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## Heat treatment of concentrated milk protein system affect viscosity and enzymatic coagulation properties

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# Context



- High added value products

- Expanding market

- Many Properties

- Coagulation
- Setting agent
- Emulsifier...

Dairy protein ingredients

- Many outlets

- Infant formulas
- Cheese making
- Bakery...



Highly concentrated protein product with increasingly complex physico-chemistry

Process key stage :  
Heat treatment

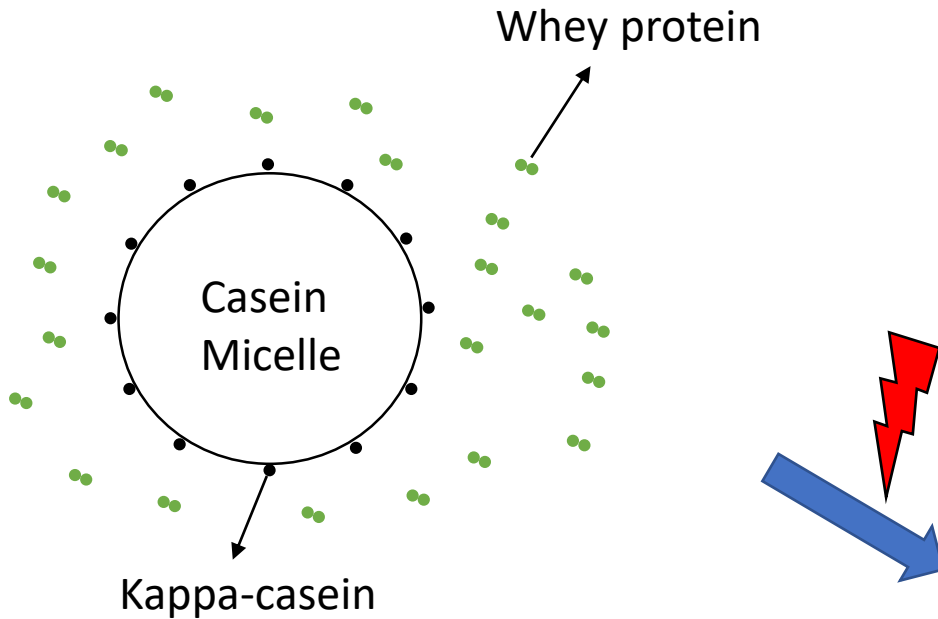
More knowledge needed for controlling functionalities

Impact of heat treatments on highly concentrated dairy protein systems ?



# ➤ Scientific Background

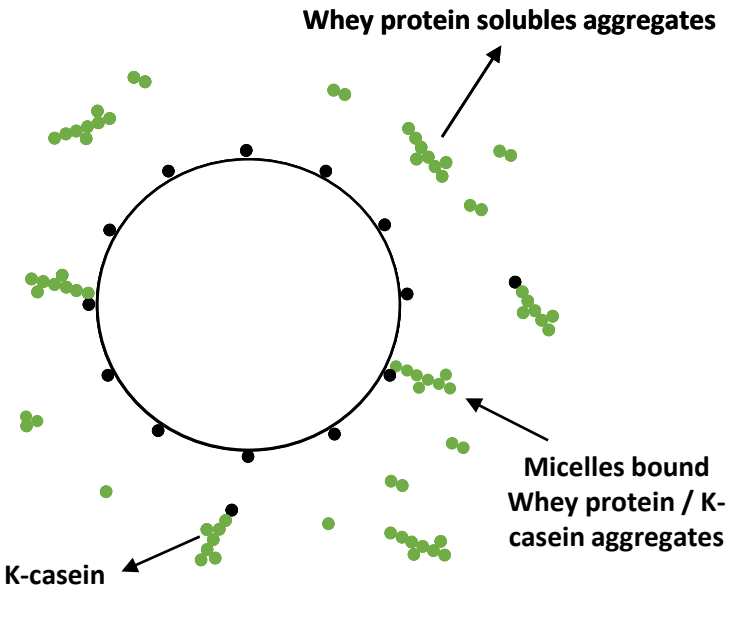
Heat treatment of protein solution :  
what happens in the case of milk?



Process : Temperature /  
duration of heat  
treatment

Physicochemical  
conditions : pH, ionic  
strength, whey  
protein/Caseins ratio...

(Smits and Van Brouwershaven .1980 ; Singh and Fox, 1985,1987 ; Anema and Klostermeyer, 1997 ; Oldfield et al. 2000 ; Anema and Li. 2003, Anema, 2009 ; O'Connell and Fox. 2003 ; Singh 2004)

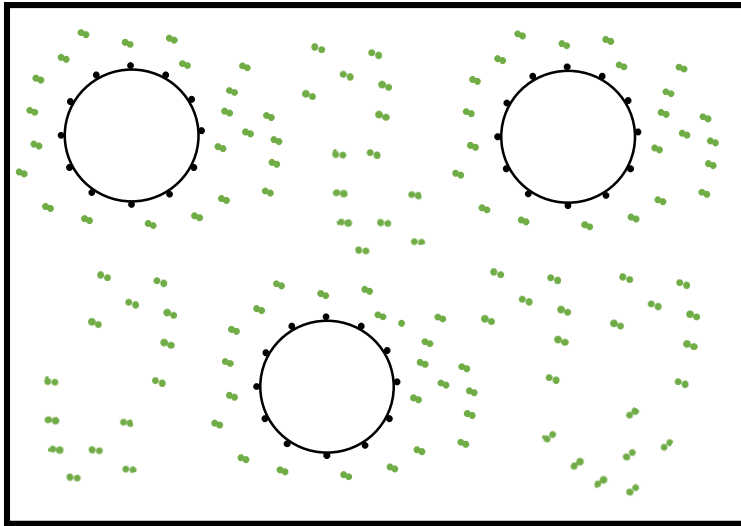


Solubles Whey protein / K-casein  
aggregates

# ➤ Scientific Background

## Heat treatment of dairy solution with different protein concentration :

Heat treatment of milk

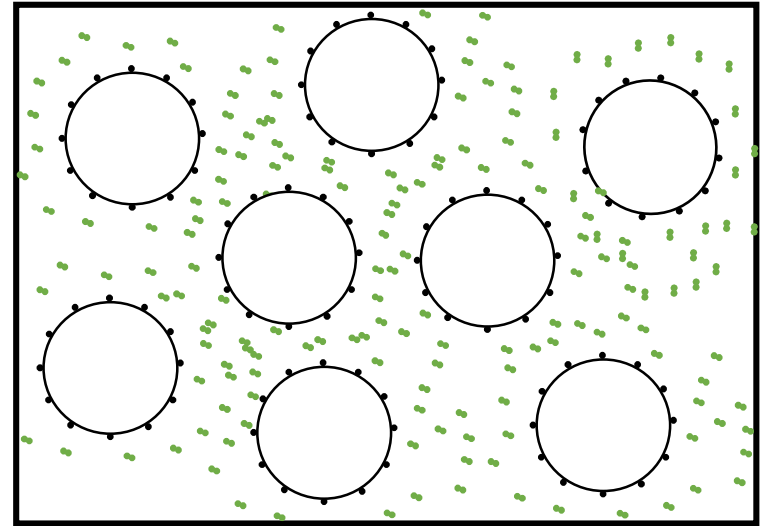


➤ [Protein] : 33 g/L



➤ [Protein] : 100-200 g/L

Heat treatment of highly concentrated dairy protein systems



**Many studies**

**Only parcellar understanding**

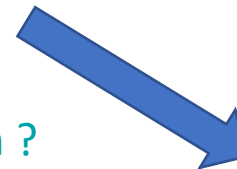
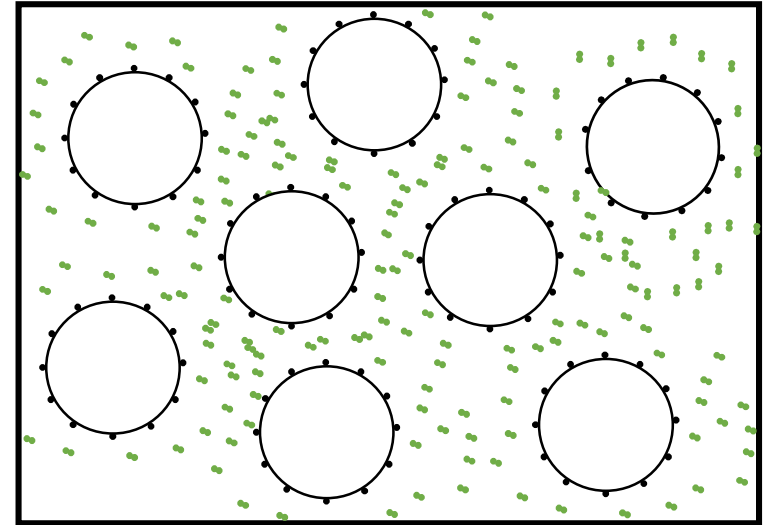
*(Smits and Van Brouwershaven .1980 ; Singh and Fox, 1985,1987 ; Anema and Klostermeyer, 1997 ; Oldfield et al. 2000 ; Anema and Li. 2003, Anema, 2009 ; O'Connell and Fox. 2003 ; Singh 2004)*

# ➤ Research questions

## Heat treatment of highly concentrated dairy protein systems

1/ Impact on heat-induced WP/Casein aggregation mechanisms ?

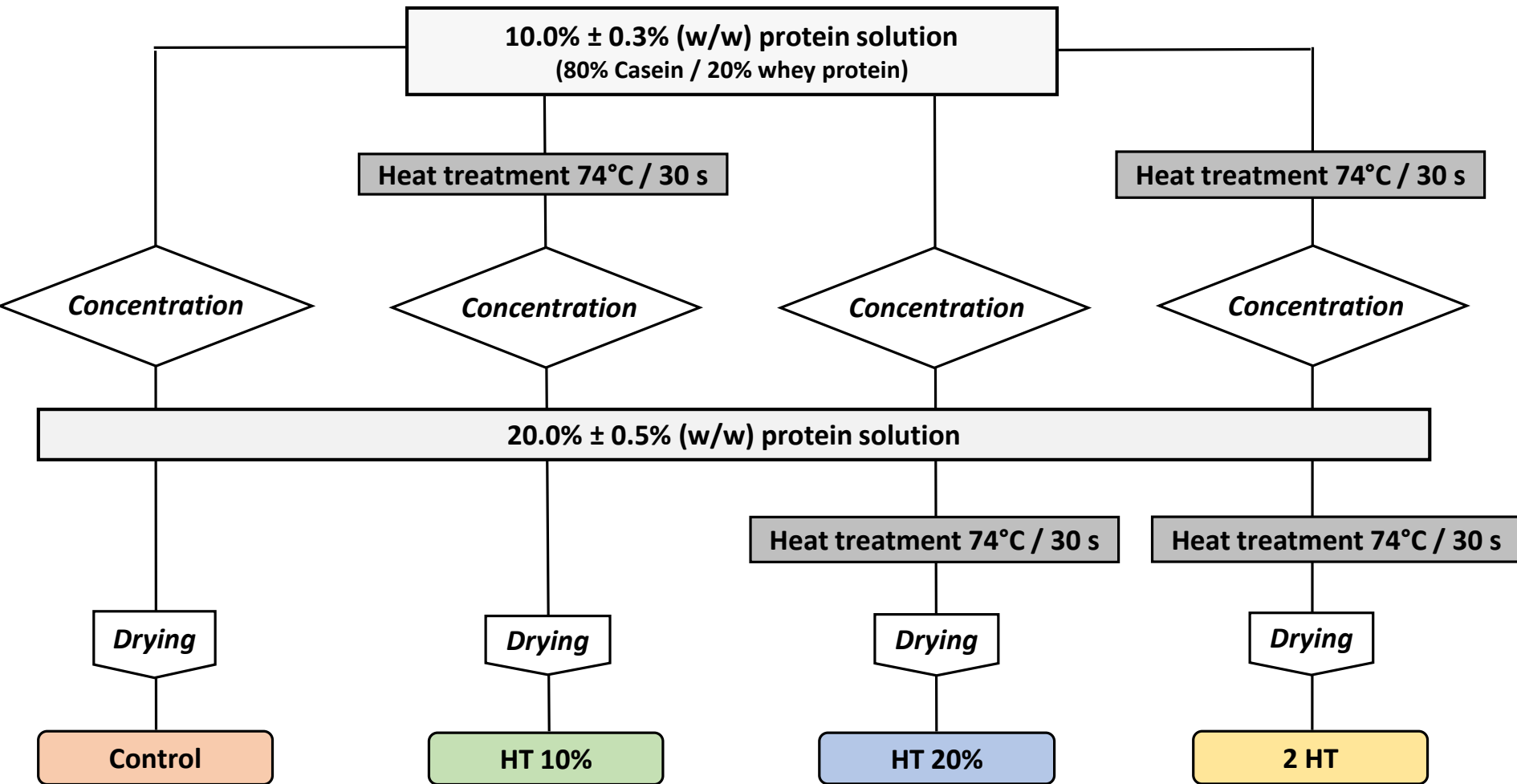
- Increase of collision probability
- Reaching of close packing of protein
  - ❖ Increasing formation of aggregates ?
  - ❖ Changes in whey protein/k-casein interaction ?
  - ❖ Changes in spatial location of protein complexes formed



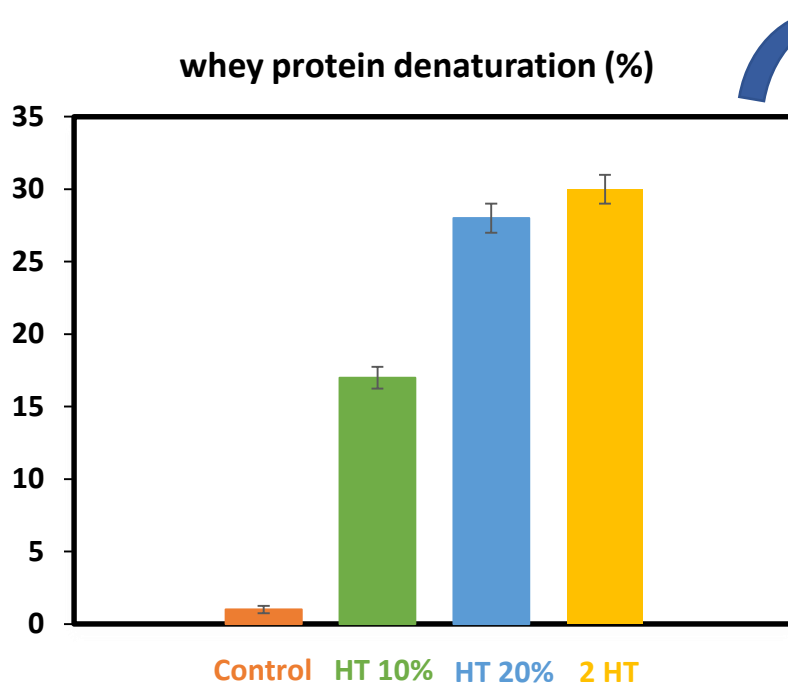
2/ What are the viscosity and coagulation properties of the resulting heat-treated solution ?

Experimental strategy

# ➤ Experimental strategy



# ➤ Results : Protein denaturation / aggregation



- HT 20% / 2HT :**
- Highest whey protein denaturation level
  - 2 times more than **HT 10%** (17% -> 30 %)

Protein concentration -> parameter that influences whey protein denaturation / aggregation

**HT 20% / 2HT :**  
Very similar whey protein denaturation level (28 and 30 %)

No additive effect

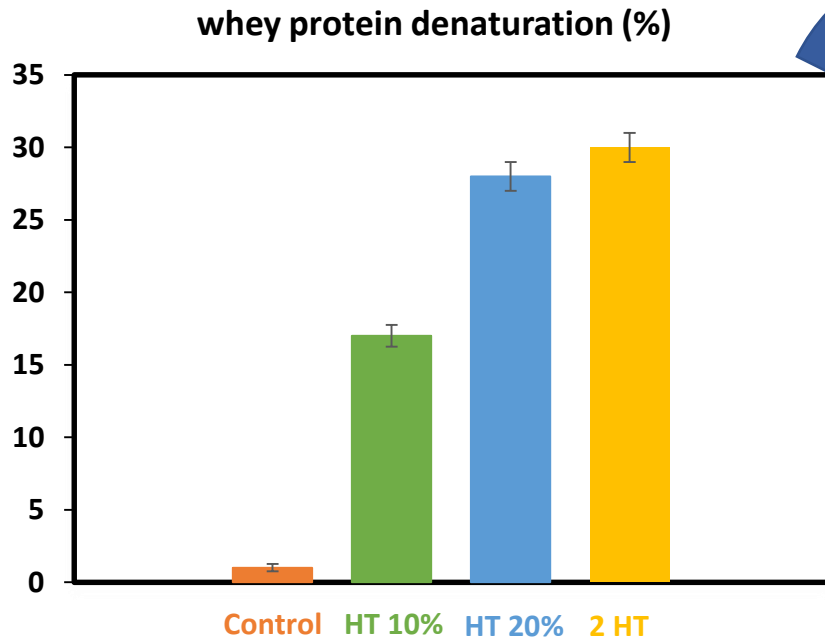
Literature data

Denaturation level : 30/35 % ->  
Heat treatment 80°C / 6 min

(Giroux et al. 2020) (Milk)



# ➤ Results : Protein denaturation / aggregation



Type of aggregates formed ?



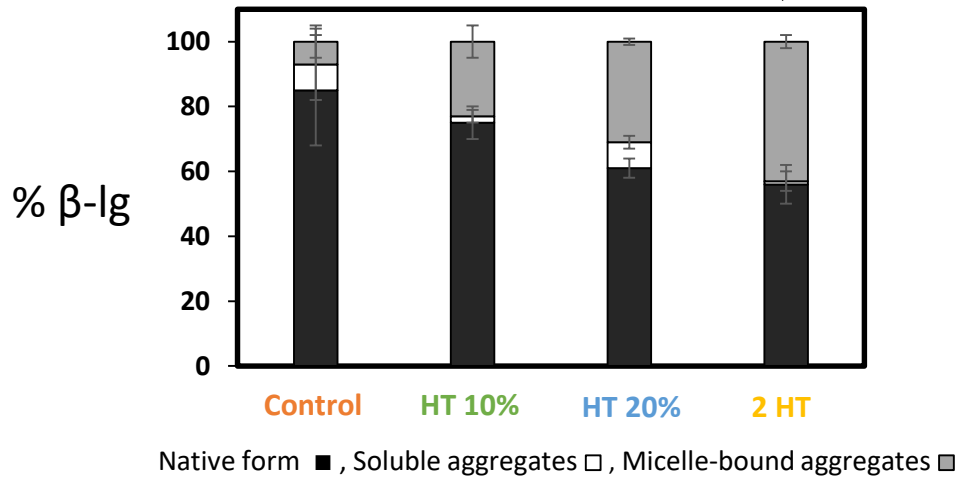
Whey proteins on the micelles or not ?

Coupled enzymatic and acid  
protein fractionation

*(Noh et al.1989 ; Vasbinder et al. 2003)*

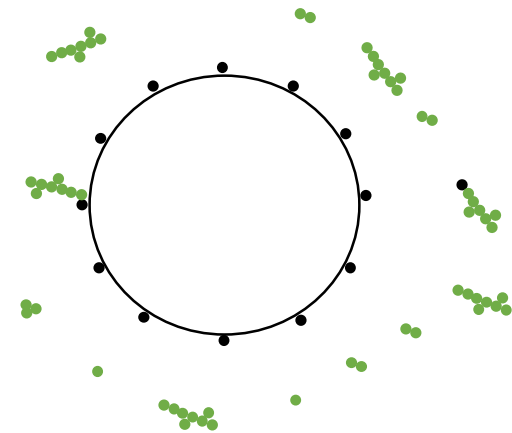
# ➤ Results : Protein denaturation / aggregation

pH Heat treatment	10% protein	6.77	/	6.77
	20% protein	/	6.68	6.70



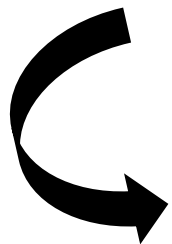
Literature data

~ 6.70/6.75 -> 80 % soluble aggregates  
(Anema et al.2003)

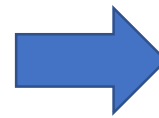


High protein concentration system

Different mechanism of k-casein / whey protein association

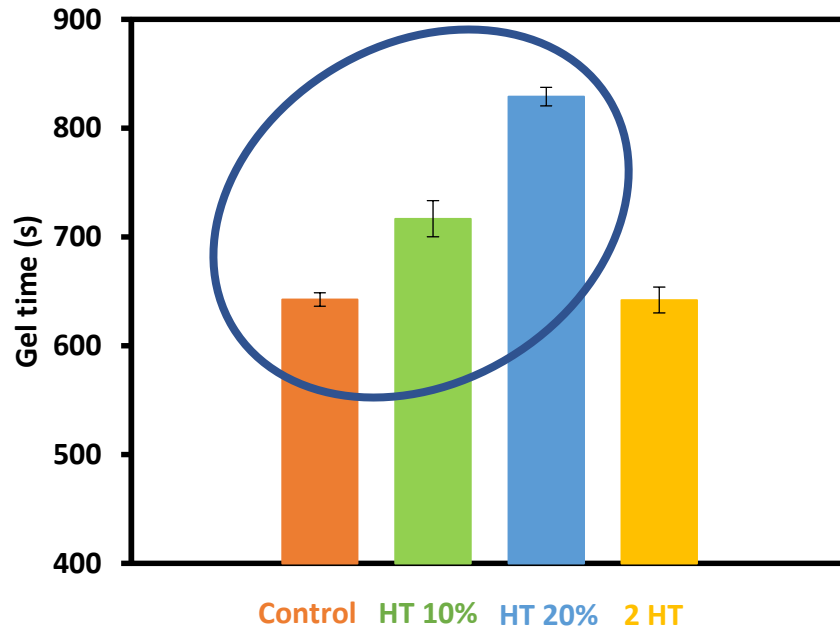


At least, 75 % micelle-bound aggregates



# ➤ Result : Enzymatic coagulation properties

1/ Gel time



In agreement with milk based studies

K-casein/Whey protein complexes disrupt hydrolyzed casein micelle aggregation

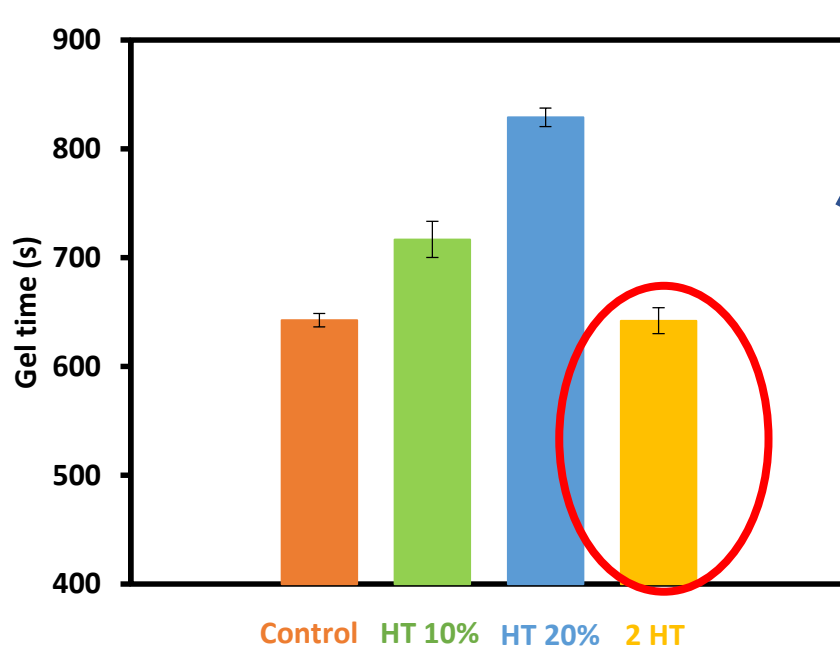
- Steric hindrance
- Electrostatic repulsion

↗ protein aggregates formation  
↳ ↗ gel time

*(Kethireddipalli et al. 2015; Vasbinder et al. 2003 ; Donato et Guyomarc'h. 2009)*



# ➤ Result : Enzymatic coagulation properties



**2HT singularity** : More protein denaturation/aggregation than HT 20% but same gel time than **Control**

Mechanisms involved ?



Investigation of :

- Para casein micelles aggregation time
- Firming time

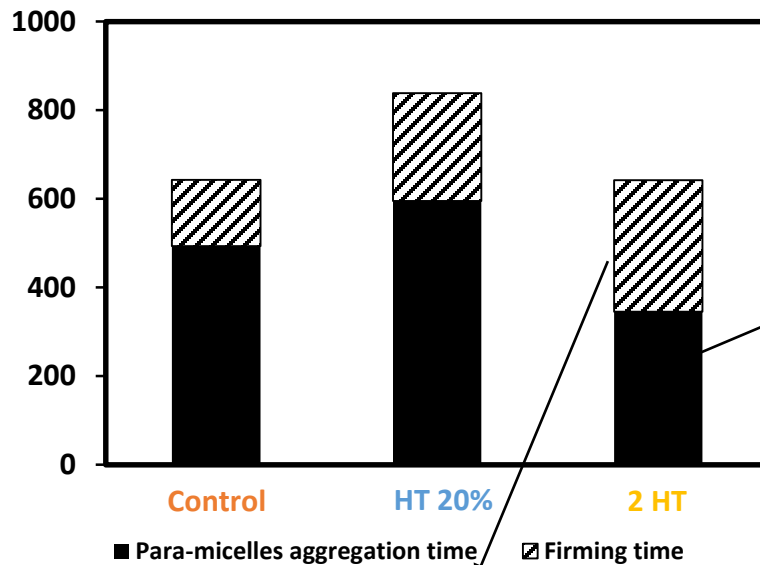
# ➤ Result : Enzymatic coagulation properties

Investigation of :

- Para casein micelles aggregation time
- Firming time

Using a light backscattering technique (Payne and Castillo. 2007 ; Bauland and al. 2020)

Time (s)



Shortest para-micelles aggregation time

Longest firming time

Impact of the 2 HT process scheme on :

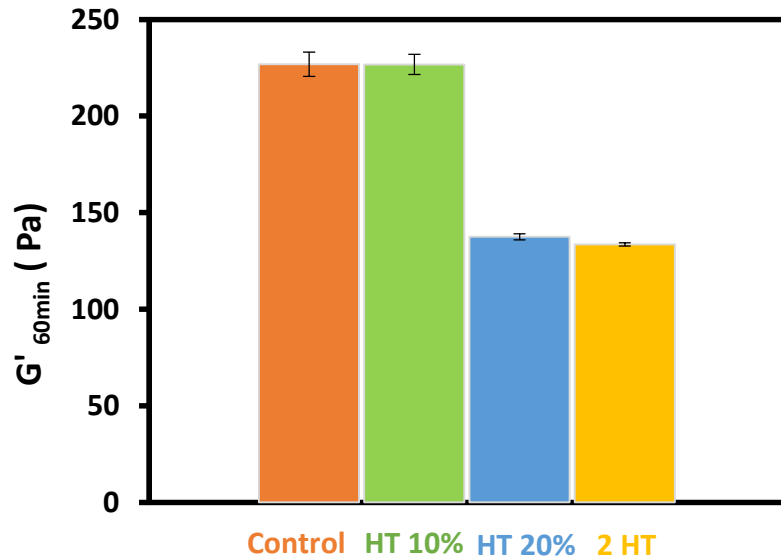
- Casein micelles structure ?
  - ❖ *Futur outlook : SAXS study and K-casein hydrolysis*
- Physico-chemical environment ?

- Large number of K-casein/whey protein complexes on micelles

- steric hindrance

# ➤ Result : Enzymatic coagulation properties

## 2/ Storage Modulus 1h of coagulation



✓ **HT 20% / 2 HT** : Whey proteins aggregates and k-casein/WP complexes disrupt gel reorganisation and generate very weak gels

*(Giroux et al. 2015 ; Perreault et al. 2017)*

✓ **HT 10%** : Same gel strength as **Control** -> amount of whey proteins aggregates and k-casein/WP complexes seems to be insufficient to disrupt gel reorganisation

↗ protein aggregates formation

↘ G'

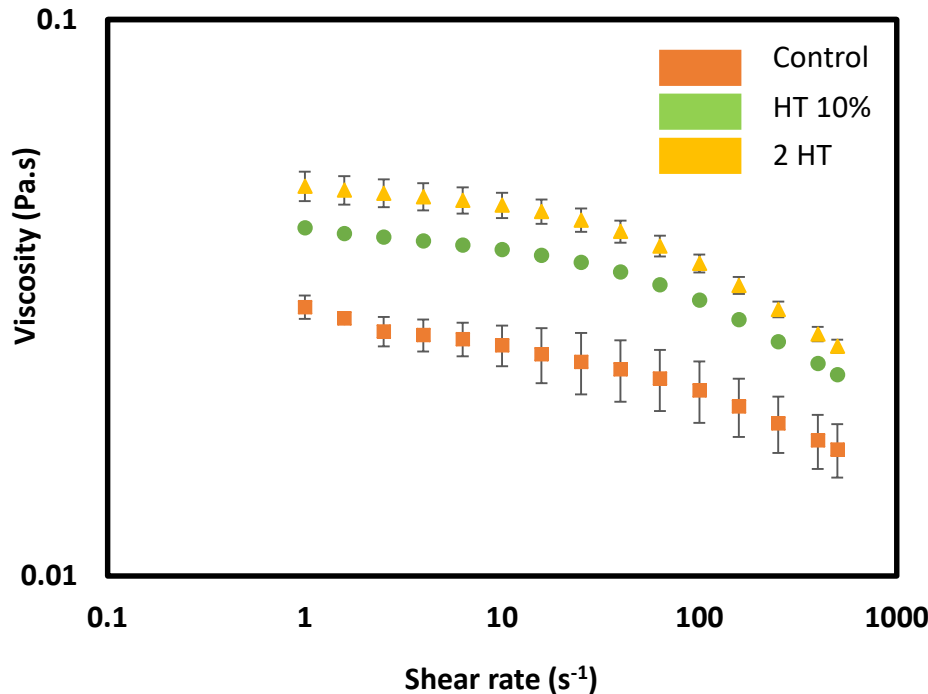


Aggregates quantity effect / threshold effect

# ➤ Results : Viscosity changes

19,5 % protein

- Control
- HT 10%
- 2 HT



All samples -> **shear thinning behavior**

- The difference between the control and heat-treated products is greater than differences among heat-treated products

Higher protein denaturation level -> higher viscosity during shearing



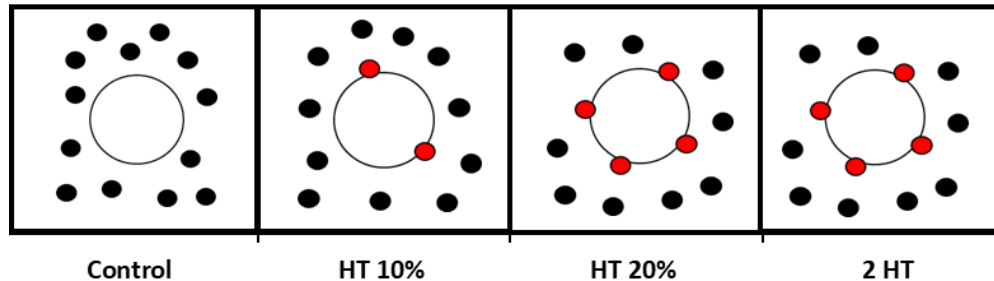
- Higher casein micelles voluminosity
- Higher water-binding capacity of whey protein aggregates >> native proteins



## ➤ Take home message

Heat treatment of highly concentrated dairy protein systems :

1/ Impact on heat-induced WP/Casein aggregation mechanisms ?



- Increase denaturation/aggregation of whey protein at 20 % (w/w) protein
- Protein denaturation -> no additive effect of heat treatment
- Drive K-casein/whey protein association toward major micellar aggregates

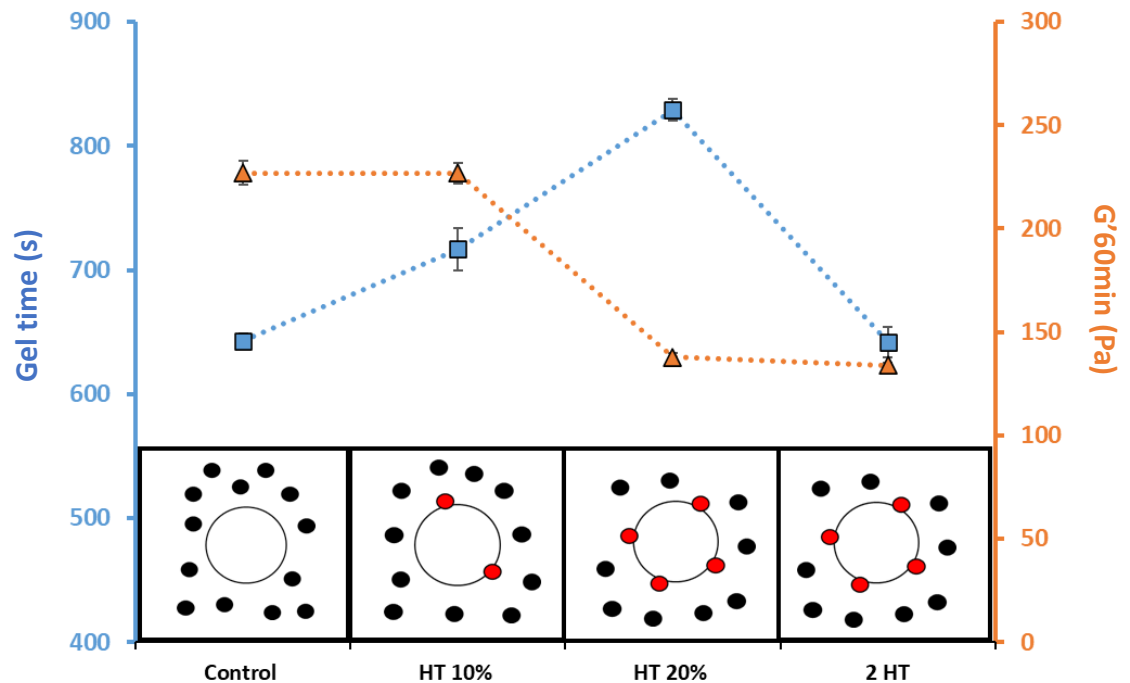


# ➤ Take home message

## Heat treatment of highly concentrated dairy protein systems :

### 2/ Impact on the coagulation properties of the resulting heat-treated solution ?

- K-casein/whey protein complexes -> increase gel time (especially by increasing the firming time), and lead to the formation of weak gels
- Few aggregates in the bulk -> gel produced have the same  $G'$  than control gel



*Lines are guides for the eyes*

# ➤ Take home message

## Heat treatment of highly concentrated dairy protein systems :

### 3/ Impact on viscosity of the resulting heat-treated solution ?

- Higher protein denaturation level -> higher viscosity during shearing
- The difference between the control and heat-treated products is greater than differences among heat-treated products

INRAE



➤ Thanks

