Heat treatment of concentrated milk protein system affect enzymatic coagulation properties

François Martin, Luisa Azevedo-Scudeller, Arnaud Paul, Romain Jeantet, Gaëlle Tanguy, Serge Mejean, Cécile Le Floch-Fouéré

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Context

- High added value products
- Expanding market

Dairy protein ingredients

- Many Properties
  - Coagulation
  - Setting agent
  - Emulsifier…
- Many outlets
  - Infant formulas
  - Cheese making
  - Bakery…

Highly concentrated protein product with increasingly complex physico-chemistry

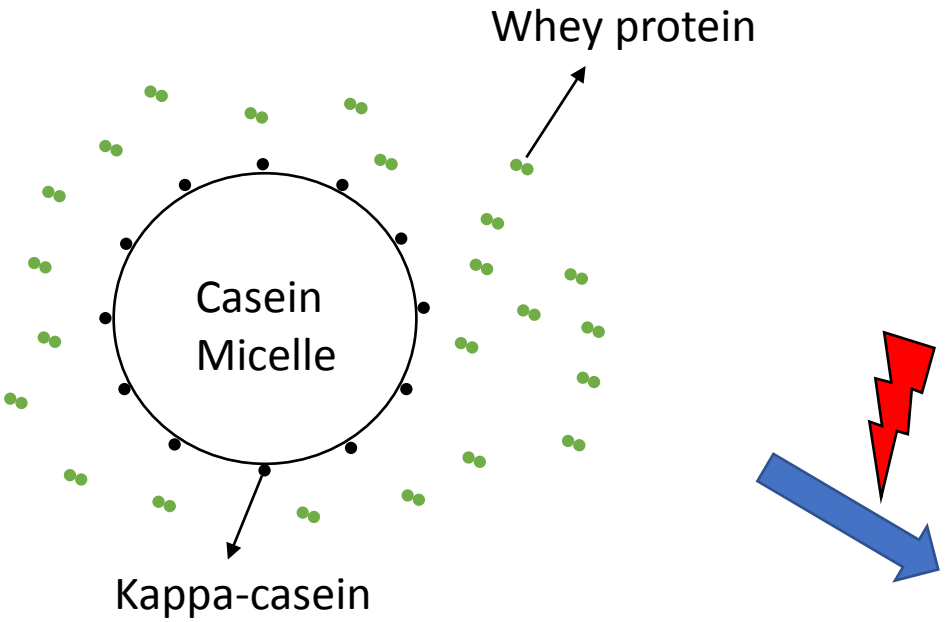
Process key stage : Heat treatment

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)

Scientific Background

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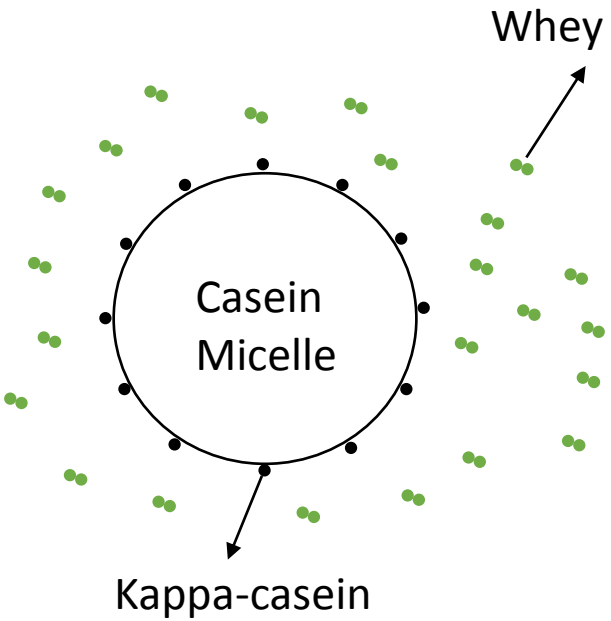

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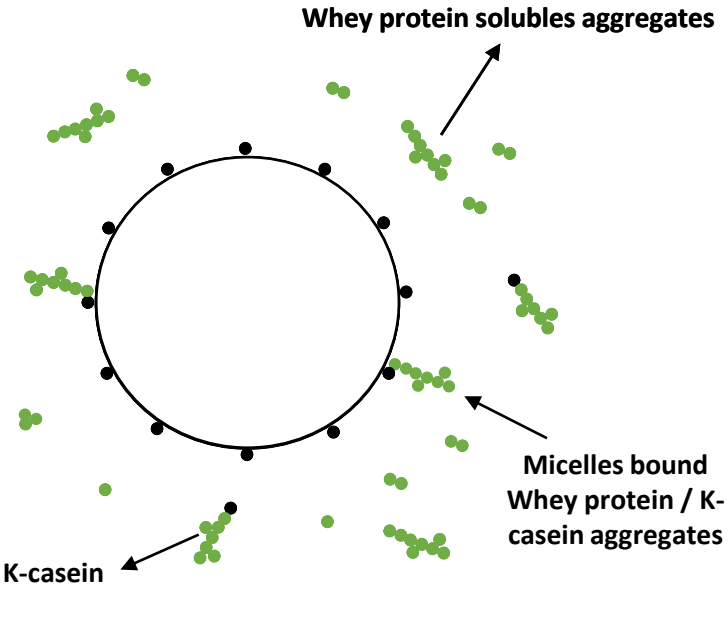

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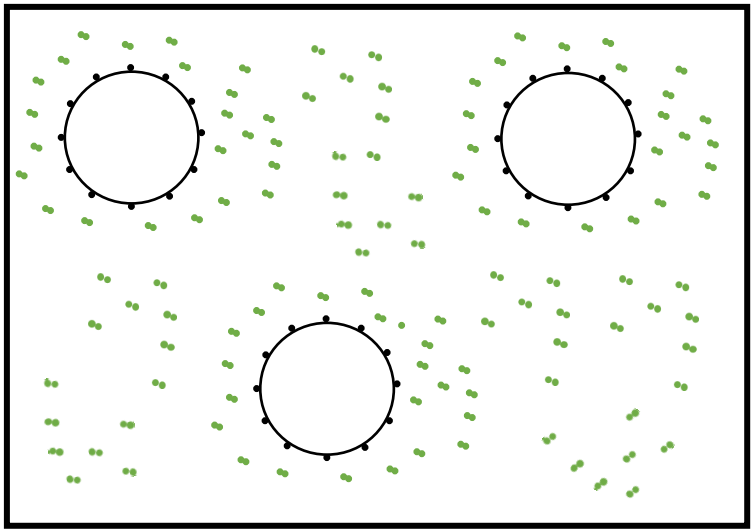
Scientific Background

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

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)

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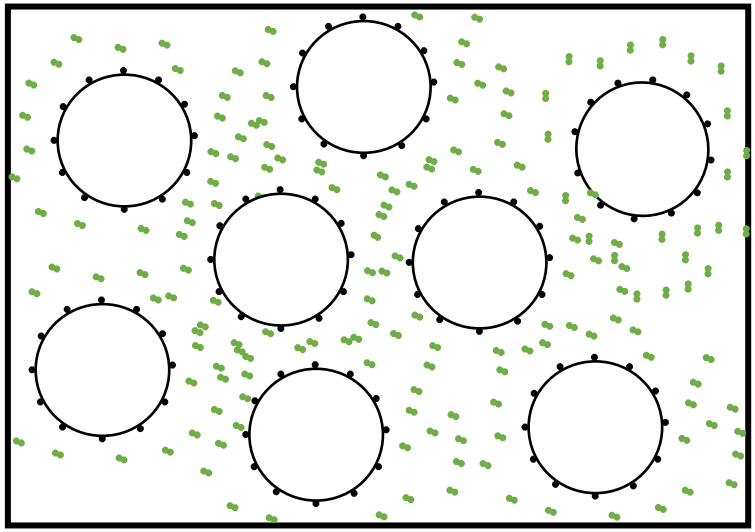
Heat treatment of highly concentrated dairy protein systems



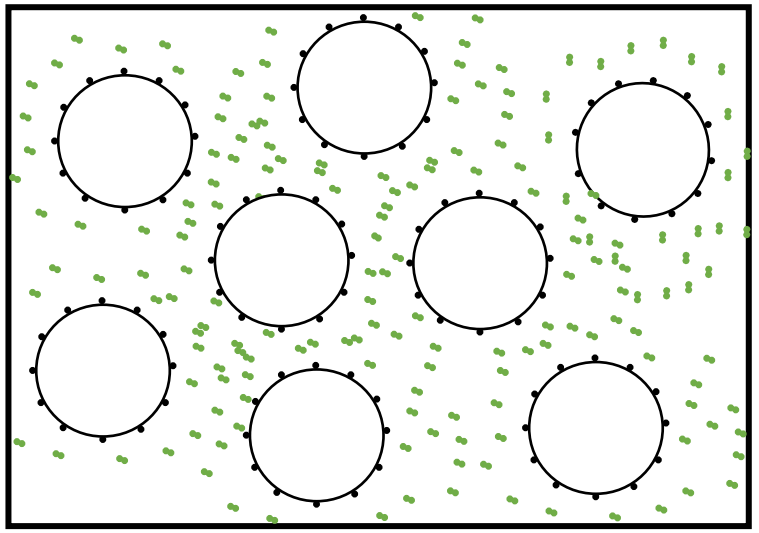
- [Protein] : 100-200 g/L
- Ionic strength : 150 - 300 mM
- [Lactose] : 2- 4 g/L

Ie : Solution of dairy protein isolate

Only parcellar understanding

Research questions

Heat treatment of highly concentrated dairy protein systems



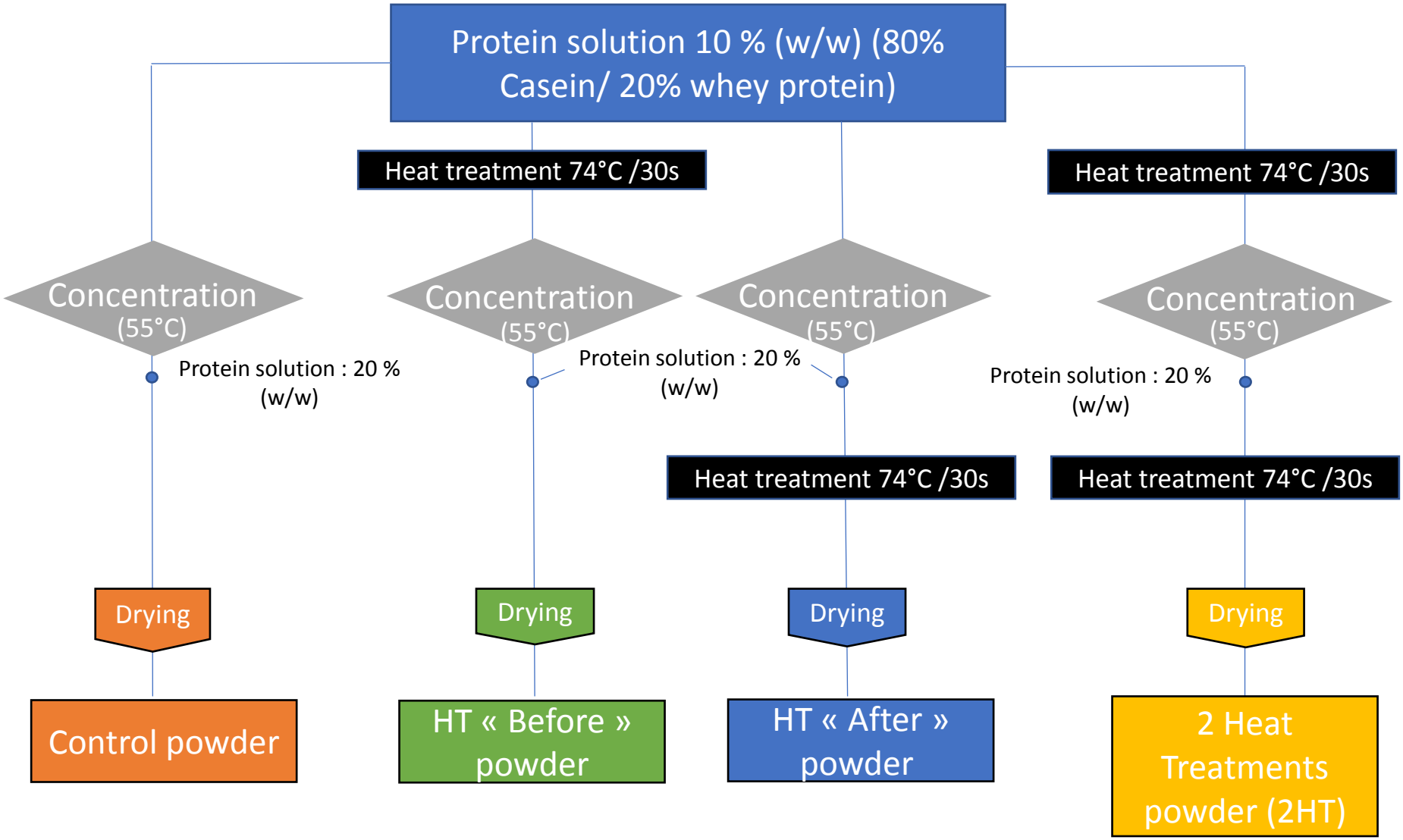
1/ Impact on heat-induced WP/Casein aggregation mechanisms ?

- 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 ?

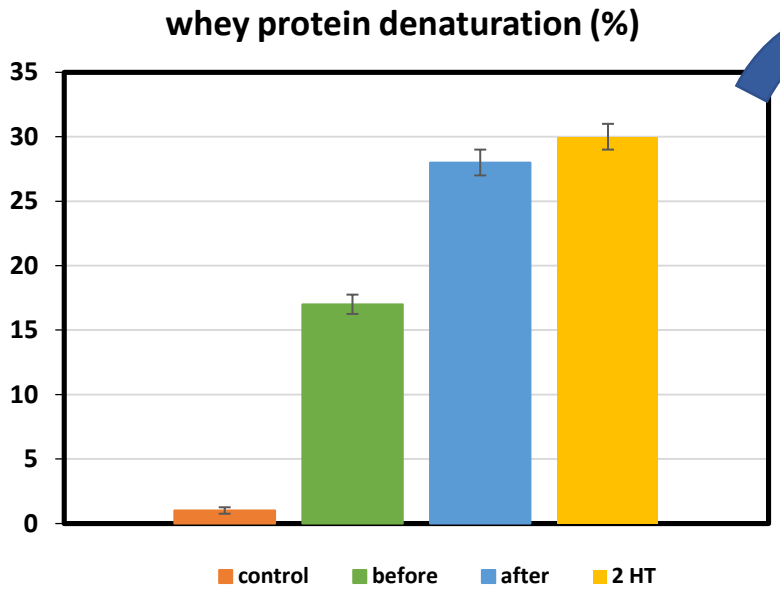
Experimental strategy

Experimental strategy



Results on next slides are obtained based on analysis of reconstituted powders

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 %)

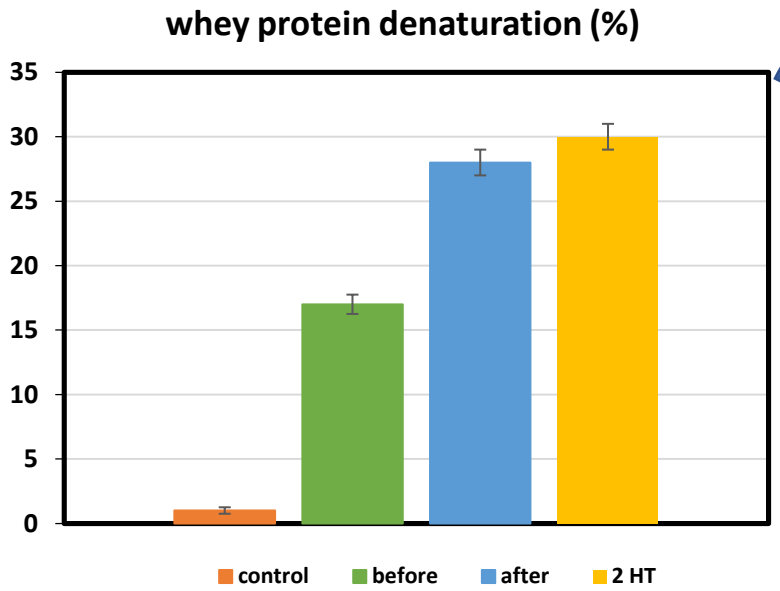
Literature data

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 ?

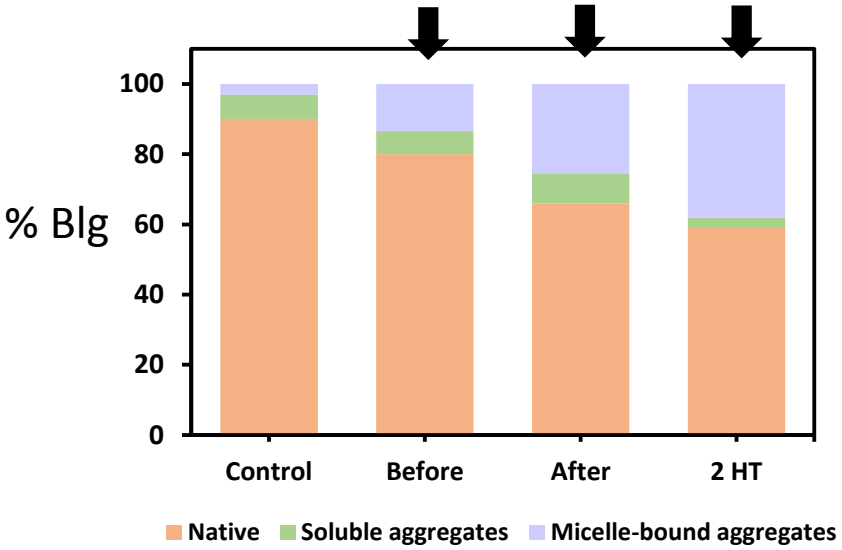
Quantitative analysis on the distribution of denatured whey proteins over WP aggregates and WP associated with the casein micelles

Coupled enzymatic and acid protein fractionation

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

Results : Protein denaturation / aggregation

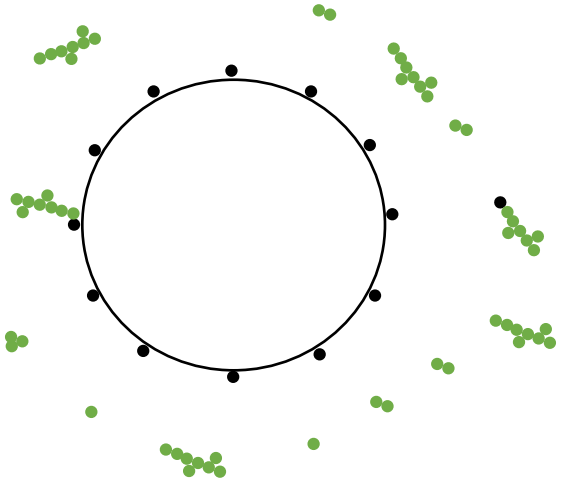
pH Heat treatment	Before	6.77	/	6.77
	After	/	6.68	6.70

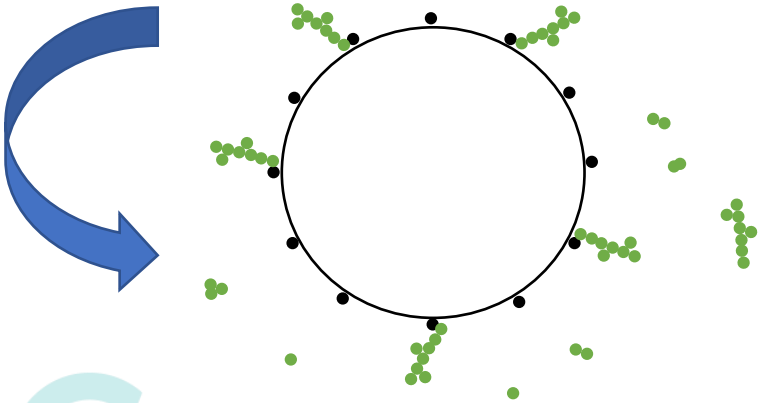


Literature data

~ 6.70/6.75 -> 80 % solubles aggregates

(Anema et al.2003)



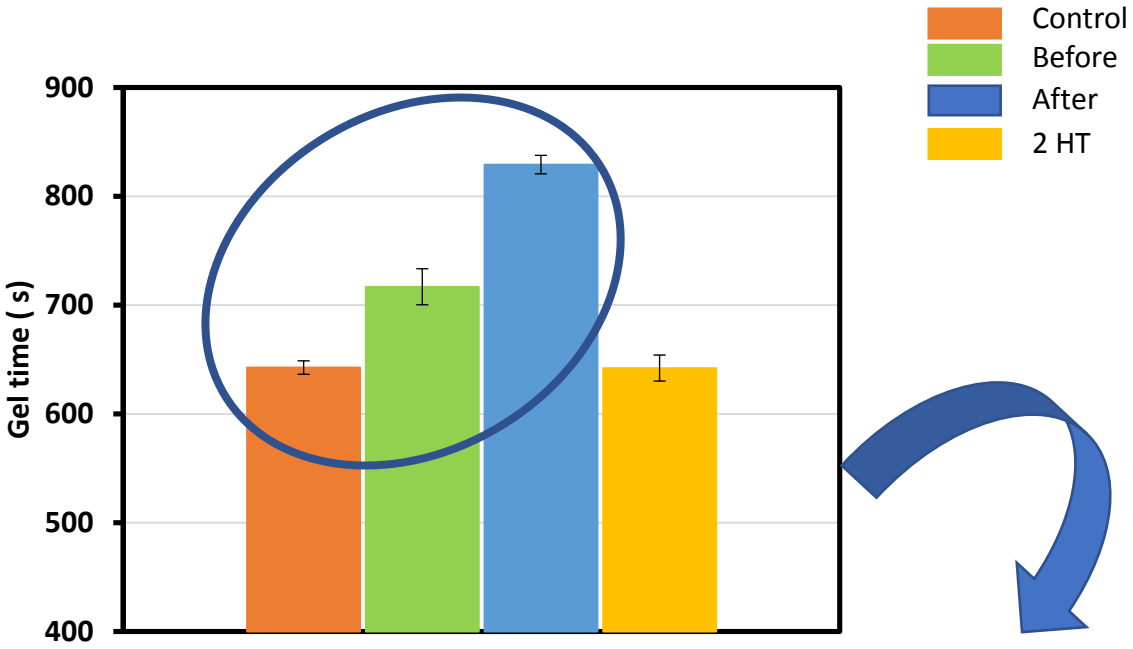


At least, 75 % micelle-bound aggregates

High protein concentration system

Different mechanism of k casein /whey protein association

Result : Enzymatic coagulation properties



↗ protein aggregates formation
↳ ↗ gel time

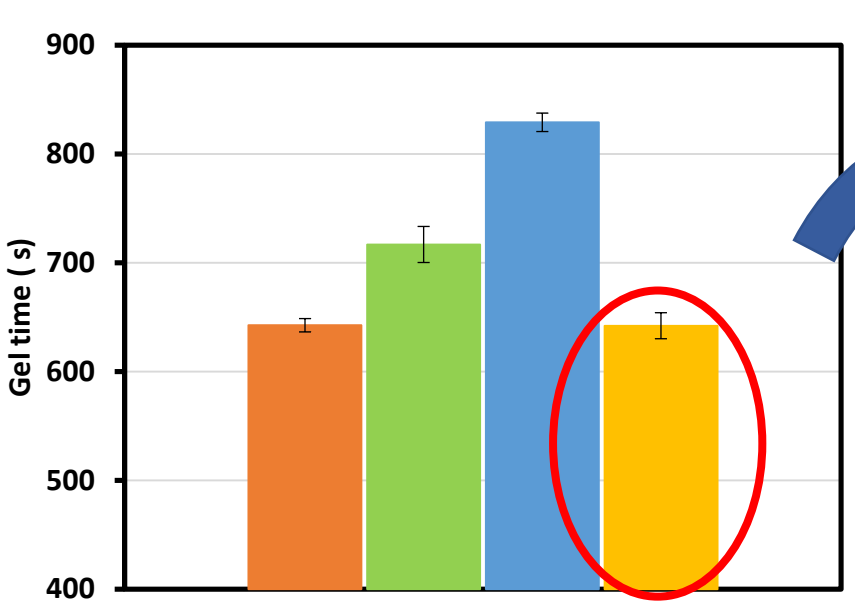
In agreement with milk based studies

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

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

- Steric hindrance
- Electrostatic repulsion

Result : Enzymatic coagulation properties



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

Mechanisms involved ?

Changes in K-casein/WP complexes ?

Investigation of :

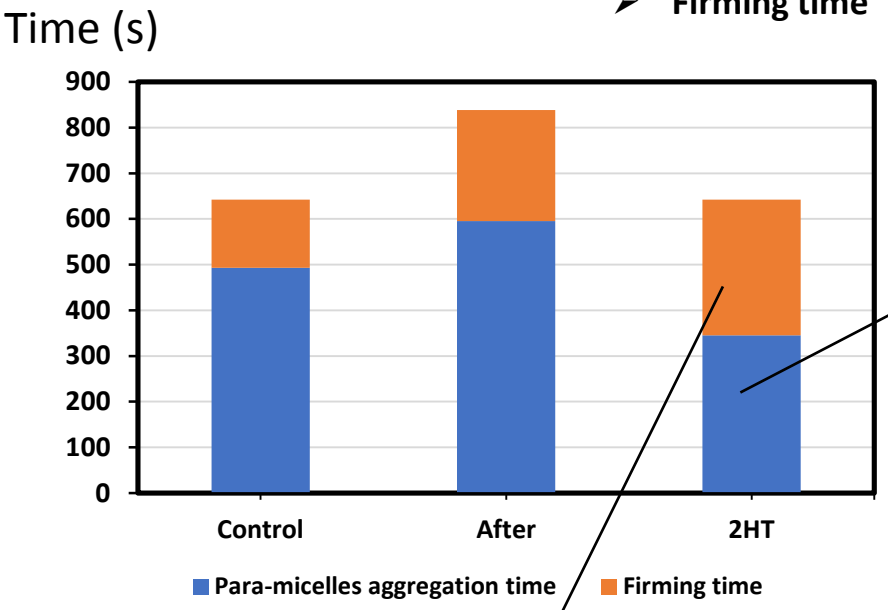
- Para casein micelles aggregation time
- Firming time

Result : Enzymatic coagulation properties

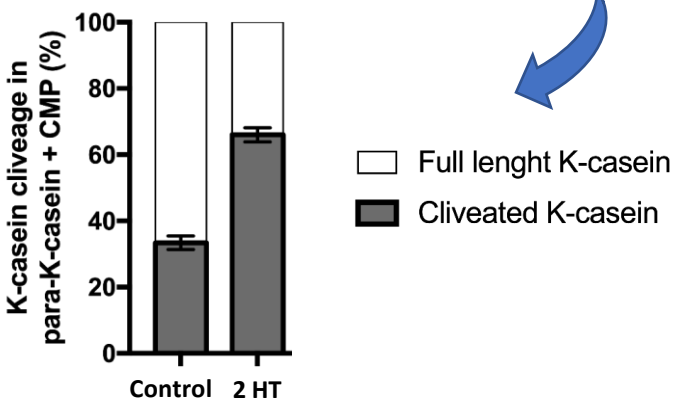
Investigation of :

- **Para casein micelles aggregation time**
- **Firming time**

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



Shortest para-micelles aggregation time



Longest firming time

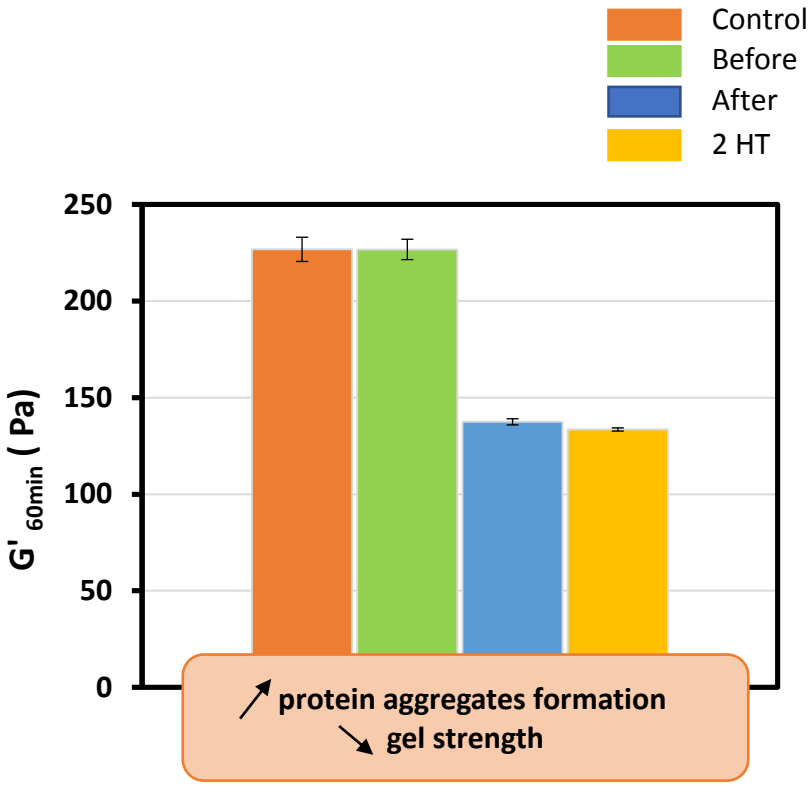
- Large number of K casein/whey protein complexes on micelles

➢ 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

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Thanks

Any questions ?