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

Thibault Loiseleux, Agnès Rolland-Sabaté, Thomas Croguennec, Catherine Garnier, Sophie Guilois-Dubois, Marc Anton, Alain Riaublanc

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Thibault LOISELEUX<sup>1</sup>, Agnès ROLLAND-SABATE<sup>1</sup>, Thomas CROGUENEC<sup>2</sup>, Catherine GARNIER<sup>1</sup>, Sophie GUILLOIS<sup>1</sup>, Marc ANTON<sup>1</sup> and Alain RIAUBLANC<sup>1</sup>

<sup>1</sup> UR1268 INRA, Biopolymers Interactions Assemblages, Nantes, France

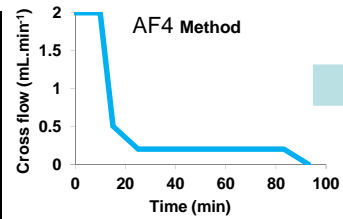
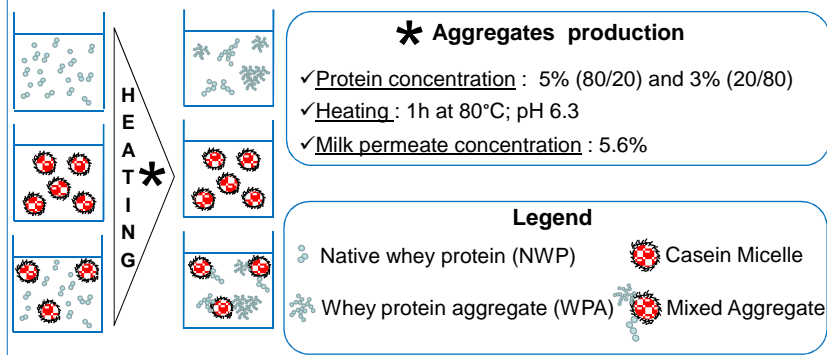
<sup>2</sup> UMR 1253 INRA, Science and technology of milk and egg, Rennes, France



**Introduction :** Currently, most of dairy emulsions at neutral pH contain thickening or gelling agent 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<sup>1</sup>. 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



- Detectors**
- ✓ MALLS
  - ✓ UV Detector
  - ✓ Differential Refractometer (DRi)

Eluent		NaCl 45mM; pH6.3 or 7; CaCl <sub>2</sub> 0 or 10mM
Membrane		Cellulose
Spacer (µm)		350
Temperature (° C)		25

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

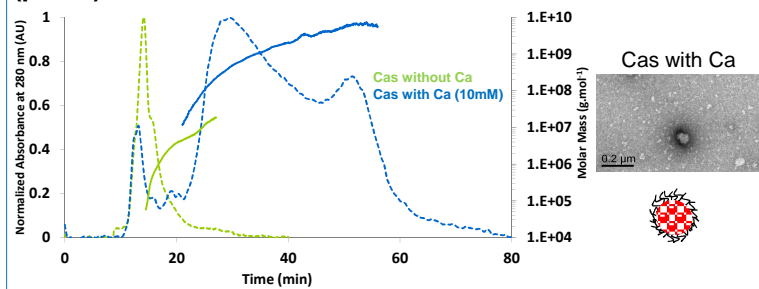


Figure 1: AF4 elugrams (UV; dotted line) and molar mass (continuous line) of casein micelles with 10mM Ca (blue) and without Ca (green)

- Without calcium: casein micelles are dissociated
- With calcium: the integrity of casein micelles is maintained ( $1.6 \cdot 10^9$  g.mol<sup>-1</sup>)

## IV. Effect of pH on the stability of MA 80/20

Mixtes 80/20	Rg (nm)	Mw (g.mol <sup>-1</sup> )
pH 7	184.9	$4.0 \cdot 10^9$
pH 6.3	190.6	$3.7 \cdot 10^9$

Micelle	Rg (nm)	Mw (g.mol <sup>-1</sup> )
pH 7	147	$1.6 \cdot 10^9$

pH: MA 80/20 are stable regardless the pH

- Radius:  $Rg_{MA\ 80/20} > Rg_{Cas}$
  - Molar Mass:  $Mw_{MA\ 80/20} > Mw_{Cas}$
- AdSORption of WPA on the Cas

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

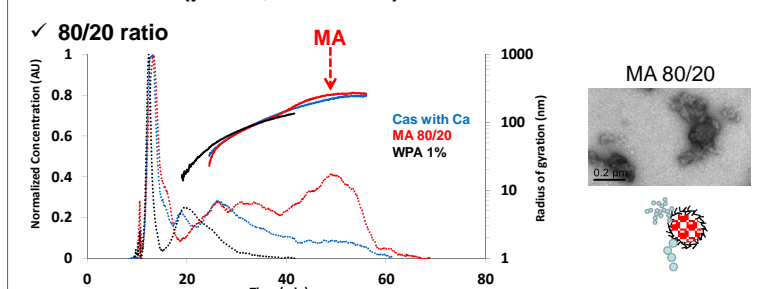


Figure 2: AF4 elugrams (DRi; dotted line) and radius of gyration (continuous line) of casein micelles (blue), MA 80/20 (red) and WPA 1% (black)

- Concentration: MA 80/20 are mainly composed of large aggregates
- Between 40 - 60 min :  $Rg_{MA\ 80/20} > Rg_{Cas}$  ----> MA formation

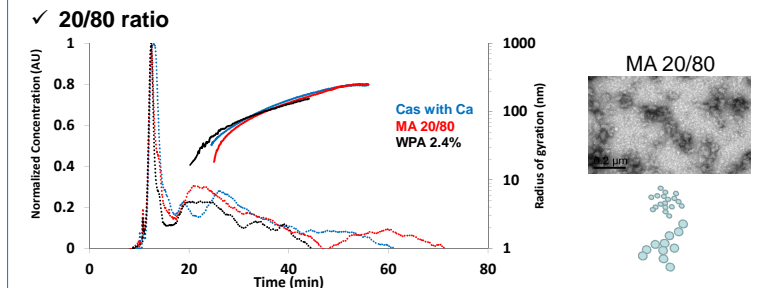


Figure 3: AF4 elugrams (DRi; dotted line) and radius of gyration (continuous line) of casein micelles (blue), MA 20/80 (red) and WPA 2.4% (black)

- Concentration: MA 20/80 are mainly composed of small aggregates
- Between 40 - 60 min:  $Rg_{MA\ 20/80}$  close to  $Rg_{Cas}$  ----> MA

**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. However, in the absence of calcium, casein micelles are dissociated and no MA is observed. On the contrary, 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.

**Reference:** 1. Surel, C., Fouquier, J., Perrot, N., Mackie, A., Garnier, C., Riaublanc, A., & Anton, M. (2014). Composition and structure of interface impacts texture of O/W emulsions. *Food Hydrocolloids*, 34, 3-9.