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Effect of pasteurization and starter cultures on physicochemical and microbiological properties of costeño cheese

Efecto de pasteurización y cultivos lácticos en las propiedades fisicoquímicas y microbiológicas del queso costeño

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ABSTRACT

Key words:
Mashed cheese
Starter culture
Microbial analysis
Sensorial analysis

The effect of pasteurization and starter cultures on physicochemical, microbiological and sensorial characteristics of costeño cheese was determined. A completely randomized design was conducted, three treatments (T) and three replicates: Treatment 1 (T1): cheese manufactured with pasteurized milk without starter cultures, Treatment 2 (T2): cheese manufactured with pasteurized milk with *Lactococcus lactis* and *Lactococcus cremoris* (1:1) and Treatment 3 (T3): cheese manufactured with pasteurized milk with *Lactococcus lactis, Lactococcus cremoris* and *Streptococcus thermophillus* (0.5:0.5:1). Treatments were compared to a control sample that was prepared with raw milk without starter cultures. Concentration of 1.5% (v/v) of culture was used in relation to the amount of used milk in each treatment. Moisture content was higher in all treatments compared to the control and protein and fat content were significantly lower. Acidity was significantly higher in samples from T2 y T3 compared to T1 and control, due to the metabolism of starter cultures. Total coliforms, yeast and mold counts showed a significant reduction due to pasteurization process in all treatments. Regarding sensorial analysis, hedonic test showed a greater preference in cheese manufactured with T2 ($P<0.05$). There were no significant preferences between T1, T3 and control. Additionally, yield was significantly higher with T1 (22%) and T3 (23%) compared to control.

RESUMEN

Palabras claves:
Queso amasado
Cultivos
Análisis microbiano
Análisis sensorial

Esta investigación estudió el efecto del empleo de pasteurización y cultivos lácticos en los parámetros fisicoquímicos, microbiológicos y sensoriales del queso costeño. Se realizó un diseño completamente aleatorio, tres tratamientos (T) y tres repeticiones: Tratamiento 1 (T1): queso elaborado con leche cruda y sin cultivos, Tratamiento 2 (T2): queso elaborado con leche pasteurizada con *Lactococcus lactis* y L*actococcus cremoris* (1:1) y Tratamiento 3 (T3): queso elaborado con leche pasteurizada y *Lactococcus lactis, Lactococcus cremoris* y *Streptococcus thermophillus* (0,5:0,5:1). Se utilizó una concentración de 1,5% (v/v) de cultivo en relación con la cantidad de leche en cada tratamiento. El contenido de humedad fue significativamente mayor en todos los tratamientos con respecto al control y el contenido de proteínas y grasa fue significativamente menor. La acidez fue significativamente mayor en T2 y T3 comparado con T1 y el control, debido al metabolismo de los cultivos lácticos. El recuento de coliformes totales, mohos y levaduras fue significativamente menor debido al proceso de pasteurización en todos los tratamientos. En cuanto al análisis sensorial, la prueba hedónica mostró una mayor aceptación en quesos de T2 ($P<0,05$). No hubo diferencias significativas en cuanto a preferencia en T1, T3 y control. Adicionalmente, el rendimiento del proceso fue significativamente mayor en T1 (22,5%) y T3 (23,2%) en comparación con el control (16,6%).

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Raw milk production in Colombia reached 6710 million of liters per year in 2015 (Fedegan, 2016); 50% of this volume was transformed into dairy products and 18% for cheese production. The Atlantic Coast produced 40% of raw milk national production according to the Agricultural National Survey (DANE, 2015). The department of Sucre produced 3.2% of the national production where approximately 166,216 liters are used on a daily basis to produce dairy products such as costeño cheese (DANE, 2015).

Costeño cheese is produced and commercialized on the Atlantic Coast. This is a traditional product highly recognized as part of its own gastronomy. Costeño cheese is a fresh cheese, made in an artisanal way with raw milk where inadequate hygienic conditions are present, thus affecting its shelf life (Chávez and Romero, 2006). Traditionally, costeño cheese has a high fat content with a rectangular appearance and a creamy color. Its internal appearance has an open texture, dried consistency and salty flavor (Calderón *et al.,* 2011; López-Tenorio *et al.,* 2012).

While in Colombia there are governmental regulations to ensure food safety conditions (i.e., Resolution 2310 (1986) and Decree 616 (2006)), the costeño cheese is still currently made with raw milk following a non-standardized process without controlling variables. This practice results in a final product with non-homogeneous textural and organoleptic characteristics, with low microbial quality, thus counts above the permissible limits (Hernández *et al.,* 2011). In fact, the presence of *Salmonella* has been reported in costeño cheese; which is a biohazard for the consumer (Chávez and Romero, 2006). Moreover, contamination of *Listeria spp. Listeria ivanovii and Listeria innocua* has been found frequently in this product. This fact clearly indicates the lack of hygienic practices during cheese manufacturing thus, this product has been catalogued as unsafe for human consumption (Gallego and Arrieta, 2007).

Sensory quality of cheese is affected by heat treatment and causes changes in physicochemical properties of milk compounds (Atasoy *et al*., 2008). However, the pasteurisation process is essential to meet food safety standards. Furthermore, cheese flavour is the result of the enzymes from cheese-related microorganisms particularly, lactic acid bacteria (Martinez-Cuesta *et al*., 2001). Additionally, natural microflora of raw milk is destroyed due to pasteurization processes therefore, incorporation of starter cultures is fundamental to ensure acidification of raw milk (Bruno *et al*., 2011). Starter cultures can be prepared from either pure bacteria strain or from a predetermined proportion mixture. They have two main goals: 1) pH reduction and 2) production of lactic acid from lactose (Powell *et al.,* 2011). As a consequence, the selection of starter bacteria is crucial for production of flavour compounds in cheese made from pasteurized milk in order to succeed consumer expectations.

The healthy properties of goat milk and dairy products (Slacanac *et al*., 2010; Yangilar, 2013) as well as the importance of the use of lactic acid bacteria (LAB) in the process, and their optimal technological properties as potential starter cultures in dairy making are well established (González and Zárate, 2012; Mangia *et al*., 2014). Among the main microorganisms considered as starter cultures, which ferments milk, *Lactococcus lactis* is found. Its potential is associated with the fast production of lactic acid contributing in this way to the curdle formation and, at the same time, avoiding pathogenic bacteria growth (Fernández *et al*., 2011). Studies have reported a great growth and acidification activity of *L. lactis* and *S. thermophilus* in dairy products like cheese (Madrau *et al*., 2006; Mangia *et al*., 2013).

The objective of this study was evaluate the effect of pasteurization and lactic acid bacteria on physicochemical, microbiological and sensorial characteristics of costeño cheese**;** therefore, improving the quality of a traditional product of the Colombian Caribbean region as part of the region's cultural heritage.

MATERIALS AND METHODS

Materials

Raw milk used to elaborate cheese was obtained from Brahman breed cows located in Sucre, Colombia. Samples were taken at seven months of lactation. Lyophilized Starter cultures were used (BIOLACT AEB Argentina S.A). Culture substrate was prepared using commercial ultra pasteurized milk (Colanta UHT 2.0% Fat).

Starter Culture Preparation

In order to prepare the starter cultures, 1 g of Lyophilized culture in 1 L of ultra pasteurized milk was used. Two starter cultures were inoculated; one had *S. thermophillus* and the other one had a mixture of *L. lactis* and *L. cremoris*, at 42 °C and at room temperature (27 °C), respectively. Both were incubated for 2 h.

Raw Milk Characterisation

Raw bovine milk was delivered from a local dairy farm. Raw milk physicochemical parameters were determined using the LAC-S Milk Analyzer (BOECO, 100-240 V, 50-60 Hz) calibrated following methods established by Association of Official Analytical Chemists (AOAC). The titratable acidity of the raw milk was calculated according AOAC 947.05/90.

Cheese Manufacturing Process and Sampling

Cheese making procedure was conducted as described by Jimenez and China, 2006 (Figure 1). Treatments were applied as follows: Treatment 1 (T1): cheese manufactured with pasteurized milk without starter cultures, Treatment 2 (T2): cheese manufactured with pasteurized milk with *L. lactis* and *L. cremoris* (1:1) and treatment 3 (T3): cheese manufactured with pasteurized milk with *L. lacti, L. cremoris* and *S. thermophillus* (0.5:0.5:1). Treatments were compared to a control sample that was prepared with raw milk without starter cultures.

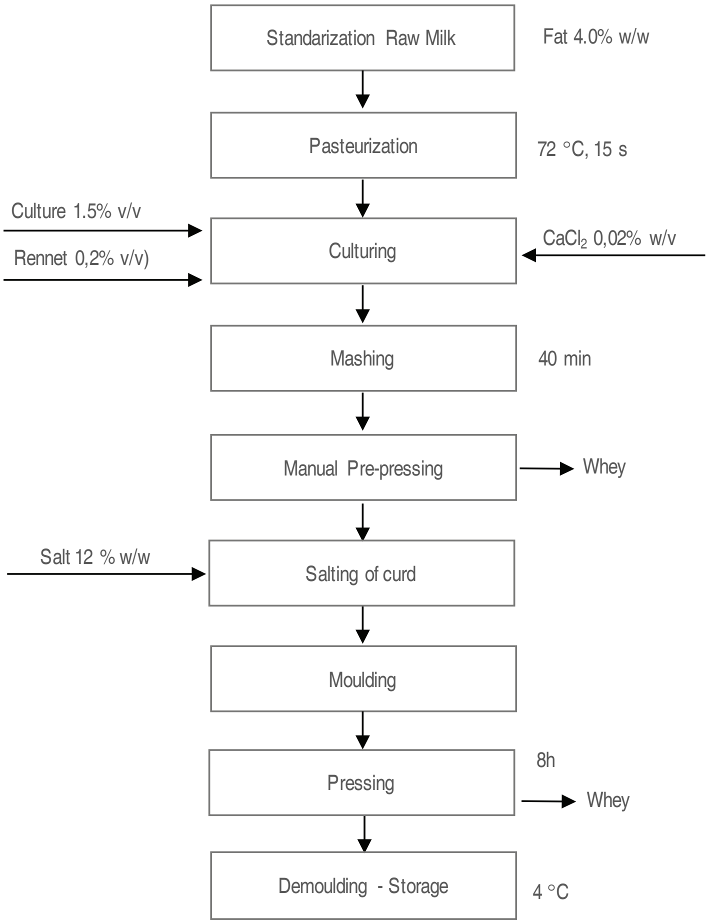

Figure 1. Cheese Manufacturing Process.

Physicochemical Analysis of Cheese

Physicochemical characteristics in cheese were analyzed as described by the AOAC. Acidity was established using a titration method as described by AOAC, 497.05, 2000. Moisture was determined following the procedure established by AOAC 934.06, protein content according to AOAC 920.152 and fat content as described by AOAC 989.04/90. The pH of the samples was measured with a ph-meter (Metrohm swiss made) (AOAC 981.12/90). Cheese yield was calculated as kg of cheese per 100 kg of milk.

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Microbial analysis of cheese

The presence of coliforms, yeast and molds was assessed after five days of refrigerated storage (4 °C). Total coliform bacteria were detected following the procedure established in NTC 4516, yeasts and moulds were determined using NTC 4132. Counts of *Staphylococcus auerus* and *Salmonella* were determined according to NTC 4779 and NTC 4574, respectively.

Sensory analysis

Sensory evaluation was carried out by acceptance test using a nine-point hedonic scale. Scores were established as follows, like extremely (9), like very much (8), like moderately (7), like slightly (6), neither like nor dislike (5), dislike slightly (4), dislike moderately (4), dislike very much (2), dislike extremely (1). Evaluation was performed by 60 untrained panelists, who differentiate samples from all treatments based on general acceptability.

Statistical analysis

A completely randomized design was conducted. Data was subjected to an analysis of variance (ANOVA) using R v.2.9.1. A randomized block design was used to evaluate sensorial parameters. Significant differences were established using Friedman test. Least squares means separation was performed using Tukey's test. All analyses were performed in triplicate. Significance was assessed at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Raw milk physicochemical and microbiological properties

Physicochemical parameters of raw milk were in compliance with the permissible range reported by the decree 616 (2006) of the Ministry of Social Protection (Table 1).

Table 1. Raw Milk Composition.

Analysis	Average
Acidity (%)[1]	0.17 ± 0.01
Density (g mL^{-1})	1.03 ± 0.00
Fat (%)	4.47 ± 0.15
Protein (%)	3.65 ± 0.07
Total Solids (%)	13.27 ± 0.21
Non Fat Solids (%)	8.80 ± 0.10
Lactose (%)	4.81 ± 0.12

[1] Described as a percentage of Lactic acid.

Microbiological counts in both raw and pasteurized milk were within the permissible range established by the NTC399 and NTC506, respectively. Microbiological Data of raw and pasteurized milk is reported in table 2.

Physicochemical analysis of cheese

Physicochemical characteristics for all treatments are reported in table 3. Acidity was significantly higher in T3 compared to the control (0.84 *vs.* 0.75). The ANOVA showed significant differences of acidity between T1 and T3, with the lowest acidity in T1. Results may indicate that acidity is lower when pasteurized milk is used without any starter culture. However, when starter cultures were used, acidity tended to increase. pH, on

Table 2. Microbial Analysis of Raw and pasteurized milk.

Raw Milk Microbial Analysis					
Mesophilic aerobic UFC mL^{-1}					*Listeria* spp.
650,000					Not Found
Pasteurized Milk Microbial Analysis					
Mesophilic aerobic UFC mL^{-1}	Total Coliforms Bac per mL	Fecal Coliforms Bac per mL	Yeast and Molds UFC mL^{-1}	*S. aureus* UFC mL^{-1}	*Salmonella* spp. UFC mL^{-1}
7,000	4	<1	Not Found	Not Found	Not Found

the other hand, tends to be higher when starter cultures are not added and pasteurization occurs. There were no significant differences among cheeses from either T2 or T3. The use of commercial lactic acid cultures produced a greater acidity in the final product compared to the control. These results may be explained by the fact that there is a relationship between acidity and lactic acid production derived from the presence of lactic acid bacteria (Powell *et al.*, 2011). Other studies have shown a similar trend for the acidity when lactic acid bacteria have been used in the production of different type of cheeses (Ballesta, 2014; Ruggirello *et al.*, 2016; Tabet *et al.*, 2016).

Tabla 3. Physicochemical Characteristics of Costeño Cheese[1].

	Acidity (%)[2]	pH	Moisture (%)	Protein (%)	Fat (%)
Control	0.75 ± 0.07^{b}	6.15 ± 0.51^{ab}	39.25 ± 2.50^{b}	14.33 ± 1.50^{a}	17.5 ± 2.38^{a}
T1	0.65 ± 0.02^{c}	6.57 ± 0.03^{a}	43.50 ± 1.29^{a}	11.83 ± 1.38^{b}	13.0 ± 2.68^{b}
T2	0.78 ± 0.01^{ab}	5.77 ± 0.23^{bc}	45.75 ± 1.26^{a}	12.97 ± 0.35^{ab}	14.4 ± 0.48^{ab}
T3	0.84 ± 0.02^{a}	5.44 ± 0.08^{c}	46.00 ± 0.82^{a}	11.06 ± 0.74^{b}	13.25 ± 1.26^{b}

[1] Least squares means within the column with no common superscript are significantly different (P<0.05).
[2] Described as a percentage of Lactic acid.

Moisture, protein and fat content in cheese samples from T1, T2 and T3 were not significant different. In this context, moisture content in cheese from T1, T2 and T3 was higher compared to control (P<0.05). Protein and fat content was significantly lower in cheese from T1 and T3 compared to the control. Results indicate that moisture content is higher and protein and fat content are lower when milk is pasteurized. The increase in moisture content may suggest a decrease in protein and fat content in all treatments. Furthermore, heat treatment during pasteurization generates a denaturation and interaction of serum proteins with k – casein, thus greater whey retention may also found a decrease in fat content of costeño cheese manufactured with pasteurized milk due to the rupture of fat globule membranes (Faria and Hennet, 2008; Ramírez and Vélez, 2012; Morales *et al.*, 2012; Buffa, 2001 and Recinos, 2007). However, no significant differences in the physicochemical properties of pH, acidity, humidity, protein and fat of treatment T2 compared with the traditionally manufactured costeño cheese (control).

Percent yield (w/w) in the manufacturing process of cheese

Cheese yield ranged from 16.6 to 23.1%; the lowest percentage was obtained in control samples, when raw milk without starter cultures was used (Figure 2). Yield was significantly higher in T1 and T3 compared to the control. All treatments with starter cultures had a statistically significant increase in yield with the exception of T2. This may be related to the fact that moisture content in T1 and

T3 was significantly higher compared to moisture content in control samples. Additionally, there could be greater whey retention and the higher yield may also be related to the serum protein bindings to casein (Galicia, 2005; Menz, 2002; García, 2006; Ramírez *et al.*, 2012; Faria and Hennet, 2008; Morales *et al.*, 2012). Consequently, it could be recommended to follow either T1 or T3 for industrial cheese production when the major goal is related to yield.

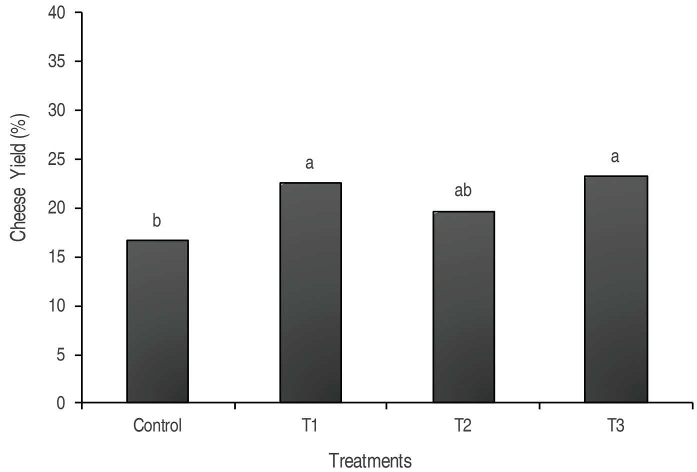

Figure 2. Percent yield (w/w) in the manufacturing process of costeño cheese [1].

[1] Least squares means in the graphic with no common superscript are significantly different (P<0.05).

Sensory Analysis of Cheese

Panel group expressed a significant greater preference for cheese manufactured with T2 (Figure 3). There were no significant differences among T1, T3 and control. Conversely, Galicia (2005) found a more intense flavor of Cheddar cheese produced with raw milk which may be an advantage if consumers prefer this strong flavor. Sameh (2006) reported similar findings regarding flavor in cheese manufactured with raw milk.

Cheese manufactured with starter cultures from T2 had better sensory scores on general acceptability compared to other treatments. The usage of this specific type of lactic acid bacteria seems to enhance sensorial characteristics

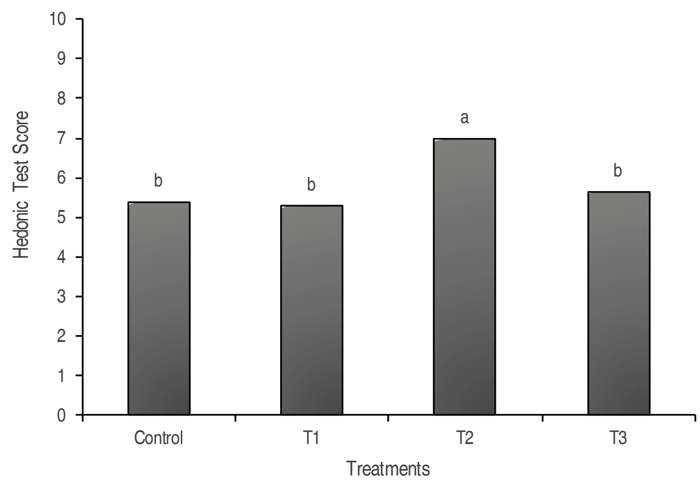

Figure 3. Hedonic Test Score[1].

[1] Least squares means in the graphic with no common superscript are significantly different (P<0.05).

of costeño cheese. Results proved that the type of starter culture have a marked influence upon the flavor, aroma and texture of cheese.

Microbiological analysis of cheese

All microbiological counts were not significantly different among treatments (Table 4).

Table 4. Microbial Analysis of Cheese[1].

Treatment	Total Coliforms (Bac per 100 g)	Fecal Coliforms (Bac per 100 g)	*S. aureus* (UFC g^{-1})	Yeast and Molds (UFC g^{-1})	*Salmonella* spp. (Bac per 25 g)
Control	28^{a}	$<3^{a}$	$<100^{a}$	20×10^{2a}	Negative
T1	$<3^{b}$	$<3^{a}$	$<100^{a}$	$<100^{b}$	Negative
T2	$<3^{b}$	$<3^{a}$	$<100^{a}$	$<100^{b}$	Negative
T3	$<3^{b}$	$<3^{a}$	$<100^{a}$	$<100^{b}$	Negative

[1] Least squares means within the column with no common superscript are significantly different (*P*<0.05

As expected, most of the bacteria counts were significantly higher when raw milk was used (Control). Only counts of fecal coliforms and *S. auerus* did not show a significant difference compared to the control. Microbiological counts met the national standard reported by NTC 750. Results are in agreement with Ballesta (2014).

CONCLUSIONS

Cheese made by the addition of mixed culture consisting of *L. lactis* and *L. cremoris* (1:1) and *L. lactis, L. cremoris* and *S. thermophillus,* had higher yield than samples of cheese made with raw milk and without starter cultures. Nevertheless, protein and fat content decreased with the addition of starter cultures in cheese manufacturing process by increasing moisture content. The type of starter culture did affect consumer preferences, cheese manufactured with pasteurized milk and *L. lactis* plus *L. cremoris* (T2) had the greatest acceptance. This study demonstrated that this combination of starter cultures allows the production of cheese with similar physicochemical properties (pH, acidity, moisture, protein and fat) to those from traditional costeño cheese, maintaining at the same time an acceptable yield for cheese producers. Moreover, pasteurization improved microbial counts, such as, total coliforms, yeast and molds, producing a reliable product with excellent quality.

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