Analysis of Major Caprine Milk Proteins by Reverse-Phase High-Performance Liquid Chromatography and Electrospray Ionization-Mass Spectrometry

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ABSTRACT

Major proteins from caprine milk were separated by preparative gel permeation and cation-exchange fast protein liquid chromatography and were characterized by flow injection analysis by electrospray ionization mass spectrometry. In addition, proteins from whole skim milk and whole casein were analyzed by coupling reverse-phase HPLC and electrospray ionization mass spectrometry by two different chromatographic methods. These methods successfully resolved the major caprine milk proteins and main casein variants. The experimental molecular masses of major milk proteins and variants were: 19,302 for κ-CN 2P; 25,599 for α_{s2}-CN A-11P; 25,514 for α_{s2}-CN B-10P; 23,370 for α_{s1}-CN A-8P; 23,345 for α_{s1}-CN B-8P; 23,264 for α_{s1}-CN E-8P; 18,817 for α_{s1}-CN F-3P; 23,835 for β-CN 6P; 18,181 for β-LG; 14,180 for α-LA and 66,318 for serum albumin. (**Key words:** caprine milk proteins, mass espectrometry, electrospray source)

Abbreviation key: cDNA = copy DNA, **HPLC-ESI-MS** = coupling HPLC and electrospray ionization mass spectrometry, **FIA** = flow injection analysis, **FPLC** = fast protein liquid chromatography, **SA** = serum albumin.

INTRODUCTION

The proteins of goat milk, like those of other mammal species, are classified into two groups: caseins (α_{s1}-, α_{s2}, β-, and κ-CN) and whey proteins (α-LA, β-LG, serum albumin [**SA**] and immunoglobulins). In recent years, researchers have clearly demonstrated that genetic polymorphism in goat milk is strongly related to the casein content, with an impact on milk production traits, technological properties of cheese milk, flavor, and proteolysis in cheese (36). In general, α-LA and β-LG have been considered to be monomorphic in the caprine species. However, caprine caseins (especially (α_{s1}-CN) exhibit a great variability, qualitative as well as quantitative. The most recent genomic data (18) show the existence of at least 14 alleles at the α_{s1}-CN locus, distributed in seven different classes of protein variants (A to G), associated with four levels of expression ranging from 0 (null α_{s1}-CN) to 3.6 g/L per allele (α_{s1}-CN A, B, and C), with E as an intermediate variant (1.6 g/L) and F associated with a reduced α_{s1}-CN content (0.6 g/L). The AA sequence of caprine α_{s1}-CN and the differences that exist between the A, B, C, D, E, F, and G variants have been described (18). The variants A, B, C, and E are distinguished by AA substitutions, whereas the D, F, and G variants exhibit more profound structural alterations such as internal deletions. In addition to the polymorphism of α_{s1}-CN, three variants of α_{s2}-CN (A, B, and C) and β-CN (A, B, and 0) have been identified and studied (4, 5, 29). Results obtained by Di Luccia et al. (12) suggest that a genetic polymorphism also exists for κ-CN.

To analyze caprine milk proteins and genetic polymorphism of caseins, various electrophoretic (1, 17) and chromatographic techniques (21, 23) have been used. Recently, the main protein fractions and casein polymorphism have been identified and studied in goat milk by capillary electrophoresis (32).

Determining the primary structure of a protein molecule facilitates understanding of its functions at the molecular level. Two general methods are employed, but both suffer from several limitations. The first, AA sequencing by the Edman degradation technique, relies on a free primary or secondary amino group at the amino terminus and, thus, cannot deal with blocked or certain modified AA, or indeed any nonprotein modification. The second method, deducing the sequence indirectly through the DNA base sequence of the cloned gene cannot predict any posttransitional process in the

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final recombinant protein product. In addition, predicted sequences may not reflect the sequences that are actually expressed, either because of heterogeneity in the code or because of errors in sequencing the copy DNA (**cDNA**).

In recent years, electrospray ionization mass spectrometry (**ESI-MS**) has become a powerful alternative for determining the relative molecular mass of milk proteins (25); detection of any changes in proteins caused by, e.g., insertion, deletion, or modification of single AA (14); identification of genetic variants (15, 38); and posttranslational modifications such as phosphorylation (9) and glycosylation (8).

In this work, we studied the application of flow injection analysis (**FIA**) and the combination of HPLC with ESI-MS (**HPLC-ESI-MS**) to investigate genetic variants and posttranslational modifications of major caprine milk proteins and to determine their molecular weights. The analytical utility of two reverse-phase HPLC methods combined with ESI-MS are discussed.

MATERIALS AND METHODS

Preparation of Caprine Milk Proteins

Raw bulk milk and milk samples from individual goats (Murciano-Granadina breed) homozygous or heterozygous at the α_{s1}-CN (AA, BB, EE, FF, AE, BE, BF, and EF) and α_{s2}-CN (AA and AB) loci were obtained from the Experimental Farm of Universitat Autònoma de Barcelona (Spain). Animal genotyping was carried out by Unitat de Genètica (Facultat de Veterinària) as described by Jordana et al. (22).

Skim milks were prepared by centrifugation at 2500 $\times g$ and 30°C for 30 min. Whole caseins were prepared by isoelectric precipitation (3.3 *M* Na-acetate buffer, pH 4.6) from skim milks, previously diluted with an equal volume of distilled water and centrifuged for 20 min at 500 $\times g$. The caseins were dispersed in distilled water, precipitated again, and washed three times in succession. Finally, caseins were freeze-dried.

Major whey proteins (β-LG, α-LA, SA, and immunoglobulins) were purified from whey skim milk by gel permeation fast protein liquid chromatography (**FPLC**) according to Felipe and Law (13).

Caprine casein samples were fractionated by cation-exchange FPLC on a Mono S HR 5/5 column as Hollar et al. (19) described.

Reverse-Phase HPLC Separations

Method 1. A modification of the method proposed by Visser et al. (39) was used to separate the major proteins of goat milk (caseins, β-LG, and α-LA) in a single run. Proteins were separated on an Apex WP ODS reverse-phase column (7 μm, 4.6 $\times$ 250 mm, Jones Chromatography Ltd., Mid-Glamorgan, UK) at 46°C with a gradient of acetonitril in 0.1% (vol/vol) trifluoroacetic acid. The acetonitril gradient was 33 to 44% for the first 15 min, followed by 44 to 49% over a further 15 min. Flow rate was 1 ml/min, and the eluted peaks were detected by UV-absorbance at 214 nm.

Method 2. The second method was chosen because of its capacity to separate the main α_{s1} and α_{s2}-CN variants of goat milk in a single run. Whole casein from bulk or individual goat milk was separated by reverse-phase HPLC according to the procedure developed by Jaubert and Martin (21) with a Vydac C_4 column (5 μm, 4.6 $\times$ 150 mm, Teknokroma S. Coop C. Ltda, Spain) at 40°C. Solvents were: solvent A, 0.1% (vol/vol) trifluoroacetic acid in water, and solvent B, 0.1% (vol/vol) trifluoroacetic acid in 80% (vol/vol) acetonitrile. For casein separation, elution was achieved with a linear gradient from 37 to 53% solvent B for 30 min at a flow rate of 1 ml/min, and the eluted peaks were detected by UV-absorbance at 214 nm.

FIA-ESI-MS

To study the molecular weight of the milk proteins, the corresponding peaks from FPLC analysis were collected and lyophilized.

Each isolated milk protein was dissolved with a solution of acetonitrile and water (acidified with 0.1% (vol/vol) trifluoroacetic acid) at a ratio 1:1 (vol/vol) and then introduced in the fluidic way via FIA, with the same solvent as carrier, to a mass spectrometer Micromass, model Platform II (Micromass, Manchester, UK), fitted with an electrospray source. A Phoenix 20-syringe pump (C. E. Instruments, Milan, Italy) set at 50 μl/min was used. Ten microliters of each sample solution, containing approximately 0.1 mg of protein per milliliter, was injected into the fluidic way with a Rheodyne 7125 injector (Rheodyne, Cotati, CA).

The main working conditions of the source and mass spectrometer were the following: capillary at 4.5 kV, counter-electrode at 500 V, sample cone at 80 V, source temperature at 80°C, scan range for the m/z from 1000 to 3000 for 2 s with an interscan time of 0.25 s, scan mode continuum, and photomultiplier at 650 V. The spectrometric data were collected and analyzed by with the MassLynx software (Micromass).

HPLC-ESI-MS

The combination of HPLC with ESI-MS allowed us to obtain information about the molecular weight of the proteins as soon as they were eluted from the reverse-phase columns. An Alliance Waters 2690 separation

Table 1. Molecular masses of major caprine milk proteins.

Proteins	Experimental M_r[1]	Number of samples	Calculated M_r[2]
κ-CN 2P	19,302	7	19,306
α_{s2}-CN A-11P	25,599	5	25,599
α_{s2}-CN B-10P	25,514	2	25,518
α_{s1}-CN A-8P	23,370	4	23,362
α_{s1}-CN B-8P	23,345	4	23,347
α_{s1}-CN E-8P	23,264	2	23,259
α_{s1}-CN F-3P	18,817	2	18,816
β-CN 6P	23,835	7	23,821
β-LG	18,181	4	18,185
α-LA	14,180	2	14,186
Serum albumin	66,318	2	—

[1]The average relative molecular mass of the analyzed samples.
[2]Obtained from the AA or nucleotide sequence of proteins.

module (Waters Chromatography, Milford, MA) fitted with the appropiate column was used as chromatograph. The elution was performed with both gradient modes described above at a flow of 1 ml/min. That flow was too strong to reach the desired sensitivity with the ESI source used. A self-made flow diverter with a 1:20 ratio was added to the end of the chromatographic column, which allowed us to have greater spectroscopic signals for protein peaks. A T-union of zero dead volume (Waters) was used. To one end of the T-union a PEEK (254 μm diameter) tubing from the column was connected, to the other end fused silica capillary tubing (50 μm $\times$ 1 m) connected the T piece with the ESI source, and in the third end a coil of approximately 3 m length of PEEK tubing (254 μm diameter) was connected to divert the main part of the liquid to waste.

Some of the samples were too rich in urea and mercaptoethanol. This led to the crystallization of the urea at the top of the sample cone, inside the ESI source, with the consequent obstruction of its capillary hole; this made it difficult to obtain any signal from mass spectrometer. To solve this problem a pneumatically assisted Rheodyne 7010 valve was introduced between the column and the flow divisor. The valve allowed us to expulse the eluate from the injection time to the complete elution of the urea (~5 min).

RESULTS AND DISCUSSION

The measured relative molecular masses of major caprine proteins from several determinations obtained by FIA-ESI-MS and by HPLC-ESI-MS are shown in Table 1.

In Figure 1 we can see the chromatographic patterns obtained from a skimmed milk sample (HPLC method 1) and its whole casein (HPLC method 2).

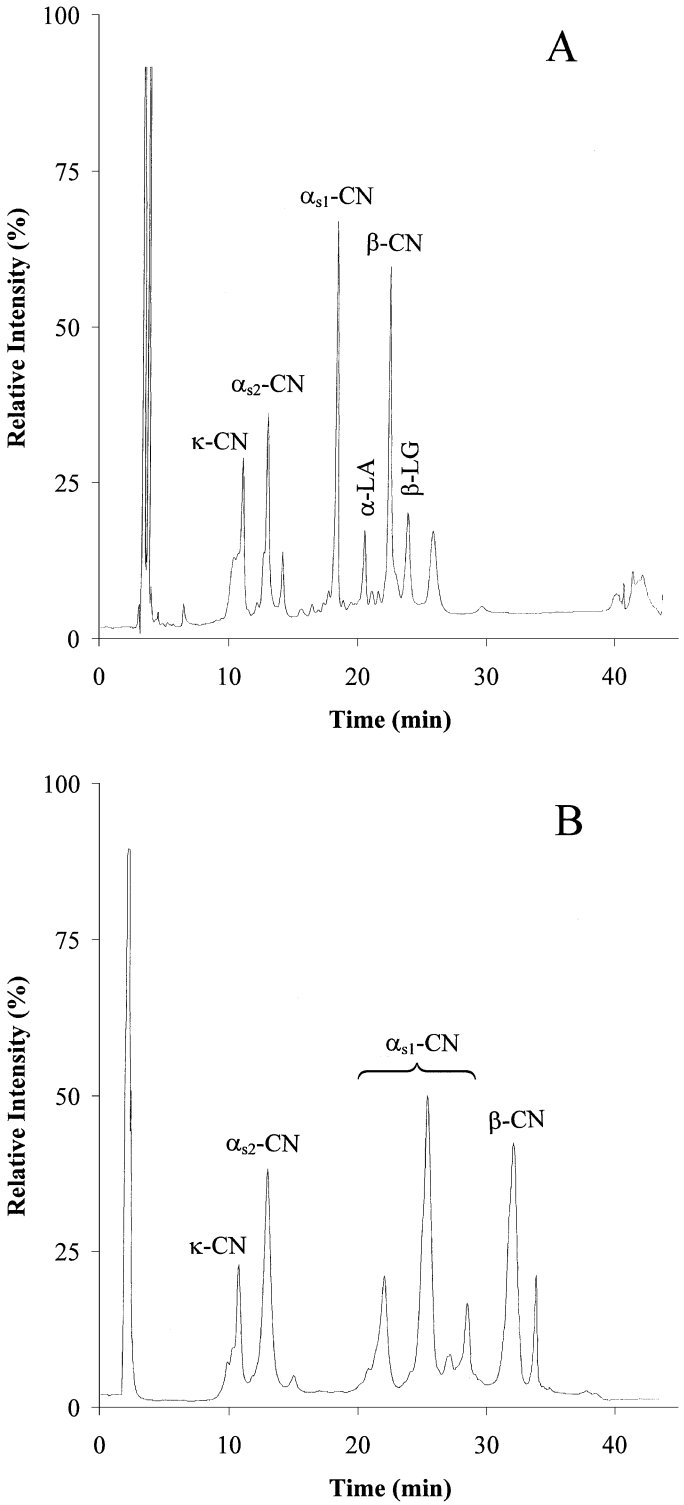

Figure 1. Chromatographic patterns of skimmed milk (A) and whole casein (B) obtained from caprine bulk milk sample by the HPLC method 1 and 2 (see reverse- phase HPLC separation section), respectively.

κ-CN

The primary structure of caprine κ-CN has been determined (30), and it has been recently confirmed by the corresponding sequences of the cDNA (10) except for the AA in position 113 (Asp→Asn). This protein contains 171 AA (two more than the bovine), two phosphate residues, and it has 84% homology with its bovine counterpart (variant A).

Figure 2 shows the ESI-MS spectrum of isolated κ-CN, which corresponds to the nonglycosylated form of κ-CN 2P (M_r 19,302.03 ± 3.25). The relative molecular mass is consistent with that expected from the known sequence (M_r 19,306).

In both chromatographic methods (Figure 1) κ-CN was composed of a series of minor peaks and a major peak. It appears that caprine κ-CN, like cow, buffalo, and ewe κ-CN, is composed of several fractions that have identical peptide chains and different carbohydrate content (2). These glycosylated forms are eluted just before the major chromagraphic peak (25). However, these forms that are visible in HPLC, were not detected by ESI-MS under the conditions used, possibly because of either heterogeneity or because of the low concentrations present in the sample or failure to ionize them. The major chromatographic peak had a relative molecular mass identical to those obtained from isolated caprine κ-CN.

The occurence of polymorphism in κ-CN has been demonstrated by isoelectrofocusing (12), reverse-phase HPLC (21), and FPLC (24) but not by genetic sequencing. From the analyzed samples, only two samples with M_r 19,375.53 ± 3.16 and 19,378.19 ± 2.84, respectively, differed considerably from the relative molecular mass found for κ-CN. These results may be connected with the occurence of polymorphism.

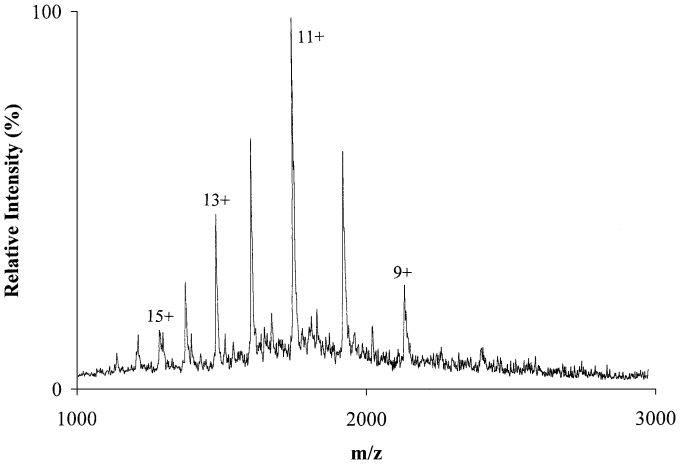

Figure 2. Electrospray mass spectrum of isolated caprine κ-CN. m/z = Mass to charge ratio.

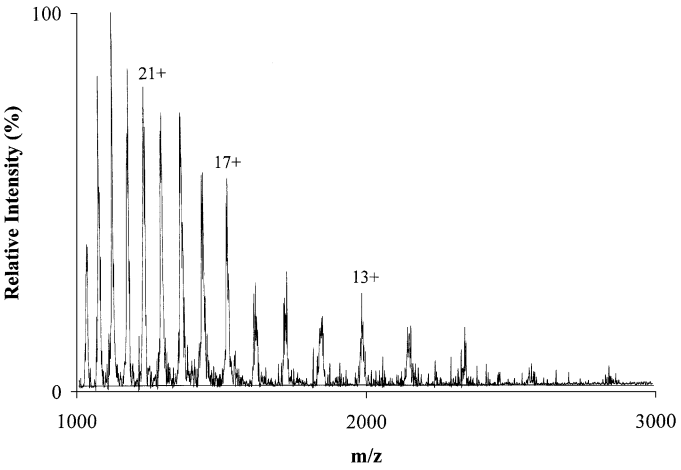

Figure 3. Electrospray mass spectrum of isolated caprine α_{s2}-CN A. m/z = Mass to charge ratio.

α_{s2}-CN

α_{s2}-Casein is the most phosphorylated casein, with up to 13 phosphate residues. This heterogeneity and the presence of polymorphism leads to a very complex ESI mass spectra. The cDNA and protein have been sequenced (3, 4), showing that it contains 288 AA (one AA more than the bovine counterpart) and a homology of 88% between these two types. α_{s2}-Casein exists as three allelic forms, A, B, and C (4, 5). α_{s2}-Casein B differs from its predominant counterpart by an AA substitution Ser-Ala-Lys(B)/$SerP_{62}$-Ala-Glu_{64}(A). The lack of a phosphate group on Ser_{62} in variant B can be explained by the Lys/Glu replacement, which affects the Glu determinant in the tripeptide phosphorylation recognition site (4). α_{s2}-Casein C differs from α_{s2}-CN A by the AA substitution Lys_{167} (A)→Ile_{167} (C).

The main components of isolated α_{s2}-CN A detected by ESI-MS (Figure 3) had M_r values of 25,597.90 ± 2.52, 25,673.07 ± 7.70, and 25,758.49 ± 5.56. The mass difference between the diferent α_{s2}-CN components can be accounted for by the presence of a different number of phosphate residues in the same protein. These relative molecular masses correspond to the calculated mass from the sequences of α_{s2}-CN A 11P (M_r 25,599), 12P (M_r 25,679), and 13P (M_r 25,759), respectively.

The cDNA sequence of α_{s2}-CN A (3) indicates that residue number 7 should be Val rather than Ile found in the protein sequence (4). The ESI-MS analysis of α_{s2}-CN A appears to confirm the cDNA sequence (difference in mass of 14) and, thus, the presence of Val at position 7.

Similar relative molecular masses were obtained for α_{s2}-CN by on-line HPLC-ESI-MS. Both HPLC methods were able to discern A and B variants of α_{s2}-CN when

milk from individual goats heterozygous (AB) at the α_{s2}-CN locus was analyzed. Chromatographic patterns (HPLC method 1) of a casein sample from individual goat heterozygous (AB) at the α_{s2}-CN locus is presented in Figure 4. The two main components found in the first peak of α_{s2}-CN (variant B) had M_r values of 25,437.17 ± 10.15 and 25,513.29 ± 4.82, which correspond to calculated mass from the sequences of α_{s2}-CN B 9P (M_r 25,438) and 10 P (M_r 25,518), respectively. α_{s2}-Casein C was not observed in any of the samples analyzed.

α_{s1}-CN

The caprine α_{s1}-CN B contains 199 AA like its bovine counterpart and shows 80% similarity. This casein varies in the degree of phosphorylation (between 8 and 10), which in part explains the origin of the different bands observed in electrophoresis at alkaline pH. The great heterogeneity observed in caprine α_{s1}-CN by electrophoresis cannot be accounted for only by the different levels of phosphorylation. The nature of this heterogeneity has been investigated by Ferranti et al. (14) through the reexamination of the primary structure of variants A, B, and C already reported by Brignon et al. (6). Mature caprine α_{s1}-CN exists as a mixture of at least four molecular species, which differ in peptide chain length. The main component corresponds to the 199-residue form. The other three are shorter forms of casein and differ by the deleted peptides 141-148, 100-117 or change in Gln_{78}. Moreover, the main form accounts for about 80%, whereas the internally deleted forms represent only about 20%.

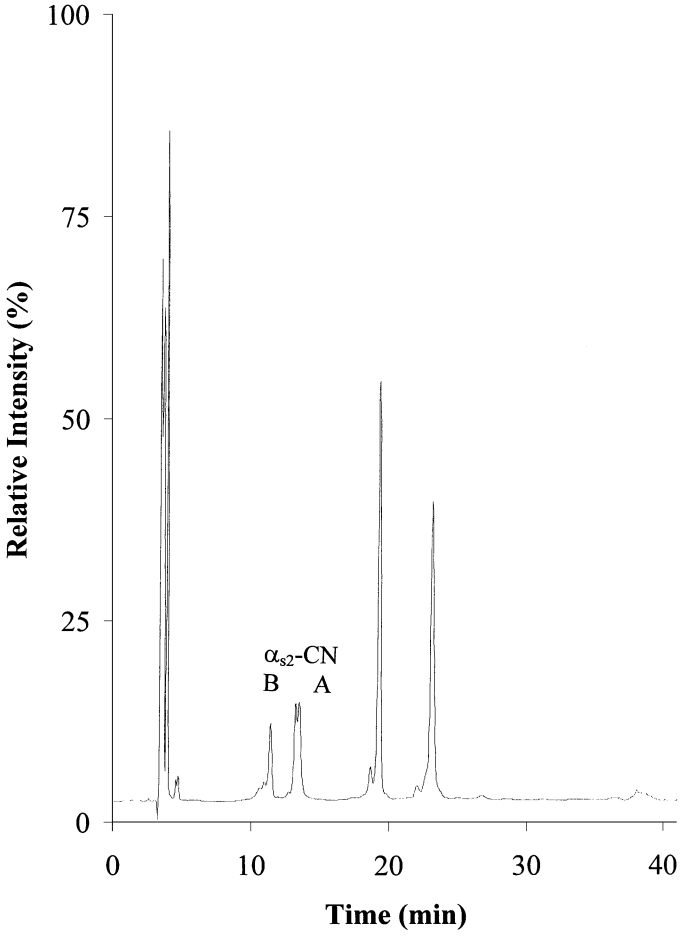

Figure 4. Chromatographic pattern (HPLC method 1) of a casein sample from individual goat heterozygous (AB) at the α_{s2}-CN locus.

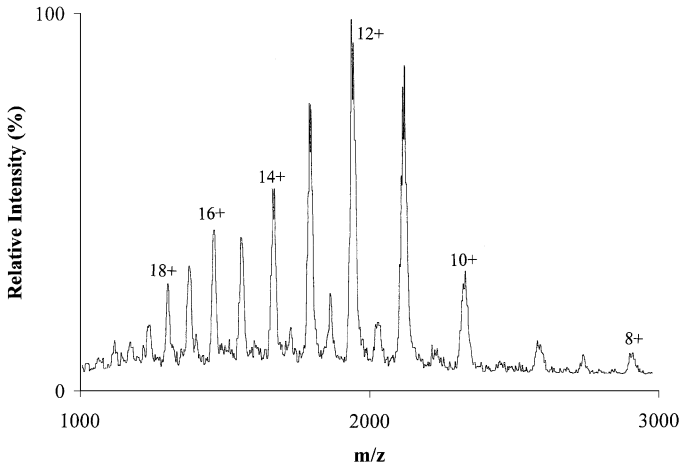

Figure 5. Electrospray mass spectrum of isolated caprine α_{s1}-CN B. m/z = Mass to charge ratio.

The relative molecular mass found for the isolated α_{s1}-CN B agreed with that from the protein sequence (6). The ESI mass spectrum from α_{s1}-CN B is showed in Figure 5. The main components found for this casein had M_r values of 23,345.15 ± 4.45, 23,422.05 ± 4.41, and 23,508.58 ± 7.26 which correspond to the calculated mass from the sequences of α_{s1}-CN B 8P (M_r 23,347), 9P (M_r 23,427), and 10P (M_r 23,507), respectively, from the 199-residues chain or main component described by Ferranti et al. (14). However, other components were observed too; these components had M_r values of 23,229.21 ± 7.68, 23,301.98 ± 7.79, and 23,376.34 ± 9.38, which correspond to the calculated mass from the sequences of the 198-residues form of α_{s1}-CN B 8P (M_r 23,219), 9P (M_r 23,299), and 10P (M_r 23,379). No other forms were observed in the ESI-MS conditions used.

The α_{s1}-CN variants A, B, E, and F, the variants most normally found in many European dairy goats, were examined by coupling HPLC and ESI-MS (both methods). The α_{s1}-CN A variant had components with M_r values of 23,295.18 ± 4.15 and 23,371.17 ± 3.62, which correspond to the calculated mass from the sequences of α_{s1}-CN A 7P (M_r 23,282) and α_{s1}-CN A 8P (M_r 23,362).

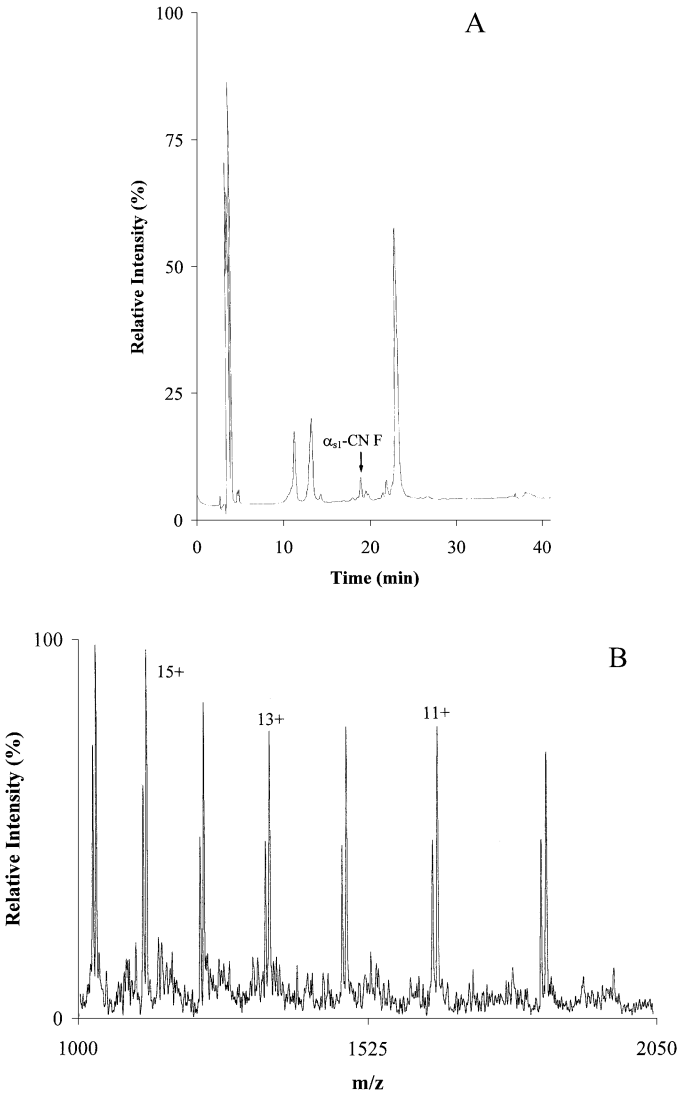

Figure 6. Chromatographic pattern obtained by HPLC method 2 (A) and electrospray mass spectrum of α_{s1}-CN F (B) of a casein sample from an individual goat homozygous (FF) at the α_{s1}-CN locus.

Variant B showed a relative molecular mass identical to the isolated protein.

The principal components obtained from α_{s1}-CN E had M_r values of 23,264.42 ± 7.46 and 23,349.97 ± 5.84, which agree with the calculated mass from the cDNA sequence of α_{s1}-CN E 8P (M_r 23,259) and α_{s1}-CN E 9P (M_r 23,339) elucidated by Jansà et al. (20).

Figure 6 shows the chromatographic pattern (A) and ESI mass spectrum (B) from an individual goat homozygous (FF) at the α_{s1}-CN locus. This casein had two different components with M_r values of 18,736.98 ± 1.63 and 18,817.46 ± 1.80, which correspond to the phosphorylated forms 2 and 3 of α_{s1}-CN F, respectively, confirming the sequence data reported by Brignon et al. (7) for α_{s1}-CN F 2P (M_r 18,736) and α_{s1}-CN F 3P (M_r 18,816). Variant F is characterized by 59 to 95 AA sequence deletion, causing the loss of the hydrophilic cluster of six continuous phosphoseryl residues. This shortened protein is not due to genomic deletion but to mutations inducing incorrect splicing with loss of exons during premessenger RNA processing, which in turn produces multiple forms of α_{s1}-CN F messenger RNA (26). Although it has to be determined if these transcripts are translated into proteins of different lengths, it appears that some of them might be translated (37). A chromatogram of α_{s1}-CN F (Figure 6A) showed other minor peaks in the chromatographic area of α_{s1}-CN, but none of them were detected under the ESI-MS conditions.

When the HPLC method 2 was used to analyze caseins, α_{s1}-CN variants could be identified from individual goats homozygous or heterozygous at the α_{s1}-CN locus (21). Coupling HPLC (method 2) and ESI-MS allowed us to determine with confidence the α_{s1}-CN genotype of individual casein samples and to determine the main α_{s1}-CN variants in a sample of a bulk milk. However, HPLC (method 1) does not appear to be fully satisfactory for the resolution of milk samples heterozygous at the α_{s1}-CN locus.

β-CN

Caprine β-CN consists of two components of similar molecular weights and two phosphorylation levels (5 and 6), it exhibits 207 AA, two fewer than its bovine counterpart, due to the deletion of the dipeptide Pro_{179}-Tyr_{180} and there is a homology between both of 90% (bovine variant A[2]) (33, 34).

Isolated β-CN A analyzed by ESI-MS presented two different components (M_r values of 23,756.44 ± 5.15 and 23,834.53 ± 5.65). The mass difference between the β-CN components can be accounted for by the presence of a different number of phosphate residues (5 and 6). The relative molecular masses found by ESI-MS (Figure 7) agree with those obtained by Chianese et al. (9) from isolated β-CN by ESI-MS. However, the observed relative molecular masses were a little larger than those of the theoretical values calculated from the primary structure sequence of the gene (M_r 23,741 and 23,821) elucidated by Roberts et al. (34). The primary structure of caprine β-CN has been determined for its cDNA precursor but not for the protein. The study of the primary structure of caprine β-CN could explain the differences in mass between the relative molecular masses obtained by ESI-MS and those obtained from gene sequencing.

Coupled HPLC-ESI-MS (both chromatographic methods) gave satisfactory results in analyzing β-CN,

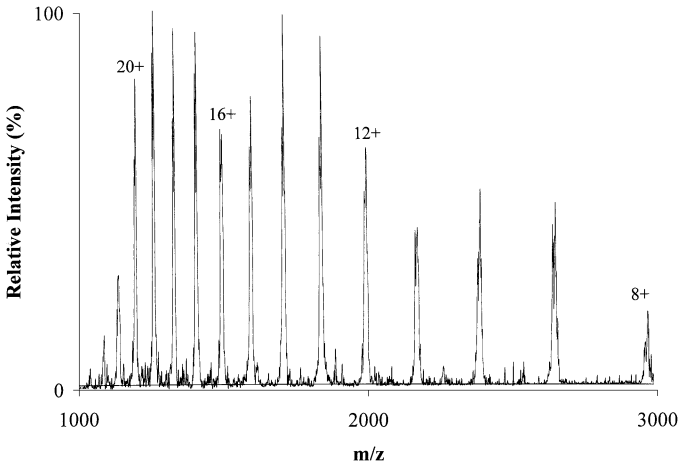

Figure 7. Electrospray mass spectrum of isolated caprine β-CN. m/z = Mass to charge ratio.

which showed identical relative molecular masses to those obtained from the isolated protein.

The caprine β-CN had been considered to be monomorphic for a long time until an analysis of goat milk of the Garganica breed revealed the existence of a probable null allele (11) confirmed later in other dairy breeds (29). In addition, these authors have described a new variant (β-CN B) with a high electrophoretic mobility with respect to variant A equivalent to the charge of one phosphate group. Figure 8 shows the HPLC pattern (method 1) of an individual milk sample with low β-CN content which was detected by electrophoresis (urea-PAGE) at pH 8.9 (results not shown), and in addition the HPLC pattern of a whey milk sample. From the chromatograms we can see a peak between α-LA and β-LG which had similar retention times to β-CN. The HPLC-ESI-MS analysis revealed a relative molecular masses closer to β-CN A 5P and 6P (M_r values of 23,755.42 ± 4.58 and 23,831.93 ± 4.00). These data indicate that the milk sample could belong to a goat heterozygous (A0) at the β-CN locus with very low content of β-CN in milk.

From the milks analyzed, a sample with altered relative molecular mass appeared for the β-CN 5P and 6P components with respect to the homologous components in the other samples. The relative molecular masses obtained for these components were 23,782.59 ± 8.01 and 23,865.89 ± 7.76, respectively. These results may be connected to those of Mahé and Grosclaude (29), who first detected, by electrophoretic techniques, the presence of a novel variant (β-CN B) in Creole goat breed.

β-LG

The major whey proteins in caprine milk are β-LG and α-LA and the minor fractions SA, immunoglobulins, and enzymes. The complete β-LG sequence (31) that was confirmed by its genomic sequence (16) is well known.

Like its bovine counterpart, caprine β-LG has 162 AA and has similar physical and chemical characteristics. Figure 9 shows the ESI mass spectrum of isolated β-LG. From the spectrum it is clear that β-LG exhibits heterogeneity with two different components. The main component had a M_r 18,180.14 ± 0.63, which corresponds to that obtained from the protein sequence (M_r 18,185) and the minor component had a M_r 18,504.02 ± 2.42. The observed difference in mass of 324 between components is consistent with the covalent linkage of a lactosyl residue to the protein. Coupled HPLC (method 1) and ESI-MS were efficient in separating and detecting β-LG.

Macha (28) described genetic variability for this protein, finding two visible variants through electrophoretic techniques. However, we have not observed other heterogeneity than the lactosylation in the samples analyzed.

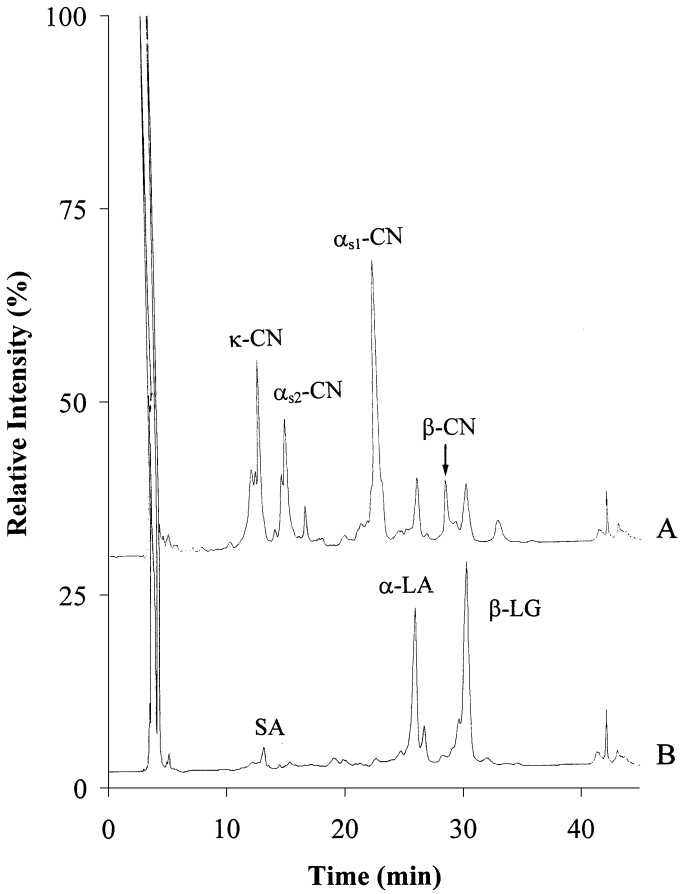

Figure 8. Chromatographic patterns (HPLC method 1) of an individual milk sample with low β-CN content (A) and a whey milk sample (B).

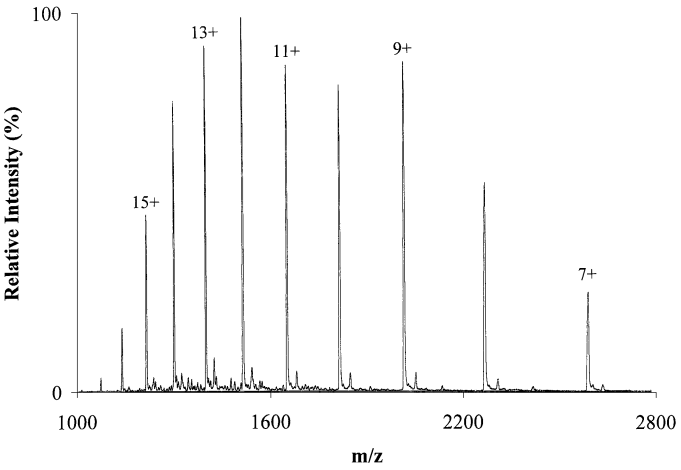

Figure 9. Electrospray mass spectrum of isolated caprine β-LG. m/z = Mass to charge ratio.

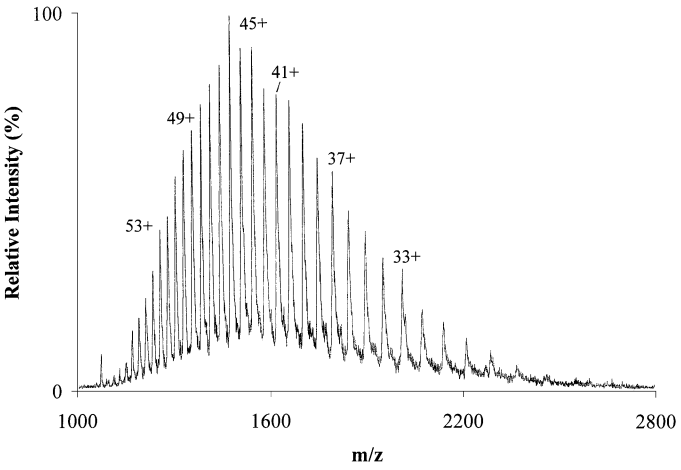

Figure 11. Electrospray mass spectrum of isolated caprine serum albumin. m/z = Mass to charge ratio.

α-LA

The sequence of the α-LA was determined by McGillivray et al. (27), corrected by Shewale et al. (35), and finally confirmed by its genomic sequence (23).

Figure 10 shows the ESI mass spectrum of purified α-LA. Like β-LG, this protein presented heterogeneity. The main component had a M_r 14,179.43 ± 5.82, which corresponds to the protein sequence (M_r 14,186) and the minor component had a M_r consistent with the presence of a lactosyl residue linked with α-LA (M_r 14,509.07 ± 12.27). However, it was not possible to detect α-LA from skim milk samples by on-line HPLC-ESI-MS in the conditions used, perhaps due to the low concentration found in milk.

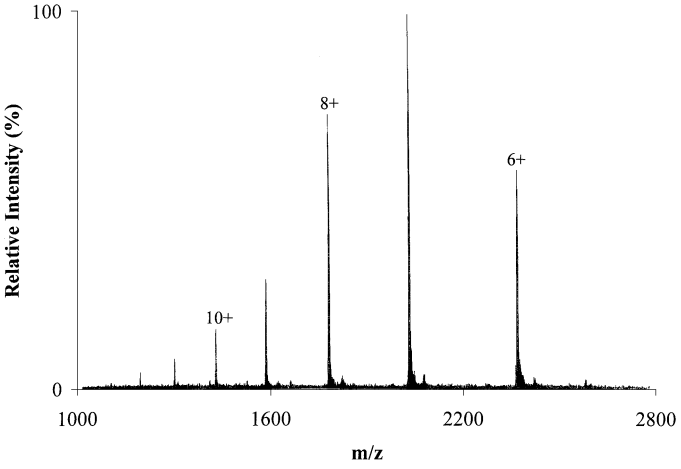

Figure 10. Electrospray mass spectrum of isolated caprine α-LA. m/z = Mass to charge ratio.

Serum Albumin

To our knowledge, no information on the protein or gene sequences of caprine SA is available. ESI mass spectrum of purified SA (Figure 11) gave a M_r 66,318.45 ± 30.52. This protein was not detected in milk by HPLC-ESI-MS (method 1) possibly due to the partial overlapping with κ-CN (Figure 8) and to the low concentration present in milk.

We were also unable to detect immunoglobulins from the purified fraction obtained by gel permeation FPLC neither by FIA-ESI-MS nor by HPLC-ESI-MS in the electrospray conditions used.

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REFERENCES

1 Addeo, F., R. Mauriello, and A. Di Luccia. 1988. A gel electrophoretic study of caprine casein. J. Dairy Res. 55:413–421.
2 Addeo, F., S. Soulier, J.-P. Pélissier, J.-M. Chobert, J.-C. Mercier, and B. Ribadeau-Dumas. 1978. Preparation and fractionation of goat κ-casein: analysis of the glycan and peptide components. J. Dairy Res. 45:191–196.
3 Bouniol, C. 1993. Sequence of the goat α_{s2}-casein-encoding cDNA. Gene 125:235–236.
4 Bouniol, C., G. Brignon, M.F. Mahé, and C Printz. 1993. Characterization of goat allelic α_{s2}-caseins A and B: further evidence of the phosphorylation code of caseins. Protein Seq. Data Anal. 5:213–218.
5 Bouniol, C., G. Brignon, M. F. Mahé, and C Printz. 1994. Biochemical and genetic analysis of variant C of caprine α_{s2}-casein (*Capra hircus*). Anim. Genet. 25:173–177.
6 Brignon, G., M. F. Mahé, F. Grosclaude, and B. Ribadeau-Dumas. 1989. Sequence of caprine α_{s1}-casein and characterization of those of its genetic variants which are synthesized at a high level, α_{s1}-CnA, B and C. Protein Seq. Data Anal. 2:181–188.

7 Brignon, G., M. F. Mahé, B. Ribadeau-Dumas, J.-C. Mercier, and F. Grosclaude. 1990. Two of the three genetic variants of goat α_{s1}-casein which are synthesized at a reduced level have an internal deletion possibly due to altered RNA splicing. Eur. J. Biochem. 193:237–241.
8 Burr, R., C. H. Moore, and J. P. Hill. 1996. Evidence of multiple glycosylation of bovine β-lactoglobulin by electrospray ionisation mass spectrometry. Milchwissenschaft 51:488–492.
9 Chianese, L., G. Garro, M. A. Nicolai, R. Mauriello, P. Ferranti, R. Pizzano, U. Cappuccio, P. Laezza, F. Addeo, L. Ramuno, A. Rando, and R. Rubino. 1993. The nature of β-casein heterogeneity in caprine milk. Lait 73:533–547.
10 Coll, A., J. M. Folch, and A. Sánchez. 1993. Nucleotide sequence of the goat κ-casein cDNA. J. Anim. Sci. 71:2833.
11 Dall'Olio, S., R. Davoli, and V. Russo. 1989. Una nuova variante di β-caseina caprina. Scienza e Tecnica Lattiero-Casearia 40:24–28.
12 Di Luccia, A., R. Mauriello, L. Chianese, L. Moio, and F. Addeo. 1990. κ-Casein polymorphism in caprine milk. Scienza e Tecnica Lattiero-Casearia 41:305–314.
13 Felipe, X., and A.J.R. Law. 1997. Preparative-scale fractionation of bovine, caprine and ovine whey proteins by gel permeation chromatography. J. Dairy Res. 64:459–464.
14 Ferranti, P., F. Addeo, A. Malorni, L. Chianese, C. Leroux, and P. Martin. 1997. Differential splicing of pre-messenger RNA produces multiple forms of mature caprine α_{s1}-casein. Eur. J. Biochem. 249:1–7.
15 Ferranti, P., A. Malorni , G. Nitti, P. Laezza, R. Pizzano, L. Chianese, and F. Addeo. 1995. Primary structure of ovine α_{s1}-casein: localization of phosphorylation sites and characterization of genetic variants A, C and D. J. Dairy Res. 62:281–296.
16 Folch, J. M., A. Coll, and A. Sánchez. 1994. Complete sequence of the caprine β-lactoglobulin gene. J. Dairy Sci. 77:3493–3497.
17 Grosclaude, F., M. F. Mahé, G. Brignon, L. Di Stasio, and R. Jeunet. 1987. A Mendelian polymorphism underlying quantitative variations of goat α_{s1}-casein. Génét. Sél. Evol. 19:399–412.
18 Grosclaude, F., G. Ricordeau, P. Martin, F. Remeuf, L. Vassal, and J. Bouillon. 1994. Du gène au fromage: le polymorphisme de la caséine α_{s1} caprine, ses effets, son évolution. INRA Prod. Anim. 7:3–19.
19 Hollar, C. M., A.J.R. Law, D. G. Dalgleish, and R. J. Brown. 1991. Separation of major casein fractions using cation-exchange fast protein liquid chromatography. J. Dairy Sci. 74:2403–2409.
20 Jansà, M., C. Leroux, A. Sánchez, and P. Martin. 1994. Occurence of a LINE sequence in the 3′ UTR of the goat α_{s1}-casein E-enconding allele associated with reduced protein synthesis level. Gene 147:179–187.
21 Jaubert, A., and P. Martin. 1992. Reverse-phase HPLC analysis of goat caseins. Identification of α_{s1} and α_{s2} genetic variants. Lait 72:235–247.
22 Jordana, J., M. Amills, E. Díaz, C. Angulo, J. M. Serradilla, and A. Sànchez. 1996. Gene frequencies of caprine α_{s1}-casein polymporphism in Spanish goat breeds. Small Ruminant Res. 20:215–221.
23 Kumagai, I., E. Tamaki, S. Kakinuma, and K. Miura. 1987. Molecular cloning and sequencing of cDNA enconding goat pre α-lactalbumin. J. Biochem. 101:511–517.
24 Law, A.J.R., and A. Tziboula. 1993. Fractionation of caprine κ-casein and examination of polymorphism by FPLC. Milchwissenschaft 48:68–71.
25 Léonil, J., D. Mollé, F. Gaucheron, P. Arpino, P. Guénot, and J. L. Maubois. 1995. Analysis of major bovine milk proteins by online high-performance liquid chromatography and electrospray ionization-mass spectrometry. Lait 75:193–210.
26 Leroux C., N. Mazure, and P. Martin. 1992. Mutations away from splice site recognition sequences might cis-modulate alternative splicing of goat α_{s1}-casein transcripts. J. Biol. Chem. 267:6147–6157.
27 MacGillivray, R. T., K. Brew, and K. Barnes. 1979. The amino acid sequence of goat α-lactalbumin. Arch. Biochem. Biophys. 197:404–414.
28 Macha, J. 1970. Protein polymorphism in goat's milk. Zivocisna Vyroba 15:801–805.
29 Mahé, M. F., and F. Grosclaude. 1993. Polymorphism of β-casein in the Creole goat of guadeloupe: evidence for a null allele. Génét. Sél. Evol. 25:403–408.
30 Mercier, J.-C., F. Addeo, and J.-P. Pélissier. 1976. Structure primaire du caséinomacropeptide de la caseine κ caprine. Biochimie 58:1303–1310.
31 Préaux, G., G. Braunitzer, B. Schrank, and A. Stangl. 1979. The amino acid sequence of goat β-lactoglobulin. Hoppe-Seyler's Physiol. Chem. 360:1595–1604.
32 Recio, I., M. L. Pérez-Rodríguez, L. Amigo, and M. Ramos. 1997. Study of the polymorphism of caprine milk caseins by capillary electrophoresis. J. Dairy Res. 64:515–523.
33 Richardson, B. C., and L. K. Creamer. 1974. Comparative micelle structure. III. The isolation and chemical characterization of caprine β_1-casein and β_2-casein. Biochim. Biophys. Acta 365:133–137.
34 Roberts, B., P. Di Tullio, J. Vitale, K. Hehir, and K. Gordon. 1992. Cloning of the goat β-casein-encoding gene and expression in transgenic mice. Gene 121:255–262.
35 Shewale, J. G., S. K. Sinha, and K. Brew. 1984. Evolution of α-lactalbumins. The complete amino acid sequence of the α-lactalbumin from a marsupial (*Macropus rufogriseus*) and corrections to regions of sequence in bovine and goat α-lactalbumins. J. Biol. Chem. 259:4947–4956.
36 Trujillo, A. J., J. Jordana, B. Guamis, J. M. Serradilla, and M. Amills. 1998. Review: Polymorphism of the caprine α_{s1}-casein gene and its effect on the production, composition and technological properties of milk and on cheese making and ripening. Food Sci. Technol. Int. 4:217–235.
37 Trujillo, A. J., G. Miranda, D. LeBars, and A. Delacroix-Buchet. 1998. Proteolytic specificity of chymosin on caprine α_{s1}-caseins A and F. J. Dairy Res. 65:233–241.
38 Visser, S., C. J. Slangen, F. M. Lagerwerf, W. D. Van Dongen, and J. Haverkamp. 1995. Idenfication of a new genetic variant of bovine β-casein using reversed-phase high-performance liquid chromatography and mass spectrometric analysis. J. Chromatogr. A 711:141–150.
39 Visser, S., Ch. J. Slangen, and H. S. Rollema. 1991. Phenotyping of bovine milk proteins by reversed-phase high-performance liquid chromatography. J. Chromatogr. 548:361–370.