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Bebida probiótica de leite fermentado adicionado de polpa de caju (*Anacardium occidentale*): formulação, características físico-químicas, microbiológicas e sensoriais

Probiotic fermented milk drink added with cashew pulp (*Anacardium occidentale*): formulation, physicochemical, microbiological and sensory characteristics

Bebida de leche fermentada probiótica añadida con pulpa de anacardo *(Anacardium occidentale*): formulación, características fisicoquímicas, microbiológicas y sensoriales

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Resumo

Os aspectos de saúde associados aos produtos lácteos fermentados resultaram no aumento do consumo desta bebida. Foram produzidas três bebidas fermentadas adicionadas à polpa de caju e diferentes concentrações de soro (10, 20 e 30%), sendo armazenadas por um período de 28 dias e avaliadas quanto às suas características físico-químicas, microbiológicas e sensoriais. Cinquenta e dois provadores avaliaram a aceitação das bebidas usando escalas hedônicas híbridas de 9 pontos. Verificou-se que quanto menor a proporção de soro de leite utilizada nas bebidas, maiores os valores de sólidos totais, proteínas, gorduras e calorias (p <0,05). Em relação aos valores de cinzas, não foram observadas diferenças significativas (p> 0,05). Os valores de pH das bebidas não diferiram quando avaliados no mesmo dia de armazenamento (p> 0,05). Após 7, 14, 21 e 28 dias de armazenamento, foram observadas diferenças (p <0,05) em relação aos valores de acidez, exceto para o tempo 0. As bebidas apresentaram viabilidade probiótica durante o período de armazenamento, sendo a contagem acima do mínimo recomendado pela corrente legislação Brasileira. Embora as bebidas adicionadas com 10% de soro de leite apresentem um melhor índice de aceitabilidade, as outras bebidas desenvolvidas neste estudo apresentaram boa aceitação pelos provadores. As bebidas probióticas aromatizadas com caju foram viáveis nutricional e tecnologicamente e apresentaram estabilidade físico-química e microbiológica durante o armazenamento refrigerado por 28 dias.

Palavras-chave: Leite; Produtos lácteos; Fruta tropical; Alimento funcional.

Abstract

The health aspects associated with fermented milk products have resulted in increased consumption of this beverage. Three fermented beverages added with cashew pulp and different concentrations of whey (10, 20 and 30 %) were produced, being stored for a period of 28 days and evaluated regarding their physicochemical, microbiological and sensory characteristics. Fifty-two tasters evaluated acceptance of the beverages using hybrid 9-point hedonic scales. It was found that the lower the proportion of whey used in the beverages, the higher the total solids, proteins, fat and caloric values (p<0.05). Regarding ash values, no significant differences were observed (p>0.05). Beverages pH values did not differ when evaluated on the same day of storage (p>0.05). After 7, 14, 21 and 28 days of storage, differences were observed (p <0.05) regarding acidity values, except for the time 0. The beverages presented probiotic viability during the storage period, being the count above the minimum recommended by the current Brazilian legislation. Even though the beverages added with 10 % of whey presented a better acceptability index, the other beverages developed in this study presented good acceptance by the tasters. The probiotic beverages flavored with cashew fruit were nutritionally and technologically viable and presented physicochemical and microbiological stability during refrigerated storage for 28 days.

Keywords: Milk; Dairy products; Tropical fruit; Functional food.

Resumen

Los aspectos de salud asociados con los productos lácteos fermentados dieron como resultado un mayor consumo de esta bebida. Se agregaron tres bebidas fermentadas a la pulpa de anacardo y diferentes concentraciones de suero (10, 20 y 30%), se almacenaron durante un período de 28 días y se evaluaron sus características físico-químicas, microbiológicas y sensoriales. Cincuenta y dos catadores evaluaron la aceptación de bebidas usando escalas hedónicas híbridas de 9 puntos. Se encontró que cuanto menor es la proporción de suero utilizado en las bebidas, mayores son los valores de sólidos totales, proteínas, grasas y calorías ($p < 0.05$). En cuanto a los valores de cenizas, no se observaron diferencias significativas ($p > 0.05$). Los valores de pH de las bebidas no fueron diferentes cuando se evaluaron el mismo día de almacenamiento ($p > 0.05$). Después de 7, 14, 21 y 28 días de almacenamiento, se observaron diferencias ($p < 0.05$) en relación con los valores de acidez, excepto el tiempo 0. Las bebidas mostraron viabilidad probiótica durante el período de almacenamiento, siendo el recuento anterior El mínimo recomendado por la legislación brasileña vigente. Aunque las bebidas agregadas con un 10% de suero tienen un mejor índice de aceptabilidad, las otras bebidas desarrolladas en este estudio mostraron una buena aceptación por parte de los catadores. Las bebidas probióticas aromatizadas con anacardos eran nutricional y tecnológicamente viables y mostraron estabilidad físico-química y microbiológica durante el almacenamiento refrigerado durante 28 días.

Palabras clave: Leche; Productos lácteos; Fruta tropical; Comida functional.

1. Introduction

Functional foods, in addition to providing basic nutrition, promote health through mechanisms not foreseen in conventional nutrition, restricted to the promotion of health rather than curing diseases (Nazir et al., 2019). Among functional food components are fermented milk and probiotics, which are living microorganisms that beneficially affect the human intestinal microbial flora (Amara & Shilb, 2015). Milk drink is defined as a dairy product resulting from the mixture of milk at a minimum of 51% (wt/wt) and whey, with or without addition of yeast extract or other dairy products (Brazil 2005). As an important vehicle of probiotics, other health aspects are associated with fermented milk products, which resulted in increased consumption of this beverage (Nazir et al., 2019).

The partial replacement of milk by whey in milk drink formulations can be a rational alternative to the incorporation of this secondary product obtained from curd during cheese-making process or during casein production (Saad, Da Cruz, & Faria, 2011). Athought whey products show important functional properties for human health (Thamer & Penna, 2006). It may represent a major problem for the environment if not properly disposed since its Biological Oxygen Demand (BOD) is greater than 30,000 mg $O_2 \cdot l^{-1}$ (Ahmad et al., 2019).

Often, in addition to whey, milk drinks are flavored with food sources including tropical fruits. Brazil offers a wide variety of fruits with different tastes that can be used to flavor milk drinks. Among the fruits with the greatest potential for exploitation is cashew fruit (Rufino et al, 2010). Cashew fruit (*Anacardium occidentale*), from the indigenous word "acaiu", which in Tupi means "nut that produces itself" is easily found in the North and Northeast regions of Brazil (Brazil, 2015). Large population segments of the Brazilian Northeast have the cashew as an important source of funds, however, only a small amount of its crops is used for fresh or industrially consumption, being most of it wasted in the field itself (Menezes & Alves, 1995). Thus, the aim of the present study was to formulate and evaluate physicochemical, microbiological and sensorial characteristics of a probiotic fermented milk drink added with cashew pulp and whey which are rich in several nutrients and until now, underexploited by the local industry.

2. Material and Methods

2.1 Materials

The raw materials used in the production of the milk drink were: pasteurized milk donated by IFRN- Campus Ipanguaçu (Rio Grande do Norte, Brazil), whey obtained from cheese production (Estrela®, Ares, RN), Dairy Stabilizer (Rica NATA®), cashew fruit pulp donated by the Cooperative of Energy and Rural Development of Seridó and two lyophilized lactic cultures - a probiotic one containing Lactobacillus acidophilus (LYO 50 DCU – S,

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FERMENTECH®, Tatuapé, SP) and a traditional for yogurt containing Streptococcus thermophilus and Lactobacillus delbrueckii spp. bulgaricus (YO-MIX 499 LYO 100 DCU, FERMENTECH®, Tatuapé, SP)

All reagents used were of analytical grade. Solutions and culture media were properly prepared and, if necessary, standardized.

2.2 Preparation of dairy cultures

The envelope containing the lyophilized Lactobacillus acidophilus probiotic culture was opened and dissolved in 1 L of UHT (Ultra High Temperature) milk in a laminar flow hood. One mL aliquots were kept in sterile tubes and stored at -20°C. For milk beverage, 50 ml of the aliquot were used per 50 L of milk, following manufacturer's instructions. In the same way, the traditional culture was aliquoted for milk drink.

2.3 Pasteurization of cashew pulp

Cashew pulp was transported in isothermal packaging to the IFRN's Cheese Technological Center of Seridó where it was subjected to pasteurization. Seven and a half kilos of cashew pulp (2.5 kg for each formulation) were pasteurized, with addition of 1.5 kg of Estrela® brand sugar. The mixture was heated at 85°C for 20 minutes and taken to cooling before to be used in the production of the milk drink.

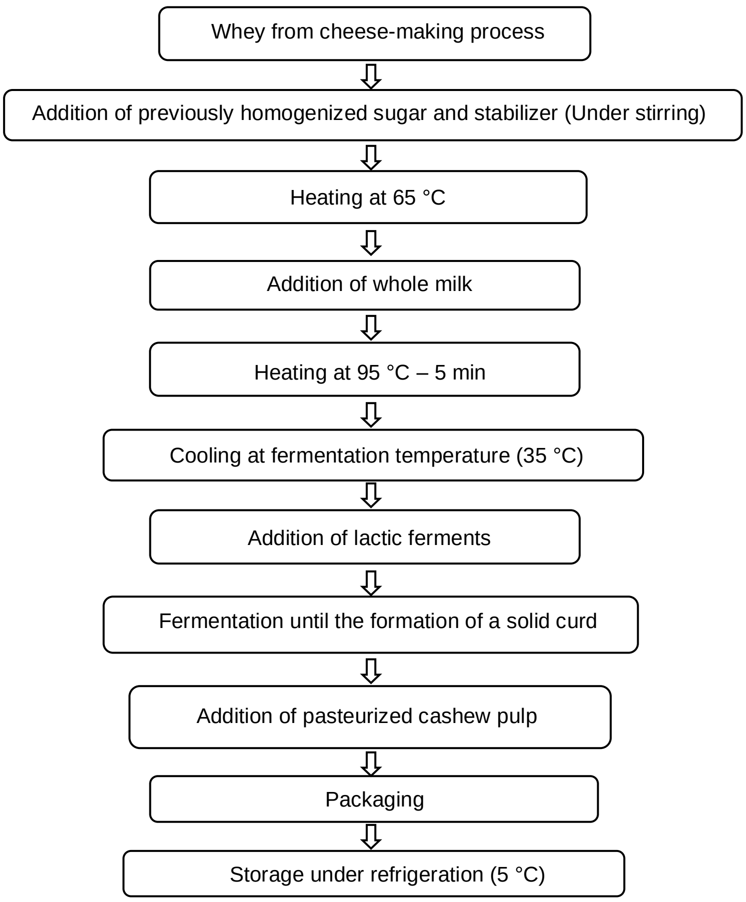
2.4 Obtaining whey

Whey was obtained from curd cheese production. To produce curd cheese, 150 L of filtered milk added with 60 ml of calcium chloride (Proregi® - Food Additives, Rio Pomba, MG) followed pasteurization was used. Coagulation was performed at 32 to 34°C using 3.5 ml of coagulant (Albamax®, 1800 IMCU, Caglificio Clerici SpA®, Campinas,SP). After the time required for coagulation, the curd was slowly cut to small-sized grains. The first mixture was set at 20 minutes, and then the second one was started with slow mass heating and indirect steam up to 50°C. The mixing process was continued until reaching the point. The whey, approximately 80% of the milk volume in the tank, was then removed for fermented milk drink production.

2.5 Stages of the milk drink production

The production of the milk drinks from milk and whey was based on the methodology proposed by Almeida, Bonassi and Roca (2001) with modifications. The milk drinks developed had the following ingredients: whey obtained from cheese-making process, whole milk, sugar, stabilizer (starch), lactic ferments (traditional and probiotics) and pasteurized cashew pulp. The beverage preparation steps are represented in Figure 1.

Figure 1. Preparation steps of the milk drink.



Source: Authors.

First, the milk was collected in bulk using an isothermal truck and the whey was removed directly from the curd cheese tank, as early described.

Three formulations containing different concentrations of 10, 20 and 30% of whey were used to prepare the fermented milk drinks, being milk and whey the only variable ingredients. Thus, the milk drink formulations were named as MD10 (10% whey), MD20

(20% whey) and MD30 (30% whey). The amount of the other ingredients was the same for the three formulations as shown in Table 1.

Table 1 - Fermented milk drink ingredients wt/v (%).

Ingredients	Milk Drinks		
	MD10	MD20	MD30
Sugar	10	10	10
Starch	1	1	1
Cashew fruit pulp	5	5	5
Whey	10	20	30
Milk	74	64	54

Source: Authors.

Solid ingredients were premixed and dissolved in whey using an industrial blender. The mixture was heated up to 65°C. Then, the whole milk was added and pasteurized by heating at 95°C for 5 minutes, followed by cooling to the fermentation temperature (≥ 37°C), growth temperature of the microorganisms used. Lactic ferments (traditional and probiotics) were added, being previously prepared according to the manufacturer's recommendation, as described in section 2.2.

Then, 2.5kg of pasteurized cashew pulp was added to each formulation. The milk drink was packed in 1 l plastic bottles and were stored under refrigeration at 5ºC. For sample collection, the vials were homogenized, and the aliquots required for analysis were collected.

2.6 Physicochemical analyses of milk, whey and cashew pulp

The whole milk and whey used to produce the milk drinks were analyzed for acidity (Dornic degrees), density and fat content according to the Gerber Method, cryoscopy (MK 540 Flex, MILKLAB, Itaquera) and pH meter (DM-22 DIGIMED®, São Paulo,SP). The pasteurized cashew pulp was analyzed for acidity and pH. All analyses were made according to IAL (2008).

2.7 Nutritional composition of milk drinks

Analysis of total solids, ashes, lipids and protein contents were performed only on the first day of storage. All analyses were performed according to Instituto Adolfo Lutz [IAL] (2008). Total solids (%wt/wt) were assessed by drying the samples to a constant weight and ash content, using the muffle furnace under heating conditions up to 550°C for approximately 3 hours. Fat content was determined using a Gerber butyrometer. Proteins were estimated by measuring the nitrogen content according to the principle of the Kjeldahl method and multiplying it by the conversion factor of 6.38.

Total carbohydrate values were obtained by difference. The caloric values of the samples were calculated using the Atwater factors: proteins = 4.0 (kcal·g-1), carbohydrates = 4.0 (kcal·g-1) and lipids = 9.0 (kcal·g-1) (De Angelis, 1997).

All analyses were performed in triplicate.

2.8 Physicochemical and microbiological analyses

The milk drinks produced were subjected to physicochemical analysis such as pH and titratable acidity at 0, 7, 14, 21 and 28 days. The pH was determined by using a calibrated research benchtop DM-22 DIGIMED® (São Paulo/SP). The titratable acidity technique was based on the potentiometric titration principle using a pH turning point of 8.3. The results were expressed as percentages of lactic acid (IAL, 2008).

The formulations were evaluated at 0, 14 and 28 days of storage under refrigeration for total and faecal coliform analysis following the Most Likely Number (MLN) method. Molds and yeasts were assayed using the standard plate count (SPC) agar method, which determines the number of colony-forming units (CFU) through a surface plate. All analyses were performed according to Silva, Junqueira and Silveira (2010).

The fermented milk were evaluated for the viability of probiotic bacteria by using a modified MRS culture medium with the addition of 0.15 % (wt/v) bile (MRS-Bile) for 0.1 mL of L. acidophilus count (Vinderola & Reinheimer, 2000). Serial dilutions of the fermented beverages and plated dilutions and incubated under aerobiosis at 37°C for 72 hours and were done in triplicate. After this period, the total probiotic bacteria count (log CFU·ml-1) was determined. All analyses were performed after 1, 14 and 28 days of storage.

2.9 Sensory analysis

Sensory analysis was performed according to the procedures described by IAL (2008). Prior execution the present study was approved by the Ethics Committee for Research Involving Human Subjects of the Potiguar University (CAAE 18754913.3.0000.5296). Only samples in accordance with the legal standards were used in the experiments.

The acceptability evaluation was performed after 7 days of the product's production. Sensory analysis was performed with 52 untrained testers, aged 18-50 years of both sexes. The participants signed an Informed Consent Form before starting the analysis.

Each taster received 50 ml of each beverages (4 ± 1°C) in plastic cups randomly encoded with three-digit numbers, along with water at room temperature, crackers and the acceptability analysis form.

Color, aroma, flavor, texture and overall quality attributes were evaluated using a hedonic scale of nine points, containing the terms as described above, ranging between "I liked very much" and "I disliked very much". At the end of the test, an optional response field was left for general comments regarding the product.

2.10 Statistical analyses

All data were submitted to the Shapiro-Wilk and Bartlett tests to confirm the symmetry of the sample distribution and homoscedasticity, respectively. The values of moisture, total solids, lipids, proteins, carbohydrates, acidity and all attributes of the sensory tests were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with a p-value <0.05. Statistical Analysis Software (SAS Institute Inc., Cary, NC) was used.

3. Results and Discussion

Physicochemical analysis results of the raw materials used in the fermented milk production are shown in Table 2.

Table 2 - Physicochemical characteristics of pasteurized milk and whey used in the production of the probiotic milk drinks.

	Milk	**Whey**
Density ($g \cdot l^{-1}$)	1033.5 ± 0.3^a	1028.3 ± 0.5^a
Acidity (10 $g \cdot l^{-1}$)	0.2 ± 1.0^a	0.1 ± 1.0^b
Cryoscopy (ºC)	0.5 ± 0.0^a	0.5 ± 0.0^a
Fat content (%)	3.2 ± 0.0^a	0.7 ± 0.1^b

[a-b] Means with different letters in the same column are significantly different (p <0.05). Expressed as mean ± SD. Values in the same column with different superscript letters are significantly different (p<0.05, Tukey's test was used for post-hoc comparison).
Source: Authors.

It was observed that the values of acidity, freezing point and fat content for the pasteurized milk used were within the range established by the Brazilian current legislation, which is 0.14 to 0.18 for acidity, maximum of -0.512ºC for freezing point and 3.0% fat content for standardized milk (Brazil, 2002). When using fresh whey obtained in the process of cheese production, the legislation requires a maximum acidity of 0.13, in the present study, the acidity observed was 0.11 (Damin, Sivieri, & Lannes, 2009).

Considering fat content and acidity, it was observed that milk showed high values (p <0.05) when compared to whey. According to Almeida et al. (2001) whey lipid content could be five times lower than milk's content. Otero, Rodriguez, Camejo, & Cardoso (1995) research with fermented milk produced with whey or water found that the average fat content for whey-based drinks were slightly higher than those produced with water. Regarding acidity, Oliveira, Cortez, Freitas, & Franco (2006) states that milk consumes more NaOH spent in titration for having higher levels of casein, an acidic protein, and, consequently, promotes an increase in final acidity.

The cashew pulp used presented values of 0.49 for total acidity (expressed as citric acid per 100 g) and pH of 4.08, values that are within the current legislation, which establishes minimum acidity of 0.30 and maximum pH of 4.6 (Brazil, 2000).

Table 3 shows the results of the physicochemical analyses and mean caloric values for the MD10, MD20 and MD30 milk drinks formulations.

Table 3 - Physicochemical parameters of probiotic fermented milk drinks added with cashew pulp.

Variables	**Milk drinks**		
	MD10	MD20	MD30
Total solids (% m/m)	20.13 ± 0.00[a]	19.40 ± 0.10[b]	18.13 ± 0.06[c]
Fat (% m/m)	2.55 ± 0.01[a]	2.13 ± 0.06[b]	1.57 ± 0.06[c]
Protein (% m/m)	2.46 ± 0.01[a]	2.24 ± 0.01[b]	1.98 ± 0.01[c]
Ash (% m/m)	0.63 ± 0.01[a]	0.61 ± 0.01[a]	0.62 ± 0.01[a]
Total carbohydrates (% m/m)	14.49 ± 0.06[a]	14.42 ± 0.15[a]	13.65 ± 0.05[b]
Total caloric content (kcal/100g)	90.78 ± 0.25[a]	85.84± 0.24[b]	79.53 ± 0.22[c]

Expressed as mean ± SD.Values in the same column with different superscript letters are significantly different ($p<0.05$, Tukey's test was used for post-hoc comparison).
Source: Authors.

It can be observed that there was statistical difference ($p <0.05$) for total solids among the samples. The milk drink with the lowest proportion of whey (MD10) presented higher total solids content. These data corroborate with the data observed in previous scientific reports (Almeida et al. 2001; Santos, Costa, Fontan, Fontan, & Bonomo, 2008; Cunha, Ilha, Amboni, Barreto, & Castro, 2009) which show that the proportion of whey increased when the total solids content decrease.

In MD30 milk drink, the lowest fat and protein contents were observed ($p <0.05$), probably due to the lower concentration of these components in whey compared to milk. The reduction in fat content, with larger proportions of whey were also observed by other authors (Almeida et al. 2001; Santos et al., 2008; Cunha et al., 2009) possibly due to the low fat observed in whey. Although milk fat is an important ingredient for the physiological and sensory aspects of dairy products, its consumption in large quantities is related to the increase of obesity and coronary diseases (Dewhurst, Shingfield, Lee, & Scollan, 2006). Thus, Tsuchiya, Almiron-Roig, Liuch, Guyonnet, & Drewnowski (2006) reinforce the importance of consuming fermented milk with low lipid content.

The milk drink produced with the lowest proportion of whey (MD10) showed the highest protein content. Similar results were observed by Cunha et al. (2009) and Reis (2011) with milk drinks. Almeida et al. (2001) state that this fact is explained due to the higher protein content presented in milk and not due to the hydrolysis of whey proteins as affirmed by Cinbas & Kilic (2006) and Donkor, Henriksson, Vasiljevic, & Shah (2006). It should also be noted that all fermented milk milk drinks produced in this study have a protein content greater than 1g/100g, the minimum value required by the Brazilian legislation for fermented milk drinks added with food products and food susbstances (Brazil, 2000).

Statistical differences ($p<0.05$) were observed regarding the carbohydrate content between MD10 and MD30, as well as, MD20 and MD30 milk drinks, but there was no difference ($p>0.05$) when comparing MD10 and MD20. Almeida et al. (2001) in their study with probiotic dairy drinks did not observe carbohydrate content statistical differences in the drinks with different concentrations of whey. On the other hand, Yet, Vinderola & Reinheimer (2000) and Cunha et al. (2009) observed significant differences regarding carbohydrate levels in probiotic fermented milk drinks and probiotic milk drinks, respectively.

It can be observed in Table 2 that replacing milk with whey contributed to obtain drinks with lower calorie values ($p <0.05$). Thus, the milk drink with 30% of whey presented lower total caloric content compared to the milk drinks developed with lower concentrations of whey. Similar results were also observed by Cunha, Castro, Barreto, Benedet, & Prudêncio (2008) and Cunha et al. (2009) studying fermented milk

The pH and acidity values of the milk drinks during the 28 days of storage are shown in Table 4.

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Table 4 - Physicochemical parameters of fermented milk drinks during refrigerated storage at 5 °C.

Variables	Time (days)	Milk Drinks		
		MD10	MD20	MD30
pH	1	4.25 ± 0.03[a]	4.22 ± 0.02[a]	4.22 ± 0.03[a]
	7	4.24 ± 0.12[a]	4.21 ± 0.02[a]	4.21 ± 0.11[a]
	14	4.24 ± 0.04[a]	4.20 ± 0.08[a]	4.21 ± 0.06[a]
	21	4.21 ± 0.02[a]	4.19 ± 0.07[a]	4.20 ± 0.05[a]
	28	4.20 ± 0.02[a]	4.10 ± 0.01[b]	4.15 ± 0.01[c]
Acidity	1	0.72 ± 0.03[a]	0.68 ± 0.00[ac]	0.66 ± 0.01[bc]
	7	0.83 ± 0.01[a]	0.79 ± 0.01[b]	0.72 ± 0.00[c]
	14	0.83 ± 0.01[a]	0.78 ± 0.03[b]	0.73 ± 0.00[c]
	21	0.92 ± 0.00[a]	0.88 ± 0.01[b]	0.84 ± 0.00[c]
	28	1.00 ± 0.00[a]	0.94 ± 0.01[b]	0.89 ± 0.00[c]

[a-c] Means with different letters on the same line are significantly different (p <0.05), according to Tukey's tests. Expressed as mean ± SD.Values in the same column with different superscript letters are significantly different (p<0.05, Tukey's test was used for post-hoc comparison).
Source: Authors.

When evaluated on the same day of storage, the pH values in the milk drinks did not differ (p> 0.05), only after 28 days of storage it was observed a significant difference between the formulations.

The values observed for the formulations MD10, MD20 and MD30 during the storage period did not change the standard of the product, since the pH generally found for milk beverages is around 4.5 (Oliveira et al., 2006).

The pH values found in the three formulations were close to those found by Cunha et al. (2009) for probiotic fermented milk drinks after 28 days of storage (4.09 to 4.27) and Buriti, Freitas, Egito, & Dos Santos (2014) for probiotic milk drinks flavored with guava and soursop during 21 days of storage (4.10 to 4.20).

During the entire storage period, differences ($p<0.05$) were observed in the acidity values among the samples, exception for time 0, in which there was no statistical difference between samples MD10 and MD30, or MD20 and MD30.

According to Dave and Shan (1997) *Lactobacillus delbrueckii* ssp. *bulgaricus* produces lactic acid during refrigerated storage, a phenomenon known as post-acidification. Kailasapathy (2006) states that post-acidification of fermented milk occurs due to the maintenance of β-galactosidase activity at temperatures ranging from 0 and 5 ºC. On the other hand, other authors report that high protein contents would favor the activity of lactic acid bacteria, thus higher milk contents would be responsible for the acidification of lactic acid derivative (Raynal-Ljutovac, Gaborit, & Lauret, 2005). As observed in our study, Cunha et al. (2009) found elevated acidity in milk drinks with higher total solids content. All the values found for acidity in the milk drinks were within the established limit (0.6 to 1.5% of lactic acid) by the current Brazilian legislation (Brazil, 2005).

In the present study there was a decrease in pH values and an increase in acidity values, which were also observed in other studies analyzing milk drinks (Birollo, Reinheimer, & Vinderola, 2000; Almeida et al. 2001; Bonczar, Wszolek, & Siuta, 2002; Cunha et al., 2009; Buriti et al, 2014; Silveira et al., 2015). Regarding the results found in this study, it can be explained using whey instead of milk. Kailasapathy (2006), replacing part of the milk by whey for yoghurt production observed a decrease in pH and an increase in acidity.

Concerning microbiological analysis, the presumptive test for total and faecal coliforms was negative in all samples of the three formulations throughout the whole storage period, thus it was not necessary to perform a confirmatory test. The results found may be related to good hygienic-sanitary conditions during all the stages of processing, since the coliforms are sensitive to the use of sanitizers and detergents used in the hygienic procedures, in addition to the heat treatment used. Another unfavorable condition for the development of these microorganisms is the low pH values presented in milk drinks (Ferreira, 2005).

Regarding mold and yeast counts, no colonies growth was observed for the three formulations of fermented milk drinks throughout the whole storage period, being then in compliance with the current Brazilian legislation (Brazil, 2000).

The count of probiotics is extremely important for assessing whether the product is in accordance with the current legislation, in addition to its influence on the flavor and aroma of the product. It is observed in Table 5 that the viability of the *L. acidophilus* probiotic culture was greater than 8 log CFU·ml^{-1} in the three formulations of milk drink throughout the whole storage period. However, in all formulations a significant decrease ($p < 0.05$) in *L. acidophilus* concentration happened after the 28th day of storage. According to Macedo, Luchese, Guerra, & Barbosa (2008), this behavior may be related to several factors such as acidification of the product.

Table 5 - Viability of *L. acidophilus* in the different formulations of fermented milk MD30, MD20 and MD10 at 0, 14 and 28 days of storage at 5°C.

Probiotic strain	**Time (days)**	**Milk Drinks**		
		MD10	MD20	MD30
	0	9.05 ± 0.04^{a}	8.96 ± 0.01^{a}	9.03 ± 0.02^{a}
L. acidophilus	14	8.74 ± 0.04^{b}	8.58 ± 0.02^{b}	8.72 ± 0.03^{b}
	28	8.28 ± 0.06^{c}	8.13 ± 0.01^{c}	8.22 ± 0.02^{c}

$^{a-c}$ Means with different letters on the same line are significantly different ($p<0.05$). Expressed as mean ± SD.Values in the same column with different superscript letters are significantly different ($p<0.05$, Tukey’s test was used for post-hoc comparison).
Source: Authors.

Despite the reduction observed in the current experiment, the values obtained after 28 days of storage were within the minimum limit established by the current Brazilian legislation for probiotic milk drinks, which is 10^{6} CFU·g^{-1} or CFU·ml^{-1} of viable cells.

Buriti et al. (2014) developed a probiotic milk drink with goat milk in addition to guava and soursop pulps, finding a significant reduction in the probiotic cultures after 21 days of storage. Kongo, Gomes, & Malcata (2006) produced fermented milks also with goat milk using *Bifidobacterium animalis* and *Lactobacillus acidophilus* and observed that after 10 days of storage the values of probiotic cultures were higher than 7 log CFU·ml^{-1}. Ramos et al. (2013) analyzing the viability of *L. acidophilus* probiotic fermented milk drink flavored with caja fruit found that the microorganism count was between 11.6 and 10.2 log CFU·ml^{-1}.

Vinderola, Costa, Reinheimer, & Reinheimer, (2002) reported that the addition of fruit pulp in food products may impair the survival of certain species of probiotic microorganisms due to their high acidity and the presence of antimicrobial compounds. In the present study, even though there was a reduction in the microorganism count during the storage period, it was found that *L. acidophilus* maintained a good viability even in the presence of the cashew pulp.

When developing a new product, one of the key points is to evaluate its acceptability in order to predict its performance in the consumer market (Moscatto, Prudêncio-Ferreira, & Hauly, 2004). The results of the sensory analysis can be found in Table 6. The evaluated parameters presented differences ($p < 0.05$) between the formulations MD30 and MD20, and between MD30 and MD10, except for the aroma parameter, in which no significant differences ($p > 0.05$) were observed among all formulations.

Table 6 - Sensory evaluation values for the formulations of the fermented milk drinks MD10, MD20 and MD30 after 7 days of storage at 5 ºC.

Formulations	Color	Aroma	Flavor	Texture	Overall quality
MD10	7.67 ± 1.17^{b}	7.10 ± 1.66^{a}	7.50 ± 1.20^{b}	7.73 ± 1.36^{b}	7.65 ± 1.34^{b}
MD20	7.67 ± 1.12^{b}	6.96 ± 1.60^{a}	7.52 ± 1.51^{b}	7.62 ± 1.29^{b}	7.50 ± 1.46^{b}
MD30	7.10 ± 1.38^{a}	6.50 ± 2.17^{a}	6.37 ± 2.17^{a}	6.39 ± 2.03^{a}	6.71 ± 1.83^{a}

[a-b] Means with different letters on the same line are significantly different ($p < 0.05$). Expressed as mean ± SD.Values in the same column with different superscript letters are significantly different ($p<0.05$, Tukey's test was used for post-hoc comparison).
Source: Authors.

In general, the milk drink formulation with the highest whey content (MD30) received the lowest scores for all attributes analyzed. Similar results were also found by Castro et al. (2013) evaluating the acceptability of probiotic milk drinks with different concentrations of whey, observing that the milk drink with the highest whey concentration (80 %) had the lowest acceptability index (5.2).

In a study with chocolate flavored probiotic milk drink made with goat milk whey and prebiotics, Silveira et al. (2015) observed that milk drinks with higher concentrations of whey and prebiotics had the highest mean values for flavor and aroma attributes, a fact not observed in the present study, in which milk drinks with the higher whey concentrations presented to have lower values for all attributes analyzed.

Among the comments registered by the tasters, the most common ones were regarding the consistency and texture of the sample with the highest concentration of whey, formulation MD30, being cited the following characteristics: "thin", "not very viscous" and "not very consistent". This fact may be related to the lower casein content in the MD30 formulation, as a consequence of the high replacement levels of milk by whey. The consistency of the fermented milk drinks is directly related to the formation of casein protein gel; thus, with lower concentrations of caseins, the protein gel becomes weaker, with consequent reduction in the consistency or viscosity of the product (Amatayakul, Sherkat, & Shah, 2006).

Viscosity is a parameter that is directly related to the texture attribute in a sensory analysis, which in turn is a key factor for the consumers' choice of a product (Damin et al. 2009). Gomes et al. (2013) studying milk drinks made with milk and whey from cow's and goat's milk found significant differences ($p<0.05$) regarding uniformity, consistency and viscosity, and the beverage produced exclusively from goat milk presented lower acceptance values. The use of goat milk in the production of dairy drinks resulted in a less viscous product, possibly as a result of the protein composition of this type of milk (Vargas, Chafer, Albors, Chiralt, & Gonzalez-Martinez, 2008).

According to Teixeira, Meinert, & Barbeta (1987) for a product to be considered accepted in terms of its sensory properties, it is necessary to obtain an Acceptance Index (AI) of at least 70 %, which can be found in all formulations evaluated, since they presented an AI higher than the minimum value established (Table 7).

Table 7 - Acceptance index [%] for the different formulations of fermented milk drinks.

Formulation	Color	Aroma	Flavor	Texture	Overall quality
MD10	85.3	78.8	83.3	85.9	85.0
MD20	85.3	77.4	83.5	84.6	83.3
MD30	78.8	72.2	70.7	70.9	74.6

Source: Authors.

Santos et al. (2008) evaluated the influence of whey concentrations on the sensory acceptance of fermented milk drinks made with mango pulp. Similar results were found in the present study, that is, the partial substitution of milk by whey is a viable alternative both sensorially and technologically.

Thus, even though the milk drink formulation with the highest concentration of whey had lower acceptance rates, this drink was well-accepted by the tasters, since its acceptance rate was greater than 70%.

4. Conclusion

Regarding the physicochemical analyses, the lower the proportion of whey used in fermented milk milk drinks, the higher the total solids and caloric contents, also reflecting on protein, lipid and total carbohydrate values. The ash content in the beverages did not present significant differences.

During the 28-day storage period, pH and acidity remained within acceptable values for this type of product. The pH values decreased during storage but did not present differences when evaluated on the same day.

Microbiological analysis showed absence of contamination by bacteria from mold and yeast groups showing good hygienic-sanitary quality of the final products. The beverages presented satisfactory results for *Lactobacillus acidophilus* count, being above the minimum recommended by the current Brazilian legislation.

Although the milk drink formulation with 10% of whey had higher acceptance rates by the tasters, the fermented milk produced with 20% and 30% of whey were evaluated with acceptance rates greater than 70%.

Considering all studied aspects, the development of a probiotic fermented milk drink produced with milk, whey and cashew fruit pulp may represent an interesting option as a dairy product with excellent nutritional quality and satisfactory evaluation from the consumers.

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