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Detection and quantification of true proteins, casein fractions and their genetic variants, and whey proteins in goat milk by reverse-phase high-performance liquid chromatography

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ABSTRACT

The protein profile of milk is an increasingly important research topic in relation to the technological properties of milk for cheesemaking and its value for human nutrition. Goats are a valuable source of milk worldwide, but there is no precise and rapid method for the simultaneous identification and quantification of the genetic variants of the major protein fractions in goat milk. In this study, we devised a method for the rapid identification and quantification of the main genetic variants of α_{S1}-, α_{S2}-, β-, and κ-CN, and of α-LA and β-LG (whey proteins) in caprine milk using an HPLC-UV reverse-phase C8 column. The R^2 of the calibration equations was >0.99 for all 15 protein fractions identified. A total of 520 milk samples were taken from 260 goats (in duplicates) of 6 different breeds reared on 22 farms operating different farming systems, ranging from traditional or extensive to modern or intensive, and analyzed them to externally validate the method. Milk samples from DNA-genotyped goats were used to identify the genetic variants of the caseins. Compared with other studies, using our method, we were able to separate the main genetic variant of caseins within a total run time of 24 min and with a repeatability >92% for all fractions except the C variant of κ-CN. Farms were found to be the major source of the variability in the contents of true proteins (56.1% of total variance), true caseins (48.6%), κ-CN C (50.6%) and whey proteins (52.9%–62.9%) in goat milk, whereas individual goat within farm was the most important source of variability in the contents of individual caseins and their genetic variants (53.9%–94.7%).

Key words: detailed protein profile, genetic variants, goat milk, high-performance liquid chromatography

INTRODUCTION

The total protein of milk consists of many different nitrogenous substances, the most important being the caseins and whey proteins. Caseins (from the Latin *caseus* = cheese) are the protein fractions that coagulate under the action of rennet enzymes to produce curd and subsequently cheese, whereas the whey proteins are the non-rennet-coagulating protein fractions. The major caseins (α_{S1}-, α_{S2}-, β- and κ-CN) are known to differently affect the quantity of protein in bovine milk (Pegolo et al., 2018), as well as the cheesemaking process and cheese yield (Cipolat-Gotet et al., 2018; Amalfitano et al., 2019; Mariani et al., 2022). Both caseins and whey proteins have several different genetic variants (Farrell et al., 2004; Caroli et al., 2009), which contribute to modifying the role of caseins during cheesemaking (Amalfitano et al., 2022). Moreover, the genetic variants of caseins and whey proteins in goat milk also affect its nutritional value for human nutrition (Albenzio et al., 2016; Mal et al., 2018; Dhasmana et al., 2022), which was also found to be the case when it was compared with the milk of other dairy species (Guha et al., 2021). As reported by (ALKaisy et al., 2023) the functional properties of goat milk proteins can push the development of new food products with better emulsifying stability.

Although we have extensive knowledge of the protein fractions and their genetic variants in bovine milk, less is known about the milk of small ruminants (Montalbano et al., 2016). In particular, whereas the genotyping of animals for different genetic variants is widely studied at the DNA level, less attention is paid to quantifying the contents of protein fractions and their genetic vari-

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ants in milk, also because of its analytical difficulty. A few studies have shed light on the effects of seasonal variation (Brodziak et al., 2014) and goat breed (Clark and Sherbon, 2000; Moatsou et al., 2008) on the protein profile of milk. Unlike studies on bovine species, those on goats have focused in particular on genetic polymorphisms at the level of the *CSN1S1* gene (Mestawet et al., 2013; Santillo et al., 2022) and its effect on milk technological properties (Clark and Sherbon, 2000; Johansson et al., 2023), as well as the presence of some null variants that inhibit synthesis of α_{S1}-CN (Guan et al., 2019). Different protein profiles may be an important source of the variation in cheesemaking efficiency observed in different goat breeds (Cosenza et al., 2007; Devendra and Haenlein, 2011; Stocco et al., 2018; Vacca et al., 2018b)

The availability of analytical methods able to quantify the contents of the major protein fractions in milk and simultaneously identify and quantify their genetic variants without the need for prior analysis of the DNA of the animals is very important for research in different branches of animal science, such as animal genetics, animal feeding, milk technological properties, and the nutritional value of milk and dairy products. Of course, these protein-based methods present some limitations in genotyping compared with DNA analysis, which can identify also variants not affecting protein sequence, as well as post-translational modifications, and can be performed directly on males object of selection and young females. Moreover, the rapidity and repeatability of the protein-based method are very important factors. The methods frequently used for analyzing bovine milk are based on capillary zone electrophoresis (Cattaneo et al., 1996; Heck et al., 2008) and HPLC (Bonfatti et al., 2008). We devised an improved and more rapid HPLC method for bovine milk in our laboratory (Maurmayr et al., 2013), which allowed us to perform large surveys of cows of different breeds and breed combinations (Maurmayr et al., 2018; Amalfitano et al., 2020). In the case of caprine species, some available HPLC methods concentrate their attention on casein fractions and relative genetic variants (Pierre et al., 2001; Montalbano et al., 2014), whereas others also consider whey proteins (Moatsou et al., 2006), but all of them with an analysis runtime between 30 and 60 min. Moreover, these methods have been applied to a small number of protein fractions, animal breeds, and farming conditions (Montalbano et al., 2016).

Therefore, the objective of this study was to establish a rapid (<30 min) HPLC method with high repeatability for identifying and quantifying the protein fractions in goat milk and their major genetic variants.

MATERIALS AND METHODS

Experimental Design: Farming Systems, Farms, and Breeds of Animals

The farms and goats sampled represent a wide variety of environments, farming systems, and genetic types. Thirty-five farms located in Sardinia, an insular region of Italy in the Mediterranean Sea, were selected for the study. These farms covered 3 different types of management system: traditional-extensive, intermediate-semi-intensive and modern-intensive. Details of these systems have been previously reported by Vacca et al. (2018a).

A total of 1,272 goats were involved in the project; 823 goats reared on 23 farms were individually sampled and analyzed to obtain detailed milk protein fraction profiles. The goats sampled belonged to 6 breeds: 2 internationally well-known Alpine types, namely Saanen (**SA**, 41 samples) and Camosciata delle Alpi/Alpine (**CA**, 166 samples), as well as 4 Mediterranean types, namely Murciano-Granadina (**MG**, 210 samples), Maltese (**MA**, 141 samples), Sarda (**SR**, 110 samples), and Sarda Primitiva (**SP**, 155 samples). For the present work, 260 goats from 22 farms were sampled in duplicate (for a total of 520 milk samples) to validate the results and reproducibility estimates (SA, 11 goats; CA, 74 goats; MG, 50 goats; MA, 53 goats; SR, 25 goats; SP, 47 goats).

Materials and Reagents

The following reagents were used to prepare the samples for the laboratory analyses: guanidine hydrochloride (**GdnHCl**; G4505, purity >99%), Bis-Tris buffer (B9754, >98%), sodium citrate (71498, >99%) and DL-dithiothreitol (**DTT**; D0632, >99%). Acetonitrile (CH_3CN; 34851, ≥99.9%) and trifluoroacetic acid (**TFA**; 302021, >99%) are suitable for HPLC and were used for the mobile phase. All the reagents were purchased from Merck KGaA (Darmstadt, Germany), and ultra-pure water (Milli-Q System, Merck Millipore, Burlington, MA; >18.2 MΩ cm) was obtained in the laboratory.

Milk Samples

Individual goat milk samples were collected on the farms with preservative (bronopol, 2-bromo 2-nitropropane-1,3-diol) added to the raw milk to prevent microbial growth. During milk collection, one 1.8-mL aliquot was taken from each milk sample and frozen at −20°C, then transferred to the laboratory and stored at −80°C until the HPLC analyses were performed. All milk samples were prepared following the method described by Bobe et al.

(1998). Briefly, a solution containing 0.1 *M* Bis-Tris buffer (pH 6.8), 6 *M* GdnHCl, 5.37 m*M* sodium citrate, and 19.5 m*M* DTT (pH 7) was added to individual 500-µL aliquots of milk at a 1:1 ratio (vol/vol) at room temperature. Each mixture was shaken for 10 s, incubated for 1 h at room temperature, and centrifuged at room temperature for 10 min at 13,000 × *g* in a microcentrifuge. The fat layer was removed with a spatula, then the remaining solubilized sample was diluted 1:3 (vol/vol) with a solution containing 4.5 *M* GdnHCl and HPLC solvent A consisting of ultra-pure water, acetonitrile, and TFA in a ratio of 900:100:1 (vol/vol/vol; pH 2). No preliminary separation or precipitation of the casein fraction was required.

Preparation of Standard Solutions and Construction of Calibration Curves

Because commercial standards for goat milk caseins and their genetic variants were not available, freeze-dried casein and whey protein samples obtained from previously DNA-genotyped goats were used as calibration standards (see the section "DNA Genotyping and Purified Protein Extraction" for details). Fifteen individual genotyped goat casein samples were selected and analyzed by reverse-phase HPLC (**RP-HPLC**) to map the detectable genetic variants of the proteins. The freeze-dried samples were kept at a constant temperature of −20°C until use. Standard stock solutions of the caseins were prepared by dissolving ~8 mg in 1 mL of 6 *M* GdnHcl solution, and standard stock solutions of the whey proteins were prepared with 4 mg. From each stock solution, 5 solutions in decreasing concentrations were obtained (Table 1), constituting the 5 points of the calibration curves for each protein fraction and its genetic variants. All the solutions were analyzed by HPLC, as described in the next section, and the resulting chromatograms were integrated. The total amount of protein injected into the column was divided between the peaks identified in the chromatogram in proportion to the area under the peak. Calibration curves were computed for each protein fraction and its genetic variants by applying a linear regression of the peak area to the amount of protein injected into the column.

Reverse-Phase HPLC Equipment and Chromatographic Conditions

The HPLC system consisted of an Agilent1260 Series chromatograph (Agilent Technologies, Santa Clara, CA) fitted with a quaternary pump (Agilent 1260 Series, G1311B) and a diode array detector (Agilent 1260 Series, DAD VL+, G1315C). The equipment was controlled by the Agilent ChemStation for LC System software (version B.04.03 [16]), which determines the solvent gradient and acquires and processes the data. Separation was performed with a C8 reverse-phase analytical column (Aeris Widepore XB-C8, Phenomenex) with large pore core-shell packing (3.6 µm, 200 Å, 250 × 2.1 mm i.d.). Samples were injected into the instrument with an autosampler (Agilent 1100 Series, G1313A). The HPLC system was installed in the La-Chi Laboratory at the Department of Agriculture, Food, Natural Resources and Environment of the University of Padua (Legnaro, Italy).

Our method of analysis was a modified version of that proposed by Maurmayr et al. (2013) for cow's milk. After comparing different chromatographic conditions, the following procedure was adopted to optimize analytical quality and time required: (1) gradient elution was carried out with a mixture of 2 solvents, with solvent A consisting of 94.9% ultra-pure water, 5.0% acetonitrile, and 0.1% TFA, and solvent B consisting of 0.1% TFA in

Table 1. Concentration of the 5 levels (A, B, C, D, and E) of standard genotyped casein fraction genetic variants and their phosphorylation level (1st or 2nd ph)[1] used for calibration of HPLC method (mg/mL)

Item	A	B	C	D	E
α_{S1}-CN-B, 1st ph	0.062	0.154	0.260	0.461	0.877
α_{S1}-CN-B, 2nd ph	0.039	0.053	0.127	0.316	0.695
α_{S1}-CN-A, 1st ph	0.060	0.142	0.266	0.509	0.997
α_{S1}-CN-A, 2nd ph	0.039	0.044	0.128	0.286	0.597
α_{S1}-CN-F	0.009	0.017	0.037	0.071	0.139
α_{S2}-CN-A/F, 1st ph	0.014	0.026	0.048	0.090	0.175
α_{S2}-CN-A/F, 2nd ph	0.100	0.195	0.389	0.767	1.532
α_{S2}-CN-C	0.046	0.089	0.172	0.341	0.684
β-CN-A	0.219	0.452	0.911	1.936	4.157
β-CN-C	0.192	0.407	0.834	1.743	3.630
κ-CN-A	0.034	0.068	0.134	0.269	0.543
κ-CN-B	0.045	0.090	0.183	0.362	0.734
κ-CN-C	0.016	0.031	0.057	0.115	0.220
α-LA	0.038	0.079	0.154	0.309	0.613
β-LG	0.102	0.211	0.423	0.888	1.848

[1]ph = phosphorylation level.

acetonitrile; (2) goat milk protein fractions were separated according to the following program: linear gradient of 27% B for 4 min, from 27% to 33% B in 1 min, from 33% to 36% B in 7 min, from 36% to 45% B in 6 min, return linearly to the starting condition within 1 min and a linear gradient of 27% B for 3 min; (3) the column was re-equilibrated under starting conditions for 2 min before injecting the next sample; (4) the total analysis time per sample was 24 min, the flow rate was 0.5 mL/min, the column temperature was maintained at 74°C, detection was carried out at a wavelength of 214 nm, and the injection volume was 2 µL.

DNA Genotyping and Purified Protein Extraction

Blood samples for DNA genotyping of the genes encoding the casein fractions were collected from a population of Sarda goats as described by Dettori et al. (2024). Individual milk samples were also collected from these animals to extract the purified proteins used for calibration. Blood samples for DNA extraction and the procedures were approved by the Ethical Animal Care and Experimental Use Committee (Organismo Preposto al Benessere e alla Sperimentazione Animale, OPBSA) at the University of Sassari (Sassari, Italy; protocol number 0122930, approved on September 28, 2021). The Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from leukocytes, and purity and concentration were measured using an Eppendorf BioPhotometer (Eppendorf, Hamburg, Germany). Whole caprine casein was separated by isoelectric precipitation according to Aschaffenburg and Drewry (1959). Briefly: (1) each milk sample was homogenized by inversion and gentle shaking; (2) 14 mL of milk were placed in a Falcon tube (Corning Inc., Corning, NY), then (3) centrifuged at 1,780 × *g* for 10 to 15 min at 8°C to separate the fat from the milk; (4) the fat was removed with a vacuum pump, leaving 7 mL of skim milk in the tube, which was then (5) placed in a thermostatic shaking bath for 10 min at 40°C; (6) 700 µL of 10% acetic acid was added to each sample, which was shaken vigorously and left in the thermostatic bath for at least 10 min at 40°C to reach a final pH of 4.6; (7) the acetic acid was neutralized with 700 µL of 1N sodium acetate; (8) the sample was centrifuged at 1,780 × *g* for 10 to 15 min at 8°C; (9) the corpuscles were removed from the supernatant with a vacuum pump and the serum was transferred to dialysis membranes; (10) the casein pellet was suspended in buffer (50 mL 10% acetic acid and 50 mL 1N sodium acetate; (11) this suspension was shaken with a mini stirrer, then (12) centrifuged at 1,780 × *g* for 2 min at 8°C; (13) the supernatant was discarded by inversion and the pellet was resuspended in double-distilled water and shaken; (14) steps 12 and 13 were repeated, then step 12 again; (15) the supernatant was discarded and the pellet was resuspended in double-distilled water to 8 mL; (16) the samples were brought to pH 7 with NaOH 0.5N. The casein obtained was freeze-dried and stored at −20°C until use. From step 9, the serum was kept overnight in the dialysis membranes, then freeze-dried.

Quantitative Analysis

Individual goat milk samples were quantified by RP-HPLC. The external standard method was used to calibrate the chromatographic system for quantification of the genetic variants of the 4 major caseins. Five-point calibration curves (Table 1) were generated for each genetic variant by estimating the parameters of the linear regression of the peak area on the amount injected.

Repeatability

The precision of the method was evaluated by estimating the repeatability of the analysis on the 520 duplicated milk samples obtained from 260 goats. The repeatability (%) of the total true protein (as the sum of all the HPLC protein fractions), the true casein (as the sum of all the HPLC casein fractions), each protein fraction, and the genetic variants of the caseins was calculated on the basis of the variance components estimated using mixed models with all factors treated as random factors. The repeatability was expressed as the total variance of the trait (sum of all variance components) minus the residual variance and was expressed as a percentage of the total variance.

RESULTS AND DISCUSSION

Separation of the Genetic Variants

All the major caseins of goat milk can be considered highly polymorphic (Marletta et al., 2007), especially in the case of α_{S1}- and κ-CN (Feligini et al., 2003; Rahmatalla et al., 2022; Tumino et al., 2023). This makes identification and quantification of the genetic variants of the protein fractions of goat milk more complex compared with bovine milk. However, the large majority of variants are very rare in goat populations, so only a few of them are important for farming and industry. It is important to keep in mind that even if some variants are rare in worldwide populations, they can be still characteristic of specific breeds, as with the α_{S1}CN N variant in Tunisian Arbi goats (Vacca et al., 2009) and the α_{S1}-CN D variant in Swedish goats (Johansson et al., 2023). Further research is needed to test the adequacy of this method for quantifying genetic variants of milk proteins not present in the goats sampled in this study. Our method allowed

us to identify and quantify 13 peaks corresponding to 10 genetic variants of caseins (3 of them presented a phosphorylated form), including those that occur more frequently in many goat populations. Figure 1 shows an example of chromatograms in which it was possible to identify not only the major peaks of the 4 caseins, but also their genetic variants. It is worth noting that 7 casein variants were identified and quantified in bovine milk using a similar method (9 peaks including the phosphorylated forms; Maurmayr et al., 2013).

Because commercial standards are not available, for this study we used individual freeze-dried casein samples from genotyped goats of a previous study (Dettori et al., 2024) as standards. Identification of the genetic variants of the 4 major caseins for the 6 breeds was based on the elution order and the results of other studies, but was confirmed through the use of these standards. As expected, with homozygous animals, the genetic variants gave rise to a single peak for a given protein fraction, whereas with heterozygous animals, they gave rise to smaller, double peaks. Therefore, the peaks of the genetic variants were assigned by comparing the chromatograms of individual milk samples from homozygous animals with those from heterozygous animals. The A, B, and C genetic variants of κ-CN (Figure 1A), the C genetic variant of α_{S2}-CN (Figure 1B), the A and B variants of α_{S1}-CN (Figure 1C), and the A and C variants of β-CN (Figure 1D) were well resolved with the method adopted in this study. Despite gradient optimization, we were unable to obtain a clear separation of all the fractions and genetic variants. In particular, in a few samples, the F genetic variant of α_{S1}-CN and α-LA co-eluted, making it impossible to quantify precisely this genetic variant in the heterozygous or homozygous animals. This is a pitfall for the present method that leaves space for future improvement. It is noteworthy to say that the F variant is a variant that causes very low production (0.7 g/L per allele) of the α_{S1}-CN (Montalbano et al., 2014), from 4 to 5 times less than the more frequent A and B variants observed in the present study (2.98 g/L and 3.54 g/L, respectively). Thus, its appearance can not only cause a lack of the easy-to-spot peaks of the A and B variants in the chromatogram, but also a substantial drop of the α_{S1}-CN production as for the null variant. Also in the case of the E variant of the α_{S1}-CN, it was not an easy task to differentiate from the B variant, having the same protein sequence (Dettori et al., 2009) and so the same elution time. In this case, the identification of the E variant was left to the quantity of protein produced by the allele, having a peak one-quarter of the peak of the B variant (easier to spot when in heterozygosis with the A variant, as in Figure 1C). Moreover, the A and F genetic variants of α_{S2}-CN are partly co-eluted and will therefore be referred to as a single A/F variant in this study.

Quantification of the Protein Fractions and Their Individual Genetic Variants

The regression analysis summarized in Table 2 shows the very good results we obtained for our calibration equations. The very high coefficients of determination (R^2) confirm the strict linearity of the relationship between the chromatographic peak area and the weight of the corresponding protein fraction, as also found by Montalbano et al. (2016). The root mean square error (**RMSE**) was always in the range of 0.36% to 1.51% of the mean and confirms the sensitivity of the method, whereas the intercept was lower in absolute value than twice the RMSE (with the sole exception of the B variant of α_{S1}-CN), confirming the absence of appreciable biases in the estimations (Bonfatti et al., 2008).

Validation at the Population Level

Table 3 reports the descriptive statistics of the casein and whey protein contents in the milk samples from individual goats collected at the population level and analyzed with our HPLC method. Our results confirm that unlike bovine milk (Bonfatti et al., 2008), in goat milk, β-CN is the most abundant protein fraction, constituting half or more of the total milk protein (Park, 2017). Although α_{S1}-CN has a similar content to β-CN in bovine milk (Gustavsson et al., 2014), it constitutes a much smaller proportion of the total protein in caprine milk and differs little from α_{S2}-CN. Lastly, κ-CN is the least abundant casein fraction in both species.

It is worth noting that the 2 major whey proteins are also present in very different proportions in the milk of these 2 dairy species. Even though whey proteins represent a quarter to one-fifth of the total protein in both species, the β-LG content is far higher than the content of α-LA in bovine milk, whereas the difference is smaller in caprine milk (Table 3).

A single casein in the milk sample of an individual goat yields a single chromatographic peak in the case of homozygous animals, but 2 peaks in the case of heterozygous animals (e.g., κ-CN chromatographic peaks showed in Figure 1A in homozygote animals AA and BB and in an heterozygote animal AB). In the latter case, the sum of the area of the 2 peaks is, of course, expected to be roughly equal to the area of the single peak in homozygous animals; hence there is little difference in the total casein content in the 2 cases. The average content of a given genetic variant of a casein is therefore expected to depend on the proportion of homozygous and heterozygous individuals in the population. This could also explain why the variability in the content of a single genetic variant of a casein fraction may be greater than the variability in the total casein fraction. The average

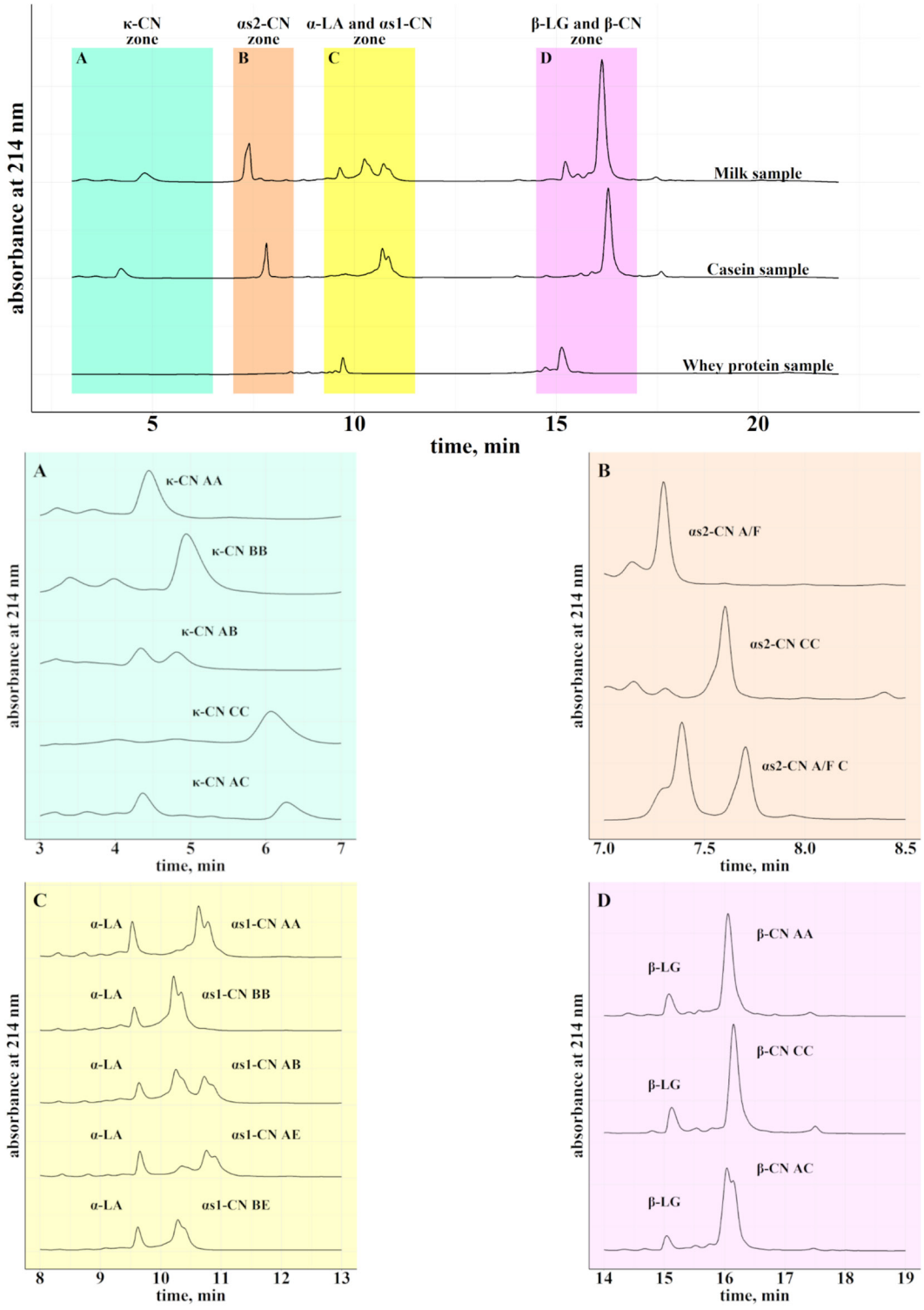

Figure 1. Chromatograms relative to an individual milk sample, a purified casein sample, and a purified whey protein sample, and detailed enlargements of the κ-CN (A), α_{S2}-CN (B), α-LA and α_{S1}-CN (C), and β-LG and β-CN (D) elution zones with all identified genetic variants of the caseins.

Table 2. Calibration linear equations (Y = a + bX ± RMSE) and their coefficient of determination (R^2) for predicting the content of protein fractions of goat milk according to their genetic variant and phosphorylation level (1st or 2nd ph)[1]

Protein	Mean	SD	a	b	RMSE	R^2
α_{S1}-CN						
α_{S1}-CN-A, 1st ph	1,300	1,239	3.12	3,287	14.8	0.99989
α_{S1}-CN-A, 2nd ph	722	769	2.23	3,285	7.8	0.99992
α_{S1}-CN-B, 1st ph	1,096	1,006	−31.10	3,108	12.8	0.99988
α_{S1}-CN-B, 2nd ph	747	848	−13.87	3,094	5.0	0.99997
α_{S1}-CN-F	181	177	−2.00	3,345	1.6	0.99994
α_{S2}-CN						
α_{S2}-CN-A/F,1st ph	233	221	−6.60	3,396	3.3	0.99983
α_{S2}-CN-A/F, 2nd ph	1973	1974	−50.89	3,392	29.9	0.99983
α_{S2}-CN-C	878	852	2.13	3,286	9.9	0.99990
β-CN						
β-CN-A	4,905	5,182	−44.82	3,224	50.3	0.99993
β-CN-C	4,484	4,601	13.21	3,285	49.7	0.99991
κ-CN						
κ-CN-A	668	663	−3.45	3,204	2.4	0.99999
κ-CN-B	910	914	−13.52	3,264	7.4	0.99995
κ-CN-C	282	266	0.02	3,205	2.3	0.99994
Whey proteins						
β-LG	1,223	1,255	−0.50	1,763	7.8	0.99997
α-LA	420	411	−0.19	1,763	2.9	0.99996

[1]ph = phosphorylation level.

Table 3. Descriptive statistics of traits obtained on goat milk using the HPLC method for quantifying the protein fractions in total and according to their genetic variants and the number of alleles of animals (homozygotes and heterozygotes)

Protein	Type	Samples (n)	Mean	SD	Minimum	Maximum
True proteins	Total	520	34.32	6.34	20.35	55.55
True caseins	Total	520	26.95	5.02	12.14	43.10
α_{S1}-CN	Total	520	4.77	2.38	0.00	10.46
α_{S1}-CN-A	Homozygous	116	6.10	1.33	3.47	9.88
	Heterozygous	178	2.98	0.82	0.33	5.15
α_{S1}-CN-B	Homozygous	86	7.10	1.72	3.57	10.45
	Heterozygous	140	3.54	1.18	1.01	7.28
α_{S1}-CN-E	Homozygous	30	1.79	0.46	0.96	2.75
	Heterozygous	116	1.29	0.32	0.88	2.36
α_{S1}-CN-0	Homozygous	2	0.00	0.00	0.00	0.00
	Heterozygous	6	0.00	0.00	0.00	0.00
α_{S2}-CN	Total	520	4.23	0.91	1.72	7.02
α_{S2}-CN-A/F	Homozygous	318	4.21	0.97	1.72	7.02
	Heterozygous	178	2.29	0.51	1.09	3.60
α_{S2}-CN-C	Homozygous	24	4.07	0.69	2.65	4.93
	Heterozygous	178	1.99	0.43	1.15	2.86
β-CN	Total	520	15.56	3.79	0.00	23.89
β-CN-A	Homozygous	120	15.31	3.15	8.96	23.49
	Heterozygous	200	9.39	1.67	5.96	14.06
β-CN-C	Homozygous	178	15.78	2.72	7.53	23.26
	Heterozygous	204	7.66	1.72	4.14	13.44
β-CN-0	Homozygous	6	0.00	0.00	0.00	0.00
	Heterozygous	28	0.00	0.00	0.00	0.00
κ-CN	Total	520	2.39	0.53	0.79	4.69
κ-CN-A	Homozygous	60	2.28	0.39	1.47	3.13
	Heterozygous	256	1.16	0.25	0.53	2.01
κ-CN-B	Homozygous	178	2.40	0.55	0.79	3.92
	Heterozygous	264	1.25	0.32	0.48	2.72
κ-CN-C	Homozygous	—	—	—	—	—
	Heterozygous	44	1.26	0.34	0.68	2.05
Whey proteins	Total	520	7.37	2.67	2.51	16.90
α-LA	Total	520	3.02	1.12	0.80	7.17
β-LG	Total	520	4.34	1.82	0.97	10.42

Table 4. Variance components of traits obtained on goat milk using the HPLC method for quantifying the protein fractions in total and according to their genetic variants

		Variance component (% of total variance)			
Protein	Total variance	Farm	Goat	Residual	Repeatability
True proteins	37.05	56.1	36.8	7.2	92.8
True caseins	24.83	48.6	45.2	6.3	93.7
α_{S1}-CN	5.61	23.2	75.4	1.4	98.6
α_{S1}-CN-A	3.37	17.1	79.9	3.0	97.0
α_{S1}-CN-B	4.97	35.3	63.6	1.0	99.0
α_{S1}-CN-E	0.21	40.6	53.9	5.5	94.5
α_{S2}-CN	0.87	36.3	57.8	5.9	94.1
α_{S2}-CN-A/F	1.59	27.6	69.4	3.0	97.0
α_{S2}-CN-C	0.66	32.7	66.2	1.2	98.8
β-CN	13.00	40.9	54.4	4.7	95.3
β-CN-A	13.75	0.0	94.7	5.3	94.7
β-CN-C	21.74	18.9	77.8	3.2	96.8
κ-CN	0.29	31.1	62.1	6.7	93.3
κ-CN-A	0.27	14.7	82.7	2.6	97.4
κ-CN-B	0.52	27.7	70.0	2.3	97.7
κ-CN-C	0.14	50.6	22.6	26.8	73.2
Whey proteins	5.97	62.9	32.6	4.5	95.5
β-LG	3.00	52.9	42.7	4.4	95.6
α-LA	1.03	61.3	33.3	5.4	94.6

content of a casein fraction could be much lower than expected in the case of genetic variants that determines a low synthesis level (see the α_{S1}-CN-E variant, Table 3). The average values reported in Table 3 are raw means not corrected for any major source of variation (e.g. farming system, individual farm, breed of goat, parity, lactation stage), but the data provide a useful basis for further studies on the protein profile of goat milk at the phenotypic and genetic levels.

The use of the HPLC method at the population level allowed us to quantify the most important sources of variation in protein fractions and in their genetic variants in the milk of individual goats. The sources of variation we quantified were farm, individual goat, and the residual variation between replicate samples of the same goat. The true protein content of milk was more affected by the variability among farms than by individual goats within farm (Table 4). This is due especially to the variability in whey proteins. In the case of true caseins (sum of all individual caseins), the 2 sources of variation were of similar importance, but for all the individual caseins, the effect of individual goat was much more important than that of farm. This means that the variability in the content of a given casein is partially compensated for by other caseins at the level of farms, but not of individual goats. However, the variability of individual goats is also strongly affected by breed, genetics within breed, parity, and lactation stage, and we hope that the availability of this HPLC method will stimulate studies on different protein fractions and their genetic variants at population level.

Quantification of the residual variance allowed us to calculate the repeatability of the analytical procedure adopted for each protein fraction and genetic variant. This can be considered very good (>90%, see Table 4) for all the protein fractions and genetic variants with the sole exception of κ-CN-C, which is a very rare genetic variant in this population. It is worth pointing out that repeatability in this case includes the variability due to the sampling, aliquoting, storing, and processing of the replicates, and not only the strictly instrumental one.

CONCLUSIONS

The HPLC method used in this study proved to be reliable and rapid. It allowed us to identify and quantify the content of the major genetic variants of α_{S1}- α_{S2}- β-, and κ-CN, and to quantify the content of β-LG and α-LA in goat milk samples. We tested the method at the population level across several farms covering different farming systems and many goats of different breeds, parities and lactation stages, allowing us to quantify also the variance components of the traits studied. The method represents a good compromise between repeatability and rapidity and can be considered a valuable tool for large studies on phenotypic and genetic variations in goat populations and for evaluating the cheese-making aptitude of milk.

NOTES

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Nonstandard abbreviations used: CA = Camosciata delle Alpi/Alpine; DTT = DL-dithiothreitol; GdnHCl = guanidine hydrochloride; MA = Maltese; MG = Murciano-Granadina; ph = phosphorylation level; RMSE = root mean square error; RP-HPLC = reverse-phase HPLC; SA = Saanen; SP = Sarda Primitiva; SR = Sarda; TFA = trifluoroacetic acid.

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