



**PHYSICOCHEMICAL QUALITY AND FATTY ACID PROFILE OF TRADITIONAL COLOMBIA
(COSTEÑO CHEESE) CHEESSE ADDED WITH LACTIC AND MICROBIONIC RENNET**

**CALIDAD FÍSICOQUÍMICA Y PERFIL DE ÁCIDOS GRASOS DEL QUESO COLOMBIA
TRADICIONAL (QUESO COSTEÑO) AÑADIDO CON CUAJO LÁCTICO Y
MICROBIÓLOGICO**

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ABSTRACT

This article is developed with the aim of characterizing physicochemically and analyzing the fatty acid profile of Costeño cheese. Methodologically, this was an experimental study, which analyzed physicochemical variables such as pH, acidity, protein content, fat, moisture, total solids, water activity and ash, and performed a fatty acid analysis through gas chromatography (GC). The analysis showed significant differences ($P < 0.05$) respecting to the variables acidity, pH, moisture, total solids, ash, water activity, intensity of acidic and bitter taste, in firmness and grainy texture; while the variables protein, fat and salty flavor intensity had no significant differences ($P > 0.05$). Nine (9) fatty acids per CG can be identified; the fatty acid profile indicates that palmitic acid is the



most abundant and was the only one with significant differences ($P < 0,05$); Appreciable levels of oleic acid, linoleic acid and linolenic acid were also found. It is concluded that the process of physicochemical characterization and fatty acid analysis allows a better recognition of the composition of milk-derived foods such as Costeño cheese.

Keywords: cheeses, dairy products, fatty acids, gas chromatography.

RESUMEN

Este artículo se desarrolla con el objetivo de caracterizar fisicoquímicamente y analizar el perfil de ácidos grasos del queso Costeño. Metodológicamente, se trató de un estudio experimental, en el que se analizaron variables fisicoquímicas como pH, acidez, contenido de proteína, grasa, humedad, sólidos totales, actividad de agua y cenizas, y se realizó un análisis de ácidos grasos mediante cromatografía de gases (GC). El análisis mostró diferencias significativas ($P < 0.05$) respecto a las variables acidez, pH, humedad, sólidos totales, cenizas, actividad de agua, intensidad de sabor ácido y amargo, en firmeza y textura granulosa; mientras que las variables proteína, grasa e intensidad del sabor salado no tuvieron diferencias significativas ($P > 0.05$). Se pueden identificar nueve (9) ácidos grasos por GC; el perfil de ácidos grasos indica que el ácido palmítico es el más abundante y fue el único con diferencias significativas ($P < 0,05$); También se encontraron niveles apreciables de ácido oleico, ácido linoleico y ácido linolénico. Se concluye que el proceso de caracterización fisicoquímica y análisis de ácidos grasos



permite un mejor reconocimiento de la composición de alimentos derivados de la leche como el queso Costeño.

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Palabras clave: quesos, productos lácteos, ácidos grasos, cromatografía de gases

INTRODUCTION.

The production of milk derivatives has a significant share in the dynamics of the diet of humans; this being a very representative part of the gastronomic elements of any culture and nation; so studying it is a contribution in terms of understanding foods that belong to the diet of millions of people around the world; representing 46% of Global dairy production by 2008 and have had a 3% annual increase according to information provided by the IFCN in The Alltech Ideas Conference 2019.

In this case, it emphasizes the importance of one of the milk derivate products: cheese. This product has the characteristic of being universal because it is produced in all regions of the world from milk from various species of mammals (Vásquez *et al.*, 2018).

This by-product has the same nutritional properties as milk but has a higher content of concentrated fats and proteins (Ramírez and Vélez, 2012). Cheeses are an important source of calcium and phosphorus,

necessary for a balanced diet (Cuentas and Díaz, 2006; Michaelidou, *et al.*, 2003; Pérez, 2008).

In Colombia, especially on the Atlantic Coast, Costeño cheese is produced and marketed, an unripe product, typical of the gastronomy of this region of the country (Chávez y Romero, 2006). This food is distributed nationally and is used as the main ingredient in the production of fritter and bono bread, typical and very consumed foods in the country (López-Tenorio *et al.*, 2012; Acevedo, Jaimes and Espitia, 2015).

In cheese production, is common the use of renin or rennet extracted from the stomachs of lactating calves and is composed of an acidic protease called chymosin (Mohamed, 2016). In recent years due to the high demand for rennet worldwide, there has been a shortage of this enzyme because its obtaining involves the slaughter of nursing calves and therefore a decrease in the foot of bovine breeding (Osorio *et al.*, 2008). For this



reason, it is necessary to look for other cheaper alternatives and apply ecofriendly technologies. Research has now been increased to develop substitute sources of the enzyme, where those of microbial origin are of great interest (Osorio *et al.*, 2008).

The addition of lactic cultures to milk for cheese making has also taken a lot of interest in recent years, these starter cultures, help the transformation of lactose into lactic acid and the unfolding of proteins and fats to improve the digestibility of the final product, as well as its nutritional value. In addition, they are used to extend the shelf life of cheeses, because they help inhibit the growth of unwanted bacteria and provide

characteristic aromas to these foods (Vanegas *et al.*, 2017).

The joint use of lactic cultures and microbial rennet represents a technological alternative to improve the quality of Costeño cheese considered a national cultural heritage, so that their autochthonous characteristics are preserved, as well as standardizing the process for use by small, medium and large producers. In addition, there is little precedent in matter of studies conducted (Ballesta, 2014). This article is present with the aim of characterizing physicochemically and analyzing the fatty acid profile of Costeño cheese.

MATERIALS AND METHODS.

1. Materials and equipment.

To conduct this study, materials and equipment were needed that served as raw materials and supports for the research exercise. On the raw materials that were used for the production of Costeño cheese types, it is emphasize that these were: Raw cow's milk provided by a company of the Colombian Atlantic, animal rennet, microbial rennet, calcium chloride, sodium chloride and lactic cultures (*Lactococcus lactis* subsp. *Lactis*, *Lactococcus lactis* subsp. *cremoris*).

Next, about the equipment these were a milk analyzer (MA), a fixed marmite with a steam cover, a cheese incubator, cutting lire, mixer, Colander, Cheese molds, a stainless-steel table, a cooler, electronic balance, potentiometer, texturometer, gas chromatograph and an analytical Balance.

2. Experiment process.

Cheeses were made considering the Good Manufacturing Practices, milk was obtained from a company in the Colombian Atlantic



cheese-maker sector. All samples obtained were tested for acidity, pH, moisture, aw, protein content, fat percentage, total solids, ash, fatty acid profile four days after processed. All determinations were made in triplicate. Liquid animal rennet granulated microbial rennet and lactic cultures from a commercial house were used for the brewing process.

The research work was carried out at the pilot plants of the University of Cartagena. The physicochemical characterization and fatty acid profile were performed in the chemistry laboratory of the University of Cartagena. The determination of the water activity of the cheeses was carried out in the Physicochemistry Laboratory of the Universidad Nacional de Colombia in Medellín.

In this sense, a completely random experimental design was made, in laboratory conditions with 2x2 factorial arrangement with 3 repetitions, to see the effect of the absence or addition of microbial rennet and lactic cultures. This arrangement resulted in four (4) treatments listed 1 to 4 as shown below:

- **TREATMENT 1:** Costeño cheese made with animal rennet.

- **TREATMENT 2:** Costeño cheese made with microbial rennet.

- **TREATMENT 3:** Costeño cheese made with animal rennet and lactic cultures.

- **TREATMENT 4:** Costeño cheese made with microbial rennet and lactic cultures.

In this sense, the following methodologies were carried out in order to perform the Physicochemical Characterization of Costeño Cheese:

- **Acidity determination:** AOAC 920.124 method was used.

- **pH determination:** 10g of cheese were diluted in 50ml of distilled water. The measurement was made by potentiometry.

- **Moisture Determination:** the amount of water was quantified by direct drying at constant weight in a Chamber Furnace at 100oC by 5h. AOAC 926.08.

- **Water activity:** It was measured with a dew point hydrometer at 25°C (Aqualab 3TE series, Decagon, Devices, Pullman, WA, USA).

- **Total protein:** The total protein was determined using the Kjeldahl method with an average correction factor, AOAC 984.13.

- **Fat:** the determination of fat was made by the extraction method– Soxhlet.

- **Total solids:** for this determination, the moisture percentage was first taken and



calculated by subtracting the moisture percentage from 100% of the mass. AOAC 925.23/90.

- **Ashes:** direct incineration method, AOAC 942.05.

Next, on the profile analysis of fatty acids by gas chromatography. This test was performed by gas chromatography (GC) using the Agilent 48900 brand gas chromatograph.

The results obtained throughout the process were analyzed on the basis of the statistical program SPSS 15.0, variance analyses (ANOVA) were performed with the Tukey separation test and were observed between which means, statistically significant differences were presented at a confidence level of 95%.

RESULTS AND DISCUSSION.

Physicochemical characterization of cheese. The results obtained for the physicochemical characterization of the different treatments are presented in Table 1; where protein and fat content are observed to be homogeneous with respect to treatments.

Table 1. Physicochemical characterization of cheeses Average values \pm Standard deviation. Average values within the same row without any common letters have significant differences at a confidence level ($P < 0,05$)

	TREATMENTS			
	1	2	3	4
pH	6,61 \pm 0,1 ^{ab}	6,78 \pm 0,09 ^a	6,66 \pm 0,3 ^{ac}	6,50 \pm 0,3 ^{bc}
Acidity	0,08 \pm 0,01 ^a	0,12 \pm 0,01 ^a	0,15 \pm 0,01 ^a	0,23 \pm 0,05 ^b
Moisture (%)	51,75 \pm 0,3 ^a	50,6 \pm 0,2 ^{be}	50,7 \pm 0,09 ^{ce}	55,25 \pm 0,5 ^d
Water activity	0,95 \pm 0,0005 ^a	0,94 \pm 0,005 ^b	0,96 \pm 0,001 ^a	0,95 \pm 0,01 ^a
Protein (%)	18,84 \pm 0,4 ^a	19,4 \pm 0,3 ^a	20,48 \pm 1,4 ^a	18,42 \pm 0,29 ^a
Fat (%)	21,33 \pm 0,8 ^a	23,1 \pm 1,0 ^a	21,53 \pm 0,8 ^a	22,75 \pm 0,5 ^a
Total solids (%)	48,12 \pm 0,3 ^a	49,3 \pm 0,2 ^{be}	49,28 \pm 0,09 ^{ce}	44,74 \pm 0,5 ^d
Ashes (%)	4,48 \pm 0,02 ^a	5,12 \pm 0,1 ^{be}	5,53 \pm 0,3 ^{ce}	4,24 \pm 0,1 ^a
Fat in dry extract (%)	44,31 \pm 1,5 ^a	46,8 \pm 2,3 ^{ac}	43,69 \pm 1,7 ^a	50,85 \pm 1,6 ^{bc}



Moisture fat free Basis (%)	65,93±0,7 ^a	65,8±1,1 ^a	64,63±0,7 ^a	71,53±1.0 ^b
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Different letters in the same row differ significantly (p<0.05). The values represent the mean ± S.E. (n = 3)

Protein value is between 18.426% and 20.486%. These values match those reported by Gómez (2005) who cited ranges between 19% and 20%. According to García (2006) panela cheese a product made in a similar process as Costeño cheese has a protein percentage between 16.1% and 20.9%, results that match those obtained in this study. González (2010) reported protein concentrations between 14.2% and 21.425% in fresh cheese, data like those found in this research.

The fat content found was between 21.33% and 23.15%. These results are among those obtained by Chávez and Romero (2006) who reported ranges between 19% and 26% Costeño cheese produced in the municipality of Sincé- Sucre (Colombia) and slightly differ to those reported by Morales *et al.* (2012) and López *et al.* (2012) who cited values of 25.5% and 31% respectively.

Costeño cheese is classified as a fatty cheese (Gómez, 2005) or semi-fat (Chávez y Romero, 2006). According to NTC 750, fatty cheese contains fat in the dry extract greater than or equal to 45% and less than 60% and semi-fat greater than or equal to 25% and

less than 45%. Treatments 2 and 4 are classified into fatty cheeses with 46.88% and 50.85% respectively; and treatments 1 and 3 in semi-fats with percentages of fat in the dry extract below 45%.

Figure 1 shows that the percentage of moisture is directly proportional to the percentage of this no fat in the dry extract. Moisture is between 50.62% and 55.25%. These results differ slightly to those reported by Chávez and Romero (2006), where an average moisture content of 39.02% was obtained.

Also, Gómez (2010) cited values of 45%-47%, Morales *et al.* (2012) of 45.19% and López *et al.* (2012) ranges of 40% moisture. Variations of these values for the same product can be observed in all studies, this is because the process of making Costeño cheese is not standardized and is hand-made with traditional methods, obtaining cheeses with different physicochemical characteristics but under the same name. According to the handbook of making kneaded Costeño cheese developed by the ICTA and the Junta del Acuerdo de Cartagena, the average moisture rate of this cheese is 51.98% and

the fat-free moisture is 65.52%, data similar to those reported in treatments 1, 2 and 3 of this investigation. Differences in moisture

and MFFB are influenced by curd cutting, the draining rate process, cheese pressing and salt content.

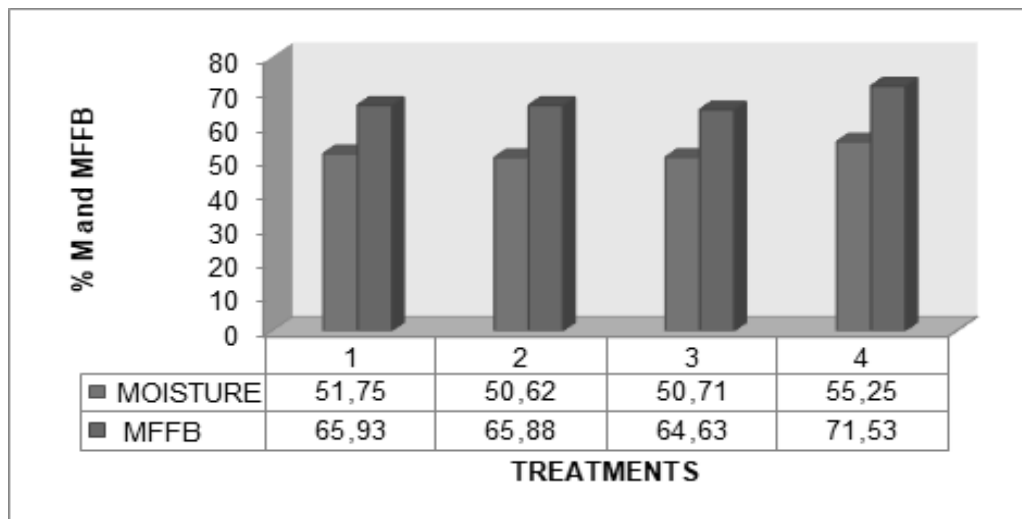


Fig. 1. Average values of moisture and MFFB

Table 2 shows the average values of pH, acidity, and water activity, which show significant differences between treatments.

Table 2. Cheeses pH, acidity and water activity. Average values \pm Standard deviation

	TREATAMENTS			
	1	2	3	4
pH	6,61 \pm 0,10 ^{ab}	6,78 \pm 0,09 ^a	6,66 \pm 0,3 ^{ac}	6,50 \pm 0,3 ^{bc}
Acidity	0,08 \pm 0,01 ^a	0,12 \pm 0,01 ^a	0,15 \pm 0,01 ^a	0,23 \pm 0,05 ^b
Water activity	0,95 \pm 0,0005 ^a	0,94 \pm 0,005 ^b	0,96 \pm 0,001 ^a	0,95 \pm 0,01 ^a

Different letters in the same row differ significantly ($p < 0.05$). The values represent the mean \pm S.E. ($n = 3$).

The pH obtained was between 6.50 and 6.78. These values are within those obtained by González (2010) in fresh cheese, who reported values between 5,415 and 6.95. Chávez and Romero (2006) reported pH

values between 5.50 and 6.51; the latter like treatment 4. Acidity results show that processed cheeses are not acidic, characteristic of this type of product. The values obtained for this physicochemical

characteristic were between 0.08 and 0.23. These results differ slightly with those reported by Chávez and Romero (2006), who reported ranges of 0.369 and 1.14 for this type of cheese.

The water activity of the different cheeses has high levels, so these are very susceptible to microbial development. The results obtained for water activity are between 0.948 and 0.96. García (2006) reported an average value of 0.988 in panela cheese and Vasquez *et al.* (2012) ranges between 0.92 and 0.94; results that differ slightly to those found in this research.

The percentage of total solids are between 44.747% and 49.379%. These values are similar to the results obtained by Pianta, *et al.*, (2004) in fresh colonial cheese made in Brazil, who reported ranges between 47.6%

and 53.2%; and they differ slightly with those determined by Garcia (2006) who obtained a dry extract percentage of 27.65% and 42, 425%.

The values for the ash fraction are between 4.245% and 5.534%. These results differ slightly with those reported by Garcia (2006) in panela cheese and Pianta, *et al.*, (2004) in colonial fresh cheese; with average values of 2.5% and 3.73% respectively.

Fatty acid profile analysis.

The fatty acid profile of processed cheeses is presented in Table 3; where nine (9) fatty acids were identified, Butyric acids (C4), Caproic (C6), Caprylic (C8), Capric (C10), Myristic (C14), Palmytic (C16), Oleic (C18:1), Linoleic (C18:2) and Linolenic (C18:2).

Table 3. Fatty acid profile of cheeses. Average values \pm Standard deviation.

Fatty acids (%)	TREATMENTS			
	1	2	3	4
C _{4:0} , Ác. Butyric	1,0 \pm 0,04 ^a	1,02 \pm 0,09 ^a	1,15 \pm 0,08 ^a	1,12 \pm 0,09 ^a
C _{6:0} , Ác. Caproico	2,67 \pm 0,02 ^a	2,70 \pm 0,2 ^a	2,82 \pm 0,1 ^a	2,87 \pm 0,2 ^a
C _{8:0} , Ác. Caprylic	3,37 \pm 0,02 ^a	3,39 \pm 0,2 ^a	3,52 \pm 0,2 ^a	3,56 \pm 0,09 ^a
C _{10:0} , Ác. Capric	12,44 \pm 0,1 ^a	12,60 \pm 0,5 ^a	12,53 \pm 0,4 ^a	12,42 \pm 0,1 ^a
C _{14:0} , Ác. Myristic	1,78 \pm 0,01 ^a	1,77 \pm 0,03 ^a	1,76 \pm 0,03 ^a	1,76 \pm 0,03 ^a
C _{16:0} , Ác. Palmitic	35,08 \pm 0,2 ^a	34,55 \pm 0,4 ^{ade}	34,05 \pm 0,2 ^{bdf}	33,96 \pm 0,1 ^{cef}
C _{18:1} , Ác. Oleic	22,72 \pm 0,4 ^a	23,62 \pm 0,9 ^a	23,69 \pm 0,3 ^a	24,05 \pm 0,3 ^a
C _{18:2} , Ác. Linoleic	6,68 \pm 0,02 ^a	6,83 \pm 0,09 ^a	6,63 \pm 0,4 ^a	6,32 \pm 0,3 ^a
C _{18:3} , Ác. Linolenic	14,22 \pm 0,3 ^a	13,48 \pm 0,6 ^a	13,81 \pm 0,4 ^a	13,89 \pm 0,2 ^a

Different letters in the same row differ significantly ($p < 0.05$). The values represent the mean \pm S.E. ($n = 3$)

The results obtained differ slightly with the fatty acid profile of milk fat according to McGibbon and Tylor (2006). The fatty acid content was similar for all treatments; except, palmitic acid that had significant differences between processed cheeses. The value of this fatty acid is between 33.96% and 35.08%. These values are higher than those reported by Perotti, *et al.*, (2008) who report a value of 31.4% of palmitic acid in Reggianito cheese made in Argentina.

Palmitic acid is the main saturated fatty acid in most diets and its high consumption in the diet is related to hypercholesterolemia disease (Food and Agriculture Organization/Worlds Health Organization). In the research this was the fatty acid that was found in greater proportion in cheeses.

Myristic acid like palmitic acid is considered harmful to health because its high consumption causes hypercholesterolemia problems. The value of this fatty acid is between 1.76% and 1.78%. These results are below those obtained by Sánchez (2004), who reported an average value of 49.2% in Semiduro cheese made in Venezuela.

The study obtained butyric acid values between 1.00% and 1.15%, caproic acid of 2.67% and 2.82%, caprylic acid of 3.37% and

3.56% and capric acid of 12.42% and 12.60%. These make up 8% to 12% of total fatty acids and have no effects on blood cholesterol (Parodi, 2004). The results of butyric acid coincide with those obtained by Chavarría, *et al.*, (2006) who reported values between 1.1% and 1.4% in different cheeses made in a Costa Rican company.

Butyric acid contributes to the stale taste and is considered an indicator in milk fat, since it is found practically only in this food, it is also very useful in the production of butter (Chavarría *et al.*, 2006). Its anti-tumor properties are also highlighted (Maier, 2000; Coradini *et al.*, 2008) and its synergistic capacity in the treatment of hypercholesterolemia (Menzel, 2002).

The percentages of caproic acid and caprylic found differ from those achieved by Sánchez (2004), who reported average values of 2.3% and 2.8% respectively in Venezuelan telita cheese. The ranges obtained for capric acid are above those reported by Perotti, *et al.*, (2008), which obtained a value of 3.1% in Argentine Reggianito cheese.

Caproic, caprylic and capric acids have antibacterial and antiviral properties highlighting monocaprin, monoFDycerid of

capric acid with activity against HIV (Thormar, Isaacs, Kim and Brown, 1994).

On the other hand, table 4, monounsaturated and polyunsaturated fatty acids obtained in processed cheeses are presented.

Table 4. Monounsaturated and polyunsaturated fatty acids.

Fatty acids (%)	TREATMENTS			
	1	2	3	4
C_{18:1}, Ác. Oleic	22,72±0,4 ^a	23,62±0,9 ^a	23,69±0,3 ^a	24,05±0,3 ^a
C_{18:2}, Ác. Linoleic	6,68±0,02 ^a	6,83±0,09 ^a	6,63±0,4 ^a	6,32±0,3 ^a
C_{18:3}, Ác. Linolenic	14,22±0,3 ^a	13,48±0,6 ^a	13,81±0,4 ^a	13,89±0,2 ^a

Different letters in the same row differ significantly (p<0.05). The values represent the mean ± S.E. (n = 3)

The oleic acid content is between 22.72% and 24.05%. These results differ from those reported in analyzed milk fat by McGibbon and Tylor (2006), who obtained 17.2% of this fatty acid. Oleic acid is the main component of olive oil and avocado, and it has been reported as beneficial to health because it is effective for lowering plasma cholesterol in the blood (Osorio, 2008).

The values of linoleic fatty acids (n-6) and ∞ - (n-3) obtained were between 6.32% and 6.83% and 13.48% and 14.22% respectively.

CONCLUSIONS.

Having completed the research, it is concluded that in relation to the physicochemical characterization of Costeño

These results are above those found by Chavarría *et al.*, (2006), which reported ranges of 1.5% and 3.2% linoleic acid and 0.4% to 2.8% linolenic acid.

These fatty acids are considered essential and must be obtained from the diet because the human body cannot synthesize them (Sources, 2009). They have also been reported as beneficial to health as well as oleic acid (Pariza and Park, 2001 cited by Avilez *et al.*, 2009; Ward *et al.*, 2002 cited by Fuentes, 2009).

cheese; it is provided that the average protein and fat values did not have significant differences between treatments, so the



proposed factors do not mark any influence on these variables, while the acidity, pH, moisture, water activity, total solids and ashes were significantly different.

Then, in the analysis of the profile of the fatty acids of the Costeño cheese; it is provided that nine (9) fatty acids were identified, with a high content of palmitic acid, which is associated with cardiovascular disease. Appreciable levels of oleic acid, linoleic acid

and linolenic acid were also found, which are attributed great benefits to human health.

It is essential that processes are carried out in the dairy sector to determine the lifespan and the various characteristics of dairy products. In this way, it is suggested to determine the fatty acid profile in the milk used for cheese making and thus compare the results with those obtained in the final product.

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