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Heat treatment of concentrated milk protein system affect enzymatic coagulation properties

François Martin^{1 2}, Luisa Azevedo-Scudeller³, Arnaud Paul⁴, Romain Jeantet¹,
Gaëlle Tanguy¹, Serge Mejean¹, Cécile Le Floch-Fouéré¹

1 STLO, INRAE, Institut Agro, 35042 Rennes, France

2 CNIEL, Paris, France

3 UMET, INRAE, PIHM Team, 59651 Villeneuve d'Ascq

4 Nancy Université, LIBio-ENSAIA-INPL, 54505 Vandoeuvre les Nancy, France

3 Novembre 2021, Biarritz

Email adress : francois.martin@inrae.fr



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

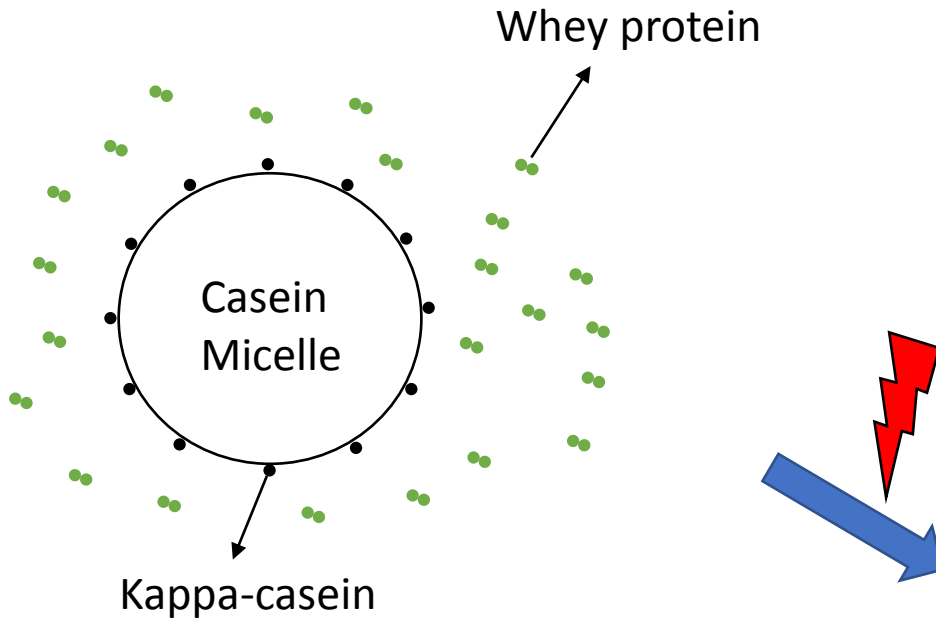
Process key stage :
Heat treatment

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)

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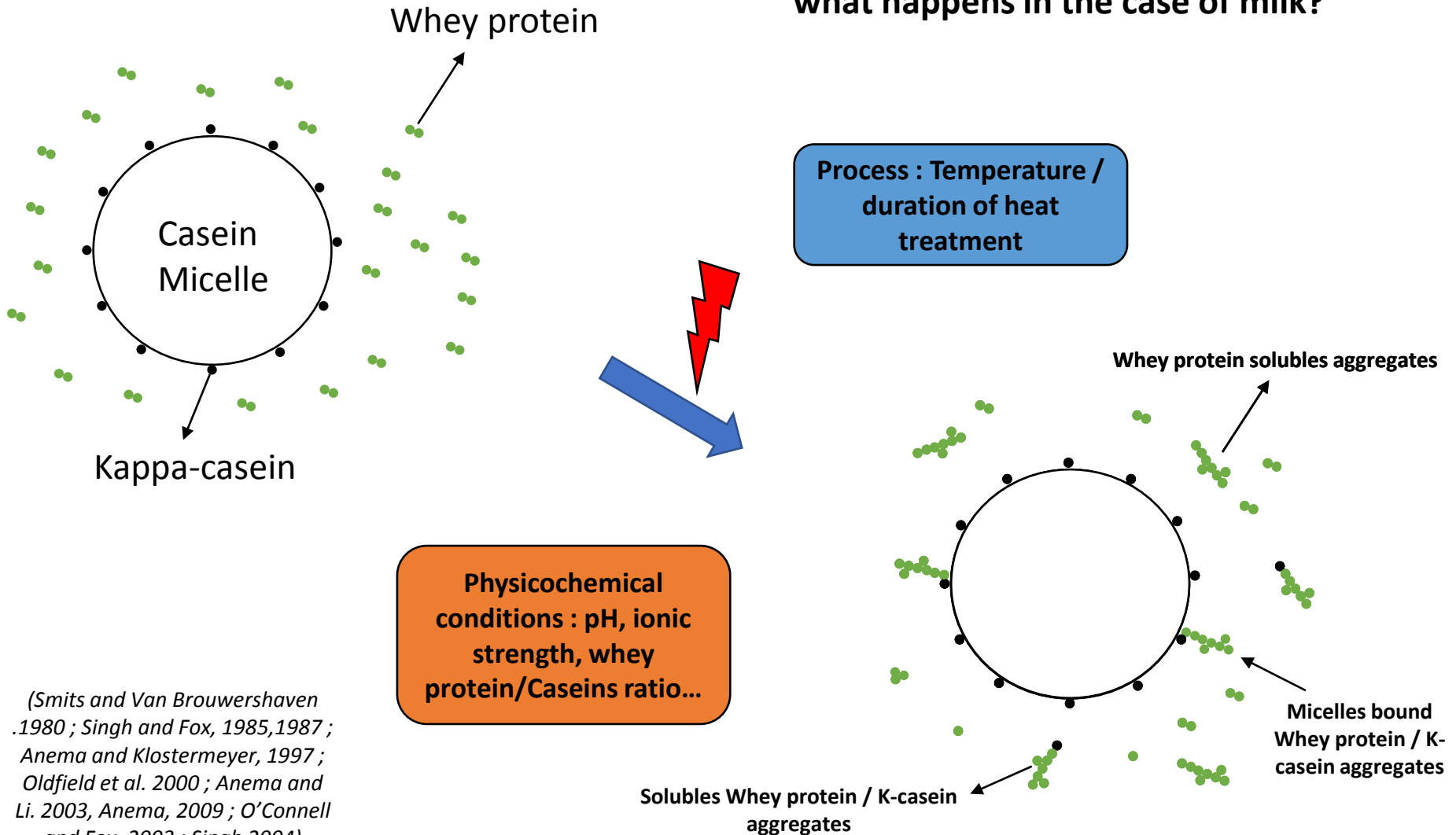
AGRO
CAMPUS
EUROPE

LIBio
Laboratoire Français de Démolécules

Cniel
Centre national interprofessionnel
de l'économie laitière

➤ Scientific Background

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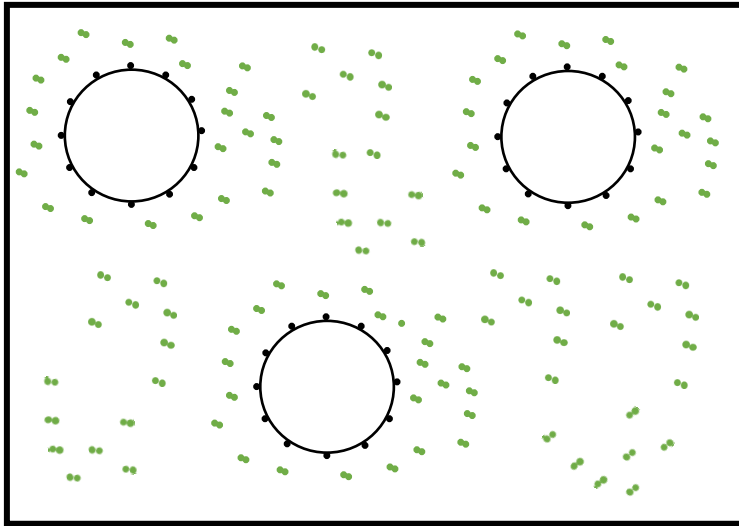
LIBio
Laboratoire Français de Biochimie



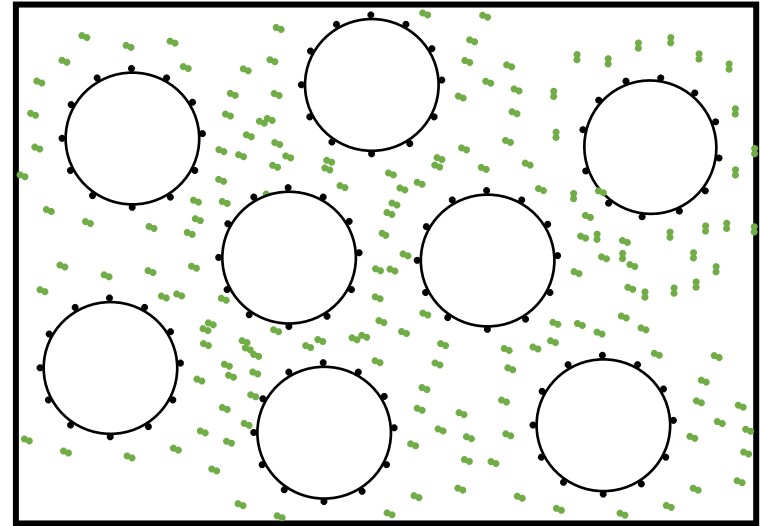
➤ Scientific Background

Heat treatment of dairy solution with different protein concentration :

Heat treatment of milk



Heat treatment of highly concentrated dairy protein systems



- [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

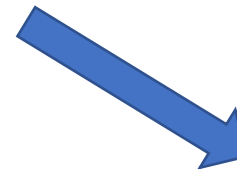
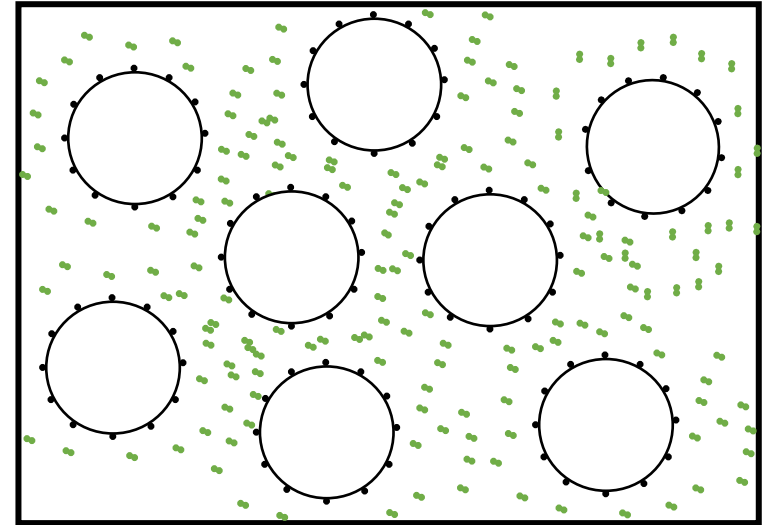


➤ Research questions

Heat treatment of highly concentrated dairy protein systems

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

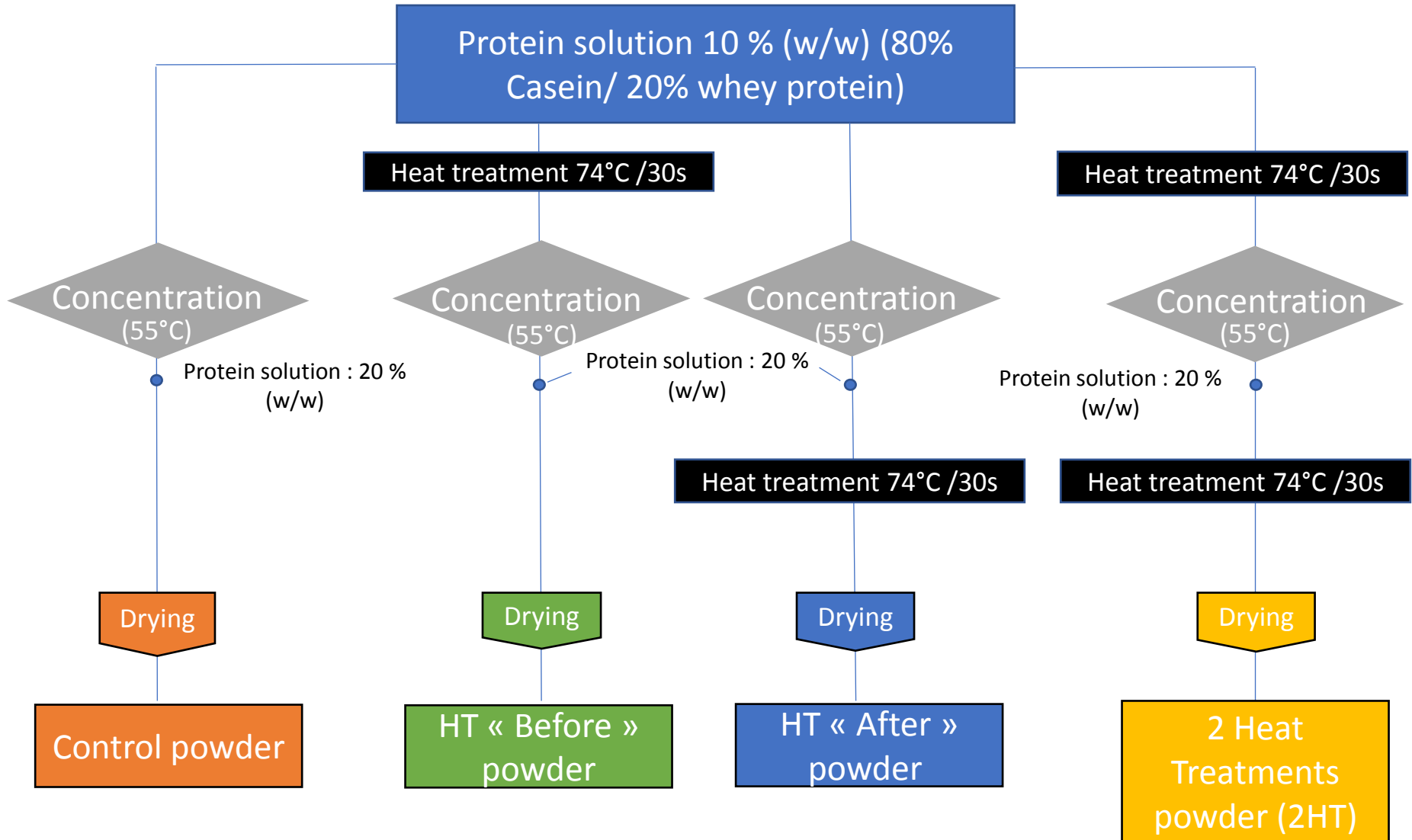
- Increase of collision probability
- Reaching of close packing of protein
 - ❖ Increasing formation of aggregates ?
 - ❖ Changes in whey protein/k-casein interaction ?
 - ❖ Changes in spatial location of protein complexes formed



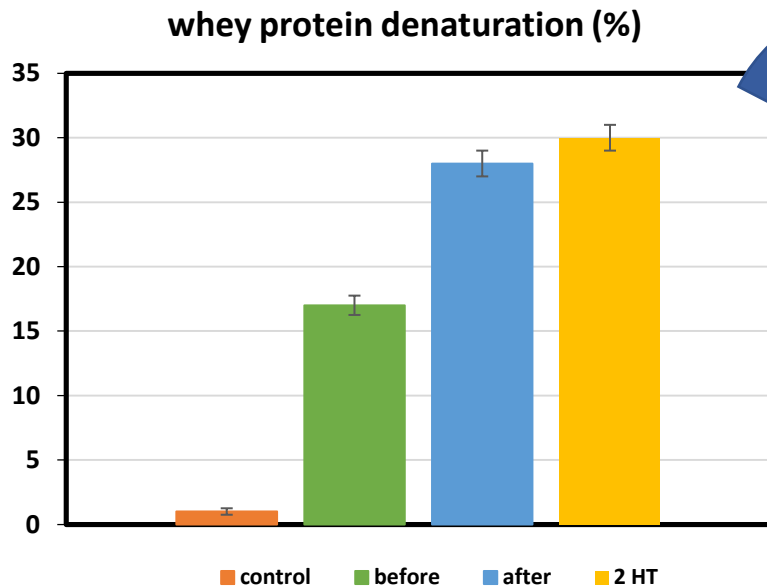
2/ What are the coagulation properties of the resulting heat-treated solution ?

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 %)

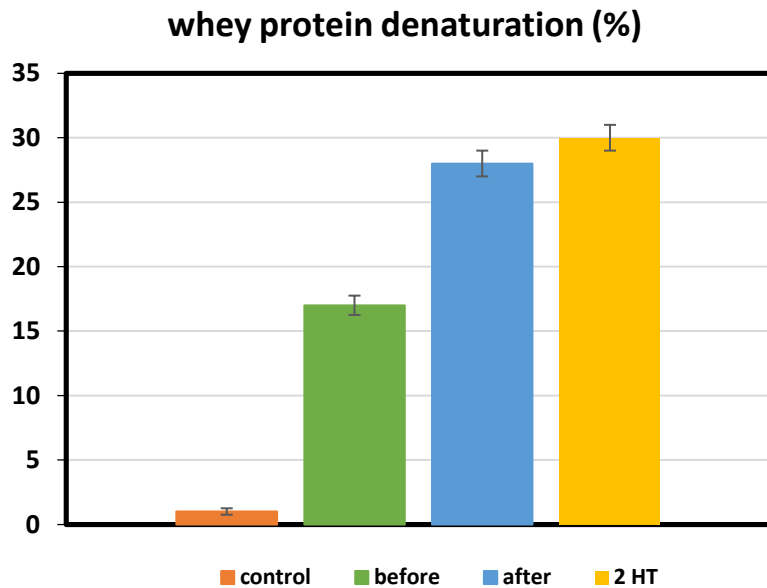
Literature data

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



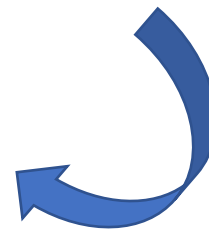
Type of aggregates formed ?



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

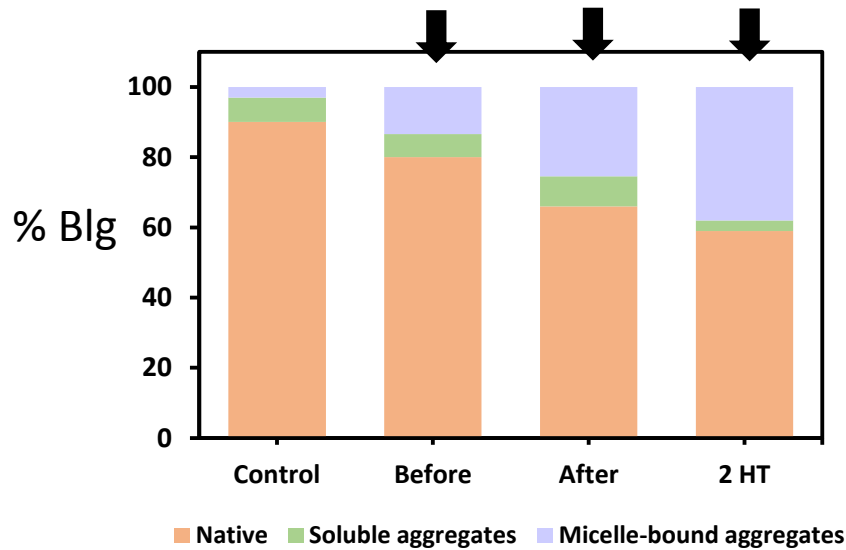
Coupled enzymatic and acid protein fractionation

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



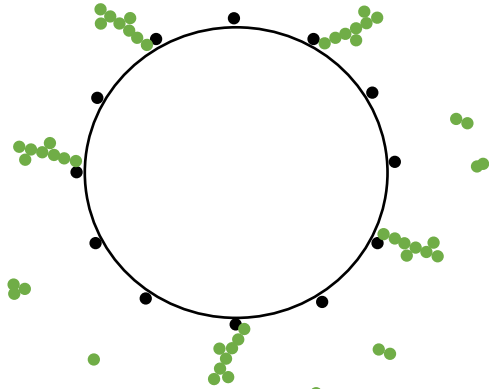
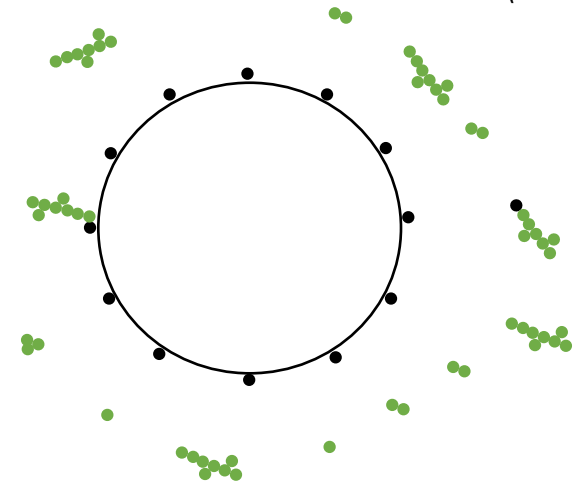
➤ Results : Protein denaturation / aggregation

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)



At least, 75 % micelle-bound aggregates

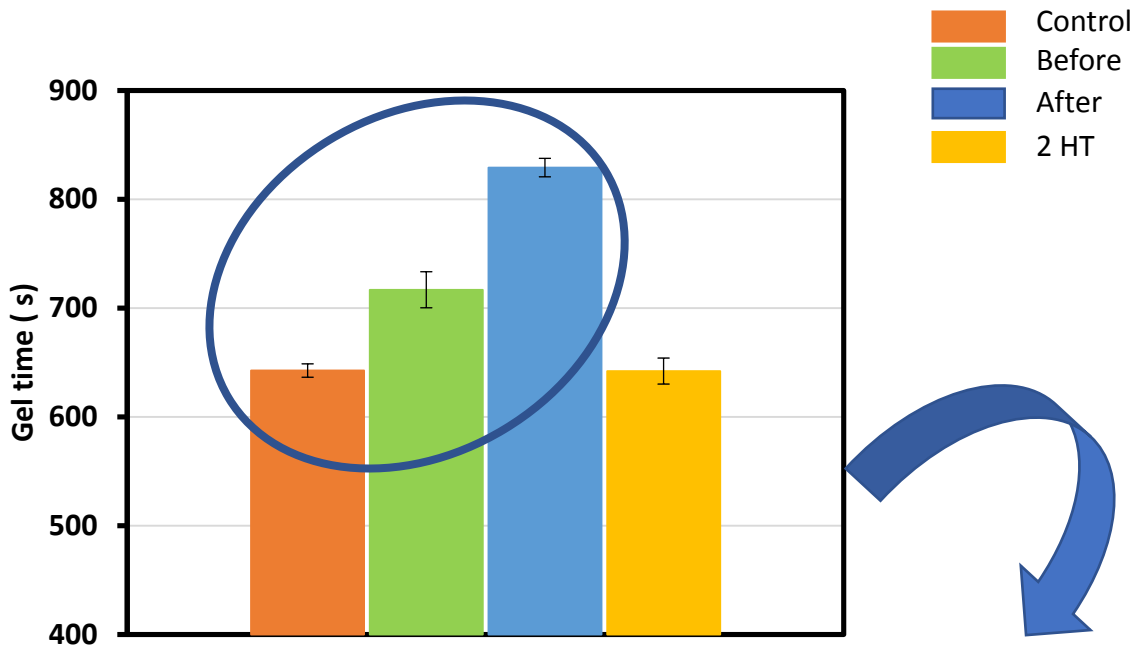


High protein concentration system

Different mechanism of k casein /whey protein association



➤ Result : Enzymatic coagulation properties



↗ protein aggregates formation
↳ ↗ gel time

In agreement with milk based studies

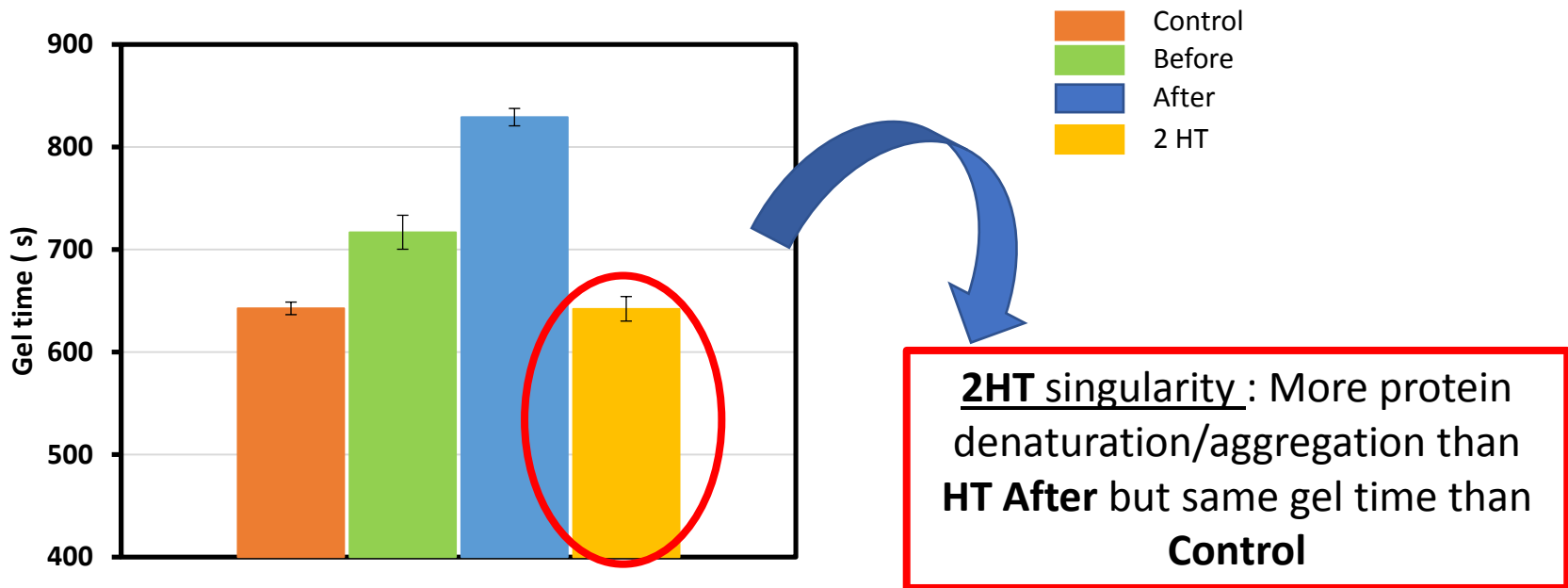
K-casein/Whey protein complexes disrupt hydrolyzed casein micelle aggregation

- 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

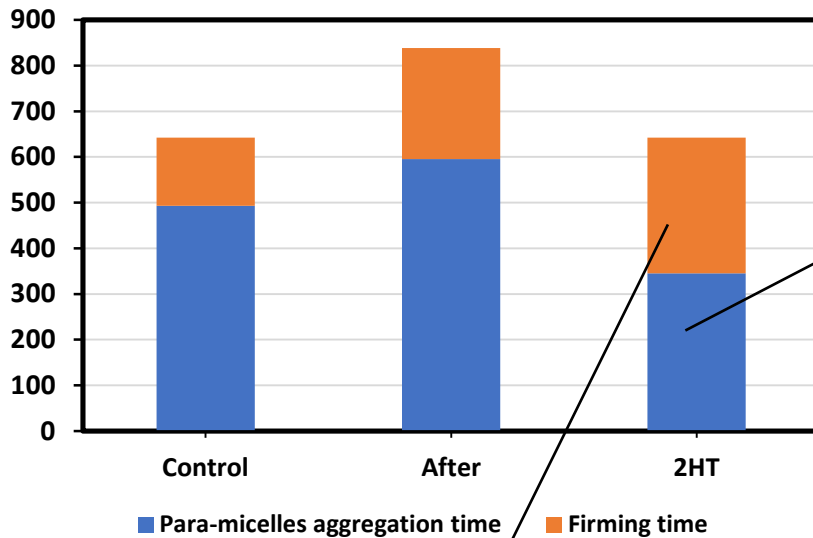
➤ Result : Enzymatic coagulation properties

Investigation of :

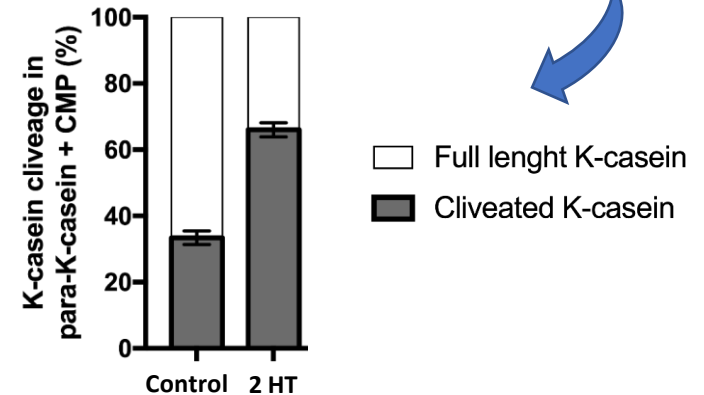
- Para casein micelles aggregation time
- Firming time

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

Time (s)



Shortest para-micelles aggregation time



Longest firming time

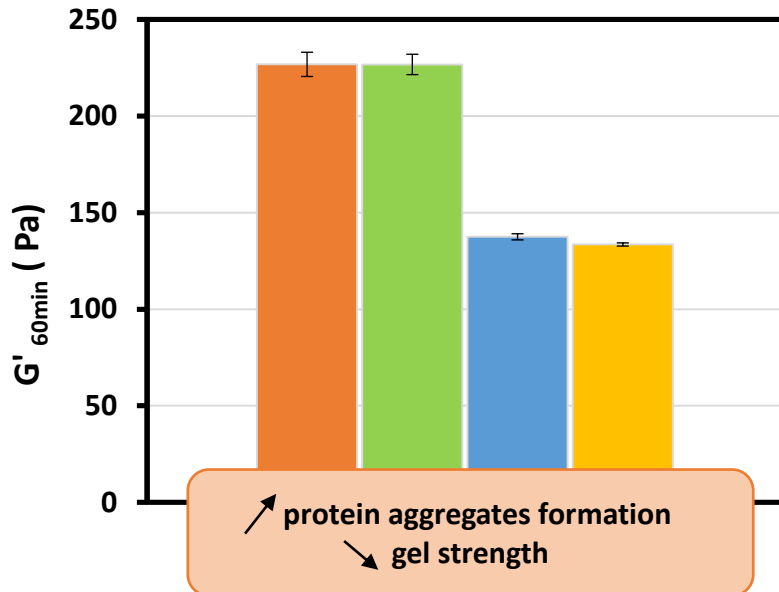
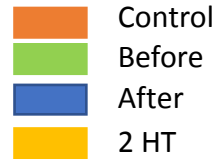
2 HT -> preliminary hydrolyse of K-casein

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

- steric hindrance

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



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➤ *Thanks*

Any questions ?