, the heat treatment received, and handling, among others.

Characterization of fermented milk mrinks

Table 3 presents the average values of acidity, pH, °Brix, water activity, and syneresis obtained in the formulations of fermented dairy drinks.

There was no significant difference ($p > 0.05$) in acidity values between formulations and storage time; acidity tended to stabilize. This result can be interpreted by the non-interference of whey in the activity of lactic acid cultures. The acidity of the formulations ranged from 1.0 to 1.3 g of lactic acid/100g, according to the legislation, which indicates 0.6 to 2.0 g of lactic acid/100g [26].

It can be stated that there was no significant interaction between the amount of dairy base, syrup, and chia seed within the treatments and between storage times when analyzing the pH results of fermented dairy drinks. However, different concentrations of dairy base influenced the fermentation time, and the end of the fermentation process was determined when the dairy drinks reached a pH between 3.86 and 4.02, which occurred after 4 hours of fermentation. The pH analysis of the raw materials (Table 2) showed average rates of: 3.67 for acerola pulp, following the established by the legislation [24]; 6.5 for whey, being between 5.3 to 6.6 obtained by [27]; 7.73 for sugar; and 3.76 for syrup. Thus, the acerola pulp may have been the ingredient that influenced the low pH values obtained in the milk drink tested.

It can be affirmed that there was a significant difference in the test time within E1, between day 0 and day 21, with the increase in the soluble solids content when analyzing the results of this research. A significant interaction was also observed between treatments in all trials. On day 0, there was a considerable difference in the values of total soluble solids (ºBrix) between E1 and E5, E6, E7, and E8. On day 7, values significantly differed between E7 and E1, E2, E3, E4, E5, E6, and E8. On the 14th day, there was a significant difference between E8 and E4, E3, E2, and E1; on the 21st day, there was a substantial difference between E1 and E7 and E8.

The variations of E5, E6, E7, and E8 tests concerning the others can be explained by their higher values of total soluble solids because they contain higher concentrations of syrup (Table 1), which may have caused an increase in the ºBrix value. In this experiment, the water activity was similar ($P<0.05$) in all treatments used, obtaining constant values during the 21 days of storage under refrigeration.

On average, the water activity for the metabolic action of most microorganisms is from 0.90–0.99, thus showing that the elaborated product has high water activity and is favorable to microbial development in the long term. [28]. In this research, syneresis was similar ($P<0.05$) in all treatments used, obtaining constant values during the 21 days of storage under refrigeration. The syneresis values shown can be explained by the percentage of the milk base, which presented 85.62% moisture; because no thickener was used to increase the consistency and viscosity of the beverage and because of the low pH values found in the formulations.

Table 3. Average values of acidity (g of lactic acid/100g), pH, degree Brix, water activity (Aw), and syneresis samples of fermented milk drinks over 21 days of storage

Formulation	Parameter	Time (in days)			
		0	7	15	21
E1	Acidity	1.0^{Aa} ± 0.05	1.1^{Aa} ± 0.05	1.2^{Aa} ± 0.08	1.2^{Aa} ± 0.17
	ph	4.02^{Aa} ± 0.06	4.02^{Aa} ± 0.06	4.03^{Aab} ± 0.01	4.07^{Aab}± 0.05
	°Brix	15.67^{Aa} ± 0.80	17.00 ^{ABa} ±0.43	17.13^{ABa} ± 0.77	17.73 ^{Ba} ±0.80
	Aw	0.97^{Aa} ± 0.02	0.98 ^{Aa} ± 0.01	0.97 ^{Aa} ± 0.01	0.93 ^{Aa} ± 0.03
	Syneresis	51.1^{aA}± 5.39	54.16 ^{aA} ± 7.34	51.84 ^{aA} ± 8.99	63.43 ^{aA} ± 8.75
E2	Acidity	1.0^{Aa} ± 0.02	1.0 ^{Aa} ± 0.09	1.1 ^{Aa} ± 0.11	1.2 ^{Aa}± 0.24
	pH	4.03 ^{Aa} ± 0.08	4.05 ^{Aa} ± 0.05	4.09 ^{Aa}± 0.03	4.11 ^{Aa} ± 0.04
	°Brix	16.87 ^{Aa} ± 0.77	16.27 ^{Aa} ± 1.14	17.13^{Aa} ± 0.30	17.27 ^{Aa} ± 0.66
	Aw	0.96 ^{Aa} ± 0.04	0.98 ^{Aa} ± 0.02	0.98 ^{Aa} ± 0.01	0.94 ^{Aa}± 0.02
	Syneresis	51.84 ^{aA} ±14.13	50.22 ^{Aa} ± 6.14	47.78 ^{aA} ± 5.57	50.00 ^{aA} ± 9.10
E3	Acidity	1.0 ^{Aa} ± 0.07	1.1 ^{Aa} ± 0.09	1.1 ^{Aa} ± 0.14	1.2 ^{Aa} ± 0.17
	pH	3.93 ^{Aab} ± 0.09	3.97 ^{Aab} ± 0.02	4.00 ^{Aab} ± 0.08	4.02 ^{Aab} ± 0.04
	°Brix	17.63 ^{Aa} ± 0.70	17.73 ^{Aa} ± 1.20	18.47^{Aa} ± 0.86	18.00 ^{Aa} ± 0.93
	Aw	0.96 ^{Aa} ± 0.04	0.98 ^{Aa} ± 0.01	0.98 ^{Aa} ± 0.01	0.94 ^{Aa} ± 0.03
	Syneresis	51,84 ^{aA} ±14.13	50.38 ^{aA} ± 6.13	49.64 ^{aA} ± 5.72	50.73 ^{aA} ± 8.96
E4	Acidity	1,0 ^{Aa} ± 0.08	1.0 ^{Aa} ± 0.11	1.2^{Aa} ± 0.15	1.2 ^{Aa} ± 0.14
	pH	3.97 ^{Aab} ± 0.07	4.00 ^{Aab} ± 0.08	4.04^{Aab} ± 0.02	4.06 ^{Aab} ± 0.01
	°Brix	18.03 ^{Aab} ± 0.77	18.20 ^{Aa} ± 0.70	18.27^{Aa} ± 0.63	18.13 ^{Aa} ± 0.96
	Aw	0.96 ^{Aa} ± 0.05	0.98 ^{Aa} ± 0	0.99^{Aa} ± 0.01	0.95 ^{Aa} ± 0.02
	Syneresis	50.02 ^{aA} ± 13.47	48.16 ^{aA} ± 7.41	50.05 ^{aA} ± 7.75	48.13 ^{aA} ± 4.48
E5	Acidity	1.1 ^{Aa} ± 0.03	1.1 ^{Aa} ± 0.05	1.3 ^{Aa} ± 0.14	1.2 ^{Aa} ± 0.18
	pH	3.90 ^{Aab} ± 0.12	3.92 ^{Aab} ± 0.06	3.94 ^{Ab} ± 0.03	3.98 ^{Aab} ± 0.03
	°Brix	19.10 ^{Ab} ± 1.17	19.73 ^{Aab} ±0.83	19.67 ^{Aab} ± 1.43	18.60 ^{Aa} ± 0.86
	Aw	0.94 ^{Aa} ± 0.07	0.98 ^{Aa} ± 0.01	0.99 ^{Aa} ± 0.01	0.96 ^{Aa} ± 0.02
	Syneresis	49.27 ^{aA} ± 12.82	50.38 ^{aA} ± 5.57	47.78 ^{aA} ± 6.21	53.69 ^{aA} ± 8.46
E6	Acidity	1.1 Aa ± 0.04	1.1 ^{Aa} ± 0.07	1.2 ^{Aa} ± 0.16	1.2 ^{Aa} ± 0.16
	pH	3.90 ^{Aab} ± 0.07	3.95 ^{Aab} ± 0.06	3.98 ^{Aab} ± 0.01	3.99 ^{Aab} ± 0.02
	°Brix	19.33 ^{Ab} ± 0.50	19.87 ^{Aab} ±0.84	19.67 ^{Aab} ± 1.17	20.00 ^{Aab} ± 1.27
	Aw	0.95 ^{Aa} ± 0.07	0.98 ^{Aa} ± 0	0.97 ^{Aa} ± 0.02	0.96 ^{Aa} ± 0.02
	Syneresis	48.89 ^{aA} ± 13.48	49.64 ^{aA} ± 4.61	48.87 ^{aA} ± 14.17	45.93 ^{aA} ± 4.48
E7	Acidity	1.1 ^{Aa} ± 0.01	1.1 ^{Aa} ± 0.07	1.2 ^{Aa} ± 0.14	1.3 ^{Aa} ± 0.21
	pH	3.86 ^{Ab} ± 0.08	3.86 ^{Ab} ± 0.06	3.90 ^{Ab} ± 0.04	3.94 ^{Ab} ± 0.03
	°Brix	19.63 ^{Ab} ± 0.77	21.40 ^{Ab} ± 0.84	20.80 ^{Aab} ± 1.30	21.07^{Ab} ± 1.36
	Aw	0.94^{Aa} ± 0.07	0.98 ^{Aa} ± 0.01	0.98 ^{Aa} ± 0.01	0.96 ^{Aa} ± 0.02
	Syneresis	51.84 ^{aA} ± 14.12	47.78 ^{aA} ± 9.62	51.75 ^{aA} ± 14.36	52.58 ^{aA} ± 10.30
E8	Acidity	1.1^{Aa} ± 0.06	1.2 ^{Aa} ± 0.09	1.2 ^{Aa} ± 0.14	1.2 ^{Aa} ± 0.20
	pH	3.87 ^{Ab} ± 0.08	3.89 ^{Ab} ± 0.06	3.92 ^{Ab} ± 0.03	3.97 ^{Aab} ± 0.04
	°Brix	20.40 ^{Ab} ± 0.87	20.20 ^{Aab} ± 0.66	21.20 ^{Ab} ± 0.90	21.60 ^{Ab} ± 1.37
	Aw	0.95 ^{Aa} ± 0.07	0.98 ^{Aa} ± 0.01	0.98 ^{Aa} ± 0.01	0.96 ^{Aa} ± 0.02
	Syneresis	49.62 ^{aA}± 12.21	44.87 ^{aA} ± 5.29	44.45 ^{aA} ± 6.94	45.56 ^{aA} ± 5.09

*Tukey test, with 5% probability (P>0.05). X1: Dairy base; X2: syrup; X3: chia seeds. Note: Different capital letters reflect significant differences between storage times for the same formulation. Different lowercase letters reflect substantial differences between formulations for the same storage time.

Characterization of optimized formulations

Table 4 shows the results obtained in the microbiological and viability analysis of *Lactobacillus acidophilus* and *Bifidobacterium* spp. of fermented milk beverage formulations and stored for 21 days.

Table 4. Research on thermotolerant Coliforms and *Salmonella* spp.; viability of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in milk beverage formulations fermented for 21 days and stored under refrigeration (4.0°C)

Microorganism	Formulation	Time (in days)			
		0	7	14	21
Thermotolerant coliforms (MPN/g-1)	E4	<3	<3	<3	<3
	E8	<3	<3	<3	<3
Salmonella spp.	E4	Absent	Absent	Absent	Absent
	E8	Absent	Absent	Absent	Absent
Lactobacillus acidophillus (CFU g–1)	E4	9.7×10^7	2.9×10^7	6.9×10^7	4.0×10^7
	E8	8.6×10^7	1.3×10^7	7.7×10^7	1.5×10^7
Bifidobacterium spp. (CFU g–1)	E4	<10	<10	<10	<10
	E8	<10	<10	<10	<10

Subtitle: E4=test 4; E8=test 8; CFU g^{-1} = Colony Forming Units per gram in logarithmic numbers. Probability. MPN g^{-1}: Most Probable Number per gram in logarithmic numbers.

Normative Instruction No. 60, December 26th, 2019 (18) recommends the microbiological analyses of the beverages prepared. The results of the research on thermotolerant Coliforms and *Salmonella* spp. showed that there was no growth in the analyzed samples. Thus, the milk drink tested is within the expected regulatory standards. In the present research, the viability of *Bifidobacterium* spp. in the treatments of the analyzed fermented milk drink was not observed. The cell viability of *L. acidophilus* in the drink proved efficient, ranging from 9.7 x 10 ^{7} to 1.3 x 10 ^{7}. Thus, the two different formulations (E4 and E8) can be considered fermented dairy drinks, as they met the requirements described in the Brazilian legislation for dairy drinks [(1)], which recommends that specific microorganisms in the final product and throughout the period shelf life for fermented lactic drinks must be viable and be present in the product in minimum amounts of 10 ^{6} CFU/mL.

Sensory analysis

In the sensory analysis, there was no significant difference ($P<0.05$) between the formulations regarding the parameters evaluated (Table 5). In the evaluations of the color, flavor, texture, and global acceptance parameters, it was observed that all formulations had a median of 7, representing on the scale the hedonic term "I liked it regularly." In purchase intent, all formulations had a median of 4, defining the hedonic term "Possibly would buy it," revealing that the product had regular acceptance by the tasters.

It can be said that the formulations had good acceptance among the judges since each formulation had an acceptance rate (AR) of 80%, demonstrating an intention to purchase the product. Among the comments recorded by the tasters, the most recurrent was about the taste and texture of the samples. Comments were reported regarding the formulations as being "very good," "very sour," "not too creamy," and "not very consistent." The natural acidity of the acerola can explain the comments about the taste of the sample and the texture, such as the low creaminess and consistency; Regardless of the use of chia seed, which could have increased the consistency, the use of milk, which has a higher concentration of water, caused a decrease in the consistency of the clot. The values obtained in the proximate analysis of the fermented milk beverage formulations are shown in Table 6.

Table 5. The statistical description of the parameters evaluated in the sensory analysis of fermented milk drinks

Parameter		E4	E8	p-value*
Color	median (IQI)	7 (2)	7 (2)	0.494
	Average (SD)	6.85 (1.38)	6.89 (1.66)	
	Min – Max	3 – 9	1 – 9	
Aroma	median (IQI)	7 (3)	7 (3)	0.775
	Average (SD)	6.38 (1.94)	6.34 (1.88)	
	Min – Max	1 – 9	1 – 9	
Flavor	median (IQI)	7 (2)	7 (2)	0.746
	Average (SD)	6.84 (1.78)	6.82 (1.84)	
	Min – Max	2 – 9	2 – 9	
Texture	median (IQI)	7 (2)	7 (2)	0.582
	Average (SD)	6.58 (1.93)	6.82 (1.84)	
	Min – Max	1 – 9	1 – 9	
Global acceptance	median (IQI)	7 (2)	7 (2)	0.390
	Average (SD)	6.9 (1.7)	7.01 (1.73)	
	Min – Max	2 – 9	2 – 9	
Purchase intention	median (IQI)	4 (1)	4 (2)	0.729
	Average (SD)	3.46 (1.21)	3.55 (1.28)	
	Min – Max	1 – 5	1 – 5	

*Kruskal-Walls test ($p < 0.05$); IQI: Interqualitative Interval SD: Standard deviation; Min: Minimum value; Max: Maximum value. E4=test 4; E8=test 8.

Table 6. Centesimal composition and energy value of the fermented milk beverage formulations

Parameter	Formulation	
	E4 (70% X1 + 29% X2 + 1% X3)	E8 (70% X1 + 28% X2 + 2% X3)
Moisture (%)	$74.21^{a} \pm 3.746$	$74,34^{a} \pm 0.453$
Ash (%)	$0.42^{a} \pm 0.012$	$0,55^{b} \pm 0.032$
Protein (%)	$2.93^{a} \pm 0.075$	$2,99^{a} \pm 0.180$
Milk fat (%)	$1.47^{a} \pm 0.047$	$0,93^{b} \pm 0.047$
Carbohydrates (%)	$20.97^{a} \pm 0.87$	$21,19^{a} \pm 0.97$
Energy value (Kcal)	$108.83^{a} \pm 0.17$	$105,09^{a} \pm 0.13$

* t-Student test ($p<0.05$). E4=test 4, E8=test 8, X1: Dairy base, X2: Syrup, X3: Chia seeds. g = gram; % - percentage. Means followed by the same letter in the lines do not differ from each other.

The values found for the moisture content were similar ($p<0.05$) in the two treatments analyzed. The high humidity and water activity (table 3) obtained in the tested formulations favor microbial multiplication and deterioration. Therefore, these beverages must be stored under refrigeration. The substitution of milk for whey in the dairy beverage favors a higher moisture content due to the reduction of total solids and consequent increase in the amount of water in the formulation [29]. However, despite using 70% of whey to prepare the dairy base used in the preparation of the formulations, the fermented milk drink presented moisture averages closer to those of milk than those of whey. The acerola syrup and chia seed possibly interfered so that the moisture observed in the ready-made fermented milk drink was lower than that of the milk base (Table 2).

It can be observed that there was a significant difference ($p<0.05$) in the formulations regarding ash

content. The fact that treatment E4 has a lower percentage of ash can be explained by the fact that the formulation has the highest rate of the dairy base, and this ingredient has a low percentage of ash (0.06%). No significant difference ($p<0.05$) was observed between the formulations regarding the protein value. All formulations presented levels of protein of dairy origin higher than the minimum recommended by the legislation, which establishes a minimum of 1g/100g of protein of dairy origin in a fermented milk drink with additions [1]. The values found in this research can be explained by the protein value found in the milk base (Table 2).

In this research, adding chia seed to the tested formulations did not cause a considerable increase in the protein values in the milk drink, even though the seed presented a substantial protein value (Table 2). There was a statistically significant difference ($p<0.05$) between formulations E4 and E8 for values of milk fat. Therefore, the possible explanation for the E4 formulation having a higher milk fat content could be that this formulation has the highest percentage of dairy base, and this ingredient has a higher rate of fat than the syrup (Table 2).

The chia used in this experiment had a lipid content of 25.49% (Table 2). The addition of chia seed did not influence the increased fat content of the tested beverage. Chia seed is rich in omega 3, corresponding to 60.35 to 64.35% of total fatty acids. No significant differences ($p<0.05$) were found between the carbohydrate values. According to [30], the higher the whey content, rich in lactose, sugar, and fructooligosaccharides, the higher the carbohydrate content. Therefore, the percentage of whey (70%) used in the preparation of the milk base and the percentage of syrup can explain the carbohydrate values found in the formulations tested in this research.

There were no significant differences ($p<0.05$) between the results found for the energy value. Therefore, the product's energy value can increase due to the addition of whey and the amount of sugar added during the product preparation [31]. The energetic values found in this research can be explained by analyzing the percentage values of whey and syrup used in the formulation of this drink. The results of the ascorbic acid analysis of the product are described in Table 7.

Table 7. Ascorbic acid (mg/100g) of fermented dairy drinks during 21 days of refrigerated storage (4.0°C)

Parameter	Formulation	Time (in days)					
		0	7	14	21	Average	CV%
Ascorbic acid	E4	$372.9^{aA}\pm5.5$	$345.7^{Ab}\pm8.0$	$241.8^{aC}\pm6.3$	$222.2^{aD}\pm2.8$	295.6	41.1%
	E8	$418.1^{bA}\pm0.3$	$366.8^{bB}\pm7.4$	$318.6^{bC}\pm14.4$	$308.1^{bC}\pm3.8$	352.9	
Average		395.5	356.32	280.24	265.17		

* t-Student test ($p<0.05$). E4=test 4, E8=test 8. Averages followed by different lowercase letters in the same column, for the same time, differ from each other. Means followed by different capital letters on the same line, for the same treatment, differ from each other.

Significant differences ($p<0.05$) were found between treatments and at all analysis times. Treatment E8 had higher levels of ascorbic acid, which can be explained by the fact that this treatment had a higher percentage of syrup containing acerola pulp. The statistical analysis performed between the analysis times showed a significant difference ($p<0.05$) between times 0, 7, 14, and 21 in treatment E4 and between times 0, 7, and 14 in treatment E8. However, there was no significant difference between times 14 and 21 in treatment E8.

The legislation's minimum value standardized for acerola pulp is 800 (mg/100g) of ascorbic acid [24]. The daily intake recommended by the legislation is 45 mg for children aged 4 to 10 years and 60 mg for adults [32]. From this point, it can be said that the ascorbic acid values found in the developed formulations are nutritionally significant and sufficient to benefit the consumer's health. The results of the β-carotene analysis of the product are described in Table 8.

A significant difference ($p<0.05$) was observed between the two trials. The E4 trial has a higher percentage of syrup, and this difference between the formulations may have caused a significant difference in the β-carotene values. Based on these data, it can be said that the value is sufficient and meets the expectations that the fermented milk drink is a source of this bioactive compound, as it is within the value found *in natura* (4.0 to 25.8 μg/g), despite the processing passed by the acerola pulp during the formulation of the fermented milk drink.

Table 8. β-carotene (µg/g) of fermented dairy drinks.

Parameter	Formulation	
	E4 (70% X1 + 29% X2 + 1% X3)	E8 (70% X1 + 28% X2 + 2% X3)
β-caroteno (µg/g)	12.33^{a}±0.737	8.19^{b}±0.214

* t-Student test (p<0.05).: . E4=test 4; E8=test 8. X1: Dairy base; X2: Syrup; X3: Chia seeds. g = gram. µg= microgram. Averages followed by different letters in the lines differ from each other.

Conclusion

Fermented dairy drinks prepared with a whey milk base added with chia seed and flavored with acerola syrup showed stability during storage, with satisfactory results for physicochemical and microbiological parameters. There was cell viability of *L. acidophilus,* and there was no viability of *Bifidobacterium* in the storage period of 21 days. The sensory evaluation showed good sensory acceptability and purchase intent; the product also proved to be a source of ascorbic acid and β-carotene. Based on this, the formulated dairy drinks are considered a viable and low-cost alternative for the food industry, as it is a way to add value to whey, avoiding its disposal and pollution of the environment and the differential of the inclusion of the seed of chia and acerola pulp, as they are foods rich in nutrients and beneficial to health.

Conflict of interests

The authors declare no conflict of interest

Author contributions

Conceptualization: J. A. Costa, R. M. Carneiro and M. M. G. P. Nóbrega; *Data curation:* J. A. Costa, R. M. Carneiro and M. M. G. P. Nóbrega; *Formal analysis*: J. A. Costa, R. M. Carneiro and M. M. G. P. Nóbrega; *Methodology*: J. A. Costa, J. T. O. Santos, R. G. A. Bacelar and D. S. N. Silva; *Writing (Original Draft):* J. A. Costa, R. M. Carneiro, J. T. O. Santos, R. G. A. Bacelar and D. S. N. Silva; *Writing (review and editing):* J. A. Costa, M. M. G. P. Nóbrega, R. G. A. Bacelar and M. C. S. Muratori; *Supervision:* M. M. G. P. Nóbrega and M. C. S. Muratori.

References

1. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa n.16, de 23 de agosto de 2005a. Aprova o regulamento técnico de identidade e qualidade de bebida láctea. Diário Oficial da República Federativa do Brasil, Brasília, 24 ago.2005a. Seção 1, p.7.

2. Moro MF, Weise AD. Produção mais limpa como alternativa para o gerenciamento de resíduos em laticínios. Revista Dellos Desarrollo Local Sostenible. 2016; 9(27): 1-20.

3. Ma Y, Liu J, Shi H, Yu LL. Isolation and characterization of anti-inflammatory peptides derived from whey protein. Journal of Dairy Science. 2016: 99(9). Available from: https://doi.org/10.3168/jds.2016-11186

4. Wijayanti HB, Bansal N, Deeth HC. Stability of Whey Proteins during Thermal Processing: A Review. Comprehensive Reviews in Food Science and Food Safety. 2014, 13. Available from: https://doi.org/10.1111/1541-4337.12105

5. De Paula JCJ, Almeida FA, Pinto MS, Rodrigues TF, Sobral D, Machado GM. Aproveitamento de soro de queijo de coalho na elaboração de bebida láctea fermentada. Rev. Inst. Latic. Cândido Tostes. 2012. 388(67): 25-33. Available from: https://doi.org/10.5935/2238-6416.20120061

6. Siqueira AMO, Machado ECL, Stamford TLM. Bebidas lácteas com soro de queijo e frutas. Ciência Rural. 2013; 43(9): 1693-1700. Available from: https://doi.org/10.1590/S0103-84782013000900025

7. Malegori JC, Grassi S, Marques EJN, Freitas ST, Casiraghi E. Vitamin C distribution in acerola fruit by near infrared -hyperspectral imaging. Journal of Spectral Imaging. 2016; 5(1): 1-4. Available from: https://doi.org/10.1255/jsi.2016.a6

8. Segura-Campos MR., Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. Chemical and functional properties of chia seed (*Salvia hispanica* L.) Gum. International Journal of Food Science, 2014: Article ID 241053. Available from: https://doi.org/10.1155/2014/241053

9. Alcântara MA, Lima AEA, Braga, ALM, Tonon RV, Galdeano MC, Mattos MC, Brígida AIS, Rosenhaim R, Santos NA, Cordeiro AMTM. Influence of the emulsion homogenization method on the stability of chia oil microencapsulated by spray drying. Powder Technology. 2019: 354, 877–885. Available from: https://doi.org/10.1016/j.powtec.2019.06.026

10. BRASIL. Agência Nacional de Vigilância Sanitária. Portaria Nº 33, de 13 de janeiro de 1998. Adota os valores constantes das seguintes Tabelas do anexo desta portaria, como níveis de IDR para as vitaminas, minerais e proteínas. Diário Oficial da União. 16 de janeiro de 1998, Seção I-E, página 5.

11. IAL - Instituto Adolfo Lutz (São Paulo) (Org.). Métodos físico-químicos para análise de alimentos. São Paulo: Instituto Adolfo Lutz, 2008; 4: 279-320.

12. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Determinação de Nitrogênio Total em Leite e Derivados Lácteos pelo método de Micro-kjedahl. 2013. Available from: < http://www.agricultura.gov.br/assuntos/laboratorios/legislacoes-e-metodos/arquivos-metodos-da-area-poa-iqa/met-poa-11-02-proteinas.pdf> Acessed March, 2020.

13. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de defesa Agropecuária (DISPOA). Instrução Normativa nº 62, de 26 de agosto de 2003. Oficializa os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Diário Oficial da União, Brasilia-DF, 26 de agosto de 2003, Seção 1, p.14, 2003.

14. Farnsworth JP, Li J, Hendricks GM, Guo MR. Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Ruminant Research. 2006; 65(1-2):113-121. Available from: https://doi.org/10.1016/j.smallrumres.2005.05.036

15. AOAC. Association of Official Analytical Chemists. Official methods of analysis. 16ª ed.,

Gaitherburg: Published by AOAC International. Washington DC, 2002.

16. Tsiaka T, Zoumpoulakis P, Sinanoglou VJ, Makris C, Heropoulos GA, Calokerinos AC. Response surface methodology toward the optimization of high-energy carotenoid extraction from *Aristeus antennatus* shrimp. Analytica chimica acta. 2015; 877: 100-110. Available from: https://doi.org/10.1016/j.aca.2015.03.051

17. Li Y, Tixier ASF, Tomao V, Cravotto G, Chemat F. Green ultrasound-assisted extraction of carotenoids based on the biorefinery concept using sunflower oil as an alternative solvent. Ultrasonics Sonochemistry. 2013; 20(1):12-18. Available from: https://doi.org/10.1016/j.ultsonch.2012.07.005

18. BRASIL. Instrução Normativa N°. 60, de 26 de dezembro de 2019. Lista de Padrões Microbiológicos para Alimentos Prontos para Oferta ao Consumidor. Diário Oficial da União. Brasília, 26 de dezembro de 2019, nº. 249, Seção 1, p. 133. 2019.

19. Lima KGC, Kruger MF, Behrens J, Destro MT, Landgraf M, Franco BDGM. Evaluation of culture media for enumeration of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium animalisin* the presence of *Lactobacillus delbrueckii* subsp bulgaricus and *Streptococcus thermophilus*. LWT –Food Science and Technology. 2009; 42(2) :491-495. Available from: https://doi.org/10.1016/j.lwt.2008.08.011

20. Vinderola CG, Reinheimer JA. Culture media for the enumeration of *Bififobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. International Dairy Journal, Barking. 1999; 9(8): 497–505. Available from: https://doi.org/10.1016/S0958-6946(99)00120-X

21. Dutcosky SD. Análise sensorial de alimentos. Revista e Ampliada. Curitiba: Champagnat, 2013; 4: 531.

22. Ayerza R, Coates W. Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). Industrial Crops and Products. 2011; 34(2): 1366-1371. Available from: https://doi.org/10.1016/j.indcrop.2010.12.007

23. Aldrigue ML, Madruga MS, Fioreze R, Lima AWO, Sousa CP. Aspecto da ciência e tecnologia de alimentos. João Pessoa. 2002; 1: 198.

24. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa N° 37, de 1 de outubro de 2018. Estabelece os parâmetros analíticos de suco e de polpa de frutas e a listagem das frutas e demais quesitos complementares aos padrões de identidade e qualidade. Diário Oficial da União, Brasília, 8 out. 2018. Seção 1, p.23, 2018.

25. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº 80, de 13 de agosto de 2020. Aprova o Regulamento Técnico que fixa os padrões de Identidade e Qualidade para o soro de leite e o soro de leite ácido. Diário Oficial da União, Brasilia-DF, 13 de agosto de 2020, Seção 1, p.2, 2020.

26. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa N° 46, de 23 de outubro de 2007. Aprova o Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. Diário Oficial da República Federativa do Brasil, Brasília, 24 out. 2007. Seção 1, p.4, 2007.

27. Poppi FA, Costa MR, Rensis CMVB, Sivieri K. Soro de Leite e Suas Proteínas: Composição e Atividade Funcional. Ciênc. Biol. Saúde. 2010; 12(2): 31-37.

28. Ferreira Neto CJ, Figueirêdo, RMF, Queiroz QJM. Avaliação sensorial e da atividade de água em farinhas de mandioca temperadas. Ciênc. agrotec. 2005; 29(4): 795-802. Available from: https://doi.org/10.1590/S1413-70542005000400011

29. Guedes, AFLM, Machado ECL, Fonseca MC, Andrade SAC, Stamford TLM. Aproveitamento de soro lácteo na formulação de bebidas com frutas e hortaliças. Arq. Bras. Med. Vet. Zootec. 2013; 65(4): 1231-1238. Available from: https://doi.org/10.1590/S0102-09352013000400040

30. Thamer KG, Penna ALB. Caracterização de bebidas lácteas funcionais fermentadas por probióticos e acrescidas de prebióticos. Ciênc. Tecnol. Aliment. 2006: 26(3): 589-595. Available from: https://doi.org/10.1590/S0101-20612006000300017

31. Miranda OC, Fonseca PL, Ponce I, Cedeño C, Rivero LS, Vázquez LM. Elaboración de una bebida fermentada a partir del suero de queso. características distintivas y control de calidad. Rev Cubana Aliment Nutr. 2007; 17(2):103-108.

32. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Resolução de diretoria colegiada – RDC nº 269, de 22 de setembro de 2005. Aprova o Regulamento técnico sobre a ingestão diária recomendada (IDR) de proteína, vitaminas e minerais. Diário Oficial da República Federativa do Brasil nº 184, Brasília, 23 set.2005b.