Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 87 (2004) 75–81
Food Chemistry
www.elsevier.com/locate/foodchem

# Casein fraction of bulk milks from different caprine breeds

G. Moatsou [a,*], M. Samolada [b], P. Panagiotou [a], E. Anifantakis [a]

[a] Laboratory of Dairy Technology, Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece
[b] Laboratory of Dairy Technology, Department of Agricultural Science, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece

Received 1 August 2003; received in revised form 15 October 2003; accepted 15 October 2003

**Abstract**

The relative amounts of the major caseins in the total casein fraction from bulk milks of four different caprine breeds (two indigenous Greek and the highly selected Alpine and Saanen) were determined by alkaline urea polyacrylamide gel electrophoresis and by reversed-phase HPLC. Both methods revealed differences with regard to the $\alpha_{s1}$-casein fraction of the different breeds. The estimated values, expressed as percentage of total caseins, ranged as follows: for $\alpha_{s1}$-casein from 9.9% to 25.6%, for $\alpha_{s2}$-casein from 12.5% to 18.9%, for β-casein from 51.1% to 58.4% and for κ-casein from 9.9% to 13.5%. The lowest $\alpha_{s1}$-casein and the highest β-casein and κ-casein percentages were found in the casein fraction of the Saanen breed.


*Keywords:* Goat's milk; Caprine casein; Caprine breeds; Chromatography; Urea–PAGE; RP-HPLC

## 1. Introduction

The protein and fat contents are variable among the different caprine breeds and they are genetically controlled, especially by the $\alpha_{s1}$-casein locus, which exhibits a high degree of polymorphism. The types of the related mutations are: single nucleotide substitution or deletion, or large insertions or deletions (Martin, 1993). Eight of the currently identified alleles (A, $B_1$, $B_2$, $B_3$, $B_4$, C, H and L) are associated with a "high" level of $\alpha_{s1}$-casein (3.5 g/l per allele), two (E and I) with a "medium" level (1.1 and 1.7 g/l) and two (F and G) with a "low" level (0.45 g/l). The $O_1$ and $O_2$ are null alleles and produce no $\alpha_{s1}$-casein (Chianese, Ferranti, Garro, Mauriello, & Addeo, 1997; Grosclaude & Martin, 1997). The relative frequencies of the $\alpha_{s1}$-casein alleles show marked differences between breeds, as well as regional tendencies. The "high" A and B alleles seem to be predominant in the local breeds of the Mediterranean area (Italy, Albania, Greece) and Africa while, in France and Spain, the "medium" E allele is the most frequent. The Swiss breeds have a high frequency of the defective alleles F, E and O. The Alpine, Saanen and Toggenburg breeds have spread those alleles all over the world (Enne, Feligini, Greppi, Iametti, & Pagani, 1997; Grosclaude & Martin, 1997).

The polymorphism of caprine $\alpha_{s1}$-casein affects (AA > EE > FF) the true protein, the casein and the fat content of milk, the maximum gel strength and the gel strengthening rate (Remeuf, 1993). Mean micellar size is lower in A milks (Pierre, Michel, & Le Graët, 1995; Pierre, Michel, Le Graët, & Zahoute, 1998b; Remeuf, 1993). Lipolysis is lower in A milk and the relative proportions of FFA were different in A and O or F milks (Lamberet, Degas, Delacroix-Buchet, & Vassal, 1996; Pierre et al., 1998a). Total solids, fat recovery and gross yield are significantly higher in cheese made from milks of goats with high rate of $\alpha_{s1}$-casein synthesis (Pirisi, Colin, Laurent, Scher, & Parmentier, 1994; Remeuf, 1993; Vassal, Delacroix-Buchet, & Buillon, 1994). Cheeses made from FF milks exhibit a more intense "goat flavour" than that made from AA milks (Delacroix-Buchet, Degas, Lamberet, & Vassal, 1996; Lamberet et al., 1996; Vassal et al., 1994). Also, caprine milk, with a very low $\alpha_{s1}$-casein content, was found to be less allergenic than that with a high level of $\alpha_{s1}$-casein (Bevilacqua et al., 2001).

* Corresponding author. Tel.: +30-210-5294630; fax: +30-210-5294630.
*E-mail address:* mg@aua.gr (G. Moatsou).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.10.020

Greece produces 31.1% of the total European Union output of caprine milk. The majority of greek goat flocks consists of a rather small number of indigenous animals, with moderate production of milk, that are mainly fed by pasturing in the mountainous and semi-mountainous regions and exhibit great adaptability and tolerance to the environmental conditions. Their milk has higher total solids, total proteins, casein and fat content than the milk of highly selected breeds (Anifantakis & Kandarakis, 1980; Morgan et al., 2003; Voutsinas, Pappas, & Katsiari, 1990).

The majority of caprine milk produced in Greece is used for cheesemaking, mainly as a supplement to ovine milk for cheese production. Since the characteristics of the casein fraction are important for the cheesemaking properties of caprine milk, the aim of the present study was to study the composition of the casein fraction of bulk milks of four different caprine breeds: two indigenous and two highly selected, using urea–polyacrylamide gel electrophoresis (urea–PAGE) and reversed-phase HPLC (RP-HPLC).

## 2. Materials and methods

### 2.1. Preparation of whole caseins

Bulk milks from four different flocks that corresponded to two different Greek indigenous caprine breeds, named Attiki and Skopelos, and to the highly selected breeds Apline and Saanen, were collected. After centrifugation at 2000*g* for 30 min at 4 °C, the skim milks were acidified at pH 4.5 with acetic acid (1 M) under continuous stirring at 25 °C. After settling for 20 min, the mixture was filtered through Whatman No. 40 paper. The precipitated casein was washed with distilled water, dissolved with the addition of NaOH 10 g/l to pH 7.0, and precipitated again. Four successive cycles of precipitation and washing were carried out. The final precipitate was dissolved in 20 mM phosphate buffer, pH 7.0, heated at 80 °C for 30 min to inactivate plasmin, dialysed (MWCO 12 kDa, Sigma–Aldrich Chemie Gmbh, PO 1120 89552 Steinheim, Germany) against distilled water and lyophilised.

### 2.2. Urea–polyacrylamide gel electrophoresis of casein (urea-PAGE)

Whole caseins were analysed by the urea–polyacrylamide gel electrophoresis (urea-PAGE) method of Andrews (1983) with direct staining using Coomassie Brilliant Blue G-250 (Blakesley & Boezi, 1977). Electrophoresis was carried out on a vertical slab unit (LKB 2001, Amersham Pharmacia Biotech SE-75184, Uppsala, Sweden) in slabs of $140 \times 160$ mm, with thickness 1.5 mm. Ten microliters of whole casein solutions (2 mg/ml) in stacking gel buffer containing 8 M urea and 0.1 M β-mercaptoethanol, after incubation at 40 °C for 20 min, were analysed. After destaining with water, gel slabs were scanned using a scanner (Hewlett Packard, ScanJet 4c/T) and the protein bands were quantified by an image processing system (GelCompar v. 4.0, Applied Maths, Belgium). Duplicates of each sample were analysed in each gel and eight independent electrophoretic runs were carried out for each sample.

### 2.3. Cation-exchange chromatography of whole caseins (CEC)

Lyophilised whole caseins were fractionated by cation-exchange chromatography (CEC) on a SP-Sepharose Hiload 26/10 column (Amersham Pharmacia Biotech SE-75184, Uppsala, Sweden) using a Waters 650E automated chromatographic system with a tunable absorbance detector (Waters Model 486, Waters, 34 Marple Street, Milford, MA 01757, USA). A modification of the method of Hollar, Law, Dalgleish, and Brown (1991) was applied. Buffer A was 20 mM Na acetate–6 M urea, pH 5.0 and buffer B was similar to A but also contained 1 M NaCl. Sixty milliliters of lyophilised casein solution (7 mg/ml), prepared according to Hollar et al. (1991), were applied onto the column. First the sample was applied onto the column at a flow rate of 2 ml/min and was washed with buffer A at a flow rate of 3 ml/min for 15 min. Then a NaCl gradient was formed by mixing buffer A with buffer B at a flow rate of 3 ml/min, as follows: from 0 to 0.08 M NaCl within 15 min, from 0.08 to 0.10 M within 45 min, from 0.10 to 0.12 M within 15 min, from 0.12 to 0.14 M within 50 min, from 0.14 to 0.20 M within 20 min and from 0.20 to 0.30 M within 90 min. Finally, the gradient was followed by isocratic elution with 0.30 M NaCl for 30 min and the column was washed with 1 M NaCl for 30 min at the same flow rate. Eluate was monitored at 280 nm and the fraction size was 6 ml. The collected peaks were dialysed (MWCO 12 kDa, Sigma–Aldrich Chemie Gmbh, PO 1120 89552 Steinheim, Germany) against distilled water and lyophilised. Both buffers and samples were filtered through a 0.45 μm membrane (Millipore Corporation, Bedford MA 01730, USA). Their identity and purity were established by the reversed-phase HPLC method of Jaubert and Martin (1992) and the urea–PAGE method of Andrews (1983), performed on a Hoefer miniVE Vertical Electrophoresis System (Amersham Pharmacia Biotech SE-75184, Uppsala, Sweden) in gel slabs of $100 \times 100 \times 1$ mm. Proteins were fixed onto the gel by trichloroacetic acid (150 g/l) and stained by a Coomassie R-250 solution (2.5 g/l) in methanol, acetic acid, water (25:5:20). Gels were destained by a solution of glycerol, acetic acid, methanol and water (3:10:40:47).

### 2.4. Reversed-phase HPLC (RP-HPLC)

Reversed-phase HPLC analyses were performed by the method of Jaubert and Martin (1992) on a Vydac C4 214 TP 5415 column (Separations Group, Hesperia, CA 92345, USA) at room temperature. The HPLC system consisted of a pump capable of mixing four solvents (Waters 600E, Waters, 34 Marple Street, Milford, MA 01757, USA), a photodiode array detector (Waters 996), a helium degasser, a Rheodyne 7125 injector (Rheodyne Inc., Cotati, CA, USA) and Millenium v.3.05.01 software (1998, Waters). Solvent A was 1 ml/l trifluoroacetic acid (TFA, Serva Electrophoresis Gmbh, D-69115 Heidelberg, Carl-Benz-Str. 7, Germany) in ultra pure water and solvent B was 0.96 ml TFA, 800 ml acetonitrile (Lichrosolv grade, Merck KGaA, Darmstadt, Germany) and 200 ml ultra pure water. Solvents were filtered through 0.45 μm filters (Millipore Corporation, Bedford MA 01730, USA). Flow rate was 1 ml/min. A linear gradient, from 370 to 530 ml/l solvent B, within 30 min, was applied. Samples were prepared according to Pierre et al. (1995) as follows: 30 mg lyophilised casein were dissolved in 2 ml buffer, pH 7.0 (100 mM, 8 M urea, 13 g/l trisodium citrate, 10 mM dithiothreitol adjusted to pH 7.0 by HCl) at 37 °C for 60 min; then 18 ml of solvent A, containing 6 M urea, were added and pH was adjusted to 2.2 by 50% trifluoroacetic acid. After filtration through a 0.45 μm filter (Millipore Corporation, Bedford MA 01730, USA), 50 μl were analysed. The protein content of the samples was adjusted after preliminary analyses. The heights of the peaks at 214 nm, corresponding to the major casein fractions, were significantly linearly correlated with whole casein concentrations ranging from 20 to 100 μg per injection. Three independent preparations of each sample were analysed.

## 3. Results and discussion

### 3.1. Cation-exchange chromatography

The cation-exchange chromatogram of Skopelos casein is shown in Fig. 1. According to urea–PAGE analysis of the collected peaks, peak 1a consisted mainly of β-caseins and of bands with electrophoretic mobility higher than that of $\alpha_s$-caseins, probably degradation products. Peak 1b was β-caseins. Peak 2 had the characteristic diffused pattern of κ-casein. Peaks 3, 4 and 5 were mixtures of $\alpha_{s1}$- and $\alpha_{s2}$-caseins. In order to interpret the urea–PAGE patterns, these peaks were further analysed by RP-HPLC. Peak 3 was $\alpha_{s1}$-casein. Peak 4 contained both $\alpha_{s1}$- and $\alpha_{s2}$-caseins and fraction 5 was $\alpha_{s2}$-casein. Therefore, by this method, whole caprine casein was essentially separated into three fractions, β-, κ- and $\alpha_s$-caseins, although a satisfactory separation of caprine $\alpha_{s1}$- and $\alpha_{s2}$-caseins has been reported by other researchers who have used a cation-exchange MonoS column at pH 5.0 (Brown, Law, & Knight, 1995; Law & Tziboula, 1992; Recio, Pérez-Rodríguez, Amigo, & Ramos, 1997).

The relative amounts of these three main casein groups were calculated by the method of Law and Tziboula (1992), using the peak area and the respective absorbance coefficients ($A^{1\%}$) at 280 nm: 4.4 for β-casein, 8.2 for κ-casein and 9.9 for $\alpha_s$-caseins. The results are shown in Table 1 and they are presented as supportive data, since they are single observations.

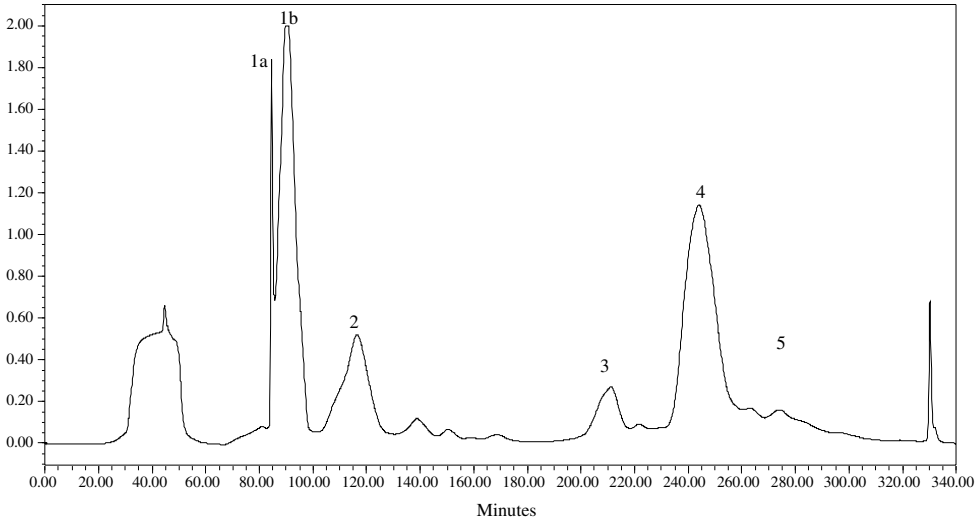


Fig. 1. Cation-exchange chromatography ($A_{280}$) on a SP-Sepharose Hiload 26/10 column of whole caprine casein at pH 5.0 (conditions and sample preparation are described in detail in Section 2).

Table 1
Relative percentages[a] of individual caseins in whole casein from indigenous and highly selected caprine breeds estimated by different analytical methods

| Breed | | β-Casein | κ-Casein | $\alpha_{s2}$-Casein | $\alpha_{s1}$-Casein | $\alpha_s$-Casein[b] | $\alpha_s/\beta$ |
|---|---|---|---|---|---|---|---|
| Attiki | CEC[c] | 52.2 | 11.7 | | | 36.1 | 0.69 |
| | PAGE[d] | 51.1 (1.5) | 10.3 (10.3) | | | 38.7 (2.3) | 0.76 |
| | RP-HPLC[e] | 51.0 (3.2) | 10.9 (5.6) | 12.5 (2.1) | 25.6 (6.8) | 38.1 (5.2) | 0.75 |
| Skopelos | CEC | 53.4 | 11.6 | | | 35.0 | 0.66 |
| | PAGE | 52.5 (2.9) | 9.9 (12.8) | | | 37.6 (2.9) | 0.72 |
| | RP-HPLC | 52.4 (1.0) | 11.0 (8.4) | 16.0 (4.7) | 20.7 (0.5) | 36.7 (2.3) | 0.70 |
| Alpine | CEC | 53.5 | 11.2 | | | 35.3 | 0.66 |
| | PAGE | 51.1 (2.6) | 11.3 (10.5) | | | 37.6 (4.6) | 0.74 |
| | RP-HPLC | 51.6 (1.5) | 11.1 (7.4) | 15.3 (4.4) | 22.0 (1.5) | 37.3 (1.0) | 0.72 |
| Saanen | CEC | 53.7 | 16.5 | | | 29.8 | 0.55 |
| | PAGE | 55.8 (3.3) | 13.5 (6.3) | | | 30.7 (5.4) | 0.55 |
| | RP-HPLC | 58.4 (1.9) | 12.7 (14.2) | 18.9 (10.3) | 9.9 (10.5) | 28.8 (4.8) | 0.49 |

[a] Expressed as percentage of total $\alpha_s$-, β- and κ-caseins.
[b] $\alpha_{s1} + \alpha_{s2}$ caseins.
[c] Cation-exchange chromatography.
[d] Urea–polyacrylamide gel electrophoresis, means of eight determinations (in parentheses the relative standard deviations).
[e] Reversed-phase HPLC, means of three determinations (in parentheses the relative standard deviations).

### 3.2. Urea–polyacrylamide gel electrophoresis

The electrophoretic profiles of the whole caseins from the four different breeds are presented in Fig. 2. Caprine β-casein consisted of two components with different electrophoretic mobilities corresponding to $\beta_1$- and $\beta_2$-caseins, that have the same amino acid composition but different phosphorylation levels, 6 and 5 for $\beta_1$ and $\beta_2$, respectively (Richardson & Creamer, 1974). Chianese et al. (1993) describe a more complex β-casein pattern in individual caprine milk samples because of multiple phosphorylation of the peptide chain giving, apart from $\beta_1$- and $\beta_2$-forms, the additional 3P and 4P forms. A variant named B (Mahé & Grosclaude, 1993) and, recently, a new variant named C (Neveu, Mollé, Moreno, Martin, & Léonil, 2002) have been described. Also, a null genetic allele has been reported (Dall'Olio, Davoli, & Russo, 1989; Mahé & Grosclaude, 1993). In the present profiles, only $\beta_1$- and $\beta_2$-caseins were clearly evident.

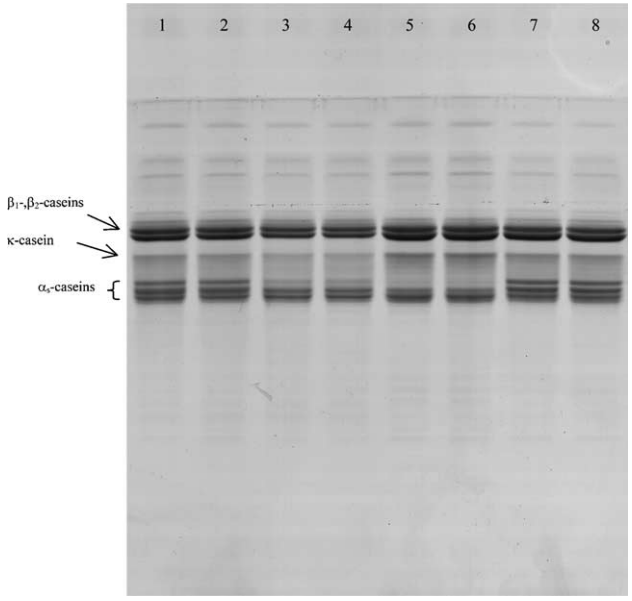


Fig. 2. Urea–PAGE profiles of whole casein fractions from indigenous and highly selected caprine breeds. Lanes 1–2, Attiki breed; lanes 3–4, Skopelos breed; lanes 5–6, Saanen breed and lanes 7–8, Alpine breed.

κ-Casein is composed of several fractions with the same peptide chain and different carbohydrate contents, like bovine and ovine κ-caseins. Therefore, it appeared as a diffuse band that migrated ahead of β-casein. Three genetic variants of caprine κ-casein have been described (Yamauchi, Coll, Sanchez, & Folch, 2001). No clear evidence of genetic variability existed in the profiles of the present study. In urea–PAGE analysis of the κ-casein fraction obtained by cation-exchange chromatography, a minor component of κ-casein co-migrated with β-casein was not expected to affect the results because of its low concentration.

The bands of $\alpha_{s1}$- and $\alpha_{s2}$-caseins were not clearly distinguished from each other because they are partly overlapped (Addeo, Mauriello, & Di Luccia, 1988; Chianese, Mauriello, Intircia, Moio, & Addeo, 1992; Trujillo, Guamis, & Carretero, 1997). Both caprine $\alpha_{s1}$- and $\alpha_{s2}$-caseins are very heterogeneous; 14 alleles have been described for caprine $\alpha_{s1}$-casein (Chianese et al., 1997; Grosclaude & Martin, 1997) and three for $\alpha_{s2}$-casein (Martin, 1993), which is the most multiphosphorylated casein. Further sources of $\alpha_{s1}$-casein heterogeneity are the deleted forms observed in at least the three common variants A, B and C that co-exist with the main full-length component and also the differences regarding the discrete phosphorylation of seryl residues

(Ferranti et al., 1997; Ferranti, Lilla, Chianese, & Addeo, 1999).

There were very pronounced differences among the profiles of the different breeds concerning the $\alpha_s$-casein group of bands. In the Saanen breed, the third band was absent, while the κ-casein band was the most intense. In the Skopelos breed, the third $\alpha_s$-casein band was weak. This variability was also depicted in the quantification results of Table 1. In the Saanen casein, the $\alpha_s$-casein relative content was the lowest (about 20% lower than that of the other breeds), κ-casein was the highest and β-casein the highest of the different breeds. These differences could be attributed to the distribution of certain $\alpha_{s1}$-casein alleles within the herds, since certain alleles are connected with high, low and medium levels of $\alpha_{s1}$-casein (Grosclaude & Martin, 1997).

### 3.3. Reversed-phase HPLC

The chromatographic profiles of the different breeds are presented in Fig. 3 and the quantification results in Table 1. Many differences, were observed among the chromatograms of the different breeds, with regard to $\alpha_{s1}$-casein (similar to the electrophoretic profiles of Fig. 2). It was clear from the profiles that different $\alpha_{s1}$-casein variants dominated the bulk milks of each breed. In the Attiki breed there were at least three $\alpha_{s1}$-casein peaks, which, according to Jaubert and Martin (1992), correspond to different genetic variants, while in Saanen there were two peaks with lower chromatographic areas. The $\alpha_{s2}$-casein consists of two well-separated peaks, with the exception of Saanen milk, where there was only one main peak. It was considered that the different peaks correspond to different caprine $\alpha_{s2}$-casein variants that can be separated by the present method (Jaubert & Martin, 1992; Pierre, Mollé, & Zahoute, 2001; Trujillo, Casals, & Guamis, 2000). Therefore, the differences in $\alpha_{s1}$- and $\alpha_{s2}$-caseins among the caseins of the different breeds were depicted by both urea–PAGE and RP-HPLC. Also, by both methods, the Saanen casein was the most diverse. β-casein was presented as a single peak that dominated the profiles. κ-casein was resolved as a single peak with uncertain shape that could be attributed to the glycosylated components, which are expected to elute earlier than the non-glycosylated ones.

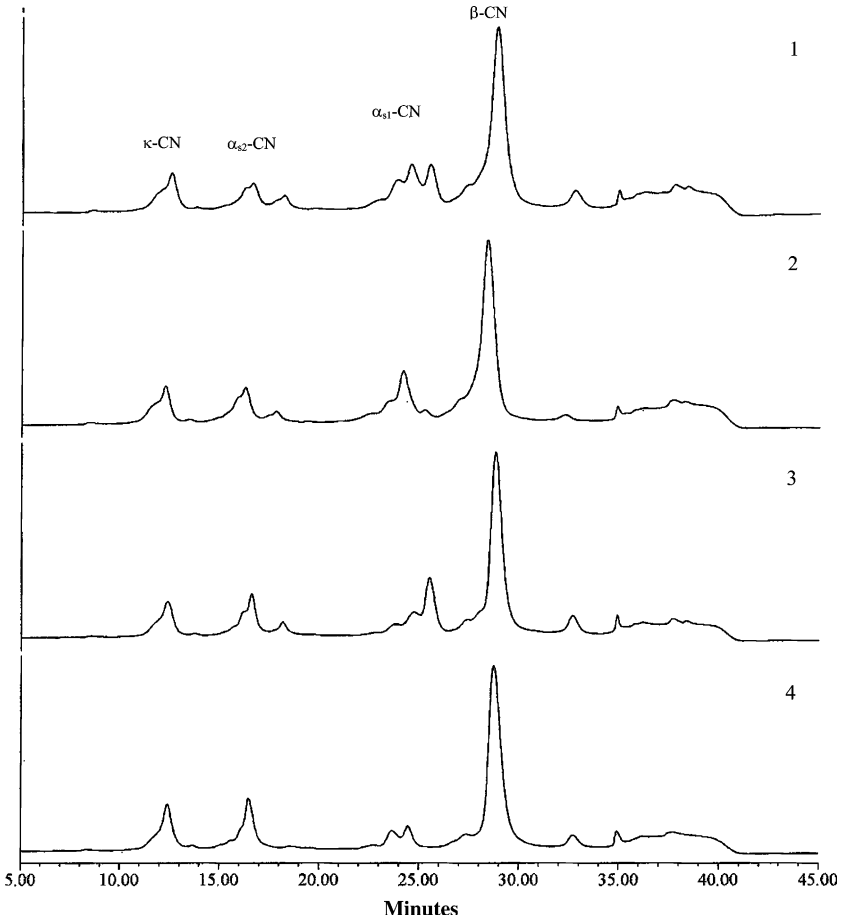


Fig. 3. Reversed-phase HPLC profiles ($A_{214}$) of whole caprine caseins from indigenous greek and highly selected breeds (1, Attiki; 2, Skopelos; 3, Alpine; 4, Saanen). Separations were performed on a Vydac C4 214 TP 5414 column using a linear gradient of acetonitrile (conditions are described in detail in Section 2).

### 3.4. Quantification of casein fractions

The relative percentages, estimated by urea–PAGE and RP-HPLC, were in general similar but the relative standard deviations of RP-HPLC estimations were better. In all caseins, the relative percentage of β-casein, estimated by both methods, was greater than 50%.

The lowest values of $\alpha_s$-caseins and the greatest values of β-caseins were observed in Saanen casein. From the very low level of $\alpha_{s1}$-casein, it can be concluded that the allelic frequencies of "weak" $\alpha_{s1}$-casein alleles were high in this milk. Among the four casein fractions, the content of $\alpha_{s1}$-casein was the most variable (Table 1). Regarding the two indigenous Attiki and Skopelos breeds, the $\alpha_{s1}$-casein relative percentage was at the level of "high" alleles, as reported by researchers who have used the RP-HPLC method (Jaubert, Bonnin, & Boisseau, 1997; Morgan, Fauquant, Micault, Bonnin, & Jaubert, 2000; Pierre et al., 1995, 1998a, 1998b). Regarding the relative percentage of $\alpha_{s1}$-casein of highly selected breeds, it was found that Alpine casein contained more $\alpha_{s1}$-casein than Saanen, which could be attributed to the predominance of "low" alleles in the latter. A similar trend for Alpine milks in comparison to Saanen has been reported by Jaubert et al. (1997) and Pierre et al. (2001).

Also $\alpha_{s2}$-casein and β-casein contents were higher in casein from the Saanen breed than the Alpine as has been reported (Jaubert et al., 1997). The $\alpha_{s2}$-casein relative content was also variable, from 12.5% to 18.9% of total casein, and it was inversely related to the $\alpha_{s1}$-relative percentage among the different breeds. A negative correlation between $\alpha_{s1}$- and $\alpha_{s2}$-contents, in individual goat milks, has been presented by Pierre et al. (1998b). In general, the $\alpha_{s2}$-casein relative percentage was in accordance with the findings of other researchers who have used the RP-HPLC method (Jaubert et al., 1997; Morgan et al., 2000; Pierre et al., 2001; Pierre et al., 1998a, 1998b) or a cation-exchange chromatography method (Brown et al., 1995; Law & Tziboula, 1992).

The relative content of κ-casein was also variable among the different breeds. In general, it was close to the lowest values reported for caprine κ-casein (Brown et al., 1995; Jaubert et al., 1997; Law & Tziboula, 1992; Morgan et al., 2000; Pierre et al., 2001). It was the highest in Saanen casein that had the lowest $\alpha_{s1}$-casein content. This trend has also been observed between "high" and "low" $\alpha_{s1}$-casein caprine milks (Pierre et al., 1995, 1998a, 1998b).

## 4. Conclusions

The casein of the two indigenous breeds had an $\alpha_{s1}$-casein relative percentage at the level reported for caprine milks with "high" alleles (20.7% and 25.6%) and β-casein was their main casein fraction (>50%). Regarding the highly selected breeds, the casein from the Alpine breed was richer in $\alpha_{s1}$-casein than that of Saanen. The variability of $\alpha_{s1}$- and $\alpha_{s2}$-fractions was clearly depicted by both urea–PAGE and RP-HPLC methods. However, the RP-HPLC method was more suitable for the estimation of the relative casein percentages, because it estimated both $\alpha_{s1}$- and $\alpha_{s2}$-caseins, which were not clearly resolved by urea–PAGE. Moreover, the relative standard deviations of the RP-HPLC means were lower.

## References


Addeo, F., Mauriello, R., & Di Luccia, A. (1988). A gel electrophoretic study of caprine casein. *Journal of Dairy Research, 55*(3), 413–421.

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research, 50*(1), 45–55.

Anifantakis, E. M., & Kandarakis, J. G. (1980). Contribution to the study of the composition of goat's milk. *Milchwissenschaft, 35*(10), 617–619.

Bevilacqua, C., Martin, P., Candahl, C., Fauquant, J., Piot, M., Roucayrol, A.-M., Pilla, F., & Heyman, M. (2001). Goat's milk of defective $\alpha_{s1}$-casein genotype increases intestinal and systematic sensitization to β-lactoglobulin in guinea pigs. *Journal of Dairy Research, 68*(2), 217–227.

Blakesley, R. W., & Boezi, J. A. (1977). A new staining technique for protein in polyacrylamide gels using Coomassie Brilliant Blue G-250. *Analytical Biochemistry, 82*(2), 580–582.

Brown, J. R., Law, A. J. R., & Knight, C. H. (1995). Changes in casein composition of goats' milk during the course of lactation: Physiological inferences and technological implications. *Journal of Dairy Research, 62*(3), 431–439.

Chianese, L., Ferranti, P., Garro, G., Mauriello, R., Addeo, F. (1997). Occurrence of three novel $\alpha_{s1}$-casein variants in goat milk. In Milk protein polymorhism, proceedings of the IDF Seminar, Palmerston North, New Zealand, February 1997, IDF S.I. 9702 (pp. 259–267). Brussels: IDF.

Chianese, L., Garro, G., Nicolai, M. A., Mauriello, R., Ferranti, P., Pizzano, R., Cappucio, U., Laezza, P., Addeo, F., Rammumno, L., Rando, A., & Rubino, R. (1993). The nature of β-casein heterogeneity in caprine milk. *Le Lait, 73*(5), 533–547.

Chianese, L., Mauriello, R., Intircia, N., Moio, L., & Addeo, F. (1992). New $\alpha_{s2}$-casein variant from caprine milk. *Journal of Dairy Research, 59*(2), 299–305.

Dall'Olio, S., Davoli, R., & Russo, V. (1989). A new variant of caprine β-casein. *Scienza e Tecnica Lattiero-Casearia, 40*(1), 24–28.

Delacroix-Buchet, A., Degas, C., Lamberet, G., & Vassal, L. (1996). Influence des variants AA et FF de la caséine $\alpha_{s1}$ caprine sur le rendement fromager et les caractéristiques sensorielles des fromages. *Le Lait, 76*(2), 217–241.

Enne, G., Feligini, M., Greppi, G.F., Iametti, S., Pagani, S. (1997). Gene frequencies of caprine $\alpha_{s1}$-casein polymorhism in dairy goat. In Milk protein polymorhism, proceedings of the IDF Seminar, Palmerston North, New Zealand, February 1997, IDF S.I. 9702 (pp. 275–279). Brussels: IDF.

Ferranti, P., Addeo, F., Malorni, A., Chianese, L., Leroux, C., & Martin, P. (1997). Differential splicing of pre-messenger RNA produces multiple forms of mature caprine $\alpha_{s1}$-casein. *European Journal of Biochemistry, 249*(1), 1–7.

Ferranti, P., Lilla, S., Chianese, L., & Addeo, F. (1999). Alternative nonallelic deletion is constitutive of ruminant $\alpha_{s1}$-casein. *Journal of Protein Chemistry, 18*(5), 595–602.

Grosclaude, F., Martin, P. (1997). Casein polymorphisms in the goat. In Milk protein polymorhism, proceedings of the IDF Seminar, Palmerston North, New Zealand, February 1997, IDF S.I. 9702 (pp. 241–253). Brussels: IDF.

Hollar, C. M., Law, A. J. R., Dalgleish, D. G., & Brown, R. J. (1991). Separation of major casein fractions using cation exchange fast protein liquid chromatography. *Journal of Dairy Science, 74*(8), 2403–2409.

Jaubert, A., Bonnin, V., Boisseau, N. (1997). Goat farm bulk milk: Analysis of $\alpha_{s1}$-casein content. In Milk protein polymorhism, proceedings of the IDF Seminar, Palmerston North, New Zealand, February 1997, IDF S.I. 9702 (pp. 292–293). Brussels: IDF.

Jaubert, A., & Martin, P. (1992). Reverse-phase HPLC analysis of goat caseins. Identification of $\alpha_{s1}$and $\alpha_{s2}$ genetic variants. *Le Lait, 72*(2), 235–247.

Lamberet, G., Degas, C., Delacroix-Buchet, A., & Vassal, L. (1996). Influence de caractères liés aux allèles A et F de la caséine $\alpha_{s1}$caprine sur la flaveur chèvre: Fabrications fromagères avec échange de protéines et de matières grasses. *Le Lait, 76*(3), 349–361.

Law, A. J. R., & Tziboula, A. (1992). Quantitative fractionation of caprine casein by cation-exchange HPLC. *Milchwissenschaft, 47*(9), 558–562.

Mahé, M. F., & Grosclaude, F. (1993). Polymorphism of β-casein in the Creole goat of guadeloupe: Evidence for a null allele. *Génétique Sélection et Évolution, 25*(5), 403–408.

Martin, P. (1993). Polymorhisme génétique des lactoprotéines caprines. *Le Lait, 73*(5), 511–532.

Morgan, F., Fauquant, J., Micault, S., Bonnin, V., Jaubert, A. (2000). Thermal coagulation of goat milk: Biochemical mechanisms. In Proceedings of the technical symposium held within the 7th international conference on goats (pp. 27–44).

Morgan, F., Massouras, T., Barbosa, M., Roseiro, L., Ravasco, F., Kandarakis, I., Bonnin, V., Fiskatoris, M., Anifantakis, E., Jaubert, G., & Raynal-Ljutovac, K. (2003). Characteristics of goat milk collection from small and medium enterprises in Greece, Portugal and France. *Small Ruminant Research, 47*(1), 39–49.

Neveu, C., Mollé, D., Moreno, J., Martin, P., & Léonil, J. (2002). Heterogeneity of caprine beta-casein elucidated by RP-HPLC/MS: Genetic variants and phosphorylations. *Journal of Protein Chemistry, 21*(8), 557–567.

Pierre, A., Le Quéré, J.-L., Riaublanc, A., Le Graët, Y., Demaizières, D., & Michel, F. (1998a). Composition and physico-chemical characteristics of goat milks containing the A or O $\alpha_{s1}$ casein variants. *Le Lait, 78*(2), 191–202.

Pierre, A., Michel, F., & Le Graët, Y. (1995). Variation in size of goat milk casein micelles related to casein genotype. *Le Lait, 75*(5), 489–502.

Pierre, A., Michel, F., Le Graët, Y., & Zahoute, L. (1998b). Casein micelle size in relation with casein composition and $\alpha_{s1}$, $\alpha_{s2}$, β and κ casein in goat milk. *Le Lait, 78*(6), 591–605.

Pierre, A., Mollé, D., & Zahoute, L. (2001). Characterization of the casein variants in goat bulk milks using on-line RP-HPLC/ESI-MS. *Le Lait, 81*(5), 667–678.

Pirisi, A., Colin, O., Laurent, F., Scher, J., & Parmentier, M. (1994). Comparison of milk composition, cheesemaking properties and textural characteristics of the cheese from two groups of goats with a high or low rate of $\alpha_{s1}$-casein synthesis. *International Dairy Journal, 4*(4), 329–345.

Recio, I., Pérez-Rodríguez, M. L., Amigo, L., & Ramos, M. (1997). Study of the polymorphism of caprine milk caseins by capillary electrophoresis. *Journal of Dairy Research, 64*(4), 515–523.

Remeuf, F. (1993). Influence du polymorphisme génétique de la caséine $\alpha_{s1}$ caprine sur les caractéristiques physico-chimiques et technologiques du lait. *Le Lait, 73*(5), 549–557.

Richardson, B. C., & Creamer, L. K. (1974). Comparative micelle structure. III. The isolation and chemical characterization of caprine $\beta_1$-casein and $\beta_2$-casein. *Biochimica et Biophysica Acta, 365*(1), 133–137.

Trujillo, A. J., Casals, I., & Guamis, B. (2000). Analysis of major caprine milk proteins by reversed-phase high performance liquid chromatography and electrospray ionization-mass spectrometry. *Journal of Dairy Science, 83*(1), 11–19.

Trujillo, A. J., Guamis, B., & Carretero, C. (1997). Proteolysis of goat casein by calf rennet. *International Dairy Journal, 7*(8/9), 579–588.

Vassal, L., Delacroix-Buchet, A., & Buillon, J. (1994). Influence des variants AA, EE et FF de la caséine $\alpha_{s1}$ caprine sur le rendement fromager et les caractéristiques sensorielles de fromages traditioannels: Premières observations. *Le Lait, 74*(1), 89–103.

Voutsinas, L., Pappas, C., & Katsiari, M. (1990). The composition of Alpine goats' milk during lactation in Greece. *Journal of Dairy Research, 57*(1), 41–51.

Yamauchi, M. H., Coll, A., Sanchez, A., & Folch, J. M. (2001). Genetic polymorphism of the caprine kappa casein gene. *Journal of Dairy Research, 68*(2), 209–216.