DETERMINATION OF PROTEINS FROM BUFFALO MILK USING HIGH PERFORMANCE LIQUID CROMATOGRAPHY RP-HPLC

DETERMINAREA PROTEINELOR DIN LAPTELE DE BIVOILŢĂ PRIN UTILIZAREA CROMATOGRAFIEI IN FAZA INVERSA, RP- HPLC

PECE AURELIA*, COROIAN C.*, GHIRILĂ BIANCA*, MUREŞAN G.*, MIREŞAN VIOARA*

*University Agricultural Science and Veterinary Medicine, Faculty of Animal Husbandry and Biotehnology, 3 – 5 Mănăstur Street, Cluj-Napoca, Romania

In the hereby paper, we have undertaken a study on buffalo milk proteins, employing high performance liquid chromatography (HPLC). This RP-HPLC technique is commonly employed in the separation and assessment of caseins K, and in the fresh, as well as processed milk. These methods are also successfully applied in the authenticity and origin assessment of certain cheese products and the qualitative analysis of milk in bubalines, ovines, caprines and bovines (Ferreira şi Cacote, 2003; Veloso şi colab., 2002). In order to identify the main protein types, we found support in literature data (Miranda şi colab., 2004), and thus achieved the chromatographic separation on whole milk, lactoserum and casein samples.

Key words: buffalo, proteins, HPLC

Introduction

The classification of milk proteins, on an overall, resides in caseins and lactoserum (buttermilk). Caseins are represented by several families of molecules (β, αs1, αs2, k), that show a genetic polymorphism and post-translational modifications, namely phosphoralytions and/or glycosilation. Furthermore, in identifying milk-specific caseins, we made use of data presented by Morra-Gutierrez et al. (1991).

In order to identify lactoserum- specific proteins, the studies of Trujillo et al. were employed, and also those of Moatsou et al. (2005). These studies certify the fact that milk proteins were separated using the same RP-HPLC technique, while their identification was eventually performed by means of mass spectrometry. In order to analyze milk proteins, we employed a gradient separation, thus modifying the mobile phase during the analysis. This modification involves
the employment of at least 2 solvents, in two different reservoirs, while their flow was regulated by two pumps. Before entering the column, there is a mixture "chamber" where solvents are mixed, in well-defined ratios. There are two basic types in HPLC chromatographic separation: isocratic and gradient separation. The insertion of samples on columns involves a volume of 20µl, with the help of a microsyringe through special devices to be found at the top of each column.

**Results and Discussions**

The analysis of milk proteins was conducted using reverse-phase chromatography (RP-HPLC - reverse phase HPLC). In this case, a Vydac V8 column was employed. It is a stationary unpolar phase, C₈ (for example C₈H₁₇) groups attached to silicate derivates. The mobile phase employed had a polar character, as a mixture of water, acetonitril and with the presence of the trifluoracetic acid (TFA). Polar compounds are first eluted and homologous compounds are retained. The stronger they are retained, the longer their catena is.

The order of casein separation was established in accordance with specific retention times and by comparing these times with literature data. Therefore, we were able to identify separated milk proteins by employing data by Miranda et al. (2004) in the analysis of buffalo milk, as compared with the data we obtained, in the same chromatographic conditions.

The only modification resides in the type of column employed: the research group at INRA Paris employed a C4 Vydac column, while we employed a C8 Vydac column. This explains the slight deviation (of about 2-3 minutes) of retention times (figure 1). In order to perform the separation, a gradient system was employed, where solvent A (fixation solvent) was a water solution TFA 0.01% and solvent B (elution solvent) was acetonitril 90% (v/v) and TFA 0.01% in water (Miranda et. al., 2004).

The separation of milk samples proteins and lactoserum was conducted in the gradient, according to the following conditions: 31% solvent B for 5 minutes; linear increase from 31% to 46% solvent B for 40 minutes, 100% solvent B for 5 minutes, 100% solvent A for 10 minutes, at a flow of 1 ml/minute.

The separation of milk samples proteins and lactoserum was conducted in the gradient, according to the following conditions:

- 31% solvent B for 5 minutes;
- linear increase from 31% to 46% solvent B for 40 minutes;
- 100% solvent B for 5 minutes;
- 100% solvent A for 10 minutes;
- at a flow of 1 ml/minute;
Fig. 1. The identification of proteins separated in the whole milk
1 - k casein; 2 - αs2 casein; 3 - αs1 casein; 4 - β casein; 5 α lactalbumine and β lactoglobulines; 6 - β lactoglobulines

In order to achieve an efficient separation, within a short-time analysis, the (HPLC – High performance pressure liquid chromatography) techniques has enforced itself lately;

Conclusions

► Among the advantages of this method we mention: the reduced quantity of the sample necessary for analysis;
► Substances for analysis should not be volatile; the short analysis times; simple working conditions for sample preparation;
► The analysis and data processing, the polarity of the mobile and stationary phases, that leads to the possibility of an efficient separation of the mixtures to be analyzed;
► Separations may be conducted regularly at a normal temperature;

Bibliography


