Original article

Casein fraction of ovine milk from indigenous Greek breeds

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Abstract – Whole casein fractions isoelectrically prepared from bulk milks from four different indigenous Greek ovine breeds were analysed by urea-PAGE and reversed-phase HPLC. Individual caseins prepared by fractionation on a cation-exchange column were used to locate the peaks on the chromatograms. Apart from heterogeneity that was depicted in the peak shape of caseins, there was also quantitative variability regarding the α_s- and β-casein contents. According to the RP-HPLC results, the relative percentages of individual caseins ranged as follows: κ-casein from 9.1% to 10.8%, α_{s2}-casein from 12.0% to 16.4%, α_{s1}-casein from 33.9% to 39.9% and β-casein from 37.0% to 42.3%. According to the results obtained by both methods, the composition of the casein fraction from the Karagouniko breed was the most different, since it had the greatest β-casein content and the lowest α_s-casein to β-casein ratio. The relative percentages of the individual caseins in two of the four breeds (Frisarta and Chios) were similar. The quantification results of urea-PAGE and RP-HPLC analyses were in general similar with respect to the means. However, the relative standard deviations of the chromatographic method were better and this was also true for the resolution of the components of the whole casein fraction.

Ovine casein / indigenous ovine breeds / chromatography / RP-HPLC / electrophoresis

Résumé – Fractions caséiques des laits de mélange provenant de troupeaux de brebis indigènes grecques. Les fractions caséiques des laits de mélange de quatre troupeaux de brebis indigènes grecques ont été analysées par électrophorèse en gel de polyacrylamide à pH alcalin en présence d'urée (urée-PAGE) et par chromatographie liquide haute performance en phase inversée (RP-HPLC). L'identification des caséines a été effectuée par l'analyse des caséines individuelles préparées par chromatographie d'échange de cations. Les résultats des analyses par RP-HPLC ont démontré que le contenu en caséines principales variait dans les proportions suivantes: caséine κ, de 9,1 à 10,8 %, caséine α_{s2}, de 12,0 à 16,4 %, caséine α_{s1}, de 33,9 à 39,9 % et caséine β, de 37,0 à 42,3 %. Les résultats obtenus par les deux méthodes ont démontré que la fraction caséique du troupeau Karagouniko était la plus différente parmi les laits analysés ; elle a présent la plus haute teneur en caséine β et le plus faible rapport caséine α_{s1} sur caséine β. Les pourcentages relatifs des caséines individuelles de deux des quatre troupeaux étudiés étaient en général similaires. La résolution et les écarts types relatifs estimés par la chromatographie, étaient meilleurs que ceux estimés par urée-PAGE.

Caséine de brebis / troupeaux de brebis indigènes / chromatographie / électrophorèse

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1. INTRODUCTION

The composition of ovine milk, which is rich in proteins and fat, results in excellent cheesemaking properties. A great variety of ovine cheeses with particular characteristics are produced in the Mediterranean area. Caseins are responsible for the enzymatic coagulation of milk and their hydrolysis pattern is a very important characteristic of cheese ripening.

Although bovine and more recently caprine caseins have been extensively characterised by a variety of analytical methods, the publications about ovine casein are more limited. They are for the most part studies on the characterisation and the polymorphism of individual caseins [1, 2, 7–11, 15–17, 34, 36, 37]. Limited information exists regarding the relative percentages of the individual caseins in the whole casein fraction of ovine milks [8, 21, 27, 33]. The influence of the α_{s1}-casein genotype and therefore of the composition of the casein fraction on the micelle structure and the cheesemaking properties of caprine milk has been studied quite extensively [28–30, 35, 39]. The influence of genetic polymorphism of the proteins on the technological properties of ovine milk has been reviewed by Amigo et al. [3]. Regarding the influence of the casein genotype, it has been reported that the α_{s1}-casein genotype affects milk production [12], total protein and casein content, the casein micelle diameter and the composition of the whole casein fraction [31–33]. Consequently, it affects the cheesemaking properties of ovine milk with respect to renneting performance and cheese yield [12, 23, 32, 33].

The purpose of the present work was to study the casein fractions of four Greek indigenous ovine herds by estimating the relative amounts of the individual caseins in the whole casein fraction. For this purpose electrophoretic and chromatographic methods were used.

2. MATERIALS AND METHODS

2.1. Preparation of whole casein

Bulk milks from four flocks that corresponded to different Greek indigenous ovine breeds named Karagouniko, Boutsiko, Chios and Frisarta were collected. After centrifugation at $2000 \times g$ for 30 min at 4 °C, the skim milks were acidified at pH 4.5 with acetic acid (1 mol·L^{-1}) 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^{-1} until it reached pH 7.0 and precipitated again. Four successive cycles of precipitation and washing were carried out. The final precipitate was dissolved in 20 mmol·L^{-1} phosphate buffer, pH 7.0, heated at 80 °C for 30 min to inactivate plasmin, dialysed (MWCO 12 kg·mol^{-1}, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) against distilled water and lyophilised. The same procedure was followed for the preparation of whole bovine casein from a Holstein-Friesian flock.

2.2. Casein hydrolysis by chymosin

Whole casein was treated with fermentation-produced chymosin (Maxiren®150, Gist-Brocades, Delft, The Netherlands) according to McSweeney et al. [24]. Solutions of ovine whole casein (30 mg·mL^{-1}) in 0.1 mol·L^{-1} phosphate buffer, pH 6.6, containing sodium azide (5 mg·100 mL^{-1}) were heated to 30 °C. The clotting activity of chymosin solutions in 0.1 mol·L^{-1} phosphate buffer, pH 6.6, was determined in 10 mL ovine skim milk supplemented with 10 mmol·L^{-1} CaCl$_2$ at 30 °C. One unit of the enzyme was determined as the amount of enzyme required to coagulate 10 mL of milk in 100 s at 30 °C. The casein solutions were incubated with chymosin solution in a ratio of 0.5 enzyme units per mL of casein solution at 30 °C for 20 and 180 min. At the end of each incubation time, the chymosin

was inactivated by heating at 65 °C for 30 min. Hydrolysates were dissolved in urea-polyacrylamide gel electrophoresis (urea-PAGE) sample buffer in a ratio 1:4 and they were analysed by alkaline urea-PAGE.

2.3. Casein hydrolysis by plasmin

The treatment of whole casein with bovine plasmin (E.C. 3.4.21.7, Boehringer, Mannheim, Germany) was carried out according to the Commission Regulation [13]. Ovine casein (10 mg·mL^{-1}) was dissolved in 0.2 mol·L^{-1} ammonium hydrogen carbonate buffer, pH 8.0, and 5 μL bovine plasmin were added. After incubation at 40 °C for 60 min, aminocaproic acid was added to inactivate the enzyme. Hydrolysates were dissolved in urea-PAGE sample buffer in a ratio 1:4 and they were analysed by alkaline urea-PAGE.

2.4. Urea-PAGE of casein

Whole caseins and their hydrolysates were analysed by the urea-PAGE method of Andrews [4] with direct staining using Coomassie Brilliant Blue G-250 [6]. Electrophoresis was carried out on a vertical slab unit (LKB 2001, Amersham Pharmacia Biotech, Uppsala, Sweden) in slabs 140 × 160 × 1.5 mm. Separation gels of T = 12.5% and C = 4% were used. Ten μL of whole casein solutions (2 mg·mL^{-1}) were put in stacking gel buffer containing 8 mol·L^{-1} urea and 0.1 mol·L^{-1} β-mercaptoethanol. Then after incubation at 40 °C for 20 min, they were analysed. The gels were run under constant current of 60 mA at 10 °C and the duration of the analysis was about 2.5 h. After destaining with water, the gel slabs were scanned using a scanner (Hewlett Packard, ScanJet 4c/T, USA) and the protein bands were quantified by an image processing system (Gel-Compar v. 4.0, Applied Maths, Kortrijk, Belgium). Duplicates of each sample were analysed in each gel and four independent electrophoretic runs were carried out for each sample.

2.5. Cation-exchange (CE) chromatography of whole caseins

Lyophilised whole caseins were fractionated by cation-exchange chromatography on a SP-Sepharose Hiload 26/10 column (Amersham Pharmacia Biotech) using a Waters 650E automated chromatographic system with a tunable absorbance detector (Waters Model 486, Waters, Milford, MA, USA). A modification of the method of Hollar et al. [19] was applied. Buffer A was 20 mmol·L^{-1} Na acetate 6 mol·L^{-1} urea, pH 5.0, and buffer B was similar to A but also contained 1 mol·L^{-1} NaCl. Sixty mL of lyophilised casein solution (7 mg·mL^{-1}) prepared according to Hollar et al. [19] were applied onto the column. Firstly the sample was applied onto the column at a flow rate of 2 mL·min^{-1} and it was washed with buffer A at a flow rate of 3 mL·min^{-1} for 15 min. Then a NaCl gradient was formed by mixing buffer A with buffer B at a flow rate of 3 mL·min^{-1}, as follows: from 0 to 0.08 mol·L^{-1} NaCl within 15 min, from 0.08 to 0.10 mol·L^{-1} within 45 min, from 0.10 to 0.12 mol·L^{-1} within 15 min, from 0.12 to 0.14 mol·L^{-1} within 50 min, from 0.14 to 0.20 mol·L^{-1} within 20 min and from 0.20 to 0.30 mol·L^{-1} within 90 min. Finally, the gradient was followed by an isocratic elution with 0.30 mol·L^{-1} NaCl for 30 min and the column was washed with 1 mol·L^{-1} NaCl for 30 min at the same flow rate. The eluate was monitored at 280 nm and the fraction size was 6 mL. The collected peaks were dialysed (MWCO 12 kg·mol^{-1}, Sigma-Aldrich Chemie) against distilled water and lyophilised. Both buffers and samples were filtered through 0.45 μm (Millipore Corporation, Bedford, USA). Their identity and purity were established by the reversed-phase HPLC method of Jaubert and Martin [20] and the urea-PAGE method of Andrews [4], performed on a Hoefer miniVE Vertical Electrophoresis System (Amersham Pharmacia Biotech) in gel slabs 100 × 100 × 1 mm. Proteins were fixed onto the gel by trichloroacetic acid (150 g·L^{-1}) and stained by a Coomassie

R-250 solution (2.5 g·L⁻¹) in methanol, acetic acid and water (25:5:20). Gels were destained by a solution of glycerol, acetic acid, methanol and water (3:10:40:47).

2.6. Reversed-phase HPLC (RP-HPLC)

Reversed-phase HPLC analyses were performed by the method of Jaubert and Martin [20] on a Vydac C4 214 TP 5415 column (Separations Group, Hesperia, USA), at room temperature. The HPLC system consisted of a pump capable of mixing four solvents (Waters 600E, Waters), a photodiode array detector (Waters 996), a helium degasser, a Rheodyne 7125 injector (Rheodyne Inc., Cotati, CA, USA) and Millenium software (v.3.05.01, Waters). Solvent A was 1 mL·L⁻¹ trifluroacetic acid (TFA, Serva Electrophoresis Gmbh, Heidelberg, Germany) in ultrapure water and solvent B was 0.96 mL TFA, 800 mL acetonitrile (Lichrosolv grade, Merck KGaA, Darmstadt, Germany) and 200 mL ultrapure water. Solvents were filtered through 0.45 µm filters (Millipore Corporation). The flow rate was 1 mL·min⁻¹. A linear gradient from 370 mL·L⁻¹ to 530 mL·L⁻¹ solvent B within 30 min was applied. Samples were prepared according to Pierre et al. [28] as follows: 30 mg lyophilised casein were dissolved in 2 mL buffer, pH 7.0 (0.1 mol·L⁻¹ tris, 8 mol·L⁻¹ urea, 13 g·L⁻¹ trisodium citrate and 10 mmol·L⁻¹ dithiothreitol adjusted to pH 7.0 by HCl), at 37 °C for 60 min, then 18 mL of solvent A containing 6 mol·L⁻¹ urea were added and pH was adjusted to 2.2 by 50% TFA. After filtration through a 0.45 µm filter (Millipore Corporation), 50 µL were analysed. The protein content of the samples was adjusted after preliminary analyses. The heights of the major casein peaks at 214 nm, were linearly correlated with the concentration of whole ovine casein in the sample, providing that the latter ranged from 20 to 100 µg casein per injection. Four independent preparations of each sample were analysed.

2.7. Isoelectric focusing (IEF) of caseins

Whole and individual caseins obtained by CE chromatography were analysed by isoelectric focusing on ultrathin (0.20 mm) polyacrylamide gel (C = 5%, T = 3%, 6 mol·L⁻¹ urea) carried out in a LKB 2117 Multiphor II Electrophoresis Unit (Amersham Pharmacia Biotech). The gel layers were prepared with a mixture of ampholytes consisting of Pharmalyte™ pH 2.5–5.0, Pharmalyte™ pH 4.5–5.4 and Ampholine™ pH 4.0–6.5 (Amersham Pharmacia Biotech) in a 1.6:1.4:1 ratio, according to Ferranti et al. [17]. Staining with Coomassie Brilliant Blue G-250 and destaining were according to the Commission Regulation [13].

3. RESULTS AND DISCUSSION

3.1. Electrophoretic and chromatographic profiles of whole caseins

The fractionation of ovine whole casein of the Frisarta flock on a SP-Sepharose cation-exchange column is presented in Figure 1. According to urea-PAGE and RP-HPLC analyses, the first eluted group of substances consisted of β-mercaptoethanol and non-casein components. Peak 1 was β-casein. In peak 2, nothing else but different forms of κ-casein were found and in peak 3, κ-casein coexisted with bands that migrated in the region of γ-caseins. The content of peak × was not visualised by urea-PAGE, but in its RP-HPLC profile there was a hydrophobic peak with high retention time similar to that of β-casein. It can be attributed to γ-caseins that are expected to be co-eluted with β-caseins [18, 40]. Peaks 4 and 5 consisted mainly of α_{s2}-casein and also contained traces of α_{s1}-casein. The last peak, 6, was α_{s1}-casein. In a cation-exchange Mono S column at 5.0, ovine α_{s1}- and α_{s2}-caseins are co-eluted [9, 21], but by the present chromatographic analysis they were separated from each other.
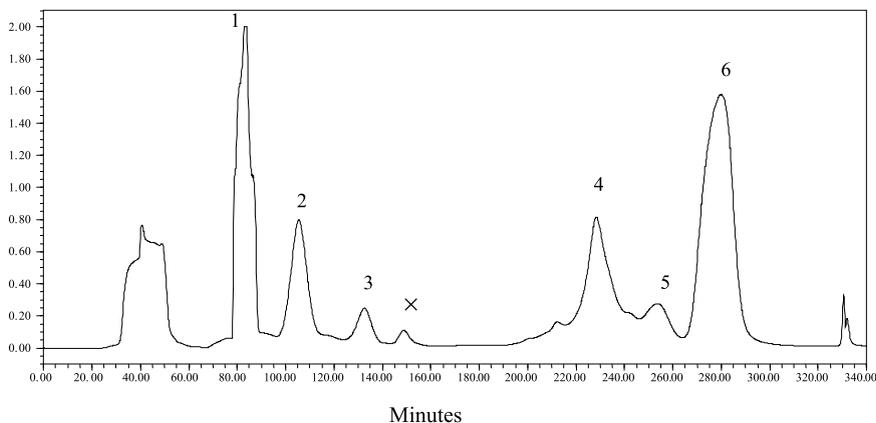
Figure 1. Cation-exchange chromatography (A_{280}) on a SP-Sepharose Hiload 26/10 column of whole ovine casein of the Frisarta breed (conditions and sample preparation are described in detail in Materials and methods).
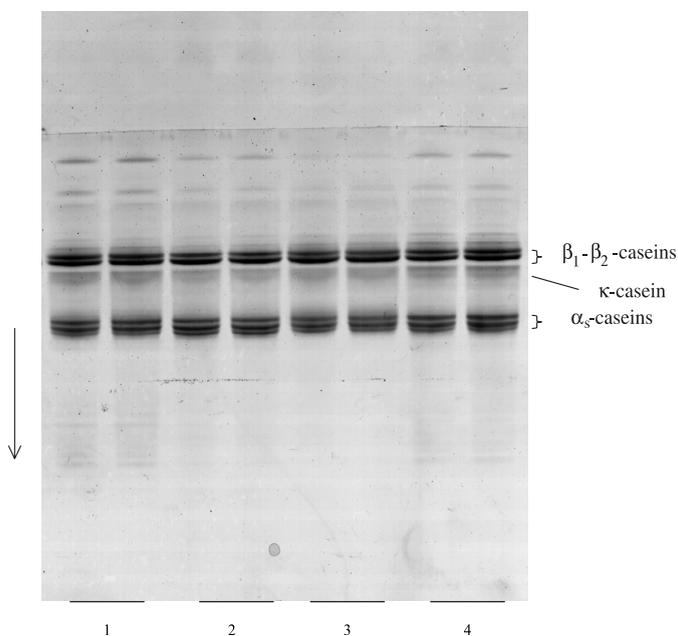
Figure 2. Urea-PAGE profiles of whole casein fractions from indigenous Greek ovine breeds. Lanes from 1 to 4, whole ovine casein fractions of the Frisarta, Chios, Karagouniko and Boutsiko breeds, respectively.

Urea-PAGE profiles of whole caseins are shown in Figure 2 and those of the chymosin and plasmin hydrolysates in Figure 3 a and b. In Figure 4, the reversed-phase HPLC profiles are presented. The identity of each peak was established after analysis of the individual caseins obtained by cation-exchange chromatography (Fig. 1).

The elution pattern of α_{s1}-casein was the most variable among the RP-HPLC
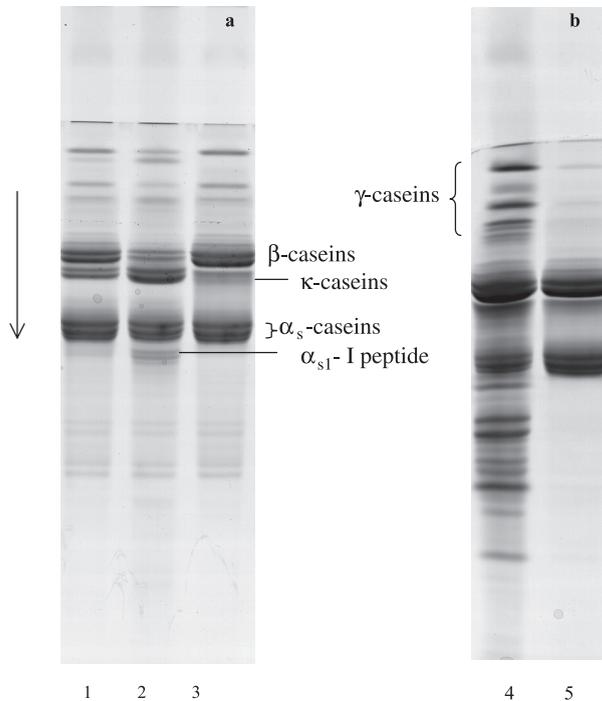
Figure 3. Urea-PAGE of whole ovine casein hydrolysates after treatment with chymosin (a) and plasmin (b). Lanes 1 and 2, whole ovine casein after 20 and 180 min of hydrolysis, respectively, with chymosin at pH 6.6 (as described in Materials and methods); lane 3, unhydrolysed whole ovine casein; lane 4, whole ovine casein hydrolysate after treatment with plasmin (as described in Materials and methods); and lane 5, unhydrolysed whole ovine casein.

profiles of the different breeds (Fig. 4). In the Frisarta, Chios and Boutsiko profiles, there were two different α_{s1}-casein peaks in different ratios, corresponding to the most frequent allelic forms within the breeds. It seemed that in the Karagouniko casein profile, there was one predominant variant of α_{s1}-casein. There are six genetic variants of ovine α_{s1}-casein [11, 33], with co-existence of short non-allelic forms and differences in the phosphorylation level of each form. Mature α_{s1}-casein is a mixture of at least seven molecular species [15–17]. The short forms of each variant are eluted earlier and the long forms follow in RP-HPLC profiles, while the full-length protein has higher electrophoretic mobility [14–16, 37]. Two of the six genetic variants, C and B, are the most frequent in Italian and Spanish breeds, while E and F are very rare [3]. Apparently, all this heterogeneity was also depicted in the IEF profiles of α_{s1}-casein (Fig. 5). In the IEF pattern of α_{s1}-casein several bands appeared, two of them being predominant, corresponding to the most frequent allelic forms. Despite the fact that the α_{s1}-casein profile of the Karagouniko breed was found to be different from that of the other three breeds by the RP-HPLC method, the IEF profiles of the four breeds resembled each other (Fig. 5).

Regarding the α_{s2}-peaks, there were differences between the RP-HPLC profiles. α_{s2}-casein of the samples of the present
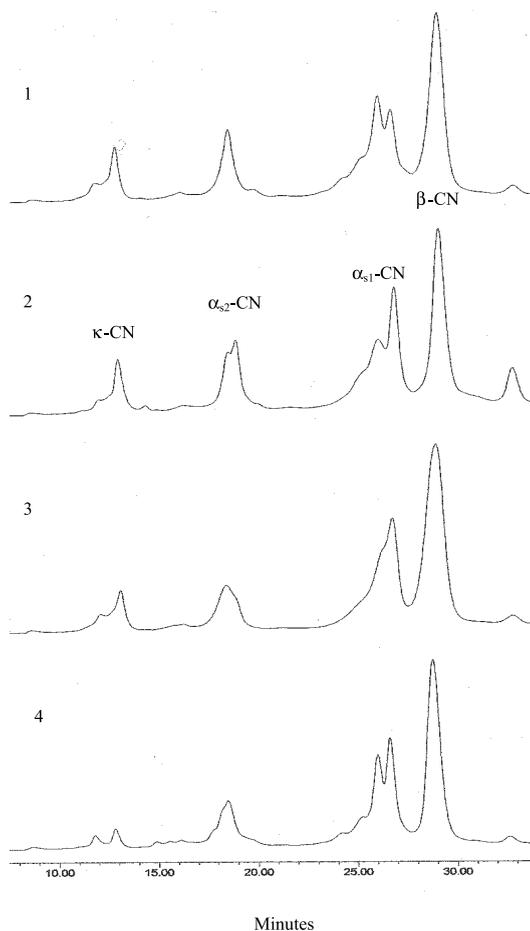
Figure 4. Reversed-phase HPLC profiles (A_{214}) of whole ovine caseins from indigenous Greek breeds (1, Frisarta; 2, Chios; 3, Karagouniko; and 4, Boutsiko).

study was resolved as two distinct but not well-resolved peaks or as a peak with rather broad shape on the reversed-phase column, as was the case of the Chios casein (Fig. 4). It is the most multisphorylated casein and similarly to its bovine counterpart, it displays genetic polymorphism expressed into two genetic variants and a non-allelic deleted form co-exists with the full-length protein [7, 17]. Eleven components and four main forms have been identified in the α_{s2}-casein peak [17, 37]. Moreover, IEF analysis of α_{s2}-casein obtained by cation-exchange chromatography revealed many bands (Fig. 5). Due to their heterogeneity,

ovine α_{s1}- and α_{s2}-caseins partly co-migrated and they appeared as a group of three bands in the electrophoretic profiles (Fig. 2), as has also been reported by other researchers [11, 14, 38].

Ovine β_1- and β_2-caseins with 6 and 5 phosphates, respectively, migrated as two separated bands in the urea-PAGE profiles (Fig. 2) [36]. According to Chianese et al. [10] and Ferranti et al. [17] there are also multiphosphorylated forms with different mobilities containing 1–7 phosphates occurring simultaneously in individual ovine milks. Ovine β-casein was eluted as a single peak in all RP-HPLC profiles (Fig. 4).
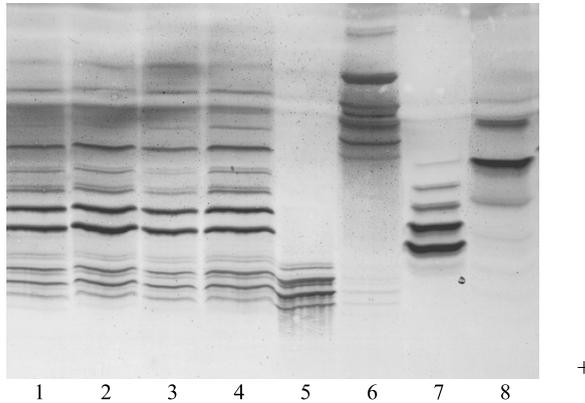
Figure 5. Isoelectric focusing (IEF) profiles on ultrathin gel, pH 2.5–6.5, of whole ovine caseins from indigenous Greek breeds. Lane 1, whole Boutsiko casein; lane 2, whole Karagouniko casein; lane 3, whole Chios casein; lane 4, whole Frisarta casein; lane 5, ovine α_{s1}-casein; lane 6, ovine α_{s2}-casein; lane 7, ovine β-casein; and lane 8, ovine κ-casein (conditions are described in detail in Materials and methods).

However, it seemed to be very heterogeneous in the IEF patterns (Fig. 5), where the β_1- and β_2-casein bands were clearly demonstrated. The less intense bands could be attributed to the other multiphosphorylated forms [10, 17].

Ovine κ-caseins appeared as a diffused band in the electrophoresis profiles (Fig. 2). Apart from Alais and Jollès [2], who have reported A and B κ-caseins in ovine milk, no genetic polymorphism has been reported for this casein. The high degree of κ-casein heterogeneity derived from the differences in phosphate and carbohydrate content is responsible for its shape in the RP-HPLC profiles of Figure 4 [17, 37]. It was eluted as two rather overlapped peaks. However, its IEF profile was more complex, presenting three main bands (Fig. 5). This complexity was in accordance with the reported IEF patterns of native and dephosphorylated ovine κ-casein [8, 17].

The position of κ-casein on the electrophoretic profiles migrating just ahead of β-casein (Fig. 2) as a diffused band was located after the analysis of the chymosin and plasmin hydrolysates by the same urea-PAGE method (Fig. 3 a, b). The disappearance of bovine κ-casein after hydrolysis with chymosin was not clearly evident in ovine casein hydrolysates because ovine κ-casein had the same mobility as proteolysis products of ovine β-casein (Fig. 3a). Hydrolysis of bovine whole casein by chymosin is accompanied by the appearance of the faster-moving α_{s1}-I peptide derived from bovine α_{s1}-casein (α_{s1}-CN f 24-191) in the urea-PAGE profiles [24]. The respective peptide in ovine casein hydrolysates became clearly evident after 180 min of hydrolysis as a couple of bands moving just ahead of the α_s-casein bands. The rate of hydrolysis of ovine β-casein and the appearance of the couple of β-I peptides (β-CN f1-192) [26] in the area of the κ-casein, seemed faster than that of ovine α_{s1}-casein. The appearance of β-I and α_{s1}-I peptides as a couple of bands has been attributed to the phosphorylation differences in the respective sequences of the parent caseins [15, 36, 38]. The presence of two bands of ovine α_{s1}-I peptide could also be attributed to the different mobilities of the full-length and the deleted forms of ovine α_{s1}-casein and of the different α_{s1}-casein genotypes [16, 17, 33]. Plasmin treatment of whole casein gave rise to many new bands (Fig. 3b).

Table I. Means[1] and relative standard deviations (RSDs)[2] for the relative percentages[3] of α_{s1}-, α_{s2}-, β- and κ-caseins in whole casein from indigenous Greek ovine breeds estimated by urea-PAGE and RP-HPLC.

Breed	Method	κ-casein	α_s-casein[4]	α_{s2}-casein	α_{s1}-casein	β-casein	α_s-casein[4]/ β-casein
Frisarta	Urea-PAGE	10.4 (12.6)	51.7 (3.4)			37.9 (3.4)	1.36
	RP-HPLC	10.2 (3.3)		14.0 (10.3)	38.8 (1.8)	37.0 (5.3)	1.43
Chios	Urea-PAGE	9.8 (21.0)	53.2 (2.4)			37.0 (4.4)	1.44
	RP-HPLC	10.2 (5.6)		16.4 (2.9)	36.3 (3.5)	37.1 (4.4)	1.42
Karagouniko	Urea-PAGE	10.3 (26.5)	48.3 (4.7)			41.4 (2.5)	1.17
	RP-HPLC	10.8 (8.0)		13.0 (3.9)	33.9 (2.2)	42.3 (3.4)	1.11
Boutsiko	Urea-PAGE	10.4 (9.9)	50.2 (3.0)			39.4 (4.4)	1.27
	RP-HPLC	9.1 (1.5)		12.0 (8.3)	39.9 (5.6)	39.0 (3.6)	1.33

[1] Means of quantification of four independent sample preparations (n = 4); [2] standard deviation expressed as percentage of the mean; [3] expressed as percentage of total α_{s1}, α_{s2}-, β- and κ-caseins; [4] α_{s1}-casein + α_{s2}-casein.

Apart from γ-caseins [5] many new distinct bands with mobility greater than that of α_s-caseins appeared, that could result from the hydrolysis of α_{s1}-casein by plasmin, as is reported for its bovine counterpart [22, 25]. The ovine κ-casein band was clearly demonstrated in the plasmin hydrolysates, since it is quite resistant to plasmin action, according to the data reviewed by Bastian and Brown [5].

3.2. Quantification of casein fractions

The quantification results of the urea-PAGE profiles expressed as relative percentages of α_s-, β- and κ-caseins are presented in Table I. Regarding the results of urea-PAGE analyses, α_{s1}- and α_{s2}-caseins were estimated as the group of α_s-caseins because they co-migrated, as explained in the previous section. A part of the κ-casein components was not expected to be quantified because they are overlapping with β-casein bands [8, 14]. The overestimation of β-casein bands was negligible, since its content was much higher in comparison with that of κ-casein. The quantification of γ-caseins was uncertain and since they are greatly affected by the treatment of milk

samples, they were excluded from the quantification results of Table I. In Table I, the results from the RP-HPLC analyses are also presented. The relative quantities of different caseins were estimated by calculating the individual peak areas as a percentage of the sum of all peaks.

The relative percentages of the individual ovine caseins estimated by PAGE and RP-HPLC were similar (Tab. I). However, there were differences between the breeds estimated by both methods, especially concerning the α_s- and β-caseins content. The relative standard deviations (RSDs) of κ-casein means estimated by urea-PAGE were very high, because of its appearance as a diffused band, its partial co-migration with β-casein and its low content.

The results of Table I were in some cases different from those reported for ovine casein fractions, especially regarding, α_s- and β-caseins. The percentage of κ-casein, ranging from 9.1% to 10.8%, was similar to that reported for the milk of Sardinian ewes by Law et al. [21] and Papoff et al. [27] who used cation-exchange chromatography. Similarly, Pirisi et al. [33] have estimated by RP-HPLC the κ-casein relative percentage as 9.8% in ovine milks

from Sarda sheep of CC and CD geno-types. However, it was much lower than that reported for the casein of Lacha Spanish sheep, by Calavia and Burgos [8]. They have estimated the κ-CN percentage to total ovine casein to be 17%, by densitometric analysis of the PAGE electrophoregrams of the SH containing casein.

Both α_{s1}- and α_{s2}-casein percentages were quite variable among the different breeds. The α_{s2}-casein percentage of the ovine samples, ranging from 12.0% to 16.4%, was higher than that reported by Calavia and Burgos [8]. Pirisi et al. [33] have reported that the relative α_{s2}-casein percentage ranges from 11.9% to 13.9% depending on the α_{s1}-casein genotype of ovine milk. The relative quantity of α_{s1}-casein was also variable (from 33.9% to 38.8%) among the different breeds and the lowest value was observed in the Karagouniko breed (33.9%). Total α_{s}-casein percentage ranged from 46.9% to 52.8% according to RP-HPLC and from 48.3% to 53.2% according to the PAGE results. These values were in general higher than those reported for ovine α_{s}-caseins by other researchers. Addeo et al. [1] and Calavia and Burgos [8] have estimated the ovine α_{s}-caseins relative percentage as 45% and 43%, respectively. Law et al. [21] and Papoff et al. [27] report very low values of this percentage, 34.2% and 26.8%, respectively. However, Pirisi et al. [33] report relative percentages of α_{s}-caseins from 45.3% to 50.2%, depending on the α_{s1}-casein genotype, which are close to the values of the present study.

β-Casein of the studied milks ranged from 37.0% to 42.3% of total casein according to RP-HPLC and from 37.0% to 41.4% according to the PAGE results. The highest value was estimated by both methods in Karagouniko casein. Calavia and Burgos [8] found that the β-casein percentage in the Spanish Lacha milk is about 38% in whole casein. Law et al. [21] and Papoff et al. [27] found that in the whole casein fraction of Sardinian ewes, β-casein

accounts for about 50%, but Pirisi et al. [33] report percentages of β-casein from 40% to 43%.

There is a link between α_{s1}-casein genetic variants and milk composition, which affects cheese yield [12, 31, 32]. According to the conclusions of Amigo et al. [3], the α_{s1}-casein D variant, even in association with others, results in lower casein content and poorer technological properties of ovine milk. From the results of Pirisi et al. [33], it is obvious that a lower α_{s}-casein/β-casein ratio in ovine milk, similar to that estimated in Karagouniko casein, is related to lower total protein and casein content of milk, greater micellar size, and longer clotting time and curd firming rate. From the results of the present study, Karagouniko milk would be expected to have inferior technological properties, compared with the milk of the other three breeds, although its IEF profile was not different regarding the α_{s1}-casein bands. Therefore, a further study has to be undertaken to relate the casein characteristics and technological properties of the milks of the present study.

4. CONCLUSIONS

Regarding the differences between the indigenous Greek ovine breeds, it seemed that in addition to the heterogeneity that was depicted in the peak shape of caseins, there was also quantitative variability, especially concerning the main casein groups (α_{s}- and β-caseins). Casein from the Karagouniko breed had the greatest β-casein content and the lowest α_{s}- to β-casein ratio, while the relative percentages of the individual caseins in the Frisarta and Chios breeds were very similar. The effect of this variability on the technological properties and especially on the cheesemaking properties of ovine milk has to be studied.

The RP-HPLC method of Jaubert and Martin [20] was appropriate for the analyses of ovine casein as it is for caprine

caseins. The heterogeneity of the individual caseins was depicted more clearly than by urea-PAGE. Furthermore, α_s-caseins were resolved in α_{s1}- and α_{s2}-peaks. Although the estimated relative percentages by both methods were similar, the RSDs of RP-HPLC means were much better. Fractionation by cation-exchange chromatography resulted in a very satisfactory separation of whole ovine casein components.

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