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

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3 Novembre 2021, Biarritz

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# Context

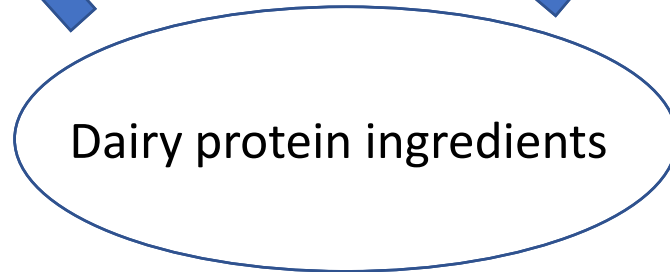


- High added value products

- Expanding market

- Many Properties

- Coagulation
- Setting agent
- Emulsifier...



- Many outlets

- Infant formulas
- Cheese making
- Bakery...



Highly concentrated protein product with increasingly complex physico-chemistry

Process key stage : Heat treatment



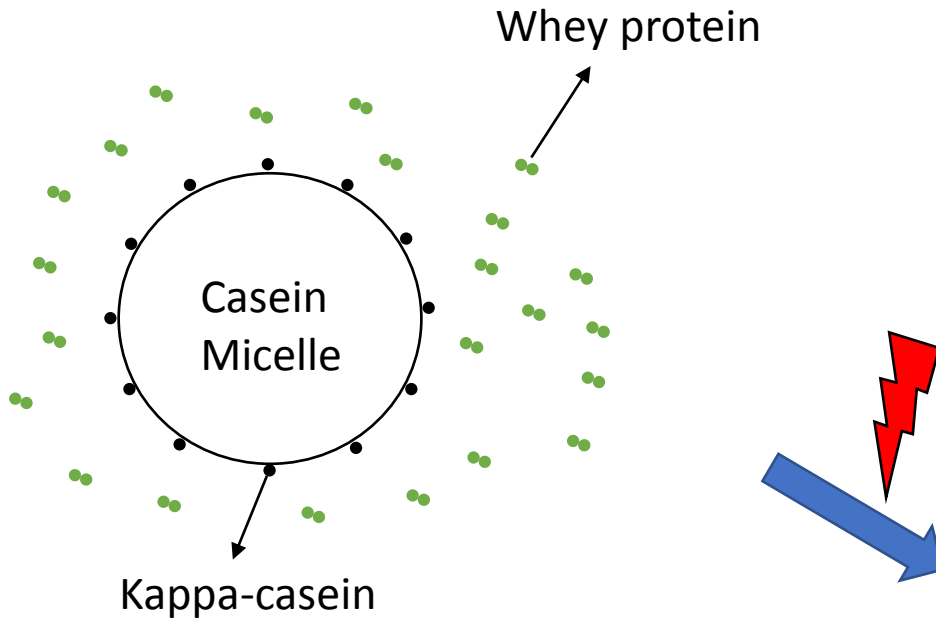
More knowledge needed for controlling functionalities



**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)*

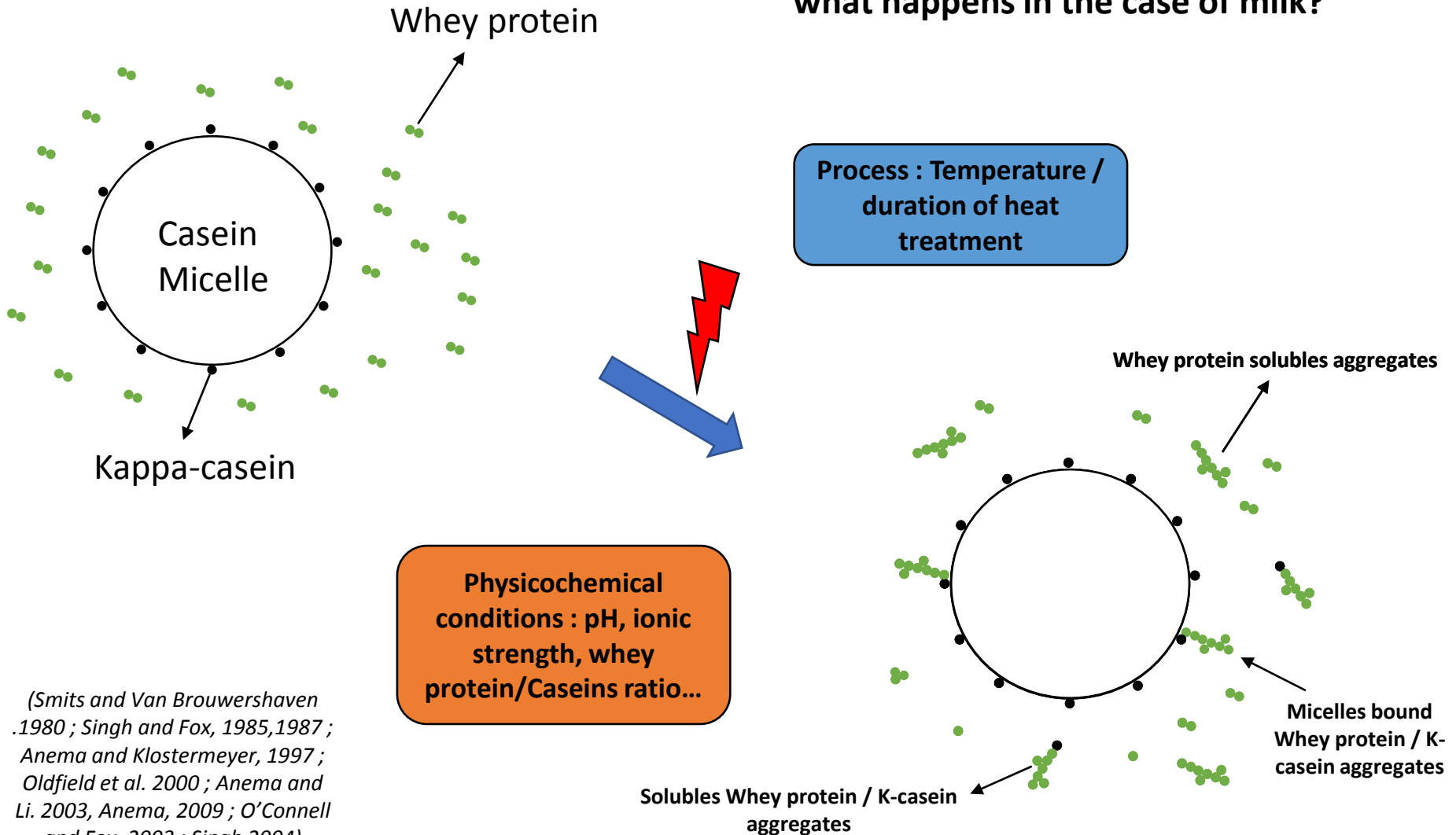
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de l'économie laitière

# ➤ Scientific Background

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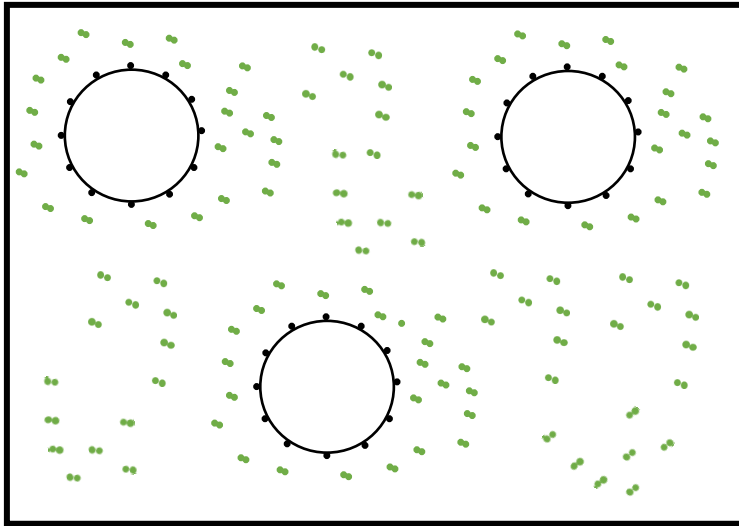
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# ➤ Scientific Background

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

Heat treatment of milk



- [Protein] : 33 g/L
- Ionic strength : 80 mM
- [Lactose] : 50 g/L



- [Protein] : 100-200 g/L
- Ionic strength : 150 - 300 mM
- [Lactose] : 2- 4 g/L

*ie : Solution of dairy protein isolate*

### Many studies

*(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)*



### Only parcellar understanding

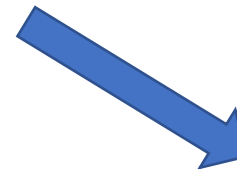
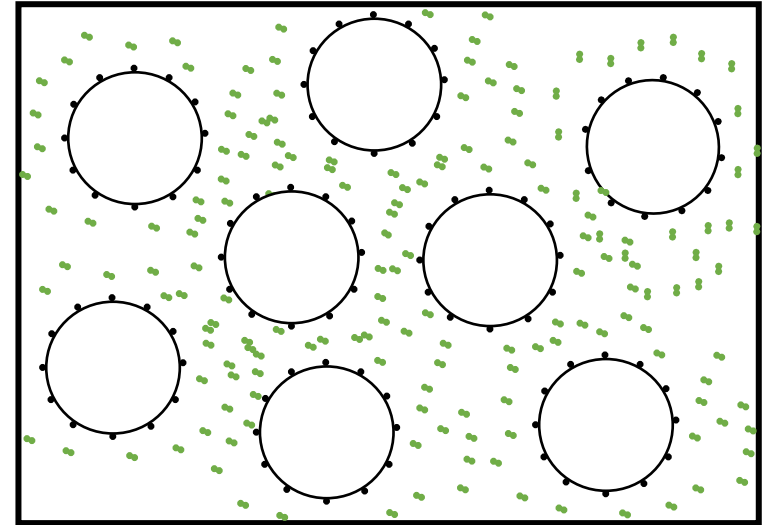


# ➤ Research questions

## Heat treatment of highly concentrated dairy protein systems

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

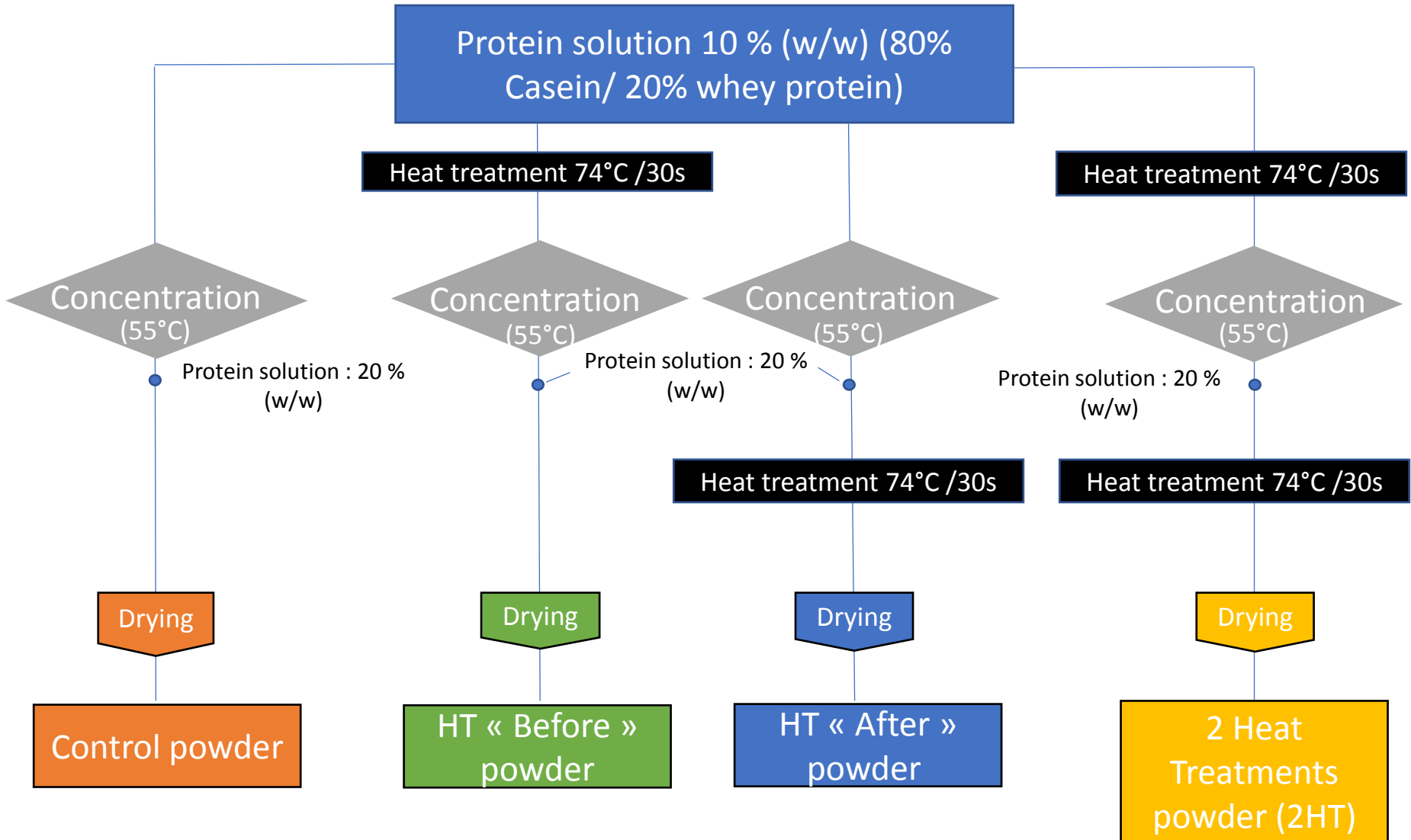
- Increase of collision probability
- Reaching of close packing of protein
  - ❖ Increasing formation of aggregates ?
  - ❖ Changes in whey protein/k-casein interaction ?
  - ❖ Changes in spatial location of protein complexes formed



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

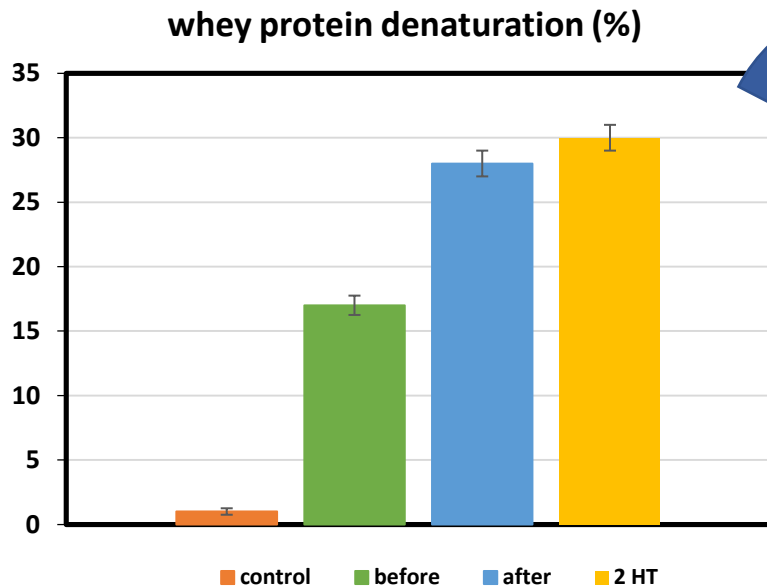
Experimental strategy

# ➤ Experimental strategy





# ➤ Results : Protein denaturation / aggregation



**HT After / 2HT :**

- Highest whey protein denaturation level
- 2 times more than **Before** (ie 16% -> 30 %)

**HT After / 2HT :**

Very similar whey protein denaturation level (ie 27 and 30 %)

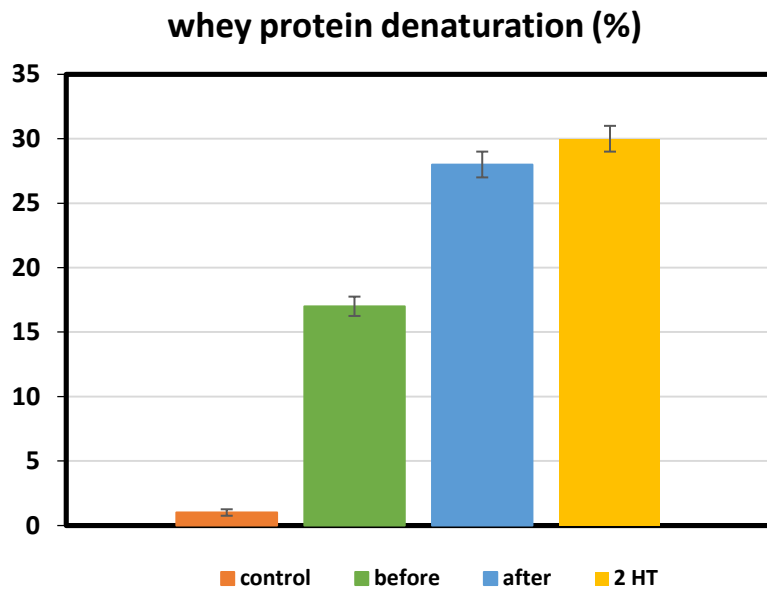
Literature data

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

(Giroux et al. 2020)

Protein concentration > parameter  
accelerating the whey protein  
denaturation / aggregation

# ➤ Results :Protein denaturation / aggregation



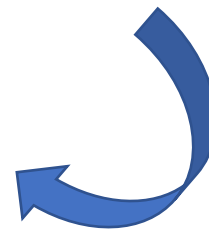
Type of aggregates formed ?



Quantitative analysis on the distribution of denatured whey proteins over WP aggregates and WP associated with the casein micelles

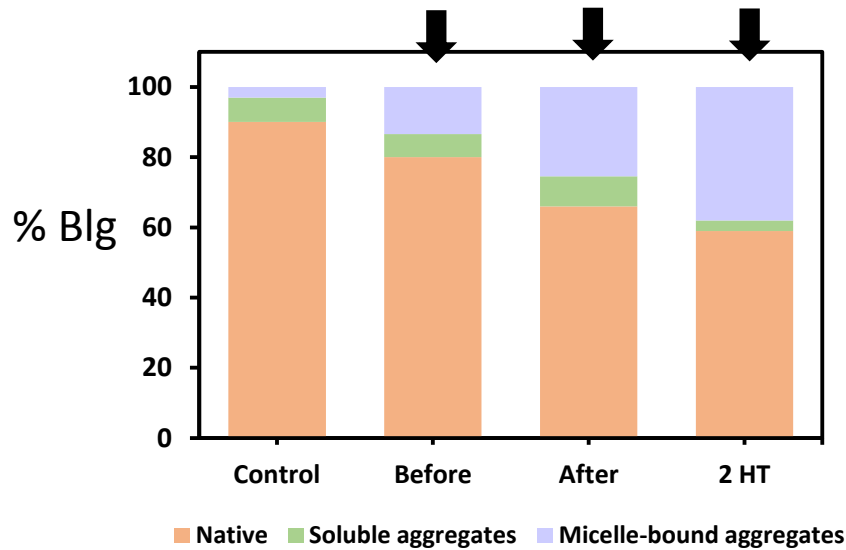
Coupled enzymatic and acid protein fractionation

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



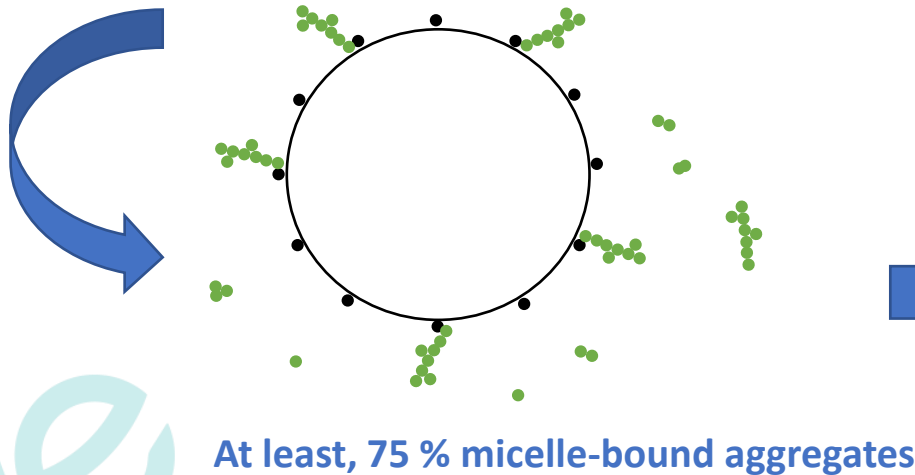
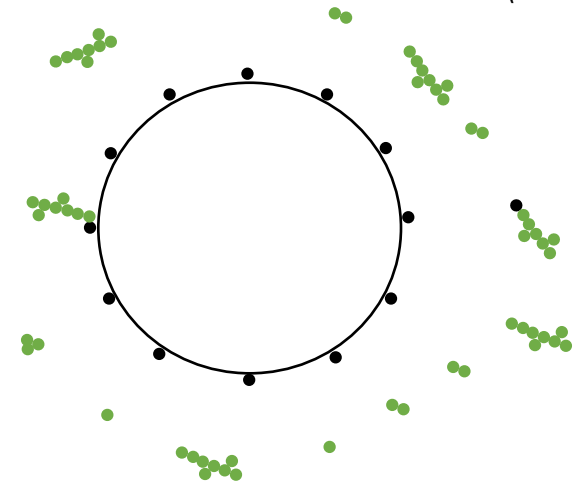
# ➤ Results : Protein denaturation / aggregation

pH Heat treatment	Before	6.77	/	6.77
	After	/	6.68	6.70



Literature data

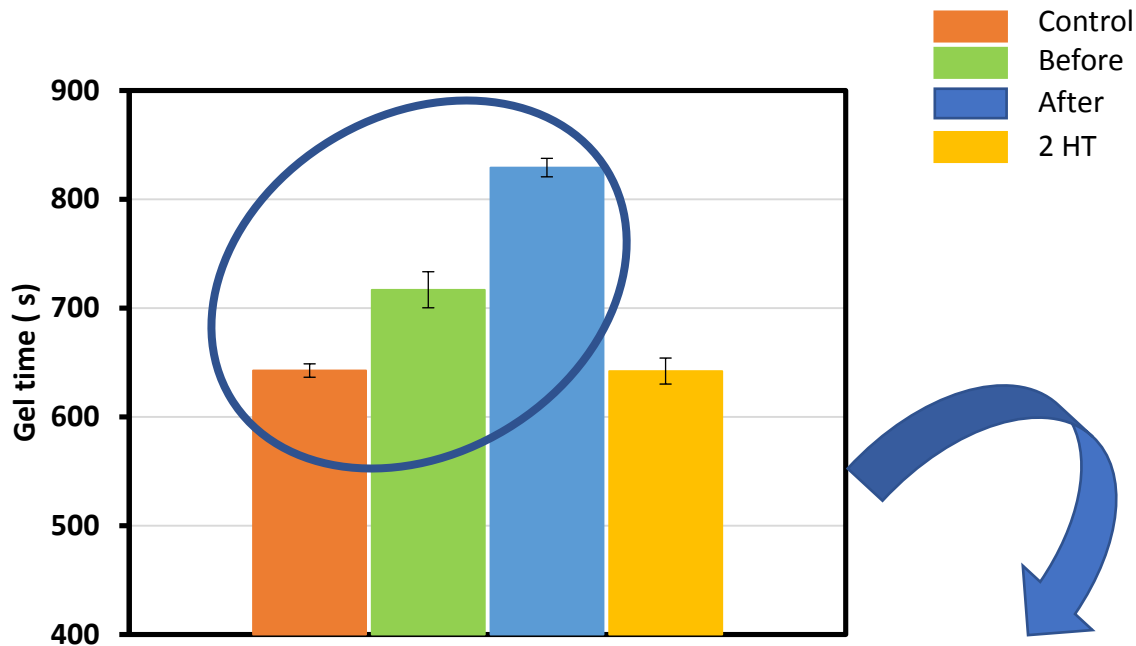
~ 6.70/6.75 -> 80 % solubles aggregates  
(Anema et al.2003)



High protein concentration system

Different mechanism of k casein /whey protein association

# ➤ Result : Enzymatic coagulation properties



↗ protein aggregates formation  
↳ ↗ gel time

In agreement with milk based studies

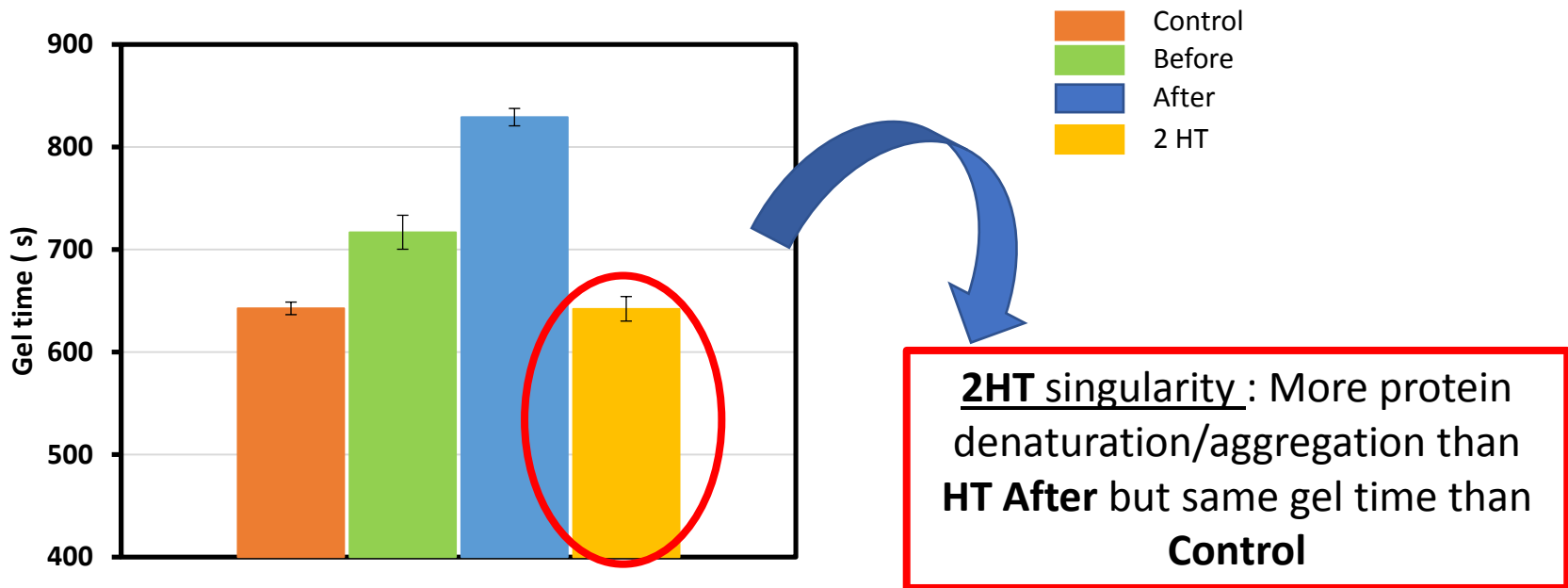
K-casein/Whey protein complexes disrupt hydrolyzed casein micelle aggregation

- Steric hindrance
- Electrostatic repulsion

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



# ➤ Result : Enzymatic coagulation properties



Changes in K-casein/WP complexes ?



Mechanisms involved ?

Investigation of :

- Para casein micelles aggregation time
- Firming time

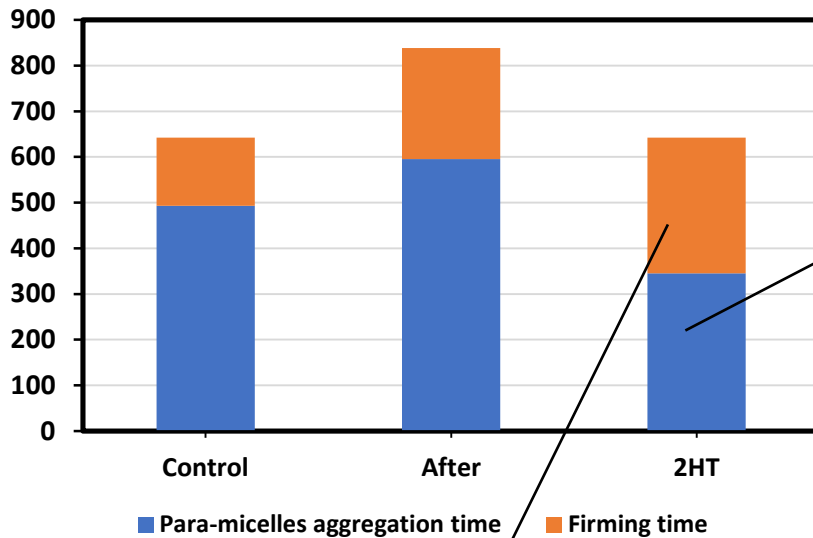
# ➤ Result : Enzymatic coagulation properties

Investigation of :

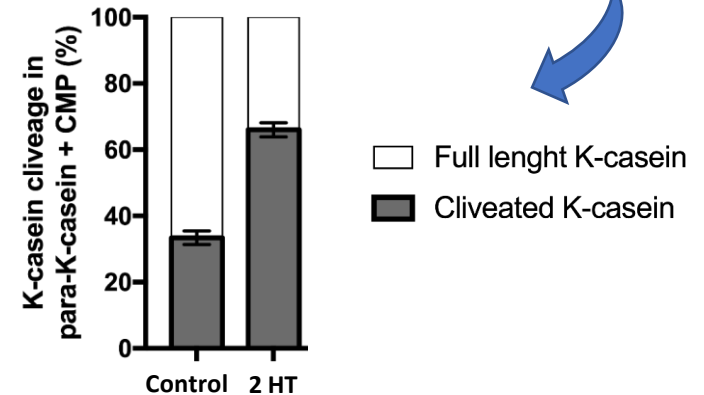
- Para casein micelles aggregation time
- Firming time

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

Time (s)



Shortest para-micelles aggregation time



Longest firming time

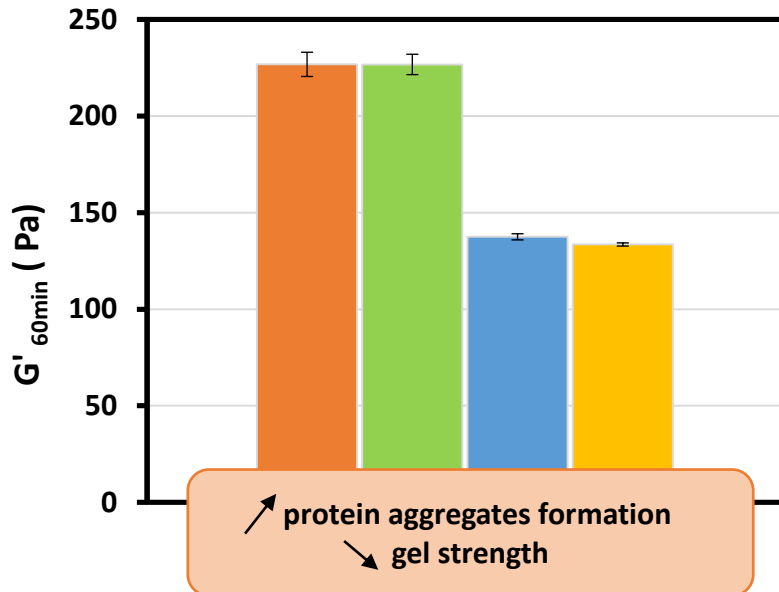
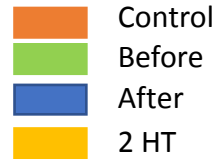
- Large number of K casein/whey protein complexes on micelles

- steric hindrance

2 HT -> preliminary hydrolyse of K-casein

Time to get 80% of micelles hydrolyse and start their aggregation is reduced

# ➤ Result : Enzymatic coagulation properties



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



Aggregates quantity effect / threshold effect

# ➤ 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
- Drive K-casein/whey protein association toward major micellar aggregates

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

- 2 Heat treatments -> generate K-casein hydrolysis -> reduce para-micelles aggregation time
- K-casein/whey protein complexes generated during heat treatment increase firming time, and lead to the formation of weak gels
- When only few aggregates are present in the bulk, gels produced have the same firmness than control gels





INRAE



➤ *Thanks*

*Any questions ?*