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Characterization of Casein Fractions from Algerian Dromedary (*Camelus dromedarius*) Milk

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Abstract: To characterize casein fractions in Algerian dromedary's milk, samples from two breeds, Larbaa and Targui, were analyzed using electrophoretic and chromatographic techniques. Analysis performed by isoelectric focusing showed heterogeneity within the same breed and between the breeds. Some samples featured a reduced beta-lactoglobulin content and one sample from Larbaa breed showed the lack of bands in the most acidic region, where kappa-casein focuses. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of this sample allowed identifying the alpha$_{s1}$-, alpha$_{s2}$- and beta-casein fractions only. High performance liquid chromatography analysis of precipitated casein showed three main peaks. In order to characterize them, electrospray ionization-mass spectrometry was exploited. Their measured molecular masses were 24,760, 22,060 and 24,970, corresponding to alpha$_{s1}$-, alpha$_{s2}$- and beta-casein. Analytical results suggested the absence of kappa-casein in this sample. Nevertheless, investigations at DNA level are necessary in order to better characterize kappa-casein locus in Larbaa breed and to define this species's milk peculiar properties.

Key words: Camelus dromedarius, milk, casein

Introduction

Dromedary milk contains approximately 3.2% nitrogen (N x 6.38) (Attia *et al*., 2000) but total casein content and total casein/total nitrogen content ratio are relatively low (about 2.2% and 68.5%) compared to the milker's ones, ranking 2.8 and 79% (Farah, 1993). Dromedary's caseins showed electrophoretic characteristics and protein amounts different from other ruminants (Kappeler *et al*., 1998), featuring high amounts of beta-casein and low amounts of kappa-casein in contrast to the milk from ruminants traditionally used in technological processing. The low content of kappa-casein and the relatively large size of casein micelles may act as an obstacle during the coagulation process; salt, citrate and nitrogen concentration in aqueous and micellar phases bestow particular characteristic to the curdling process, affecting acidification and inducing minerals' release from micelles, therefore promoting their destabilization (Farah, 1993; Attia *et al*., 2000). Recent studies made on camel cheese made by skimmed milk pointed out that some important chemical characteristics of the cheese depend upon milk's salt content (Inayat *et al*., 2003).

The goal of this work was to characterize casein fractions from Algerian dromedary's milk. Special attention was dedicated to the study of differences in caseins' composition between samples. Results were compared with existing data concerning several other dromedary breeds from Tunisia, Kenya, Egypt and Somalia.

Materials and Methods

Sampling: Six individual dromedary milks from Larbaa (D1, D2, D3, D4) and Targui (D5, D6) breeds were collected in Laghaouat and Ghardaia regions. Sample collection was carried out in the morning, manually, from one 2-yr-old primipara and four multiparas aging 15, 5 and 7 respectively. Milk samples were immediately frozen at -20 °C without preservatives.

Milk was defatted by centrifugation and casein was precipitated with 1 M HCl at pH 4.3. The precipitation procedure was repeated and the precipitation product was washed three times with doubly-distilled water.

Isoelectric focusing (IEF): Milk samples were defatted by centrifugation at 2000g for 15 min at 4°C. Skimmed milk was diluted 1:1.5 (vol/vol) with a denaturing solution prepared according to Krause and Belitz (1985). The polyacrylamide gel matrix (30% T; 2.7% C) was prepared with 8 M urea and 12.2% (wt/vol) glycerol (87%) (Sigma Chemical Co., St. Louis, MO, USA). The pH gradient was obtained by mixing 2.5% (vol/vol) Pharmalyte ampholynes (Amersham Biosciences, Uppsala, Sweden) pH 2.5-5 and 4-6.5 in a 2:1 (vol/vol) ratio. The final volume of gel solution was 15 mL. Electrophoretic run was carried out in a Multiphor II apparatus (Amersham Biosciences) for 2.5 h at 4 mA, max 250 V cm^{-1} and 20 W. The plate was cooled to 10°C with a thermostatic circulator. The gel was stained in Coomassie blue G-250 according to Blankesley and Boezi (1977). IEF profiles were image-processed with a

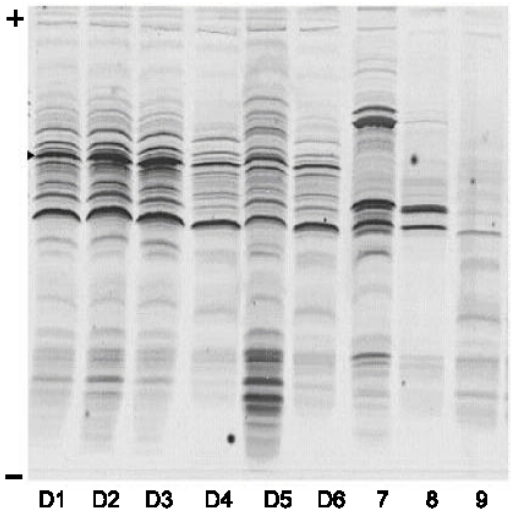

Fig. 1: Isoelectric focusing of dromedary milk from two Algerian breeds (D1, D2, D3 and D4: Larbaa; D5 and D6: Targui). Lane 7: bovine milk; lane 8: whey from bovine milk; lane 9: whey from dromedary milk. Bracket indicates the acidic region where the greatest heterogeneity was observed; arrow indicates the band recognized as beta-casein, featuring a marked variability in intensity.

GS 800 densitometer and Quantity One 1-D Analysis software (Bio-Rad, Richmond, CA, USA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): Casein was diluted 1:10 (wt/vol) in a reducing buffer containing SDS according to Laemmli (1970). Acrylamide/bisacrylamide (29:1, wt/wt) gel was prepared at 14% T with 5% acrylamide stacking gel. Electrophoresis was carried out in a Mini Protean II (Bio-Rad) cell at constant 120 V for 1 h. Gel was stained with Coomassie R-250 for 1 h. Identification of the main proteins was done on the basis of the relative migration of known markers (RNP 800, LMW Calibration kit, Amersham Biosciences; alpha$_s$-, beta- and kappa-CN from bovine milk, Sigma). Computerized densitometry (Bio-Rad) was used to calculate bands' molecular weight (MW). Electrophoresis was repeated 6 times for each sample, resulting in a total of 36 analyses.

Urea-PAGE: Dromedary's caseins were separated by a non-dissociating discontinuous buffer system (pH 9.5) according to Medrano and Sharrow (1988). Running and stacking gel contained 8% and 4.6% polyacrylamide respectively. Electrophoresis was performed in a Mini Protean II (Bio-Rad) cell loading 4 µl per lane of each sample; run was conducted at constant 50 V for 30 min after which voltage was increased to 100 V and mantained until 35 min after the dye front reached the anodic end of the gel. The gel was stained for 1 h in Coomassie R-250. Markers included alpha$_s$-, beta- and kappa-CN from bovine milk (Sigma). Casein ratios were calculated by densitometry and software image processing (Bio-Rad).

Reversed phase high performance liquid chromatography (HPLC): Casein samples were reduced with 1 mL of 8 M urea, 0.1 M Bis-TRIS, 0.3% beta-mercaptoethanol, 1.3% sodium citrate for 1 h at room temperature. Reduced samples were diluited (1:5, vol/vol) with 6 M urea and 0.1% trifluoroacetic acid and filtered through a 0.45 µm filter (Visser *et al.*, 1991; Sala *et al.*, 1993). The chromatographic system used was equipped with a 20 µl loop and an UV detector (Waters Corporation, Milford, MA, USA). Separation was performed in a 250 x 4,6 mm reversed-phase C4 analytical column, 300Å pore diameter and 5 µm particle size (Jupiter, Phenomenex®, Torrance, CA, USA). The mobile phase was made of 0.1% (vol/vol) trifluoroacetic acid in ultra pure water (eluant A) and 0.1% trifluoroacetic acid in acetonitrile (eluant B). Analyses were run following this elution programme: linear gradient, starting at 30% to 50%, eluant B from 0 to 40 min; 50% to 100% B from 40 to 46 min; 100% B from 46 to 48 min; 30% B from 48 to 55 min. The flow rate was 0.8 mL/min and the analytical wavelength was 220 nm. The column was kept at room temperature. Data were processed with the chromatographic system's software Millennium® 32 (Waters).

Molecular mass determination: The combination of HPLC with electrospray ionization mass spectrometry (ESI-MS) (Gaskell, 1997) allowed obtaining information about the molecular weight of the casein fractions eluted from the C4 column. The HPLC used is a Surveyor LC system (Thermo Finnigan, San Jose, CA, USA). All experiments were run using an LCQ DECA ion trap mass spectrometer equipped with ESI ion source and controlled by Xcalibur software 1.1 (Thermo-Finnigan, San Jose, CA, USA). Experiments were carried out in positive ion mode under constant instrumental conditions: source voltage 4.5 kV, capillary voltage -20 V, sheet gas flow 70 (arbitrary units), auxiliary gas flow 20 (arbitrary units), capillary temperature 200°C, tube lens voltage -5 V. The acquired spectra were deconvoluted using the Bioworks 3.0 program (Thermo Electron Corporation, Boston, MA, USA). Determinations were performed on sample D4 and were repeated 3 times.

Results and Discussion

Electrophoretic analysis of individual dromedary milk performed by IEF is shown in Fig. 1. Basing upon the isoelectric points (Ip) calculated by Kappeler *et al.*, (1998) for Somali dromedary (4.41 for alpha$_{s1}$-, 4.58 for alpha$_{s2}$-, 4.76 for beta-, 4.11 for kappa-CN) and the whey

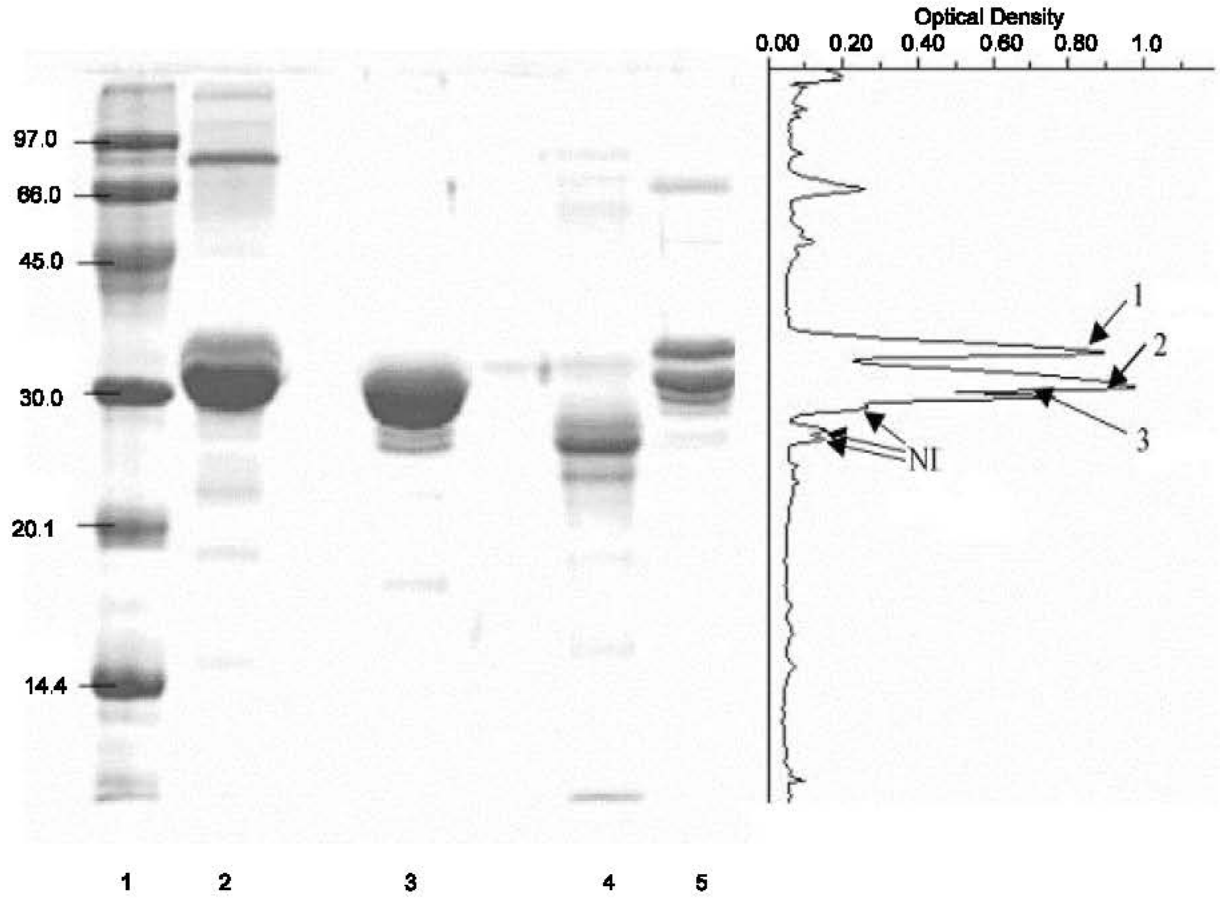

Fig. 2: SDS-PAGE and densitometric analysis of individual dromedary casein. Lane 1: marker; lane 2: bovine alpha$_s$-CN; lane 3: bovine beta-CN; lane 4: bovine kappa-CN; lane 5: dromedary's casein (sample D4). Peak 1: alpha$_{s1}$-CN; peak 2: alpha$_{s2}$-CN; peak 3: beta-CN. NI indicates not identified peaks.

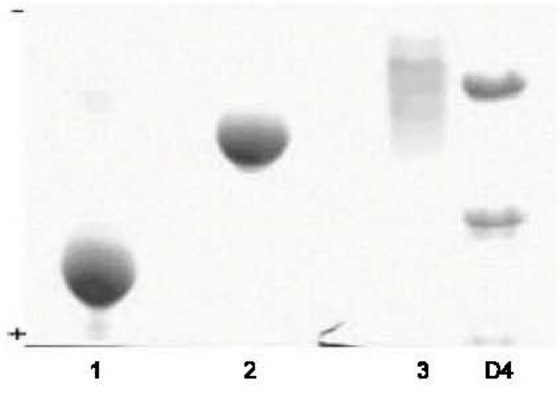

Fig. 3: Urea-PAGE of casein from Larbaa breed (sample D4). Lane 1: bovine alpha$_s$-CN; lane 2: bovine beta-CN; lane 3: bovine kappa-CN.

sample's IEF profile, casein fractions were discriminated. The heterogeneity in the same breed and between breeds was related to the acidic region of electrophoretic patterns, as observed by Wangoh *et al.*, (1998). In particular, the absence of bands with apparent Ip ranging 4.35 to 4.40 (referable to kappa-casein) was observed in one sample from Larbaa (D4), whereas their intensity was very low in one from Targui (D6). Moreover, in the less acidic region a band with apparent Ip of 4.75, corresponding to beta-casein, showed a marked difference in intensity between samples.

Further characterization of casein samples was performed by discontinuous SDS-PAGE. Eight bands were observed in all samples except D4, which lacked the lightest two (Fig. 2); their MWs were estimated as the mean value over 6 repeats. Three major bands were identified as alpha$_{s1}$-, alpha$_{s2}$- and beta-casein by comparing the observed electrophoretic profiles with those reported by Ochirkhuyag *et al.* (1997) for Egyptian dromedary and by Larsson-Raznikiewicz and Mohamed (1986) for Somali dromedary. Identified bands' MWs resulted 34.4 ± 0.3 kD, 29.8 ± 0.2 kD and 31.0 ± 0.3 kD (alpha = 0.05) respectively. In addition, five other bands were recognized; their mean MWs were found to be 28.5 ± 0.3 kD, 26.8 ± 0.3 kD, 25.8 ± 0.3 kD, 22.7 ± 0.3 kD and 21.7 ± 0.2 kD (alpha = 0.05). The last two ones

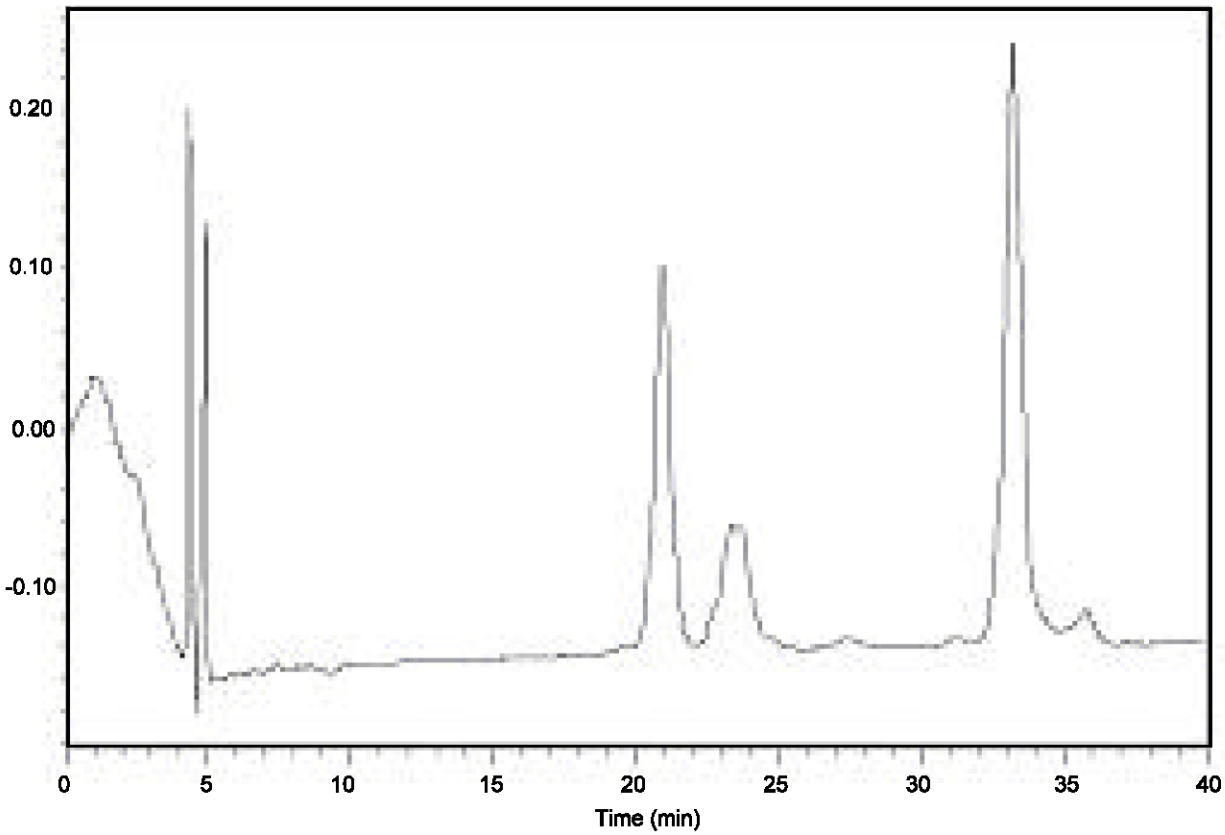

Fig. 4: RP-HPLC profile of individual casein sample (D4). Peak 1: alpha$_{s1}$-CN; peak 2: alpha$_{s2}$-CN; peak 3: beta-CN

corresponded to the Kenyan dromedary kappa-casein's size, estimated to be 22,294 D and 22,987 D as reported by Kappeler *et al.* (1998).

In relation to its different IEF (Fig. 1) and SDS-PAGE (Fig. 2) profiles, further investigation focused on sample D4. Urea-PAGE pattern of sample D4's casein showed only three bands (Fig. 3) representing alpha$_{s1}$-, alpha$_{s2}$- and beta-CN (Ochirkhuyag *et al.*, 1997). Neither a band corresponding to kappa-CN, nor proteins with mobility similar to bovine casein fractions could be detected. Cited authors suggested beta-CN's band be constituted by 12% of unresolved kappa-CN.

Fig. 4 shows the HPLC analysis of isoelectric point-precipitated casein from sample D4. The chromatogram featured three peaks (indicated in Fig. 4 as 1, 2 and 3); in comparison to the results from Kappeler *et al.* (1998), in which kappa-casein was eluted faster than other caseins, the most prominent finding is the absence of a chromatographic peak referable to kappa-casein. In order to characterize the observed peaks, casein was analyzed by HPLC-ESI-mass spectrometry. The analysis was repeated 3 times for each peak. The mean relative molecular masses resulted 24,760 for peak 1, 22,033 for peak 2 and 24,970 for peak 3 allowing their identification as: alpha$_{s1}$-, alpha$_{s2}$- and beta-CN respectively.

Conclusion: The electrophoretic and chromatographic analyses performed on Algerian dromedary's casein showed heterogeneity between samples under both qualitative and quantitative aspects. Interestingly, analytical results suggested the absence of kappa-casein in one sample from Larbaa breed. The compositional characteristics of its casein make this milk close to the human one and could therefore be useful to promote this product as a substitute of bovine milk for allergic people. Nonetheless, investigations at DNA level are necessary in order to better characterize dromedary's genetic structure at the loci involved in caseins' biosynthesis.

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