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# Effect of the climatic period on the nutritional quality of cow's milk in Antioquia

## Efecto del período climático sobre la calidad nutricional de la leche de vaca en Antioquia

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Laura Marcela Bustamante-Córdoba[1*]; José Uriel Sepúlveda Valencia[1] and Diego Alonso Restrepo Molina[1]

## ABSTRACT

**Key words:**
Dairy
Nutritional quality
Quality based payment system
Caseins
Amino acids

This research was carried out in the milk production plants for 10 weeks (5 during the dry season and 5 during the rainy season in Medellín, Colombia). Samples of raw (fresh) milk were taken from Holstein cattle and fresh farmer cheese was produced through enzymatic coagulation. For the milk and the cheese, the physicochemical characteristics and nutritional quality, were evaluated for both seasons. For parameters such as the casein and the ash content, the non-fat milk solids showed a confidence interval that fluctuated between 8.3% and 8.7% (with an $\alpha$=0.05), which is comparable to that reported by the Colombian legislation; however, unusual results were identified within the registered periods, especially at the transition period between rainy and dry period (7.89% non-fat solids). The obtained yield averages were significantly higher in regards to those reported by various authors, while those found for the other studied parameters had results similar to those proposed in the literature. The evaluation and determination of high nutritional value factors in food, such as the protein percentage and the essential amino acids profile, allow for the improvement of quality based milk payment systems that can be applied nationwide and that are based on the initial proposal of *Fedegan* and the Republic of Colombia.

## RESUMEN

**Palabras claves:**
Lácteos
Calidad nutricional
Sistemas de pago por calidad
Caseínas
Aminoácidos

El presente trabajo de investigación se llevó a cabo en la Planta de Leches, durante 10 semanas (Cinco correspondientes a período seco y cinco correspondientes a período lluvioso en Medellín, Colombia) se tomaron muestras de leche cruda (fresca) provenientes de Ganado Holstein y se realizó queso fresco campesino mediante coagulación enzimática. Características fisicoquímicas y calidad nutricional fueron evaluadas a la leche y al queso para las muestras obtenidas en ambos periodos. Los análisis estadísticos de parámetros cómo caseína y ceniza; sólidos no grasos lácteos arrojaron un intervalo de confianza que fluctúa entre 8.3% y 8.7% ($\alpha$ = 0.05) comparables con lo reportado por la legislación colombiana, sin embargo se identificaron períodos con resultados atípicos, especialmente al presentarse la transición entre invierno y verano (7.89% de sólidos no grasos). Las medias de rendimiento obtenidas son significativamente mayores con respecto a las reportadas por diversos autores, mientras que las encontradas para los demás parámetros estudiados tuvieron resultados similares a los propuestos en la literatura. La evaluación y determinación de factores de alto valor nutricional en los alimentos como el porcentaje de proteína y el perfil de aminoácidos esenciales permiten mejorar el sistema de pago por calidad para la leche que aplica al territorio nacional, con base a lo inicialmente propuesto por Fedegan y el Ministerio de Agricultura de la República de Colombia.

[1] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. A.A. 1779, Medellín, Colombia.
* Corresponding author <lmbustam@unal.edu.co>

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The production of fresh milk in Colombia has been gaining increasing importance in the national economy due to its increasing demand in the domestic market, making dairy activities a mitigating factor in the agricultural sector crisis and creating jobs. The value of the production of fresh milk represented 4.8% of the gross agricultural domestic production in 1980, increasing to 6.3% in 1990, 10.0% in 1997, and almost 20.1% in 2012. In 2012, the participation of the agricultural sector in the country's GDP was 7.0%, with a contribution from cattle to the livestock sector of 50% (Salcedo, 2012).

The flexibility that currently exists in the production systems allows for adaptation to all agricultural ecologic environments that exist in the country, forcing milk and its derivatives to retain their nutritional characteristics regardless of the weather or time of the year, making dairy activities an alternative for regions where agricultural production has been affected. Milk production has been one of the few activities that has continued during this decade, with growth rates around 5% (per year, from approximately 1990 until now), even during the years when the agricultural sector, as a whole, declined.

Because milk is considered one of the most nutritional foods and is raw material for a great amount of products of high consumption in Colombia, it is necessary to provide consumers a detailed description of its composition and its nutritional and industrial value. High quality dairy products possess an adequate industrial yield and a good composition of nutrients, such as vitamins, proteins and amino acids, among others, regardless of the time of the year or the specific climate conditions (Tamime, 2009).

The quality of these foods plays a very important role for companies (speaking in terms of competitiveness), given that consumers are looking to acquire products that guarantee a necessary input of nutrients required by the body; producers want the efforts made to improve their harvests to be reflected in the prices that are paid for the raw material. The determination of non-fatty solid dairy products (especially the protein portion), for example, is quite important nowadays because it is a parameter that plays a special role in the calculation of the price in the global market and because it acts as an indicator of adulteration (represents the protein, lactose and minerals portion) and establishes the nutritional value and the yield in the processing of dairy products, such as yogurt and cheese, among others (Santoyo *et al.*, 2001).

Therefore, it is desirable for the purchased product to have acceptable sensory characteristics that are free of physical (external material), chemical, and microbiological pollution, and, at the same time, it is expected to be economically and nutritionally beneficial, with a long shelf-life, combined with products that are exclusive in quality and designed for high added value (Analdex, 2010). Fresh milk must be perfect when used as raw material for the production of quality products, not only nutritionally but also industrially, in order to satisfy consumer preferences and increase the profitability of the processes, providing better payments for producers regardless of factors such as climate that may affect quality.

Therefore the aim through this study is to evaluate the effect of climatic period on the nutritional quality of cow's milk in Antioquia.

## MATERIALS AND METHODS

### Obtaining samples

The milk of Holstein cattle were collected from the Paysandú property in the municipality of Santa Elena and transported to the laboratory. The milk was used, which had good quality and was in a good physic-chemical and microbiological condition.

### Sampling

Randomly, 10 milk samples were chosen (approximately 11 kg each), of which 10 kg were transformed into cheese during the dry and rainy seasons of Colombia.

By these means, samples were analyzed under certain parameters of interest: Total protein and casein, essential amino acids profile (5 out of 8: phenylalanine, isoleucine, leucine, methionine and tryptophan), and ash, calcium, phosphorus and fat percentages through standardized methods of the National Technical Standards and the AOAC 994.12 and AOAC 982.30 (AOAC, 1998).

### Cheese making

The coagulation of the cheese was done with an enzymatic mechanism according to the process described by Fox *et al.* (2004).

**Experimental design**

A $2^k$ ($2^2$) factorial design was developed with five replications in a random complete experimental design. The response variables were: ash, calcium, phosphorus, fat, protein content, casein content and amino acid profile of raw milk and cheese. The study factors corresponded to: A type of milk (at two levels: raw and cheese); and B season (at two levels: rainy and dry.

Analysis of variance (ANOVA) was applied to the data using MiniTab (V14) in order to detect differences between the means (Significance level of $\alpha$=0.05). Pareto charts were obtained, showing the vital Xs that acted on the response variable; graphs of the main effects showed how the data behaved under each of the factors, which allowed us to identify the incidence of each of the factors in the variables of interest.

**Determination of essential amino acids**

High performance chromatography (HPLC) with a BAS® chromatographer (California, USA) and a Water 474 fluorescence detector was used through standardized methods of AOAC 994.12 and AOAC 982.30 (AOAC, 1998).

**Protein, fat and casein determination**

To obtain the protein and casein content, the AOAC 991.20 method was employed (AOAC, 1998). To obtain the fat content the methods were AOAC 989.04 (AOAC, 1998) and AOAC 974.09 (AOAC, 1998).

## RESULTS AND DISCUSSION

Table 1 shows the overall average for ash in the milk, with results within the minimum value established by the current Colombian standard: NTC 399 (2008) and others national technical standards, decrees and resolutions; this result is also in accordance with the data reported which indicate that, for Holstein cattle, this value must be 0.74% ± 0.02% (Canilec, 2011; Hadjipanayiotou, 1994).

For the cheese, according to the analysis, an average concentration of 1.70% with a 95% confidence interval that fluctuated between 1.611% and 1.814% was seen, in accordance with the values reported by Cunningham (2000) and Alais (1985), where the result of the concentration of this variable was between 0.9% and 1.9%.

The design of the experiments carried out for this variable indicated that the factor types, milk and season, had an effect on the final percentage of ash in the samples, as well as the interaction between the two variables as shown below:

**Table 1.** Overall average for quality variable of samples.

| Variable | Sample | Mean | SE | CI of 95% | |
|---|---|---|---|---|---|
| Ash (%) | Milk | 0.7350 | 0.010 | 0.7281 | 0.7420 |
| | Cheese | 1.712 | 0.142 | 1.611 | 1.814 |
| Calcium (%) | Milk | 0.112 | 0.008 | 0.106 | 0.118 |
| | Cheese | 0.540 | 0.047 | 0.507 | 0.573 |
| Phosphorus (%) | Milk | 0.080 | 0.005 | 0.077 | 0.083 |
| | Cheese | 0.334 | 0.022 | 0.318 | 0.349 |
| Fat (%) | Milk | 3.230 | 0.125 | 3.141 | 3.319 |
| | Cheese | 21.485 | 1.726 | 20.250 | 22.720 |
| Total Protein (%) | Milk | 2.906 | 0.085 | 2.845 | 2.967 |
| | Cheese | 16.082 | 1.203 | 15.221 | 16.943 |
| Total Casein (%) | Milk | 2.477 | 0.085 | 2.306 | 2.648 |
| | Cheese | 12.983 | 2.962 | 10.864 | 15.102 |

Figure 1 shows that the ash percentage, both for the milk and cheese, was affected by the season factor, with higher values in the rainy season (Figure 2). This result can be due to the change in the quality of the grass with which the cattle was fed, based on what was reported by various authors (Zeng *et al.*, 2007; Hadjipanayiotou, 1994), who indicated that cattle milk is more stable in terms of composition in the colder months. During this time, fat, minerals (expressed as percentage of ash), lactose and proteins are concentrated.

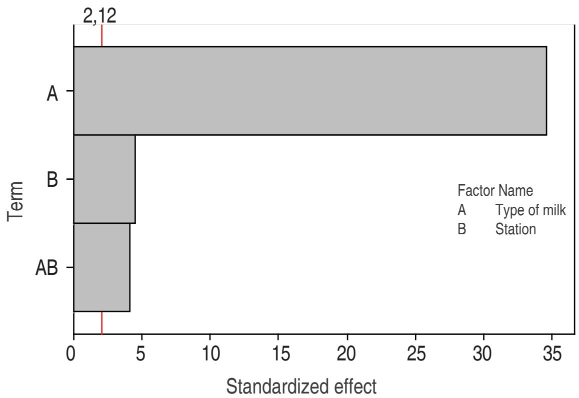


**Figure 1**. Pareto chart of standardized effects and main effects: ash percentage.

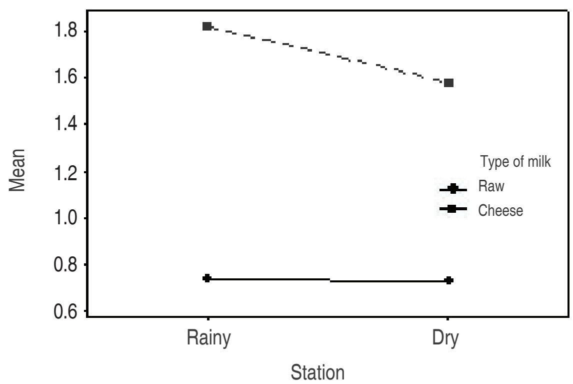


**Figure 2**. Interaction plot: ash percentage.

## Minerals: calcium and phosphorus

Particularly for the calcium percentage, the analysis indicated that this variable was only affected (according to the statistical evidence) by the type of milk factor, as shown in Figure 3; this observation is consistent with the report of Mapekula *et al.* (2011), who indicated in one of their studies that the calcium composition does not vary significantly (P> 0.05) nor does it show a marked trend or variation for any season of the year (Figure 4).

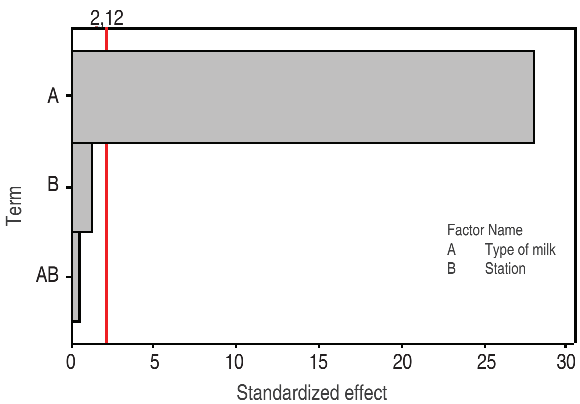


**Figure 3.** Pareto chart of standardized effects: calcium percentage.

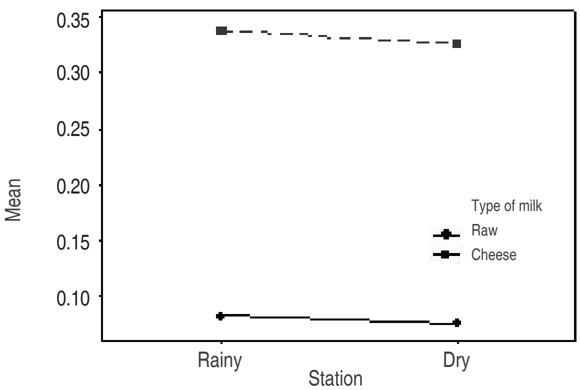


**Figure 4.** Interaction plot: calcium percentage.

The percentages reported for the calcium in the fresh milk (Table 1) showed a significance level of $\alpha$=0.05 in an interval between 0.106% and 0.118% and, for the fresh cheese, between 0.507% and 0.573%; these data are close to those reported in research carried out by Sepúlveda (2007) and Menz (2002), who indicated that, for the first type of milk, the calcium percentage must be at least 0.08% and, for cheese, between 0.45% and 0.55%, values that are between our 95% confidence interval.

As in the observations made for the calcium, the Pareto chart of standardized effects showed the phosphorus to be a response variable that was only significantly affected by the type of milk factor and the interaction: type of milk and season factor did not significantly affect the concentration of the mineral as indicated by the statistical analysis.

The graphs of the average profiles for the interaction of the factors: type of milk and season (Figure 5) show that the double interaction was not significant, since, for both the level of raw milk and cheese, the data varied slightly in the dry season and rainy season levels, showing straight lines with a similar slope for both (Figure 6).

Basic statistics performed on the phosphorus composition variable for both fresh milk and cheese are presented in Table 1. For the phosphorus, we found that its concentration fluctuated between 0.077% and 0.083% for the raw milk and from 0.318% to 0.349% for the fresh farmer cheese; as compared with the values proposed by Sepúlveda (2007) and Badui (2006): between 0.07% and 0.09% for raw milk and between 0.26% and 0.36% for fresh farmer cheese.

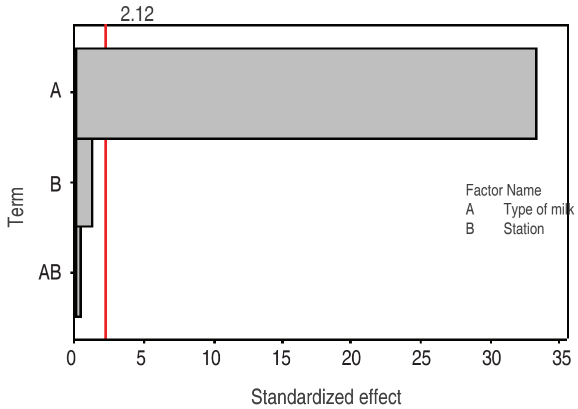


**Figure 5.** Pareto chart of standardized effects and profile of averages: phosphorus percentage.

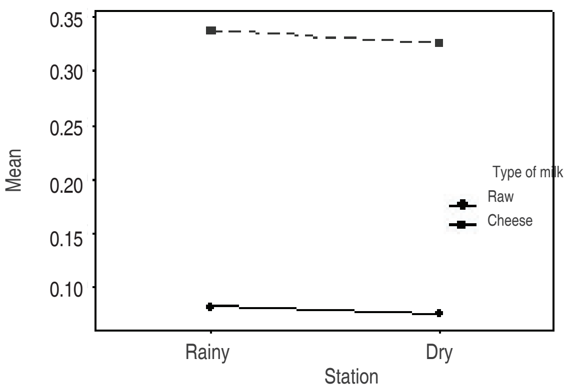


**Figure 6.** Interaction plot: phosphorus percentage.

## Fat and total protein

The percentage of fat was not significantly affected by the effects of the seasons (Figure 7), as has been reported in writings by Zeng *et al.* (2007) and Wedholm *et al.* (2006).

The interaction diagram allows us to conclude that, during the seasons, the fat percentage did not have a significant statistical variation (Figure 8).

The statistical analysis (as described in Table 1) allowed for the statement that the percentage of fat in the analyzed milk had an average of 3.23% and 21.5% for the cheese; average fat values of 22% have been reported; for example, Jaramillo *et al.* (1993) made reports similar to what we found in our samples; however, these results had a slight difference from the contents of Decree 616 of 2006 and Moreno and Ruiz (2007), who reported values of 28% in fresh farmer cheese.

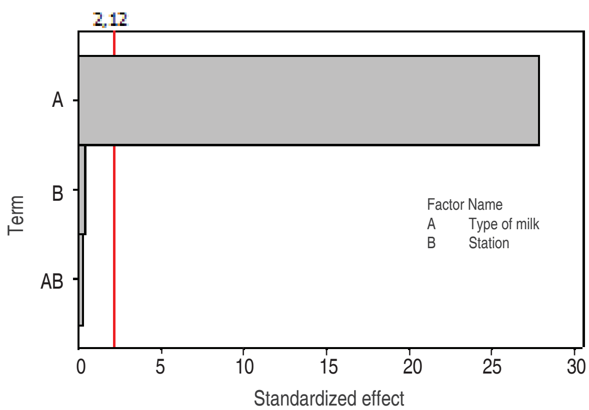


**Figure 7.** Pareto chart of standardized effects: fat percentage.

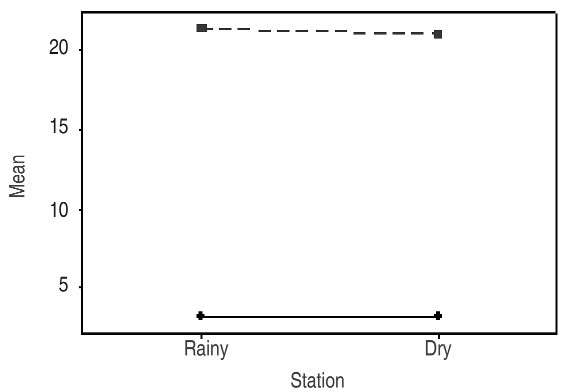


**Figure 8.** Interaction plot: fat percentage.

For the analyzed milk, there were similar values to those reported by the Ministerio de Agricultura y Desarrollo rural (1989); Furtado (2005) and Corrales, and Sepulveda and Higuera (2005), who proposed an average percentage of total fat of 3%.

The results obtained in the design of the experiments showed that, in the dry season and rainy season, there were no significant differences as shown in the Pareto Effects chart (Figure 7), where the only bar that goes through the P-defined value is the one that represents the type of milk factor, leading to the conclusion that there was statistical equality of the averages between both climatic periods.

The basic statistical analysis presented the following values for the raw milk and fresh cheese, taking into account its influence on the outcome of the variable (Figures 9 and 10).

The percentage of total protein showed a range between 2.85% and 2.97% with a confidence of 95% for the raw milk, an interval that differs slightly from that proposed by NTC 399 (2008); Sepúlveda (2007); Decree 616 of 2006; Páez *et al.* (2002), who reported values close to 3.2%, this was probably due to the low composition of the total non-fat solid milk identified in the samples.

For the cheese, the average score for the percentage of total protein was 16.1% (95% CI 15,221% to 16,943%);

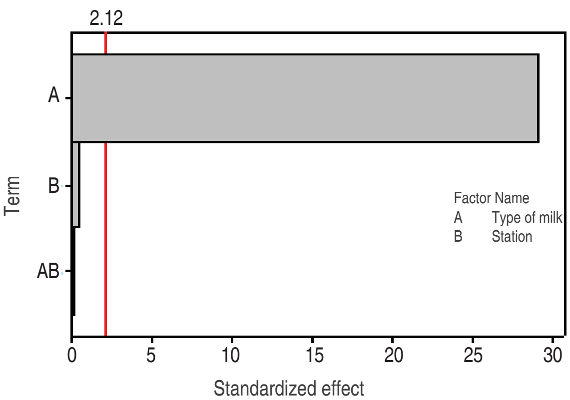


**Figure 9.** Pareto chart of Standardized effects: percentage of total Protein.

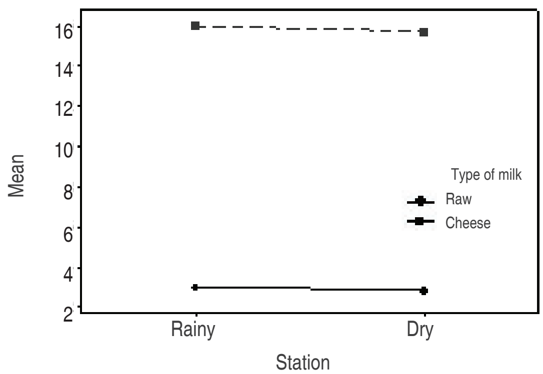


**Figure 10.** Interaction plot: percentage of total Protein.

this value, as seen in the raw milk, differed slightly from those reported by Moreno and Ruiz (2007): 19.95% to 20.56%; FAO (2002): 22.0% to 24.0% for fresh cheese and Jaramillo *et al.* (1997), who reported up to 24.0%; this difference occurred because the raw material used for the production of the cheese was low in the non-fat solids variable.

## Total casein

The statistical analysis for the casein percentage variable indicated that the factors type of milk, season and the interaction of both had a significant effect, which means that a change in any of these factors will generate a change in the variable (Figure 11).

The interaction chart (Figure 12) shows how, for the cheese factor, the season had a very marked effect by maximizing the value in the dry season level; for raw milk, the variation was not as marked but was statistically significant (with the same behavior).

The basic statistical analysis (Table 1) indicated that the casein showed significant differences between the observed seasons, this agrees with the observations of Bernabucci *et al.* (2002) and Callanan (1991), who agreed that the amount of casein increases considerably in the dry season.

The average was 2.48%, with a 95% confidence interval ranging between 2.3058% and 2.6482%; this range can be compared to what was reported by Walstra *et al.* (2006), who proposed a much wider range of between 1.7% and 3.5%. For the cheese, a similar situation was observed, where the average of this variable has been reported in the literature, values close to 13% (Cunningham, 2000), which

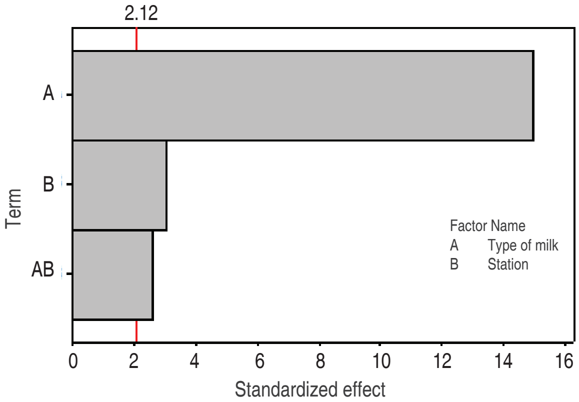


**Figure 11.** Pareto chart of Standardized effects: percentage of total Cassein.

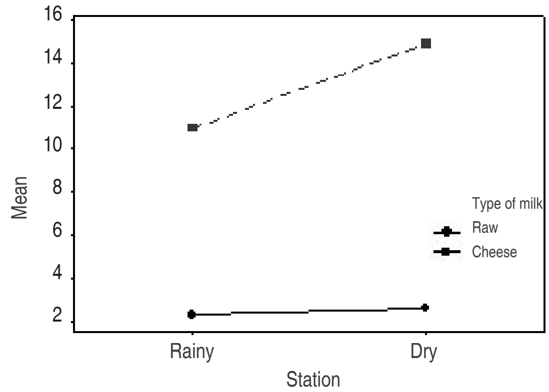


**Figure 12.** Interaction plot: percentage of total Cassein.

corresponds to the average found with our data (12.76 % with an α=0.05). However, it is important to emphasize that, in the dry season, the average for this variable was found to be around 14.54%, while, for the rainy season, it was 10.98%. The loss of serum proteins in the production of cheese explains why higher average values of total percentage of total casein can be found in the dry season in contrast to those found in the rainy period and, therefore, significantly higher values of cheese yield in this period; this phenomenon is linked to the differences in the ways of feeding livestock: in the rainy period, the concentration of serum proteins in the milk is favored, which are lost in the production of cheese, decreasing the percentage of casein in the total protein portion; which would lead to a decrease in the cheese yield (Olson, 1977).

## Amino acids

In 4 out of the 5 studied amino acids, there were observed behaviors in which only the type of milk factor had an impact on the concentration of amino acids. The only one for which the climatic periods played an important role was leucine, which had a tendency to increase in the rainy time.

In the studies that have been conducted to determine and quantify amino acids in milk and its derivatives, which are relatively few, we found average values that are detailed below (for the 5 amino acids studied).

As can be observed in Mapekula *et al.* (2011) and Walstra (2006), reported slightly higher values for 3 amino acids, a variation explained by the total protein parameter, while the average for methionine and isoleucine is within, or near, the confidence interval of the experimental data.

On the other hand, for the cheese, there are a few theories about how amino acids behave after coagulation processes that must be performed, taking into account the loss of serum and casein protein. In the following table, one can observe a comparison of the average proposed by Ramo (2009) with the experimental results (95% confidence).

Individual Analysis of the amino acids is detailed as follows:

The values show the same trend in the milk and cheese, so there was preservation of the amino acids in the production of cheese (Mapekula *et al.*, 2011; Walstra, 2006).

### Phenylalanine

The graphic analysis carried out with Minitab indicated that the phenylalanine response variable was significantly affected by the type of milk factor, while the interaction between the defined factors (type of milk and season) and the season factor did not significantly affect the outcome of the final concentration individually, as shown in Figure 13.

In the raw milk, Phenylalanine presented a similar behavior for both seasons, while, for the cheese, the Phenylalanine composition was slightly higher for the

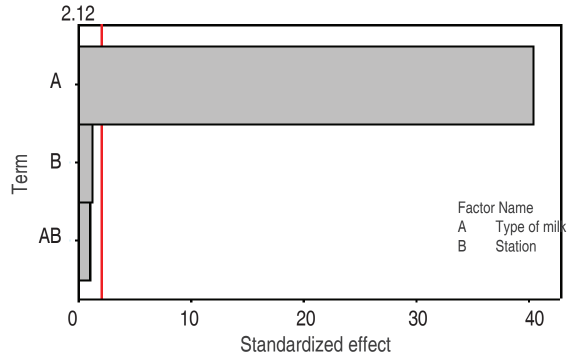


**Figure 13.** Pareto chart of Standardized effects: Phenylalanine percentage.

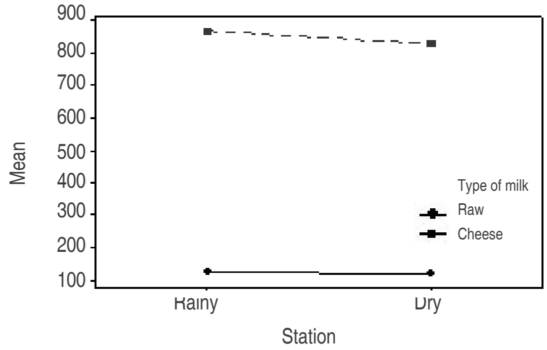


**Figure 14.** Interaction plot: Phenylalanine percentage.

rainy period (slight significant difference) as shown in Figure 14.

## Isoleucine

The isoleucine variable presented a similar behavior to that of Phenylalanine; this response variable was significantly affected by the type of milk factor, while the interaction between the defined factors (type of milk and season) and the season factor (individually) did not significantly affect the value of the variable, as shown below (Figure 15).

However, graphically, the values are very close to the reference value, which tells us whether the change in the levels of the experiment design affected the response variable. According to the factor

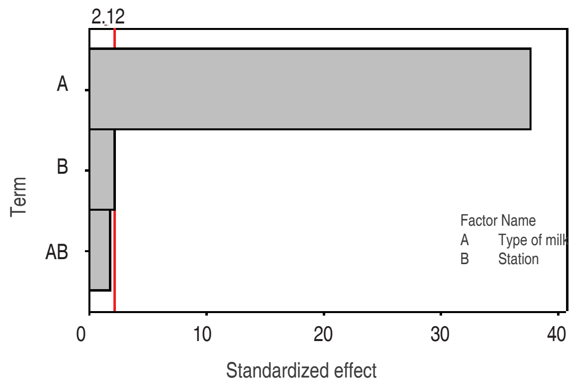


**Figure 15.** Pareto chart of Standardized effects: Isoleucine variable.

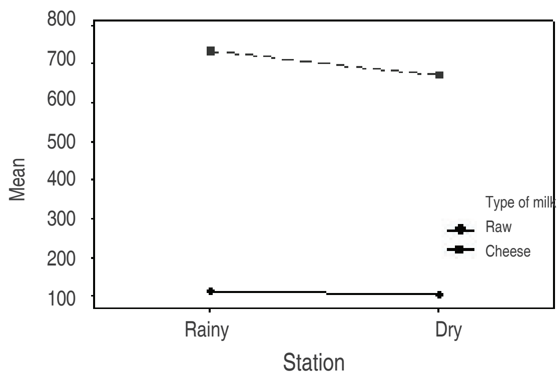


**Figure 16. I**nteraction plot: Isoleusine variable.

interaction graph (Figure 16), the season factor had a considerable slope for the cheese level, which indicates higher values of the amino acids in the rainy period, indicating a possible effect of the type of milk factor on the others; more data is needed to discard this hypothesis.

## Methionine

The Methionine tended (slightly significant) to increase in the dry period; however, this situation was not considered statistically significant so it is possible to regard it as having had a constant observation in the two seasons (Figure 17). In the slopes observed in the interaction graph for both the cheese level and the season factor, as well as for the raw milk level, there are parallel lines, which indicate that the interaction between the two cannot be given as shown in Figure 18. There was a slight trend for the data to take higher values in the dry period level (Figure 18).

## Tryptophan

The graphic analysis indicated that the tryptophan response variable was significantly affected by the type of milk factor, while the interaction between the

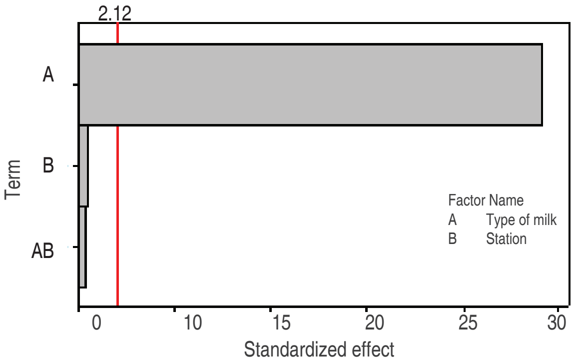


**Figure 17.** Pareto chart of Standardized effects: Methionine.

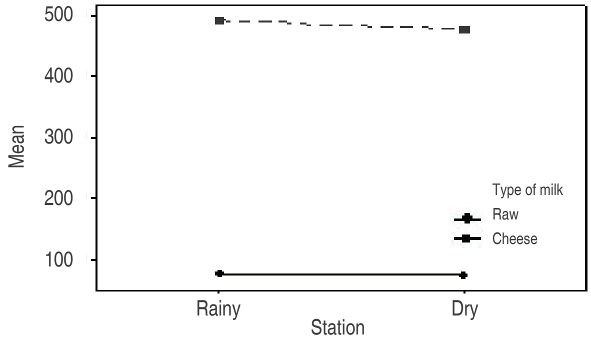


**Figure 18.** Interaction plot: Isoleusine variable.

defined factors (type of milk and season) and the season factor (individually) did not affect the value of the response variable (Figure 19). The main effects chart indicates that the season factor did not present significant differences in its levels, although, for the fresh cheese, a small tendency to increase in the dry period was observed (graphically) as shown in Figure 20.

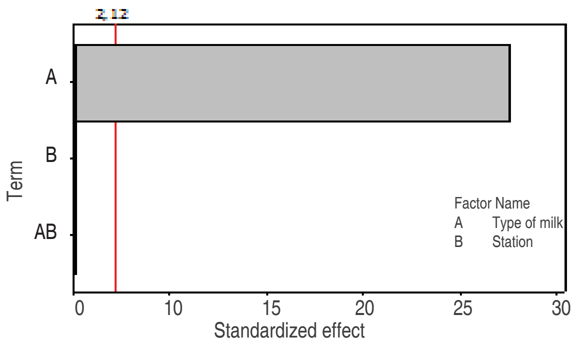


**Figure 19.** Pareto chart of Standardized effects: Tryptophan.

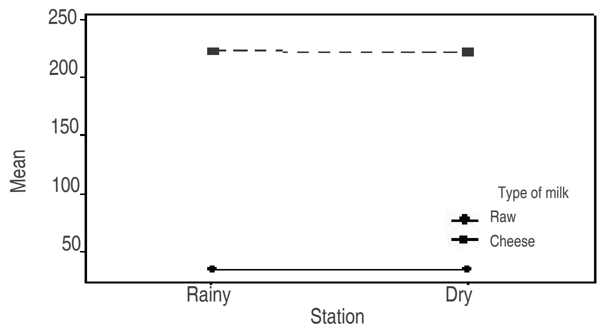


**Figure 20.** Pareto chart of Standardized effects and interaction: Tryptophan

**Table 2.** Comparison of the average proposed by Ramo, 2009 with the experimental results (95% confidence) for amino acids.

| Variable | Sample | Reference Value | Mean (mg/100mL) | SE | CI of 95% (mg/100mL) | |
|---|---|---|---|---|---|---|
| Phenylalanine | Milk | 143 | 124.24 | 4.28 | 121.18 | 127.31 |
| | Cheese | 801 | 855.9 | 50.3 | 819.9 | 891.8 |
| Isoleucine | MIlk | 175 | 109.86 | 6.93 | 104.91 | 114.82 |
| | Cheese | 663 | 709.6 | 52 | 672.4 | 746.7 |
| Leucine | Milk | 286 | 271.24 | 25.06 | 253.31 | 289.16 |
| | Cheese | 1659 | 1696.3 | 236.5 | 1527.2 | 1865.5 |
| Methionine | Milk | 71 | 76.69 | 5.74 | 72.59 | 80.8 |
| | Cheese | 423 | 491.7 | 47.4 | 457.8 | 526.6 |
| Tryptophan | Milk | 39 | 35.2 | 3.92 | 32.71 | 38.32 |
| | Cheese | 242 | 224.8 | 23.1 | 208.3 | 241.3 |

**Leucine**

For the Leucine variable, it can be observed in Figure 21 that both factors (type of milk, season and the interaction of the two) had a significant effect. The graph of the interaction shows how, for the cheese factor, the season had a significant impact, maximizing the value in the rainy season, while, for the milk, there was a slight variation with the same trend (Figure 22).

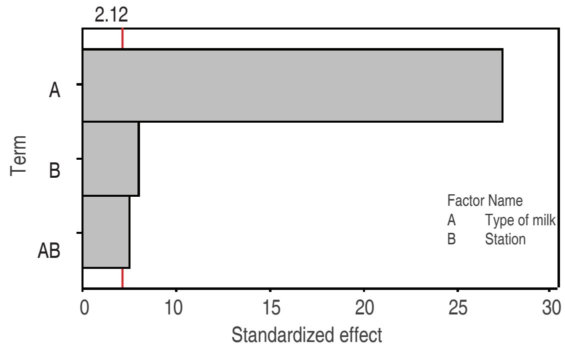


**Figure 21.** Pareto chart of Standardized effects and interaction: Leucine.

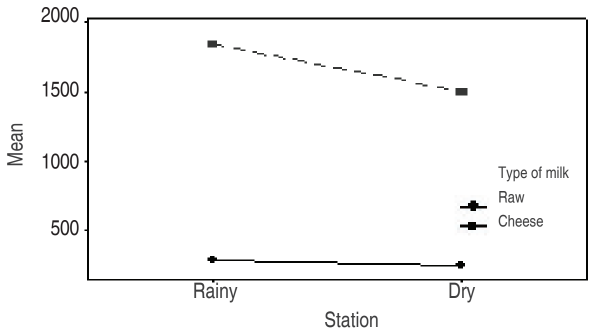


**Figure 22.** Pareto chart of Standardized effects and interaction: Leucine.

## CONCLUSIONS

The comparison of the data in the variables of interest in relation to the season factor showed that the vast majority did not have significant differences; only the percentages of ash, casein and leucine were affected by the season factor.

The ash percentage presented a difference between the seasons, presenting higher values during the rainy period; however, the averages for both were within the ranges proposed in the literature.

The percentages of calcium and phosphorus (both for milk and cheese) had opposing behaviors. For the calcium, the higher averages were obtained in the dry season, while, for the phosphorus, the rainy season had higher values.

The percentages of fat and total protein were not significantly modified by the seasonal changes (Climatic changes in Colombia).

Maintaining the ratio between total protein and fat in the raw material (raw milk) will ensure that the final product has excellent organoleptic properties (taste, smell and texture) and concentrations of amino acids.

In the rainy period of Colombia (between October and March), the concentration of serum proteins (losses in the coagulation process) is favorable within the percentage of total protein in the milk and, for this reason, the casein composition in the cheese decreased considerably in this period, in relation to the dry period (10.98% compared to 14.54%).

The inclusion of parameters such as percentages of casein and amino acids for the representation of the nutritional quality of milk and its derivatives will lead to the improvement of product processes.

Leucine was the only analyzed amino acid that presented a particular behavior, with very marked trends (statistically significant) in the rainy period, leading to a recommendation for a more in-depth study of leucine in relation to the transformation of milk into cheese and the transformation processes that proteins suffer, particularly caseins.

In general, the literature has not yet reported on changes seen in amino acids between climatic periods, which makes it difficult to have a structured framework in which a comparison can be made with the results obtained at this point in the research.

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