Tropical and Subtropical Agroecosystems

SHORT NOTE [NOTA CORTA]

ISOLATION, MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF GOAT MILK CASEIN AND ITS FRACTIONS

[AISLAMIENTO, CARACTERIZACIÓN MOLECULAR Y BIOQUÍMICA DE LA CASEINA DE LA LECHE DE CABRA Y SUS FRACCIONES]

Samir A. Salem; Elsayed I. El-Agamy; Fatma A. Salama and Nagwa H. Abo-Soliman

Department of Dairy Science, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt.
samirsalem51@yahoo.com, eielagamy@yahoo.com
**Corresponding author*

SUMMARY

The SDS-PAGE electrophoretic pattern of goats´ milk has a unique pattern compared to those of cow and human milk. β-casein is the major fraction and comprises 70.2% of total goat-milk caseins, while α_s- is a minor fraction (29.85 %). This pattern is similar to that of human casein but different to that of cow casein. Purified casein fractions of goat milk showed different electrophoretic migration compared to those of bovine milk. The corresponding Mr(s) of goat α_s- and β-casein were estimated at 30.2 for α_s and 26.6 & 23.9 for β_1 and β_2 versus 32.6 and 26.6 for bovine α_s- and β-casein, respectively. The amino acid composition of goat-milk whole casein appeared to be similar to those of cow, sheep and camel caseins. Meanwhile, goat casein has the satisfactory balance of essential amino acids equal to or exceeding the FAO/ WHO/ UNU requirements for each amino acid. Goat α_s-casein was characterized by the presence of higher contents of both acidic and basic amino acids than β-casein. Peptide mapping profiles of goat, cow and human caseins were completely different. This means that each protein has its own unique peptide mapping.

Key words: *Goat milk casein, fractions of casein, human casein, essential amino acids*

INTRODUCTION

Milk as a biological fluid is well designed to the requirements of the specific offspring. Therefore, the composition of milk differs markedly among different species. Milk proteins as a major component of milk constituents play different important roles not only in nutrition and growth of the offspring but also in the different technological aspects as heat treatment, coagulation and rate of digestion. Milk proteins from cow (Swaisgood,1992), buffalo (Shamsia et al., 2008) sheep (Haenlein and Wendorff, 2006), camel (El-Agamy, 2006), goat (Park, 2006), human (El-Agamy et al., 1997), mares (El-Agamy et al., 1997) and donkey (El-Agamy et al., 1997) were well studied. However, little is known about the composition and structural characterization of Egyptian goat milk. The present study was aimed to gain more information about goat milk proteins which prepared from milk of local breeds of goat in order to verify the observation of using goat milk for nutrition of infants in some areas of Egypt.

MATERIALS AND METHODS

Milk and colostrum

Cow and goat milk samples were obtained from the herds of the Faculty of Agriculture, Alexandria University, Egypt. Composite human milk samples were collected from healthy mothers at El-Shatby Hospital, Alexandria, Egypt.

Isolation of casein and its fractions (α- and β-caseins)

1. Whole Casein preparation. The whole casein was prepared from raw skim-milk by slow acidification with 0.1N HCl to pH 4.6 at 25^0C (Warner, 1944).

2. α- and β-casein preparation. Whole α-casein was prepared by the urea method of Hipp et al. (1952). The β-casein was prepared by urea fractionation method of Aschaffenburg (1963).

Alkaline native-polyacrylamide gel electrophoresis (Alkaline native- PAGE)

Prepared proteins were separated on polyacrylamide gel in the absence of SDS and β-mercaptoethanol and the discontinuous buffer system (Hames and Rickwood, 1990). An appropriate volume of the sample was mixed with an equal volume of sample buffer (0.0625 M Tris-HCl, pH 6.8, 10 % glycerol and 0.002% bromophenol blue). After gel polymerization, 20μg protein were applied to each lane in the gel. The electrophoresis was performed using Mini-

PROTEAN II cell (Bio-Rad) at 75V through stacking gel followed by 125V to the end of electrophoresis (2hr). After electrophoresis gels were stained for 30 min using 0.1% Coomassie blue R-250 (Bio-Rad) and then distained using a distaining solution of glacial acetic acid, methanol and water (1:4:9).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (10 and 12.5%T) was carried out using the discontinuous buffer system described by Laemmli (1970). An appropriate volume of the protein sample was mixed with an equal volume of sample buffer (0.0625 M Tris-HCl, pH 6.8, 2 % SDS, 10 % glycerol, 0.002 % bromophenol blue, with 5 % β-mercaptoethanol) and submitted to heat treatment for 5 min in a boiling water bath prior to be applied to the gel. Samples allowed to cool to room temperature, finally centrifuged at 10000 **g** for 5 min to remove any insoluble materials causing streaking during electrophoresis. After gel polymerization, 30 µg protein were applied to each lane in the gel. The electrophoresis was performed at the same conditions of native-PAGE.

Protein molecular weight determination

Isolated proteins were applied to SDS-PAGE to determine the molecular weights using standard protein marker, molecular weight range: 14.2-66 kDa, Sigma, according to the method described by Weber and Osborn (1969).

Gel scanning

Protein bands revealed on gels were scanned with Video Copy Processor P65E (Appligene). Quantitative determination of the resolved protein bands was carried out using the Molecular Dynamic Image Quant V3.3 Program (Appligene) and Total lab soft ware (V1.11).

Peptide mapping and two-dimensional gel electrophoresis of proteins

The basic method of Grandier-Vazeille and Guerin (1996) was used for comparison or identification of isolated proteins. The method is summarized as: 500 µl of isolated protein is treated with 5 µl of trypsin (2.07 U/µl) and incubated at 37^0C for 2 hrs then the reaction was stopped by adding sample buffer (0.125M Tris-HCl pH 6.8, glycerol 10%, 0.001% bromophenol blue). Protein mixture was separated by alkaline native-PAGE (12.5%T) in the first dimension using a 0.75 mm thick slab gel with the discontinuous buffer system. At the end of the electrophoresis, the gel lane containing the separated sample components was cut out and equilibrated in 50 ml of β-mercaptoethanol 5 %, 0.125M Tris-HCl pH 6.8, SDS, 0.1 %, glycerol, 10 % for 30 min at room temperature with gentle swirling. For the second dimensional gel electrophoresis, a slab gel (15 %T) of 1.5 mm thick. After the polymerization of stacking gel, the first-dimensional gel strip placed between the slab gel glass plates and quickly aligned horizontally in close contact with stacking gel. The electrophoresis was performed using Mini-PROTEAN II cell (Bio-Rad) at 100V to the end of electrophoresis (2.5 hr). After electrophoresis, gels were stained with Coomassie blue R250 to visualize the spot positions.

Amino acid composition of purified proteins

Amino acid composition of proteins was determined after hydrolysis with 6N HCl at 110 ^{o}C for 18 hrs according to the method of Ozols (1990) using a Beckman Amino Acid Analyzer model 119C1.

RESULTS AND DISCUSSION

Protein composition of goat milk

The SDS-PAGE electrophoretic patterns of cow, goat and human milks are presented in Figure 1. Each type of milk has a unique electrophoretic pattern. In cow's milk, casein was separated into two major fractions, α_s- and β-caseins. They are quite similar in ratios 56.5 and 43.5% of total casein, respectively (Mora-Gutierrez et al., 1995). In goat milk, also two casein fractions were remarked; however, β-casein is the dominant (70.2%), while α_s- is minor (29.8%) (Montilla et al., 1995**;** Mora-Gutierrez et al., 1995**;** Jin and Park, 1996; Anema and Stanley, 1998).

In human milk, α_s-casein was appeared as a faint band. While β-casein represented the major fraction (69%). This result agrees with other reported data **(**Mohran, 1990; Darwish et al., 1996**,** Fox and McSweeney, 1998). Meanwhile, human milk pattern is free of β-lactoglobulin (β-lg) and α-lactalbumin (α-lac) is the main whey protein, comprise 33.5% of total whey proteins, this result coincides with other reports **(**Mohran, 1990**;** Susan et al., 1992**;** Park, 1994**;** Selo et al., 1999**;** Afify et al., 2003**).** It was noticed also that β-lg in goat milk was faster but α-lac was slower in migration mobility on the gel comparing to those of cow milk proteins. This result means that their corresponding molecular weights are different. Other proteins like serum albumin and lactoferrin showed also the marked differences in migration mobilities of these different proteins. Human serum albumin was the fastest and bovine one was the slowest in migration mobility on the gel. Cow lactoferrin was also slowest in migration, while goat and human lactoferrins have the same migration position, i.e., equal in molecular weights.

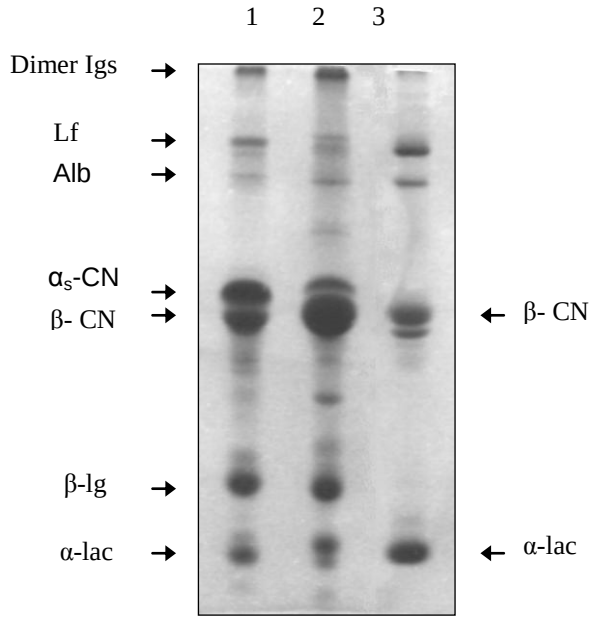

Figure 1: SDS-PAGE (10%T) of cow, goat and human milks. Lanes 1→3: Cow, goat and human milk, respectively; Dimer Igs: Dimer Immunoglobulins; Lf: Lactoferrin; Alb: Albumin; α_s-CN: α_s-casein; β-CN: β-casein; β-lg: β-lactoglobulin; α-lac: α-lactalbumin; Anode is toward bottom of the photo.

Based on these findings, it is expected that the corresponding proteins in the three types of milk having different net charges and amino acids in their compositions.

Molecular characterization of goat-milk caseins

SDS-PAGE electrophoretic pattern of purified goat-milk α_s-casein (Figure 2) showed that there was a marked difference in migration position compare with bovine-milk α_s-casein.

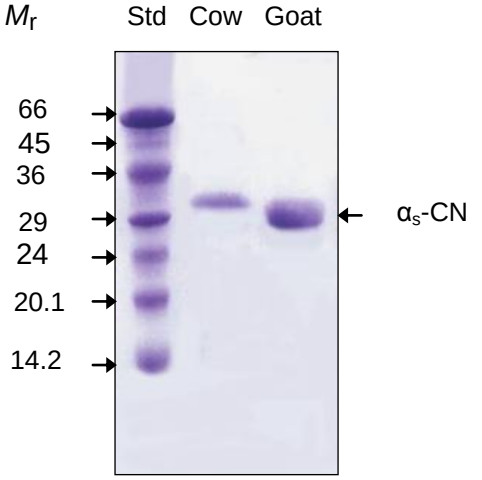

Figure 2: SDS-PAGE (12.5%T) of purified goat-milk α_s- and β-caseins; Std: Standard protein marker; α_s-CN: α_s-casein; β-CN: β-casein; Anode is toward bottom of the photo.

Since, the goat-milk α_s-casein was faster in migration than that of bovine-milk α_s-casein. The corresponding molecular weights of α_s-caseins of goat and bovine milks were estimated at 30.2 and 32.6, respectively **(**El-Agamy et al., 1997).

SDS-PAGE electrophoretic pattern of purified goat-milk β-casein (Figure 3) showed the presence of two subunits of purified goat-milk β-casein. One of them has the same migration position, i.e., equal molecular weight with that of purified bovine-milk β-casein (26.6). While, the other subunit of goat-milk β-casein was faster in migration and lower in molecular weight (23.9).

These results are in agreement with that reported by **(**Dall' Olio et al., 1988; Kaminarides and Anifantakis, 1993**).** Richardson and Creamer (1974) stated that goat pure β-casein had molecular weight of about 24,500 as determined by gel filtration on sepharose 6B in guanidine-HCl. Trujillo et al. (2000) estimated the molecular mass of caprine β-casein 6P at 23,835.

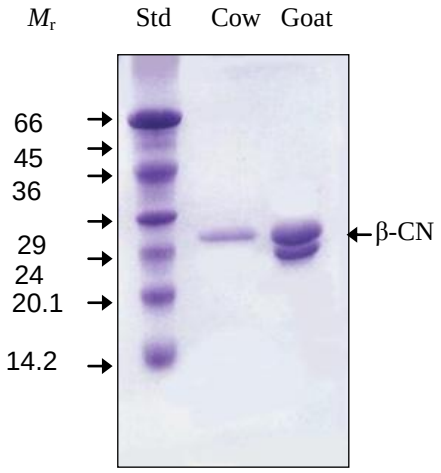

Figure 3. SDS-PAGE (12.5%T) of purified goat – milk α_s- and β-caseins; Std: Standard protein marker; α_s-CN: α_s-casein; β-CN: β-casein; Anode is toward bottom of the photo.

Peptide mapping and two-dimensional gel electrophoresis of goat milk casein

In general, the proteins in milks of different animals share a large number of characteristics. Many of these proteins have approximately the same molecular weight across species. However, milks from different mammals also present differences in relative proportions and characteristics of caseins and whey proteins and in the amino acids composition of similar

proteins. These milk protein differences and similarities are difficult to analyze by a single-dimensional technique alone; therefore modern techniques of protein analysis, such as two-dimensional gel electrophoresis has been used to characterize and to compare individual milk proteins of mammals. These two-dimensional gels can be used as comparative maps for milk proteins of major mammals. Figure 4 shows the peptide mapping (fingerprints) of goat, cow and human-milk caseins treated with trypsin. Each casein has its own unique peptide mapping, since; goat-milk casein map showed the appearance of 8 spots (peptides) on the gel differ completely in migration positions and spot intensity than those of cow or human-milk casein. The fingerprints of each casein confirmed our previous results of trypsin-treated caseins and analyzed by Native-PAGE.

Amino acid composition of goat-milk caseins

Table 1 shows amino acid composition of goat-milk casein and its purified fractions. Results showed that glutamic and leucine are the major amino acid in whole casein, while methionine and glycine are the minor amino acids. These results are in agreement with that reported by Abd-El- Salam et al., 1992; El-Agamy et al., 1997**).** Lysine is present in low level in goat casein. Overall, the amino acid composition of goat casein appears to be similar to those of cow, sheep and camel **(**El-Agamy et al., 1997). The ratio of essential to non essential amino acids was 1.01 and is closer to those of camel, cow, buffalo, sheep, ass, mare and human casein 0.93, 1.0, 1.6, 0.95, 0.99, 1.03, 1.07, respectively. Data revealed also that goat casein has the satisfactory balance of essential amino acids equally or exceeding the FAO/ WHO/ UNU/ (1985) requirements for each amino acid. It was documented that several amino acid differences exist between human and cow caseins that can present problems in feeding cow milk or its formulas to certain infants. One of these problems is the concentration of phenylalanine and tyrosine. Since infants have limited ability to metabolize these amino acids which, can build up and cause phenylketonuria (PKU babies) (Jelliffe and Jelliffe, 1978). Human milk has low levels of both phenylalanine and tyrosine and the ratios of phenylalanine to tyrosine in human milk were found as 0.7 versus 2.5 and 2.7 for camel and cow casein, respectively. According to the results of our study, the corresponding ratio of phenylalanine to tyrosine in goat milk is 0.96. This means that the goat casein has a property very closer to that of human-milk casein than that of cow or camel.

Data in Table 1 showed also that glutamic and lysine are the major amino acids in α_s-casein fraction; however, in β-casein glutamic, proline and leucine are the major amino acids. Arginine was present in the lowest level in α_s-casein versus glycine in β-casein. Goat α_s-casein was characterized by the presence of high contents of both acidic and basic amino acids than β-casein.

CONCLUSIONS

Goat milk proteins have a unique electrophoretic pattern comparing with that of cow milk. The molecular weights of goat casein fractions were smaller than those of cow milk. The amino acid composition of goat-milk casein appeared to be similar to those of cow caseins. Meanwhile, goat casein has the satisfactory balance of essential amino acids equally or exceeding the FAO/ WHO/ UNU requirements for each amino acid. Peptide mapping profiles of goat, cow and human caseins were completely different. This means that each protein has its own unique structure.

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Table 1. Amino acid composition of goat-milk casein and its purified fractions (g/100g protein)

Amino acids	whole casein	α_s-casein	β-casein
Threonine	4.9	2.3	6.2
Valine	6.7	6.1	8.0
Methionine	2.7	2.6	1.9
Leucine	13.6	6.4	10.8
Isoleucine	4.2	5.3	5.7
Phenylalanine	4.4	2.9	3.8
Histidine	3.2	2.7	1.8
Lysine	6.7	11.6	5.9
Arginine	3.9	1.8	1.9
Aspartic	4.7	8.7	4.8
Serine	3.6	6.1	9.2
Glutamic	20.3	23.8	19.5
Proline	9.3	6.8	14.3
Glycine	2.8	2.8	1.2
Alanine	3.5	5.9	2.9
Tyrosine	4.6	3.9	2.1

Essential amino acids of casein (%) = 50.3
Non-essential amino acids of casein (%) = 49.7

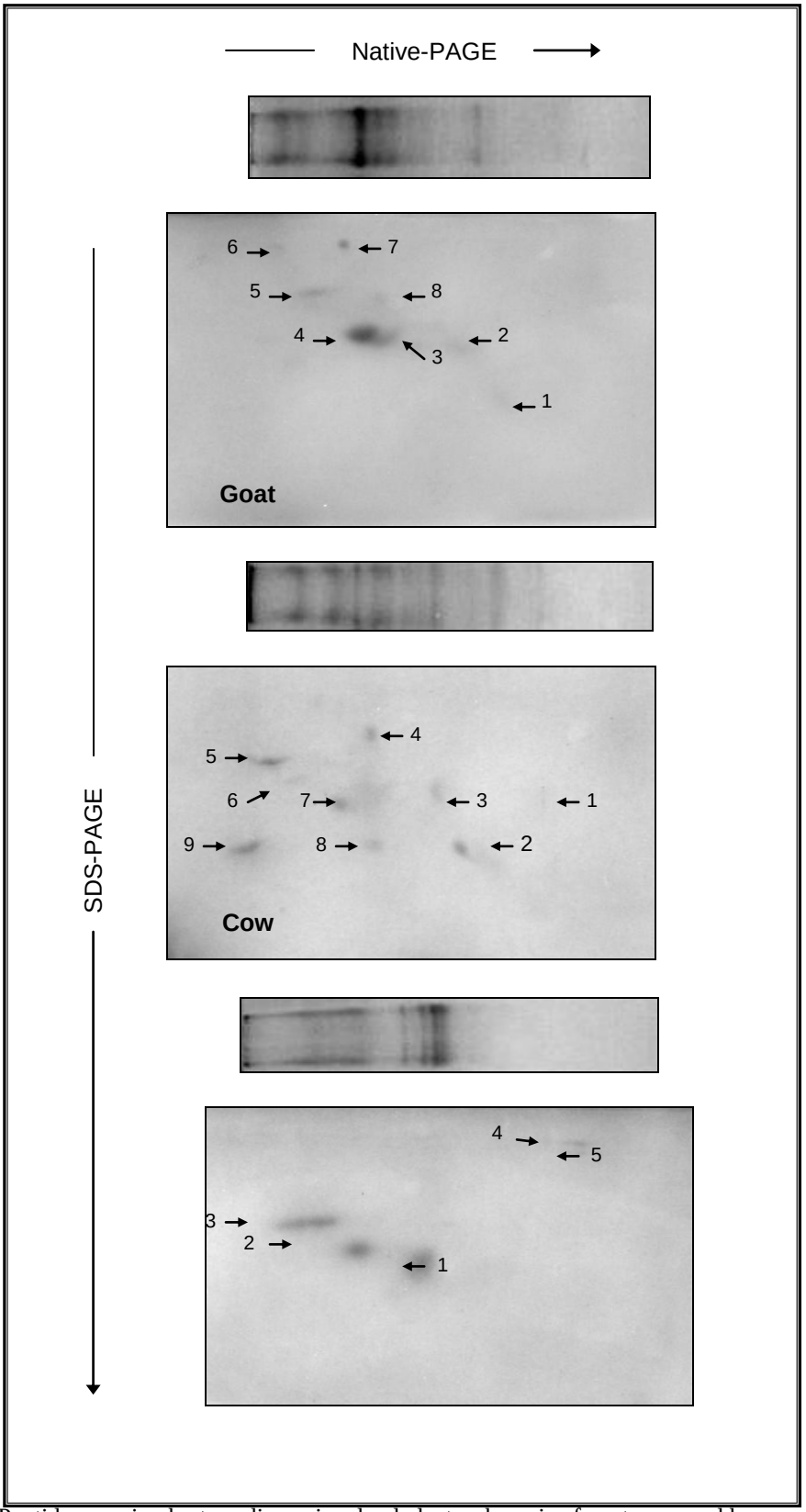

Figure 4: Peptide mapping by two-dimensional gel electrophoresis of goat, cow and human caseins treated with trypsin at pH 7.0 for 2 hrs. Arrows indicate the spots of separated peptides.

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