

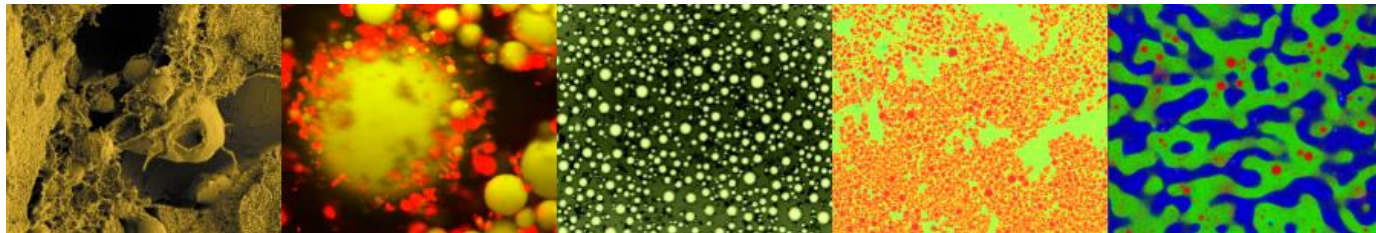
# Protein aggregates modulate the texture of emulsified and acidified dairy model systems

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*8<sup>th</sup> – 11<sup>th</sup> April 2018 – The University of Leeds, UK*



**17th Food Colloids Conference :  
Application of Soft Matter Concepts**



# Context

- **PROFIL** : Assemblages **PRO**téiques multi-**Fonctionnels** pour l'Innovation en industrie Laitière

(MultiFunctional **Protein** assemblies for Innovation in miLk industry)

- Answer to **consumer expectations** with dairy products based on **100% milk ingredients** and "clean label" products (removal of texturing agents...)
- Creating new products



- **Texturizing properties**

- *Texturizing emulsion at **neutral pH** with protein aggregates*
- **Texturizing ACID** milk gels using protein aggregates



# Scientific context

- Obtaining a milk acid gel:

  **Select fat and protein content** .....→

↓  
**Homogenization**

↓  
**Heat treatment**

↓  
**Acidification kinetics  
(GDL)**

↓  
**Cooling and cold storage**



↗ **protein concentration**: increase in gel strength and stabilization of the system (CHEFTEL and LORIENT, 1982 ; ANDOYO *et al.*, 2015)

↗ **fat concentration**: increase in gel strength and decrease gelation time (AGUILERA *et al.*, 1993)

↓  
**Protein and fat concentration will be modulated during this work**



**3.5 to 5%**



**0 to 10%**



# Scientific context

- Obtaining a milk acid gel:

  **Select fat and protein content**



**Homogenization**



Size of fat droplets, prevent separation of fat and whey (LUCEY and SINGH, 1998)



**Homogenization pressure will be modulated**



**50 – 100 or 500 bars**

Heat treatment

Acidification kinetics  
(GDL)

Cooling and cold storage






# Scientific context


- Obtaining a milk acid gel:

  **Select fat and protein content**

  
**Homogenization**

  
**Heat treatment**

  
**Acidification kinetics  
(GDL)**

  
**Cooling and cold storage**

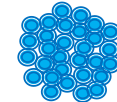


## Influence of protein aggregates addition:

- **Best reactivity to gelation** → higher pI and hydrophobicity (LUCHEY *et al.*, 1997 ; ANDOYO *et al.*, 2015)
- **Functionalization of micelles by protein aggregates** → gelation at higher pH (LUCHEY and SINGH, 1998 ; FAMELART *et al.*, 2011)



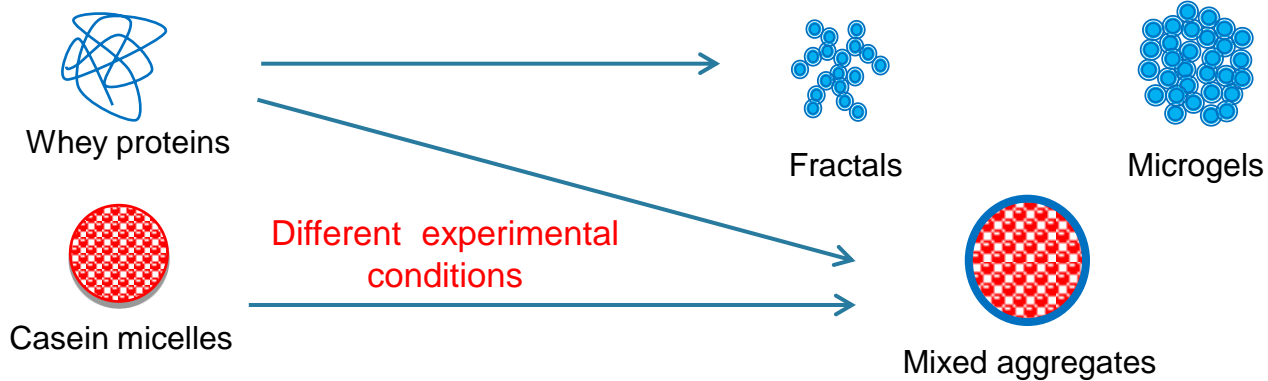
**Various protein aggregates will be used to improve texture of acid milk gel**



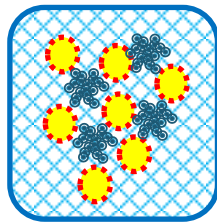
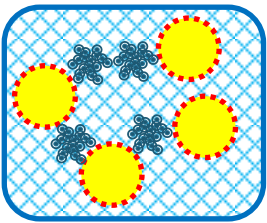
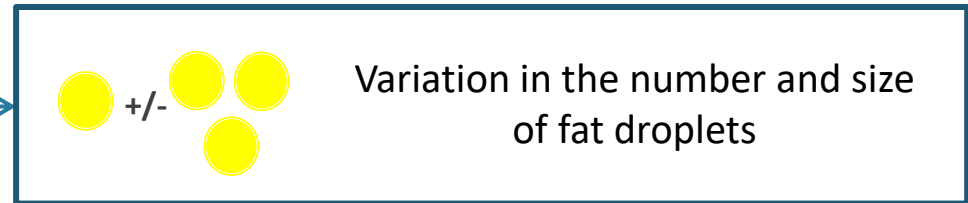


# Objectives

## Preparation of different types of protein aggregates



Use in acidified systems: yoghurt (pH 4.6, gel formation by casein precipitation)



### Objectives :

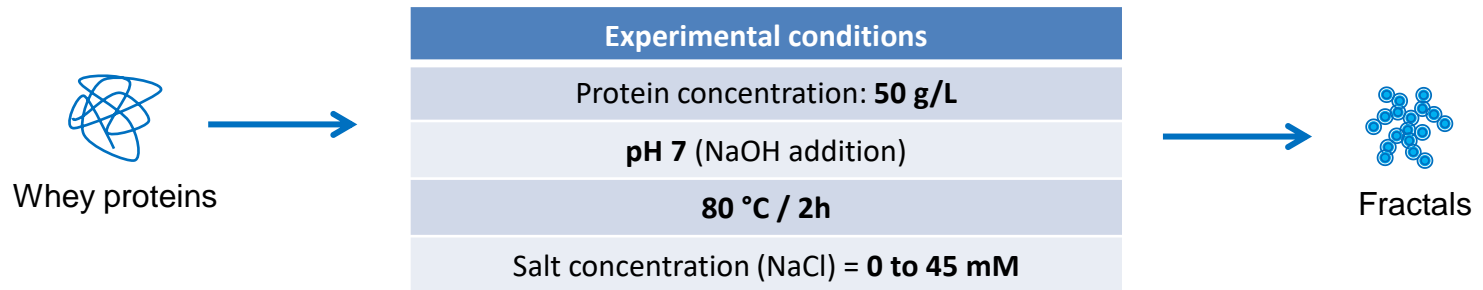
Understanding the **interactions** between **protein aggregates** at the **interface** and **proteins** in the **continuous phase** of the acid gel

Use protein aggregates to **control and vary the texture** of acidified systems

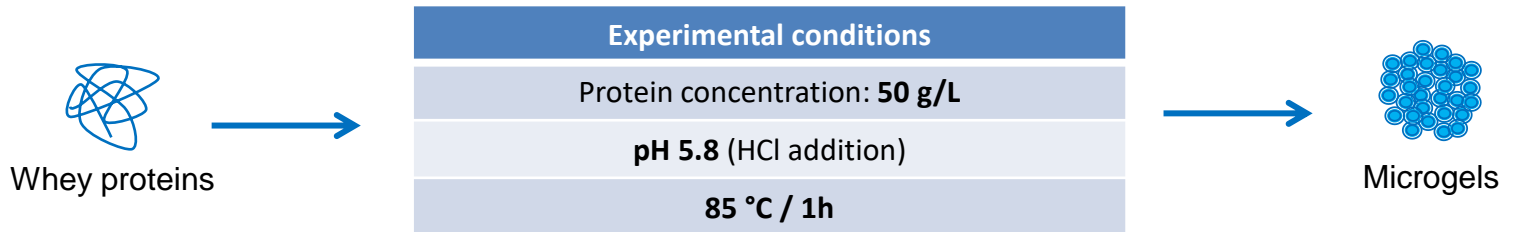


# Methods

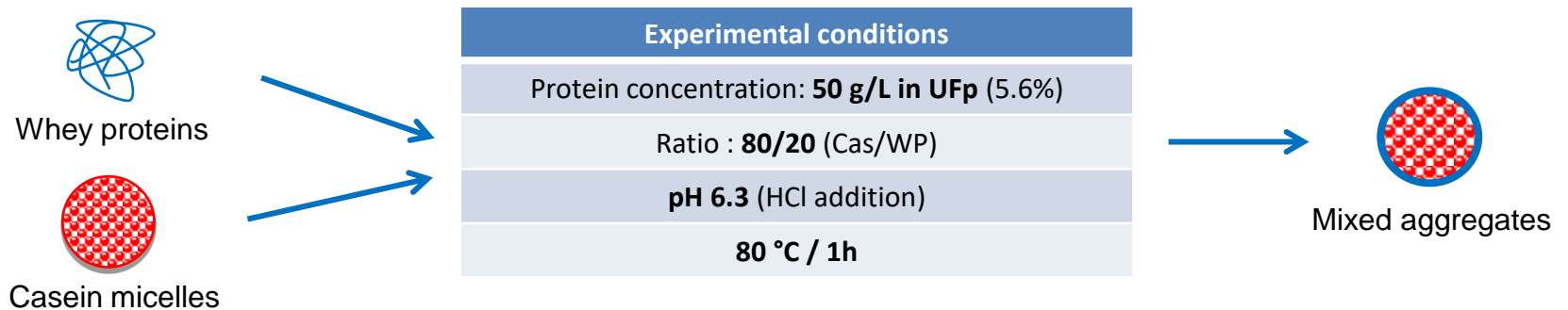
- Preparation of fractal aggregates (MAHMOUDI *et al.*, 2007):



- Preparation of microgel aggregates (SCHMITT *et al.*, 2010):

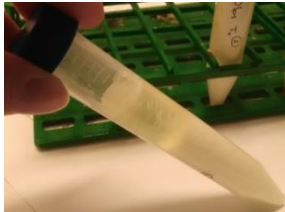
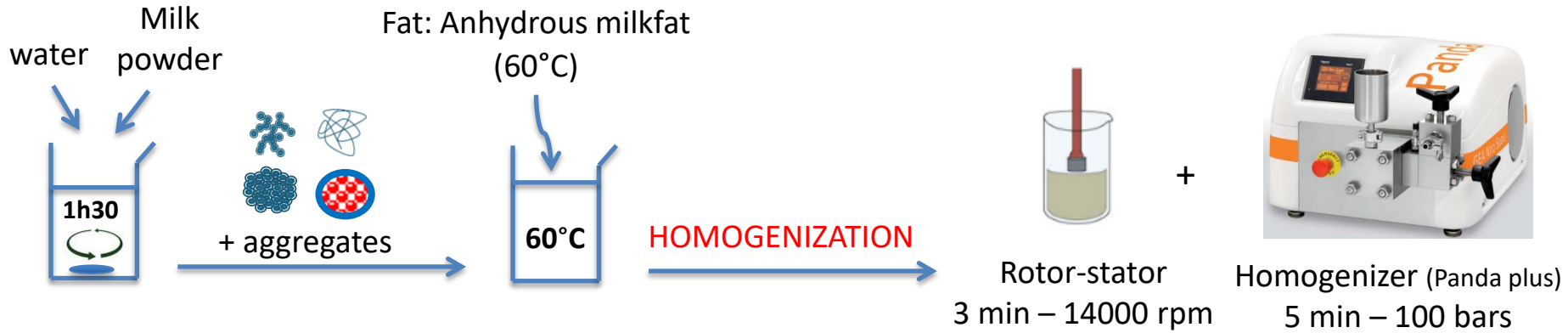


- Preparation of mixed aggregates (ANEMA and LI, 2000):

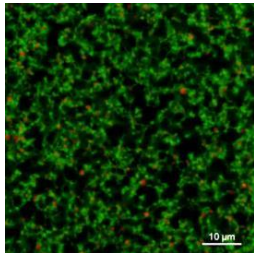




# Methods



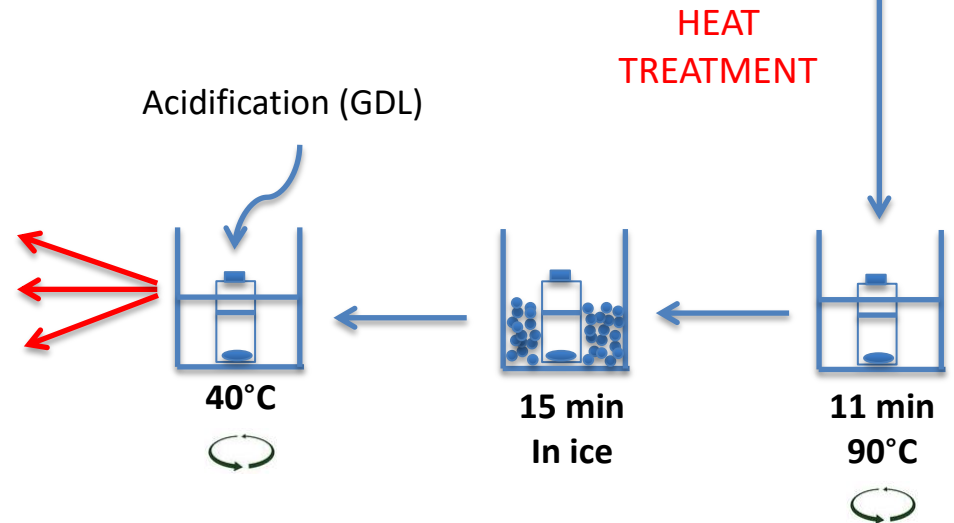
Analysis of syneresis



Microscopy



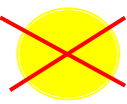
Rheological measurements



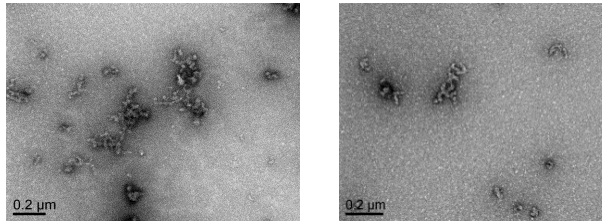




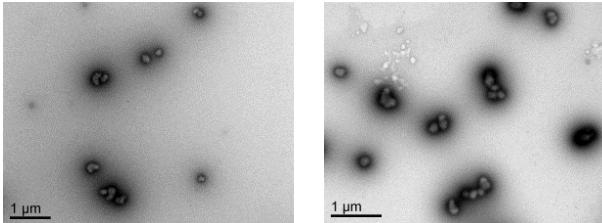
# Acid milk gel **without fat**



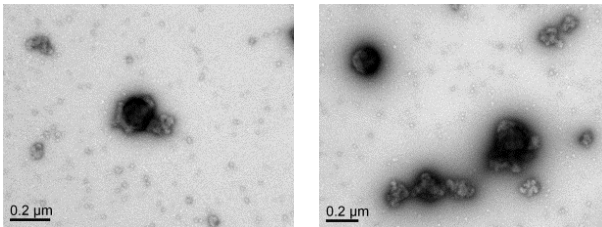
Better structuring and firmness,  $\searrow$  gelling time with **increasing concentration**  
Decrease of syneresis



Same results than with WP but **less efficient**



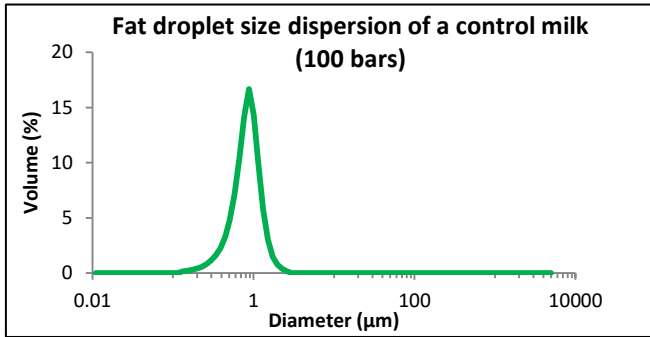
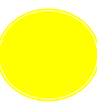
No improvement of firmness and no decrease of syneresis



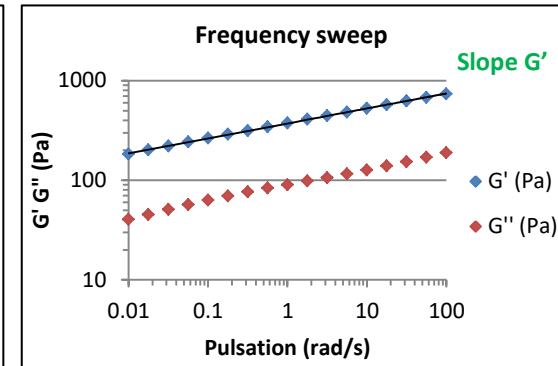
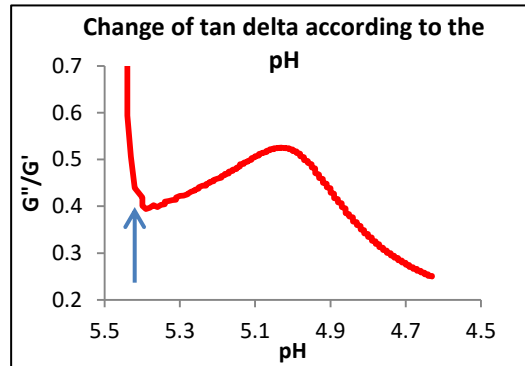
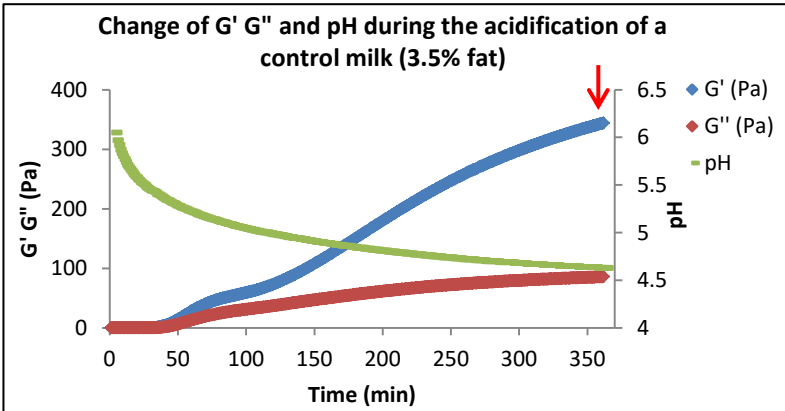
**→ Protein enrichment of products**



# Control acid milk gels with 3.5% fat (100 bars)



- Average diameter of fat droplets : **0.8 μm**

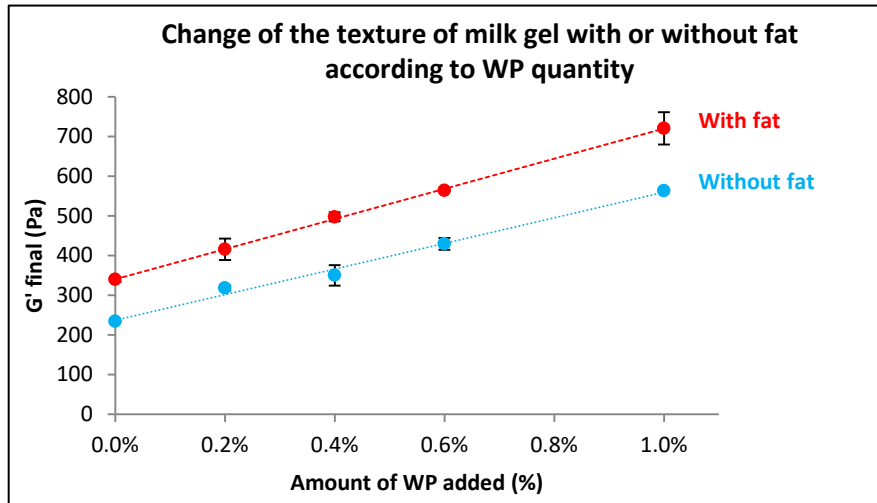
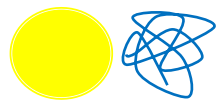


Sample	Gelation time (min)	Gelation pH	G' max (Pa)	Final pH	Slope G' (FS)
Without fat	29	5.44	234	4.60	0.164
<b>3.5% fat</b>	<b>29.4</b>	<b>5.45</b>	<b>340</b>	<b>4.63</b>	<b>0.150</b>

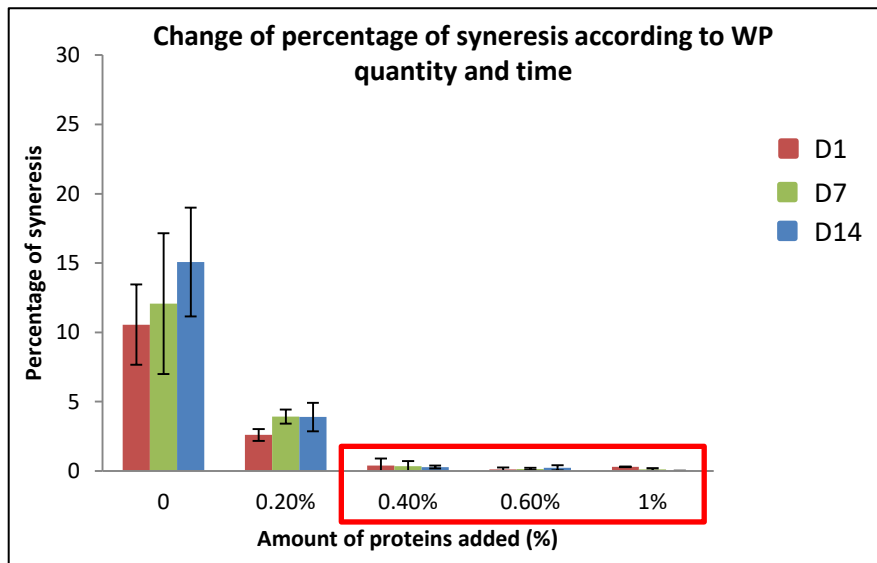
- **Time and pH of gelation similar** for control acid milk gels with or without fat
- **3.5% fat: 100 Pa more** than control acid gel without fat



# Acid milk gels with 3.5% fat and WP



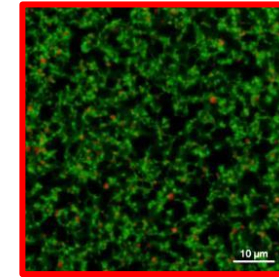
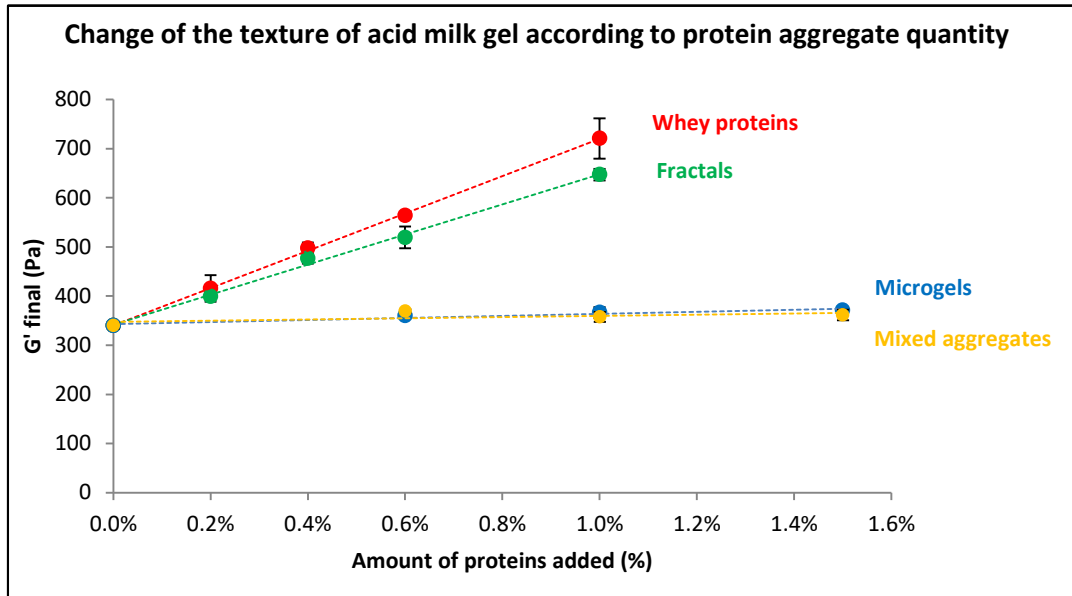
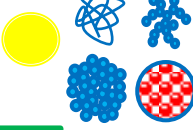
- Linear increase of the gel strength (final G') with WP concentration (up to 1% WP)
- Decrease of tan (delta) amplitude and FS slope with increasing concentration of WP → **more structured protein network**
- Similar evolution with or without fat → **implication of the interface ?**



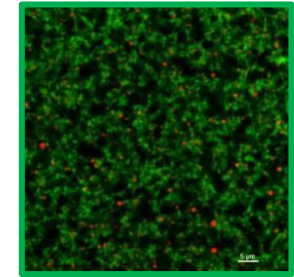
- Syneresis: close to 0% from 0.4% added WP and **stable over time** (D1, D7 and D14)
- Without fat: 50%



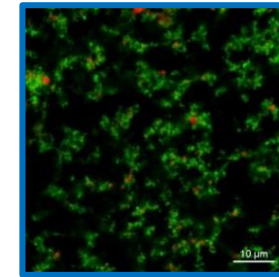
# Acid milk gels with 3.5% fat and aggregate



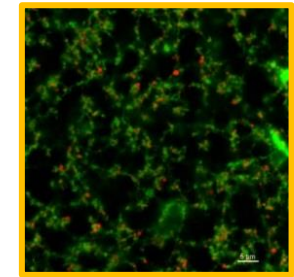
1% WP



1% fractals



1% microgels



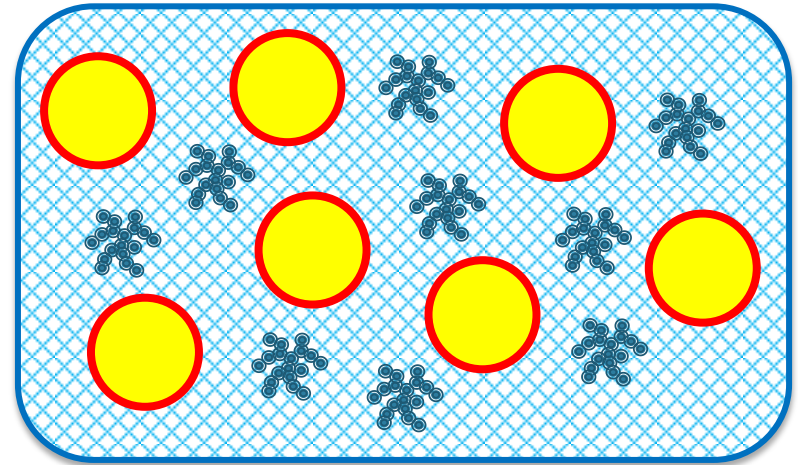
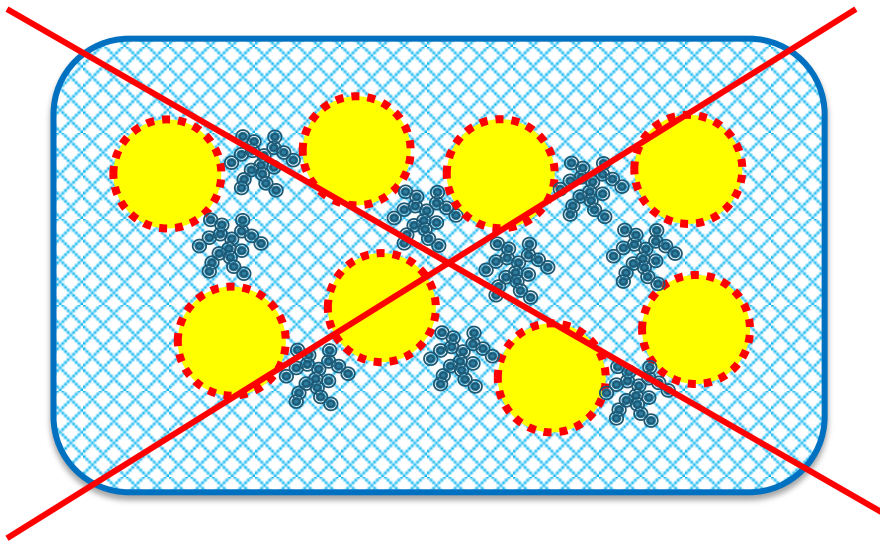
1% mixed aggregates

- + Fractals: **Increase of the gel strength** (slightly lower than for WP addition)
  - Same evolution WP/fractals → **impact on the interface, role in the continuous phase ?**
- + Microgels and mixed aggregates: **constant** final  $G'$ , no modification with increasing concentration → **connection with the network ?**



# Hypothesis on modulation of the texture

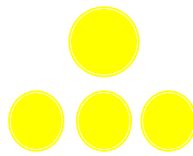
- **Hypothesis**: Modulation of the texture by control of the continuous phase
  - Aggregates in the continuous phase (connected or not to the protein network)
  - Saturation of the surface of fat droplets by caseins



- **Modulation of the interface surface**



# Modulation of the interface



Classic protocol



Modulation of the interface

Milk with **3.5% fat (100 bars)**



Variation in the concentration of aggregates

Milk with **3.5% fat (50 - 100 or 500 bars)**



Constant concentration in aggregates (1%)

Milk with **10% fat (100 or 500 bars)**

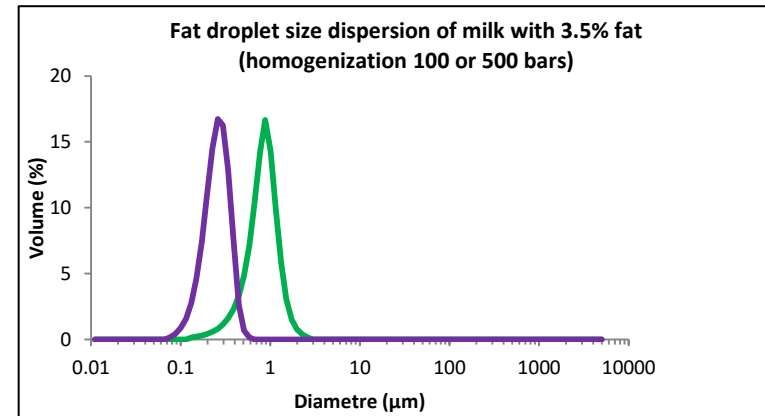


Constant concentration in aggregates (1%)

Milk with **3.5% or 10% fat (100 or 500 bars)**



Constant concentration (1%) but variable fractal size

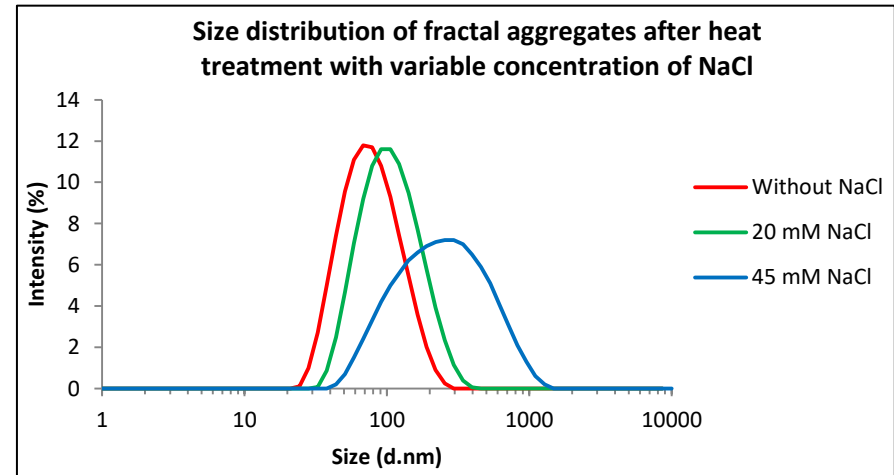




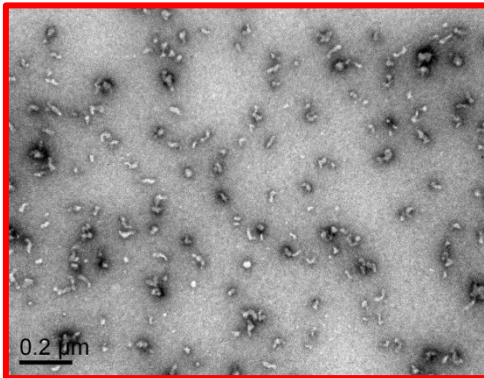
# Variation of fractal aggregate size



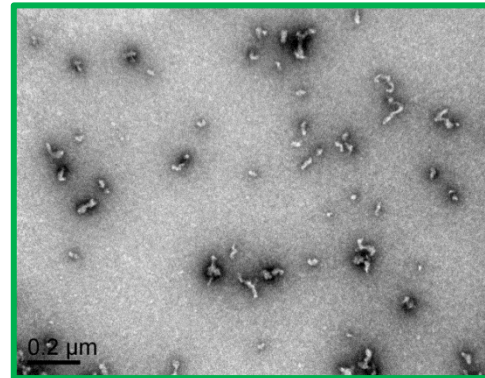
- Preparation of fractal aggregates:
  - Heat treatment: **2h at 80°C**
  - **Variable concentration of NaCl** (to change the size of aggregates)



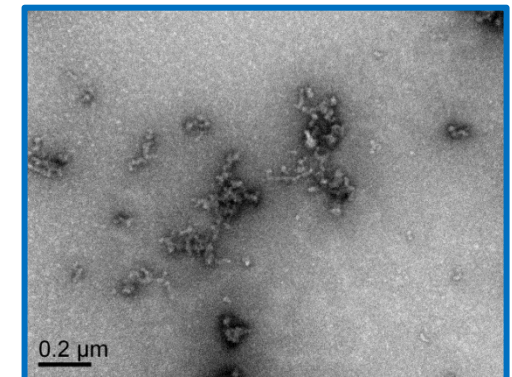
- Observation of fractal aggregates by Transmission Electron Microscopy (TEM):



Without NaCl  
**68 nm**



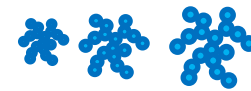
20 mM NaCl  
**93 nm**



45 mM NaCl  
**228 nm**



# Variation of fractal aggregate size



Milk with **3.5% fat**  
(100 or 500 bars)



228 nm



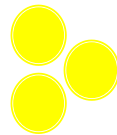
93 nm



68 nm

Constant concentration  
(1%) but variable fractal size

Milk with **10% fat**  
(100 or 500 bars)



228 nm

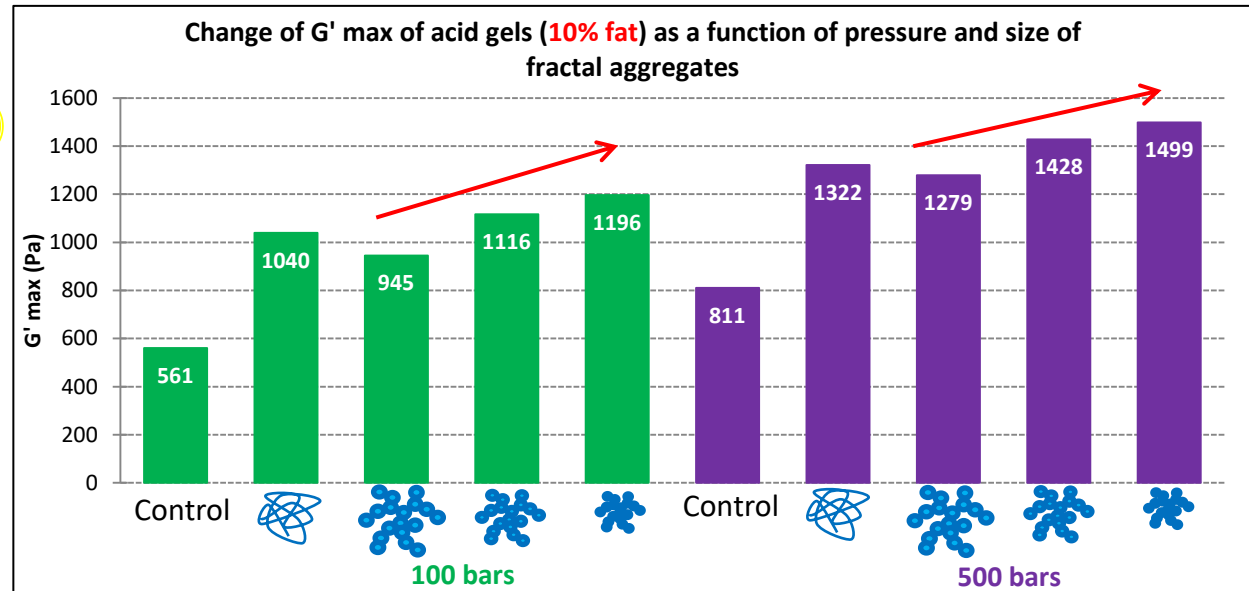
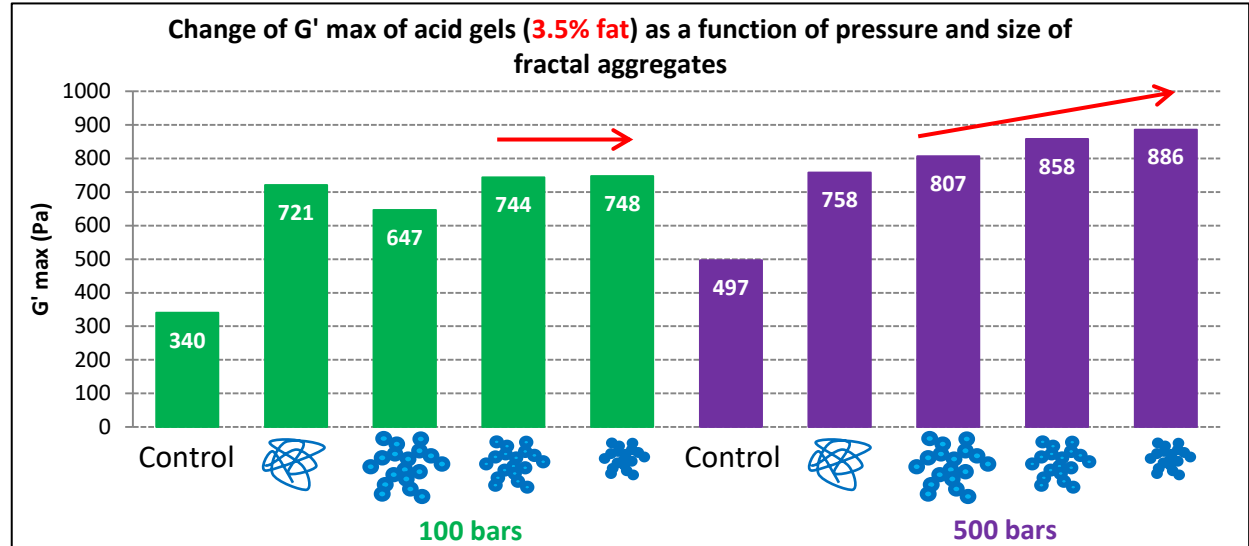


93 nm



68 nm

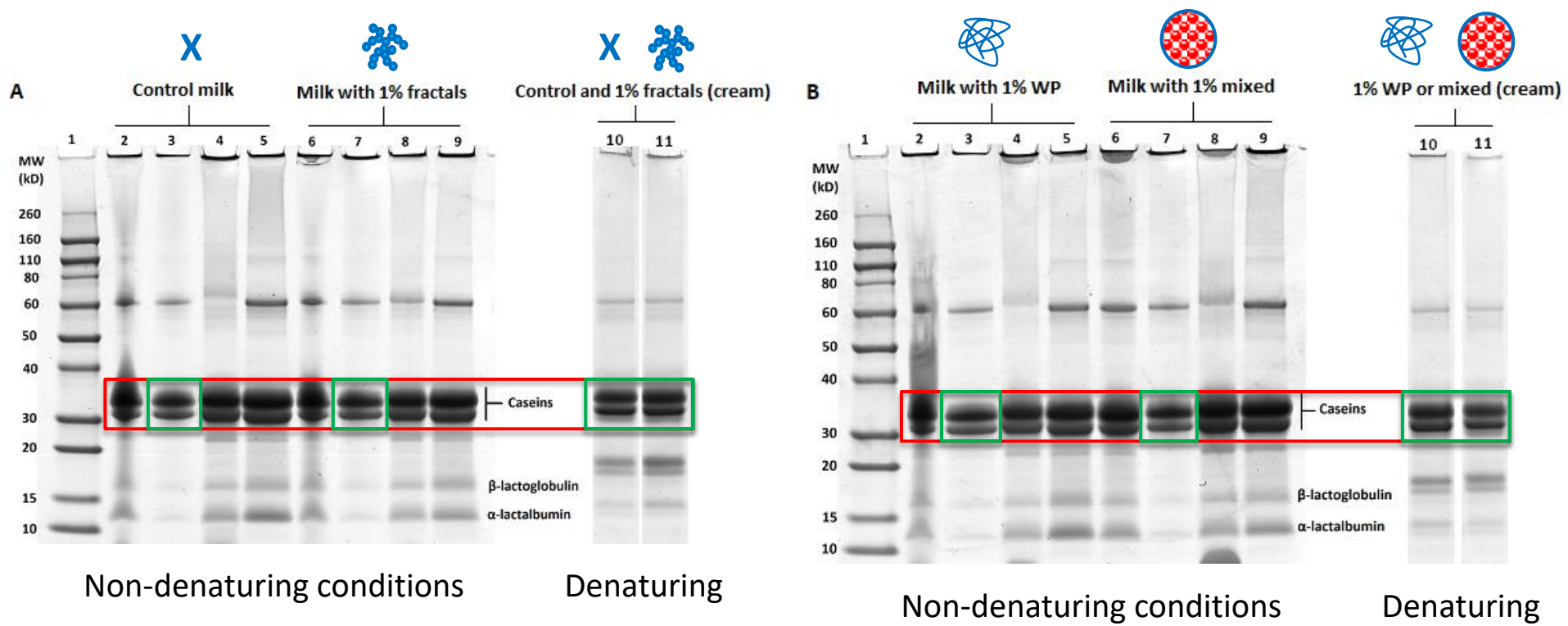
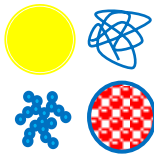
Constant concentration  
(1%) but variable fractal size







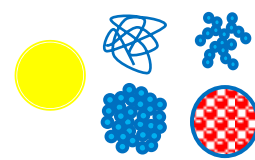
# Interfacial composition of acid milk gels



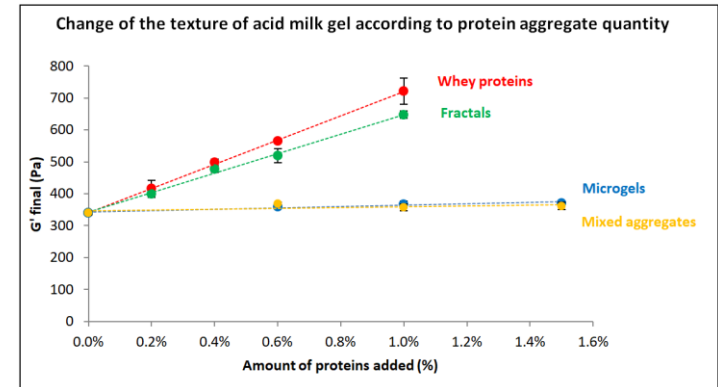
→ **Saturation of the interface by milk caseins** (aggregates can not access the interface)



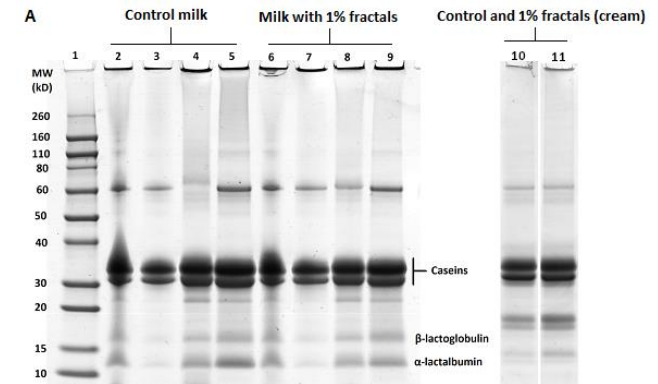
# Conclusions on acid milk gels with fat and aggregates



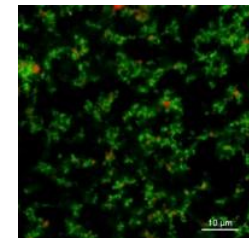
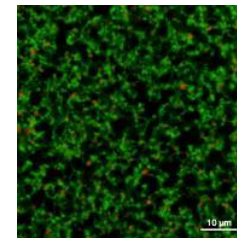
- Results dependent on the type of added aggregates  
→ **Modulation of the texture** (specific products)



- Similar changes for systems with or without fat  
→ **saturation of the interface by milk caseins**

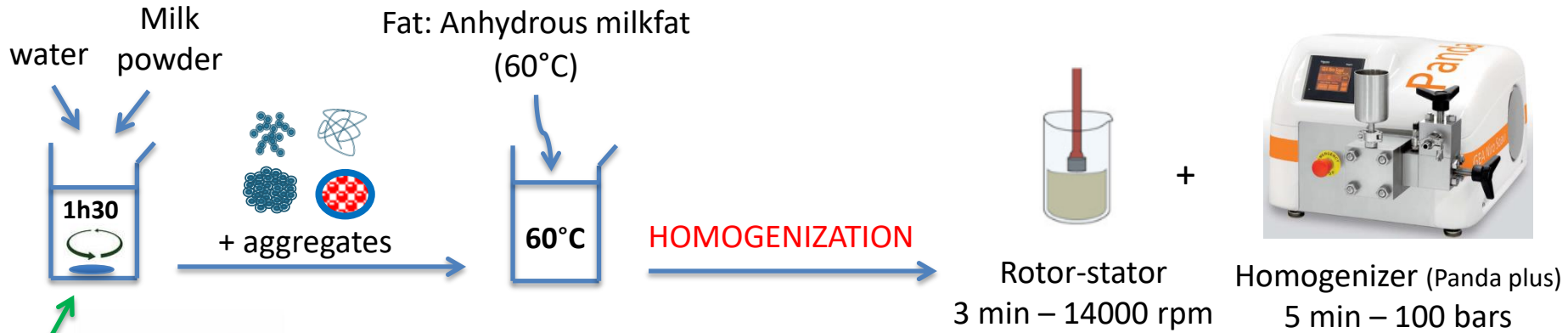
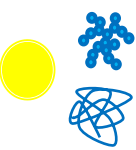


- Different results depending on the type of aggregates  
→ **role in the continuous phase**





# Modulation of the interaction by NEM



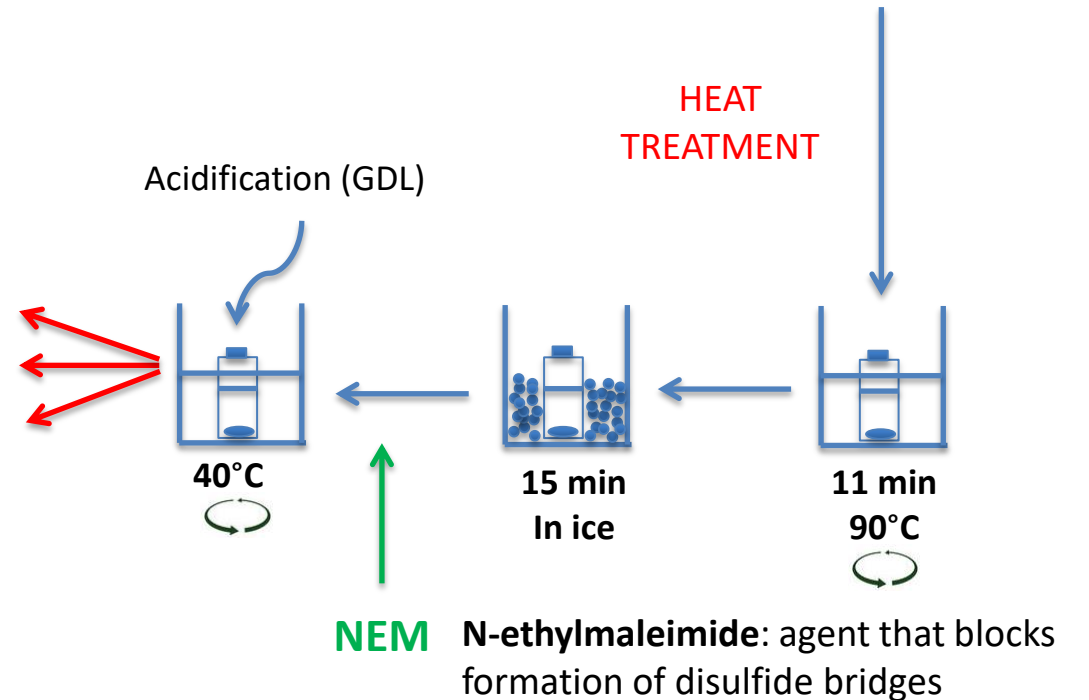
**NEM**

Analysis of syneresis

Microscopy

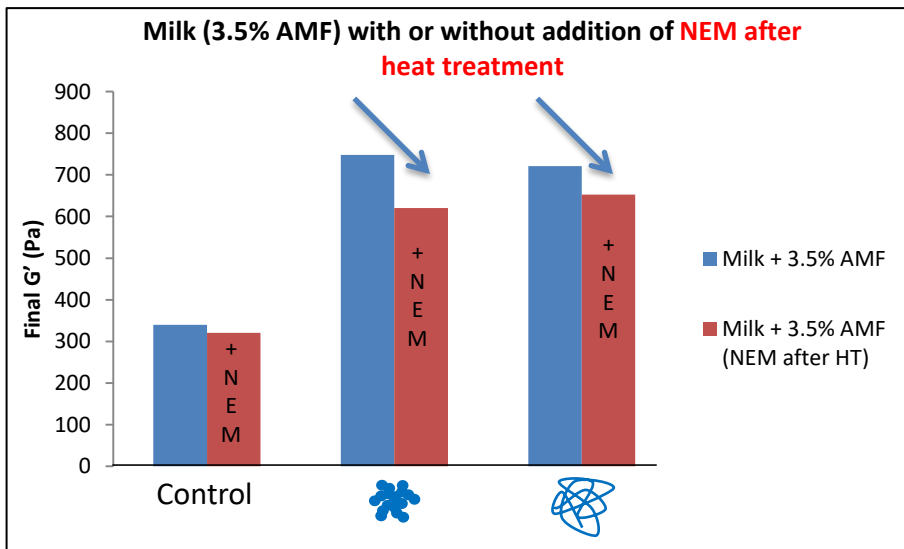
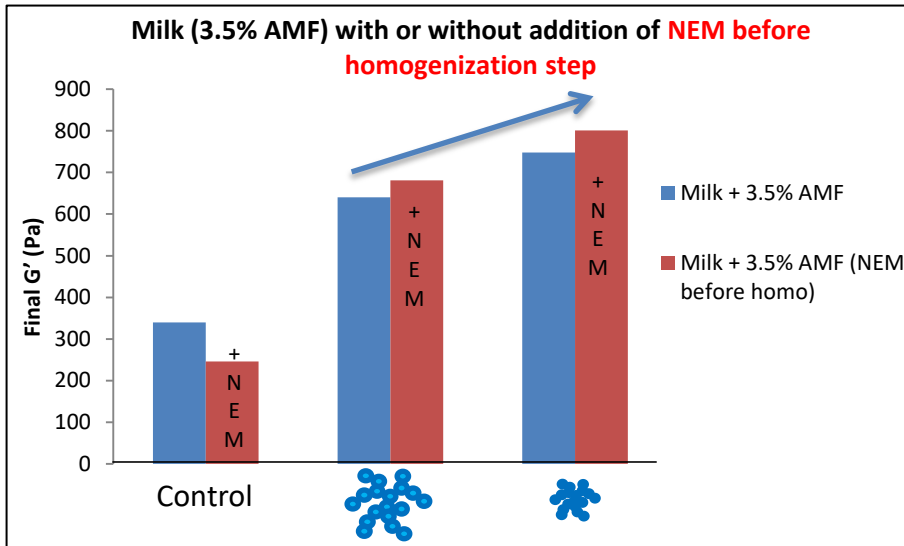
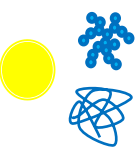


Rheological measurements





# Modulation of the interaction by NEM

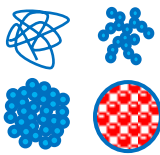



- WP: measurements are not possible → gels break during experiments
- Fractals: gel strengthening with NEM → **formation of a large number of low energy bounds** (fractals/protein network)
- **Rupture of gel at low deformation**
- **Same evolution (fractals/WP)** → decrease of final  $G'$  with NEM (after HT)
- Possible **formation of disulfide bridges** during acidification and formation of the protein network

**Fractal aggregates allow the formation of many bounds (+ WP)**



# Conclusions



- Difficult to control the interface by addition of aggregates in yoghurt-like systems where caseins are predominant (80%) → **ability of caseins to adsorb preferentially on fat droplets** 
- **Fractal aggregates**: better results at high pressure and with small aggregates even at 3.5% fat
- **Microgel and mixed aggregates**: protein enrichment, no modification of texture with increasing concentration

→ **Modulation of the texture (firmness, syneresis, protein network) by the impact of aggregates in the continuous phase**

→ **Impact of small fractals → more interactions in the continuous phase**



# Perspectives

- Complete the experiments with **small fractal aggregates**: TEM of emulsions...
  - Investigate how systems are formed/structured:
    - Differences between fractal/mixed aggregates/microgels
- 
- Study interface/continuous phase **interactions**
  - Increase fat concentration (up to 15%)



# Acknowledgements

Milk Valley



- ✓ Interregional project PROFIL (supported by BbA industrial association and managed by Pôle Agronomique Ouest)
- ✓ Regional councils of Pays de la Loire and Brittany
- ✓ BIA unit



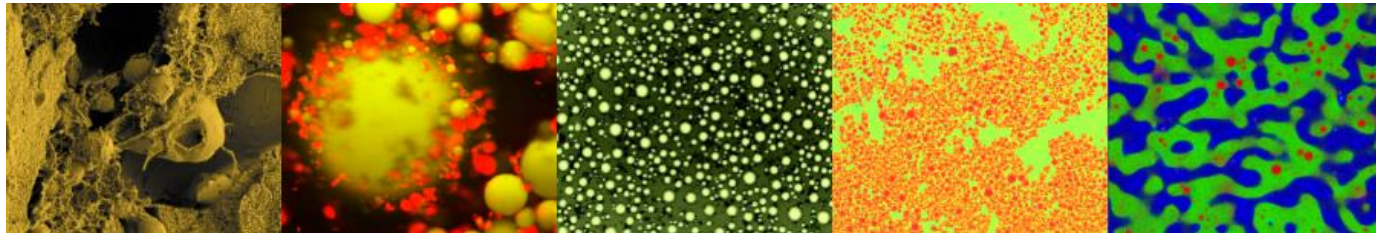
# Thank you for your attention

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*8<sup>th</sup> – 11<sup>th</sup> April 2018 – The University of Leeds, UK*