

Quantification of α_{s1} -Casein in Goat Milk from French-Alpine and Anglo-Nubian Breeds Using Reversed-Phase High Performance Liquid Chromatography

A. MORA-GUTIERREZ
Cooperative Agricultural Research Center
Prairie View A&M University
Prairie View, TX 77448

T. F. KUMOSINSKI and H. M. FARRELL, JR.¹
Eastern Regional Research Center
Agricultural Research Service, USDA
600 East Mermaid Lane
Philadelphia, PA 19118

ABSTRACT

Samples of isoelectrically precipitated goat casein from the milks of French-Alpine and Anglo-Nubian breeds were separated into four components in a single run by reversed-phase HPLC. The proportion of α_{s1} -casein thus resolved was determined quantitatively. The method uses a reversed-phase C-4 column and a linear gradient from 30 to 50% acetonitrile in 30 min with trifluoroacetic acid constant at .1%. Sodium dodecyl sulfate-PAGE was carried out to establish the identity of the isolated components. By a comparison with previously published results for caprine and bovine milk caseins, the four peaks were identified as κ -, α_{s2} -, α_{s1} -, and β -casein.

Quantitative variations in the chromatographically resolved α_{s1} -casein fraction of goat milk were evident. Some individual goat milks contained high levels of α_{s1} -casein (2.70 g/L), but others contained significantly low levels (.12 g/L). There was no statistical difference in the overall means between breeds in α_{s1} -casein composition, but cluster analysis statistics showed three distinct categories of α_{s1} -producers: high, medium, and low. Interestingly, 6 of 15 French-Alpine goats and only one Anglo-Nubian goat

fell into the "low" producer category (.38 \pm .2 g/L). Thus, expression of the α_{s1} -component may be genetically regulated but may not be a breed-specific trait. (Key words: reversed-phase, high performance liquid chromatography, casein, caprine)

Abbreviation key: RP = reversed-phase, TFA = trifluoroacetic acid.

INTRODUCTION

It has generally been accepted that the level of α_{s1} -casein, which is the major protein in cow milk, appears to be low or nonexistent in goat milk (2, 4). Such compositional differences probably contribute to the lower heat stability of goat milk and other distinctive properties compared with those of cow milk (1, 6, 9, 14).

Understanding the behavior of the milk proteins during processing has been facilitated by techniques that enable the proteins in milk to be identified, quantitatively analyzed, and fractionated into pure components. In this context, HPLC gives rapid resolution of very complex protein mixtures and has become a valuable method in the analysis of whole casein. Various reversed-phase (RP) media have found successful application in this regard (3, 12, 17) and have yielded results that are more rapid and accurate than those derived from DEAE ion-exchange chromatography (5, 15).

Our research on goat milk proteins afforded the opportunity to examine the efficacy of RP HPLC in monitoring the different components of goat milk casein. Work centered on the goat

Received December 26, 1990.

Accepted March 7, 1991.

¹To whom correspondence should be addressed.

milk α_{s1} -casein fraction, which was previously reported by Boulanger et al. (2) and was shown to be a homologue of α_{s1} -casein from cow milk. The RP HPLC was used for the identification and quantification of α_{s1} -casein in milks from French-Alpine and Anglo-Nubian breeds during the same stage of lactation and could serve as a powerful analytical tool to establish changes in caseins rapidly when investigating both functional and physicochemical properties during milk processing.

MATERIALS AND METHODS

Reagents

Chemicals and solvents were analytical reagent or HPLC grade.

Sampling

Individual goat milk samples from French-Alpine and Anglo-Nubian breeds were collected from the goat herd of the International Dairy Goat Research Center, Prairie View A&M University, Prairie View, TX. All goats were in midlactation.

Sources of Caseins

Whole casein was isoelectrically precipitated from 1 L of fresh raw skim milk. The precipitate was dissolved by addition of NaOH to yield a solution of pH 7.0. The casein was reprecipitated, washed, and then resuspended in water at pH 7.0. The sodium caseinate was subsequently cooled to 4°C, centrifuged at 10,000 × *g* for 30 min, freeze-dried, and recovery determined by weight recorded.

Purified α_{s1} -casein was obtained as described (15, 16). Crude α_{s1} -casein was prepared by urea fractionation (16) followed by column chromatography on DEAE cellulose in urea (15).

Instrumentation and Chromatographic Conditions

Chromatography was performed on a C-4 column with a Waters Division of Millipore

HPLC (Milford, MA)² with WISP 712 automatic sample injector, Model 510 pump, and system interface data module; column, 3.9 × 300 mm, butyl, substituted-RP, C-4, 15- μ particle size, 100 Å pore size, Waters (Milford, MA); mobile phase, solvent A: .1% trifluoroacetic acid (TFA), solvent B: acetonitrile, .1% TFA; gradient, 30 to 75% B, 40 min; flow rate, 1.0 ml/min; detector gain, .005 absorbance units full scale. A sample 10 mg of casein were dissolved in 1 ml of starting solvent (70% water:30% acetonitrile with .1% TFA, containing 3.3 *M* urea), then passed through a .2- μ m pore size filter, and 40 μ l injected onto the column. The eluant absorbance was continuously monitored at 280 nm with a Waters UV-481 variable wavelength detector. Volatile solvents were removed from collected fractions with an N₂ stream at 40°C in preparation for electrophoresis.

Gel Electrophoresis

The SDS-PAGE of proteins collected from the analytical column was carried out. The method of Laemmli (10) was used with 16% gels containing 1% SDS. Electrophoresis was conducted with an Ephortec vertical slab gel electrophoresis unit (Haake Buchler Instruments, Saddle Brook, NJ). Electrophoresed gels were fixed, stained, and destained as described (10).

Quantification of Goat Milk α_{s1} -Casein

The HPLC peak corresponding to α_{s1} -casein was identified by using retention time comparison with chromatographically purified α_{s1} -casein from goat milk. When this was done, standard and sample peaks for α_{s1} -casein were perfectly superimposed, confirming sample peak identification. Quantification of α_{s1} -casein for each individual milk sample was made by measurement of the peak areas with reference to standard of known concentration. Range of linearity of standards was .1 to 1.0 mg of proteins (400- μ g average sample).

Statistical Analysis of the Distributions of α_{s1} -Casein Contents

Statistical analyses of the distribution of values for α_{s1} -casein content were carried out using three forms of cluster analysis included

²Reference to firm or brand name does not constitute endorsement by USDA over other products not mentioned.

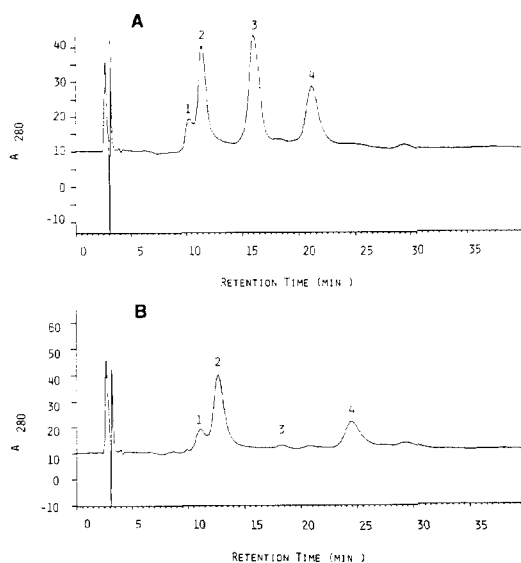


Figure 1. Reversed-phase HPLC elution profile from French-Alpine goat milk high (A) or low (B) in α_{s1} -casein. Peak 1, κ -casein; peak 2, α_{s2} -casein; peak 3, α_{s1} -casein; peak 4, β -casein. Absorbance (A) at 280 nm.

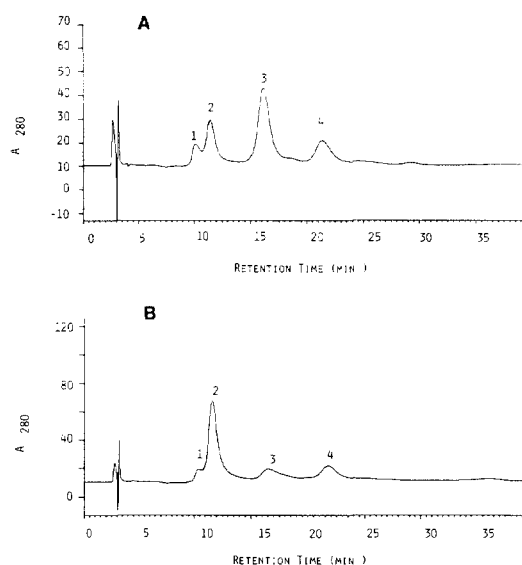


Figure 2. Reversed-phase HPLC elution profile from Anglo-Nubian goat milk high (A) or low (B) in α_{s1} -casein. Peak 1, κ -casein; peak 2, α_{s2} -casein; peak 3, α_{s1} -casein; peak 4, β -casein. Absorbance (A) at 280 nm.

in Proc-Cluster of the SAS/STAT module of SAS (SAS Institute, Cary, NC). The three forms of analysis used were average linkage cluster analysis, centroid hierarchical cluster analysis, and Wards' minimum variance cluster analysis. Each type of analysis yielded a similar result; the overall division of the population (for the 25 samples in this study) is not significantly improved beyond the cluster number of 3, and the cubic clustering criterion reaches a maximum at three groupings with values of .71 for all three methods.

RESULTS AND DISCUSSION

The elution pattern obtained for isoelectrically precipitated goat milk casein from French-Alpine and Anglo-Nubian breeds showed four major peaks (Figures 1 and 2). In order to identify the different components of goat milk casein and determine their purity, each protein fraction was analyzed by SDS-PAGE (Figure 3). The electrophoretic patterns enabled all fractions to be identified readily. As expected, the α_{s1} -casein band was very weak in caseins low in that component and considerably more prominent in goat milk caseins with higher contents (as indicated by

RP HPLC). The position of the α_{s1} -casein component was also confirmed by separately running the purified component on the column under identical conditions (Figure 4). The retention time and the integrated detector response were reproducible for both the individual α_{s1} -casein and casein mixtures.

The profiles shown in Figures 1 and 2 were common to all samples analyzed. The patterns are similar to those obtained by Mikkelsen et al. (12) but are considerably more resolved. This might be attributed to the use of an ultra-short C_4 chain length packing under the given experimental conditions. The butyl C_4 system used in the present study failed to separate γ -casein, which is usually found in low amounts in milk from most species. γ -Casein is absent in rare cases from cow milk homozygous for β -casein C; this is because γ -caseins are "proteolytic fragments" of β -caseins as determined from the primary structure (8). No identification of a goat γ -casein has been made by sequence analysis so that either this protein is absent or it is not resolved from β -casein in our system.

Although SDS-PAGE showed little difference between the α_{s1} -casein bands of the whole goat casein samples, HPLC results (data not shown) suggested that the slightly different

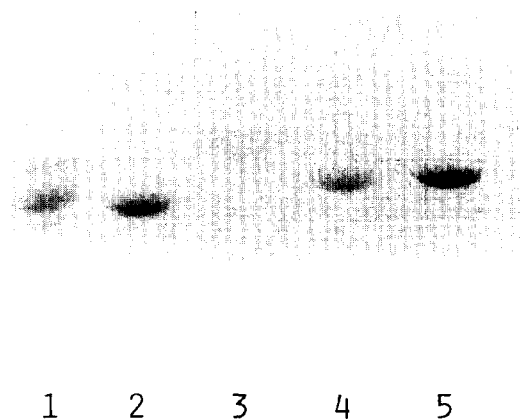


Figure 3. The SDS-PAGE of peaks 1, 2, 3, and 4 from the reversed-phase HPLC elution profile Figure 1A. Lane 1, whole goat milk casein; lane 2, peak 1, κ -casein; lane 3, peak 2, α_{s2} -casein; lane 4, peak 3, α_{s1} -casein; lane 5, peak 4, β -casein. Absorbance (A) at 280 nm.

elution rates and shapes of the α_{s1} -casein peaks were caused by different degrees of phosphorylation or by polymorphism. Other recently reported HPLC data on caseins are consistent with these observations (3, 17).

Although distinct breed differences in the relative amounts of α_{s1} -casein were not observed in the present investigation, the results for the individual goat samples from both breeds (Table 1) indicate considerable quantitative variation in the relative amount of α_{s1} -casein among samples from the same breed. Overall the standard deviation for each analysis was less than 3% of the mean. The 25 analyzed samples, without respect to breed, could be grouped statistically into three distinct categories (Table 2). Systems of seven alleles have been postulated as the genetic mechanism controlling a high, intermediate, or low α_{s1} -casein content observed in milking goat populations from European Alpine and Saanen breeds (7, 11). Our results agree in part with these observations: the occurrence of α_{s1} -casein is not restricted to certain breeds of goats, and its quantitative variation may be genetically regulated. Differences between the two breeds are not apparent in the overall mean (Table 1), although the standard deviation

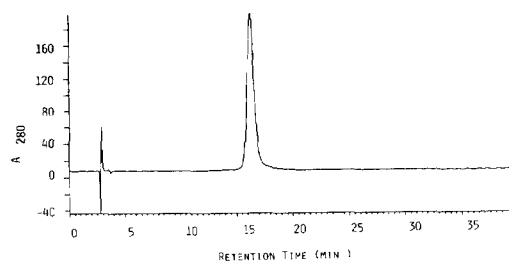


Figure 4. Analytical reversed-phase HPLC of chromatographically purified goat α_{s1} -casein. Absorbance (A) at 280 nm.

for French-Alpine is somewhat larger than that of the Anglo-Nubian breed. However, when the animals were grouped statistically by cluster analysis techniques, three distinct categories emerged. These categories have the best average nonoverlapping means. They can be classed as high, medium, and low producers of α_{s1} -casein (Table 2). Only one animal in the Anglo-Nubian breed fell into the lowest category. In contrast, 6 of 25 French-Alpine goats

TABLE 1. α_{s1} -Casein concentrations (g/L) in goat milk of French-Alpine and Anglo-Nubian breeds during midlactation.^{1, 2}

	French-Alpine		Anglo-Nubian	
No. observations	15		10	
	\bar{X}	SD	\bar{X}	SD
	2.70	.02	2.54	.02
	2.61	.01	2.51	.01
	2.30	.03	2.33	.02
	2.21	.01	1.96	.02
	1.44	.01	1.96	.01
	1.29	.04	1.47	.01
	.92	.01	1.29	.03
	.92	.03	1.10	.01
	.92	.01	.98	.04
	.55	.02	.71	.02
	.49	.02		
	.37	.03		
	.28	.01		
	.15	.01		
	.12	.02		
Overall means	1.15	.90	1.69	.63

¹All data are means and standard deviations for three determinations.

²For comparison Davies and Law (5) report $10.3 \pm .57$ g/L for the average α_{s1} -casein content cow milk.

TABLE 2. Overall mean concentration (g/L) of α_{s1} -casein in goat milk for statistically grouped¹ data of French-Alpine and Anglo-Nubian breeds.

α_{s1} -Casein		Animals tested
(g/L) ²		(no.)
\bar{x}	SD	
2.34	.25	9 ³
1.15	.24	9 ⁴
.38	.20	7 ⁵

¹Statistical grouping was by high, medium, and low amounts of α_{s1} -casein in milk.

²Cow casein is reported to contain $10.3 \pm .57$ g/L of α_{s1} -casein (5).

³Four French-Alpine and five Anglo-Nubian.

⁴Five French-Alpine and four Anglo-Nubian.

⁵Six French-Alpine and one Anglo-Nubian.

fell into the low range. Division of the data points beyond three clusters was not warranted based upon $n = 25$. It should be noted that the high α_{s1} -casein-producing animals (Table 2) still only secrete about one-fourth the amount of this protein normally found in cow milk (5).

CONCLUSIONS

The present study has separated and quantified the α_{s1} -casein component in goat milk from two different breeds by means of RP HPLC. Such a technique is at present the best method for resolving closely related hydrophobic proteins (13). With the establishment of RP HPLC as a routine analytical method, it will be possible to isolate polymorphic α_{s1} -caseins from the milk of the appropriate goats and to establish the differences that may occur in their amino acid composition or phosphorus content. The existence of some differences is suggested but not proven by the differences in shape observed for some α_{s1} -casein peaks from individual animals. Such studies could aid in the understanding of the structural features and physicochemical properties of the α_{s1} -casein present in goat milk. These studies appear to indicate a higher incidence of goats in the French-Alpine breed producing low α_{s1} -casein.

REFERENCES

- Ambrosoli, R., L. Di Stasio, and P. Mazzocco. 1988. Content of α_{s1} -casein and coagulation properties in goat milk. *J. Dairy Sci.* 71:24.
- Boulanger, A., F. Grosclaude, and M. F. Mahe. 1984. Polymorphisme des caséines α_{s1} et α_{s2} de la chèvre (*Capra bircus*). *Genet. Sel. Evol.* 16:157.
- Carles, C. 1986. Fractionation of bovine caseins by reverse phase high performance liquid chromatography: identification of a genetic variant. *J. Dairy Res.* 53:35.
- Ciafarone, N., and F. Addeo. 1984. Composizione della caseina e proprietà del latte di capra, II. Vergaro 11:17.
- Davies, D. T., and A.J.R. Law. 1980. The content and composition of protein in creamery milks in south-west Scotland. *J. Dairy Res.* 47:83.
- Fox, P. F., and M.C.T. Hoynes. 1976. Heat stability characteristics of ovine, caprine and equine milks. *J. Dairy Res.* 43:433.
- 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. *Genet. Sel. Evol.* 19:399.
- Groves, M. L., W. G. Gordon, E. B. Kalan, and S. B. Jones. 1973. TS-A², TS-B, R- and S-caseins: their isolation, composition and relationship to the β - and γ -casein polymorphs A² and B. *J. Dairy Sci.* 56:558.
- Horne, D. S., and T. G. Parker. 1982. Some aspects of the ethanol stability of caprine milk. *J. Dairy Res.* 49:459.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680.
- Mahé, M. F., and F. Grosclaude. 1989. α_{s1} -Cn^D Another allele associated with decreased synthesis rate at the caprine α_{s1} -casein locus. *Genet. Sel. Evol.* 21:127.
- Mikkelsen, J., P. Hojrup, and J. Knudsen. 1987. Purification of goats' milk casein by reversed-phase high performance liquid chromatography and identification of α_{s1} -casein. *J. Dairy Res.* 54:361.
- Nice, E. C., M. W. Capp, N. Cooke, and M. J. O'Hare. 1981. Comparison of short and ultrashort-chain alkylsilane-bonded silicas for the high-performance liquid chromatography of proteins by hydrophobic interaction methods. *J. Chromatogr.* 218:569.
- Storry, J. E., A. S. Grandison, D. Millard, A. J. Owen, and G. D. Ford. 1983. Chemical composition and coagulation properties of renneted milks from different breeds and species of ruminant. *J. Dairy Res.* 50:215.
- Thompson, M. P. 1966. DEAE-cellulose urea chromatography of casein in the presence of 2-mercaptoethanol. *J. Dairy Sci.* 49:792.
- Thompson, M. P., and C. A. Kiddy. 1964. Genetic polymorphism in caseins of cows' milk. III. Isolation and properties of α_{s1} -caseins A, B, and C. *J. Dairy Sci.* 47:626.
- Visser, S., K. J. Slangen, and H. S. Rollema. 1986. High-performance liquid chromatography of bovine caseins with the application of various stationary phases. *Milchwissenschaft* 41:559.