



Fermentation Parameters of Suero Costeño Elaborated with Lactic Acid Bacteria Strains

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Abstract

Studying the fermentation parameters of "Suero Costeño" was the focus of this research, considered a traditional product in the Caribbean region of Colombia, inoculated with lactic acid bacteria *Lactococcus Lactis* subsp. *Lactis* (ATCC29146) and *Lactobacillus paracasei* subsp. *paracasei*(ATCC 334). It was found that the percentage of lactose decreased progressively during the hours of fermentation, with a minimum lactose value of 3.1% at 15 h for the samples of Suero Costeño with *L. lactis*, while for *L. paracasei* the percentage of lactose was 3.4%. *L. paracasei* the percentage of lactose was 3.4%; on the other hand, the pH, which is a limiting factor in the fermentation process, was reported to have a considerable reduction in the samples with *L. lactis* in comparison with *L. paracasei*, at the end of the fermentation process at 15 h, with values of 4.3 and 4.5, respectively. Finally, the sensory evaluation allowed establishing that the Suero Costeño with *L. lactis* obtained similar values to that produced in a traditional way, and therefore its use can be recommended in this type of dairy products as a starter culture.



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Introduction

Milk is one of the world's most productive and important foods, providing key nutrients and is considered an important energy source of essential nutrients, which can help achieve food security, strengthen the economy and alleviate poverty.¹

Compared to cow's milk, buffalo milk has exceptional nutritional characteristics, such as higher fat, protein, lactose and some minerals (calcium, iron, magnesium and phosphorus), lower cholesterol levels and almost twice the content of conjugated linoleic acid.^{2,3}

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In addition, their higher casein, and fat contents are able to provide the final products with a better gel consistency, and more creaminess, respectively.⁴ Considering that buffaloes are raised in many parts of the world and are adaptable to different climates, the consumption of buffalo milk is not popular in Colombia compared to cow's milk, and the production of buffalo dairy products, such as Suero Costeño, can help increase demand for buffalo milk, as well as generate income for farmers.

Suero Costeño is made mainly with cow's milk, but it is also produced with goat's and buffalo's milk. It is a sour cream that is made by farmers in the Colombian Caribbean Region and other departments^{5,6} and in some municipalities of the department of Santander and represents a cultural heritage of the Coast,^{7,8} it presents a thick viscous consistency, caused by the concentration of total solids such as protein and fat, as a consequence of lactic coagulation, with the addition of sodium chloride.⁷

To obtain Suero Costeño, the first stage used is the fermentation of milk. Fermentation is an ancient method used to preserve food. When food is fermented, it is difficult to contaminate, and even more so when antimicrobial is added.⁹ In addition, this type of food improves sensory properties such as flavor and texture, and imparts a desirable characteristic to consumers.¹⁰ Today fermentation has evolved beyond food preservation to become a tool to create sensory attributes such as flavor, aroma, texture and, improve nutritional and functional aspects.^{11, 12, 13, 14, 15} Cultures used in the fermentation of dairy products play an important role in the fermentation of lactose and the production of lactic acid.¹⁶ This acid produces an improvement in sensory properties such as flavor, aroma, texture and consistency; it also increases shelf life because the pH is reduced and inhibits pathogenic microorganisms present in the food.^{17,18} Likewise, research concludes that during the fermentation process, lactic acid bacteria (LAB) also produce bioactive compounds such as peptides, immunoglobulins, among others, which have beneficial contributions to health.^{19, 20, 21}

They are available on the market in various forms, such as capsules, powders or fermented milk or food products. FAO and WHO describe probiotic organisms and probiotic foods as safe agents. The most commonly used are fermented milk products,

since milk contains the fundamental nutrients for the growth of beneficial microorganisms.^{22, 23}

Therefore, the objective of this research was to study the parameters of the fermentation process of Suero Costeño, elaborated with lactic acid bacteria strains: *Lactococcus Lactis* and *Lactobacillus paracasei* in order to select the most promising starter culture.

Materials and Methods

In this research we worked with lactic acid bacteria (LAB) collection strains, *Lactococcus Lactis* subsp. *lactis* (ATCC@29146™, Virginia, USA) and *Lactobacillus paracasei* subsp. *paracasei* (ATCC@334™, Virginia, USA). The selection was made based on the results obtained in previous isolation, selection and activation of strains applied to "Suero Costeño" produced by artisanal production.²⁴ Activation of the strains from the cryopreserved pure culture was carried out by transferring 1 mL of the culture to a test tube containing 9 mL of MRS culture medium, special for lactic acid bacteria; subsequently, the inoculated test tube was incubated for 24 h at 30 °C. After the incubation time, each of the bacteria was surface-seeded in Petri dishes with Agar-MRS (Scharlab, Spain) modified with aniline blue (medium in which the colonies of lactic acid bacteria have a characteristic blue color) and incubated for 48 h at 30 °C.

Next, purity was checked, and morphological characteristics were observed under the microscope by first focusing with the 10x and 40x objective, and then read with the immersion objective (100 X). One colony was taken from each test tube with MRS, and these were transferred to tubes containing 9 mL of milk, and incubated at 30 °C for 24 h. Finally, all cultures were adjusted to a concentration of 10⁸ CFU/mL.^{25, 26, 27} Suero Costeño was obtained from a farm in El Carmen de Bolívar and an industrially produced one purchased at a local supermarket. The Suero Costeño was obtained with the purpose of being compared for syneresis, yield and sensory analysis.

Preparation of Suero Costeño

The raw buffalo milk was obtained in the Municipality of El Carmen de Bolívar. Was pasteurized at 80 °C, for 20 min: then two samples of 300 mL of milk were inoculated at 30 °C for each microorganism *L. lactis* and *L. paracasei* (10% v/v of *L. lactis* and *L.*

paracasei) strains in a 30 L capacity fermenter, more information can be found in Yacub *et al.*, (2016).²⁸ It was incubated at the fermentation temperature of 30 °C for 15 h; after the clot was obtained, 30% of the whey was broken and separated, in relation to the initial volume of milk, by passing it through a filter; 0.12 % of sodium chloride (NaCl) was added, per 100 mL of curd and homogenized, to obtain a suitable consistency; finally, the yield was calculated.²⁹ The artisanal Suero Costeño was purchased at a neighborhood store, and the industrial commercial Suero Costeño was purchased at a local supermarket.

Fermentation kinetics and Determination of Lactose

The pH measurement was performed using a potentiometer (CAL CHECK™ HI 9126, Rhode Island, USA) (Official Method 973,41), then calibrating standard buffers of pH 4.0 and 7.030. The absorbance was measured at 610 nm in the UV-VIS spectrophotometer, 4255. The data obtained were correlated with the sugar concentration through the calibration line, previously made in the laboratory.

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Determination of Syneresis, Yield and Sensory Analysis

After two days of refrigerated storage at 5 °C, the samples of Suero Costeño were shaken for 2 min, at 400 rpm, with a magnetic stirrer and, subsequently, they were centrifuged at 5000 rpm at 20 °C for 15 min (HettichMikro 22R). The syneresis was expressed as a percentage, calculated as the amount of liquid separated from the Suero Costeño due to centrifugation in relation to the total mass that was centrifuged; this same procedure was performed on a sample of commercial Suero Costeño and was compared with those obtained previously³⁴. Yield was determined as the ratio between grams of Suero Costeño produced and grams of milk used to produce it multiplied by 100.

Samples of Suero Costeño were analyzed: artisanal, industrial commercial (purchased in a Supermarket) and inoculated with *L. lactis* and *L. paracasei*. The sensory analysis was carried out with a group of thirty trained people, attributes such as: flavor, color, texture, aroma and general acceptance were evaluated, using a 5-point hedonic scale; where 1 was the lowest rating and 5 was the highest rating.

For syneresis, yield and sensory analysis, the Suero Costeño made from these two microbial cultures were compared with Suero Costeño made in an artisanal way in El Carmen de Bolívar, and one made in an industrial commercial (purchased in a Supermarket) way from buffalo milk.

Statistical Analysis

The triplicate data of Suero Costeño were processed in the statistical package STATGRAPHICS Centurión XVI.I®, performing an analysis of variance (ANOVA), and when statistical differences were found ($p < 0.05$); Fisher's method (LSD) was applied with an error rate of 0.0535.

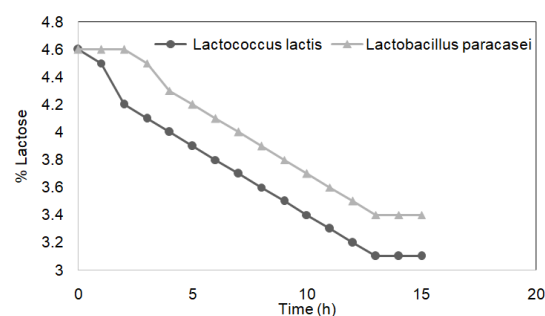


Fig.1: Lactose % decrease in the fermentation of Suero Costeño inoculated with *Lactococcus lactis* and *Lactobacillus paracasei*

Results and Discussion

Fermentation Process

The initial lactose concentration was 4.6%, as shown in Figure 1; decreasing significantly during the 12 h of fermentation, at the end a minimum value of 3.1% was obtained at 15 h for the Suero Costeño samples with *L. lactis* while for *L. paracasei* the lactose percentage was 3.4%; the decrease in the lactose percentage is explained by the metabolism of Lactic acid bacteriato obtain energy.³⁶ Several dairy products with similar lactose values to Suero Costeño were observed, such as skimmed natural yogurt (3.7%), cheese slices, condensed whole milk (5%).³⁷ Taking into account the loss of lactose due to consumption by the microorganisms for the production of lactic acid, and also during the manufacturing process, especially that which remains in the whey due to the solubilization of this reducing sugar, the final percentage is considerable in Suero Costeño. these data coincide with those obtained by Simanca *et al.*, (2010)⁷ who reported a decrease in lactose content, reaching a value of 4.44%,

consumed by the microorganisms responsible for the fermentation process. Approximately 98% of the lactose is eliminated in the whey as lactose or lactate during the production of Suero Costeño.³⁸

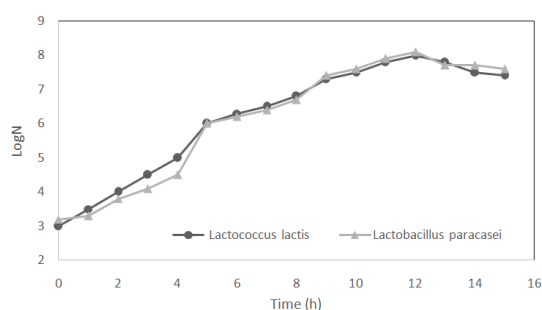


Fig.2: Viability by standard surface plate count technique on MRS for Suero Costeño inoculated with *Lactococcus lactis* and *Lactobacillus paracasei*

The viability of BAL strains in Suero Costeño has been studied and it has been found that the genus *L. lactis* has better results.^{39,40} The rate of decrease in lactose concentration is high, as is the viability (Figure 2), values that coincide with the research of Patrignani *et al.*, (2006)⁴¹ who studied a fermented milk product, using starter cultures of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus paracasei* and *Lactococcus Lactis* for its production. The reduction observed with the addition of the two lactic microorganisms was probably caused by the presence of acids, bacteriocins, peroxide hydrogen and flavor compounds that affected the cell vitality and viability of the bacteria studied.⁴² Microbiological analyses performed by Yu *et al.*, (2015)⁴³ on different fermented dairy foods showed the presence of lactic acid bacteria at levels ranging from 3.18 to 5.30 log cfu/mL, 7.30 to 8.53 log cfu/mL, 7.16 to 9.05 log cfu/g, and 7.06 to 9.23 log cfu/mL, for 3 fermented mare's milk samples, 6 sour cream samples, 8 cheese samples, and 24 fermented milk samples respectively. Fermented cow milks from two different regions, observed that the presence of *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Lactobacillus sakei* were significantly lower than those from other regions ($p < 0.001$) and *Lactobacillus sakei* counts were significantly higher than those from other regions ($p < 0.05$).

It is known that pH is a limiting factor in the fermentation process of dairy products.⁴⁴ In this research, a greater pH reduction of the Suero

Costeño samples with *L. lactis* compared to *L. paracasei* was presented during the first 7 h (Figure 3). The Suero Costeño samples reached a pH of 4.6 in the time interval from 8 to 10 h, the isoelectric point of the caseins, so the acidification rate was higher than that reported for the artisanal fermentation of Suero Costeño, which was 22 h.³⁴

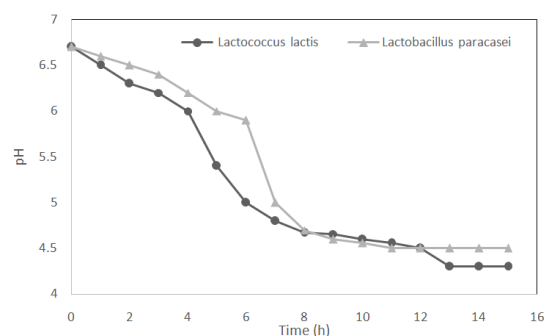


Fig.3: pH decrease in the fermentation of Suero Costeño inoculated with *Lactococcus lactis* and *Lactobacillus paracasei*

The Suero Costeño samples with *L. paracasei* behaved almost constantly after 10 h; therefore, the *L. lactis* inoculum present in the Suero Costeño decreased the pH of the samples more rapidly. At the end of the fermentation process at 15 h, values of 4.3 and 4.5 were obtained (*L. lactis* and *L. paracasei*, respectively).

It has been stated that the strains suitable for the formulation of starter cultures are those that produce a pH lower than 5.4, after 6 h of incubation at 30 °C, since they would be ensuring a correct acidification and on the other hand, avoiding the proliferation of undesirable microorganisms.⁴⁵ Analyzing these results, it can be affirmed that the variation of pH at the end of fermentation could be mainly due to the activity of the starter culture used. Bondarchuk (2018)⁴⁶ during the production of sour butter, from cream fermentation; they observed a change in titratable acidity and pH during cream fermentation. They found that during the first 2 hours of fermentation at 8 °C and then at 21 °C for 1 h, the initial pH (6.5 units) and titratable acidity (17°T) remained the same, which may be due to adaptation of the cells to the medium. During the following 21-22 h of cream fermentation, pH decreased by 5.15-5.20 units.

Syneresis and Yield Analysis

No significant differences were observed between the Suero Costeño samples prepared with *L. lactis* and *L. paracasei* cultures for syneresis and yield; however, with respect to industrial commercial whey, there were statistical differences as shown in Table 1 ($p < 0.05$). The control of syneresis is essential because it determines the moisture content which,

in turn, produces effects on the feed.⁴⁷ Hassan *et al.*, (2017)⁴⁸ observed higher syneresis values of sour cream, both in its fresh state and during cold storage, because it released a high value of whey, in the range of 2.6 ml to 3 ml. This could be due to the different total solids contents (mainly protein), and also to the presence or absence of stabilizers.

Table 1: Syneresis and yield of Suero Costeño inoculated with *Lactococcus lactis*, *Lactobacillus paracasei* and a commercial one. Different letters indicate statistical differences in the same row ($p < 0.05$)

	<i>Lactococcus Lactis</i>	Artisanal	<i>Lactobacillus paracasei</i>	Industrial commercial
Syneresis (%)	16.12 ± 0.23 ^a	26.83 ± 0.87 ^b	18.15 ± 0.41 ^a	26.20 ± 0.24 ^b
Yield (%)	50.25 ± 0.34 ^a	44.57 ± 0.45 ^b	49.33 ± 0.32 ^a	43.45 ± 0.31 ^b

Acevedo *et al.*²⁷ obtained similar results in the syneresis of Suero Costeño, stating that it was significantly affected by the level of total solids, and by the interaction of the variables between temperature and total solids. These researchers stated that, syneresis increased as temperature increased and decreased as total solids increased. It should be noted that as temperature increases,

syneresis is accelerated, due to the relaxation of the bonds and denaturation of the proteins that form the casein network.^{27, 49} Also, Tirado *et al.*, (2018)⁵⁰ analyzed by scanning electron microscopy that the coastal whey produced with low protein content, produced higher porosity and syneresis. In contrast, as the protein content increased, the porosity of the coastal whey decreased as did the syneresis.

Table 2: Sensory analysis of the samples of Suero Costeño: commercial, artisanal, inoculated with *Lactococcus lactis* and *Lactobacillus paracasei*. Different letters indicate statistical differences in the same row ($p < 0.05$)

Sample	Flavor	Color	Texture	Aroma	General Acceptance
<i>Lactococcus lactis</i>	4.3 ± 0.25 ^c	4.2 ± 0.10 ^b	4.6 ± 0.21 ^b	4.5 ± 0.19 ^c	4.4 ± 0.17 ^c
<i>Lactobacillus paracasei</i>	3.8 ± 0.21 ^b	4.2 ± 0.09 ^b	3.8 ± 0.10 ^a	3.9 ± 0.08 ^b	3.8 ± 0.05 ^b
Artisanal	4.5 ± 0.19 ^c	4.3 ± 0.12 ^b	4.5 ± 0.15 ^b	4.5 ± 0.16 ^c	4.5 ± 0.10 ^c
Commercial	3.4 ± 0.29 ^a	4.0 ± 0.05 ^a	3.8 ± 0.22 ^a	3.6 ± 0.21 ^a	3.5 ± 0.12 ^a

Sensory Analysis

The sensory analysis performed on the samples of artisanal Suero Costeño was similar to that inoculated with *L. lactis* as starter culture. Isolated strains of *Lactococcus lactis* subsp. *Lactis* have been identified in fresh artisanal goat's milk cheese, and it was observed that this type of strains contributed to the development of sensory characteristics.⁵¹

The formation of flavors and odors depends on the proteolytic system, since Lactic acid bacteria partially degrade caseins generating free peptides, which are

hydrolyzed to amino acids by the combined action of peptidases.⁵² The *Lactococcus* genus of Lactic acid bacteria has been reported as a flavor promoter, generating small amounts of acetaldehyde and large amounts of diacetyl in sour cream.⁵³ Shepard *et al.*, (2015)⁵⁴ stated that the factors that most influence consumer acceptance and preference in fermented milk cream are milk fat, cooked aromatic flavors, opacity, color intensity and viscosity.

On the other hand, the commercial Suero Costeño was the least preferred with a general acceptance of

3.5 points, compared to the artisanal Suero Costeño and the one inoculated with *Lactococcus lactis*, which showed values of 4.5 and 4.4, respectively. This can be explained by the industrial techniques used in the production and storage process, which affect some main sensory attributes. An important parameter to determine the quality of food is through sensory analysis. Salem *et al.*, (2015)⁵⁵ observed that the addition of Moringa oleifera leaves extract and Moringa oleifera oil, had a significant effect on flavor, body texture and total score of Sour Creams inoculated with 2% starter of *L. lactis* spp. *Lactis* and *L. lactis* spp. In addition, it improved flavor with no adverse effect on quality.

Pasteurization can eliminate part of the natural microflora of raw milk and consequently affect sensory characteristics.⁵⁶ In the same order, it has been reported that dairy products made with pasteurized milk have less flavor than those made with raw milk due to the reduction in the number of Lactic acid bacteria during the thermal process.⁵³

Conclusions

The use of *Lactococcus Lactis* as starter culture is recommended in the manufacture of Suero Costeño with pasteurized milk, considering that it obtained the best fermentation characteristics. These findings

can help manufacturers to produce sour creams with optimized sensory properties by modifying starter cultures and processing procedures. Suero Costeño, being a product with prebiotic characteristics, can be used for other types of dairy fermentation, due to the advantages of having this type of microorganisms, considered as a functional food; in addition, it is an important point that can imply in the selection by consumers.

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Conflict of Interest

The author(s) declares no conflict of interest.

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