Characterization of milk protein aggregates as a function of casein micelles/whey proteins ratio by Asymmetrical Flow Field Fractionation (AF4) coupled with Multiangle Laser Light Scattering (MALLS)

Introduction: Currently, most of dairy emulsions at neutral pH contain thickening or gelling agents for improving their texture. However, manufacturers are more and more seeking for the substitution of these food additives by natural ingredients like proteins. During heat treatment of milk, whey proteins are denatured and can interact with casein micelles (Cas) to form Mixed Aggregates (MA). In dairy emulsions, MA are able to adsorb at the oil-water interface and texturize emulsions by connecting oil droplets. In this way, MA could be an alternative to additives used in dairy emulsions. The casein micelles/whey proteins (Cas/WP) ratio is a key parameter for the production of MA. Asymmetrical Flow Field Flow Fractionation coupled with MALLS could be an interesting method to define the best Cas/WP ratio (80/20 or 20/80) to use. Moreover, this technique can be used to determine the stability of MA to calcium concentration change and pH variation.

I. Materials and Methods

\[ \text{MALLS} \]
\[ \text{UV Detector} \]
\[ \text{Differential Refractometer (DRI)} \]

II. Effect of calcium on the integrity of casein micelles (pH6.3)

- Without calcium: casein micelles are dissociated
- With calcium: the integrity of casein micelles is maintained (1.6.10^6 g mol⁻¹)

III. Characterization of Mixed Aggregates as a function of Cas/WP ratio (pH6.3; 10mM Ca)

- 80/20 ratio
- 20/80 ratio

Conclusions and Perspectives: MA are mainly produced using the 80/20 Cas/WP ratio whereas only pure whey protein aggregates are obtained with the other ratio. Moreover, whey proteins aggregates seem to interact preferentially with large casein micelles. However, in the absence of calcium, casein micelles are dissociated and no MA is observed.

In presence of calcium, MA are stable in the pH range 6.3-7 allowing them to be used as food additive substitutes in neutral dairy products. AF4-MALLS is an interesting alternative to characterize MA in comparison with size exclusion chromatography where caseins interact with the stationary phase and are retained on the column.


Figure 1: AF4 elugrams (UV: dotted line) and molar mass (continuous line) of casein micelles with 10mM Ca (blue) and without Ca (green).

Figure 2: AF4 elugrams (DRi: dotted line) and radius of gyration (continuous line) of casein micelles (blue), MA 80/20 (red) and WPA 1% (black).

Figure 3: AF4 elugrams (DRi: dotted line) and radius of gyration (continuous line) of casein micelles (blue), MA 80/20 (red) and WPA 2.4% (black).