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PRODUCTION OF PROBIOTIC FERMENTED MILK DRINK BASED ON WHEY

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Abstract: Functional foods are increasingly gaining ground and acceptance among consumers due to the biological activity exerted by the bioactive substances present. Functional dairy products represent the most important segment of functional foods, including various products, including fermented milks. As well as being one of the best sources of calcium, an essential nutrient that can prevent osteoporosis, these products are made with probiotic bacteria, prebiotic ingredients or both, which help with intestinal microbial balance. Using whey from the production of rennet-type cheese obtained from the Milk Laboratory of the Federal Institute of Sergipe/ Campus São Cristóvão and jenipapo kindly provided by the fruit-growing sector of the same institution, the fermentation processes were outlined and the physicochemical, microbiological and probiotic characteristics were analyzed. The favorable nutritional composition of the whey consisted of 4.95g of lactose, 076g of proteins and a pH of 5.5, titratable acidity of 0.67 to 0.88g/100g, absence of coliforms and *salmonella spp*, presence of *Bifidobacterium spp*. and *L.* acidophilus with an efficiency of 0.67 to 0.88g/100g. *acidophilus* with an efficiency of 6.98 x 10^6 to 7.62 x 10^6 and 9.^{7}x 10^7 to 1.3 x 107, respectively, together with a range of viable cells at the entrance to the stomach of 10^8 to 10^9 CFU/mL, reaching the end of the duodenum with a range of 10^4 to 10^5 CFU/mL, ensured microbial development and confirmed the probiotic potential of the natural fermentation milk drinks developed.
Keywords: cheese whey, dairy drinks, probiotic bacteria

INTRODUCTION

Milk drinks are a rational and logical way of using whey and are a reality in the Brazilian market, being processed in various ways, in various flavors, forming part of a very promising market (PFLANZER *et al.,* 2010).

According to Venturini Filho (2010), dairy drinks can be classified in different ways according to specific characteristics: refreshing drinks (low prices and short shelf life); drinks intended for sports diets or other specific diets (high prices and medium shelf life); fermented drinks (have action on the intestinal microflora, metabolic properties and great acceptance); and nutritional drinks (high nutritional value, low prices and long shelf life).

According to Nakamae (2014) and Neves (2011), in Brazil one of the main options for using whey is to produce dairy drinks, the most commercialized being fermented drinks, with sensory characteristics similar to yogurt, and non-fermented dairy drinks.

The main regions consuming dairy drinks in Brazil are the south-east, south and north--east. Because it is a fresh food with a pleasant taste and texture, rich in calcium, proteins and vitamins, consumption of dairy drinks has been growing (ALMEIDA; BONASSI; ROÇA, 2011).

According to Penna, Oliveira and Tamime (2013) this type of drink accounts for around a third of the Brazilian yogurt and fermented milk market.

The use of whey in food formulation is a good alternative for obtaining products to be introduced into programs to combat malnutrition. Fermentation by lactic acid bacteria is also expected to reduce the lactose content in whey and produce a product with a better taste and aroma and a longer shelf life.

In this way, the possibility of mixing cheese whey with fruit and/or vegetables to make drinks, dairy products, soups and desserts would benefit a product made up of fiber, vitamins and minerals, making it an alternative in the composition of diets for institutional groups, nursing homes, hospitals, nurseries, school meals, among others, as well as meeting the market demand for a greater variety of products of this nature.

FERMENTED MILK DRINK

According to Brazilian legislation, a fermented milk drink is a milk product made from a mixture of milk (fresh, pasteurized, sterilized, UHT, reconstituted, concentrated, powdered, whole, semi-skimmed or partially skimmed and skimmed) and whey (liquid, concentrated and powdered) fermented by specific microorganisms and which cannot be subjected to heat treatment after fermentation. The total lactic acid bacteria count must be between 10^6 and 10^9 CFU/mL in the final product, for the specific lactic acid cultures used during the shelf life (BRASIL, 2005).

According to Sousa (2020), data published in the literature show that, in 2019, dairy beverages accounted for 4.21 % of sales of dairy products in Brazil (EMBRAPA, 2020). The growing demands among consumers for new and interesting flavors should have an impact on dairy product innovations and also on the increase in consumption of dairy beverages. The range of products called dairy drinks can contain various constituents. The constituents associated with milk can be powdered or fluid milk with different fat contents, powdered or fluid whey, cream and other dairy ingredients such as caseinate and whey protein concentrate. Non-dairy ingredients include fruit pulp, sugar, honey, cereals, cocoa, sweeteners and flavorings, among others (REVISTA INDÚSTRIA DE LATICÍNIOS, 2023).

The increased demand for a healthy diet means that part of the population prefers fruits and fruit pulps that maintain the flavor and color of the fruit "*in natura*", preserving its nutritional and functional composition (DINIZ; SILVA; VIEIRA, 2017). For this reason, one of the viable possibilities is to produce products that use natural ingredients that are easily accessible to small producers (FREITAS; NASCIMENTO; VIEIRA, 2018) and that replace additives that are permitted by the competent bodies in relation to color and flavor attributes (SCHIOZER; BARATA, 2017).

PROBIOTICS

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) define probiotics as live microorganisms which, when administered in adequate quantities, confer health benefits on the host. Among the microorganisms most commonly used as probiotics in food are bacteria from the genera *Bifidobacterium and Lactobacillus*. These have been isolated from all parts of the gastrointestinal tract of healthy humans. Among the bacteria belonging to the genus *Bifidobacterium are B. bifidum, B. breve, B. infantis, B. lactis, B. animalis, B. longum and B. thermophilum*. Lactic *acid* bacteria belonging to the Lactobacillus genus *include L. acidophilus, L. helveticus, L. casei subsp. paracasei, L. casei subsp. tolerans, L. paracasei, L. fermentum, L. reuteri, L. johnsonii, L. plantarum, L. rhamnosus and L. Salivariu* (Arrais, 2015).

According to the World Gastroenterology Organization (WGO), the *Lactobacillus and Bifidobacterium* species are the most commonly used as probiotics, but *Saccharomyces boulardii, E. coli and Bacillus* are also used.

Strictly speaking, the term "probiotic" should be reserved for live microbes that, in controlled human studies, have been shown to produce health benefits (WGO, 2017). According to Divella et al. (2021), probiotics can exert effects in the small and large intestines if they survive the acidic gastric environment and bile. Preclinical studies have shown that probiotics can improve intestinal barrier properties, antioxidative status and attenuate inflammatory responses in rodents after exhaustive exercise (MARTTINEN et al., 2020).

One of the trends in the dairy industry has been the development of functional products, especially those containing isolated probiotic microorganisms or co-cultures of live microorganisms (lactic acid bacteria and other bacteria or yeasts) that are used in a mixed form to achieve a probiotic relationship (Mei-

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ra, 2015). The National Health Surveillance Agency (ANVISA) determines as a parameter for classifying a product as probiotic that the minimum concentration of probiotic microorganisms in the food must be between 10^8 and 10^9 CFU/mL of viable cells proven until the end of the shelf life (Brasil, 2008).

Probiotic bacteria have been widely used in the dairy industry, mainly as ingredients in products such as yogurts, fermented milks, dairy drinks, ice creams, dairy desserts and cheeses (Rolim, 2015). The fermentation of dairy products can be carried out by probiotic lactic acid bacteria, which is a technological alternative that meets the demands of consumers, who are increasingly looking for health benefits (Rolim, 2015; Barbosa & Belo, 2017).

Through the protosymbiotic or protocooperative action of the mixed culture, the development of one species of bacteria provides favorable conditions for the growth of the other, ensuring that at the end of fermentation the product obtains the flavor, acidity and aromatic compounds characteristic of the fermented milk drink.

Therefore, controlling the time-temperature binomial and the percentage of each lactic acid bacterium present in the inoculum are important tools for controlling the quality of fermented milk drinks (Recchia, 2014). In the storage of fermented milk products, important factors to consider are associated with their physicochemical characteristics of total titratable acidity, hydrogenionic potential (pH), total soluble solids (ºBrix), water activity (aw) and syneresis detected during storage, since changes in these values modify the sensory characteristics of the product, reducing its acceptability.

METHODOLOGIES

MATERIALS AND METHODS

The agro-industrial waste (whey from the production of rennet cheese) was obtained from the Milk Laboratory of the Federal Institute of Sergipe/Campus São Cristóvão. The sample was collected directly from the production tank, just after the cheese had been cut. The whey collected was filtered and placed in previously sanitized plastic containers and stored at 18ºC in a freezer in plastic packaging until it was used.

The jenipapo was kindly provided by the fruit-growing sector of the Federal Institute of Sergipe/Campus São Cristóvão. After cold blanching, it was stored under refrigeration until it was used to make the pulp.

The experiment was divided into two stages. The first stage was characterized to define the best formulations, based on the physicochemical analyses of the milk drinks developed. In the second stage, the optimized formulations were characterized on the basis of centesimal and microbiological analyses and the viability of probiotic bacteria.

PHYSICO-CHEMICAL CHARACTERIZATION:

The physicochemical analyses were carried out in triplicate at the Bromatology Laboratories of the Federal Institute of Sergipe/Campus São Cristóvão and in partnership with the Sergipe Institute of Technology and Research (ITPS).

Humidity

The cheese whey and milk drink were analyzed for moisture content by drying in an oven at 105ºC until constant weight (IAL, 2008).

To determine the moisture content, approximately 3 g of the samples were measured in crucibles which had previously been weighed and submitted to an oven at 105 °C (model SL-100). After 24 hours, the samples were weighed in triplicate and the moisture content was calculated using the gravimetric method using the following equation:

% moisture = (weight loss/weight wet sample) x 100

Fixed mineral residue (ash)

The cheese whey and milk drink were analyzed for total solids and ash content using the gravimetric method, which consists of desiccating the sample followed by incineration at 550^0C. In this way, the organic fraction is volatilized in the form of carbon dioxide and water, with the ash or mineral residue remaining fixed in the container (IAL, 2008).

After 24 hours, the samples, in triplicate, were weighed and the moisture content calculated using the gravimetric method using the following equation:

% moisture = (weight loss/weight wet sample) x 100

Total carbohydrates

The cheese whey and milk drink were analyzed for total carbohydrates using the colorimetric method based on the reaction of phenol with glucose (DANIELS; HANSON, & PHYLLIPS, 1994).

Protein content

The cheese whey and milk drink were analyzed for protein content using the Kjeldahl method, which initially consists of oxidizing the sample with hot sulphuric acid in the presence of catalysts that break down the protein structure to release nitrogen in the form of ammonium salts. Sodium hydroxide was added to the residue obtained, releasing ammonia which was distilled and captured by a boric acid solution. The ammonium metaborate formed was titrated with hydrochloric acid, representing the nitrogen fraction to be determined. The nitrogen/protein conversion factor used was 6.38, according to the methodology of the IAL - Instituto Adolfo Lutz (2008).

Determination of Titratable Acidity

The cheese whey and milk drink were analyzed for titratable acidity content by titrating 10 mL of the drink with Dornic solution (0.1 N) and 50 mL of distilled water, using three drops of phenolphthalein as an indicator until the turning point, according to the methodology of the IAL - Instituto Adolfo Lutz (2008). This was done using the equation:

% lactic acid (m/v) = $Vxfx\ 0.09\ xNx\ 100\ v$

Where:

V = volume of sodium hydroxide solution (0.1 N) used in the titration, in mL;

v = volume of the sample, in mL;

f = correction factor of the 0.1 N sodium hydroxide solution;

0.09 = lactic acid conversion factor;

N = normality of 0.1 N sodium hydroxide solution.

Determination of Soluble Solids

The cheese whey and milk drink were analyzed for soluble solids using a portable refractometer, placing a drop of the milk samples on the prism (Mendes et al, 2017). The results were expressed in ºBrix.

pH

The cheese whey and milk drink were analyzed for pH by the potentiometric method using a previously calibrated bench pH meter (Luca - 210), where 10 mL of the samples were added to a 50 mL beaker and the electrodes were immersed until the pH was completely stabilized (IAL, 2008).

Lipids

A butyrometer, 10 mL of sulfuric acid, 11 mL of the sample and 1 mL of isoamyl alcohol were used to quantify the lipid content. After mixing the sample with the other reagents mentioned above, the mixture was centrifuged for 5 min at 5000 rpm. After centrifugation, the percentage of fat was read directly on the butyrometer's meniscus.

DEVELOPMENT OF A PROBIOTIC MILK DRINK USING AGRO-INDUSTRIAL WASTE

The choice of waste for use as a substrate was based on its carbohydrate, protein and sugar content, since high levels of these constituents are promising for probiotic fermentation processes. The cheese whey was obtained from the milk coagulation process used in the production of rennet cheese.

Fermentation processes

The batch fermentations were carried out in stainless steel pans, with the following quantities of cheese whey and milk in the experiments:

- Fermentation 1: 75% cheese whey and 25% cattle milk;
- Fermentation 2: 50% cheese whey and 50% cattle milk and
- Fermentation 3: 25% cheese whey and 75% cattle milk.

In each experiment, the combination of cheese whey and cattle milk was mixed with sugar and starch, under constant and vigorous stirring, totaling approximately 6% total soluble solids. This mixture was heated to 45 °C and kept for 5 min to eliminate contaminating bacteria, promote physicochemical changes and improve the properties of the curd that will be formed during the production of the milk drink. The mixture was cooled in a water and ice bath to 35 - 38°C for slow fermentation for a period of 10 to 12 hours until a firm colloid was formed with a pH of 4.6. The mixture was then cooled to a temperature of 1 to 10°C for a period of 12 to 16 hours to stop the fermentation process. After fermentation, the clot was broken for 30 seconds by manual stirring and a 15% jenipapo pulp preparation was added. The drink was filled into plastic cups and stored in a refrigerator at a temperature of approximately 5 °C.

MICROBIOLOGICAL ANALYSIS

The microbiological analyses were carried out in triplicate at the Microbiology Laboratory of the Federal Institute of Sergipe/Campus São Cristóvão and in partnership with the H. Martins Laboratory, with the aim of verifying the hygienic and sanitary quality of the milk drink produced. Samples of the formulations were subjected to microbiological analysis to determine coliforms, mesophilic aerobes, molds and yeasts.

Sample preparation

Initially, 25 g of the sample from each formulation was weighed out aseptically and the material was homogenized by manually stirring it in 225 mL of sterilized peptone water. Serial dilutions of 0.1% (w/v) peptone water were then made to quantify the microbiological groups.

Quantification of aerobic mesophiles

In relation to the aerobic mesophile count, after preparing the sample, 1 mL was taken from each dilution and sown in petri dishes containing PCA (Plate Count Agar) culture medium, incubating them at 35 °C ± 2 °C for 48 hours (SOARES, 2013). The result was expressed as Colony Forming Unit per mL (CFU/mL).

Salmonella spp

After preparation, the diluted samples were first pre-incubated at 37 °C for 18-24 hours, and then 100 µL were aseptically added and seeded onto Compact Dry Compact SL® plates (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 41-43 °C for 24 hours. All analyses were carried out in triplicate and according to the manufacturer's instructions. The results were expressed as the presence or absence of Salmonella sp. in 25g of the sample.

Thermotolerant coliforms

Thermotolerant coliforms were quantified using the Most Probable Number (MPN) technique with series of three tubes. Aliquots of 1 mL of the sample dilutions for each formulation were individually inoculated into tubes containing lauryl sulphate tryptose broth with inverted Durhan tubes and incubated at 35°C for 48 hours. Subsequently, the tubes that tested positive for coliforms at 35 °C were transferred to tubes containing Escherichia coli (EC) broth and incubated at 44.5 °C for 48 hours. The result was expressed as MPN of thermotolerant coliforms/g of sample analyzed.

ANALYSIS OF PROBIOTIC CAPACITY USING A STOMACH-DUODENUM SIMULATOR

The fermented milk was added to a model system representing the conditions of the human stomach and duodenum, as shown in Figure 2. The system consisted of two jacketed reactors of 1 L each with the temperature maintained at 37°C. Reactor 01 (stomach reactor) contained 17.5 mL of 150 mM HCl before adding the fermented milk to simulate the cephalic phase of acid secretion. Initially, the flow rate of HCL was 3.5 mL/min until the reactor reached pH 3.0, after which the flow rate was increased to 0.9 mL/min to simulate gastric inhibition. In reactor 02 (duodenum reactor) the pH was maintained at 6.5 by adding 0.9 mL/min of 1 M NaOH. At time 0 h, the duodenum reactor contained 7 mL of 4% Oxgall bile solution, this solution was pumped into the reactor for the first 30 min at a flow rate of 0.5 mL/min, then the solution was reduced to 2% Oxgall until the end of the experiment. Surviving cells were determined in both reactors by plate counting at 30, 60 and 90 min (MAINVILLE *et al.,* 2005).

Lactic acid bacteria - probiotic viability

Lactic acid bacteria counts were carried out by standard plate counting, where 1 mL of each dilution was sown in petri dishes and 20 mL of AGAR MRS culture medium was added, homogenized, making "eight" movements, six times to the right and six times to the left until completely solidified. The plates were then overlaid, waiting for them to solidify again and inverting them before incubating them. The plates were then incubated at 30 °C for 5 days.

To count *Lactobacillus acidophilus and Bifidobacterium animalis*, MRS Agar (Kasvi®) was used, modified by the addition of maltose solution (Dinâmica®) and inhibitory compounds of lithium chloride (Dinâmica®) and sodium propionate (Sigma®). The procedure began by weighing 25g of the sample and diluting it in 225mL of 0.1% peptone water (Ion®), forming an initial dilution (10^{-1}). From this, serial decimal dilutions up to 10^{-6} were prepared. Dilutions from 10^{-4} to $10^{(-6)}$ were used for analysis.

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

For the quantification of *B. animalis*, MRS (Kasvi®) was used as the base medium and added inhibitory compounds, 0.002g/mL of lithium chloride (Dinâmica®) and 0.003g/mL of sodium propionate (Sigma®) (15). For both microorganisms, the plates were evaluated in duplicates with deep inoculation, incubated at anaerobiosis at 37°C for 72 hours. The counts were expressed in colony-forming units per milliliter (CFU/mL in log10).

The diagram below represents the stomach-duodenum simulation used in the experimental activity.

RESULTS AND DISCUSSION

The formulations were based on the minimum amount of milk base (51% of the total ingredients) that the legislation requires for the drink to be considered a milk drink (BRASIL, 2005) and a minimum amount of jenipapo pulp. After preliminary evaluation and experimental design, the fermented milk drinks formulated in this work with the respective percentages of 50 and 25% cheese whey had technical aspects that were not favorable to the evaluators and were therefore not analyzed in accordance with the principle of economical use of materials, reagents and energy, among others

EVALUATION OF THE PHYSICOCHEMICAL CHARACTERISTICS OF WHEY AND PROBIOTIC MILK DRINK

The results of the physicochemical analyses carried out on the *in natura* whey resulting from the manufacture of "Type Coalho" cheese are shown in Table 8.

Analyzing the results of the *in natura* whey analysis, it was found that the physicochemical characteristics were close to those obtained by other researchers for sweet whey, considering the Standard of Identity and Quality of Whey, which determines a pH value of between 6.0 and 6.8; titratable acidity in lactic acid (g/100g) of at least 0.08 to 0.14; total solids content (g/100mL) of at least 5.0. The small differences observed were due to the type and quality of feed offered to the cattle as well as the technological processes used, the milk used and the type of cheese made (FURTADO and POMBO, 1988; MARWAHA and KENNEDY, 1988; TEIXEIRA and FONSECA, 2008; FREITAS, 2011, COSTA, 2022).

The results of the physicochemical analyses carried out on the probiotic fermented milk drink with added jenipapo pulp and 75% cheese whey concentration are shown in Table 9.

In view of the above, there was no significant difference in the acidity values with storage time, i.e. the acidity tended to stabilize. This result can be interpreted as the whey not interfering with the activity of the lactic cultures. The acidity of the formulations ranged from 0.67 to 0.88 g of lactic acid/100g, meeting the legal requirements of 0.6 to 2.0 g of lactic acid/100g. Similar pH and acidity results were found by Cunha *et al.* (2018) in dairy drink and fermented milk samples, and by Fernandes (2003) and Henriques *et al.* (2012) in yogurt samples. The pH values were also similar to fermented milk drinks (TEIXEIRA, 2002; SOUSA, 2020; COSTA, 2022) and yogurts (MARAFON *et al.,* 2011).

According to SOUSA (2022), in Thamer's (2006) study on the characterization of functional milk drinks fermented with probiotics and added with prebiotics, although fermentation was completed and cooling began at pH 4.8, the author found pH values varying between 4.72 and 4.83. Also in the same study, the author found total titratable acidity values determined in degrees Dornic between 44.33 and 50.39 °D. Toledo (2013), evaluating the use of by-products from the industrialization of passion fruit to make yogurt, found pH values for the samples ranging from 4.05 to 4.57.

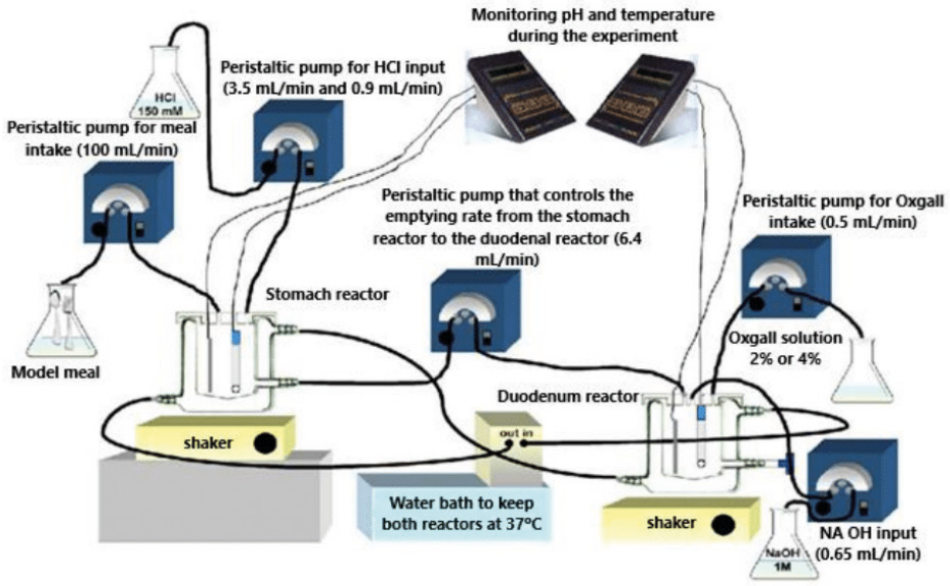

Figure 3: Experimental unit of the stomach-duodenum simulator (MAINVILLE *et al.*, 2005 with modifications)

Features	Sweet whey	
	in natura	***in natura*** **(Data from the literature**
Protein (g/100g)	0,76±0,098	0,8 - 1*
Lactose (g/100g)	4,95±0,398	4,5 -5,0*
Ash (g/100g)	0,63±0,012	0,5- 0,7*
Total Lipid (g/100g)	0,20±0,073	0,1-0,5(**
Dry extract (g/100g)	6,73±1,396	5,0-7,0(***
pH	5,52±1,043	5,2 - 6,7*
Total Acidity (g/100mL)	0,09±0,031	0,1- 0,2*
Moisture (g/100g)	94,27±1,185	93 - 94*
Density (g/mL	1,02±0,	-
*HARPER (1998), **BEHMER (1991), ***ORDÓÑEZ (2005).		

Table 8: Physicochemical characteristics of *fresh* cheese whey.

Features	**Milk drink**				
Protein (g/100g)	2,01 ± 0,02				
Total Carbohydrates (g/100g)	14,68 ± 0,04				
Ash (g/100g)	0,78 ± 0,01				
Total Lipid (g/100g)	1,75 ± 0,07				
Dry Extract (g/100g)	17,23 ± 0,06				
Moisture (g/100g)	83.94± 0.37				
o	16.56± 0.44				
Total caloric value (Kcal/100g)	82,51 ± 0,11				
	0 day	**7th day**	**14th day**	**21th day**	**28th day**
pH	4,60±0,02	4,58±0,10	4,58±0,04	4,57±0,03	4,53 ± 0,01
Total acidity (g/100g)	0,67±0,01	0,73±0,01	0,74±0,01	0,84±0,01	0,88 ± 0,01

Table 9: Physico-chemical characteristics of the milk drink with jenipapo pulp

There was no significant difference in total soluble solids (°Brix) ($p \leq 0.05$). The total soluble solids values found in this study are in line with those found by various authors. According to Sousa (2022), in the study by Rocha *et al.* (2016), the soluble solids values found in the fermented milk drink with coconut sour were 19.20, 17.15 and 17.00 ºBrix. In the study by Silva *et al.* (2014), a value 15 ºBrix was found for fermented milk drinks containing 5, 10 and 15% grape pulp.

According to the relevant legislation (BRASIL, 2005), to be considered a fermented milk drink, it must contain at least 1.7% protein and 2.0% milk-based lipids. Gerhardt *et al.,* 2013, states that Teixeira (2002) in a similar study developed fermented milk drinks with the addition of 50 to 70% ricotta whey and found protein values (2.05-1.73%) and lipids (0.22-0.13%)

As can be seen in Table 9, the protein and lipid contents found in this study are above the values recommended by legislation and did not differ significantly from one another.

Costa (2022), reports that in Thamer's research (2006), evaluating the characterization of functional milk drinks fermented with probiotics and added with prebiotics (fructooligosaccharides), the protein content found in the fermented milk drinks varied between 1.93% and 2.46%. The highest value of 2.46% was found in the experiment with the lowest percentage of whey (45%), while the experiment the lowest protein content had 55% whey in its formulation. The variation in protein content was due to the difference in the quantities of whey and skimmed milk used to make the drinks.

It can be seen in this research that the lipid content is in line with the research by Pfrimer (2018) on a fermented milk drink with buttermilk, whose formulations showed lipid content varying between 1.15 and 2.76%.

In relation to moisture analysis, the work by Pfrimer (2018) on the development and evaluation of fermented milk drinks with buttermilk and flavored with cagaita pulp, all the formulations evaluated had moisture values above 80%. The raw materials whey/buttermilk and pulp have a high moisture content, so the higher the concentration of these components, the higher the moisture content of the milk drinks.

Costa (2013) found moisture values between 80.5 and 81.04% for a fermented milk drink flavored with araticum pulp. Vieira (2016) evaluated the moisture content of different fermented milk drinks produced with fruits from the cerrado (araçá, araticum, gabiroba, mangaba, murici and pequi) enriched with passion fruit peel mesocarp flour and obtained results ranging from 80.59 to 82.85 %. Sousa (2022) quantified the moisture content of a fermented milk drink flavored with graviola and obtained results ranging from 80.63 to 83%, similar to those presented in this study.

The results for carbohydrates and caloric value were similar to those found by Cunha *et al.* (2018) in samples of fermented milk drink with 70% milk and 30% cheese whey, in line with current legislation.

EVALUATION OF MICROBIOLOGICAL ANALYSIS

The results of the microbiological tests carried out on the probiotic fermented milk drink with jenipapo pulp and 75% cheese whey concentration are shown in Table 11.

The values obtained demonstrate the hygienic-sanitary quality of the probiotic milk drink with jenipapo pulp in accordance with the specifications of Normative Instruction No·60 of December 23, 2019.

In this study, the aerobic mesophile count showed results within the range recommended by current legislation, which establishes maximum values of $7.5x10^4$ (CFU/mL) for

Parameter	Results	Unit	VMP	LQ	Methodology
Mold and Yeast Count	$2.3 \times 10^1 \pm 0.02$	CFU/g	$1{,}0 \times 10^2$	1	ICUMSA, M.GS 2/3-41(2011)
Testing for Coliforms at 45°C	absence	CFU/g	-	1	APHA-CMMEF Chap.3, 5th Ed.2015
Total Coliforms	absence	CFU/g		1	APHA-CMMEF Chap.3, 5th Ed.2015
Total Aerobic Mesophile Count	$3.5 \times 10^1 \pm 0.63$	CFU/g	-	1	ICUMSA, M.GS 2/3-41(2011)
Salmonella	absence		-	1	APHA-CMMEF Chap.3, 5th Ed.2015

Table 10: Results of the microbiological analysis of the probiotic milk drink

Description of the samples		Viable cells (CFU/mL) 0 (min)	Viable cells (CFU/mL) 30 (min)	Viable cells (CFU/mL) 60 (min)	Viable cells (CFU/mL) 90 (min)
Milk drink (commercial)	Stomach	$1{,}82 \times 10^5$	$2{,}71 \times 10^4$	$1{,}3 \times 10^3$	$3{,}2 \times 10^3$
	Duodenum	-	$5{,}5 \times 10^3$	$1{,}4 \times 10^2$	$3{,}8 \times 10^2$
Milk drink (without fruit pulp)	Stomach	$1{,}37 \times 10^7$	$2{,}04 \times 10^4$	$1{,}05 \times 10^5$	$1{,}39 \times 10^5$
	Duodenum	-	$8{,}8 \times 10^4$	$5{,}3 \times 10^5$	$1{,}4 \times 10^6$
Milk drink (with fruit pulp)	Stomach	$1{,}9 \times 10^9$	$1{,}01 \times 10^4$	$9{,}1 \times 10^5$	$4{,}2 \times 10^5$
	Duodenum	-	$1{,}4 \times 10^4$	$6{,}8 \times 10^5$	$5{,}3 \times 10^6$

Table 11: Number of viable cells in the stomach-duodenum simulator.

Microorganism	Time (days)				
	0	7	14	21	28
Bifidobacterium	$6{,}98 \times 10^6 \pm 0{,}27$	$7{,}51 \times 10^6 \pm 0{,}39$	$7{,}40 \times 10^6 \pm 0{,}76$	$7{,}37 \times 10^6 \pm 0{,}40$	$7{,}62 \times 10^6 \pm 0{,}67$
Lactobacillus acidophillus	$8{,}18 \times 10^7 \pm 0{,}11$	$7{,}34 \times 10^7 \pm 0{,}65$	$6{,}80 \times 10^7 \pm 0{,}006$	$7{,}44 \times 10^7 \pm 0{,}09$	$7{,}98 \times 10^7 \pm 0{,}54$

Table 12 - Lactic acid bacteria counts of the fermented milk drink produced

this microbial group in milk drinks (BRASIL, 2005). The aerobic mesophile count provides general information on the conditions during food processing, where values greater than 10^6 (CFU/mL) indicate inappropriate processing from a hygiene and health point of view, or the raw material may also be contaminated.

In this study, the results found for thermotolerant coliforms are in line with current legislation (BRASIL, 2005), which establishes a maximum of 10 NMP/mL. The analysis of thermotolerant coliforms is essential as it indicates the possibility of contamination by feces and consequently pathogenic microorganisms in the raw materials used to produce food (SILVA; ARAÚJO, 2003). As in the experiments by Sousa (2022) and Dos Reis *et al.* (2014), the quality and microbiological safety of fermented dairy products of bovine origin was checked by determining total coliforms and thermotolerant coliforms, and all the samples were found to be free of these bacteria.

In the present study, as well as in the research by Dos Reis *et al.* (2014) and in the studies carried out by Pfrimer (2018) and Costa (2020), *Samonella spp*. were absent for all formulations, in accordance with current legislation, showing good hygienic conditions during the preparation of dairy drinks, indicating that the products were safe to be submitted to sensory analysis.

EVALUATION OF PROBIOTIC POTENTIAL USING A STOMACH-DUODENUM SIMULATOR

Table 10 shows the probiotic potential of naturally fermented milk drinks and commercial milk drinks, using a stomach-duodenum simulator. The commercial milk drink had a probiotic potential of 10^5 CFU/mL at the entrance to the stomach. After 60 min of processing in the simulator, there was a decrease in its probiotic potential, reaching 10^2 CFU/mL at the end of the duodenum. The natural fermentation milk drinks without and with the addition of 15% jenipapo pulp showed a variation at the entrance to the stomach of 10^8 to 10^9 CFU/mL, reaching a variation of 10^4 10^5 CFU/mL at the end of the duodenum. With these results, it was observed that naturally fermented milk drinks have a greater probiotic potential when compared to the commercial milk drink used in this study and can be considered a probiotic product under Brazilian legislation.

According to the literature, in order to provide health benefits, a probiotic product must overcome physical and chemical obstacles such as acid and bile found in the gastrointestinal tract , so the microorganisms need to be viable, active and abundant, with a minimum concentration of 10^6 CFU/mL of product (GUO *et al.,* 2019). According to THAMER and PENNA (2016), developing a dairy product with a high concentration of probiotic microorganisms is essential, so that at least around 10^3 to 10^4 CFU/mL reach the intestine.

The values obtained in this study were lower than those described in Brazilian legislation, which establishes a minimum limit of 10^7 CFU/g or mL of the product (BRASIL, 2000). Based on the results obtained, it can be considered that naturally fermented milk drinks have probiotic characteristics.

According to the literature, it is essential for probiotic microorganisms to remain viable in food during its shelf life, being present in significant numbers and being viable at the time of consumption, in the recommended concentration until its last day of shelf life (ONG; HENRIKSSON; SHAH, 2006; MICHAEL, 2010 PHEBUS, 2010; RAMOS, 2013; SCHMIDT, 2010). According to Matsubara (2001), the amount of lactic acid bacteria must be very high, and the author considers that the values required by legislation are low. The author mentions that the appropriate value would be around 10^9, to have at least around 10^3 to 10^4 CFU reaching the intestine, and ideally 10^6.

Thamer (2005) analyzed the effect of whey, sugar and fructooligosaccharide content on the population of probiotic lactic acid bacteria in fermented beverages, finding that the probiotic microorganisms *Bifidobacterium spp. and Lactobacillus acidophilus* ranged from 9.00 x 10^6 to 9.65 x 10^{12} and from 1.15 x 10^8 to 2.55 x 10^{12} (CFU/mL), respectively. She also found that the highest counts of bacteria corresponded to milk drinks with a lower acidity content and a high solids content, while drinks with a higher acidity content had the lowest counts. Also according to same author, probiotic bacteria (*Bifidobacterium and L. acidophilus*) are more sensitive to lower pH values, showing lower viability.

Table 11 shows the quantification of probiotic lactic acid bacteria during the 28-day storage period.

In this study, the viability of *Bifidobacterium spp*. was observed in the fermented milk drink treatments analyzed, with an efficiency of 6.98 x 10^6 to 7.62 x 10^6. The cell viability *of L. acidophilus* in the fermented milk drink proved to be efficient, with results ranging from 9.7 x 10^7 to 1.3 x $10^{(7)}$. Similar values to the milk drink produced in this study were found by Thamer; Penna (2006) in milk drinks containing *Lactobacillus acidophilus, Bifidobacterium* and a

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prebiotic ingredient. Pelais *et al.* (2020), Ames (2019), Zarpelon (2017), Ferreira (2016), Bandiera *et al.* (2013), Faria *et al.* (2006) evaluating the development of fermented probiotic drinks found similar values to this study and reported that increased acidity contributed to the viability of the probiotic culture.

According to Coutinho (2021), the reduction in pH and the increase in titratable acidity during the storage period is due to the production lactic acid, the genus, the quantity of lactic acid bacteria, the storage time and the substrates added.

According to Table 11, the formulation proposed in this experiment can be considered a fermented milk drink, as it meets the requirements described in the Brazilian legislation for milk drinks, which recommends that the specific microorganisms in the final product and throughout the shelf life for fermented milk drinks must be viable and present in the product in minimum quantities of 10^6 CFU/mL. Therefore, the fermented milk drink had satisfactory counts for the probiotic product claim.

In this way, we can affirm that the preparation of a probiotic milk drink with the addition of jenipapo pulp complies with the relevant legislation and presents potential as an alternative way of using agro-industrial waste with a view to improving consumer health.

FINAL CONSIDERATIONS

In this study, it was shown that it is possible to obtain a dairy drink by fermenting cheese whey using the natural whey microbiota, with the addition of fruit pulp.

The whey's favorable nutritional composition of 4.95g of lactose, 076g of protein and a pH of 5.5 ensured microbial development, resulting in the biotransformation of compounds of interest.

With regard to the number of viable cells, the results found were in the range of 10^9 CFU/mL, meeting the value recommended by Brazilian legislation for a product to be probiotic, the minimum limit being 10^7 CFU/mL (BRASIL, 2000). The range of viable cells at the entrance to the stomach from 10^7 to 10^9 CFU/mL, reaching the end of the duodenum with a range of 10^4 to 10^6 CFU/mL, guaranteed the probiotic potential of the natural fermentation milk drinks developed

In view of the aim of this work, it is clear that rennet whey can be used to reduce its potential to pollute the environment and develop a new food product using fruit and organic waste.

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