α₅ₛ₁-casein in goat milk: identification of genetic variants by Capillary Zone Electrophoresis compared to Isoelectric Focusing

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INTRODUCTION – AlphaS₁ casein fraction in caprine milk is characterized by an important polymorphism due to substitution, deletion of amino acids and post transcriptional modifications (Grosclaude et al., 1994; Ferranti et al., 1997). This structural polymorphism is associated to a quantitative variability in protein expression related to different milk quality and dairy properties (Pierre et al., 1998; Remeuf, 1993; Vassal et al., 1994). Classical electrophoretic methods were applied to characterize the phenotypic variants at α₅ₛ₁-casein fraction (Addeo et al., 1988; Russo et al., 1986). During the last ten years capillary electrophoresis became an analytical technique for rapid and automated analysis requiring small sample volume and small solvent waste. These characteristics, together with the high resolution and the chance to give quantitative results, made this technique a useful tool in studying milk protein characterization and in detecting adulteration (Cattaneo et al., 1996a; 1996b) in different application fields. CZE was applied to the study of caprine milk proteins to quantify high, medium and low α₅ₛ₁-casein content and to identify genetic variants α₅ₛ₁ A, B and C on the basis of their different migration time (Recio et al., 1997). The aim of this work was to test a CZE procedure able to identify and discriminate the main α₅ₛ₁ caprine variants A, B, E and F through specific and repeatable electromigration patterns. Comparison between CZE and IEF assays is discussed.

MATERIAL AND METHODS – Samples: Forty individual raw milk samples were collected from Sarda, Nera di Verzasca, Frontalasca and Alpine goat breeds (1:1:1:1) and stored at -20°C. Before analysis milk samples were defatted by centrifugation at 1000g for 10 min at 4°C. Caseins were separated from defatted milk by precipitation at the isoelectric point.

CE Sample treatment: Sample buffer (pH 8.6±0.1) was prepared by mixing 10 M urea, 167 mM TRIS,
42 mM MOPS, 67 mM EDTA and 17 mM dithiotreitol. The solution was filtered over a 0.45 mm filter (Sartorius, Göttingen, Germany). Milk samples were diluted 1:1.5 (w/w) in sample buffer. Isoelectric casein was dissolved at 4% (w/v) in a sample buffer diluted (60% v/v) solution. Both milk and casein samples were incubated 5 minutes at room temperature, centrifuged at 10000 g for 10 minutes then analysed by CZE. (Recio et al., 1996) Capillary zone electrophoresis: Electromigrations were carried out using a Biofocus 2000 capillary system (Bio-Rad Laboratories, Hercules, Ca, USA). Separations were performed at 38°C using a 550 mm x 50 µm i.d. Bio-Rad Biocap hydrophilically coated capillary with a running electrolyte (pH 3 ±0.1) made up of 20 mM sodium citrate buffer, 0.05% MHEC and 6 M urea. Voltage was set up at 20.00 kV with polarity from positive to negative, pressure injection 10 psi*sec and UV detector at 214 nm. (Cattaneo et al., 2002).

IEF The experimental procedure is described in a previous work (Feligini et al., 2002).

Data repeatability was tested using at least five samples characterised by the same αS1-CN genotype, AA genotype at k-CN locus and AA, AC, AB genotype at αS2-CN locus. Alpha s1-CN reference samples were genotyped by DNA analysis (Spallanzani laboratories).

RESULTS AND CONCLUSIONS – A good correlation between αS1 genotype, CZE peak areas and the intensity of IEF corresponding bands was found, although not quantitative results were achieved. CZE, by the procedure here described, was found to be a suitable technique to identify αS1 A, B, E and F caprine milk genetic variants (Fig. 1), showing a good comparability with IEF results (Fig. 2). Partial discrimination was obtained between variants such as B and E, which show similar electrophoretic properties. However, the same problem occurred also when IEF was applied.

IEF confirmed to be a suitable electrophoretic technique able to give accurate results although particularly influenced by the operator effect (Feligini and Nudda, 1999), due to the manual steps. On the basis of the reduced amount of toxic solvents, the automated technology used and the simple procedure, CZE can be proposed for genetic screenings as feasible substitute of IEF method in routine labs with this specific aim.

Figure 1. Electropherograms obtained by CZE-urea of individual caprine milk samples.
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