

Comprehensive study of acid gelation of heated milk with model protein systems

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Heat treatment of milk is applied for hygienic purposes

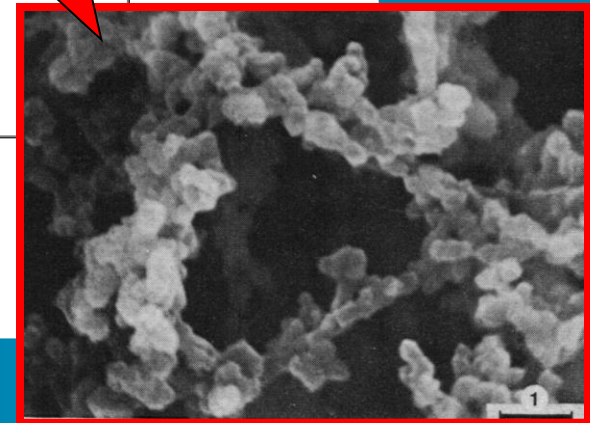
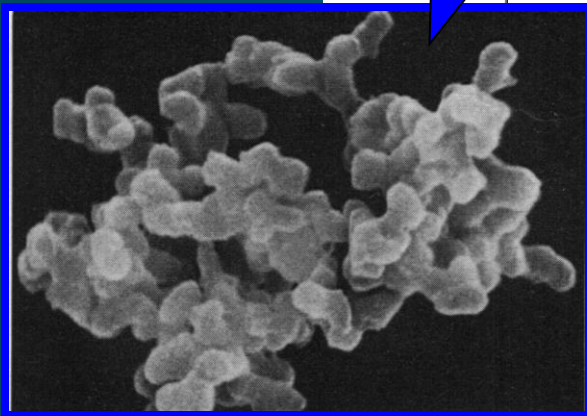
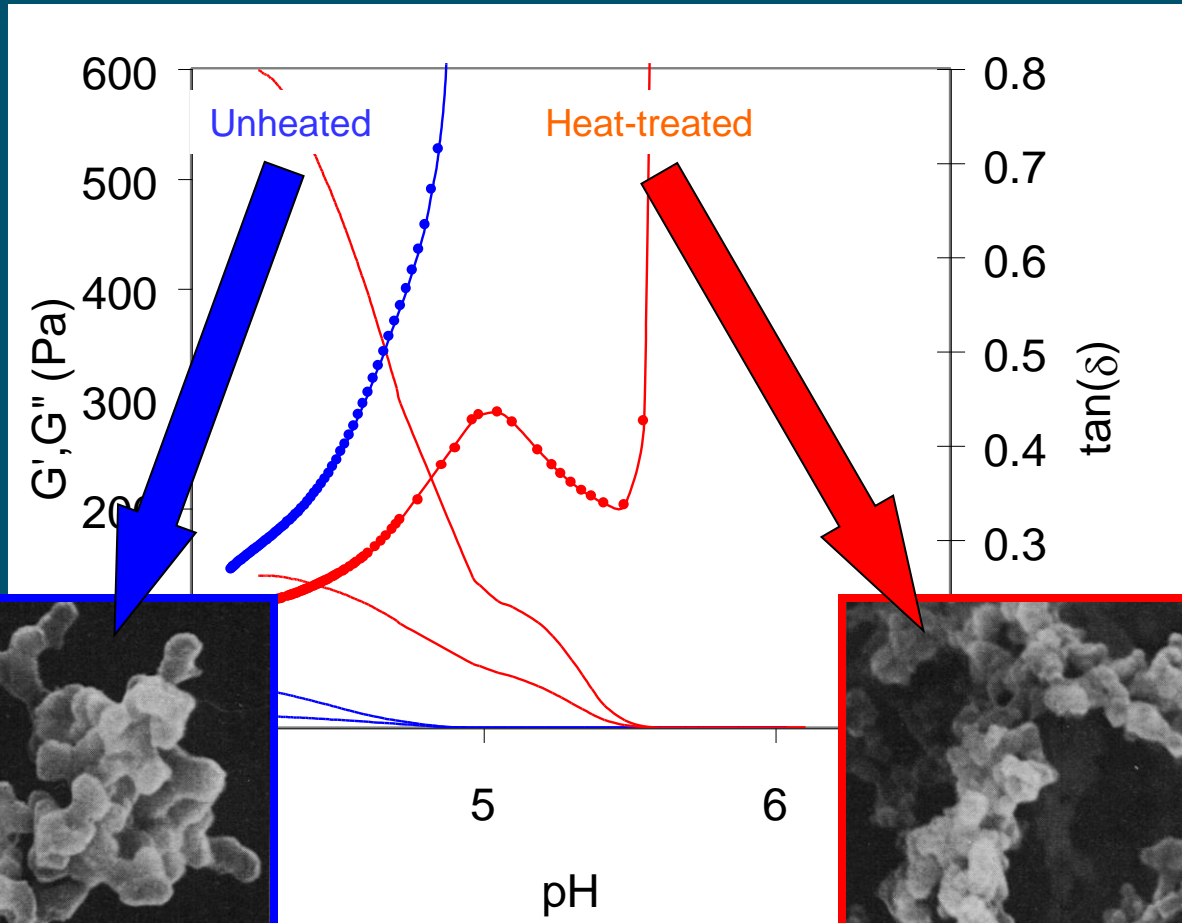


higher pH of gelation

modified pattern for $\tan(\delta)$

higher firmness

modified microstructure



Photographs from Kalab et al., Milchwissenschaft, 1976

Understand the role of heat treatment of milk on acid gel formation

Heat treatment of casein and whey proteins

- ☞ Unfolding of whey proteins

- ☞ β -Lactoglobulin (β -Lg) interacts with β -Lg, with other whey proteins and with caseins

 - ☒ Sulphydryl-disulfide exchanges,

 - ☒ Hydrophobic interaction,

 - ☒ Calcium bridging.

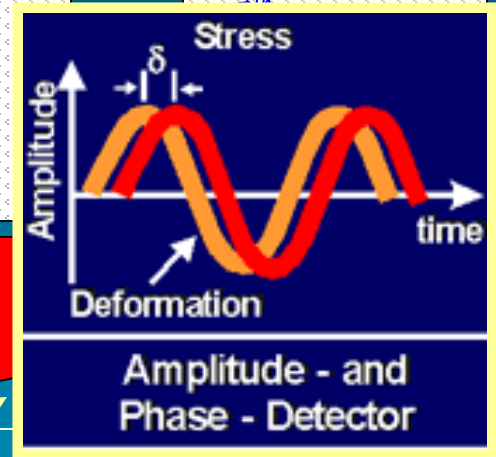
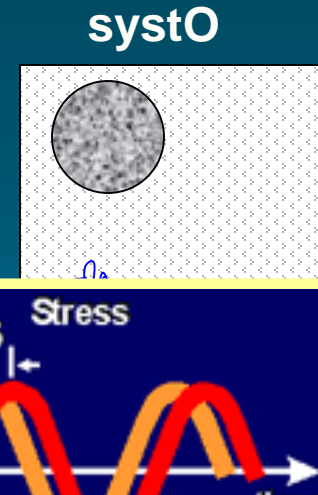
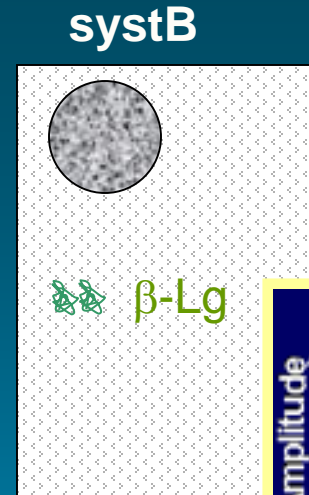
- ☞ Polymers of β -Lg, α -Lactalbumin and κ -casein

 - ☒ On casein particles

 - ☒ In the “soluble” phase (κ -casein leaves the micelle)

During acidification, denaturation of whey proteins can lead to a higher pH at gelation and to firmer gels

Casein micelles: 35 g.kg⁻¹
β-Lg or ovalbumin: 4 g.kg⁻¹
0.46 g.kg⁻¹ yeast extract



Heat treatment: 90°C -24 min (14 min from 20 to 90°C)

Gelation

After heat treatment: inoculation with yoghurt starters at 42°C

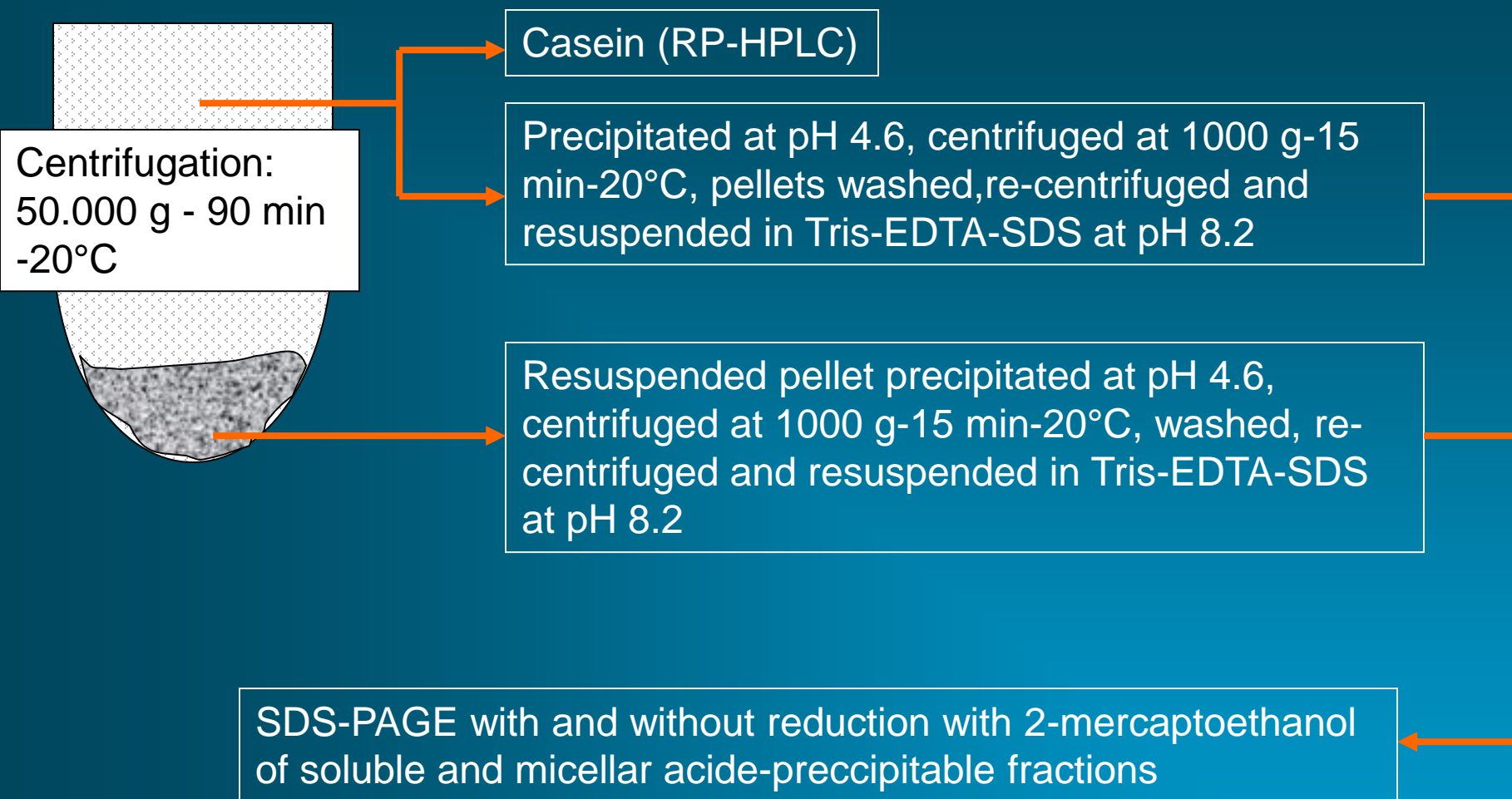
Low amplitude dynamic oscillation

Scanning electron microscopy at pH 4.6

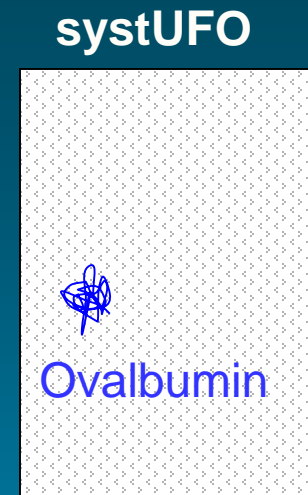
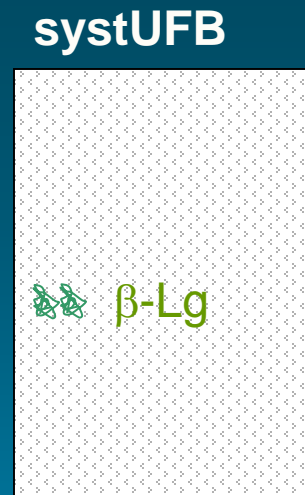
Analyses

Before and after
Partition of casein proteins into solid phases

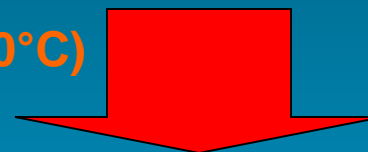




β -Lg or ovalbumin: 4 g.kg⁻¹



Heat treatment: 90°C -24 min (14 min from 20 to 90°C)



Solubility versus pH

After heat treatment: dilution into milk ultrafiltrate adjusted at pH 6.5 to 1.6

- OD at 600 nm against water
- Centrifugation (3000g-15 min-20°C), dilution in Tris-HCl, urea buffer and OD at 280 nm

pH of heated systR, systB and syst O at 6.6

SystUFR, systUFB, systUFO were translucent before heat treatment

Hazy appearance of heated systUFR and systUFB

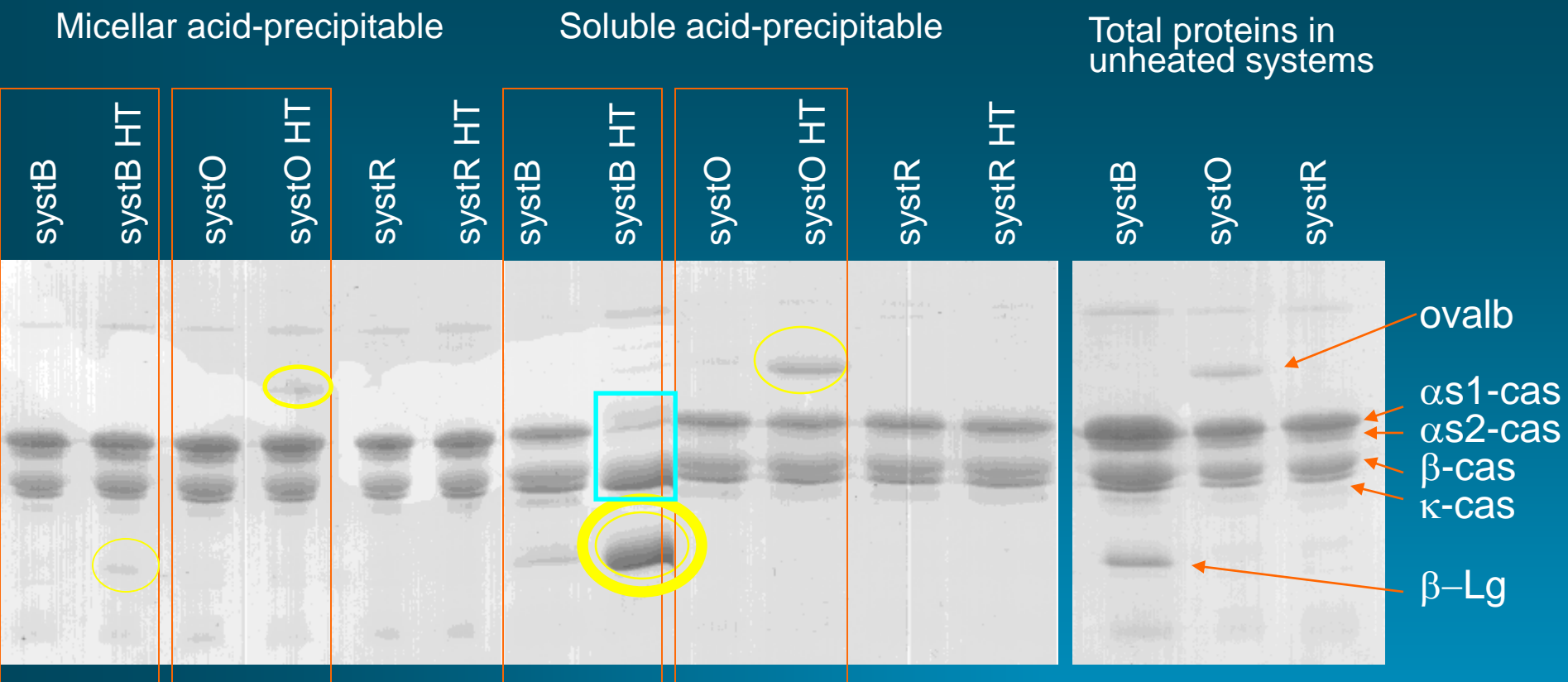
A precipitate in heated systUFO, easily resuspended

Ovalbumin aggregated in a different way than β -Lg

	Percentage of soluble caseins					
	SystR		SystB		SystO	
	NHT	HT	NHT	HT	NHT	HT
κ -cas	12.02 ± 2.01	11.68 ± 0.05	14.97 ± 1.64	41.15 ± 2.15	13.00 ± 0.94	10.90 ± 1.02
α_{s2} -cas	6.62 ± 5.16	4.83 ± 1.54	4.88 ± 1.03	1.53 ± 2.17	4.06 ± 0.71	7.22 ± 4.10
α_{s1} -cas	5.28 ± 1.52	4.79 ± 0.22	6.76 ± 0.54	1.85 ± 0.02	5.18 ± 0.06	4.44 ± 0.09
β -cas	8.22 ± 2.89	8.01 ± 0.61	12.78 ± 0.95	10.46 ± 0.51	8.28 ± 0.29	7.50 ± 0.13

Heat treatment of systB led to a considerable increase in soluble κ -casein and to a decrease in soluble α_{s1} -casein

In the other systems, soluble phases of heated and unheated were the same

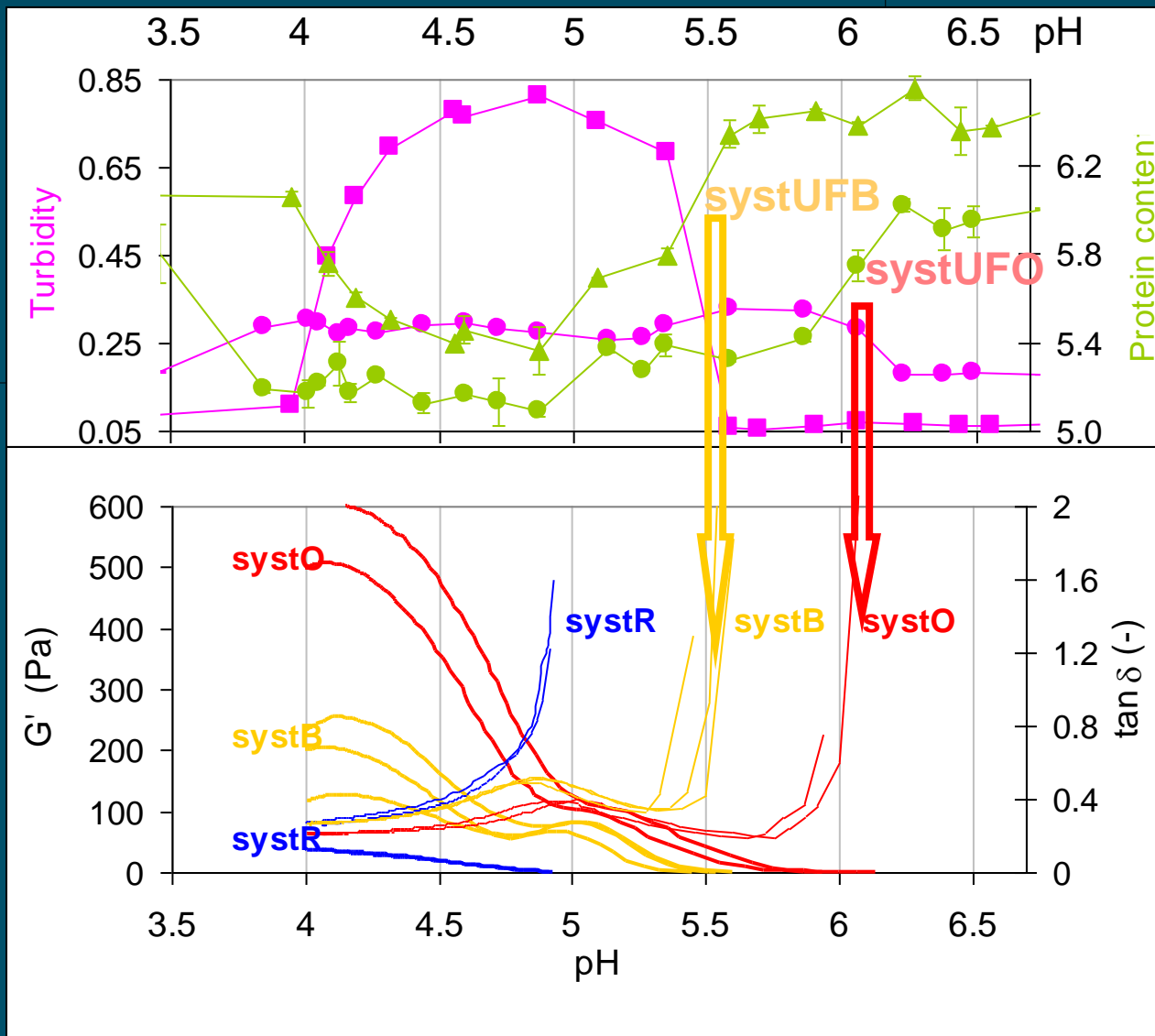


Heat treatment ⇒ Slight bands of β-Lg and ovalb in micellar acid-precipitable fractions

⇒ Large bands of β-Lg and ovalb in soluble acid-precipitable fractions

Relative intensity of β-Lg band in soluble acid-precipitable fraction from heated systB was very high

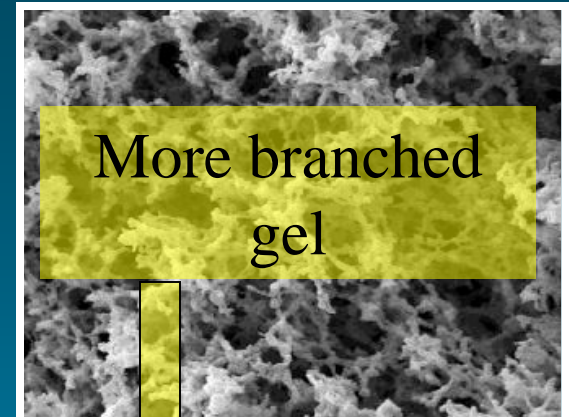
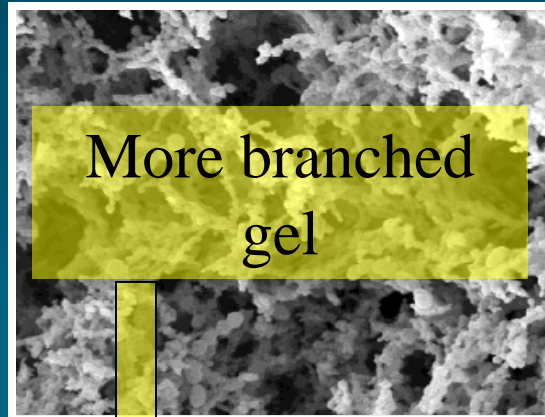
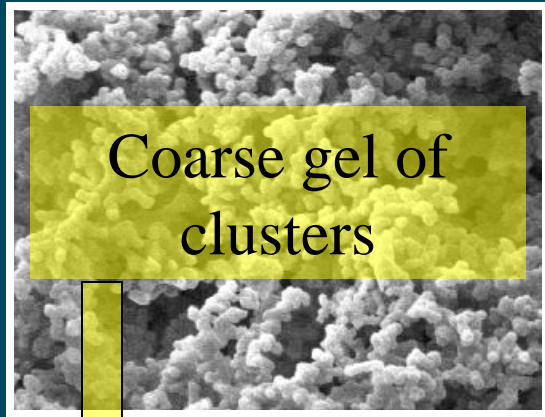
Absence of globular proteins when samples were not reduced



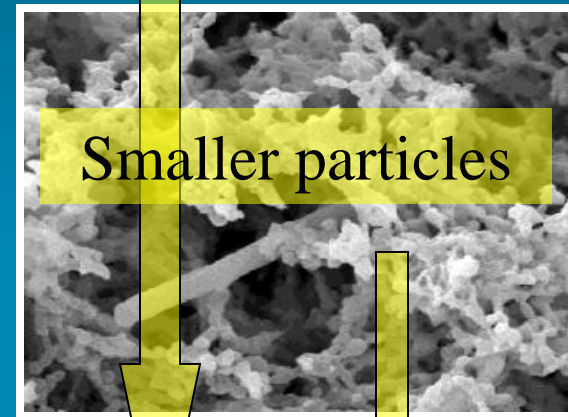
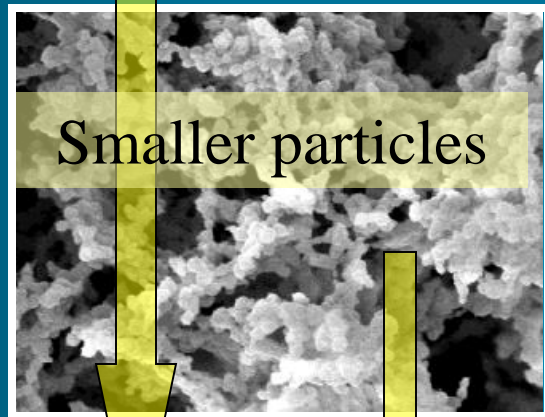
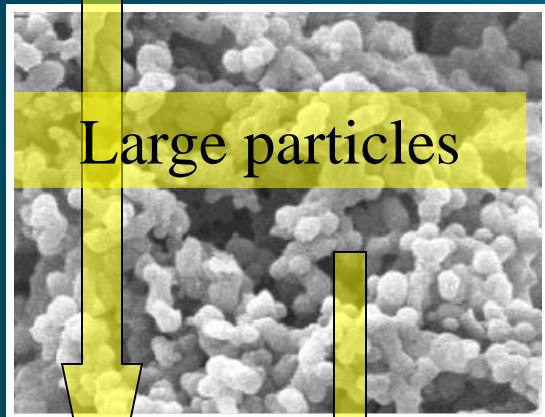
- High concentration of soluble κ -casein in the presence of β -Lg
- Not in the presence of ovalbumin

**Why does β -Lg provoke solubilisation of κ -casein?
Why ovalbumin does not?**

- Different size \Rightarrow different penetration \Rightarrow different accessibility of κ -casein
- Different molar concentration (ovalbumin: 0.08mM; β -Lg: 0.27 mM)
- Ovalbumin is already denatured



2 μ m



1 μ m

systR

systB

systO

A gel from unheated milk # B gel from heated milk

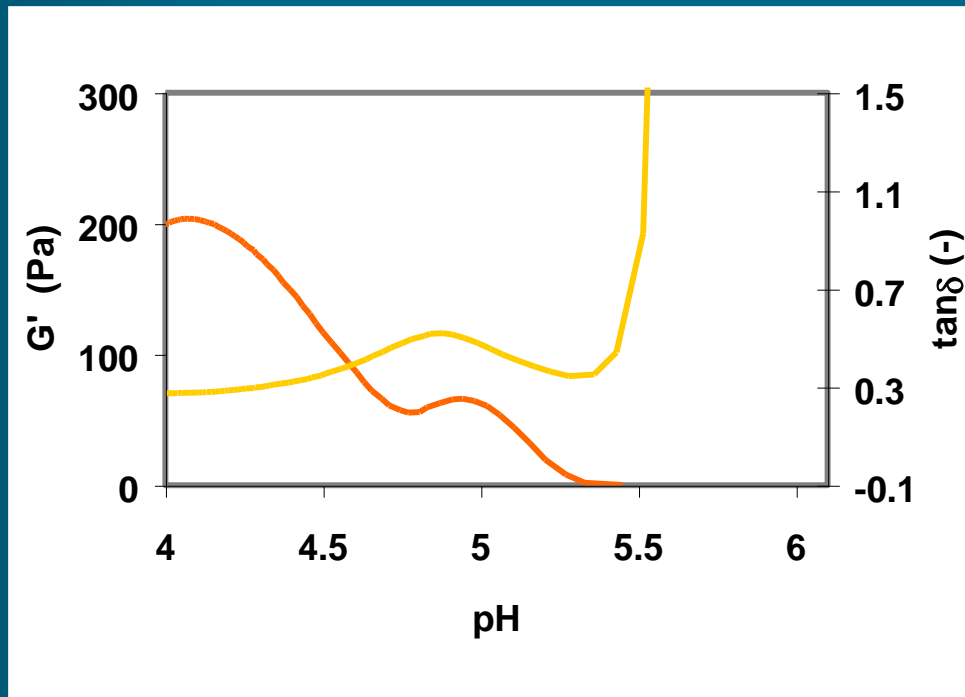
systB

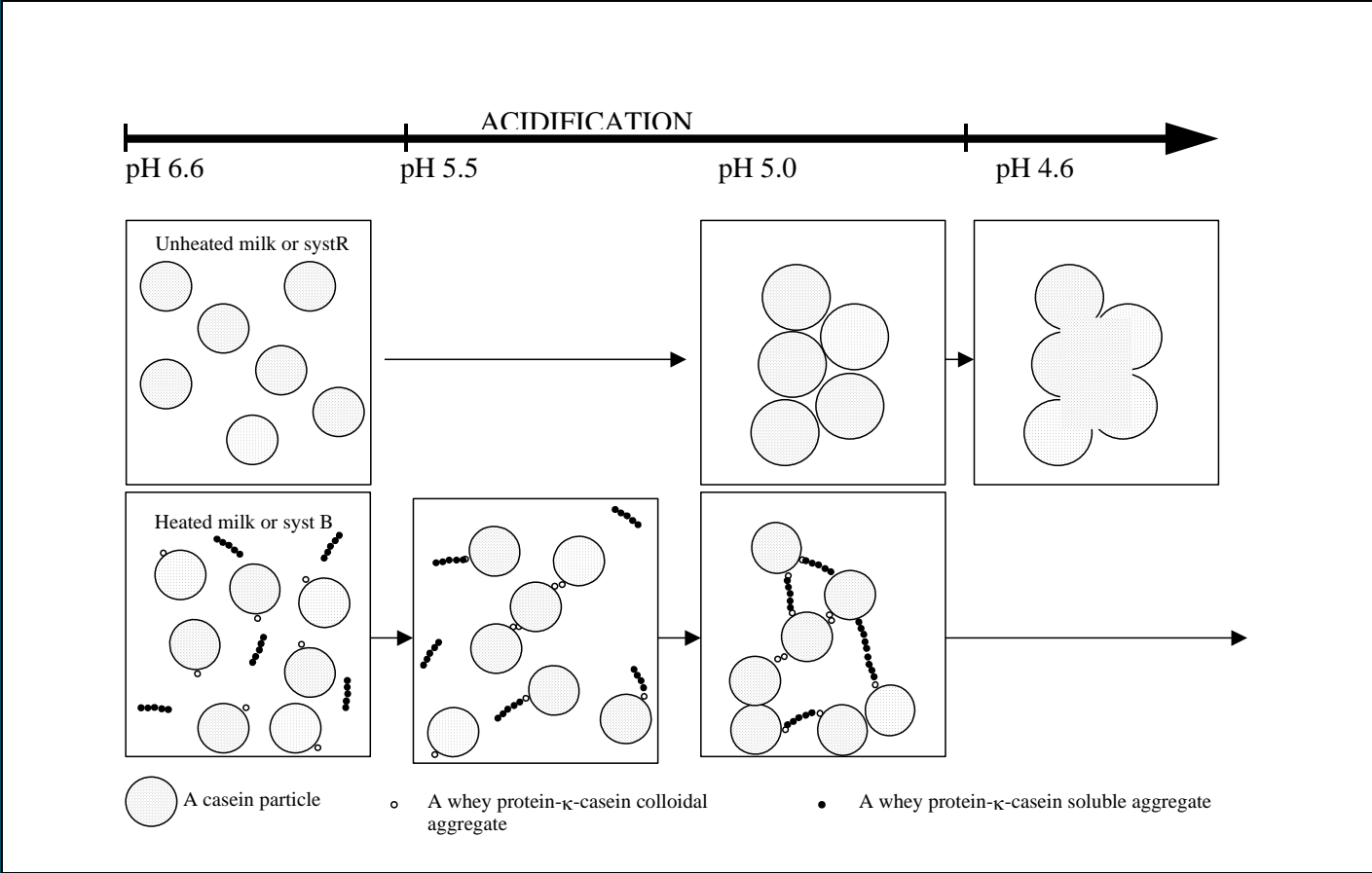
Colloidal complexes

Soluble complexes

Aggregation of

- micellar β -Lg
- soluble complexes of β -Lg- κ -cas





The globular protein confers its solubility property to the casein micelle.

The 2 globular proteins have different behaviours toward the casein micelle

Heating in the presence of globular proteins leads to higher pH at gelation and to higher G' value than without globular protein.

With β -Lg final $G' \sim 200$ Pa

With ovalbumin final $G' \sim 600$ Pa