

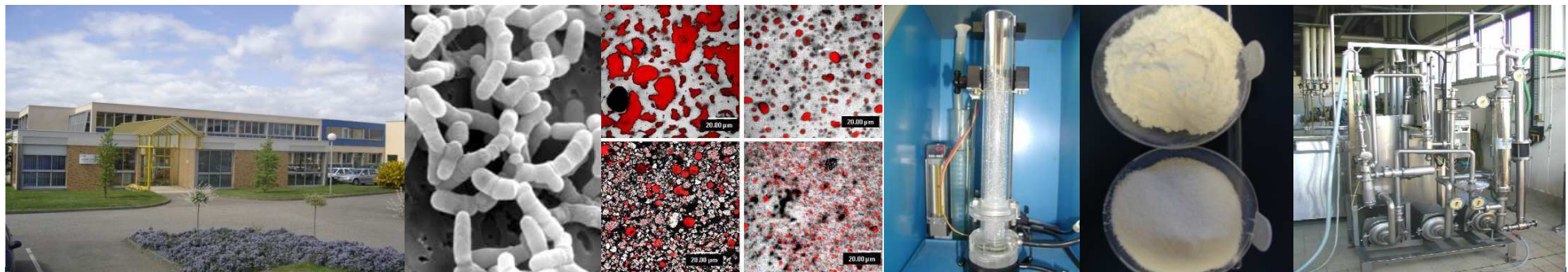
Effect of heat treatment on the formation of acid milk gel: a kinetic study using rheology and confocal microscopy

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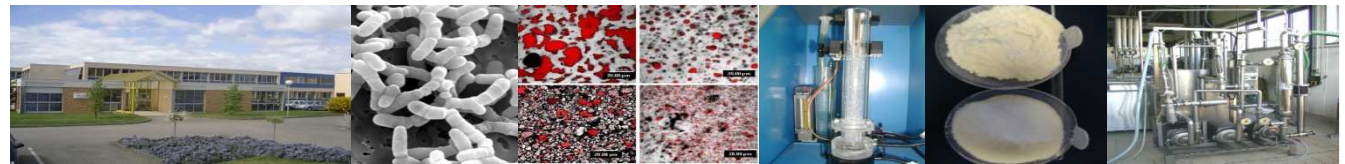
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Introduction

Why study the heat treatment of skim milk?



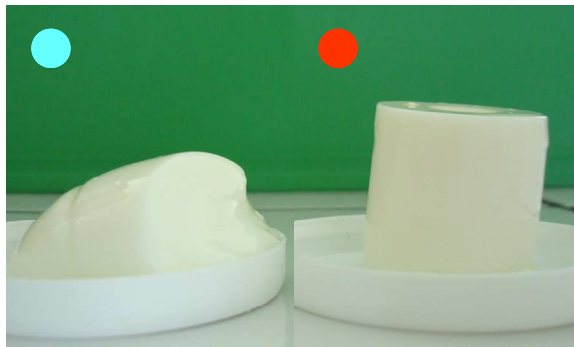
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Introduction

Heating is commonly used in the food industry, for safety purposes.

In the dairy industry, heating also induces changes in the functionality of dairy products: short and long-term stability, texture and other sensory properties.



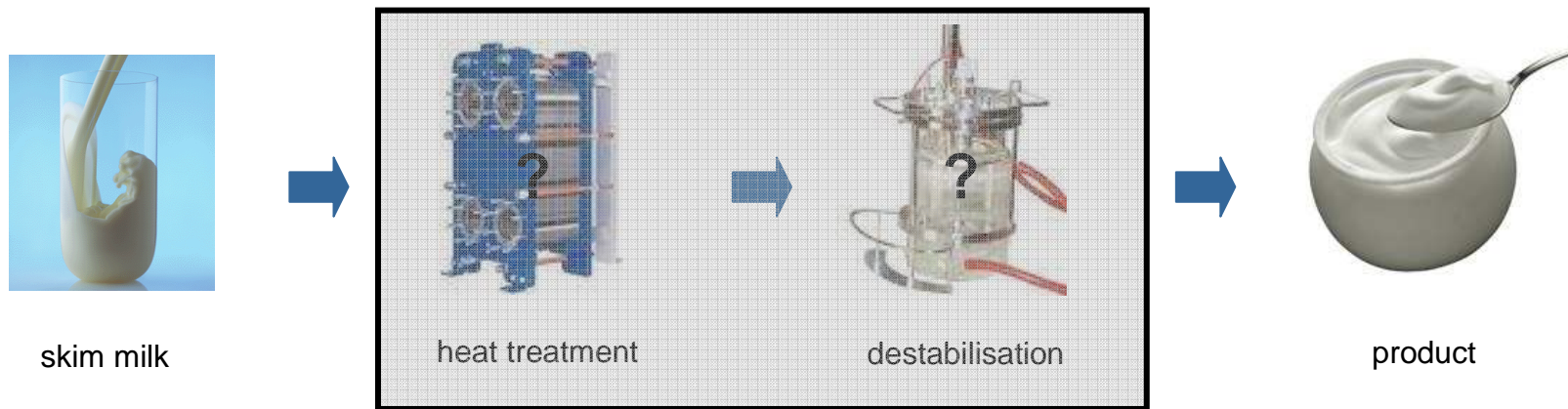
Jemin, 2008

For instance, pre-heat treatment at 85-95°C for up to ~10 min is widely applied to manufacture fermented milk e.g. yogurt or lactic cheese. Strongly heated milk indeed leads to faster gelation, firmer acid gels, with an increased retention of whey.

To a large extent, these heat-induced changes correlate with the denaturation of whey proteins and formation of whey protein/ κ -casein complexes in the milk.

Introduction

Understanding the response of milk proteins to heating is essential



~90% water
3-4% proteins
~5% lactose
~1% ashes

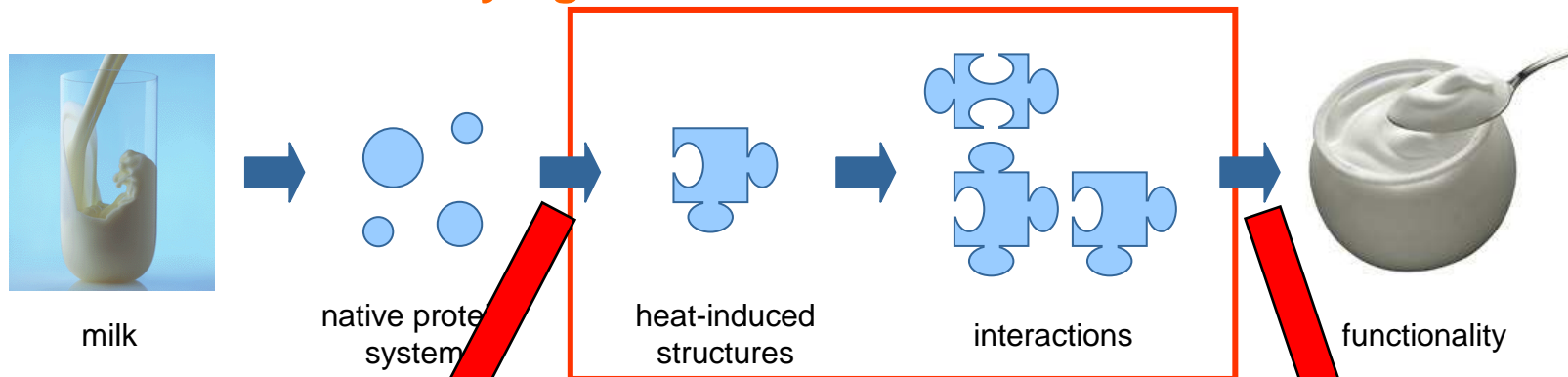
The black box

In most cases, technological operations are “black boxes”

- ▶ better control functional properties
- ▶ control over molecular and macromolecular changes during the process
- ▶ better understanding of the response of the underlying mechanisms

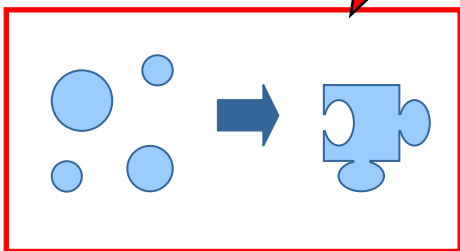
Introduction

What are the underlying mechanisms ?

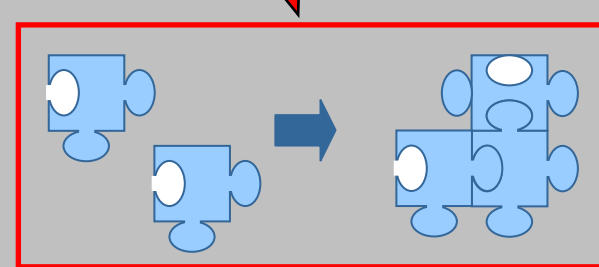


► Objectives




To master the construction of acid milk gels by the comprehension of interactions

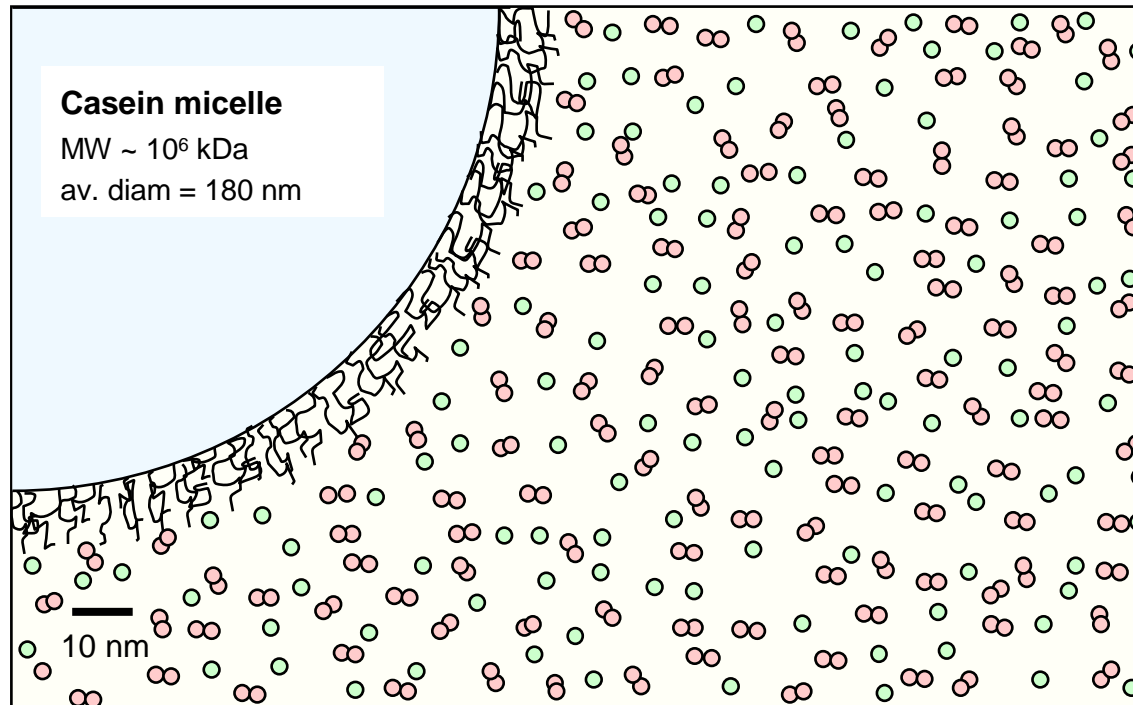


What properties do the heat-induced structures have that may explain their ability to enhance interactions on the building of the protein gel ?



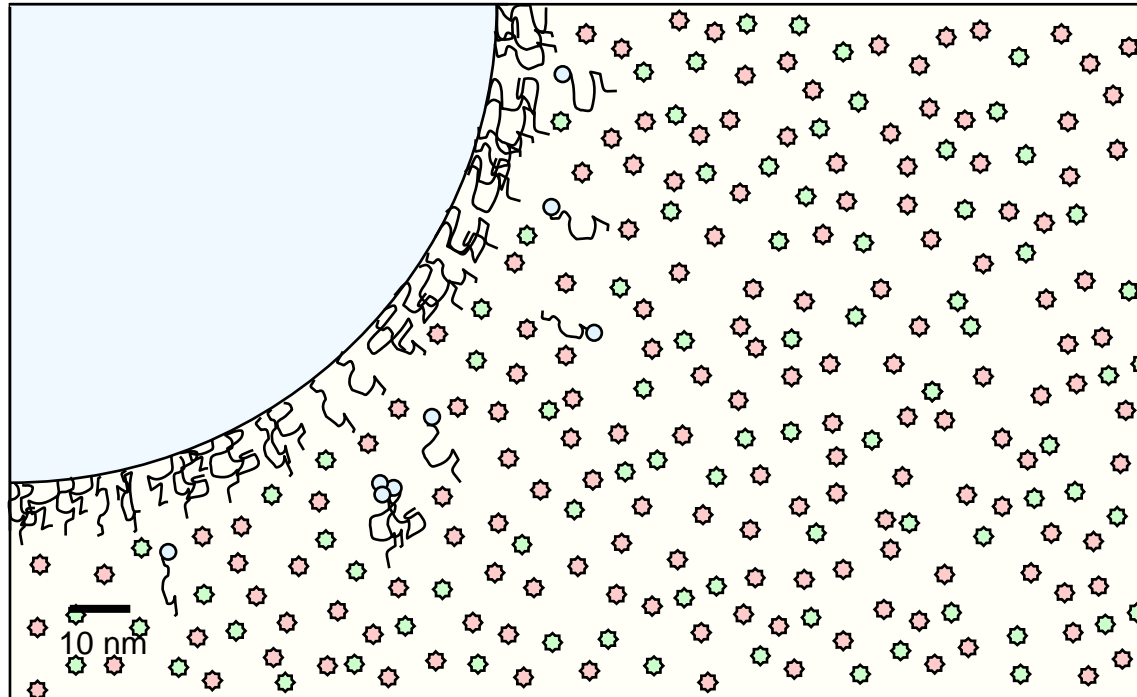
Introduction

	β-lactoglobulin
MW 18.6 kDa, 2 SS bridges, 1 SH	
	α-lactalbumin
MW 14.2 kDa, 4 SS bridges	
	κ-casein
MW 19.0 kDa, 2 ½ SS bridges involved in polymers	



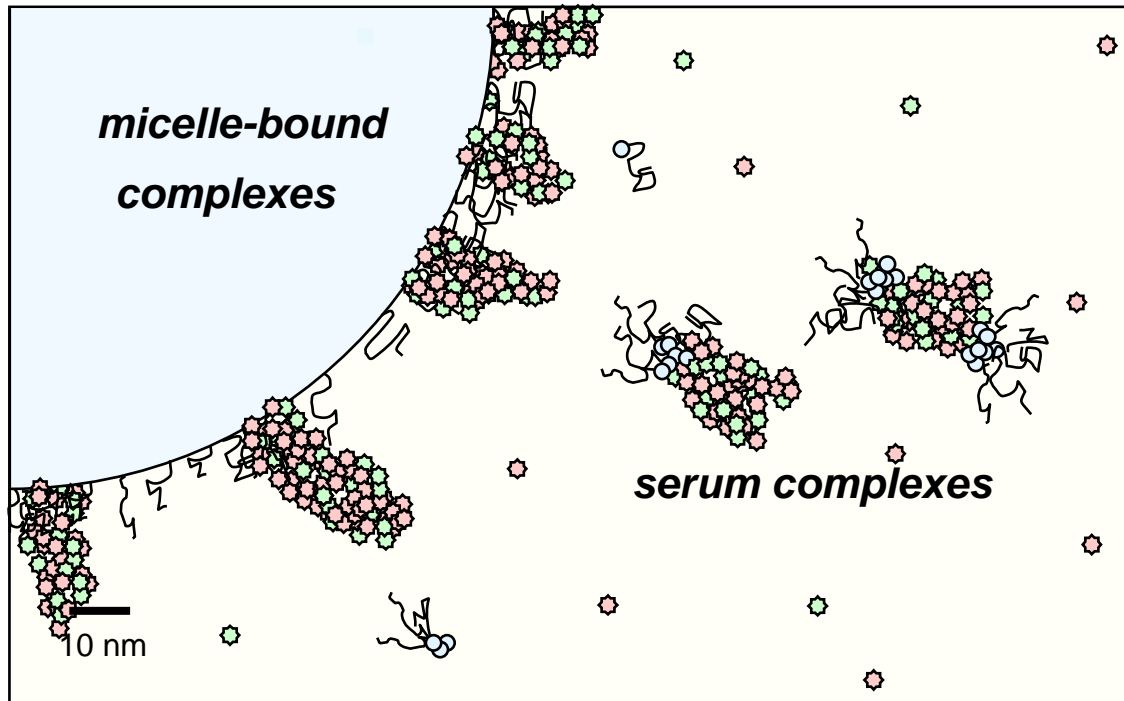
To scale schematic representation of the major whey proteins and of the casein micelle in unheated milk

Introduction

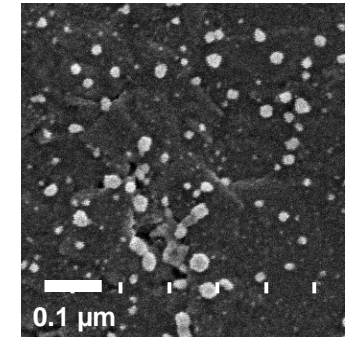


When temperature exceeds $\sim 60^{\circ}\text{C}$, the whey proteins denature, the free thiol of β -lactoglobulin is activated and caseins (especially κ -casein) dissociate from the micelle.

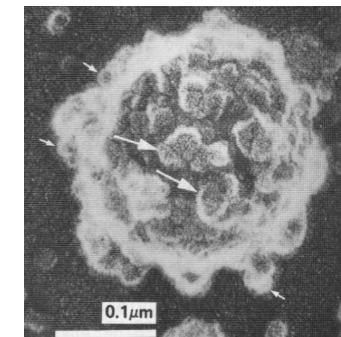
Introduction



Micelle-bound and serum complexes are formed, through hydrophobic interactions and inter-molecular SS bonds between whey proteins, κ -casein and traces of α_s -caseins (Guyomarc'h et al., 2003; Renan et al., 2006; Donato & Dalgleish, 2006).

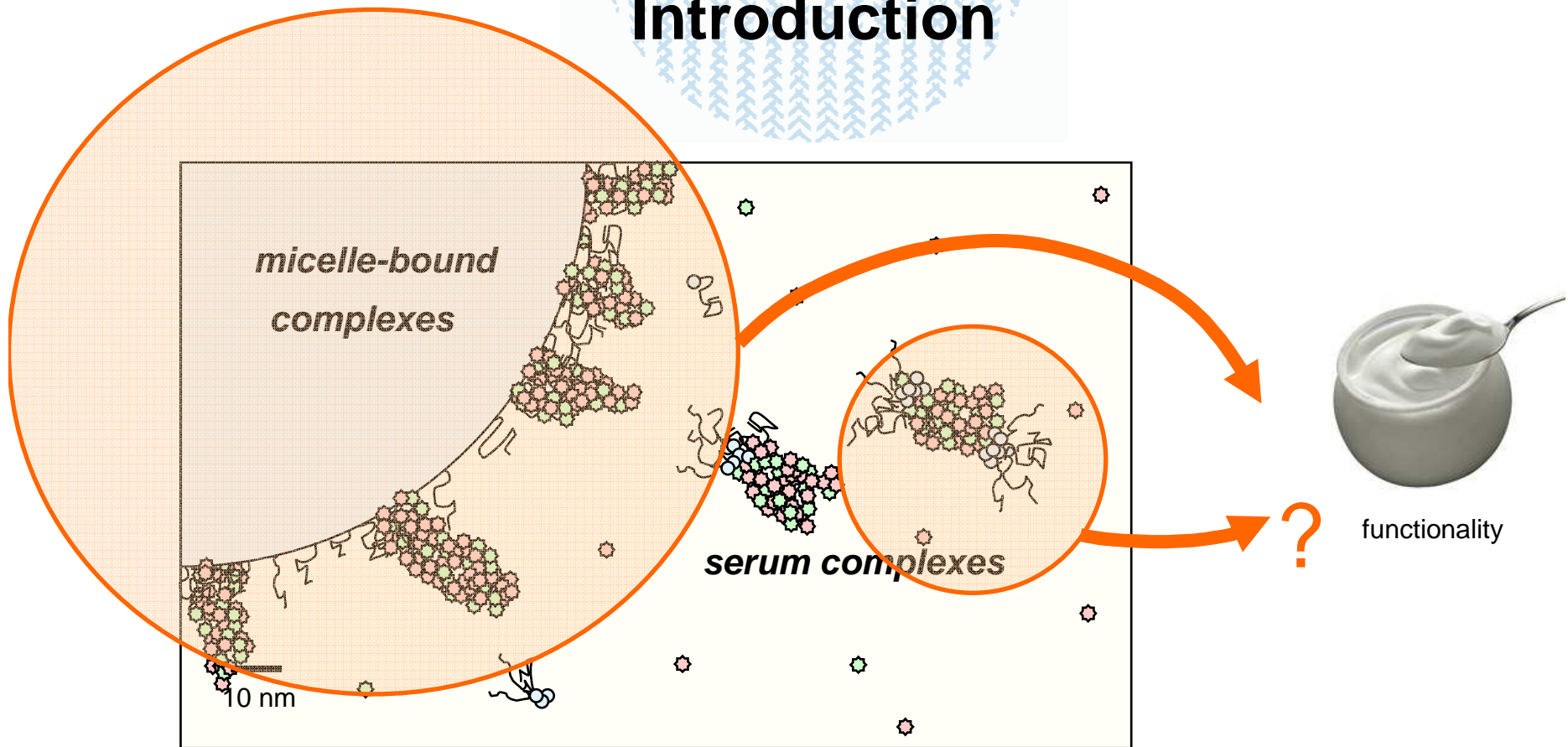


Serum of heated milk observed by SEM (Rodriguez & Dalgleish, 2006)



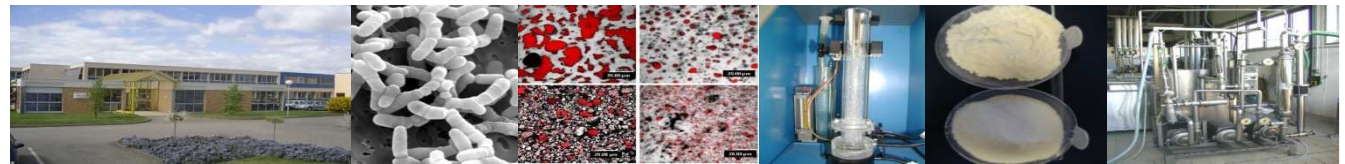
Casein micelle of heated milk observed by SEM (Harwalkar et al., 1989)

Introduction





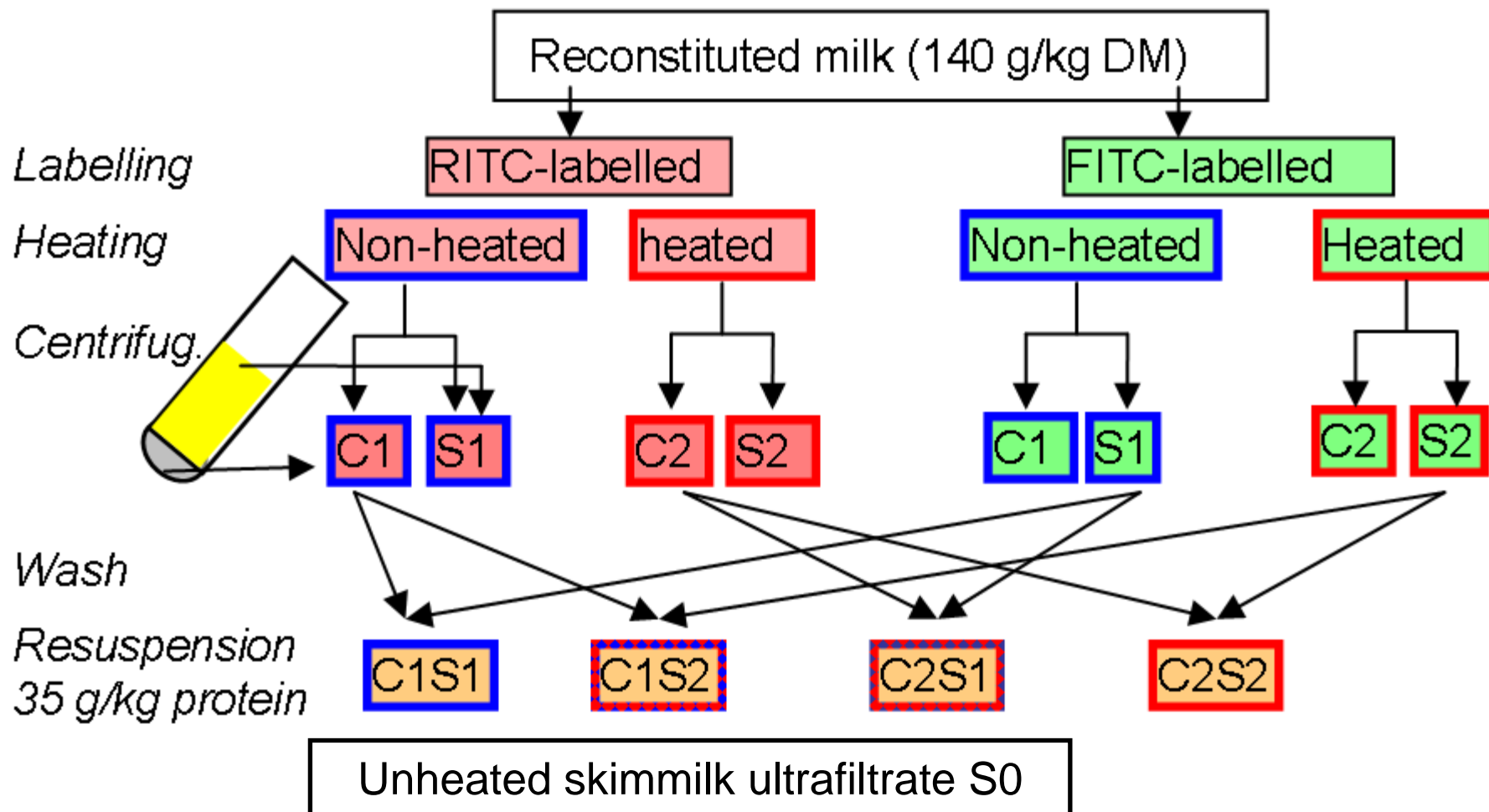
Material and methods



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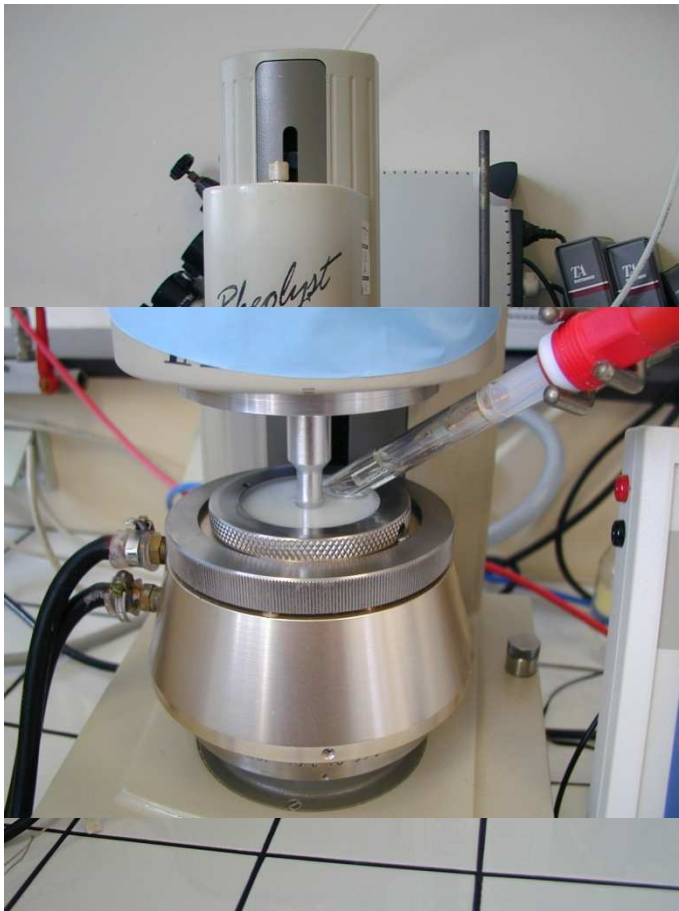


Materials

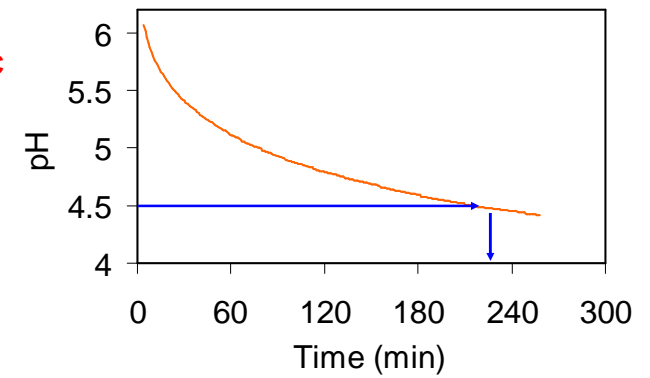


Methods

Acid gelation



35°C



GDL: Glucono-delta-lactone

Methods

Confocal scanning laser microscopy

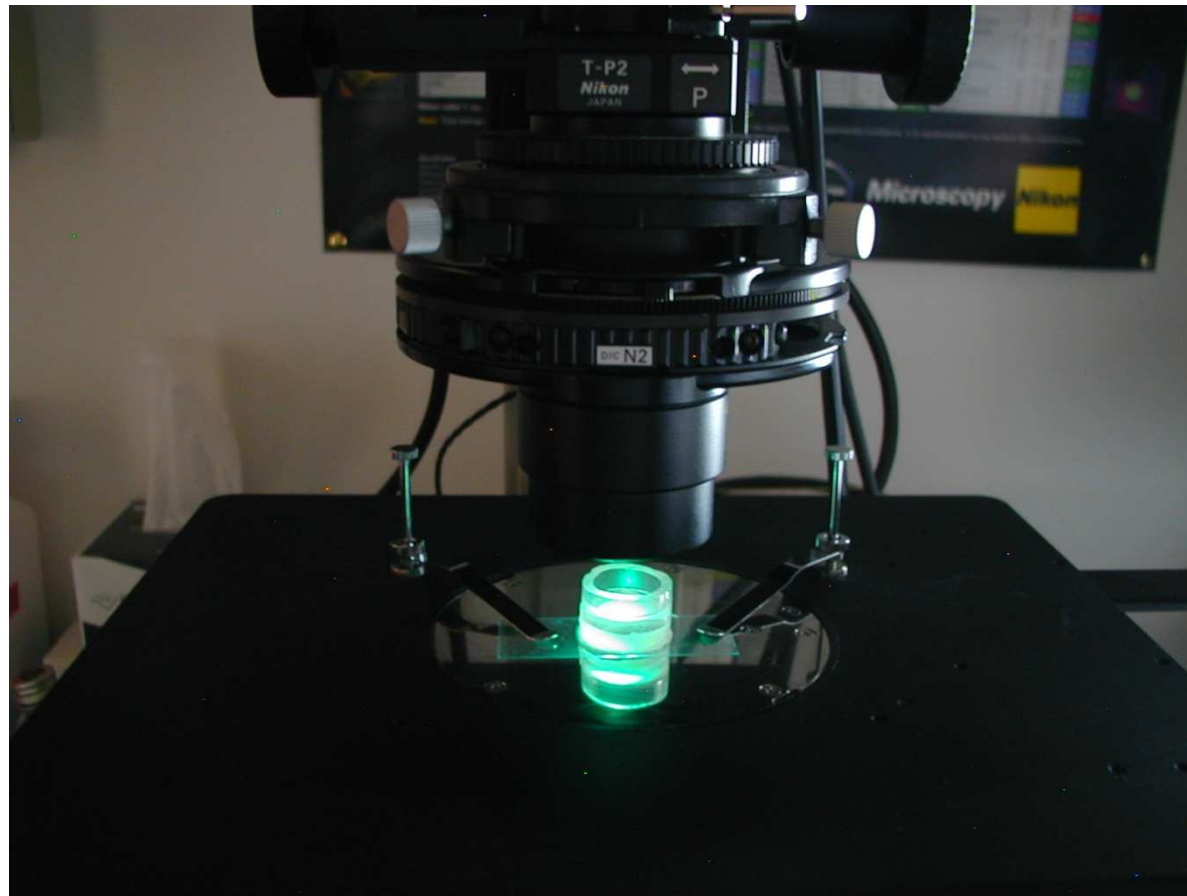
60X oil immersion
objective NA 1.40

FITC

λ_{exc} 488 nm
Emitted light
[515±15 nm]

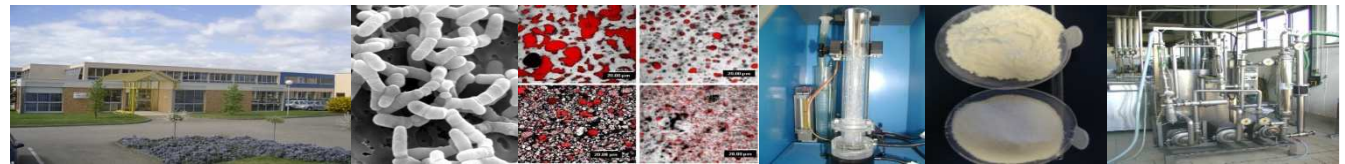
RITC

λ_{exc} 543 nm
Emitted light
[590±25 nm]





Results and discussion



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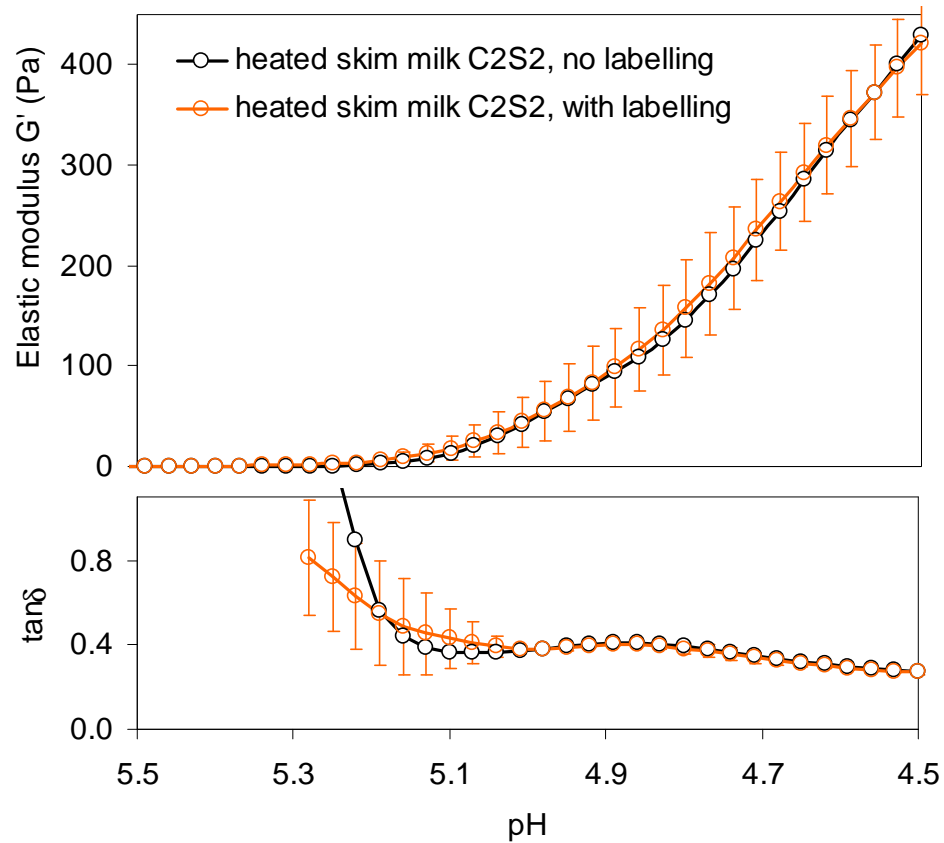


Results and discussion

Effect of labelling the 2 fractions on the C2S2 system

The labelling did not significantly change the acid gelation of C2S2 milk system

When measured by rheometry



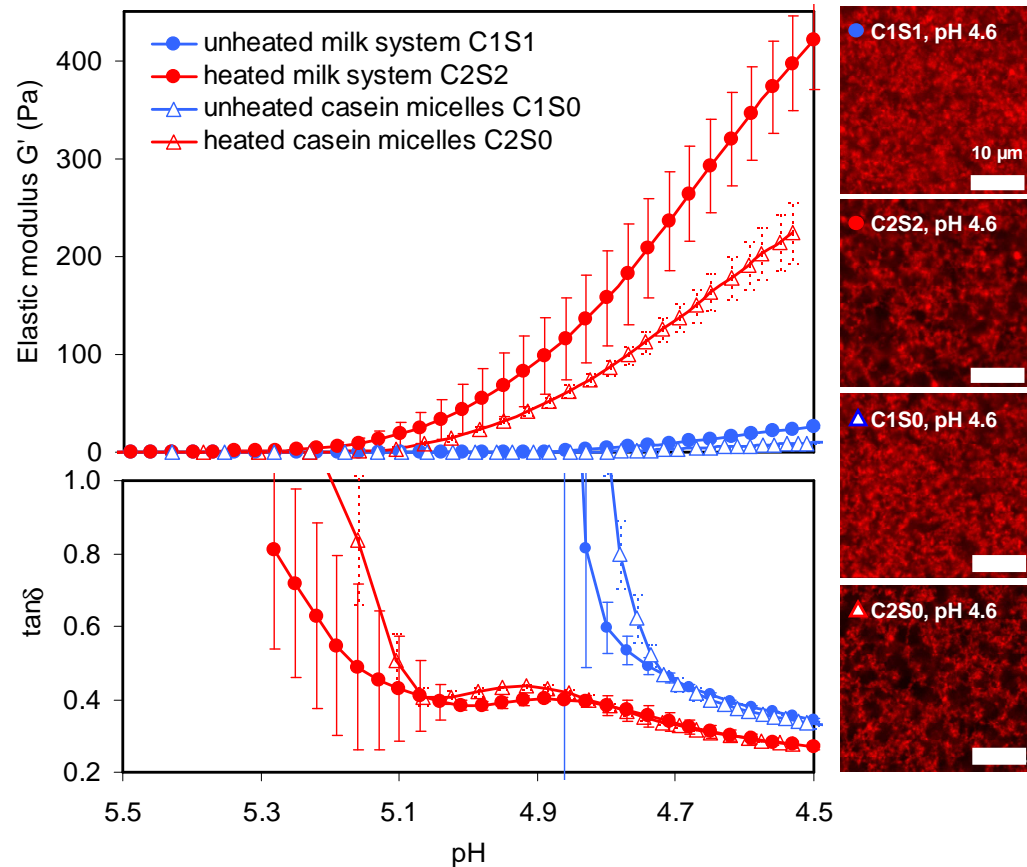
Results and discussion

Effect of the heat-induced micelle-bound complexes to the acid gelation of skim milk systems

	C1S1 (# unheated milk)	C2S2 (# heated milk)
Gelation pH	4.9	5.3
Final G'	26 Pa	420 Pa
Max tan δ	No	Yes

	C1S0	C2S0
Gelation pH	4.8	5.2
Final G'	10 Pa	223 Pa
Max tan δ	No	Yes

Micelle-bound complexes contributed to a large extent to the heat-induced improvements of the acid gel



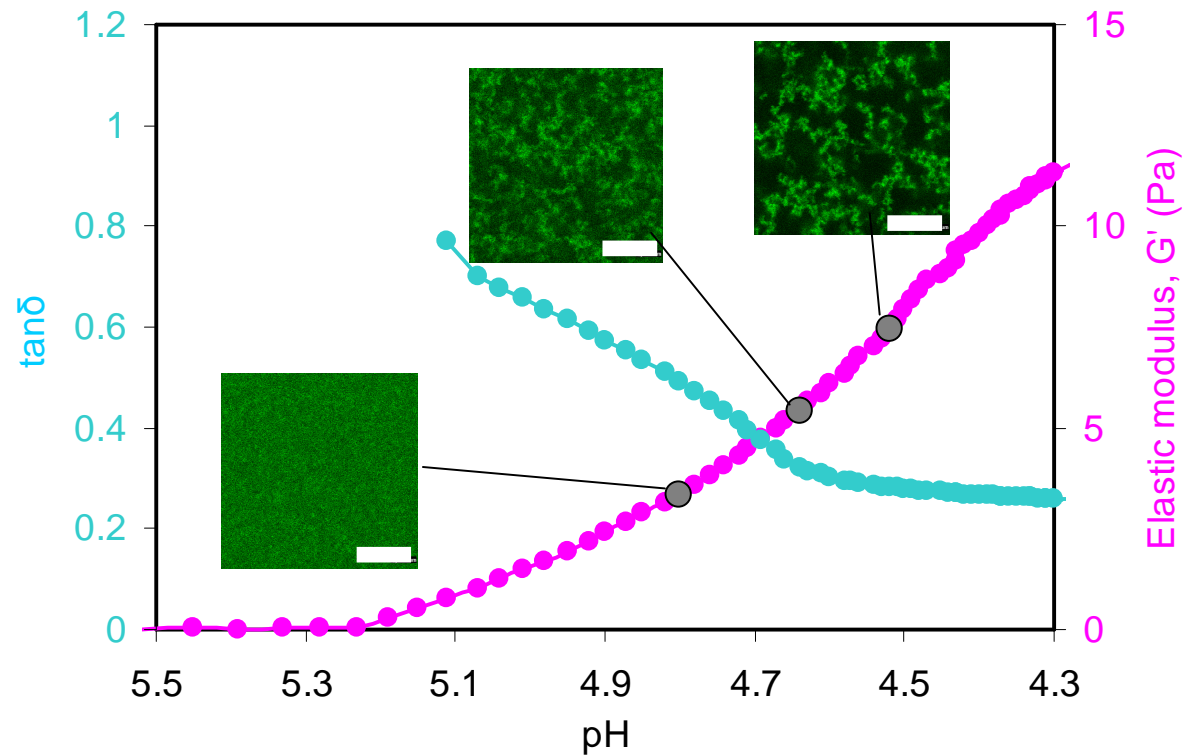
Results and discussion

Acid gelation of the soluble heat-induced complexes

Milk was labelled with only FITC, then heated and its soluble phase was obtained by centrifugation
[prot] ~ 12 g/kg

A gel was formed at pH ~5.2
 $\tan\delta \sim 0.3$

Soluble heat-induced complexes formed a gel at pH ~ 5.2



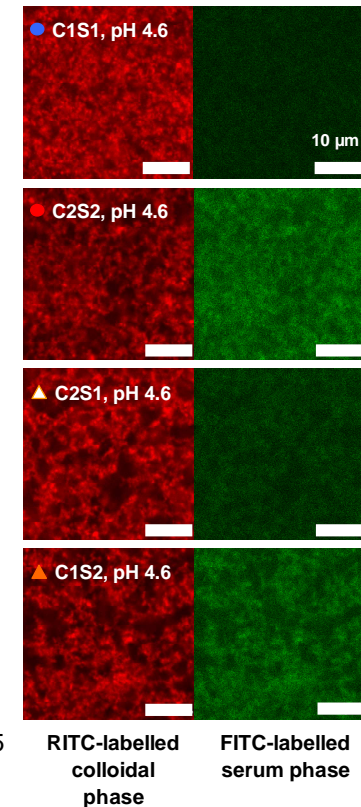
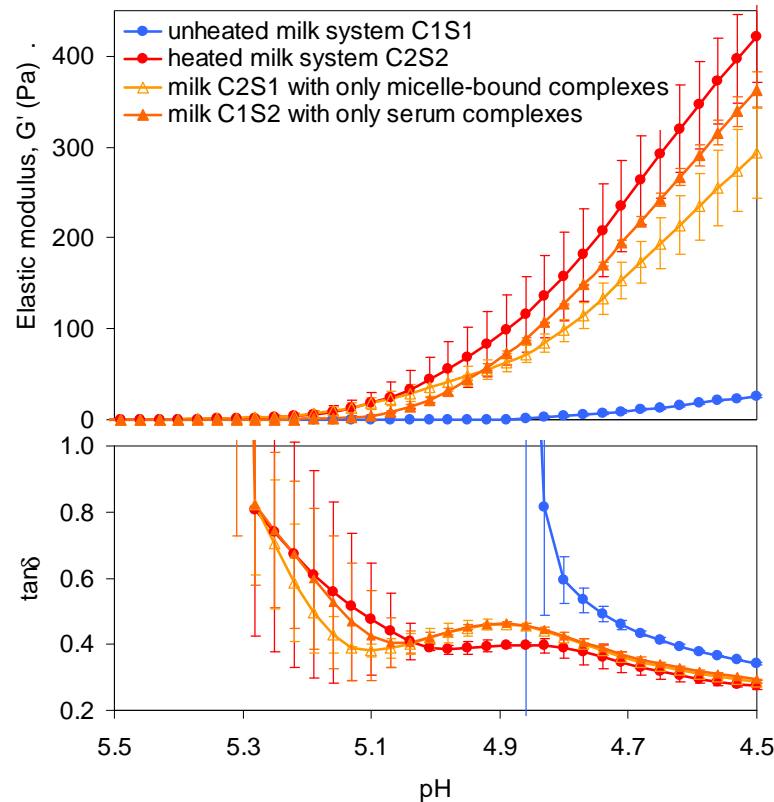
Results and discussion

Contributions of soluble heat-induced and micellar-bound heat-induced complexes to the acid gelation of skim milk systems

- C1S2 ~ C2S1 ~ C2S2

- Heat-induced complexes were responsible for the improvement of the gel
- No matter what their localisation in milk

Heat-induced soluble complexes were co-located with the casein micelles in the gel

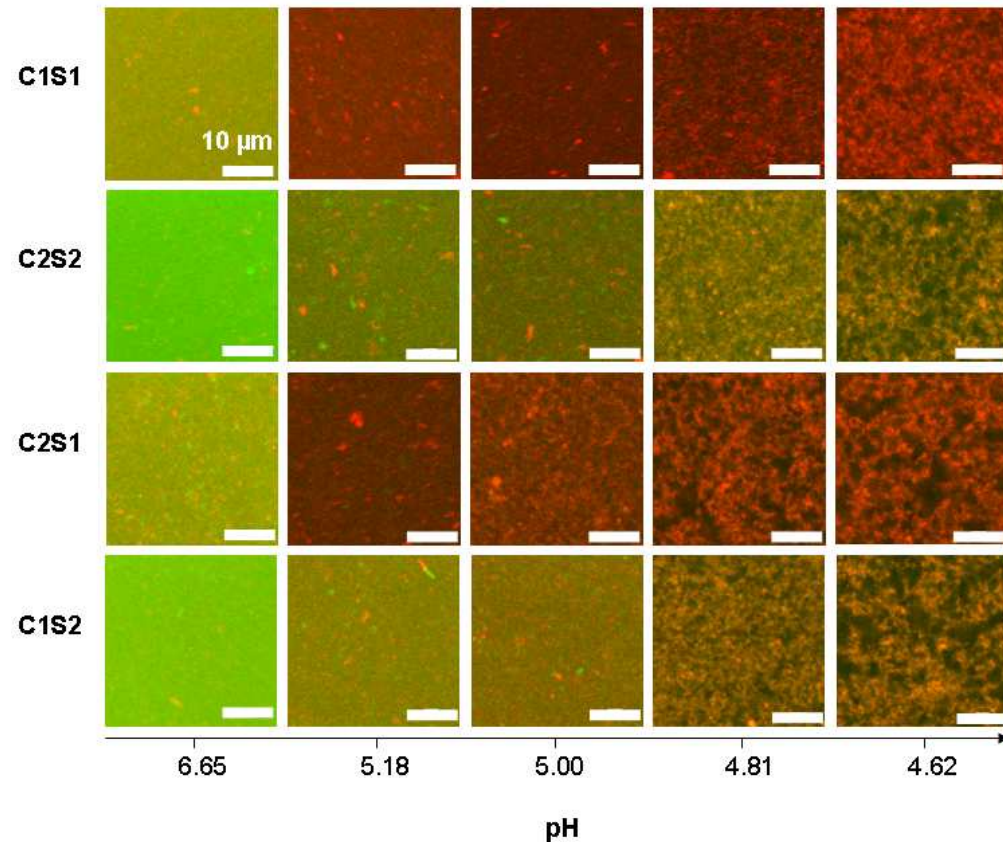


Results and discussion

Contributions of soluble heat-induced and micellar-bound heat-induced complexes to the acid gelation of skim milk systems during acidification

- Arrested diffusion:
 - C1S2, C2S1 and C2S2 at **pH 5**
 - C1S1 at **pH 4.8**
- **Gel formed at pH 4.8** for the 4 systems
- No particular sequence in the formation of the network

FITC-labelled soluble complexes and RITC-labelled micellar complexes became structured simultaneously in super-imposable images

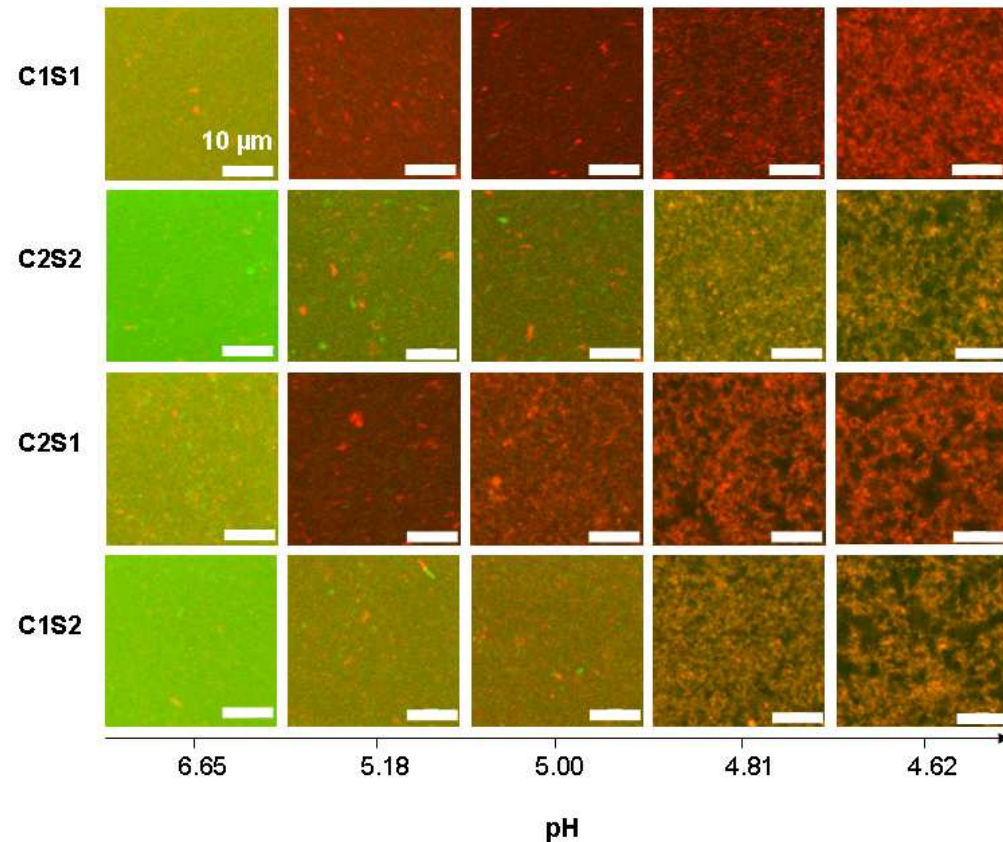


Results and discussion

Contributions of soluble heat-induced and micellar-bound heat-induced complexes to the acid gelation of skim milk systems during acidification

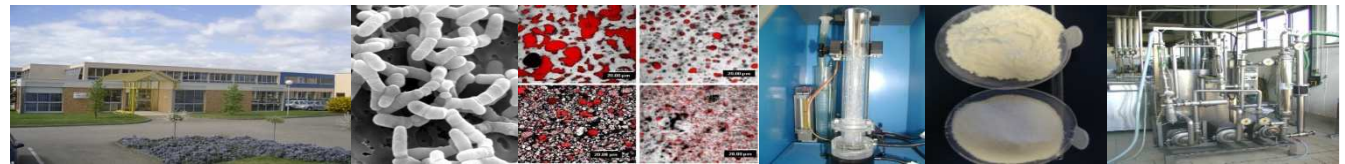
Absence of difference in the rheological properties and microstructure with the initial localisation of the heat-induced complexes and co-localization

Heat-induced soluble complexes interacted with the casein micelle before the onset of gelation ?





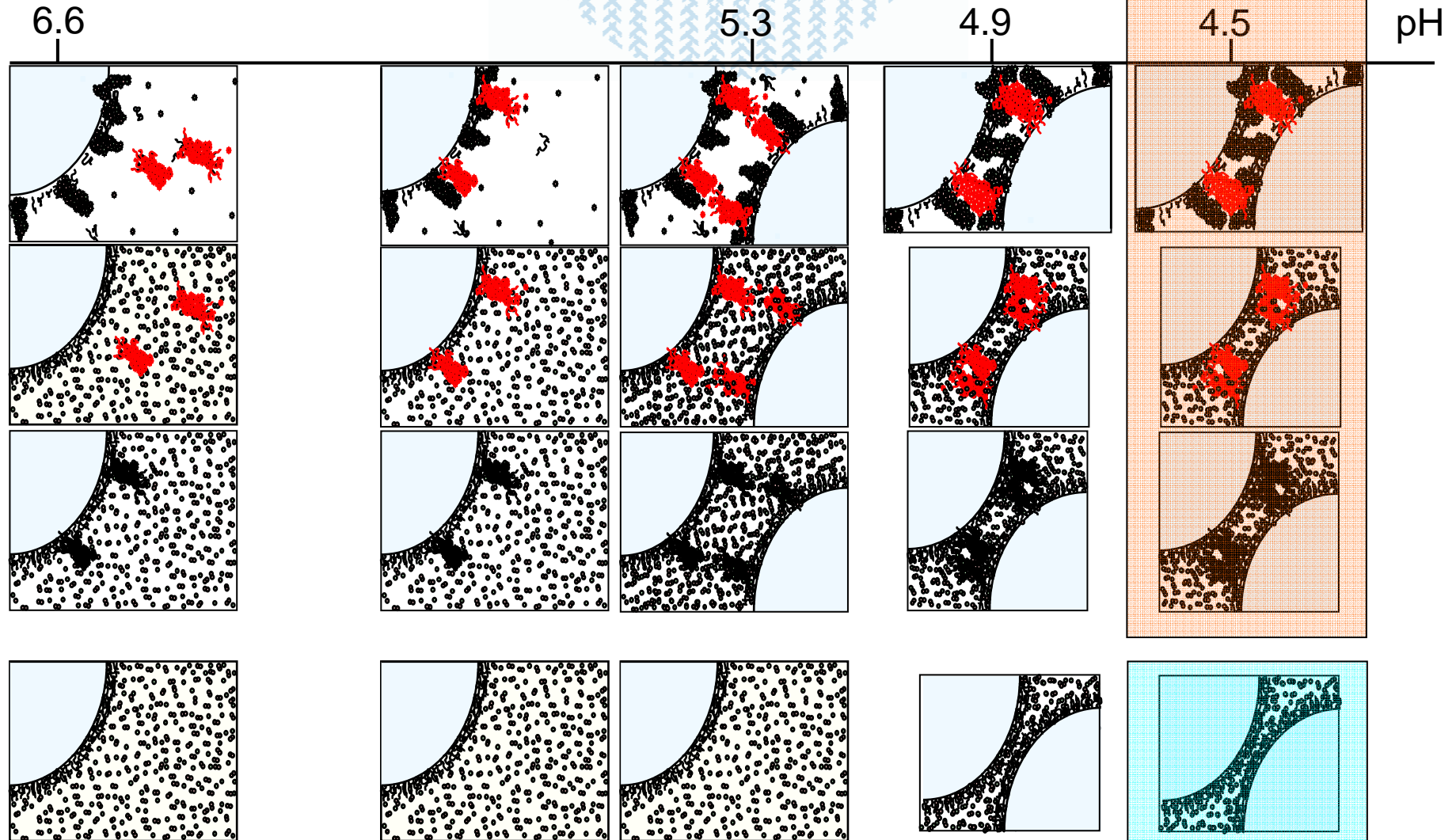
To conclude...



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Conclusion





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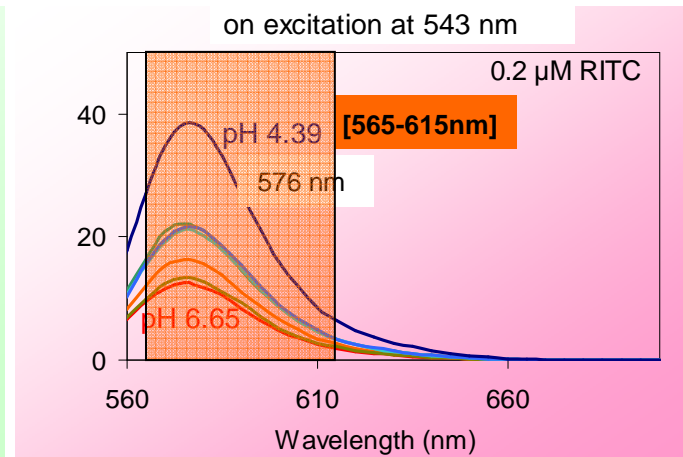
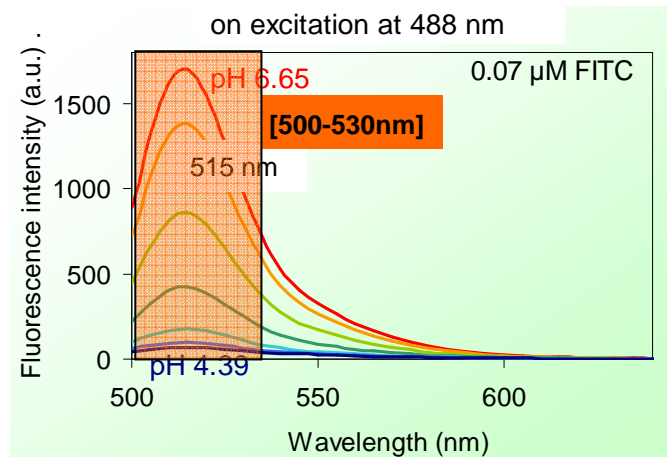
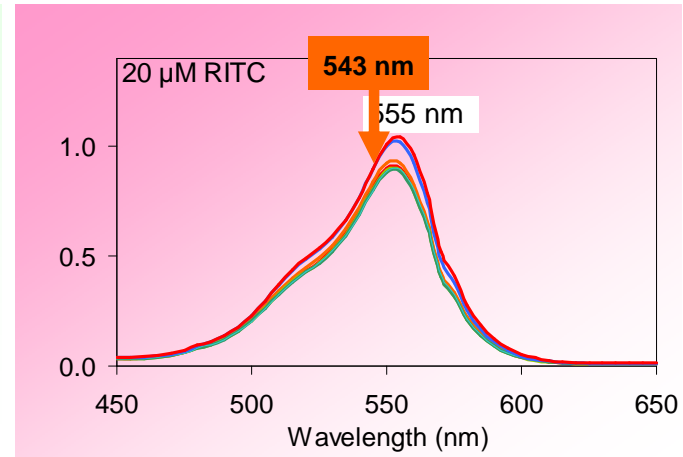
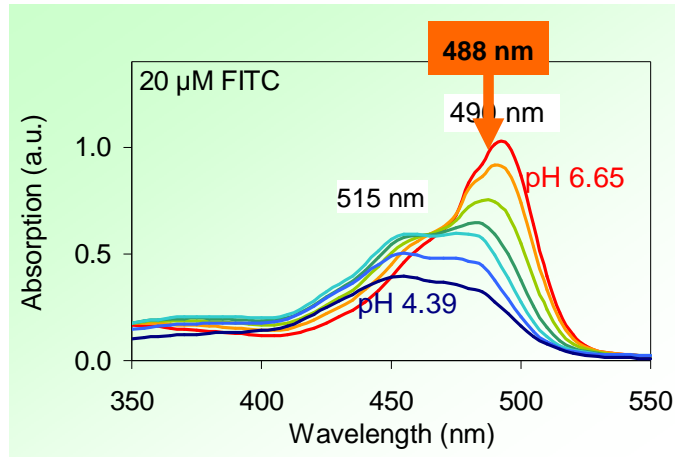


Thank you for your attention



Results and discussion

Absorption and emission of RITC and FITC as a function of pH





What else could be done?

Labelling at pH 8-8.5, because at this pH value, the casein dissociation is rather low and reversible and the binding would have been more covalent and irreversible

Instead of using a ratio of serum phase/micellar phase of 4, it would have been better using 80% of the total mass of protein as caseins and 20% as serum proteins for a total protein content of 35 g/kg

Use the reverse-phase HPLC or mass spectrometry to follow the binding of the probes along the preparation and acidification (to check if probes stayed bound or not)



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