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Characteristics of Algerian Goat's Milk

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Abstract: Goat milk, which is very popular especially in France where many famous cheeses are made, could meet the needs of people living in the mountains, and can be eaten fresh or processed. However, for the latter case, it is established that only milk with α S1-casein expressed with a high percentage could result in the manufacture of cheese. For this, we conducted a study that aims in a first step to assess the physical and chemical characteristics of goat milk collected in three regions of Tizi-Ouzou. The analysis performed has carried on the pH, acidity, total solids, proteins, fat, lactose and vitamin C. The results obtained for these parameters, including total solids (109.3 g / l), fat (30.7 g / l), lactose (39.1 g / l) and proteins (26g / l), are state of the good nutritional value of milk collected locally. However, the levels obtained were below those of bovine milk analyzed under the same conditions. In a second step, we performed the isolation and characterization of proteins. For this, we used the precipitation of the casein at their isoelectric pH (pH 4.2) followed by ion exchange chromatography on DEAE-cellulose. The fractions were then checked by polyacrylamide gel electrophoresis under different conditions (native, in the presence of urea, in the presence of SDS). The resulting electrophoretic profiles have identified similarities between goat and cow milk as they have helped to highlight some particularities including both caseins (αS) as serum proteins (PP3, α-La and β-Lg). The use of weak anionic resin in a single chromatographic separation step is advantageous in that it led to the isolation of goat protein (casein β, casein α S, PP3, SA and β-Lg) with a high degree of purity.

Keywords: Goat milk, Proteins, Phenotyping, Electrophoresis, Chromatography.

Introduction

The nutritional benefit of milk lies in its richness in basic nutrients (proteins, lipids and carbohydrates) but also in calcium, vitamins and trace elements. It is one of the rare foods that is suitable for different age groups where it can be consumed as is, fresh or in the form of a processed product, notably in cheeses and yogurt. In addition, the different ingredients constituting milk (proteins, peptides, lactose, fat, etc.) have been used wisely by the food industry for recent decades to produce products with new functionalities and best suited to the demands of the consumer. It is precisely for these reasons that the need for this material continues to increase around the world while global milk production is unable to follow this trend.

Thus, over the last quarter of a century, the milk consumption of the world population has increased by 32% while production per capita has fallen by 9%. In these ratios, cow's milk occupies the largest proportion (around 80%), the rest is made up of buffalo, goat, sheep and camel milk. This situation of deficit in milk produced is even more accentuated when we look closely at the case of our country which is rightly considered as the first

Maghreb consumer of milk (100 l/year/inhabitant) but whose milk production (1 billion l/year) does not cover needs estimated at more than 3 billion l/year. Here too, other dairy species (goat, sheep, camel) only cover around 10% of needs which are met by resorting each year to the importation of milk powder (250,000t/year) and fat. anhydrous. In order to stem this trend somewhat and restore balance, our country has put in place a strategy for developing and encouraging national production by allowing breeders in particular to import appropriate dairy breeds and to establish themselves in breeding cooperatives while the milk collection circuit has been improved by the introduction of collection centers and means of early milk refrigeration. This development plan has also recorded an increase in the production and collection of fresh milk. But until then, the bulk of efforts have been focused on the beef sector. The other sectors (sheep, goats and camels) remain marginal with production intended mainly for self-consumption. Furthermore, in mountain agriculture, the goat, renowned for its hardiness and its adaptation to this particular terrain, has always constituted an ideal solution for local populations who obtained practically all of their milk needs from this animal, which is known and prized in other countries, particularly in France for the reputation of the guaranteed appellation cheeses that are made exclusively from its milk. If the development effort in our country continues, the tonnages of goat milk, will be revised upwards, which will provide interesting prospects for the sale and consumption of this milk in its fresh state or its processing, particularly into cheese.

In this section in particular, it is established that only milks having αS1 casein expressed with a high percentage can be transformed into cheeses. Therefore, it seems obvious that the analysis of this milk and its characterization on the protein level can help to better guide technologists on the possibilities of industrial exploitation of this collection milk. From this perspective, the present study aims, on the one hand, to evaluate physico-chemical plan the goat milk collected in 3 three regions of Tizi-Ouzou (Azazga, Mekla and Larbaâ Nath Irathen) and, on the other hand, to carry out the isolation and purification of the major proteins of these milks before characterize in terms of their electrophoretic behavior.

Material And Methods

Raw Material

The milk samples analyzed are fresh, large-mix milks from herds of healthy goats, located in the Tizi-Ouzou region.

Analysis Methods

Milk Collection

Milk samples were collected from healthy goats. The milk is milked cleanly and is immediately added with 0.3 g/l of sodium azide (NaN3), in order to avoid any microbial development. The samples are transported in a cooler to the laboratory where they are immediately analyzed. On arrival, a pH measurement is immediately carried out. Depending on the experimental objective, the milk is fractionated. One part is intended for physicochemical analyzes and another for protein phenotyping. For the rest, it is divided into small fractions and frozen thus for later use.

Physico-Chemical Analyzes

The milk comes from healthy goats from the localities of Mekla, Azazga and Larbaâ Nath Irathen. As soon as it arrives at the laboratory, the milk is analyzed. Parameters such as pH and acidity (°D) are measured. The other parameters were analyzed: determination of the total dry extract (FIL 21 standard, 2010), fat content (FIL 22B, 1987), lactose content (according to the method described by AUDIGIE et al, 1978) and finally vitamin C following the AOAC 967.21 method (2006). The protein content was estimated according to the method of LOWRY et al (1951).

Protein Isolation and Purification

The proteins were separated by acid precipitation at pH 4.2 followed by centrifugation at 3500 g/20 min. The supernatants (serum proteins) were checked by polyacrylamide gel electrophoresis (PAGE-native) according to

the method of HILLIER (1976); with porosity of the gel (T = 12% and C = 2.7%); gel buffer (TRIS, 0.75 M, pH 8.9) and electrode buffer (TRIS, 5 mM; glycine, 77 mM; pH 8.3). The pellets (caseins) were analyzed in PAGE-Urea by applying the protocol described by NG-KWAI-HANG and KROEKER (1984) using a concentration gel (T = 4% and C = 2.7%, buffer: urea , 0.8 M, TRIS, 0.49 M at pH 6.8) and a separation gel (T = 13% and C = 2.7%, buffer: urea, 4 M, TRIS, 1.5 M for a pH of 8.8). The determination of the molecular weights of these proteins in PAGE-SDS is carried out following the protocol of LAEMMLI and FAVRE (1973), with a concentration gel (T = 4% and C = 2.7%, buffer: TRIS - HCl, pH 6.8) and the separation gel (T = 15% and C = 2.7%, with a buffer: TRIS - HCl, pH 8.8). For the fractionation of individual caprine caseins we applied a step•wise according to the protocol of WEI and WHITNEY (1985) (pH 7 buffer: 0.02 M, urea at 3.3 M and 2-mercaptoethanol at 0, 3% (v/v). The fractionation of individual caprine serum proteins is carried out according to the conditions established by MAUBOIS (1964) (0.01 M Tris-HCl buffer pH 6.8.

Results and Discussion

Physico-Chemical Analyzes

The pH recorded for the goat milk samples analyzed with an average of 6.61 remains relatively close to that recorded for bovine milk at 6.69. These values generally agree with those reported in the bibliography (REMEUF et al, 2001; IMRAN, 2008). The acidity follows these trends in the two milks (average: 15.5°D), this reveals their good health status in reference to the fact that certain dairies give the upper limit of acceptance of milks at 16°D (QUELLETTE, 2004). On average the contents of dry extract (109.3 g/l), fat (30.7 g/l), lactose (39.5 g/l), vitamin C (13.7 mg/l) and proteins (26.7 g/l) of goat's milk, follow a similar fluctuation consistent with the literature (VENOUGLOU et al, 1982; JAUBERT G, 1997), but remain lower than those of bovine milk (respectively: 119 g/l, 35. 7 g/l, 45.4 g/l, 18.5 mg/l and 28.5 g/l).

Table 1. Protein concentrations of LC goat's milk compared to LV cow's milk. PT: Total protein. PLS: Whey proteins CN: Caseins.

Paramètres	LC1	LC2	LC3	LV
pH	6,65 ± 0,04	6,58 ± 0,02	6,61 ± 0,06	6,69 ± 0,03
Acidité titrable (°D)	15,3 ± 1,43	16,5 ± 0,40	14,7 ± 0,85	15,5 ± 1,22
EST (g/l)	115,7 ± 3,29	110 ± 1,41	102,3 ± 1,73	119 ± 2,16
MG (g/l)	34 ± 1,24	30,4 ± 1,28	27,7 ± 0,35	35,7 ± 0,92
Lactose (g/l)	40,6 ± 1,88	42,7 ± 2,20	34,2 ± 1,53	45,4 ± 0,73
Vitamine C (mg/l)	13,9 ± 0,95	10,8 ± 1,25	16,4 ± 1,45	18,5 ± 1,25
PT (g/l)	28 ± 0,52	27,4 ± 0,85	24,8 ± 1,19	28,5 ± 1,60
PLS (g/l)	6,5 ± 1,14	6,8 ± 0,27	5,5 ± 0,85	6 ± 1,34
CN (g/l)	21,5 ± 1,03	20,6 ± 0,66	19,3 ± 0,78	22,5 ± 1,23

However, sample 3 apart from vitamin C (16.4 mg/l) stands out for the other parameters mentioned above by relatively lower averages (EST: 102.3 g/l, MG: 27.7 g/l , lactose: 34.2 g/l, proteins: 24.8 g/l) in comparison to the other two samples.

Characterization of Proteins

The serum fraction of goat milk in native PAGE presents the same protein species as its bovine counterpart. Referring to the work of MATI (1992), we can see the same level of migration of immunoglobulins and serum albumin from both milks and a different level for PP3 (bovine PP3 migrates further forward than that of goats). B-lactoglobulin and α-lactalbumin have relatively lower levels of migration.

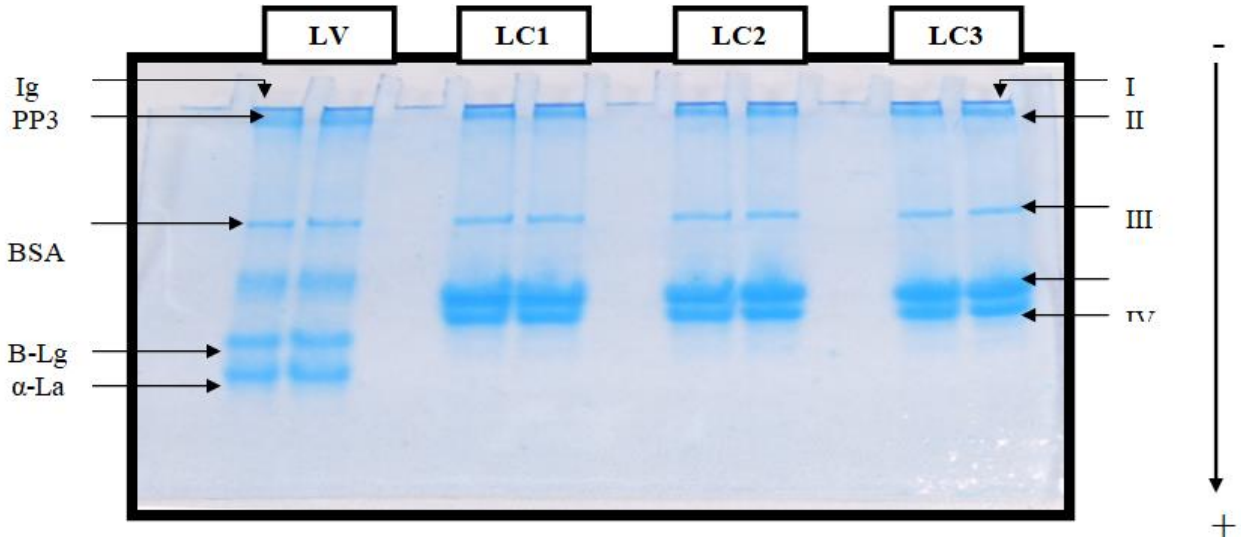

Figure 1. Electropherogram of serum proteins from goat milk compared to bovine milk (T = 12%; C = 2.7%); LV: cow's milk; LC1, LC2, LC3: goat's milk. Ig: immunoglobulin. PP3: proteose-peptone 3. BSA: Bovine Serum Albumin. B-Lg: B-lactoglobulin. α-La: α-lactalbumin.

PAGE-SDS determines three migration bands I, II and III (figure 2), the MW of these is respectively 66221 Da, 17005 Da and 13558 Da and would correspond to caprine serum albumin, B-lactoglobulin and with α-lactalbumin (Jovanovic et al., 2007).

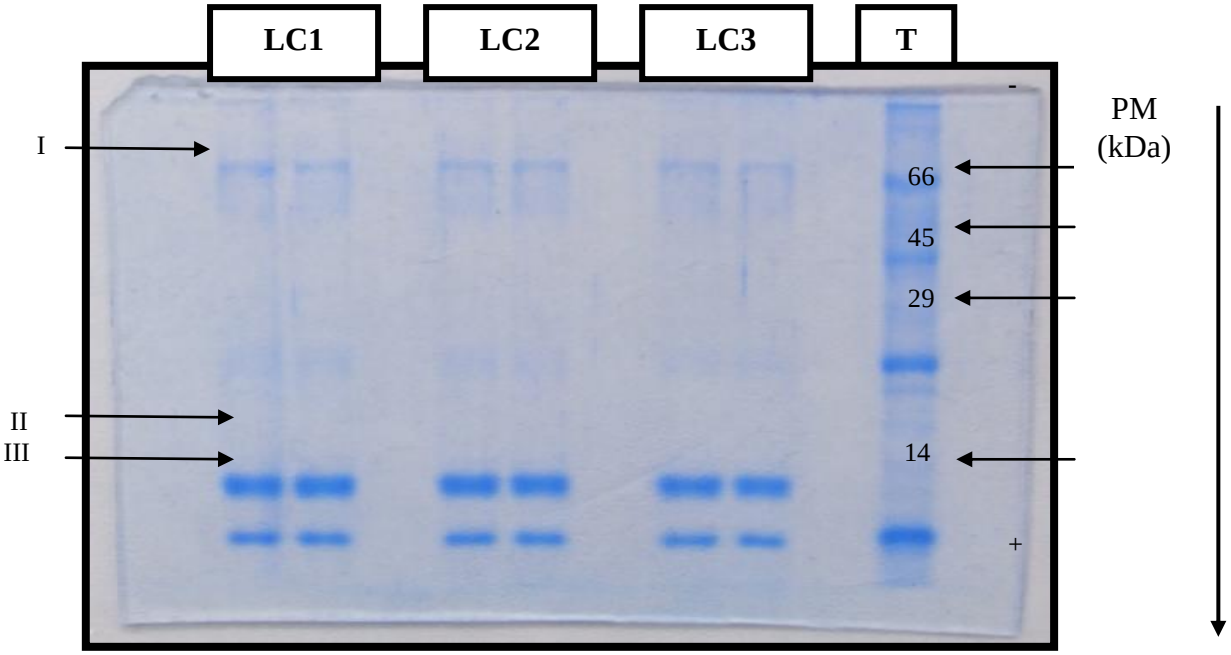

Figure 2. Electropherogram of goat milk serum proteins in PAGE-SDS; separation gel (T = 15%; C = 2.7%) and concentration gel (T = 4%; C = 2.7%); T: standard proteins; LC1, LC2 and LC3: goat's milk. CSA: Caprine Serum Albumin

The behavior of caprine caseins in PAGE-urea presents some similarities compared to their counterparts in reference milk. β-casein has the same level of migration (figure 3), while α S-caseins are more delayed, which is in line with the conclusions of LE BARS and GRIPON (1993). The presence/absence of α S1 casein has been the

subject of numerous studies in the literature which have shown that the presence of certain variants of this protein are correlated with good technological capabilities of goat milk (Piacer &Elsen, 1992; Remeuf, 1993; Remeuf et al., 2001) In PAGE-SDS, we obtain 4 migration bands (I to IV) with respective PMs of 33674 Da, 32270 Da, 28398 Da, and 27214 Da. These would correspond to α s2, α s 1, β and κ casein (Hiroyouki et al., 2006).

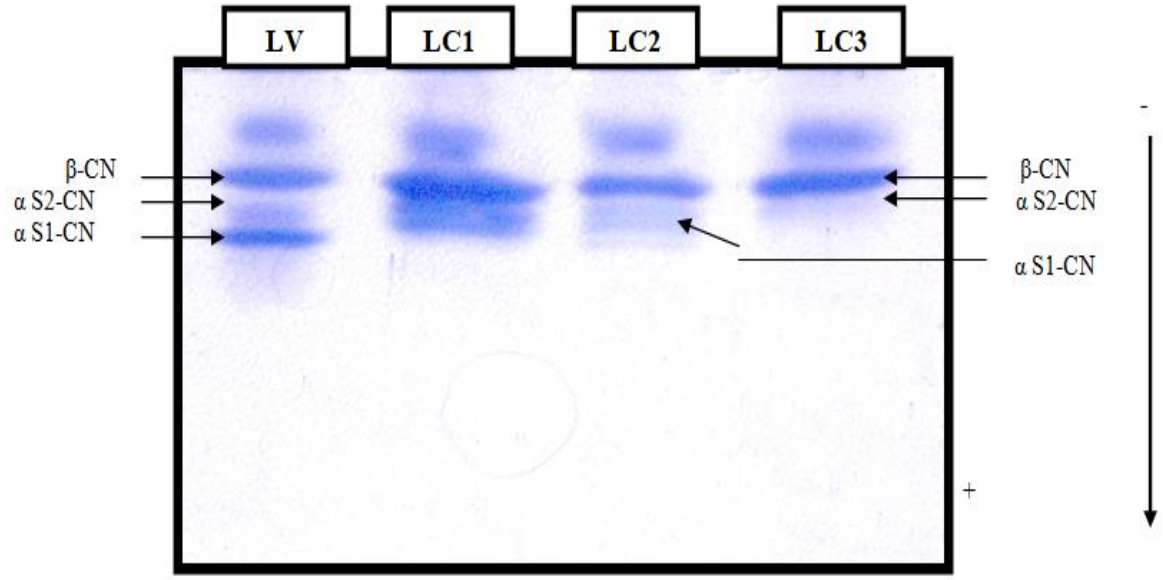

Figure 3. Electropherogram of Caseins in PAGE-urea (5.7 M); separation gel (T =13%; C =2.7%) and concentration gel (T = 4%; C = 2.7%); LV: cow's milk; LC1, LC2 and LC3: goat's milk; CN: Caseins.

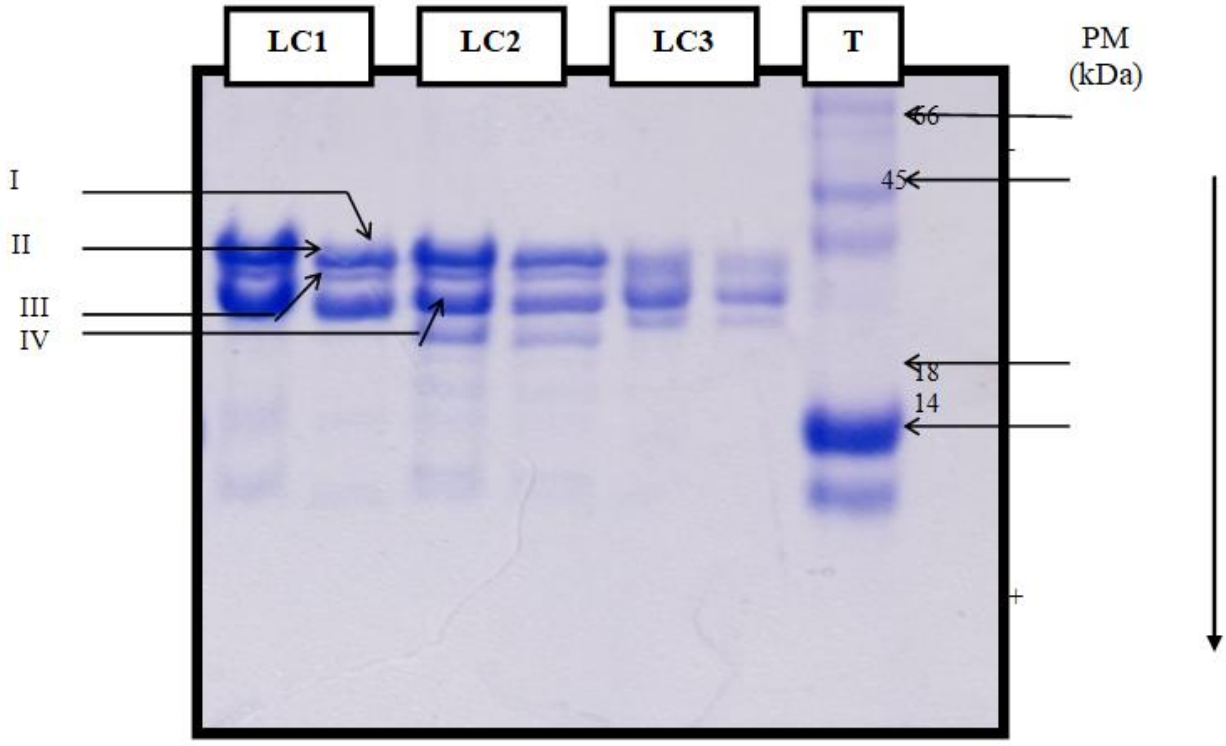

Figure 4. Electropherogram of Caseins in PAGE-SDS; separation gel (T = 15%; C = 2.7%) and concentration gel (T = 4%; C = 2.7%); T: standard proteins; LC1, LC2 and LC3: goat's milk.

Chromatographic Fractionation

The elution of caseins from goat milk is carried out by a discontinuous NaCl gradient, the elution buffer contains NaCl concentrations varying from 0.06 to 0.18 M. It is also composed of imidazole (0.02 M, pH 7; 3.3 M urea

and 2-Mercaptoethanol. We obtained five fractions (F1 to F5) (figure 5). β-casein is eluted in fraction F1, α S caseins in fraction F5 with an appreciable degree of purity . For serum proteins, the electrophorgram (figure 6) obtained by native PAGE of the collected fractions (F1 to F5) shows that alpha-lactalbumin is eluted in fraction F1. However, it remains slightly contaminated by traces of PP3. The latter is eluted in fraction F3 at a high degree of purity. Serum albumin is eluted in fraction F3 while β - Lactoglobulin is found in fraction F4.

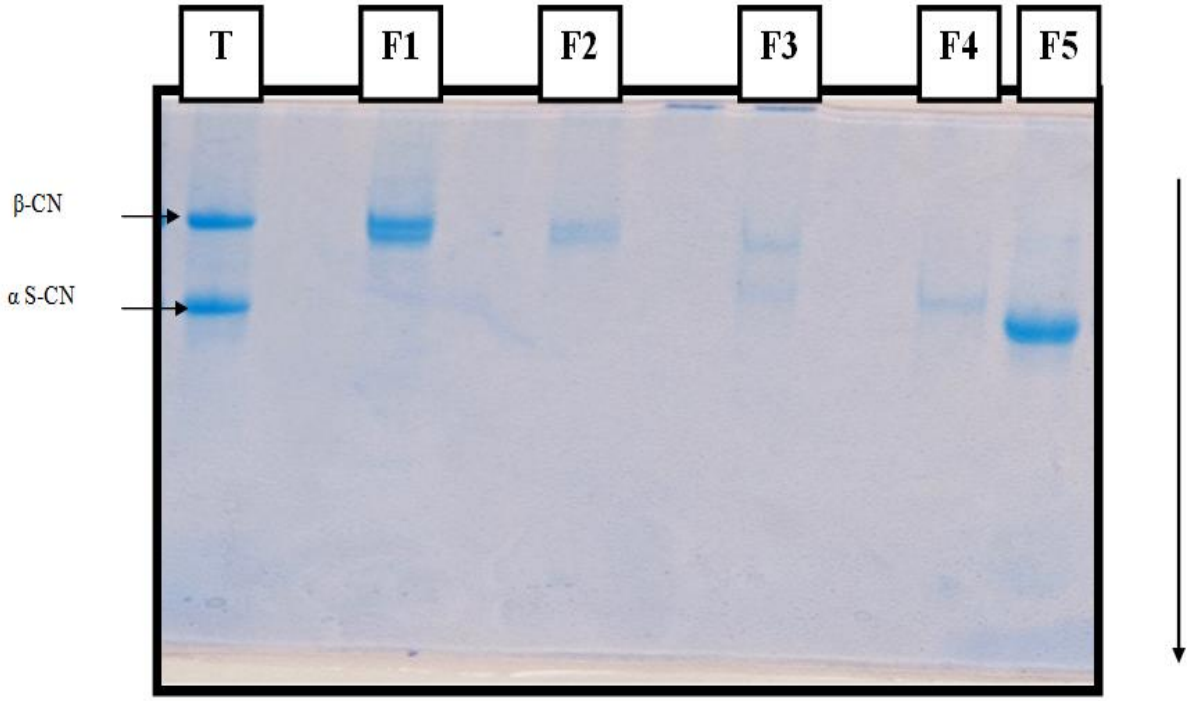

Figure 5. PAGE-urea electropherogram of the fractions collected after separation of the caprine caseins by chromatography on DEAE-cellulose; T: control (whole caseins) F1 fraction eluted at 0.06 M NaCl F2 fraction eluted at 0.09 M NaCl F3 fraction eluted at 0.12 M NaCl F4 fraction eluted at 0.15 M NaCl and F5 fraction eluted at 0.18 M NaCl.

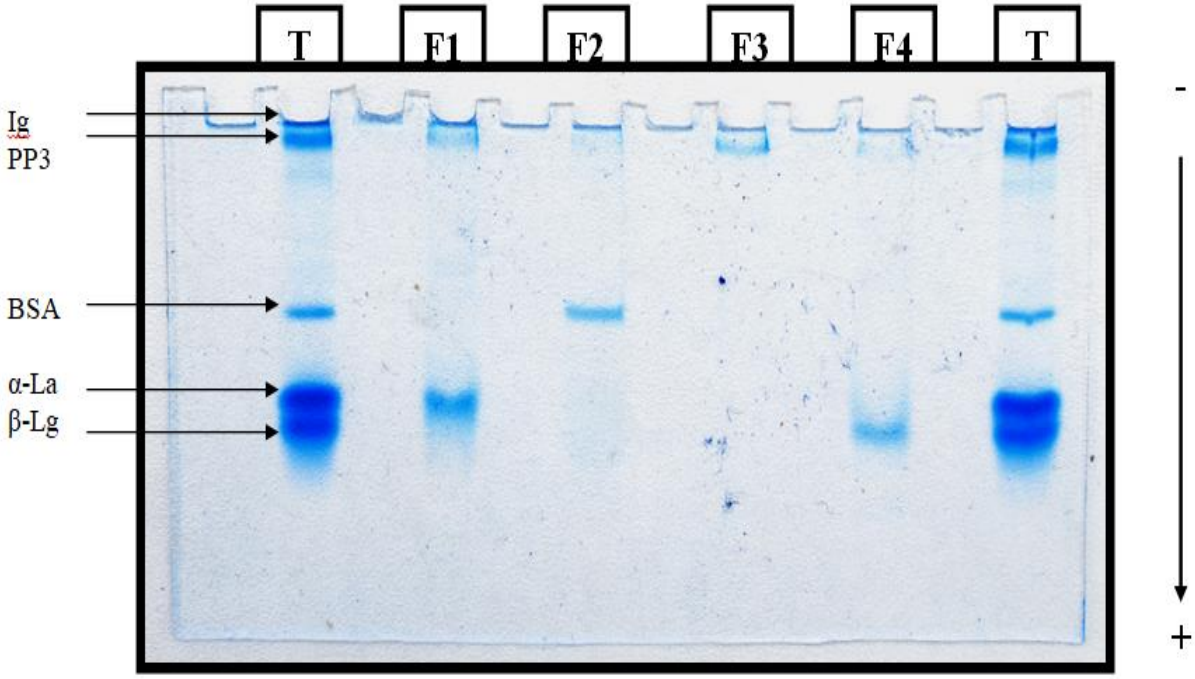

Figure 6. Native PAGE electropherogram of the fractions collected after separation of the Caprine serum proteins by chromatography on DEAE-cellulose; T: control (whole serum proteins) F1 fraction eluted at 0.08 M NaCl F2 fraction eluted at 0.11 M NaCl F3 fraction eluted at 0.13 M NaCl and F4 fraction eluted at 0.19 M NaCl.

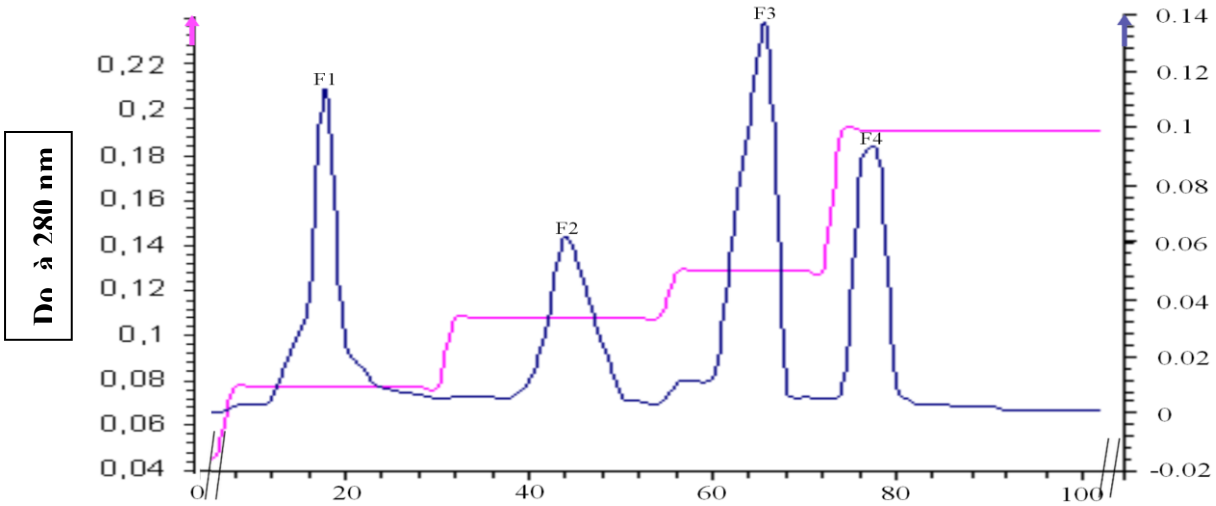

Figure 7. Chromatogram of goat milk serum proteins isolated on DEAE cellulose in 0.01 M Tris-HCl buffer, pH 6.8; flow rate 30 ml/h. F1 fraction eluted [illegible] at 0.11 M NaCl F3 fraction eluted at 0.13 M NaCl and F4 fraction eluted at 0.19 M NaCl.

Conclusion

This study, undertaken with the aim of contributing to a better knowledge of milk goat collected in the Tizi-Ouzou region, is divided into two parts, one focused on phenotyping of the protein fraction whether serum or casein and the other on the physicochemical characteristics of goat's milk, thus aiming for better exploitation of it in the cheese industry. As for physicochemical analyses, these mark a notable difference between goat's milk and cow's milk. The latter being taken as the reference milk. Thus we note relatively lower levels of dry extracts (on average 109.3 compared to 119 g/l for cattle). This trend is thus verified for fat (30.7 versus 35.7 g/l) and lactose (39.1 versus 45.7 g/l). These variations are independent of the origin of milk collection. Concerning the protein fraction, this, the importance of which in technology has been proven, is at a rate of 26g/l below the content obtained for the bovine milk sample (28g/l) which itself is quite deficit, because it is below the required standards (32g/l). In this count, and even if the rate of caseins recorded is appreciable (20g/l), this alone cannot predict an interesting cheese transformation given that precisely in this aspect it is the presence and nature of the αS1 casein which is decisive in this technology. It is in this sense that we set out to qualitatively evaluate this milk by isolating and characterizing its major proteins. For this, we implemented and adopted ion exchange chromatographic isolation techniques on DEAE-cellulose followed by electrophoretic separations on polyacrylamide gel under several conditions (native, in the presence of urea, in the presence of SDS) . This is how the application of DEAE as a separation support for caprine proteins gave good results in the sense that we were able to obtain αS and β caseins with a high degree of purity. Serum proteins, notably PP3, serum albumin and beta-lactoglobulin were also obtained with few contaminants using this technique. Concerning the electrophoretic behavior, in native PAGE, we noted a different migration of caprine PP3, α-lactalbumin and β-lactoglobulin compared to their bovine counterparts. PAGE-urea highlighted the particularity of the migration of caprine caseins where in particular the absence of αS1-CN in the case of sample № 3 suggests a low use of this milk for cheese technology purposes. These preliminary results obtained call for the implementation of more specific investigations for protein identification and phenotyping as well as the development of an exhaustive mapping between the nature of the milk produced and its technological capabilities.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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