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Carmen.llenerar@ug.edu.ec

Correspondence:

Delactosed goat's milk fermented with probiotic cultures and oat fiber addition

Leche deslactosada de cabra y fermentada con cultivos probióticos con adición de fibra de avena

Carmen Llerena
Master's Degree, Universidad de Guayaquil,
Faculty of Chemical Engineering, Guayaquil,
Ecuador
carmen.llerenar@ug.edu. ec
https://orcid.org/0000-0003-4374-8599

Aldo Hernandez
Ph.D. University of Havana, Faculty of Pharmacy
and Food, Havana, Cuba.
hernandoaldo@uh.cu
https://orcid.org/0000-0002-4259-5589

Abstract

The objective of this thesis was to develop a lactose-free goat milk fermented with a mixture of probiotic cultures in co-culture with yogurt lactic bacteria, with the addition of oat fiber with good acceptability, probiotic characteristics and a shelf life that complies with Ecuadorian standards. During the research, goat milk was characterized, two types of enzymes were evaluated for the lactose delactosate and a mixture of cultures with probiotic strains with good characteristics was designed for the elaboration of the new product. For the development of the formulations, a cross-factor mixture design was used. The fermented milk developed presented as characteristics: microorganisms with gastric barrier resistance, pathogen inhibition, viability of 109 cfu/mL, "like" acceptability, less than 0.1 % lactose, 3.9 % protein, 8.6 % dietary fiber and rich in calcium. During storage at 4 ± 1 oC it maintained its characteristics up to 70 days. The production cost for the product in 250 mL bottles was $ 1.47. The economic feasibility of the artisanal production of the developed fermented lactose-free goat milk was demonstrated.

Key words: goat milk, probiotics, prebiotics, symbiotics, lactose free.

Resumen

Esta tesis tuvo como objetivo desarrollar una leche deslactosada de cabra y fermentada con mezcla de cultivos probióticos en cocultivo con las bacterias lácticas del yogur, con la adición de fibra de avena con buena aceptabilidad, características probióticas y una vida de almacenamiento que cumpla con lo establecido en la norma ecuatoriana. Durante la investigación se caracterizó la leche de cabra, se evaluaron dos tipos de enzimas para el deslactosado y se diseñó una mezcla de cultivos con cepas probióticas con buenas características para la elaboración del nuevo producto. Para el desarrollo de las formulaciones se realizó un diseño de mezcla con factor cruzado. La leche fermentada desarrollada

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presentó como características: microorganismos con resistencia a la barrera gástrica, inhibición de patógenos, viabilidad de 109 ufc/mL, una aceptabilidad de “me gusta”, menos de 0,1 % de lactosa, 3,9 % de proteína, 8,6 % de fibra dietética y rica en calcio. Durante el almacenamiento a 4 ± 1 oC mantuvo sus características hasta los 70 días. El costo de producción para el producto en botellas de 250 mL fue de $ 1,47. Se demostró la factibilidad económica de la producción artesanal de la leche deslactosada de cabra y fermentada desarrollada.

Palabras clave: Leche de cabra, probióticos, prebióticos, simbióticos, deslactosado

Introduction

Goat milk is a product that over the years has increased its popularity for its nutritional value, qualities, components and benefits in human health, currently in one of the primary components in the diet of millions of people Bidot & Muñoz (2017) and its breeding has transcended to the present day. Ecuadorian regulations (NTE INEN 2624, 2012) defines it as a product of the normal mammary secretion of a mother goat *(Capra spp.)* after no less than five days following parturition.

Goat milk offers an alternative for people who cannot tolerate cow's milk, because it has a chemical composition that makes it more digestible, however, certain nutrients such as copper and zinc are more digestible. Park, Juarez, Ramos, & Haenlein (2007)However, certain nutrients such as copper and zinc are lower, so it is not a substitute for either breast milk or formula milk. (North Carolina Department of Agriculture and consumer services, 2010) (Lastre, et al., 2020 p. 98).

It has been estimated that there are a total of 570 goat breeds in the world of which only 69 are for milk production. Of these, 36 (approximately 52 %) originate from Europe, 25 (37 %) originate from Asia and 8 (11 %) from Africa. goat milk is marketed in fluid form and also as yogurts, fresh and mature cheeses. One of the breeds that best adapts to tropical countries such as Central and South America, the Philippines, Malaysia and Africa, is the Anglo-Nubian, which is the result of crossing the Jamnapari breed from India and Saraibi from Egypt with the native British goat. It is characterized by producing 770 kg of milk in 270 days, which corresponds to approximately 2.8 L per day. (Pulina, et al., 2018).

In Ecuador, most of the goats are found in the Sierra region, in the provinces of Loja, Imbabura, Pichincha, Azuay and Chimborazo. (INEC-ESPAC, 2019)In the Coastal region, they are found in Guayas, Manabí, Santa Elena and Esmeralda.

The consumption of goat milk in Ecuador is given by the influence of popular culture that attributes nutraceutical properties to it. Among the main ones is the recommended consumption for people with cow's milk protein allergy (CMPA), because it contains less case of goat's milk.

(Bidot, 2017) because it contains less casein α s_1 and α lactalbumin. Another reason is its calcium content and immune system enhancement. (Bidot, 2017 p. 89).

The application of goat milk for obtaining yogurts or fermented milks has become an acceptable option to increase consumption, because it contains short chain fatty acids, the use of lactic acid bacteria (LAB) such as *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus para casei* among others, have favorable aspects because, during their growth and fermentation of goat milk, they cause partial hydrolysis of lactose and proteolysis of proteins producing small peptides and free amino acids, and also improve the health of the gastrointestinal tract. The use of LAB in yogurt is important because they contribute to the development of flavors and odors, improve rheological properties and allow the formation of bioactive peptides.

Another characteristic of the development of goat milk fermented with oat fiber is based on the fact that this type of fiber is mainly composed of beta-glucans, reported as effective ingredients to reduce LDL cholesterol in humans. Studies have shown that products fermented with oats or oat fiber present formation of exopolysaccharides, these compounds are able to develop texture and viscosity to the final products. (Lambo, Oste, & Nyman, 2005).

Materials and methods

Raw materials used in the research

Goat milk. Goat milk from the Anglo-Nubian crossbreed with Creole from the Young Living farm located in Chongón - Guayaquil - Ecuador was used. The milk was obtained from healthy females and was delivered in 6 lots, applying the sampling method for three months. (NTE INEN 4, 2012) for three months. The goat milk must comply with the indicators described for raw milk and pasteurized milk. (NTE INEN 2624, 2012) and for pasteurized milk (NTE INEN 2623, 2012)

Probiotic cultures. For the preparation of the cultures, pure probiotic strains of: *Lactobacillus acidophilus* LA - 5*, Bifidobacterium bifidum* ABY-3, *Lactobacillus paracaseissp. Paracasei, Lactobacillus bulgaricus YC-380,* and *Streptococcus thermophilus YC-380,* supplied by the firm CHR Hansen.

Enzyme. Safera Pure 2600 L, from the commercial firm Novozymes.

Oat fiber. The product HF 200 with 96 % dietary fiber from the commercial firm Dannova Química was used.

Lactose measurement

Lactose was determined by the determination of sugars by HPLC (NOM 155 SCFI, 2012) with an Agilent 1260 HPLC equipment with refractive index detector. The column used was Amino Si-(CH2)3-NH2 for sugars (normal phase) 5 µm- 46-150 mm, with a mobile phase composed of hexane and isopropanol.

Control methods used in fermentation and in fermented milk

The fermentation process was controlled through the measurement of pH. (NTE INEN 1087, 1973)titratable acidity (NTE INEN 13, 1973) every hour. The fermented product was evaluated for ash (AOAC 923.03, 2005), dietary fiber (AOAC 2011.25, 2011) calcium (AOAC 985.35 , 2016). The viscosity of the finished product was measured in accordance with the standard (A.S.T.M-D1439-03, 2005)The viscosity of the finished product was measured according to the standard, in a Brookfield digital viscometer model DV, a sample volume of 800 mL was used and the measurements were made at 20 °C with the number 2 rod at a rotation speed of 20 min^{-1} .

Microbiological analysis of fermented milk

According to the standard for fermented milk (NTE INEN 2395, 2011) The following were analyzed: total coliforms (NTE INEN 1529-7, 1990)*E. coli* count (NTE INEN 1529-8, 1990)count, molds - yeasts (NTE INEN 1529-10, 1990) and the probiotic m.o. content was evaluated.

Evaluation of the viability of probiotic microorganisms.

For the quantification of lactic acid bacteria, dilutions from 10^{-6} to 10^{-8} were performed in MRS broth and were used for *Lactobacillus* agar MRS, for *Streptococcus* agar M17 and for *Bifidum* agar Bifidum. Plates were incubated inverted at 37° C for 48 h, under anaerobic conditions. (De Man, Rogosa, & Sharpe, 1960), (Guimarães, Brugnera, & Abreu, 2013)Counts were expressed in cfu/mL.

pH and bile tolerance under gastrointestinal conditions *in vitro*.

For the acidity barrier, tests were performed at different pH values (3 and 2) with an exposure of 2 h, the pH adjustment was made with 6 M HCl. Two replicates were performed in the first test using McFarland 0.5 standards with an equivalent of 10^8 to 10^9 cfu/mL. (Rivas & Rivero, 2009)**.**

For the analysis of resistance to bile salts the concentration of bile used was 0.3% with a 2 h exposure, the study was carried out according to the methodology used by Lara & Burgos, (2012). LAB were multiplied in MRS broth with 0.3% ox bile. The cultures were incubated at

37 °C for 2 h, the results were expressed as cfu/g (Rivas & Rivero, 2009), (Guimarães, Brugnera, & Abreu, 2013).. The equations for calculating the percentage of acid and bile resistance are as follows:

$$Resistencia\ \acute{a}cidos\ (\%) = \left(\frac{\log(ufc\ .mL\ pH\ 2)}{\log(ufc\ .mL\ inicial)}\right)\ x\ 100\ \text{(Ecuación 4)}$$

$$Resistencia\ bilis\ (\%) = \left(\frac{\log(ufc\ .mL\ 0{,}3\ bilis)}{\log(ufc\ .mL\ inicial)}\right)\ x\ 100\ \text{(Ecuación 5)}$$

Strains are classified as follows: resistant (R) above 68 %; tolerant (T) between 34 and 66.9 % and sensitive (S) below 33.9 %. (Vera, Ormaza, Muñoz, Arteaga, & Sanchez, 2018).

Pathogen inhibition

It consists of an inhibition or resistance test to pathogenic microorganisms such as *Staphylococcus aureus* ATCC 36862, *Escherichia coli* ATCC 10536, *Listeria monocytogenes* ATCC 10536 and *Salmonella enteritidis* ATCC 13076. They are incubated for 24 h at 37 ± 1 ºC and the complete diameter, including the well, is measured. (Rentero, 2009), (Collado & Salminen, 2007). The results are interpreted as follows: resistant (R) when the halo is less than or equal to 11 mm; intermediate (I) halo from 11 to 13 mm and sensitive (S) when the halo is greater than or equal to 14 mm.

Sensory evaluation

Quantitative descriptive test

The evaluation was carried out by trained yogurt tasters, for the selection of fermented milk, attribute intensity descriptors were used, and the characteristic herbal taste and smell of goat's milk was presented as a descriptor.

Population acceptance test

To evaluate the acceptance of the final formulation, a seven-point hedonic scalar test was applied, ranging from extremely like to extremely dislike. (Espinosa J. , 2014)The seven-point hedonic scalar test, ranging from extremely like to extremely dislike, was used to evaluate 90 potential consumers.

Design of lactose-free and fermented goat milk formulations with the addition of oat fiber.

The experimental unit was 2 kg of lactose-free goat milk, standardized to 3 % fat and pasteurized at 85º C for 30 min for each of the treatments. The selected mixture of probiotic cultures of *L. acidophilus, B. bifidum, L. paracasei was* used, which were combined in various concentrations from 84 to 90 % in co-culture with the yoghurt LAB.

For fermented milk, a cross-mix design was proposed using the Design Expert version 8.0.6.1 program. Oat fiber (4 to 10 %), mixed culture proportion (84 to 90 %), milk mass (87 to 95 %) and culture dose (1 to 3 %) were considered as independent variables. As response variables, fermentation time (until reaching 60ºD), viability of probiotic microorganisms and acceptability were taken. Annex 2 shows the experimental design matrix.

The final formulations were evaluated for pH and bile tolerance under *in vitro* gastrointestinal conditions, pathogen inhibition, population acceptability, sensory descriptive profile, nutritional composition, fatty acid profile, fiber content, lactose, calcium and sodium content was determined by atomic absorption Spectra equipment model 220 Fast sequential with sodium lamp (AOAC 985.35, 2005) and with magnesium lamp (AOAC , 1999).

Result

Annex 3 shows the results of acceptability, viability and fermentation time of the proposed mixtures. The acceptability of the formulations was liked with the exception of formulation seven, which obtained a rating of neither liked nor disliked. The result of the analysis of variance showed that this response variable did not present significant differences ($p \leq 0.05$) among the 28 mixtures or formulations, so it could not be taken as a selection criterion.

The viability [log(cfu/mL)] of the formulations moved in the interval between 8.1 and 9.5, a result that can be qualified as very good because it complies with the regulations in force for this type of product. When an analysis of variance was performed, this indicator did not show significant differences ($p \leq 0.05$), so it could not be used to discriminate the best formulation.

The fermentation time of the 28 formulations presented significant differences ($p \leq 0.05$), this variation was 300 to 480 min, which shows the incidence of yogurt bacteria in improving the fermentation process of goat milk. This decrease in the fermentation time with the incorporation of yogurt bacteria and oat fiber in the culture mixture is of vital importance to be able to introduce this research result at pilot or industrial scale, due to the symbiotic effect. (Coronel, 2018). It is noteworthy that the variable proportion of the culture mixture does not influence the fermentation time, which means that it could be worked in any of the proposed proportions, this result was given by the great incidence that the culture dose presented in the fermentation time and the narrow interval in which the yogurt culture proportion moved (from 10 to 16 %).

The fermented milk with greater than 10^8 (log (cfu/ mL)) and fermentation time less than 300 min, the following formulation was obtained: oat dose 10 %, culture 3 %, milk mass 87 % and the proportion of mixed culture 89.7 %.

Table 1 and 2 show the results of the gastric barrier and microbial antagonism tests performed on the selected fermented milk formulation. The microorganisms of the fermented milk withstood the simulated gastric barrier of pH equal to 3 and bile concentration 3 %, they maintained a high survival with a viability close to the therapeutic minimum of 10^7 cfu/mL. The antimicrobial capacity of the probiotic bacteria in the fermented milk at 24 hours showed sensitivity for all the study strains.

Table 1 *Ability of the microorganisms of the fermented milk formulation to withstand the gastric barrier in vitro*

Counting conditions	**Feasibility [log (cfu/mL)].**	**Survival of microorganisms (%)**
Initial	9,14 (0,3)	-
pH value 2	5,1 (0,1)	55,7
pH value 3	6,3 (0,1)	68,9
Bile at 0.3%.	7,5 (0,3)	82

Table 2 *Antimicrobial capacity of the microbiota of fermented milk against pathogenic bacteria*

Halo diameters (mm)			
S. aureus	*E. coli*	*L. monocytogenes*	*Salmonella*
		11,8	12,6

The population acceptability of delactosed and fermented goat milk as a final product is presented graphically in Figure 1.

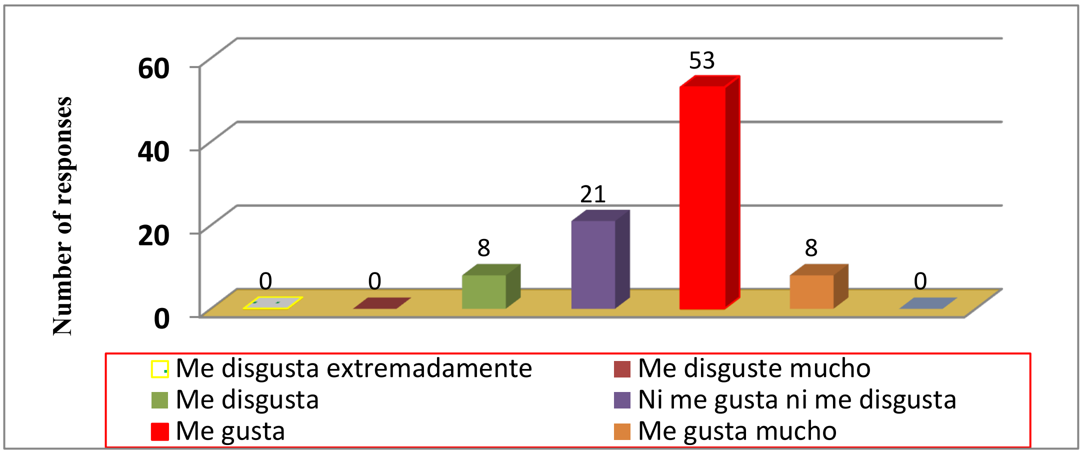

Figure 1. Behavior of responses according to degree of intensity of liking.

The rating score was 5, which corresponds to an evaluation of "I like it". Acceptance was 67.8%, which can be considered very good considering that no sugar was added and that it is a new product that the population is not used to consuming.

Figure 2 shows the result of the quantitative descriptive analysis of the selected formulation of fermented milk, including the herbal flavor and odor because goat's milk is used and because it includes oats, the color ranges from white to cream. As can be seen, the fermented milk was characterized by relevant descriptors such as the cream color (proper), given by the oat fiber that distinguishes it from a product without fiber, pronounced viscous aspect that is also due to the oat fiber for its content in β-glucans. Interesting was how homogeneity predominated over graininess despite the incorporation of a high dose of oat fiber. The fermented milk odor and flavor were well balanced, followed by the lower acid odor due to the composition of the culture mixture used.

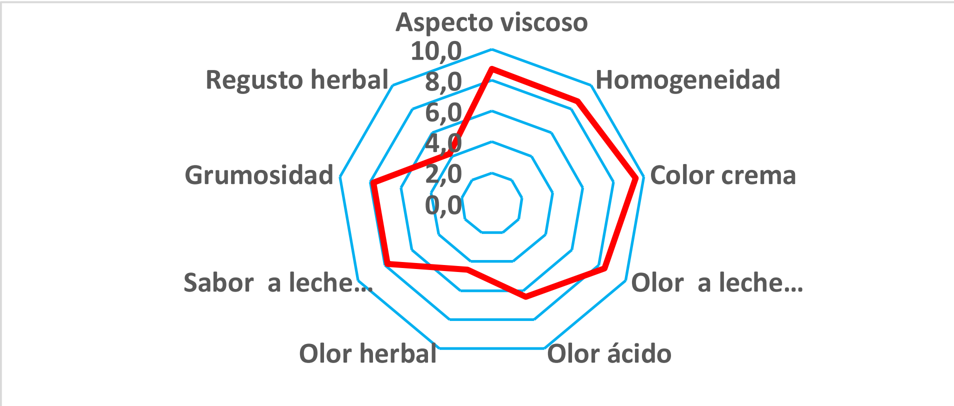

Figure 2. Results of the quantitative descriptive analysis of the selected fermented and lactose-free goat milk.

The macronutrient content of fermented goat milk is shown in Table 3.

Table 3. *Macronutrient content of goat milk.*

Indicator	Average value (g/100g)
Water	72,79
Grease	2,78
Protein	4,2
Carbohydrates	10,8
Lactose	Less than 0.01
Glucose	2,0
Ash	0,79
Fiber	8,64

Fat appeared lower in standardized milk due to the addition of oat fiber, the protein content of the formulation is slightly lower (3.90 g/100 g) than that of the initial standardized goat milk (4.2 g/100 g), this is due to the addition of oat fiber, total sugars are given by the hydrolysis of lactose into glucose and galactose in the fermented milk was obtained from 2.0 to 2.8 % of glucose and the decrease of lactose was effective in 99.9 %.According to Oliveira (2014), after hydrolyzing lactose, the metabolism becomes different according to the species of BAL, lactic acid is the main product, the acidity reached is 60 ºDornic and the pH is in a range of 4.5 to 4.6 at 24 hours of fermentation, the fiber content is in accordance with the proportion of oat fiber added. The cholesterol content of this product was 0.89 mg/ 100, the energy of 348.19 kJ/100 g (72.54 (kcalories)/100g) and the viscosity of 1 200 mPa.s the same is within the established ranges for this type of product and depends on the adjustment of non-fat solids, denaturation of proteins, the amount of inoculum added, Makinen (2015) worked with lactose-free cow's milk with other cereals and concluded that the relationship of milk with other cereal extracts has great potential given its rheological behavior, for its ability to form gels in acidic environments. (Makinen, Uniacke - Lowe, O'Mahony, & Arentdt, 2015) (Tamine & Robinson, 2000), (Lee & Lecey, 2010), (Coronel, 2018). The fatty acid profile of fresh fermented goat milk is shown in Table 4.

Table 4. *Fatty acid profile of fresh fermented goat milk*

Fatty acids	Type	Concentration (g/100 g)
Saturated fatty acids (%)	Caproico (C6:00)	2,10
	Capricorn (C10:00)	9,83
	Caprylic (C8:00)	2,84
	Lauric (C12:00)	5,04
	Myristic (C14:00)	9,74
	Palmitic (C16:00)	2,99
	Stearic (C18:00)	11,40
Mono unsaturated (%)	Oleic (C18:1)	2,42
Polyunsaturated (PUFA) (%)	Linoleic (C18:2)	0,26
	Linolenic acid (C18:3)	0,51

It is important to note that saturated fatty acids from C4 to C10 play roles in the cholesterol balance (Voblikova, Permyakov, Rostova, & Eliseeva, 2020), (Sumarmono,Sulisryowati; Soenarto 2015). Ebringer et al. (2008) explains that milk fat is not only a source of bioactive lipids, it also serves as a vehicle for nutrients such as fat-soluble vitamins, Lauric acid is known for its anti-carcinogenic effect, caprylic and caprylic acid may be associated with antiviral activity, (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018)Linoleic and linolenic acids are present in smaller quantities in fermented milk but are important because they are considered to be anticarcinogenic, antitumor, etc. (Garcia, Rovira, Boutoial, & Lopez, 2014). From a nutritional point of view, the most important fatty acids in fermented milk are palmitic, stearic, oleic, linoleic and linolenic acids, all of which are present in fermented goat milk. (Ebringer, Ferencik, & Krajcovic, 2008) (Coronel, 2018). The results of the microbiological quality of the fermented milk analyzed at 24 h comply with current regulations. (NTE INEN 2395, 2011).

The counts of probiotic microorganisms in the fermented milk are shown in Table 5.

Table 5. *Count of probiotic microorganisms in fermented milk*

Product	Requirement of the standard [log (cfu/g)	Results [log (cfu/g)
Lactobacillus		8,6 (0,3)
Streptococcus termophylus		8,6 (0,5)
Bifidobacteriun. bifidum		6,8 (0,7)
Total count of microorganisms		9.1 (calculation)

The results were higher than the specific values established in the standard for fermented milks without post-fermentation heat treatment, so they can be qualified as very good. (NTE INEN 2395, 2011)The results were higher than the specific values established in the standard for fermented milks without post-fermentation heat treatment, so they can be qualified as very good.

Goat milk fermented with probiotics in co-culture with yogurt LAB and oat fiber is considered a food that offers therapeutic and nutritional characteristics beneficial to health. The fact that it does not contain lactose allows it to be consumed by the public with lactose intolerance, also because it is not of bovine origin it does not cause allergies to cow's milk protein (Tamine & Robinson, 2000) (Bidot, 2017).

The product developed presents three probiotic bacteria (*Bifidobacterium bifidum, L. acidophilus, L. casei)* in co-culture with yogurt bacteria *(S. thermophilus, and L.. bulgaricus)*, which have demonstrated their probiotic characteristics and can therefore be considered a functional food. Each of the microorganisms offer health benefits such as: they regulate the composition of microorganisms in the intestine, the genus Bifidum in particular reduces the activity of nitroreductase and ß-glucoronidase, enzymes involved in colon cancer. They also allow the assimilation of cholesterol in the presence of trihydroxy conjugated bile salts by precipitation and assimilation. They stimulate cytokinin production and synthesize proteins, reduce ammonium concentration (which promotes colon cancer). *L. casei* can protect against diarrhea caused by *Salmonella and E. coli*. The product is considered a complete food due to the content of proteins, fats, carbohydrates, fiber and calcium. Annex 5 shows the macronutrient intake of fermented milk with a daily intake of 250 g compared to the daily nutritional requirements recommended by the (WHO-FAO, 2003). It can be observed that the consumption of a portion of 250 g of fermented milk gives a good contribution of the necessary daily protein and fiber; the contribution in fat is low, but the saturated fatty acids of short, medium and long chain that it contains facilitate the digestion of these fats without the problem of the agglutinin of the cow's milk that tends to join the fat globules, the 26.6 % of contribution of the necessary sugars are already in disposition in the form of glucose and galactose.

The physical-chemical composition of the fermented milk, lactose-free with oat fiber, presents high viability [log(cfu/mL)], this result indicates that the mixture of probiotic cultures in coculture with yogurt bacteria did not present antagonism, which is consistent with what was reported in the elaboration of fermented goat milk with *L. casei* and *L. acidophilus* in coculture with yogurt bacteria. Hernández, Torres, Duarte, & Rodríguez, (2016).. It could be observed at 24h that the *B. bifidum* count is lower compared to the growth of *Lactobacillus and S. thermophilus* used in fermentation (see appendix 6). This coincides with what was observed by Heller (2001), who mentions that when probiotics are added at the same time with the starter cultures, in this case the LAB of yogurt, the content of viable cells of bifidobacteria decreases, possibly due to inhibitory substances elaborated by. *L. delbrueckii spp bulgaricus and S. thermophilus.*

Fermentation time was influenced by microorganisms interacting with their environment by exchanging components of the medium for products of their metabolism. Essential variables are the available carbon source (type and amount), the degree of hydrolysis of proteins as a source of essential a.a. , which decreases the fermentation time. (Champagne, Da Cruz, & Daga, 2018), (Saarela, Fonden, Matto, & Mattila-Sandholm, 2000). The variables that influenced the decrease in fermentation time were the culture dose with the highest weight followed by oat fiber. It is noteworthy that the culture mixture ratio variable was not included, which means that any of the proposed ratios could be used.

Conclusions

Regarding the acceptance of the product by presenting a mixture of probiotic cultures with the LAB of yogurt, the odor and herbal aftertaste remained at a very low intensity, which differentiates it from other fermented goat milks in which these descriptors have been reported as a defect as in the case of Guerrero & Gamarra, (2006) with goat and cow milk, which used *Bifidumbacterium lactis, S. thermophilus, L. thermophilus and Lcidophilus and the product maintained the characteristic flavor of goat milk, which was considered a defect. thermophilus, L acidophilus and* the product maintained the characteristic flavor of goat milk which was considered as a defect. The fermented milk presented probiotic characteristics according to its viability, *in vitro* gastric barrier behavior and microbial antagonism against pathogenic bacteria such as *E. coli, Salmonella, S. aeurus, L. monocytogenes*.

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ANNEXES

Annex 1

Descriptive sensory analysis sheet of the product

Test the sample and indicate by means of a mark (X) on the line, the perceived intensity in each attribute.

Attribute	Attribute detail	Interval scale	Scale concept
Texture	Consistency/ viscosity	0 1 2 3 4 5 6 7 8 9 10	0 = Low viscosity 10= Very Viscous
	Homogeneity	0 1 2 3 4 5 6 7 8 9 10	0 = Non-homogeneous appearance 10= Homogeneous appearance
	Granularity	0 1 2 3 4 5 6 7 8 9 10	0 = Absence of lumps 10= Presence of lumps
Color	Uniformity/ typicity	0 1 2 3 4 5 6 7 8 9 10	0 = Tends to white 10= Tends to cream color
Odor	Characteristic odor of fermented milk	0 1 2 3 4 5 6 7 8 9 10	0 = Little noticeable odor 10= Very noticeable odor
	Acid odor	0 1 2 3 4 5 6 7 8 9 10	0 = weak odor 10= intense odor
	Herbal odor	0 1 2 3 4 5 6 7 8 9 10	0 = herbal absent 10= herbal present
Taste	Characteristic fermented milk flavor	0 1 2 3 4 5 6 7 8 9 10	0 = Flavor not very noticeable 10= Very appreciable flavor
	Herbal aftertaste	0 1 2 3 4 5 6 7 8 9 10	0= Absent 10=Intense
	Acidity	0 1 2 3 4 5 6 7 8 9 10	0 = Weak acidity 10= Intense acidity

Annex 2

Formulation	Oat fiber (%)	Milk (%)	Mixed cultivation (%)	Crop dose (%)
1	4,00	95,00	87,00	1,00
	4,00	93,00	90,00	3,00
	4,00	93,00	87,00	3,00
	7,00	91,00	87,00	2,00
5	5,50	92,50	85,50	2,00
	4,00	94,00	90,00	2,00
	10,00	89,00	87,00	1,00
	4,00	95,00	85,50	1,00
	4,00	94,00	84,00	2,00
	10,00	89,00	90,00	1,00
	4,00	95,00	90,00	1,00
	10,00	87,00	87,00	3,00
	10,00	87,00	84,00	3,00
	4,00	95,00	84,00	1,00
	4,00	93,00	84,00	3,00
	7,00	92,00	84,00	1,00
	10,00	89,00	84,00	1,00
	8,50	89,00	88,50	2,50
	7,00	92,00	90,00	1,00
	5,50	92,50	88,50	2,00
	4,00	93,00	84,00	3,00
	4,00	93,00	90,00	3,00
	8,50	89,00	85,50	2,50
	10,00	89,00	84,00	1,00
	4,00	93,00	87,00	3,00
	10,00	87,00	90,00	3,00
	10,00	88,00	87,00	2,00
	10,00	89,00	90,00	1,00

Annex 3

Design results of mixtures of pasteurized, lactose-free goat milk with a probiotic

Formula	Oat fiber (%)	Crop dose (%)	Milk mass (%)	Crop mix ratio (%)	Acceptability (points)	Feasibility [log(cfu/mL)].	Fermentation time (min)
1	4,00	1,00	95,00	87,00	4,9 (0,3)	9,1 (0,3)	
	4,00	3,00	93,00	90,00	5,0 (0,5)	9,5 (0,3)	
	4,00	3,00	93,00	87,00	4,9 (0,5)	9,3 (0,0)	
	7,00	2,00	91,00	87,00	5,0 (0,3)	9,4 (0,6)	
5	5,50	2,00	92,50	85,50	4,9 (0,5)	9,9 (0,4)	
	4,00	2,00	94,00	90,00	5,1 (0,5)	9,3 (0,2)	390
	10,00	1,00	89,00	87,00	4,5 (0,2)	9,1 (0,5)	390
	4,00	1,00	95,00	85,50	4,9 (0,5)	9,0 (0,7)	
	4,00	2,00	94,00	84,00	5,3 (0,9)	9,1 (0,6)	420
	10,00	1,00	89,00	90,00	4,7 (0,5)	8,3 (0,5)	420
	4,00	1,00	95,00	90,00	4,7 (0,4)	8,6 (0,9)	
	10,00	3,00	87,00	87,00	4,8 (0,6)	8,0 (0,7)	240
	10,00	3,00	87,00	84,00	4,9 (0,5)	8,1 (0,8)	240
	4,00	1,00	95,00	84,00	4,8 (0,3)	9,0 (0,6)	
	4,00	3,00	93,00	84,00	5,1 (0,5)	8,8 (0,6)	
	7,00	1,00	92,00	84,00	5,0 (0,4)	8,9 (0,7)	
	10,00	1,00	89,00	84,00	5,3 (0,5)	8,2 (0,4)	
	8,50	2,50	89,00	88,50	5,1 (0,3)	8,1 (0,4)	
	7,00	1,00	92,00	90,00	5,0 (0,6)	9,3 (0,8)	
	5,50	2,00	92,50	88,50	5,1 (0,4)	8,8 (0,6)	
	4,00	3,00	93,00	84,00	5,0 (0,3)	9,0 (0,6)	
	4,00	3,00	93,00	90,00	5,0 (0,2)	9,2 (0,6)	
	8,50	2,50	89,00	85,50	4,9 (0,4)	9,2 (0,5)	
	10,00	1,00	89,00	84,00	5,1 (0,3)	9,2 (0,6)	
	4,00	3,00	93,00	87,00	4,5 (0,5)	9,1 (0,8)	
	10,00	3,00	87,00	90,00	4,6 (0,2)	8,9 (0,4)	
	10,00	2,00	88,00	87,00	4,8 (0,2)	9,2 (0,4)	
28	10,00	1,00	89,00	90,00	4,9 (0,3)	9,3 (0,4)	

culture mixture in co-culture with yogurt LAB and oat fiber.

Annex 4

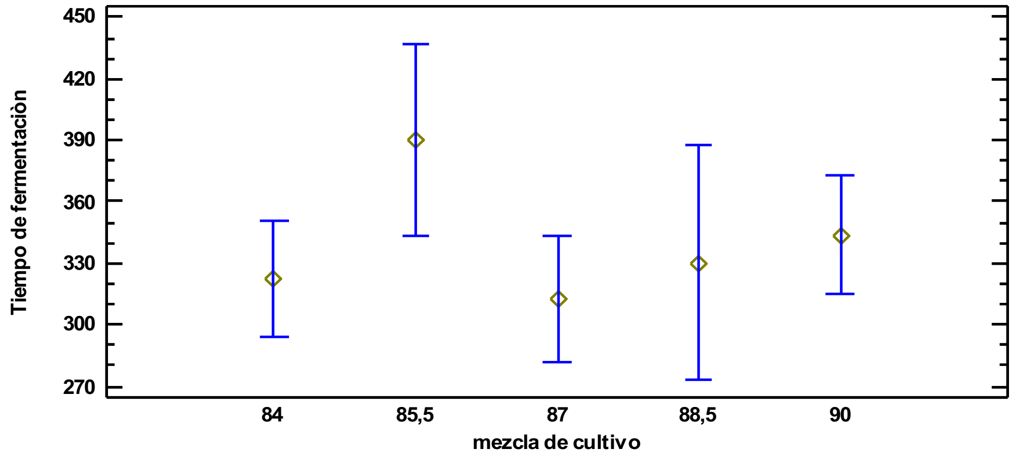

Figure of fermentation time behavior in relation to culture mix ratio.

Annex 5. Macronutrient intake of fermented milk with a daily intake of 250 g.

Indicators	Average value g/100 g	CRD WHO - FAO (g)	Amount contributed per portion of 250 g (g)	Average contribution to the CRD (%)
Protein	3,90	10 a 15	9,75	
Grease	2,78	15 a 30	6,95	12,3
Total sugars	6,93	55 a 75	17,32	26,6
Dietary fiber	8,64		21,6	86,4
kcal	72,54	2 000		2,8

Legend CRD- Recommended daily allowance

Annex 6
Log (cfu/ml) of growth of *Lactobacillus, S. Thermophilus and B. bifidum*