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Characterization of goat milk and potentially symbiotic non-fat yogurt

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Abstract

Combining prebiotics and probiotic microorganisms improve quality in the formulation of foods. In this paper, the characteristics of goat milk and symbiotic yogurt were studied. Raw goat milk was analyzed and the skimming process was optimized. For the formulation of a potentially non-fat symbiotic yogurt made with skimmed goat milk, inulin, gelatin, sugar, and *Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus* and *Lactobacillus casei subsp. rhamnoshus*. Chemical characteristics, acceptability, and viability of lactic acid bacteria and probiotic culture were assessed. The protein and fat content of the raw milk was 2.90 and 3.56 g/100 mL, respectively. The optimum skimming process was obtained at 9,800 rpm and 4 °C for 15 minutes. The product formulated had a protein and fat content of 4.04 to 0.04 g/100 mL, good sensory properties, and acceptability of 95%. The lactic bacteria count was 9×10^7 CFU mL^{-1}, and probiotic culture count was higher than 1×10^6 CFU mL^{-1}, which guarantees their effect and capacity to survive in the digestive tract and spread in the intestine. The yogurt was stable during the 21 days of storage. Therefore, this study shows that goat milk yogurt is an adequate delivery vehicle of the probiotic culture *L. casei* and inulin.

Keywords: goat milk; yogurt; symbiotic; *L. casei*, inulin.

1 Introduction

Yogurt made from cow's milk is widely consumed in the world. On the other hand, there is a desire for alternatives to cow's milk due to problems relating to gastrointestinal intolerance and market demand for the formulation of novel dairy products. Goat's milk is reported to have higher digestibility and lower allergenic properties compared to cow's milk (Senaka Ranadheera et al., 2012). It also has a higher content of short chain fatty acids in milk fat, higher content of zinc, iron, and magnesium, and antibacterial characteristics (Slacanac et al., 2010). In addition, these benefits may be further enhanced by using goat's milk as a vehicle for delivering probiotics and prebiotics.

Probiotics have been defined as selective viable microorganisms that can be incorporated into foods offering positive impact on the health and nutrition to consumers. Lactobacilli are associated with maintaining optimum microbial balance in the digestive tract with well documented health benefits such as, strengthening the immune system, reduction of lactose intolerance, decrease of serum cholesterol level, and possible anti carcinogenic effects. Thus, these organisms have been extensively incorporated into dairy foods over the last years, and yogurts containing Lactobacillus are widely marketed (Senaka Ranadheera et al., 2012). *Lactobacillus casei* increases healthy immune system response and improves healthy cellular function and the growth of healthy bacteria in the intestinal tract. Some studies have shown that *L. casei* can modify potentially harmful bacterial activities such as those of b-glucuronidase and nitroreductase (Kayanush & McGrew, 2007). Matsuzaki (2003) reviewed the biological activities of *L. casei* that favorably influence human health. Bertazzoni Minelli et al. (2004) examined four strains of *L. casei* for their potential use in new probiotic fermented milk based on their technological performances, in vitro adhesioncapacity, and intestinal transit tolerance after administration to rats. They reported that all four strains of *L. casei* are favorable for utilization in health-promoting foods.

Prebiotic substances, defined as non-digestible nutritional ingredients, affect the host in a positive way by selective stimulation of colon bacteria´s growth and/or activity (Anadón et al., 2010). Inulin is one of them (Kip et al., 2006). This non-digestible carbohydrate is widely used in the food industry and is based on the nutritional and technological properties of inulin. It can be found in dairy products, in beverages, in low-fat foods, and in ice cream. At the same time, inuline has a bifidogenic effect, increasing calcium absorption with positive effects on bone health, lowering serum lipids promoting satiety with potential positive consequences for weight management (Parnell & Reimer, 2009) and improving resistance to infections and stimulating the immune system (Lomax & Calder, 2009).

The technological use of inulin is based on its properties as a sugar replacer, as a fat replacer, and texture modifier. Inulin seems particularly suitable as a fat replacer in low-fat dairy products since it may contribute to an improved mouthfeel. Guggisberg et al. (2009), Guven et al. (2005), Kip et al. (2006) and Paseephol et al. (2008) showed that inulin addition to low-fat yogurt resulted in enhanced creaminess. When inulin is added to food in low concentrations, the rheological properties and the sensory quality of the product will not be affected

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strongly due to the neutral or slightly sweet taste and the limited effect on viscosity of this ingredient (Kalyani Nair et al., 2010). However, to make inulin based dietary fiber claims, inulin should be added in amounts that range from 1 g/100 mL (Argentina, 1969), and contents of 3-8 g per portion have to be added to assure its beneficial effect (Meyer; Stasse-Wolthius, 2009). As inulin content increases, its effect on product structure and texture becomes important. At these higher levels, and due to its physicochemical properties, inulin can modify the texture of dairy products and may significantly influence their sensory quality (Meyer et al., 2011).

Symbiotics are the combined form of a probiotic and a prebiotics, which contribute to some properties of their formulation, including the fact that they enhance the survival of live bacteria in products resulting in an extension of useful life. They increase the number of the eaten bacteria that reach the colon in a viable way; they replace fat presenting synergy with many gelantin agents, and they stabilize emulsions and foams (Kip et al., 2006).

People lifestyles today lead to high consumption of high-fat foods and low fiber resulting in an increase in cholesterol levels, overweight, and high indexes of cardiovascular disease. All of this makes it necessary to decrease the intake of trans fat and saturated fat, decrease cholesterol levels, and to increase the intake of products that produce beneficial effects for human health (Taranto et al., 2008).

Therefore, the objective of the present study was to characterize Saanen goat milk, optimize the skimming process, and develop a potentially symbiotic yogurt (*L. casei* and inulin) by evaluating its chemical properties and acceptability and the viability of lactic acid bacteria and probiotic culture.

2 Materials and methods

2.1 Chemical and microbiological characterization of goat milk

The following parameters were determined in raw goat milk of Saanen breed (40 L): pH (pH meter Hanna), acidity by titration with NaOH N/9 (Dornic solution) in the presence of phenolphthalein indicator to pH 8.0; carbohydrates, proteins, and fats using a milk analyzer (Lacto Star Funke Gerber), moisture, and total ash (Association of Official Analytical Chemists, 1998). The microbiological quality was evaluated by the methylene blue test (Association of Official Analytical Chemists, 1998); total aerobic mesophilic bacteria on Plate Count (International Commission on Microbiological Specifications for Foods, 1986); total coliforms on Violet Red Bile Lactose Agar (Mhone et al., 2011); and the somatic cell method by California Mastitis Test (CMT) using the Fossomatic electronic equipment. All determinations were performed in triplicate. Whole milk was skimmed according to the following parameters: heat treatment (raw, pasteurized at 63 °C for 30 min., and pasteurized at 72 °C for 1 min); speed centrifugation using a refrigerated centrifuge Damon/IEC Division B-20a (8,800 and 9,800 rpm) and temperature (4 and 8 °C). (Table 1) (Silveira et al., 1999).

Table 1. Combination of variables used during the standardization process of skimming.

Heat Treatment	8,800 rpm 4 °C	8,800 rpm 8 °C	9,800 rpm 4 °C	9,800 rpm 8 °C
None (raw)	A	D	G	J
63 °C 30 min.	B	E	H	K
72 °C 1 min.	C	F	I	L

When choosing the appropriate method of skimming, it was taken into account that the fat content (by Gerber) (Association of Official Analytical Chemists, 1998) was not higher than 0.5 g/100 mL of milk; and lipolysis was lower than 1.82 μeq FFA (Free Fatty Acids)/mL of milk since higher values may result in unwanted tastes like stale, soapy, or bitter (Deeth, 2006). Chemical and microbiological characteristics of skim milk were determined according to the following procedures: total aerobic mesophilic bacteria in Plate Count Agar (International Commission on Microbiological Specifications for Foods, 1986); total coliforms on Violet Red Bile Lactose Agar (Mhone et al., 2011); moisture (Association of Official Analytical Chemists, 1998); carbohydrates by difference; protein determination (formol titration method) (Egan et al., 1991); fats by Gerber (Argentina, 1969); total ash; phosphorus, and calcium (Association of Official Analytical Chemists, 1998).

2.2 Formulation of symbiotic yogurt

Skim milk yogurt formulation was selected as a suitable method (mentioned above), which was incorporated with inulin powder Raftiline HP® (Orafti, São Pablo, Brasil) (4.5 g L^{-1}), gelatin (INS 440) (0.75 g L^{-1}), and sugar (7.5 g L^{-1}). The culture starters *Streptococcus salivarius subsp. thermophilus* ST M6® and *Lactobacillus delbrueckii subsp. bulgaricus* Lb- 12® were added at 0.04 g L^{-1} with bacilo/coco 1:1, and the probiotic culture: *Lactobacillus casei subsp. rhamnoshus* was added at three concentrations: 0.025 (Lc25); 0.010 (Lc10), and 0.005 (Lc05) g L^{-1}. The milk was fermented at 42 ± 1 °C for 2 hours, up to pH 4.6. The product was then gently shaken and refrigerated at 4 ± 2 °C.

The count of *L. casei* was performed on MRS vancomycin agar. The MRS broth was prepared with about 2 mL of 0.05 g vancomycin/100 mL. The solution was then added to 1000mL of MRS broth to obtain 1mg/L final concentration. Agar was added at a concentration of 12 g/L. Final pH was 5.60. The yogurt in the cup was briefly agitated using a sterile pipette, and 1 g yogurt was taken from the center of the yogurt cup and transferred to a sterile bottle containing 99 mL of sterile peptone water. The contents were agitated with previously prepared 10 serial dilutions. Plate counts were determined by plating serial dilutions of yogurt on MRS vancomycin agar. The plates were incubated anaerobically at 37 °C for 72 h. White, shiny, smooth colonies of 1.0 mm in diameter were counted.

2.3 Sensory analyses

Sensory evaluation was performed with 100 untrained panelists aged from 20 to 25 years old. The individuals were regular consumers of yogurt, not allergic to milk, and willing to

participate. The yogurt samples were evaluated for consistency, aroma, and overall flavor using a structured 9-point hedonic scale ranging from 1 (Dislike it very much) to 9 (Liked it very much), according to the Institute of Food Technologists (2007, Part II). Each sample (15g) was coded with a 3-digit random number and served in 50 mL disposable transparent plastic cups. The yogurt samples were evaluated after 1 day of refrigerated storage at 4 °C.

2.4 Chemical characterization and viability of lactic acid bacteria and probiotic culture

The final product was tested for: moisture, carbohydrates, and total dietary fiber by enzymatic-gravimetric activity (Association of Official Analytical Chemists, 1998); inulin by HPLC (Zuleta & Sambucetti, 2001); proteins by the Kjeldhal method; fats by alkaline hydrolysis; ashes by incineration with the furnace at 550 °C, calcium by atom absorption spectrometry, and phosphorus by molecular absorption spectrometry (Association of Official Analytical Chemists, 1998).

The yogurt was evaluated for 21 days during storage at 4 ± 1 °C, and every 7 days the pH, Dornic acidity, and percentage of draining by weight difference was determined. The pH values of the yogurt samples were measured using a pH meter (Hanna) after calibration with fresh pH 4.0 and 7.0 standard buffers. Titratable acidity (TA) was determined after mixing the yogurt sample with 10 mL of hot distilled water and titrating with 0.1 N NaOH using 0.5 % phenolphthalein indicator (Dave & Shah, 1997).

2.5 Statistical analysis

To select the temperature and the revolutions per minute in which the milk got skimmed, the samples were analyzed by ANOVA and Duncan tests determining the significance level between them. The results were expressed as mean ± standard deviation (SD). After verification of a normal distribution of data, ANOVA and Duncan tests were used. Differences were considered significant at $P<0.05$.

3 Results and discussion

3.1 Chemical and microbiological characterization of goat milk

The physicochemical and microbiological composition of raw goat milk is shown in Table 2. The low acidity levels may be due to the fact that acidity is influenced by casein, mineral salts, and ions found according to the period of lactation. The pH value was 6.7, and it depends mainly on caseins´ stability. It is affected by the lactation phase, diet, goat´s breed, bubbles of carbonic gas given off during milking, and refrigeration or transportation of the milk (Albenzio & Santillo, 2011). Values of protein and fat were similar to those reported in the literature. The first nutrient may be influenced by breastfeeding and race (Albenzio & Santillo, 2011), and the second, in addition to these reasons, it is also influenced by the animals' diet. (Park et al., 2007). The methylene blue test showed that the milk had a good hygienic quality, with less than 100,000 bacteria/mL.

The count of total aerobic mesophilic and coliform bacteria established by Argentina Legislation for milk from the same dairy farm is 3.3×10^7 CFU/mL (Argentina, 1969). Both tests showed that the milk produced by the farm had good hygienic quality. The milk had no signs of mastitis, intramammary infections, and fecal contamination during milking and/or inadequate hygiene due to use of the milking machine (Kondyli et al., 2012). The somatic cell count in the milk was lower than the maximum level established by Argentina Legislation: 400,000 cells/mL (Argentina, 1969). Generally, the results in Table 2 show that levels of microbial contamination of raw goat milk are satisfactory (Kondyli et al., 2012). Table 3 shows the values of fat and lipolysis of goat milk with and without heat treatment, subjected to different spin speed and temperatures, which were used to select the best skimming process.

The fat content ranged from 0.00 (A,D,K,L) to 0.22 (I) g/100 mL, and none of the values were higher to 0.5 g/100 mL (Argentina, 1969; Güler & Gürsoy-Balc, 2011). No statistically significant differences between the samples ($P \leq 0.05$) were found.

Lipolysis values ranged from 1.15 (K) to 5.45 (G) μeq FFA/mL; the last value with a high FFA level would be unacceptable to most people due to the changes in the flavor

Table 2. Chemical composition and microbiological quality of whole raw goat milk.

	Whole Raw Goat Milk (100 mL)
pH	6.7
Acidez	10.50 ± 0.51 °D
Moisture (g)	87.50 ± 0.70
Carbohydrates (g)	5.01 ± 0.00
Proteins (g)	2.90 ± 0.00
Fats (g)	3.56 ± 0.00
Ash (g)	1.03 ± 0.00
Methylene Blue	No change in color for 5.5 hours
Aerobic Mesophilic	2.6×10^7 CFU/mL
Total Coliforms	2.5×10^4 CFU/mL
Somatic Cells	331,000 cell/mL

Means ± standard deviation ($n=3$). mL: milliliter; g: grams; CFU/mL: Colony Forming Units/ mL.

Table 3. Fat content (g/100 mL) and lipolysis (μeq of Free Fatty Acids/ mL) of skimmed milk.

Heat Treatment	Fat (g%)	FFA (μeq/mL)
A	0.00 ± 0.00^{g}	1.41 ± 0.04^{e}
B	0.05 ± 0.07^{g}	1.81 ± 0.10^{bc}
C	0.10 ± 0.00^{g}	1.67 ± 0.09^{cd}
D	0.00 ± 0.00^{g}	1.92 ± 0.04^{b}
E	0.15 ± 0.07^{g}	1.99 ± 0.04^{b}
F	0.15 ± 0.07^{g}	1.54 ± 0.09^{de}
G	0.05 ± 0.07^{g}	5.45 ± 0.09^{a}
H	0.15 ± 0.07^{g}	1.34 ± 0.04^{ef}
I	0.22 ± 0.03^{g}	1.54 ± 0.00^{de}
J	0.05 ± 0.07^{g}	1.94 ± 0.04^{b}
K	0.00 ± 0.00^{g}	1.15 ± 0.14^{f}
L	0.00 ± 0.00^{g}	1.65 ± 0.02^{cd}

Means ± standard deviation ($n=3$). Means in the same row with different letters are significantly different (P <0.05). g: grams; mL: milliliter; FFA: Free Fatty Acids; μeq: milliequivalents; min.: minutes.

which would described as rancid, butyric, astringent and even bitter. It is possible that such situation did not occur with the other samples without thermal treatment. In the samples with thermal treatment, the lipolysis could have happened before pasteurization since this mechanism activates lipases (Deeth, 2006).

It was observed that the samples that were not heat-treated, showed values of lipolysis of 1.92 (D), 5.45 (G), and 1.94 (J) μeq FFA/mL. On the other hand, the milk that was pasteurized at 63 °C for 30 minutes had a value of 1.99 (E) μeq FFA/mL, which is higher than the reference value (Argentina, 1969), and 1.81 (B) μeq FFA/mL, which is close to the maximum acceptable limit; therefore, these samples were not considered.

The presence of total free fatty acids in the raw samples (A, D, G, J) could be due to the action of lipases in milk, which are inactivated only by heat treatment (Chilliard et al., 2003).

There were difficulties in the separation of fat after the processing of the milk samples that were centrifuged at 8 °C (D, E, F and J, K, L, and for that reason, they were not considered appropriate.

The selected process for the skimmed of the goat milk was that was heat-treated at 63 °C, centrifuged at 9,800 rpm at the temperature of 4 °C for 15 minutes (H), in which the lipolysis value was lower (1.34 eq of FFA/mL milk) and fat separation was feasible. The results of chemical composition and microbiological characterization of goat milk is shown in Table 4.

Macronutrient content was similar to the reference values. The count of total aerobic mesophilic and coliform bacteria was 9×10^2 CFU/mL and 0 CFU/mL, respectively, values that are lower than the maximum allowed by Argentina Legislation (9×10^2 CFU/mL and 0 CFU/mL). These results indicate the good sanitary and hygienic quality of the milk (Kondyli et al., 2012) (Table 4).

3.2 Formulation of symbiotic yogurt

The non-fat yogurt is sensitive to mechanical defects since it has fewer dry substances. To stabilize consistency and to prevent syneresis in yogurt, a stabilizer of a hydrocolloid type (gelatin) was added (Spreer, 1991); inulin, which affects viscosity in a positive way, was also added in order to achieve high water retention capacity through hydrogen bonding with the yogurt proteins contributing to the formation of gel. A stable product was obtained at the temperature of 38 ± 1 °C, which resulted in yogurt with pH 4.5 (Martín-Diana et al., 2003) and a whipped consistency. The viability of lactic acid bacteria and probiotic culture is shown in Table 5.

The count of lactic bacteria ranged between 6×10^7 and 9×10^7 CFU mL^{-1}, values that are within those established by Argentina Legislation (10^7 CFU mL^{-1}) (Argentina, 1969)

With respect to the probiotic culture count of sample Lc25 was the only one that was between 10^6 to 10^9 CFU mL^{-1}, which guarantees its effectiveness and capacity to survive the digestive tract and spread in the intestine (Taranto et al., 2008; Mani-López et al., 2014).

Sample Lc25 had the largest probiotic culture count (3×10^7 CFU mL^{-1}), which ensures its effect and ability to survive the digestive tract and proliferate in the intestine (Taranto et al., 2008; Mani-López et al., 2014); therefore, this product was selected for further testing.

The values obtained were higher than those found by Salva et al. (2007), who obtained probiotic culture count of 1.6×10^6 CFU mL^{-1} in fermented goat milk, which shows the survival of L. casei in acidic medium.

3.3 Sensory analysis

Table 6 shows the results (%) of the acceptability test of potentially symbiotic non-fat yogurt The highest percentage obtained (35%) corresponds to the score 8 on the hedonic scale ("Like it"); the average score was 7.62 ± 1.24: 95% of the reviewers liked the product, 2% showed indifference, and the 3% remaining disliked it.

Table 4. Chemical composition and microbiological quality of pasteurized skimmed goat milk (Sample H).

	Pasteurized Skimmed Goat Milk (100 mL)
Moisture (g)	91.97 ± 0.35
Carbohydrates (g)	4.39 ± 0.00
Proteins (g)	2.63 ± 0.00
Fats (g)	0.10 ± 0.00
Ash (g)	0.91 ± 0.11
Calcium (mg)	163.57 ± 3.95
Phosphorus (mg)	108.26 ± 10.48
Aerobic Mesophilic	9×10^2 CFU mL^{-1}
Total Coliforms	0 CFU mL^{-1}

Means ± standard deviation (*n=3*). mL: milliliter.

Table 5. Viability of lactic acid bacteria and probiotic culture of a potentially symbiotic non-fat yogurt.

Samples	Lactic bacteria CFU mL^{-1}	Probiotic culture CFU mL^{-1}
Lc25	9×10^7	3×10^7
Lc10	6×10^7	8×10^5
Lc05	6×10^7	2.5×10^4

Table 6. Percentage of acceptability of potentially symbiotic non-fat yogurt.

Hedonic scale Points	Acceptability	Percentage (%)
9	Liked it very much	25
8	Liked it	35
7	Liked it regularly	27
6	Liked it little	8
5	Indifferent	2
4	Disliked it slightly	1
3	Disliked it regularly	2
2	Disliked it a lot	0
1	Disliked it very much	0
	Total	100
	Average score	7.62 ± 1.24

The consumer panel evaluated the samples for consistency, aroma and overall flavor. As for the taste, 72% of the panelists found it pleasant, 23% considered it acid, and 5% disliked it; 97% of the consumers considered the acidic taste as nice, and 94% considered consistency as adequate.

3.4 Chemical characterization and feasibility of starters and probiotic bacteria

Table 7 shows the physicochemical characteristics of potentially symbiotic non-fat yogurt. Moisture values and carbohydrates were similar to those observed by Quintana & Ramón (2007) (77.90 and 11.17 g/100 mL, respectively) in a fermented goat milk containing probiotic culture.

The inulin content was 2.15 g/100 mL; a 48% loss was observed compared to the initial content, which may be due to the fact that it is hydrolyzed into shorter chains and fructose, according to the conditions of temperature, acidity, time, and water activity (Coussement, 2010). A large peak in the chromatogram showed that inulin was hidrolyzed to fructose (Zuleta & Sambucetti, 2001). Moreover, inulin may have served as a substrate for maintaining the viability of L. casei Protein content was lower than the reference values, which could be due to its lower content in the goat milk evaluated. Fat content confirms that it is a nonfat product. The Ca/P ratio was 1.2:1. The recommended value is equal to or greater than 1, and when it is less than that, it becomes a triggering factor for bone loss (Teegarden et al., 1998) (Table 7).

The pH on day 1 was 4.5, but it decreased to 4.4 on day 7. This value was kept stable until day 21; this may be due to the fact that lactic bacteria stabilized between days 10 and 15 of storage (Minervini et al., 2009).

Dornic acidity was between 73 and 76 °D during the 21 days of storage, and it is within the range established by Argentina Legislation (60 – 150 °D) (Argentina, 1969).

The percentage of draining ranged between 0.00 and 0.38%. Yogurts made with inulin in general, do not exhibit syneresis during 21 days of refrigerated storage (4 ± 1 °C), because of the water retention capacity of this polysaccharide. This phenomenon is of great importance because the presence of exudate can cause consumer rejection (Minervini et al., 2009).

Table 7. Chemical composition of symbiotic non-fat yogurt made from goat milk.

	Symbiotic Yogurt (100 mL)
Moisture (g)	79.23 ± 0.05
Carbohydrates (g)	12.67 ± 0.00
Total Dietary Fiber (g)	3.04 ± 0.00
Inulin (g)	2.15 ± 0.00
Proteins (g)	4.04 ± 0.00
Fats (g)	0.04 ± 0.00
Ash (g)	0.98 ± 0.00
Calcium (mg)	114.80 ± 4.71
Phosphorus (mg)	94.20 ± 8.29
Total Calories	66 kcal

Means ± standard deviation (*n=3*). mL: milliliter; g: grams; mg: milligrams; kcal: kilocalories.

A study on the useful life and acceptability of a probiotic yogurt shows that it is does not undergo changes after 38 days of storage at 10 °C; thus, the sensory characteristics of the products are not modified (Minervini et al., 2009). If the product is kept refrigerated and with a pH no lesser than 4.2, the residual activity of the probiotic culture will be minimal, and its survival will be optimized. It is worth mentioning that a count higher than 10^7 CFU/mL will guarantee an adequate number of viable cells during the useful life of the product

4 Conclusions

The goat milk used showed good chemical and microbiological quality. The appropriate process conditions to obtain skim milk with a low level of lipolysis were: pasteurization at 63 °C for 30 minutes and centrifugation at 9,800 rpm at a temperature of 4 °C for 15 minutes. In addition, the temperature facilitated separation of fat.

Overall, this research shows the technological potential and adequacy of using goat milk to produce potentially symbiotic yogurt with good nutritional value with respect to protein, Ca/P, good sensory score and acceptability (95%) with good viability of lactic acid bacteria and probiotic culture. No flavors were associated to the goat milk, and satisfactory probiotic viability was maintained throughout the 21 days of refrigerated storage.

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