Performance of Filed-Flow Fractionation technique for milk protein characterization

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INP TOULOUSE PURPAN TOULOUSE FIELD-FLOW FRACTIONATION CENTER

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Toulouse FFF Center (TFFFC): Performance of Field-Flow Fractionation techniques for milk protein characterization

Marie Hennetier[1], Audrey Ric[1], Estelle N'tsiba[1], Mireille Gaucher[1], Amira Halabi[3], Thomas Croguennec[3], Audrey Romelard[4], Alain Baniel[4], Frédéric Violleau[1,2]

[1]*Toulouse FFF Center, Université de Toulouse, INP-PURPAN, Toulouse, France*
[2]*Laboratoire de Chimie Agro-industrielle, LCA, Université de Toulouse, INRA, Toulouse, France*
[3] *AGROCAMPUS OUEST-INRA Science & Technologie du Lait et de l'Oeuf (STLO), Rennes, France*
[4] *Ingredia, Arras, France*

Aims of the project:

The Toulouse FFF Center (TFFFC) project aim is the development of a shared research platform for characterization of complex samples using Field-Flow Fractionation technologies. The project is built around collaborative projects with partners from various fields of activities such as agrifood, polymer chemistry, renewable carbon valorization and analytical equipment. The financial support of the Region Occitanie, European funds and industrial partners make possible the creation of an analytical platform dedicated to FFF technologies unique in Europe and to develop innovative approaches and tools allowing the characterization of complex samples by these Field-Flow Fractionation technologies. The performance of these techniques will be evaluated for the characterization various nano and micro-structures like proteins. We will also develop analytical methodologies using different FFF techniques to better characterize complex milk protein samples used as agro-food texturing agents and lignocellulosic materials, a product of interest in renewable carbon chemistry. These projects will be carried out in collaboration with industrial partners.

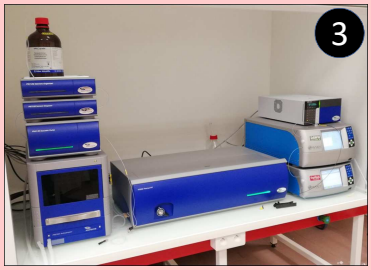
FFF techniques available:

The platform is equipped with all existing FFF technologies today (FlFFF, EAF4, SdFFF, ThFFF). The coupling of these fractionation tools with different detection tools (UV, RI, Static scattering and light dynamics, particle analyzer) will make it possible to determine essential criteria (Molecular weight distribution, Size distribution, Conformation / form, State aggregation ...) to control the quality and performance of different type of products. The platform is opened to all industrial and academic partners wishing to evaluate the performance of these technologies in fields as varied as pharmacy, cosmetics, nanotechnologies, agri-food, biotechnologies, materials ...

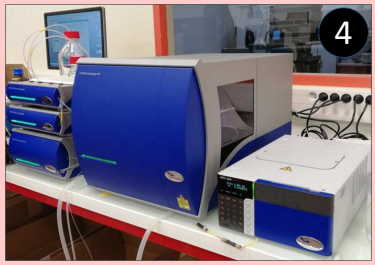
(1) AsFlFFF & EAF4-UV-MALS-RI

(2) 2D-AsFlFFF×LC-UV-MALS-RI

(3) ThermalFFF- UV-MALS-RI



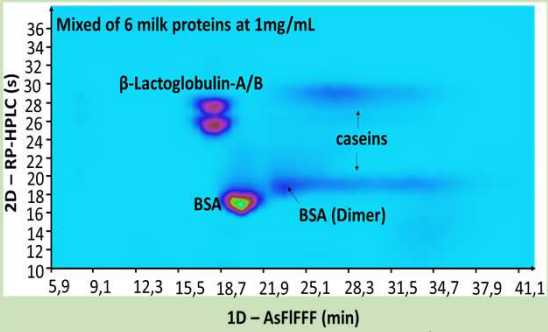
(4) CentrifugalFFF-UV



Results:

Milk Proteins standards analysis by Full comprehensive 2D – AsFlFFF × LC - UV

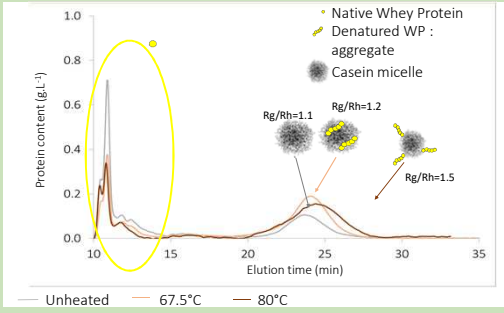
2D AsFlFFF-RP-HPLC was investigated to separate whey proteins and caseins. This technique allows to separate proteins by their size and by their polarity. A whey protein standard mix is studied: α-lactalbumine, β-lactoglobuline A and B, BSA, and 3 caseins. A 2D map is obtained with all separated proteins.



Conditions: 1D-AsFlFFF: spacer 350µm ; V_{out}=0,5mL/min ; eluant: PBS
2D-HPLC : Poroshell 300 C8 ; A: water 0.1% TFA; B: ACN 0.07% TFA

Infant milk formulation analysis by AsFlFFF-MALS-RI

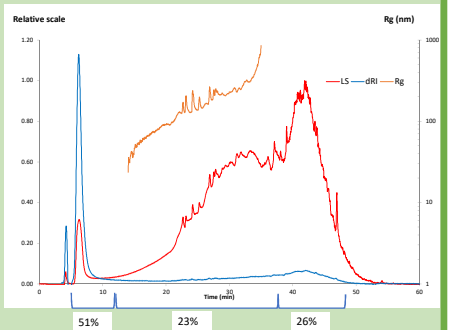
Pasteurized Infant milk formulation without fat was investigated by AsFlFFF-MALS-RI. The technique separates the non-aggregated whey proteins to the casein micelles. Native whey proteins content decreases with the pasteurization to make aggregates which fixed to the casein micelles. Shape ratio Rg/Rh of casein micelles + aggregates increases with the temperature. At 67.5°C, aggregates are fixed inside the micelle, at 80°C they are fixed outside the micelles (+TEM results).



Conditions: Spacer 250µm ; V_{out}=1ml/min Vx = 4 to 0,1mL/min ; Eluant: NaCl 50mM + $CaCl_2$ 5mM

Milk protein aggregates characterization by CentrifugalFFF-MALS-RI

Milk proteins after heat treatment were studied by Centrifugal FFF coupled with MALS and RI. Aggregates with the lowest density and size elute in the void peak and represent 54% of the mass fraction. For the bigger one, there are two populations: one smaller than 1µm and one higher, up to 4.5µm (collect + DLS batch because MALS can't calculate size after 1µm).



Conditions: Flow rate: 1,2 ml/min ; Speed rotation: from 4500 to 50 rpm ; Eluant: Water + FL70 0,1%

Summary:

These works highlight the potentiality of FFF techniques to fractionate and characterize proteins and protein aggregates. Toulouse FFF Center is opened to all partners wishing to evaluate the performance of these technologies in fields as pharmacy, cosmetics, nanotechnologies, agri-food, biotechnologies...

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