Physicochemical characterization of milk, SueroCosteño and QuesoCosteño

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Abstract—The objective of this study was to carry out the physical-chemical characterization of milk, sueroCosteño and quesocosteño from the towns of Patillal, Badillo and La Vega. The milk produced in the Department of Cesar is transformed by family businesses located in rural areas without infrastructure, previous standardization processes and hygienic conditions. A physicochemical and chromatographic characterization was carried out in which the analysis of amino acids was included, first performing hydrolysis of the protein and derivation of the sample. Data were analysed using ANOVA analysis of variance and a minimum significant difference test (MSD). It was evidenced that milk and whey from Badillo were the ones that reported the highest protein content, 3.32% and 1.20% respectively. The results showed that Badillo reported the highest protein content for milk with 3.32% and whey1.20%, with Patillal cheese showing the highest percentage of essential amino acids such as isoleucine 31.28%, tryptophan 34.92% and valine with 32.84%. The amino acid content of suerocosteño and quesocosteño can be attributed to the production process, strength, rennet type and storage time.

Key words - physicochemical analysis, profile of amino acids, milk and whey.

I. INTRODUCTION

Milk is an ideal food in nutritional terms, it is composed on average of approximately 87.3% water, 4.2% fat, 4.6% lactose, 3.25% protein and mineral substances 0.65% [1]. This is why it is susceptible to microbial contamination, forcing the dairy industry to employ different processes to preserve its nutritional qualities [1]. From the physicochemical point of view, milk is a very complex and complete product containing all the nutrients such as protein, carbohydrates and lipids, indispensable for the individual, particularly the 10 essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine[2]. The milk of the Department of Cesar is characterized physicochemically by its percentage of fat (4.3%) and total solids (12.5 and 14%), which is why multinational and regional companies have been located [3]. At present, there is a milk production of over 600,000 L day⁻¹, with a 50% decrease (Fedegan, 2016), which is distributed as follows: 52% is intended for formal processing; 43% is destined for the production of coastal cheese and 5% for local consumption [3].

Emphasizing the "sueroCosteño", is defined as a milk matrix fermented and commonly elaborated in the Colombian Atlantic Coast, produced by the acidification of raw milk, due to the action of the native microorganisms. It differs from the fermented milks because there is a break of the gel, the waste is produced and sodium chloride is added. The final product is similar to sour cream and is used as a companion. During the fermentation a system of two phases is formed: one liquid and one solid; where the liquid part is called whey and the other is known as "suero" [4]. The fermentation temperature of the "sueroCosteño" is the most important factor in the growth of microorganisms, followed by the pH, acidity and lactose obtained in the product [4].

Another derivative of the milk processing is cheese, which is defined as the product of the coagulation of casein in milk, followed by separation and removal of whey from the curd, is the generic name of a group of products fermented milk-based foods, produced in a wide range of flavours and shapes all over the world. Although the main purpose of making cheese is to conserve the main components of milk, which has evolved into a high-quality food with important qualities, as well as being very nutritious [5]. During the elaboration ofquesocosteño, several of the nutritive compounds like vitamins, proteins and milk fats are concentrated in a sub product that is the whey [6], the final fat content in the product is determined by the size of the fat globule, the kinetics of coagulation and the manipulation performed on the curd [7]. Likewise, the type of pasteurization used in the hygienization process influences the protein content of the cheeses, so the high temperatures favor the formation of a complex between the K casein and the B lactoglobulin that retains the proteins in the curd [8]. Taking into account the above, 43% of the production of quesocosteño is transformed by a guild that develops artisan practices established in rural areas without having a necessary infrastructure, without prior...
standardization of processes and quantification of yields. From this informal process is obtained approximately 4 to 6 tons in average of coastal cheese [3], being its main market place the city of Valledupar where the product is distributed to different market places and sales points to follow the distribution chain [3].

On the other hand, the methods that detect and quantify the amino acids, are of great interest for the food industry. Currently, reverse phase high performance liquid chromatography (HPLC) in combination with pre or post-column derivatization is the most widely used, "it is a separation technique in which the mixture to be solved is introduced into a system formed by a fluid (mobile phase), which circulates in contact with a phase which remains fixed or immobile during the process (stationary phase). In the case of liquid chromatography, the stationary phase is a solid or a liquid fixed in a solid and the mobile phase is a liquid. The two phases are chosen so that the components of the mixture are distributed differently between the mobile phase and the stationary phase. Those components that are strongly retained by the stationary phase move slowly with the flow of the mobile phase; on the other hand, the components that attach weakly to the stationary phase, move quickly. As a consequence of the different mobility, the components of the mixture are separated into discrete bands or zones that can be analysed qualitatively and/or quantitatively " [9]. Consequently, the amount of free amino acids in the cheese depends on the amount of milk proteins, the activity of proteolytic enzymes in dairy technologies and the microorganisms involved in these processes [10].

Multiple analytical methods have been developed for the quantification of amino acids in various biological matrices, including the combination of different chromatography techniques and various detection systems such as the amino acid analyzer by ion exchange chromatography Redruello et al., [10]. Researches as reported by Salmen et al.,[11], evaluated the amino acid content and the electrophoretic profile of casein in different bovine milks, reporting that the essential amino acids found in most of them were: lysine; 9.78 and leucine with 8.24, followed by valine with 6.17 and threonine with 4.00 mg 100g⁻¹. On the other hand, the non-essential amino acids detailed in the highest percentage were glutamic acid with 21.38, followed by aspartic acid with 7.30, serine 5.81 and tyrosine 4.97mg 100g⁻¹. Taurine and lysine, essential amino acids were considered essential nutrients for growth and development, taurine plays a key role in intestinal fat absorption and plays an important role in protecting neurons from oxidative stress [12]. The objective of this study was to physicochemically characterize the milk, Suerocosteño and QuesoCosteño from the townships of Patillal, Badillo and La Vega located in the Department of Cesar.

II. MATERIALS AND METHODS

To know the manufacturing process of suerocosteño and quesocosteño from cow milk, a processing plant was visited for each township. From each township, three samples of different batches of 500mL of milk and suerocosteño were taken, and 250g of quesocosteño, obtained the same day of milking and elaboration, respectively. Samples were taken in triplicate, one per batch of production. These products were refrigerated at 4 °C individually in hermetic Styrofoam coolers and were taken to the Laboratories of Biology and Chemistry of the Popular University of Cesar for further analysis. These townships were selected because they are in the same northeastern area and because of their high production of suerocosteño and quesocosteño. For statistical analysis of the physicochemical characteristics, an experimental design was used in divided plots, where the plot was the type of township (Patillal, Badillo and La Vega) and the plot divided the type of product (milk, suerocosteño and QuesoCosteño).

A. Physicochemical properties

The following physicochemical parameters were determined: density: by the gravimetric method [13] (925.22), the total nitrogen quantification Kjeldahl method (960.52), fat by the Gerber method (AOAC 2000.18), total solids by the modified stoving method (990.19) and ash: gravimetric method [14], all these tests were performed in triplicate [13].

B. Chromatographic analysis

This analysis was performed on a high water liquid chromatograph, equipped with a Quaternary pump model 600E, a model 997 photodiode array detector (PDA), a model 717 automatic injector, and a reverse phase column (C-18 ) for analysis of amino acids PICO-TAG model 88131 of 3.9mm × 150mm. The data were processed by an NC power MATE 433 PC with millennium 2032 software, which was performed as follows: protein hydrolysis, pre-column sample derivation and reversible HPLC phase analysis. Separation of the amino acids was obtained by gradient elution at 38 °C, using as a mobile phase the combination of effluents such as sodium acetate trihydrate (19g), water (1L), triethylamine (TEA, 0.5mL), acetonitrile 600mL and 400mL water. The total time of analysis and balance of the system was 20 min [15].

A. Reaction of hydrolysis

The acid hydrolysis process was carried out by treating the different samples; milk, Suerocosteño and quesocosteño, with HCl 6N and 1% phenol. To avoid reactions of tryptophan that might interfere with the result,
a solution of HCl 7M: 10% trifluoro-acetic acid; 20% trichloroacetic acid and 1% phenol was used. These samples consisted of 10µL: 9.5µL of reagent and 0.5µL of sample. It was then transferred into a flask and wrapped with foil, each sample was brought to a heating plate for 24 hours [15].

B. Derivation of the sample

The resulting hydrolyzate was dried under vacuum to remove any impurities and a solution with ethanol, water and triethylamine (TEA) was added in a ratio of (2: 2: 1) respectively, this sample was again dried under vacuum and 7 µL of a mixture of three solutions (OPA reagent), formulated as: ortho-phthalaldehyde, brij 35, methanol, 2-mercaptoethanol and potassium tetraborate, adjusted to a pH of 10.4 was added.

C. Statistical analysis

An analysis of variance (ANOVA) was carried out and the post hoc test of minimum significant difference (MSD) was applied, setting a level of significance at p<0.05 [16]. The results were expressed with their mean and standard deviation, using the statistical software package (Statgraphics Centurion 16.103, version 15.0, Chicago, USA). The number of samples per township was determined taking into account the total of analyses that were to be realized and the amount necessary according to previous investigations.

III. RESULTS AND DISCUSSION

A. Physicochemical composition of milk, suerocosteño and quesocosteño

Table 1 shows the results of the physicochemical characterization of the samples of milk, suerocosteño and quesocosteño obtained in the municipalities of Patillal, Badillo and La Vega. An objective observation was made of the conditions of processing of the unpasteurized type of cheese in each of the study areas. It was observed that Patillal lost about 84% of the protein content in whey during the production of suerocosteño, followed by Badillo with 63.85% and La Vega with 68%. Regarding fat content, 84.34% of the Patillal district was eliminated in whey, 77.38% for Badillo and 72.56% La Vega. Studies conducted by Bertolino et al., [17] reported values of fat and protein of 23.9% and 19.5% in the elaboration of Roman pecorino cheese. Addis et al.,[18] reported that there were no significant differences in the moisture, fat and protein content with values of 33.19%, 47.83% and 39.25%, respectively, using different types of rennet (animal, vegetal and enzymatic). Regarding the total solids, La Vega reported the lowest content of these compounds by 36.02%. Non-fatty solids were only affected in Badillo and La Vega with 4.76% and 0.70% loss, during the production of suerocosteño. Regarding the cheese production, the group that concentrated the most protein was Patillal with 83.12%, being superior that Badillo and La Vega in 7.05% and 7.56% respectively. The homogenization of the milk increases the retention of fat and moisture globules, due to the alteration of the structure of the clot. Some authors have stated that the casein / fat ratio of milk when it is close to 0.72, produces better recovery of fat and protein in cheese, being maximum, in addition the addition of calcium chloride increases the yield of the cheeses [5].

| Analysis          | Patillal | Badillo | La Vega | Patillal | Badillo | La Vega | Patillal | Badillo | La Vega | Patillal | Badillo | La Vega |
|-------------------|----------|---------|---------|----------|---------|---------|----------|---------|---------|----------|---------|---------|
| **Density (g/L)** | 1.026±0.15<sup>a</sup> | 1.028±0.17<sup>b</sup> | 1.028±0.26<sup>c</sup> | 1.023±0.42<sup>a</sup> | 1.026±0.14<sup>a</sup> | 1.024±0.42<sup>b</sup> | 1.025±0.25<sup>ab</sup> | 1.022±0.19<sup>c</sup> | 1.029±0.14<sup>a</sup> |
| **Protein (%)**   | 3.18±0.14<sup>a</sup> | 3.32±0.35<sup>b</sup> | 3.00±0.15<sup>c</sup> | 0.70±0.17<sup>a</sup> | 1.20±0.61<sup>a</sup> | 0.96±0.24<sup>b</sup> | 18.84±0.12<sup>a</sup> | 14.6±0.15<sup>b</sup> | 12.95±0.25<sup>c</sup> |
| **Fat (%)**       | 3.9±0.09<sup>a</sup> | 4.01±0.22<sup>b</sup> | 4.01±0.10<sup>c</sup> | 0.62±0.52<sup>a</sup> | 0.90±0.24<sup>a</sup> | 1.10±0.12<sup>c</sup> | 24±0.25<sup>a</sup> | 20±0.45<sup>b</sup> | 20±0.36<sup>c</sup> |
| **Ash (g/100g)** | 0.74±0.24<sup>b</sup> | 0.69±0.14<sup>a</sup> | 0.69±0.26<sup>c</sup> | 0.50±0.10<sup>a</sup> | 0.36±0.37<sup>a</sup> | 0.42±0.35<sup>c</sup> | 2.81±0.14<sup>c</sup> | 2.90±0.83<sup>b</sup> | 3.15±0.42<sup>c</sup> |
| **Total solids (%)** | 12.2±0.26<sup>a</sup> | 12.57±0.41<sup>b</sup> | 12.52±0.35<sup>c</sup> | 8.86±0.42<sup>a</sup> | 9.08±0.45<sup>a</sup> | 8.01±0.41<sup>c</sup> | 42.29±0.42<sup>a</sup> | 34.24±0.65<sup>b</sup> | 49.28±0.25<sup>c</sup> |
| **Non-fat solids (%)** | 8.2±0.28<sup>a</sup> | 8.59±0.28<sup>b</sup> | 8.51±0.30<sup>c</sup> | 8.24±0.20<sup>a</sup> | 8.18±0.47<sup>a</sup> | 8.45±0.14<sup>c</sup> | 18.29±0.63<sup>a</sup> | 14.24±0.82<sup>b</sup> | 29.28±0.14<sup>c</sup> |

In the case of fat, Patillal cheese was the one that collected the more fat globules in 83.5%, followed by Badillo and Vega with 4.07% and 4.25% less respectively. Likewise, La Vega obtained the highest amount of ash, total solids and non-fat solids, with Badillo and La Vega averaging 2.12%, 8.59% and 33.39%, respectively. This could be due to the fact that the amount of rennet that was used in this township was more precisely dosed, controlling its use in comparison with Badillo and La Vega. The time of cutting and cheese disposal are relatively equal in all three municipalities and depends on the coagulation time, which was approximately 30 to 45 minutes. Abellán[8] reported values of total solids, fat and protein of 53.96%, 58.80% and 34.59%, respectively, for the preparation of cheese obtained with natural coagulants in powder form. The content and composition of the fat exert a relevant influence on the quality of the cheese. Fat contributes to enhance the cheese texture, which is homogeneously distributed within the protein matrix Addis et al., [19]. These researchers reported a fat percentage of 47.9%, protein 37.5% and ash 0.71% in semi-hard cheese. The fat...
content of the cheese is important because defines the consistency, the flavour, the aroma and the nutritive value of the cheese [20]. In fresh cheeses, high moisture content and low pH are conditions that affect texture and taste. The acidity in the cheese is another important factor that causes changes in the curd, having a correlation in the exit of humidity. During salting whatever the technique used, there is an exchange of matter between the curd and the phase in contact with the salt, causing a loss of water, organic substances and minerals. If there are more caseins in the middle, this forms a mesh, trapping more fat and other solids in the curd. On the other hand, the presence of somatic cells is an important source of enzymes, especially proteases, which correlate negatively with cheese yield, together with calcium concentration and pH decrease [5].

B. Milk amino acid analysis

Table 2 reports the results of the chromatographic analysis performed on milk, whey and coastal cheese, which detail the essential and non-essential amino acids found in the samples. These data are compared for each product of each township. It was evidenced that Patillal reported statistically significant differences (p <0.05) in milk with the other two townships, among the following amino acids: isoleucine, valine and tyrosine (p <0.05), but between these two study areas no differences were found. Researches as reported by Mazhitova et al., [21], studied the amino acid and fatty acid profile in Yegua's milk, reporting that the most abundant amino acids were glutamic acid (16.8-21.2%), leucine (8.2-10.1%) and lysine (8.9-10.1%), these being very different from those of this study. Also Pappa et al.,[22] reported that cheeses that made with different types of milk, the concentration of amino acids showed a clear tendency to increase with maturation time, because during proteolysis, these compounds are released by proteolytic agents.

### Table 2. Amino acid variation in milk, suerocosteño and quesocosteño in the townships.

| Analysis | Amino acids | Patillal | Badillo | La Vega | Patillal | Badillo | La Vega | Patillal | Badillo | La Vega | Patillal | Badillo | La Vega |
|----------|-------------|----------|---------|---------|----------|---------|---------|----------|---------|---------|----------|---------|---------|
| Amino acids | Milk | Milk | Milk | Suero | Suero | Suero | Cheese | Cheese | Cheese |
| Isoleucine | 6.95±0.05 | 7.45±0.28 | 7.44±0.74 | 4.17±0.04 | 7.48±0.56 | 7.67±0.42 | 27.75±0.32 | 0.18±0.01 | 0.16±0.74 |
| Leucine | 3.64±0.05 | 3.87±0.15 | 3.86±0.36 | 2.22±0.02 | 3.88±0.37 | 3.99±0.63 | 12.05±0.25 | 0.09±0.02 | 1.12±0.67 |
| Lysine | 4.08±0.06 | 4.22±0.13 | 4.27±0.72 | 2.53±0.08 | 3.88±0.47 | 4.49±0.45 | 12.71±0.15 | 1.35±0.12 | 2.39±0.43 |
| Metihonine | 7.42±0.05 | 8.10±0.47 | 8.00±0.78 | 4.14±0.07 | 8.05±0.23 | 10.34±0.63 | 27.64±0.12 | 0.045±0.03 | 20.52±0.85 |
| Phenylalanine | 8.76±0.06 | 9.00±0.14 | 9.27±0.96 | 5.08±0.01 | 9.44±0.19 | 9.73±0.41 | 31.28±0.17 | 3.70±0.06 | 3.64±0.27 |
| Threonine | 3.78±0.07 | 4.01±0.25 | 3.81±0.85 | 0.24±0.09 | 4.07±0.45 | 4.07±0.23 | 30.08±0.01 | 0.84±0.04 | 2.10±0.36 |
| Tryptophan | 10.870±8 | 11.36±0.31 | 11.47±0.41 | 5.86±0.14 | 12.19±0.41 | 12.15±0.14 | 34.92±0.06 | 4.65±0.02 | 5.96±0.47 |
| Valine | 6.09±0.05 | 6.71±0.21 | 6.50±0.36 | 2.23±0.03 | 6.61±0.28 | 6.38±0.45 | 32.84±0.04 | 0.82±0.06 | 12.6±0.23 |

| Non-Essential Amino Acids |
|---------------------------|
| Tyrosine | 9.45±0.04 | 9.96±0.14 | 9.90±0.12 | 3.06±0.02 | 10.1±0.39 | 9.87±0.74 | 54.35±0.08 | 4.90±0.04 | 0.25±0.45 |
| Arginine | 6.97±0.04 | 7.29±0.78 | 7.37±0.85 | 1.37±0.07 | 7.49±0.25 | 7.47±0.24 | 47.67±0.02 | 1.61±0.08 | 0.86±0.45 |
| Histidine | 6.48±0.06 | 6.90±0.36 | 6.50±0.25 | 0.68±0.01 | 6.72±0.45 | 6.51±0.24 | 49.30±0.04 | 1.55±0.06 | 0.09±0.21 |

The data indicate the mean (±) with its respective standard deviation. Different letters in the same row indicate statistically significant differences (p <0.05).

C. Suero costeño amino acid analysis

With regard to suerocosteño, the amino acids that had statistical differences among all townships were lysine, methionine, phenylalanine, valine, tyrosine and histidine. For the case of the suerocosteño, when the milk is acidified the physicochemical properties of the casein micelles are modified. If the pH decreases, the colloidal calcium pH of the micelles dissolves, causing a destabilization and release of the caseins to the medium. During proteolysis, casein and large peptides hydrolyze, resulting in the formation of intermediate sized peptides, which are hydrolyzed to form small peptides and free amino acids. This casein hydrolysis occurs due to the presence of residual plasmin which maintains a large part of its activity after the heat treatments of pasteurization. The levels of casein can be reduced by the effect of plasmin and residual rennet, together with the proteolytic activity of microorganisms present in milk as Enterococcus [23].

D. Cheese amino acid analysis

Emphasizing cheese amino acids, only the isoleucine and phenylalanine of Patillal had differences with Badillo and La Vega, the other amino acids had statistical differences between each others. The concentrations of the different amino acids in cheese are related to the manufacturing technology (type of curd, maturation
conditions), duration of maturation and the degree and type of proteolysis, this is the most complex and the most biochemical event important during maturation [22].

On the other hand, the non-essential amino acids that predominated most in Patillal cheese were: tyrosine, arginine and histidine, being on average greater than the other amino acids from the other two municipalities in 95.26%; 97.4% and 98.33% respectively. It is important that these amino acids are concentrated in the curd, because they are important in the development of aromas and texture in cheeses. Researchers have argued that the most relevant cause of nutrient loss was possibly due to the breakdown of the peptide bond in the chain of amino acids by the action of peptidase, which acts in a non-selective way by breaking the initial structure of the protein and hydrolysing it completely in the suero costeño; as a result of this reaction, essential and non-essential amino acids are lost: as isoleucine, leucine, lysine, histidine, arginine and tyrosine [24]. Studies developed by Bertolino et al., [17] reported that glutamic acid, valine, leucine, phenylalanine and lysine were the most free amino acids found in hard cheese. Also, Diana et al., [16] performed the amino acid profile in hard cheese, where glycine, leucine, lysine and valine had the highest concentrations. The content of these amino acids from the previous study in the total samples was 99.48, 77.87, 61.60 and 52.82 g / kg, representing 16.2%, 12.7%, 10.1% and 8.62% respectively. These authors stated that the release of amino acids is significantly influenced by the salt content. On the other hand, when the salt concentration is higher there are less water molecules to interact with the charged part of the protein, and therefore the protein interaction is stronger, releasing the free amino acids. Also the type of rennet that is used influences the final amino acid content, since the cheeses elaborated with artisan rennet have greater concentration of amino acids than the cheeses made with commercial rennet.

The quantification of loss established for the township Badillo, it is detailed that the amino acids in general are lost or hydrolyzed in the course of the process, leaving little concentration of them in the cheese. The essential amino acids that are conserved in higher concentration are lysine 91.9% and tryptophan with 93.19%, and with a greater percentage of loss are isoleucine 98.8% and leucine with 99.81%. In La Vega, the essential amino acids of cheeses from the previous study in the total samples were: isoleucine 98.8% and leucine with 99.81%. In this study, the quantification was done under industrialization conditions, duration of maturation and the degree and type of proteolysis, this is the most complex and the most biochemical event important during maturation [22].

IV. CONCLUSIONS

With respect to the results of the municipality of Patillal, this is characterized by being the zone whose essential amino acids predominate in greater proportion after the process of industrialization in comparison with the other towns. On the other hand, the essential amino acids retained in the highest concentration in Badilloqueso costeño are lysine with 91.9% and tryptophan with 93.19%. Emphasis was placed on milk, whey and cheese from Badillo township, which showed the highest protein content and total solids. It was also possible to show that in these food matrices, there were statistically significant differences in protein content and total solids. Patillal reported the highest protein, fat and lowest ash content in cheese samples.

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