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# PROTEIN DISTRIBUTION IN A SUPERNATANT OF MILK ULTRA-CENTRIFUGED USING LAB-ON-A-CHIP MICROFLUID ELECTROPHORESIS

Fabiano F. Costa[1]
Maria Aparecida V. Paiva e Brito[1]
Marta F. M. Guimarães[1]
Marco Antônio M. Furtado[2]
Marcone A. L. de Oliveira[2]
and Isabella S. B. Pinto[1]

[1] Embrapa Gado de Leite, Juiz de Fora, Brazil
[2] Federal University of Juiz de Fora, Juiz de Fora, Brazil

The separation and quantification of individual milk proteins is an important issue in dairy research. SDS-PAGE, HPLC (high-performance liquid chromatography), capillary electrophoresis and immuno-based assays have also been successfully applied for the separation and/or quantification of proteins in various milk proteins systems. The disadvantages of almost all separation techniques is the time required for the preparation of the samples, the physical separation of the proteins and the final integration and quantification of the individual protein components in the sample. In recent years a novel methodology called microchip electrophoresis or lab-on-a-chip technique has been developed for the separation and quantification of proteins, as well as for DNA and RNA. For proteins, this technique has been reported to be a high-throughput, automated alternative with the separation and quantification of a number of samples (10/chip) within 30 min. The objective of this study was to determine the separation potential of the lab-on-a-chip technique in a supernatant milk ultracentrifuged sample. The protein composition of milk in the supernatant after centrifugation (40,000 × g and 20 °C for 60 min), using a Sorvall ultracentrifuge (Sorvall Centrifuges with A-841 rotor, EUA), was determined by microfluid electrophoresis (lab-on-a-chip) using an Agilent 2100 Bioanalyser system and the associated Protein 80 kit (Agilent Tecnologies, Waldbronn, Germany). The individual milk proteins αs1-casein, ß-casein, κ-casein, α-lactalbumin and ß-lactoglobulin were supplied by Sigma-Aldrich (St. Louis, MO, USA). Solutions of ~ 10 mg $mL^{-1}$ of individual protein were prepared by adding each protein to purified water and stirring until dissolved. The results showed an excellent separation for whey proteins in the supernatant (α-lactalbumin and ß-lactoglobulin). The separation times for the whey protein in the supernatant were consistent with the molecular weights of the individual proteins, with α-lactalbumin eluting first and ß-lactoglobulin in the end. For the caseins, a reasonable separation of major caseins was achieved. In order of increasing eluting time, ß-casein eluted first, αs1-casein second and κ-casein in the end, even though κ-casein has the lowest reported molecular weight. Considering that the molecular mass is based on eluting times of proteins standards, the estimated molecular masses for the whey protein and caseins proteins using this technique were in reasonable agreement with reported values. In solutions of purified standard proteins and typical milk system, all major milk proteins could be separated with high resolution. These results indicated that the microchip technology may provide a rapid and alternative method for the simultaneous separation and quantification of the major whey and caseins in milk proteins systems.

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PP-A-24

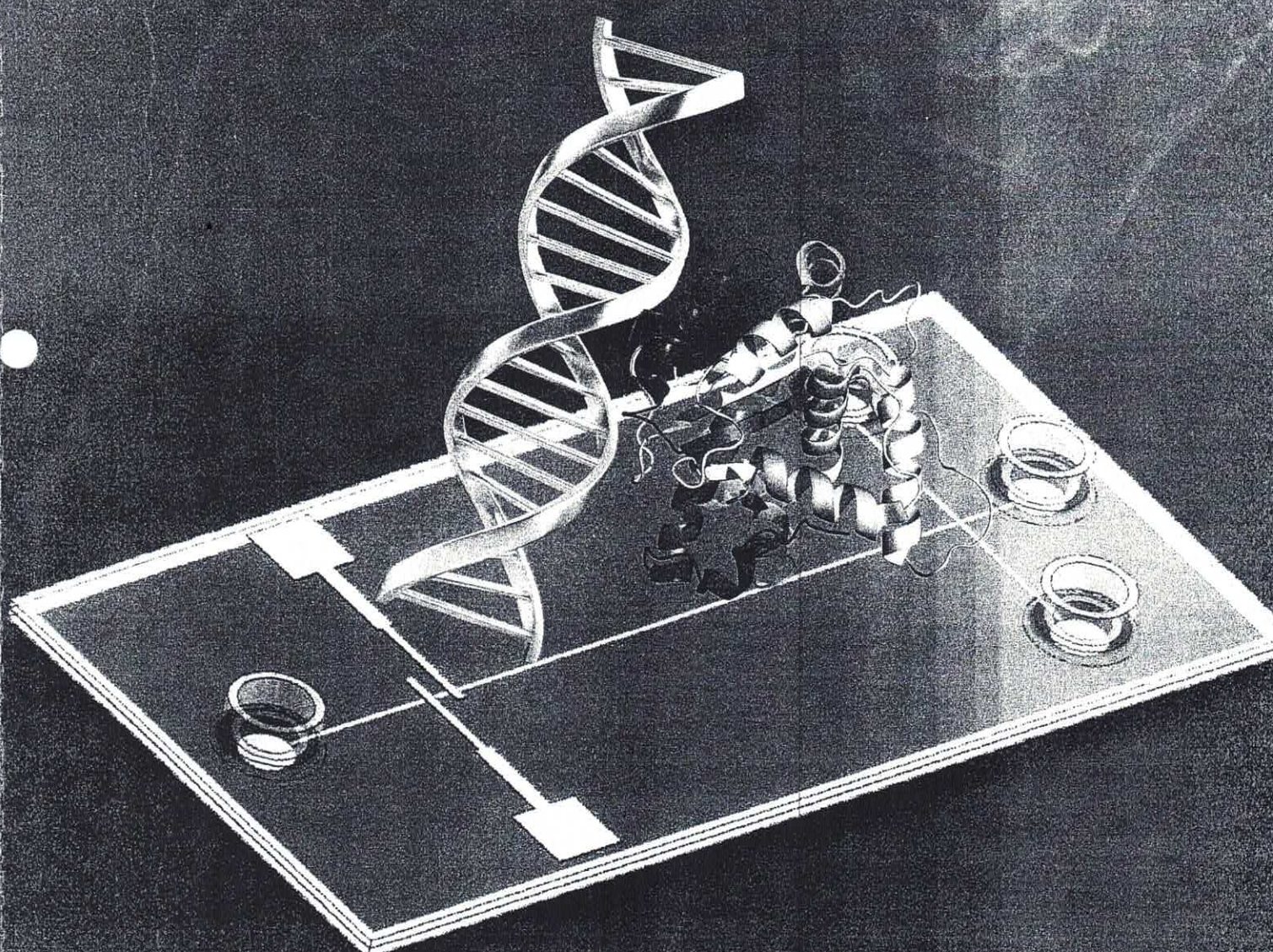
# LACE 2010

# 16th Latin-American Symposium

on Biotechnology, Biomedical, Biopharmaceutical, and Industrial Applications of Capillary Electrophoresis and Microchip Technology

## December 3 - 7, 2010

Jurere Beach Village, Florianópolis, Brazil



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