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# Pilot-scale purification of milk proteins by association of membrane filtration techniques and preparative ion-exchange chromatography.

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**Abstract**

Milk is composed of about 3.5% of proteins, which are divided in two groups: caseins and whey proteins. The present study aimed to explore and optimize membrane filtration and ion-exchange chromatography techniques in order to obtain isolates of casein micelles and of the main globular proteins of the whey: β-lactoglobulin (β-LG), α-lactalbumin (α-LA), BSA and lactoferrin.

***Key words:***

*Whey proteins, casein micelles, membrane filtration, liquid chromatography.*

## Introduction

Membrane filtration techniques allow the separation of molecules based on their size. Casein micelles, which have a hydrodynamic diameter ranging from 150 to 300 nm, can be separated from the whey by microfiltration using membranes displaying pore size around 100 nm (1). In order to isolate the whey proteins from the remaining whey components, ultrafiltration using membranes displaying pore size around 5kDa can be used (2).

To obtain isolates of the different whey proteins, their different charge can be used to allow a separation by using ion-exchange liquid chromatography (3).

In this context, the present work explored the separation of proteins from skimmed milk, avoiding the thermal and/or chemical treatments generally applied at industrial level, aiming to preserve protein's native structures.

## Results and Discussion

### STEP 1: Isolation of casein micelles and whey proteins using successive membrane filtrations

In order to obtain casein micelles isolate (CMI) and whey protein isolate (WPI), successive membrane filtrations were used, as summarized in the Image 1.

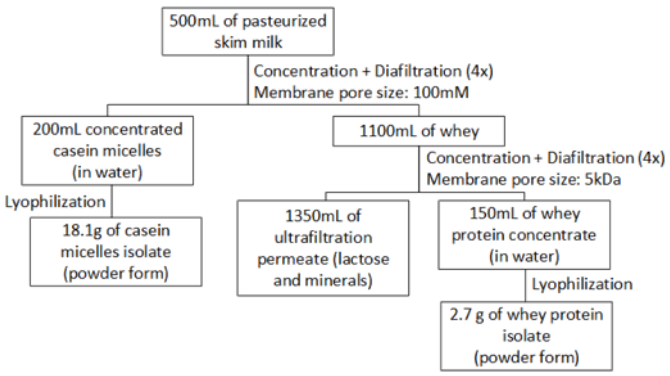


**Image 1.** Flowchart of the applied methodology.

Image 2 shows the SDS-PAGE profile (electrophoresis in gel of polyacrylamide) of the obtained WPI compared to a commercial one. Very close profiles were observed.

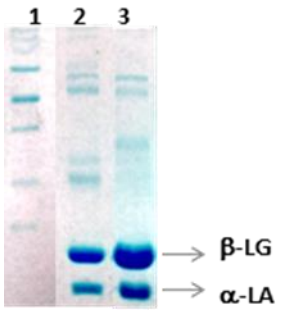


**Image 2.** SDS-PAGE. Wells: (1) size standard, (2) WPI obtained at pilot scale and (3) commercial WPI.

### STEP 2: Isolation of the different whey proteins using ion exchange liquid chromatography

In order to isolate the different whey proteins, a XK 16/20 column of 10 cm of bed height filled with of Q Sepharose Fast Flow resin was used. By applying a linear gradient of a saline buffer, it was possible to separate the two main whey proteins: β-LG, α-LA (Image 3).

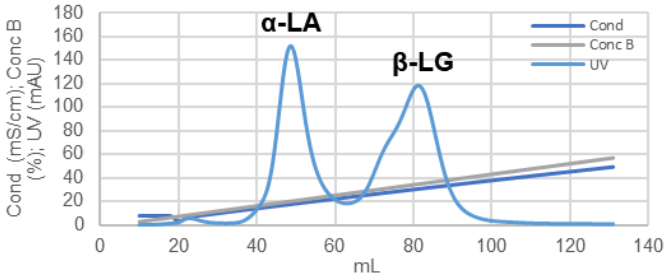


**Image 3.** Ion exchange chromatography profile (linear gradient – buffer B (1M NaCl))

## Conclusions

The adapted separation protocol is promising. Although it needs to be improved in order to magnify the amount of isolated proteins.

## Acknowledgement

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