Indigenous microorganisms for the elaboration of lactic cultures for specific use in "suero costeño"

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ABSTRACT

Suero Costeño (SC) is a fermented milk product, traditionally made in the Colombian Caribbean Region with native microorganisms. Due to its poorly controlled processes, it has undesirable microbiological and quality characteristics during storage, commercialization, and consumption. Directed fermentation processes with the usage of specific microorganisms help to improve fermented products characteristics. A mixture design using three types of indigenous lactic-acid Bacteria (LAB) was proposed in the Suero Costeño (SC) production process on a laboratory scale. The mixture that yielded the best sensory and physicochemical characteristics were selected for its scale-up on an artisan producer scale. A naturally fermented SC produced by an artisan producer was used as a control sample (SCC). The selected mixture contained *L. plantarum* 66.7%, and *L. lactis* 33.3%. This sample showed more stability during storage and the best attributes of sensory quality. Finally, the product made under the conditions of the artisan producer with this mixture had better quality and physicochemical properties than the SCC. Still, the fermentation time was longer, probably, due to the microorganism diversity that exists in natural fermentation processes, and the stress caused by freezing the strains.

Keywords: suero costeño, lactic acid bacteria, fermentation, *lactobacillus plantarum, streptococcus infantarius, lactococcus lactis.*

1 INTRODUCTION

Fermented products represent between 10 to 40% of the world diet and constitute a cultural and gastronomic heritage of high value (Talon and Zagorec, 2017). Within the traditionally fermented dairy

products, Suero Costeño (SC) is a traditional product made in the Colombian Atlantic Coast. SC is produced through the natural fermentation of raw milk due to the action of indigenous microorganisms (Cueto et al., 2007). This product has a good general acceptance. However, some of these products are reported to have unsafe microbiological and quality characteristics for storage, commercialization, and consumption, due to poorly controlled processes of processing (Granados et al., 2012) (Chams et al., 2012).

The pasteurization process and the use of starter cultures in the elaboration of dairy products, have been broadly used in the food industry (Simanca et al., 2010)(Cueto et al., 2007). However, these treatments do not have a broad sensory acceptance when used to make some products (Acevedo et al., 2013)(Granados et al., 2012). For instance, Colombian Artisanal SC is preferred over industrially produced SC. Probably, these differences are due to the diversity among the communities of microorganisms involved in fermentation, which develop under different environmental influences and exhibit variable metabolic properties (Eisenbrand et al., 2010).

The group of lactic acid bacteria (LAB) are some of the microbial communities encountered in artisanal fermented foods. These bacteria provide a series of physicochemical and sensory properties that give particular characteristics to the final products. Furthermore, the LAB has antagonical responses against undesirable microorganisms, and in some cases, functional properties attributed to its probiotic capacity (Quigley, 2019). Therefore, the potential use of these microorganisms as starter cultures for fermentation processes is of great interest (Eisenbrand et al., 2010) (Hati et al., 2013).

Mixture design for the elaboration of different products in the food industry has attracted attention among the producers. This methodology enables producers to carry out combinations of raw materials to look for new functional characteristics and optimize their use to improve the processes and thus obtain higher quality products (Salamanca et al., 2015)(Puente et al., 2015). However, this methodology has been little reported to define combinations of microbial strains that can be used as starter cultures for specific artisanal products. Additionally, the use of this strategy allows to visualize the individual effect of each type of microorganisms and in binary and tertiary mixtures to propose starter cultures, which facilitates obtaining more accurate results with a focus on resolving the immediate needs of consumers (Puente et al., 2015).

Therefore, this paper presents the evaluation of a mix design to obtain a ferment of indigenous Lactic Acid Bacteria (LAB) with application in the production of a fermented milk product, such as Suero Costeño (SC), in a laboratory and producer scale.

2 MATERIALS AND METHODS

2.1 MICROORGANISMS AND CHEMICALS

LAB strains: *Lactobacillus plantarum* (60-1), *Streptococcus infantarius* (46-3) and *Lactococcus lactis* (34-3), isolated from SC from the Colombian Caribbean Region (7°59'05 "N; 75°11'53"W) were identified in previous works, and they were deposited in the Sequence Read Archive (SRA) of the NCBI (https://www.ncbi.nlm.nih.gov/sra)(Motato et al., 2017). They were cryopreserved in the laboratory of the Biotransformation group at -80 °C. The culture medium used to activate the strain of *L. plantarum* was the MRS broth (Man, Rogosa, and Sharpe, MERCK) and MRS agar (Man, Rogosa, and Sharpe, MERCK) was used for the counts. For *S. infantarius* and *L. lactis*, BHI broth (MERCK) supplemented with glucose (13.20 g/L) (Biosystems) was used, and the counts were performed on M17 agar (Oxoid) + 1% Lactose (Merck). All cultures were incubated under micro-anaerobic conditions. The skimmed milk powder and the whole pasteurized liquid milk were purchased in local stores.

2.2 DESIGN OF MIXTURES OF INDIGENOUS LACTIC CULTURES

To establish the proportions of microorganisms to be used in the different scales of work, an experimental design of "Simplex Laticce" mixtures was proposed using the Design Expert 10 statistical program. Three LAB strains previously referenced were used in proportions that started from a minimum of 0% and a maximum of 2% of inoculum for the preparation of SC. However, for design purposes, this range was taken as 0 and 100%, respectively. The total number of experiments performed was 17, including repetitions of the central and axial points.

2.3 PREPARATION OF PRE-INOCULA

Each microorganism pre-inoculum was added as indicated in the design of mixtures, to obtain the total inoculum for each experiment. The total amount of inoculum added in each one was 300 mL (2%). To this end, pasteurized skim milk was reconstituted with sterile distilled water at 11% w/v in each batch. In the case of *L. plantarum*, the milk was supplemented at 1% w/v, with hydrolyzed casein. Subsequently, flasks of supplemented milk were subjected to a thermal treatment of 90 °C for 10 min and placed on ice for a thermal shock for 10 min. Then, the flasks were stored at room temperature to be inoculated the next day with the microorganisms. The Pre-inocula were incubated at the growth temperature of each type of microorganism overnight (32 °C for *L. plantarum*, 37 °C for *S. infantarius*, and 32 °C for *L. lactis*).

2.4 ELABORATION OF SUERO COSTEÑO AT LABORATORY SCALE.

For each treatment, 15 L of pasteurized liquid whole milk were used, which were heated to 35 ± 2 °C and inoculated at 2% v/v. The microorganisms were added separately in the indicated proportion.

Subsequently, a manual homogenization was performed, seeking an adequate distribution of each of the pre-inocula in the milk. The batches of milk were fermented at room temperature 25 ± 3 °C for 24 hours. After the fermentation time, the product was subjected to a manual dewatering process by gravity at room temperature (25 ± 5 °C) between 8-10 hours. The obtained dried product was salted at 1% (w/w), duly homogenized, and finally packed in plastic containers. Then, samples of the same product were taken every four days, starting at the initial day. These samples were stored at 4 °C to perform microbiological, physicochemical, and sensory analyses to evaluate the products.

The samples presenting significative differences in the physicochemical (Acidity, pH, Moisture, texture, and color) properties for these products were discarded. The determinations of organic compounds (HPLC and gas chromatography) were carried out only to the mixtures selected as candidates for starter cultures at producer scale.

2.5 ELABORATION OF SUERO COSTEÑO AT PRODUCER SCALE.

Based on the laboratory scale tests, one of the mixtures was selected to make an SC similar to the original product. Additionally, A naturally fermented SC produced by an artisan producer was taken as a control sample (SCC). The Suero Costeño was elaborated in one of the farms located in La Apartada (Córdoba), where the microbial strains were initially isolated (Motato et al., 2017). In this phase, three treatments were carried out: (1) Raw whole milk and 10% of natural inoculum "backslopping" (control) (SP1); (2) Pasteurized milk and 10% natural inoculum "backslopping" (SP2); (3) Pasteurized milk and 10% of the mixture obtained in the laboratory phase (SP3). All assemblies were carried out by the artisanal producer under the supervision of the project's research staff. The fermentation and maturation processes were followed as usual, and the kinetics of pH was evaluated in the three treatments. Two samples were taken at the beginning and the end of the fermentation. The samples with the different treatments were maintained at room temperature (24 ± 6 ° C) for 24 - 48h. During that time, the pH conditions were monitored to one of the batches of each treatment.

After breaking the clot, the curd was separated, and 1.2% salt was added. The product was homogenized in an electric blender and packaged in plastic containers. They were stored at 4 ± 2 °C and transported to the laboratory where the different physicochemical analyzes (acidity, pH, moisture, ash, protein, fat, and sugars) were carried out. Additionally, the organic compounds (volatile) were analyzed, and the results were compared with the selected SC, obtained at the laboratory scale. Each treatment was performed in triplicate

2.6 ANALYTICAL TECHNIQUES

Physicochemical analyzes. The titratable acidity determination was carried out according to the norm NTC 4978 and was expressed in% of lactic acid (%p/p). The pH, according to the AOAC method 981.12 and the percentage of moisture, was carried out by the AOAC method 925.45.

Texture profile analysis (TPA). For this analysis, a TA-XT2i (Stable Micro Systems) texturometer was used, provided with a 50 kg load cell and a 0.5 mm diameter probe. The operating conditions were: pre-test speed 2 mm/s, test speed 10 mm/s, post-test speed 5 mm/s, and time between compression of 2 sec. The size of the container where they were packed was 6.4 cm in diameter and 4.4 cm in height. The TPA parameters were determined from the force (N) versus time (s) graph, supplied by the Texture Expert Exceed software, version 2.54. The parameters elasticity, cohesiveness and resilience (dimensionless) were considered (Valencia et al., 2007).

Color measurement. Color measurement was done using the X-Rite portable sphere spectrophotometer (model SP-64), and the D65 illuminant, observer 10º, with included specular, and a 4 mm observation window. The color coordinates CIE-L* a* b* were determined from the reflection spectra of the samples (Valencia et al., 2013). Where the reference parameters L* 0, a* 0, and b* 0 correspond to the standard sample (artisanal SC) of the product under evaluation. Three readings of each sample were made. The results of the polar coordinates of the color were plotted by Rhinoceros 3D software.

Plate counts of lactic acid bacteria. It *was performed according* to Motato, 2017, and the result was analyzed by exponential regression with the GraphPad Prism 5.0 software, with which the first order death-rate constant (*Kd*) was determined.

Sensory analysis. We included 15 habitual consumers of SC whose ages were between 18 to 40 years of age, which included students and teachers from the School of Microbiology of the University of Antioquia. A descriptive quantitative analysis was used, the scale used was from 1 to 5, with 5 being the most optimal feature of the attribute. The granularity was the only attribute evaluated with a reversed scale (A value of 1 represented a product with lower granularity).

The evaluated attributes were appearance (homogeneity and color), smell (intensity), texture in mouth (creaminess, softness, granularity, and fat), flavor (acid, sweet, spicy, bitter, salty and rancid), and finally, aftertaste (persistence and intensity). Samples were presented to consumers in disposable spoons marked with three-digit random numbers assigned in each test. It should be noted that among the tastings of the samples, each consumer should eat crackers to cleanse their palate.

Analysis of fatty acid profile by gas chromatography. This test was performed using a gas chromatograph coupled to masses (GC / MS HP 6890-5972A); with HP-5ms column of 30 m x 0.25 mm x 0.25 um. The analytical conditions were: Injector 220C, splitless mode, Detector 280C, Oven: 40C – 5

min, 5C/min 60C - 0min, 10C/min 200C – 10 min. Solid-phase microextraction (SPME) and manual injection with 30um fiber coated with polydimethylsiloxane were used to prepare the sample.

2.7 STATISTICAL ANALYSIS

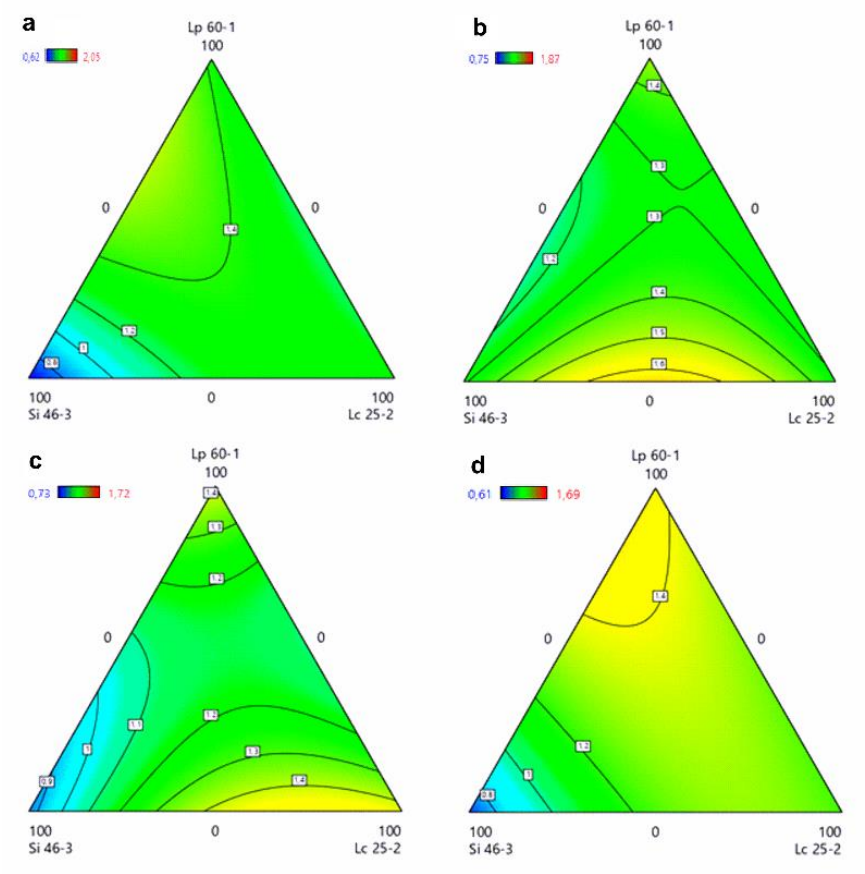
The effect of the acidity of the design of mixtures was analyzed with the statistical package Design Expert 1.0, using ANOVA. The statistical difference between treatments was determined using the 95% standard deviation limits (LSD). The other response variables were analyzed with the GraphPad Prism 5.0 software using 2-way ANOVA and the Bonferroni test to determine a significant difference between treatments.

3 RESULTS

3.1 PHYSICOCHEMICAL ANALYSIS OF SUERO COSTEÑO SAMPLES AT LABORATORY SCALE.

The effects of the different proportions of each strain of LAB in SC production at laboratory scale on acidity are showed (Fig.1). SC samples were significantly different at initial time (p-value > 0.05) (Fig. 1A). Acidity is higher when *L. plantarum* is used as an inoculum for SC production, with values of 1.44 ± 0.05% Lactic acid, which is within the reported parameters (> 1.4% Lactic acid) (Acevedo et al., 2012). Additionally, when the inoculum used contains a mixture of *L. plantarum* and *L. lactis*, acidity levels close to 1.4% Lactic acid are also observed. On the other hand, the presence of *S. infantarius* in the inoculum at higher proportions (> 33%) decreased the acidity of the sample at the initial stage of the fermentation process. Even though, *S. infantarius* is characterized as a fast fermenter, when in acidic environments (pH < 5.0) this microorganism's growth seems to be inhibited (Valencia et al., 2018).

Figure 1. Contour graphs of the acidity for the SC of the mix design with LAB strains, in different storage times. A: t0, B: t4, C: t8 and D: t12.



The sample analysis was performed during the 12 days of storage (Fig. 1A – D). These analyses indicated that the different treatments had a significant effect in the acidity of the samples, with values ranging between 1.36 ± 0.04 and 1.70 ± 0.01% w / w (Figures 1B-D). These values were within the range of values reported for these products (Acevedo et al., 2012). However, the SC inoculated with high proportions of *S. infantarius* did not reach the acid content reported in the literature. *Lactobacillus plantarum* is a microorganism that presents higher tolerance to acidity and acidification capacity (Motato et al., 2017). These characteristics allow it to predominate in adverse conditions. This resistance is critical to compete against other microorganisms, promoting control in the growth of foodborne pathogens (García et al., 2018) (De Bassi et al., 2012). Given that the acidity in a fermented product is one of the main physicochemical parameters that determines its quality, 6 mixtures were pre-selected as possible candidates for obtaining SC at the producer scale (Table 1).

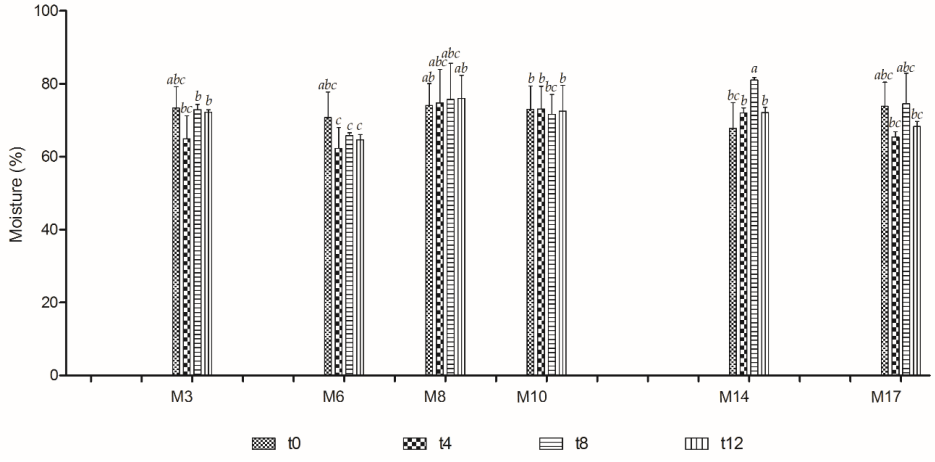
Table 1. Proportion of LAB present in the inoculum of the SC selected by the acidity criterion.

Mixture	Ratio		
	L. plantarum	***S. infantarius***	***L. lactis***
M3	0,5	0,5	2,0
M6	2,0	0,0	1,0
M8	1,0	1,0	1,0
M10	3,0	0,0	0,0
M14	2,0	1,0	0,0
M17	0,0	0,0	3,0

Regarding pH, all the SCs were below casein's isoelectric point of (pH: 4.6), responsible for the coagulation of the milk, presenting values between 3.9 and 4.5 (Data not shown). These values coincide with the reports of other authors (3.5-4.5) (González et al., 2016), (Batista, 2011).

Moisture analyses on the selected mixtures indicated that neither the time or its interaction with the type of mixture presented significant differences over the moisture. Only the type of mixture showed a significant effect. The minimum moisture value obtained was 62.6 ± 5.7% for SC with four days of storage (t4), inoculated with *L. plantarum* and *L. lactis* in a 2: 1 ratio. In contrast, the highest moisture content was presented in SC inoculated with *L. plantarum* and *S. infantarius* in a 2: 1 ratio after eight days of storage. Although this analysis is not a relevant parameter for the selection of the inoculum type, it is evident that the moisture of almost all the products is stable during storage (Fig. 2). Therefore, the samples that presented a more stable moisture content during storage were selected (M6, M8, M10, and M17) for further analyses.

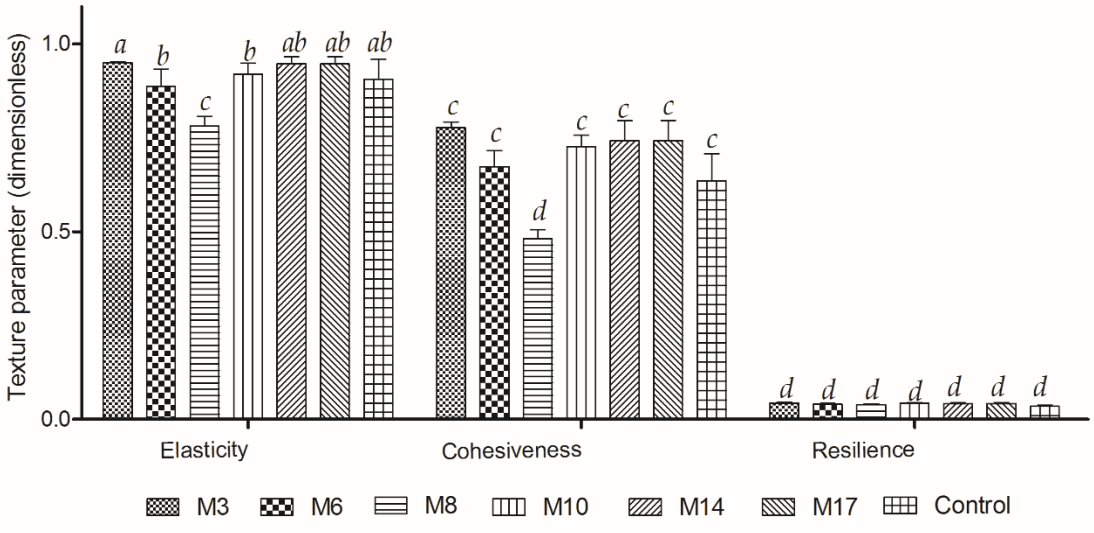
Figure 2. Moisture of SCs prepared with selected inocula. Different letters on the bars show significant difference between treatments (n = 3).



The results of elasticity, cohesiveness, and resilience (TPA) in SC of samples M3, M6, M8, M10, M14, and M17 are shown in Figure 3. These results indicated that the composition of the inoculum for the selected parameters showed significant differences between groups. The sample of SC inoculated in

equal proportions of *L. plantarum, S. infantarius, L. lactis* (M8), presented the lowest values of elasticity and cohesiveness (0.782 ± 0.025 and 0.481 ± 0.025, respectively) in comparison with the other treatments. The other mixtures presented similar behavior to the control sample. The above, probably due to the interaction of the three microorganisms on the nutritional components of milk, specifically fat and protein. Regarding resilience, there were no significant differences between the mixtures of LAB selected or concerning the controls.

Figure 3. Analysis of the texture parameters of the SC at the initial time (t0), elaborated with the selected inocula. Different letters on the bars show a significant difference between treatments (n = 3).

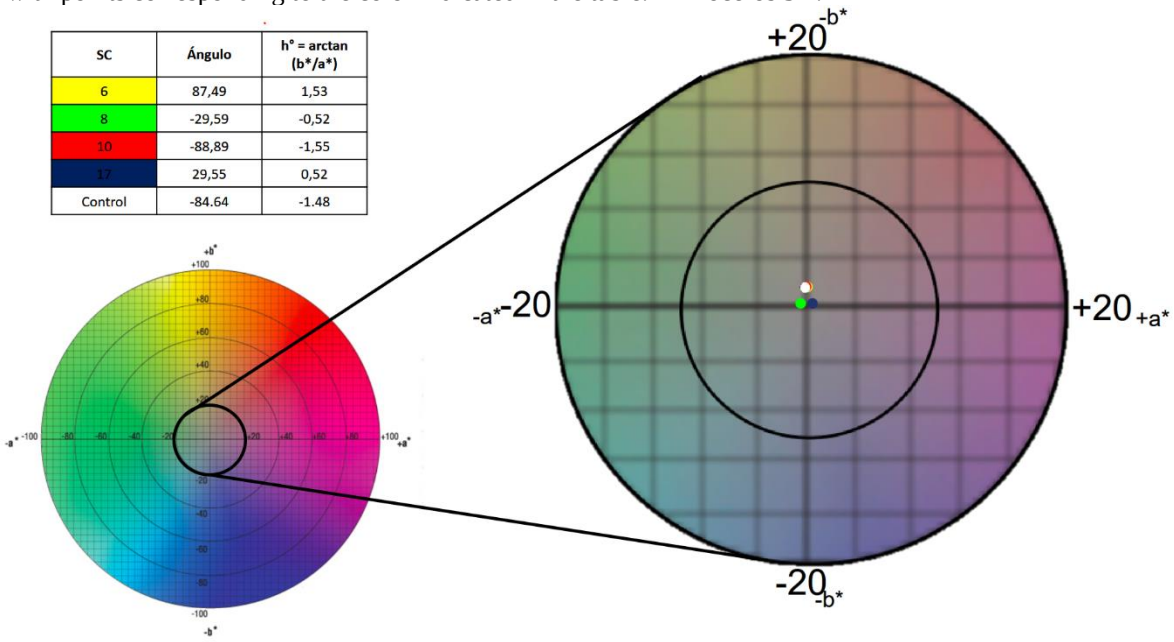


Possibly, the M8 sample had greater competition among the three microorganisms. These microorganisms could present higher enzymatic production, which favor the protein's hydrolysis. The hydrolysis would be reflected in the loss of cohesiveness and adhesiveness.

A rapid drop in the pH stimulates the demineralization of the curd. This demineralization alters the firmness of the dairy products (Morand et al., 2012). In addition, all SC were presented relatively low values in comparison to those obtained in studies of fresh cheeses (Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015)(Guzmán et al., 2015), (Guerrero et al., 2015), (Álvarez et al., 2015), (Acevedo et al., 2015), (Agudelo et al., 2015); what is explained, in the Suero Costeño, for being a product of semi-solid type that conserves a greater moisture. It has been reported that a high moisture content weakens the firmness of the structure due to continuous re-arrangements of the protein network and the distancing of the protein from each other (Guzmán et al., 2015).

The polar coordinate color analysis (Cab* and hab*) for the SC samples M6, M10, and M17 presented statistically significant differences (see Fig. 4).

Figure 4. Polar coordinate color (Cab* and hab*) of the mixtures of SC selected in the initial time (t0), represented in the diagram with points corresponding to the color indicated in the table. Rhinoceros 3D.



In general, all SC samples show a high luminosity (L *> 76), possibly due to the casein present in the milk that allows the reflection of light. The obtained total color (ΔE) values ranged between -0.73 and 0.96, values lower than the value of the visual perception of the human eye (2.0). The chromatic coordinate b * with ranges between 7.84 and 9.27, showed the presence of yellow tones, possibly attributed to the vitamin B produce by *L. plantarum* (M6 y M10), and it was different to the contribution of carotenoids present in the milk (Table 2).

Table 2. Volatile compounds presented from the CS obtained with selected inoculums at laboratory scale (M6, M8, M10, M17) and at producer scale (15L) (PS1, PS2, PS3).

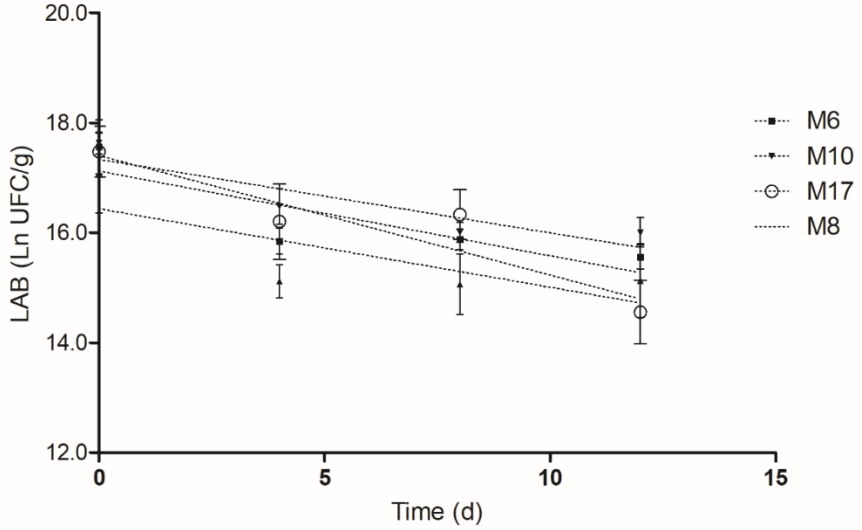
Compound	Tr (min)	Relative peak area (%)						
		M6	M8	M10	M17	PS1	PS2	PS3
Alcohols								
2,3-Butanediol	7.3	--	0.02 ± 0.01	--	0.03 ± 0.00	--	--	--
1,4-Butanediol	11,9	--	--	--	--	0.92 ± 0.05	0,89 ± 0.49	0.76 ± 0.27
Hexanol	9.4	0.01 ± 0.01	--	0.02 ± 0.01	3.10 ± 5.20	--	--	--
Furufural alcohc	9.4	--	0.01 ± 0.01	0.02 ± 0.01	--	--	--	--
Cetones								
Acetoin	5.4	0.03 ± 0.03	--	--	0.27 ± 0.17	--	--	--
Acetol	4.6	0.01 ± 0.00	--	0.02 ± 0.03	0.02 ± 0.03	11.33 ± 1.53	13.33 ± 2.31	11.67 ± 0.58
Acids								
Acetic acid	3.7	0.03 ± 0.03	0.09 ± 0.05	0.04 ± 0.04	0.01 ± 0.01	9.75 ± 2.64	6.60 ± 1.65	6.72 ± 3.12
Capric acid	19,1	--	4.70 ± 2.02	1.98 ± 0.82	--	5.12 ± 2.13	8.65 ± 2.21	6.32 ± 3.11
Butyric acid	9.0	1.73 ± 1.93	0.02 ± 0.01	0.01 ± 0.00	--	--	--	--
Aldehides								
Acetaldehyde	2.0	0.10 ± 0.03	4.26 ± 7.16	0.20 ± 0.12	0.01 ± 0.01	12.47 ± 4.25	18.30 ± 10.58	13.63 ± 6.11
Hexanal		--	0.11 ± 0.08	0.01 ± 0.01	--	--	--	--
3-Methyl butanal		0.01 ± 0.02	--	--	0.01 ± 0.00	--	--	--
Nonanal	15.0	--	0.04 ± 0.01	0.03 ± 0.03	--	6.02 ± 0.53	1.54 ± 2.67	3.62 ± 0.00
Octanal	14.5	--	0.01 ± 0.00	--	0.02 ± 0.01	--	--	--
Furfural	17.2	0.01 ± 0.02	0.02 ± 0.02	0.05 ± 0.01	0.02 ± 0.00	--	--	--
Other compounds								
Vitamin B	20.3	0.10 ± 0.15	--	0.08 ± 0.09	--	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
Dimethyl sulfide	11.2	0.01 ± 0.01	--	--	0.15 ± 0.12	0.37 ± 0.11	0.15 ± 0.03	0.15 ± 0.01
2,4-Toluene disc	18.7	0.02 ± 0.04	0.01 ± 0.01	0.04 ± 0.05	0.01 ± 0.02	0.59 ± 0.23	0.54 ± 0.15	0.37 ± 0.09

The means and standard errors of the identified compounds are expressed as a percentage of the relative peak areas. The means are derived from three repetition samples.

3.2 LAB COUNT IN SUERO COSTEÑO IN LABORATORY SCALE.

Microbial counts were performed during the 12 days of storage (at 4°C) to evaluate the viability of LAB in the selected SC samples (M6, M8, M10, and M17) (Fig. 5). Although all selected samples presented a decrease in the microbial load throughout the storage time, the concentration of LAB was maintained in the order of 10^6 CFU / g for day 12. The lowest values of death constant (Kd) were presented in the samples elaborated with a single species of LAB: *L. plantarum* or *L. lactis*, with values of -0.216 ± 0.022 d-1 and -0.246 ± 0.048 d-1, respectively.

Figure 5. Exponential regression of the LAB count for the SC samples selected in the different storage times.



On the other hand, the SC elaborated with the mixture of both strains of microorganisms in proportion *L plantarum* - *L. lactis* 2: 1, showed a higher value of Kd: -0.357 ± 0.073 d-1 (M6), indicating that it maintains for a longer time a load of lactic microorganisms. This is an essential characteristic of fermented products to guarantee a sufficient number of viable microorganisms during the product's useful life. Although the M8 sample also presented this characteristic (Kd: -0.4610 ± 0.176 d-1), it was the only sample in which the most significant difference in texture was identified.

3.3 SENSORY ANALYSIS FOR SC AT LABORATORY SCALE.

The final product sensory analysis of each of the selected SCs and the control are presented in Table 3. For all the treatments, significant differences were obtained between descriptors, the type of mixture. The SC produced with the inoculum of *L. plantarum* and *L. lactis* in a 2: 1 ratio (M6), was the most similar to the control, in terms of homogeneity, smoothness, fatty, sweetness, bitterness, salty, rancidity, persistence and intensity. Therefore, this was selected as the starter inoculum at the producer scale.

Table 3. Sensory descriptors for the different SC samples selected at the laboratory level.

Descriptor	M6	M8	M10	M17	Control
Homogeneity	3.6 ± 1.2	3.7 ± 1.2	3.5 ± 0.9	3.5 ± 1.2	3.5 ± 1.5
White color	3.6 ± 1.0	3.7 ± 1.1	3.5 ± 0.9	3.5 ± 1.0	4.6 ± 0.7
Intensity	3.1 ± 1.2	2.9 ± 1.0	3.1 ± 1.0	2.7 ± 0.9	1.9 ± 0.8
Creaminess	4.2 ± 0.7	4.6 ± 0.5	3.9 ± 0.7	4.4 ± 0.7	1.8 ± 0.8
Smoothness	3.8 ± 1.1	4.2 ± 1.1	3.6 ± 0.8	3.9 ± 0.8	4.0 ± 1.2
Granularity	1.8 ± 0.8	2.3 ± 1.5	2.3 ± 1.0	1.5 ± 0.9	3.3 ± 1.2
Fatty	2.8 ± 1.1	2.9 ± 1.2	2.3 ± 1.2	2.7 ± 1.1	3.0 ± 0.8
Acidity	3.4 ± 0.9	3.7 ± 1.0	3.4 ± 0.7	2.9 ± 0.7	1.8 ± 0.9
Sweetness	1.4 ± 0.4	1.9 ± 1.2	1.5 ± 0.9	1.3 ± 0.5	1.0 ± 0.7
Spicy	1.7 ± 0.9	1.9 ± 1.4	1.8 ± 1.2	1.7 ± 0.8	1.0 ± 0.2
Bitterness	1.8 ± 1.0	2.1 ± 1.5	1.6 ± 1.1	1.7 ± 0.8	1.6 ± 0.6
Salty	2.4 ± 1.2	2.9 ± 1.4	2.7 ± 1.2	2.4 ± 1.0	3.0 ± 1.5
Rancidity	1.6 ± 0.8	1.7 ± 1.3	1.7 ± 1.0	1.4 ± 0.7	1.4 ± 0.5
Persistence	3.3 ± 0.9	3.5 ± 0.6	3.3 ± 0.9	3.0 ± 1.1	2.8 ± 0.8
Intensity	3.2 ± 1.0	3.1 ± 0.9	3.2 ± 1.2	2.8 ± 0.9	2.7 ± 1.1

The means and standard errors of the descriptors correspond to a sample size of 17 consumers.

The results of the mixture selection are summarized in Table 4. The mixture 6 (M6) had an inoculum proportion with 66.7% of *L plantarum* and 33.4% of *L. lactis* and was selected to produce SC at a producer scale.

Table 4. Results of physicochemical tests for the selection of the microorganism mixture.

Parameter	M3	M6	M8	M10	M14	M17
Acidity	✓	✓	✓	✓	✓	✓
pH	✓	✓	✓	✓	✓	✓
Humidity	X	✓	✓	✓	X	✓
Texture	-	✓	X	✓	-	✓
Color	-	✓	-	✓	-	X
Sensory	-	✓	-	X	-	X

(✓) Fulfillment of the characteristic. (X) Non-compliance. (-) Does not apply.

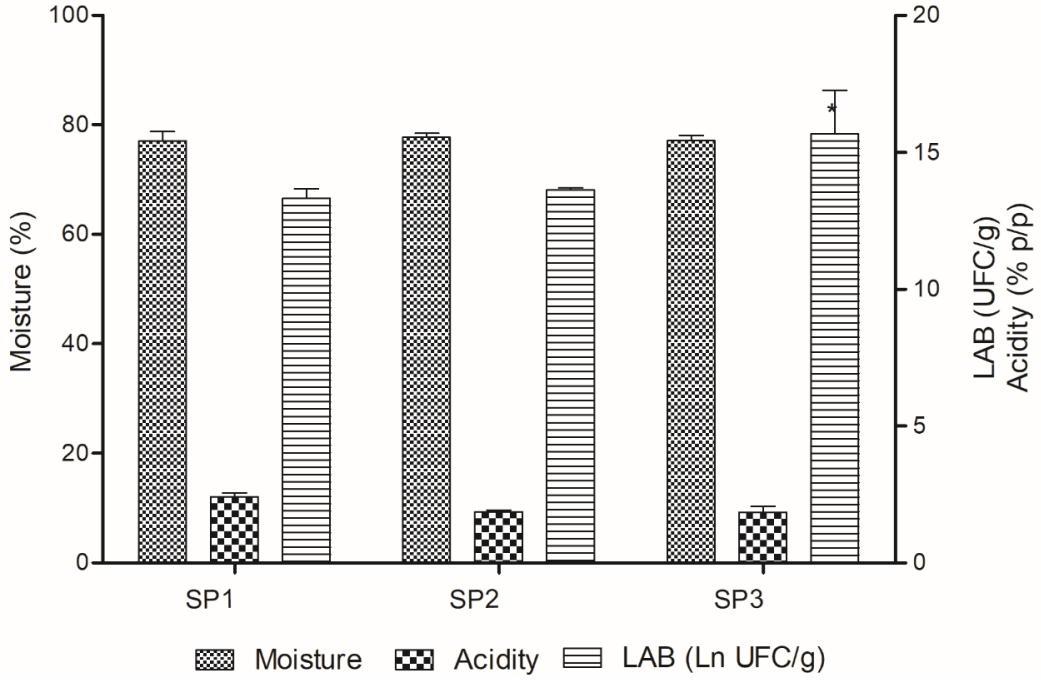
3.4 PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYZES OF SC AT THE PRODUCER SCALE.

A 10% v/v proportion of the selected mixture microorganisms was added to pasteurized whole milk (SP3). This mixture was compared with two controls (SP1 and SP2), as described in the methodology. The pH monitoring indicated that the isoelectric point of the protein was reached at different times according to the treatment used. The SP3 treatment reached the isoelectric point in 11h and 45min, while SP1 reached this point in 4h and 45 min, and SP2 in 6h and 30 min. Probably, these results were due to the effect of pasteurization and inoculum change. This difference in the fermentation time was

probably due to the lower diversity of microorganisms present by the application of pasteurization. Furthermore, the stress of the cultures caused by the freezing process could also have an impact even if they were individually activated under optimum conditions.

The results of the physicochemical and microbiological analysis of the three treatments of SC, showed that the SP3 sample did not present significant differences in the moisture or in the acidity, with respect to the controls (SP1 and SP2) (Fig. 6). Regarding the LAB count, it was observed that the SC, prepared with the selected starter inoculum (SP3) showed a significantly higher microbial load in the final product ($1.66x10^7 \pm 7.35x10^6$ CFU/g), compared to the treatments SP1 and pasteurized SP2.

Figure 6. Moisture, acidity, and LAB counts of the SC at the producer scale. The bars marked with *, indicate significant difference with 95% (n = 3).



3.5 VOLATILE COMPOUNDS OF SC ELABORATED AT LABORATORY LEVEL VS PRODUCER LEVEL.

The main volatile compounds of the SC elaborated in the different work scales: M6, M8, M10, M17, PS1, PS2, PS3 are presented in Table 2. A total of 18 volatile compounds were identified by GC-MS and classified into five functional groups: alcohols (4), ketones (2), acids (3), aldehydes (6) and other compounds (3). The samples elaborated at laboratory scale had a higher number of volatile compounds; between 11 and 12 compounds that varied according to the type of proportion of microorganisms used. Even though, laboratory tested mixtures yielded a higher diversity on volatile compounds in their products than the producer scale samples, the concentration of these compounds was less than the ones obtained for SP1, SP2 and SP3.

Aldehydes were the most varied compounds in the SC samples at the laboratory level (0.1 and 4.4%). However, the highest proportions were found in the SC of the producer (17.2 - 19.8%), whose main compounds were acetaldehyde and nonanal. On the other hand, among the mixtures, the highest production of acetaldehyde was presented by mixture 8, which was the one with the highest proportion of *S. infantarius*. This concurs with reports indicating that *S. thermophilus* is the producer of formate, acetaldehyde and diacetyl (Uriot et al., 2017), characteristics that could coincide because they are of the same genus. Ketones were the least varied compounds (2) and were present in almost all SC evaluated (0.02 and 13.3%), except for M8, a mixture characterized by the presence of the 3 microorganisms in equal proportion.

Diacetyl, acetoin and 2,3-butylene glycol are well-known compounds in the dairy industry (Bojanić Rašović, 2017). For the synthesis of these metabolites, an excess of pyruvic acid is required, which is generated by the co-metabolism of citric acid present naturally in milk (Farelo de la Hoz, 2002) (Shepard et al., 2013). The yoghurt flavor of certain products is considered as a defect and is produced by the acetaldehyde defect that can be corrected thanks to the use of *Lactococcus* species, which has the ability to reduce acetaldehyde to ethanol. However, microorganisms with alcohol dehydrogenase, by accumulating ethanol, favor the production of large quantities of ethyl-esters, which characterize fruity flavors, considered a defect in most dairy products (Ruales, 2012) (Capozzi et al., 2017).

Finally, the selected mixture at laboratory scale *(L. plantarum* 66.7% and *L. lactis* 33.3%) (M6) was related to compounds that provide health effects such as vitamin B. This factor adds value to the selection of this mixture for its usage at producer scale. However, this characteristic at the producer scale did not yielded higher concentrations in comparison to the controls SP1 and SP2. Probably, this result was due to the less controlled conditions of the process that promote the contamination with contaminant microbiota that takes advantage of indispensable nutrients for its growth such as vitamin B.

4 CONCLUSIONS

The behavior of the selected ferment allowed to obtain Suero Costeño similar to that elaborated by the producer under its environmental conditions (artisan type). In addition, this ferment (*Lactobacillus plantarum* 66% and *Lactococcus lactis* 33%) complied with the physicochemical and organoleptic characteristics of the original artisanal product and can be used as a starter culture.

Furthermore, design of mixtures is a useful tool to visualize the effect of different proportions of microorganisms to elaborate a fermented product. This based on the fact that, the results of the physicochemical characteristics of the mixtures with different proportions of three microorganisms (*L plantarum, St. Infantarius* and *L. lactis)* were decisive in selecting a mixture that would provide the characteristics most similar to those of SC elaborated in a traditional manner.

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CONFLICT OF INTEREST

All authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The [THE DATA USED TO BUILD GRAPHICS AND STATISTICAL ANALYSIS] used to support the findings of this study are available from the corresponding author upon request.

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