Gelation of whey proteins at alkaline pH

Marie-Hélène Famelart, Florence Rousseau

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MH. Famelart, F. Rousseau

STLO

STLO, UMR 1253,
INRAE, Institut Agro, 35000 Rennes, France

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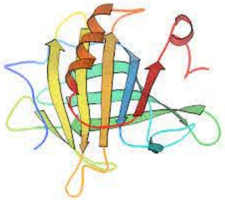
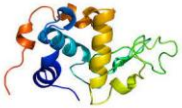
Context

- ✓ Whey proteins = globular proteins from milk (~ 6 g/L),

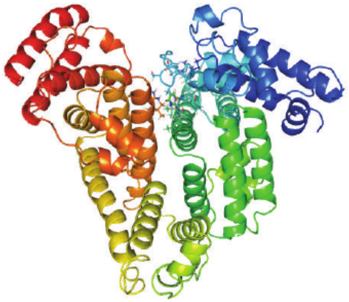
β-Lactoglobulin
3 g/L
18/36 kDa



α-Lactalbumin
1.2 g/L
14 kDa



Bovine serum albumin
0.3 g/L
66 kDa



+ others minor ones

- ✓ Widely used in the food industry for their functional properties,
- ✓ Texturing ingredients in foods,
- ✓ Whey proteins in solution are denatured/aggregated/gelled by heat treatment at neutral or acidic pH ► large interest in the food industry,
- ✓ Less is done on their gelation at alkaline pH (Mleko 2001; Mercadé-Prieto at al. 2005),
- ✓ And few without heating

Objectives:

- ✓ Study the gelation of whey proteins at alkaline pH values

What is known on exposure of whey proteins to alkaline pH?

General effect of alkaline pH on proteins (Whitaker & Feeney 2015) - more intense with heating

- Changes on solubility and aggregation: net negative charge ↑ ⇒ solvation and conformation changes, hydrophobic residues exposed, …aggregation
- Hydrolysis: amide groups (asparagine, glutamine), peptide bonds, guanidino groups (arginine);
- β-Elimination reactions (side chains of cystine, threonine, serine) ⇒ dehydroalanine
- Crosslinking reactions (as dehydroalanine + lysine ⇒ lysinoalanine…)
- Racemization of amino acids ⇒ ↑ contents in D-amino acids

Exposure of whey proteins at pH > 6 (mainly β-lactoglobulin):

- pH 6-8: reversible, Tanford transition, exposure of a glutamate residue (Tanford et al.1959)
- pH 9-13 (Roels et al.1971; Monahan et al. 1995; Partanen et al. 2011)
  - irreversible, slow,
  - partially unfolded monomers of β-lactoglobulin,
  - exposure of buried SH groups,
  - ↑ hydrophobicity,
  - polymerisation, probably by thiol oxidation + others crosslinks (such as lysinoalanine)
- Gelation of whey proteins at pH 9.6 (Mercadé-Prieto & Chen, 2005)
- At lower temperatures ?

T°C	t_{gel} WPI 160 g/kg (min)
50	75
55	25
60	7

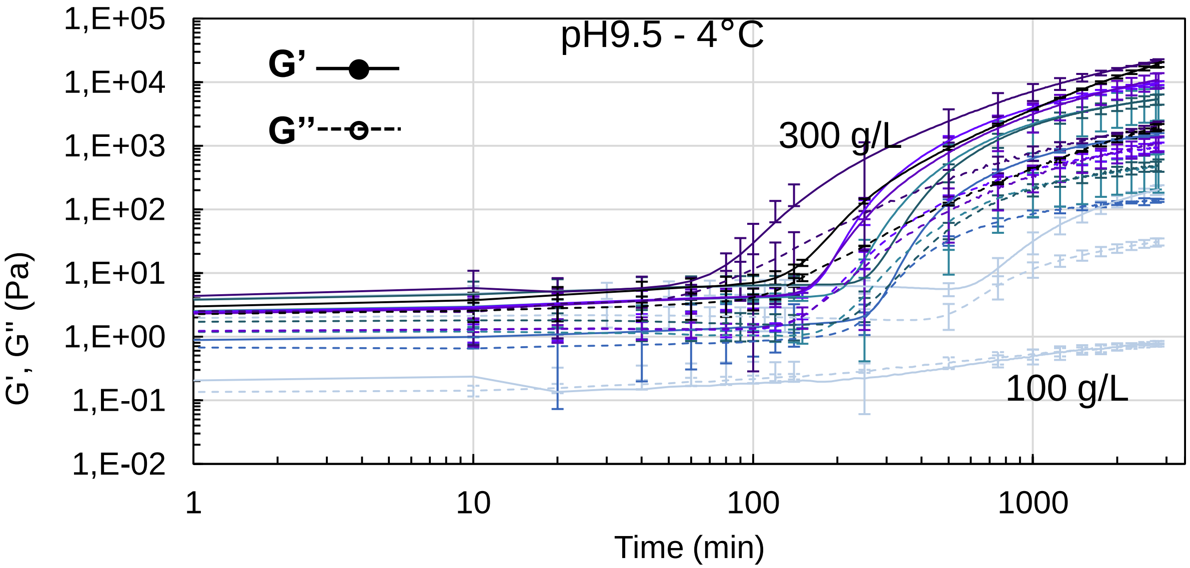
M&M

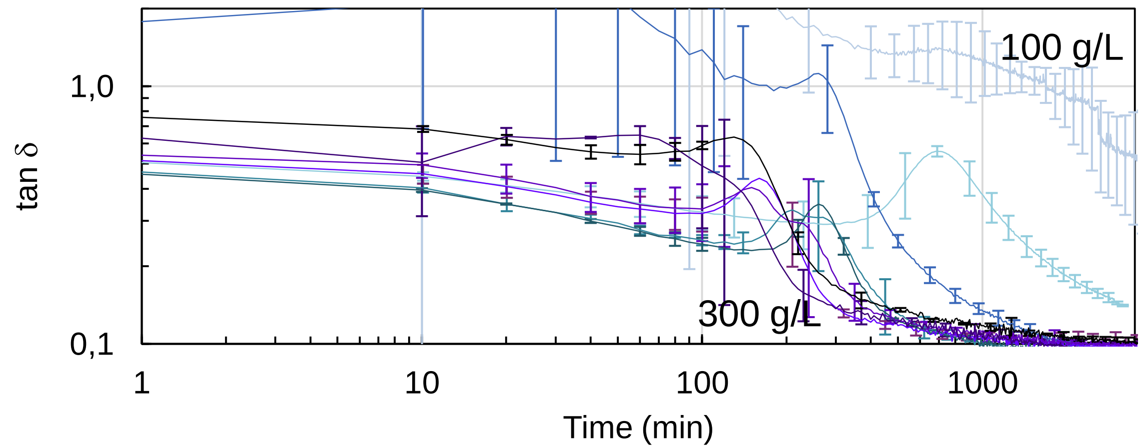
- *Whey protein isolate*
  - 890 g prot, 5.92 g lactose and 63 g water/kg powder
  - 93.5% is whey proteins (β-lactoglobulin + α-lactalbumin + bovine serum albumin + other minor ones)
  - 6.5% is a mixture of caseins
- *Concentration of whey proteins:*
  - **[100-300 g/L]** (by OD280nm)
  - + 0.02% sodium azide
- *Gelation experiments*
  - Overnight solubilization with magnetic stirrer at [protein] > $[\text{protein}]_{target}$,
  - Adjustment at **pH 9.5** and [protein] at t_{0},
  - Transfer on rheometer: DHR2 (TA Instruments) - plate-cone (5 cm diameter), T=4°C or 20°C - paraffin oil
  - Kinetics (low amplitude oscillation, γ=0.1%; frequency=1 Hz; gap_{init}=2.5 mm; cst normal force)
  - Frequency sweep ($10\text{-}10^{-3}$ Hz, 5 points/decade; γ=0.1 %)
- *Type of interactions during gelation*
  - SDS-PAGE electrophoresis (dissociant+reduction SS)

Results

Gelation at 4°C

- ✓ Slow rate$_{gel}$ (t_{gel}~100-700 min ~ 2-12 h)
- ✓ ↑ [protein] ⇒ ↑ rate$_{gel}$
- ✓ ↑ [protein] ⇒ ↑ G'_{50h}, G''_{50h}
- ✓ [protein]>120 g/L ⇒ G'>G''
- ✓ 3 apparent steps
  - 0-200 min tanδ ↓
  - tanδ ↑ (G'' & ☝' ↑): thickening
  - tanδ ↓ and G' ↑ (G'' ↑): gelation
- ✓ Final tanδ ~ 0.1

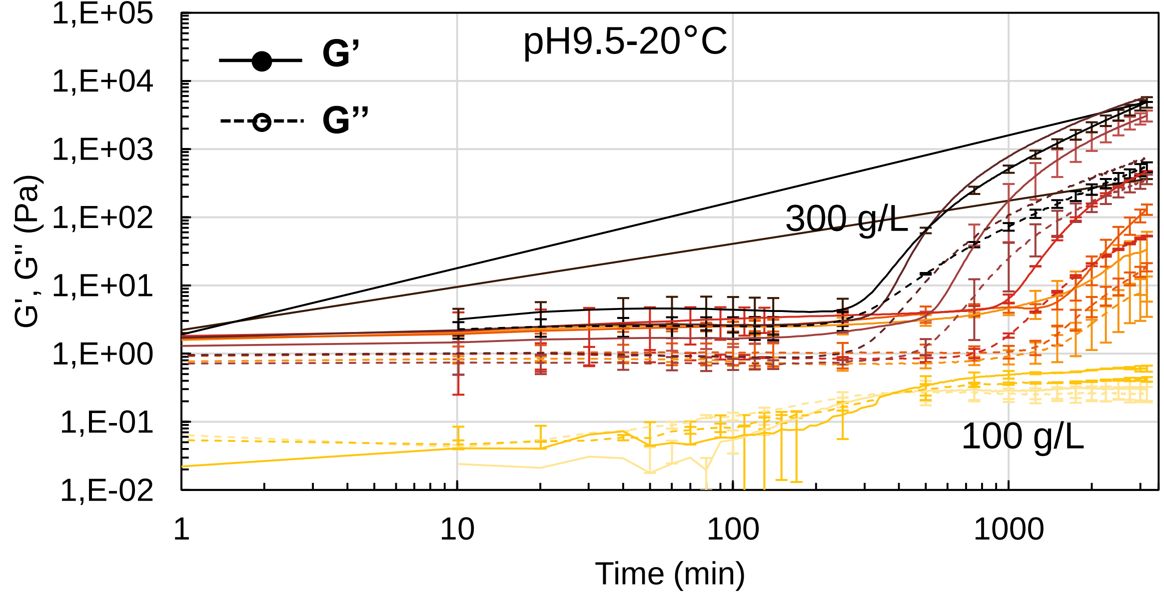


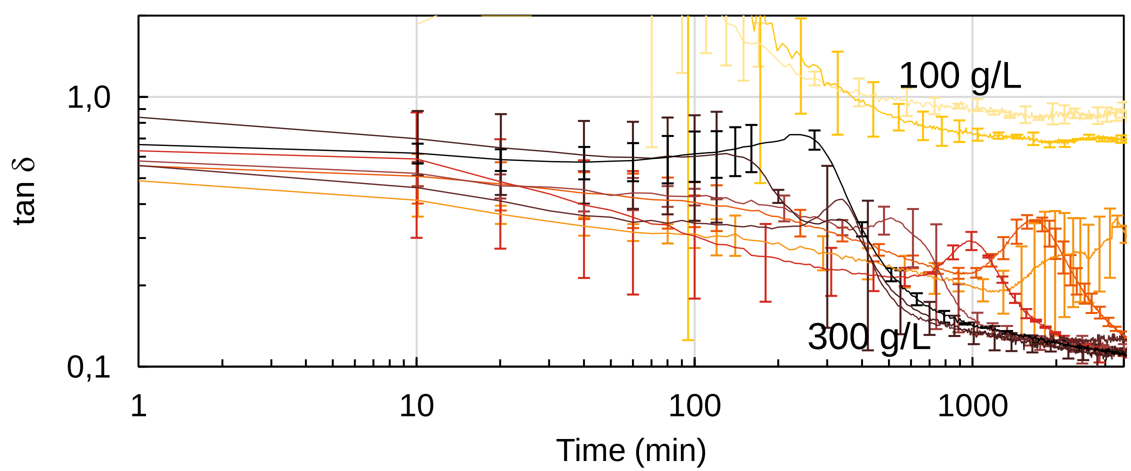


Results

Gelation at 20°C

- ✓ Same conclusions
- ✓ Slower $rate_{gel}$ (t_{gel}~300-2000 min ~ 5-30 h)

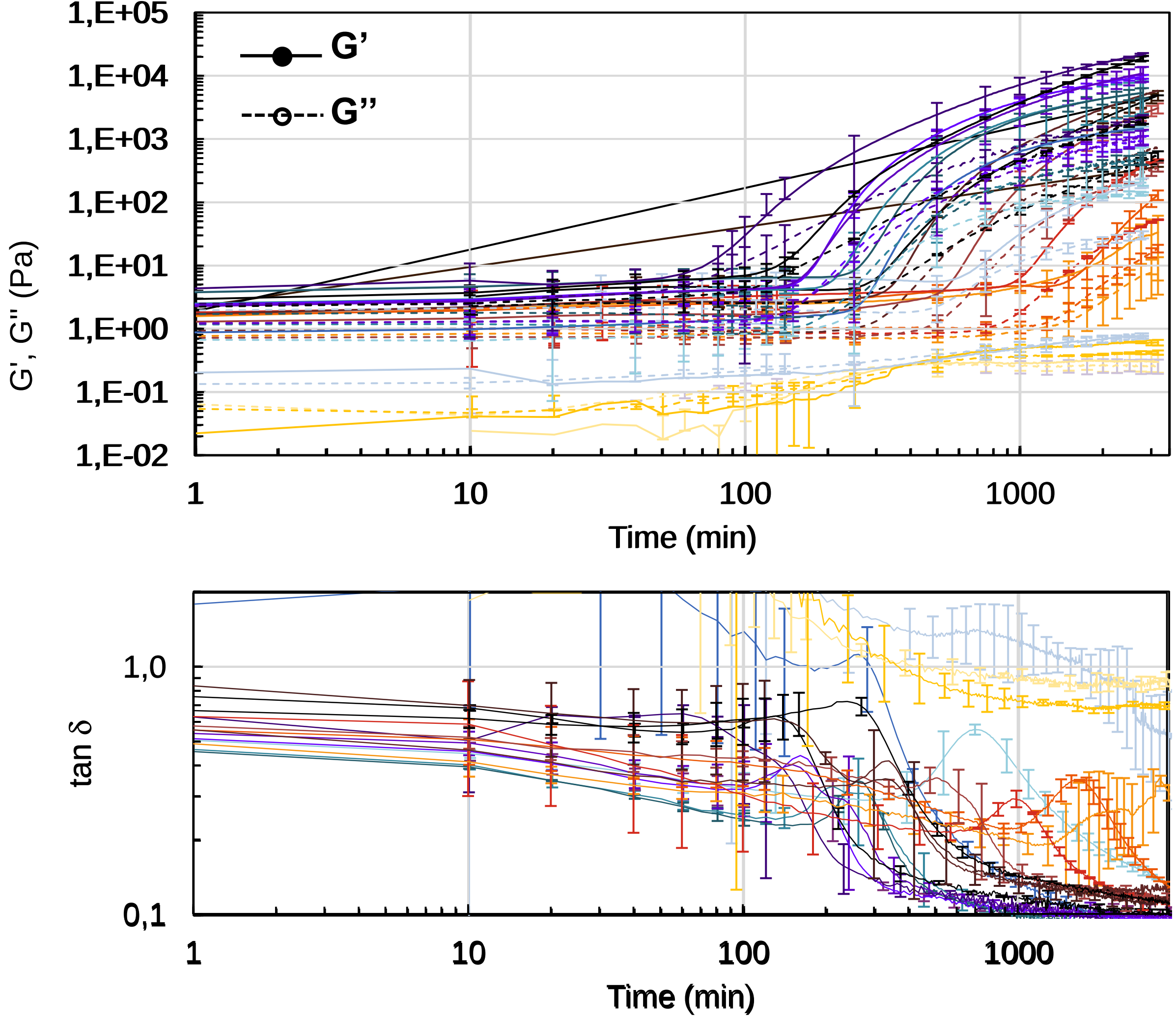




Results

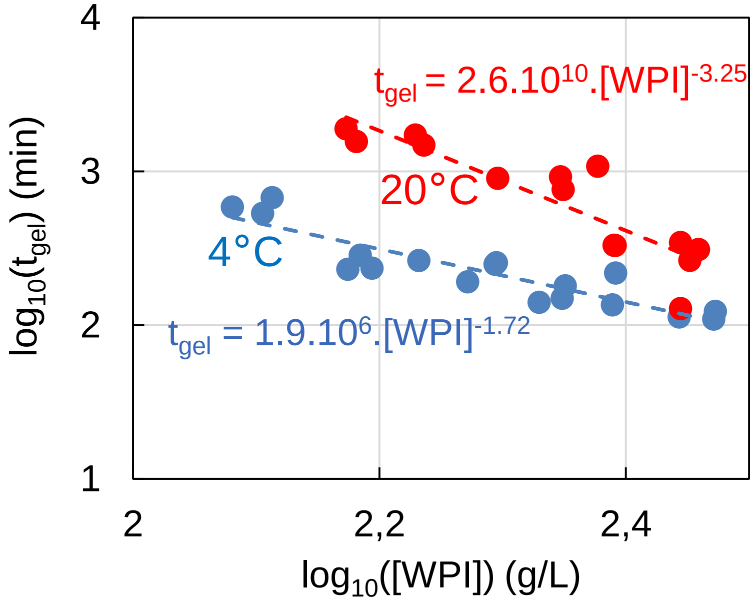
Comparison 4/20°C

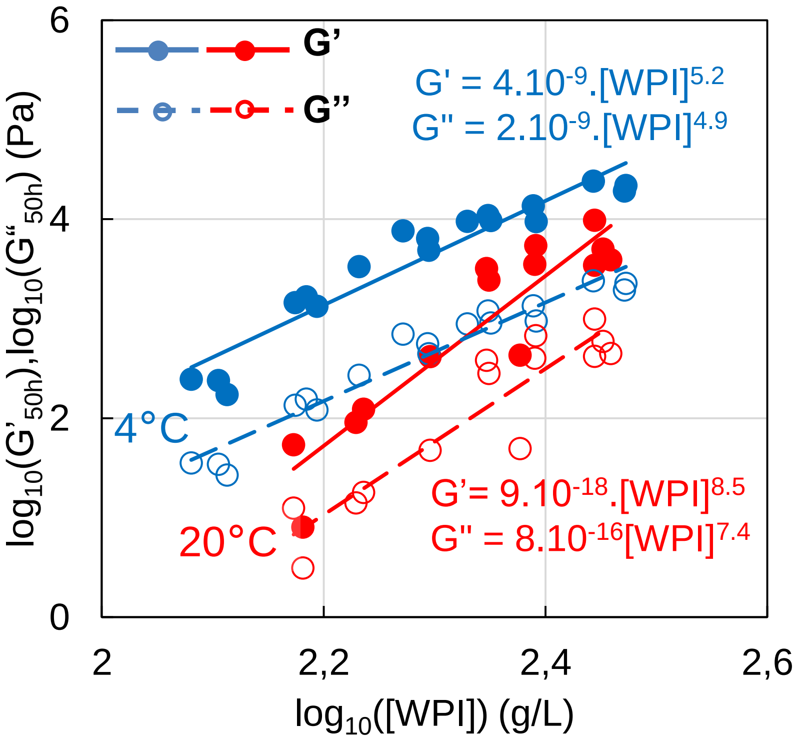
- ✓ $rate_{gel}$ 4°C>$rate_{gel}$ 20°C
- ✓ G'_{50h} 4°C>G''_{50h} 20°C



Results

Gelation at 4 and 20°C



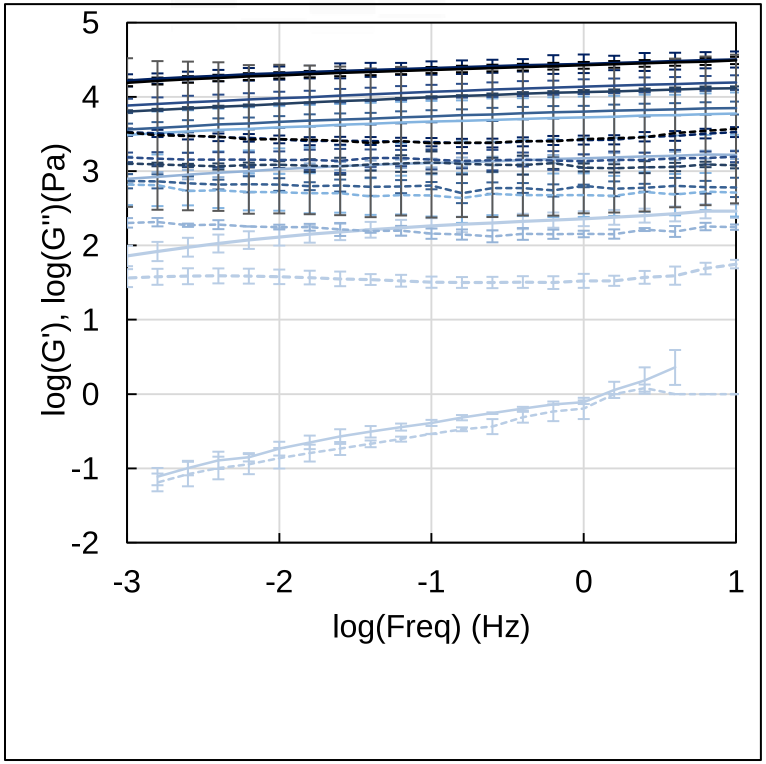


- ✓ [WPI] ↑ ⇒ t_{gel} ↓ (power law)
- ✓ Exponent 20°C > 4°C
- ✓ t_{gel} 4°C<t_{gel} 20°C

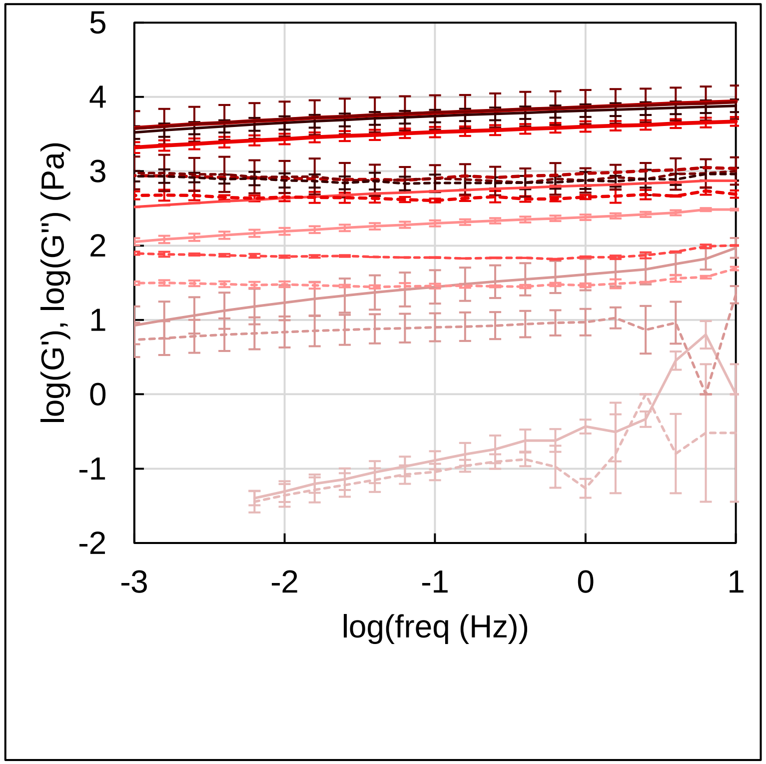
- ✓ [WPI] ↑ ⇒ G'_{50h} & G''_{50h} ↑ (power law)
- ✓ Exponent 20°C > 4°C

Results

Frequency sweep 4°C

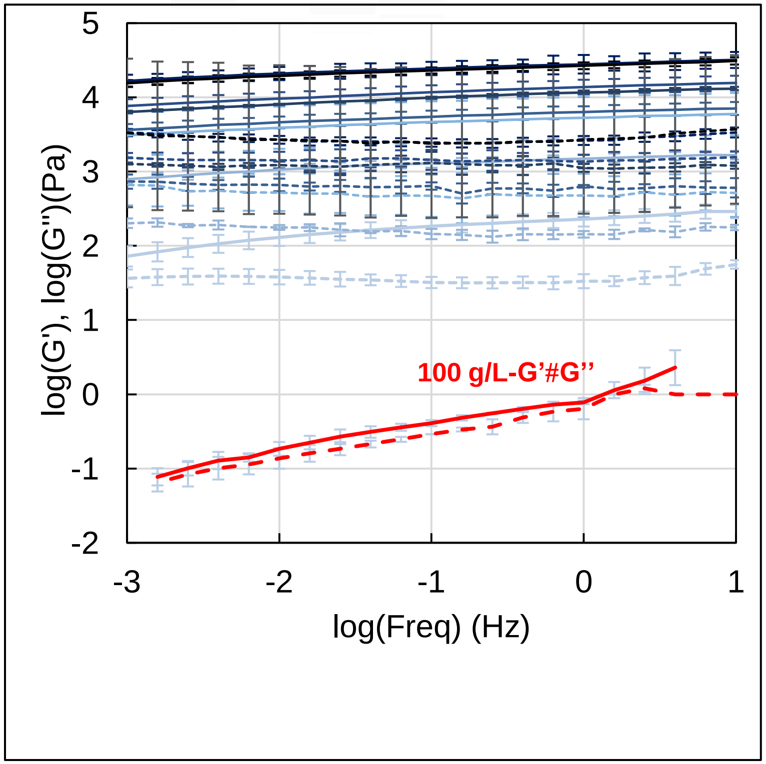


Frequency sweep 20°C

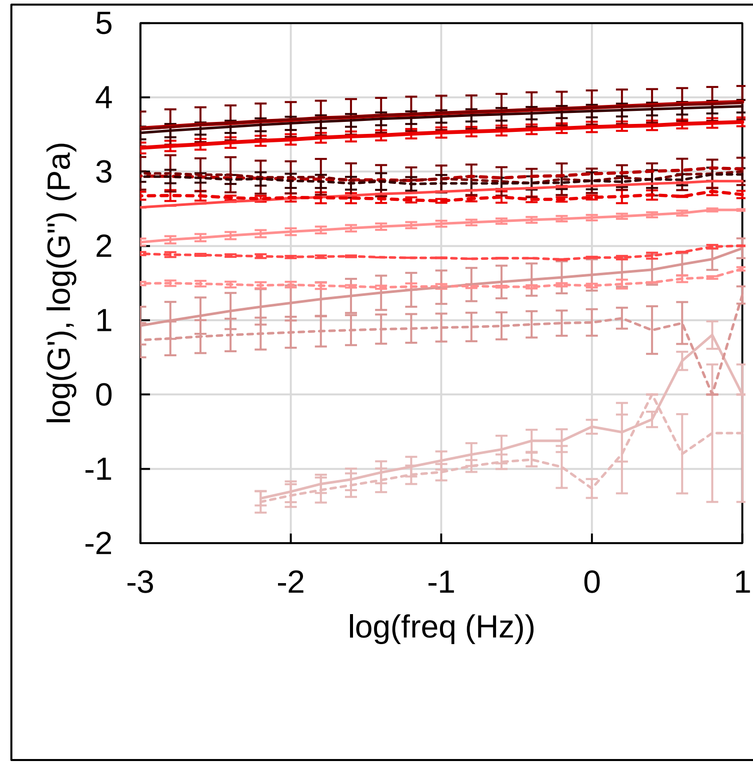


Results

Frequency sweep 4°C

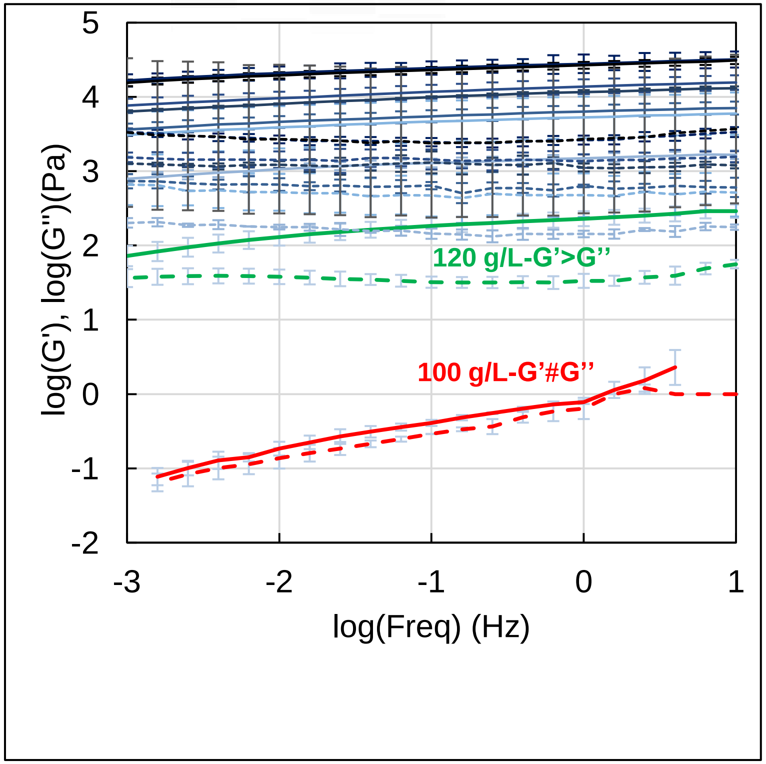


Frequency sweep 20°C

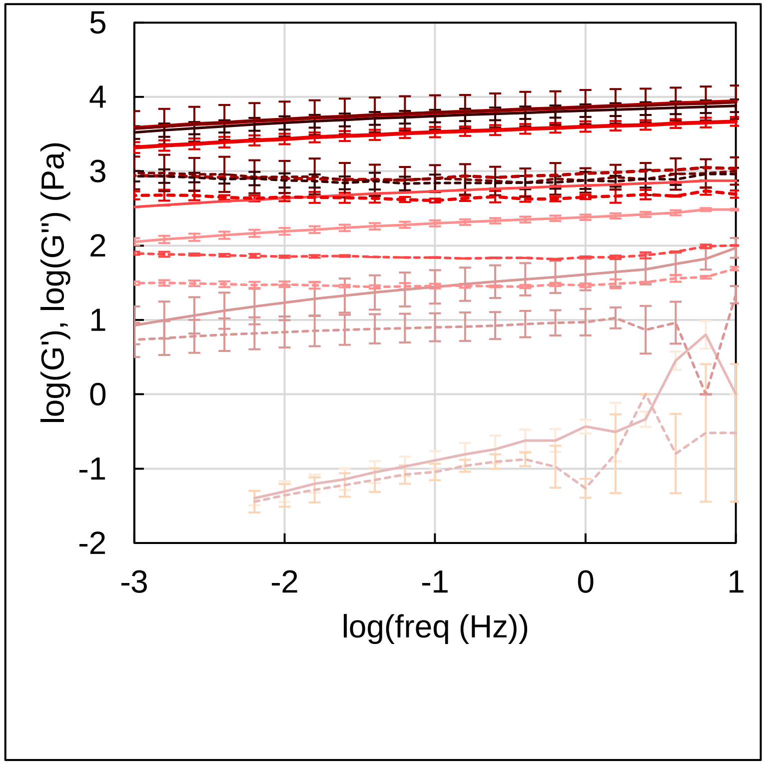


Results

Frequency sweep 4°C

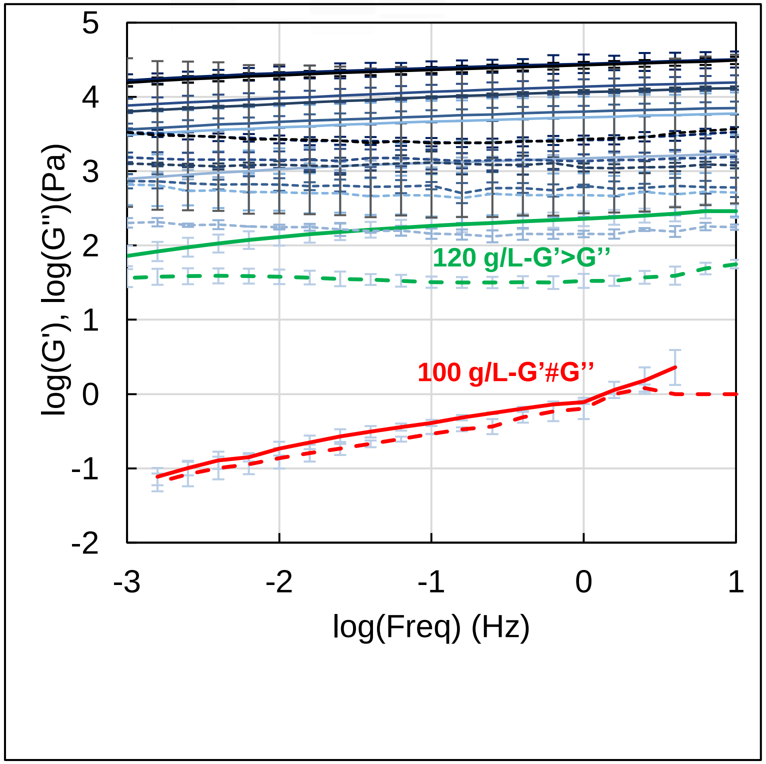


Frequency sweep 20°C

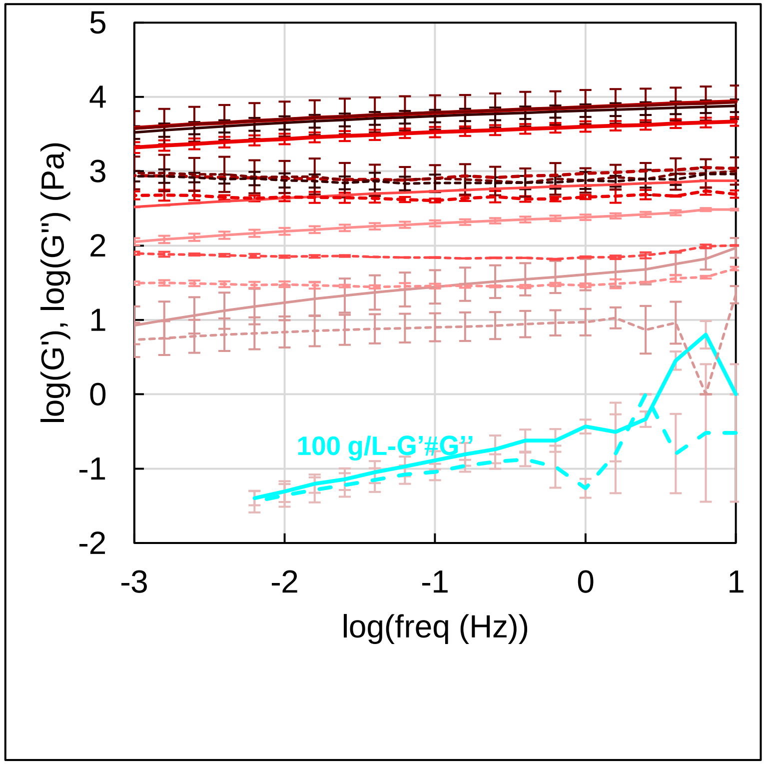


Results

Frequency sweep 4°C

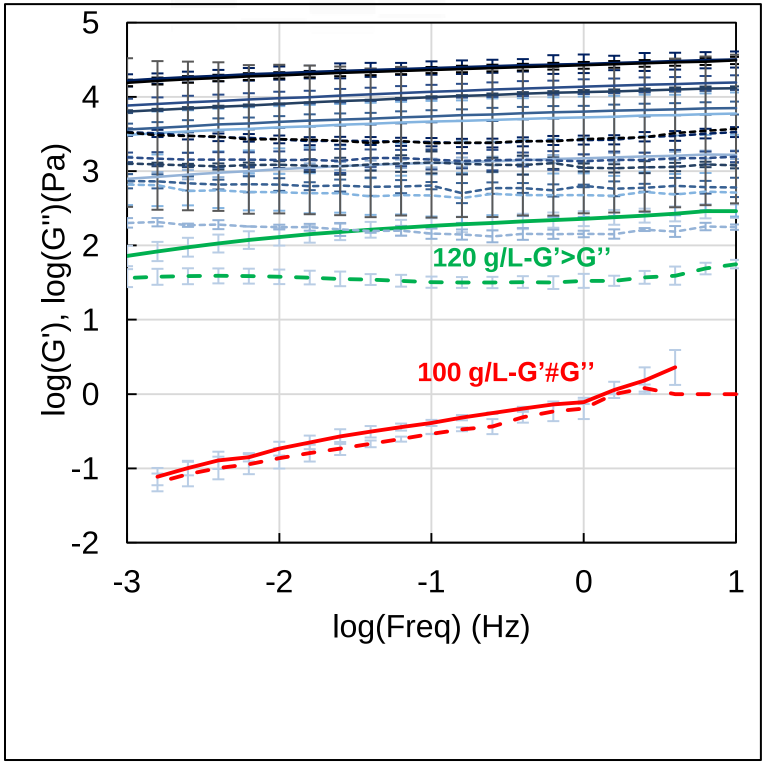


Frequency sweep 20°C

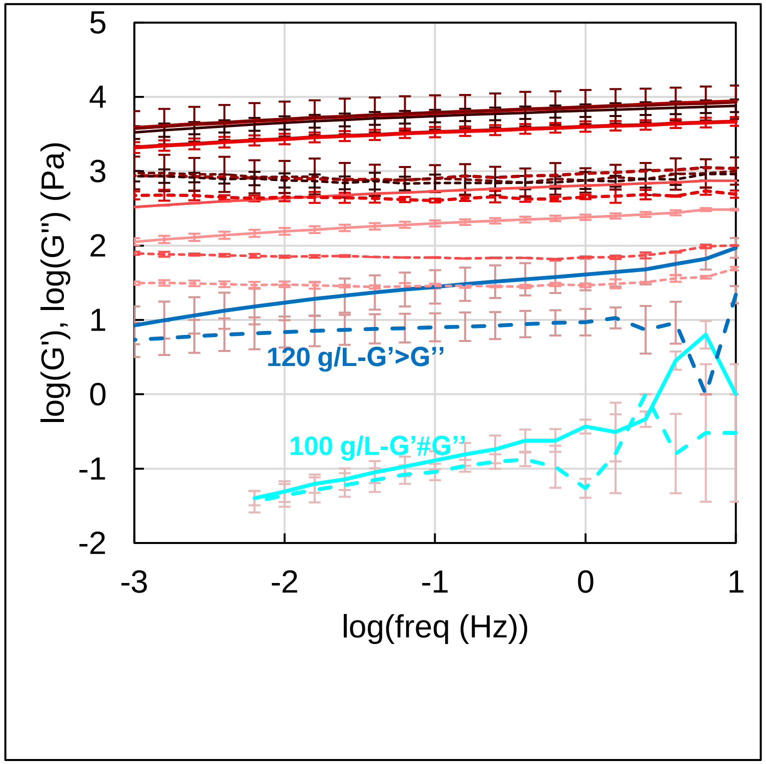


Results

Frequency sweep 4°C



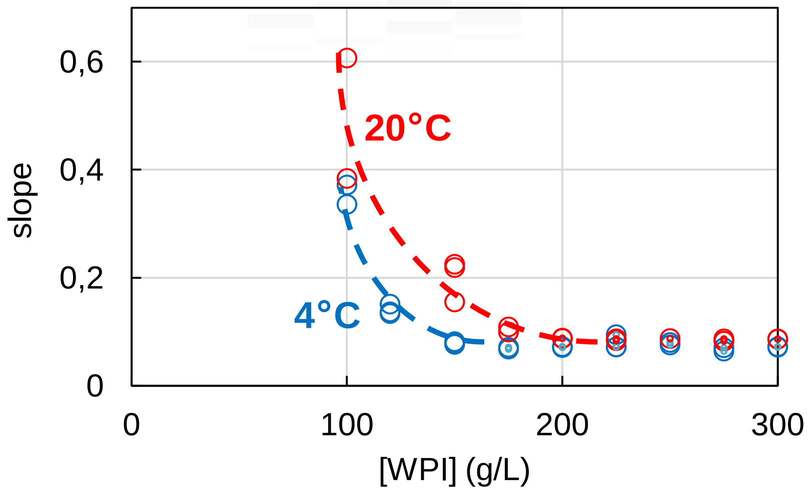
Frequency sweep 20°C



- ✓ At 100 g/L ⇒ a viscous liquid
- ✓ At 120 g/L ⇒ G'>G'', crossing point at freq <= 10^{-3} Hz
- ✓ At 150-300 g/L, no crossing in the range of measured frequencies and G', G'' more or less independent of the frequency

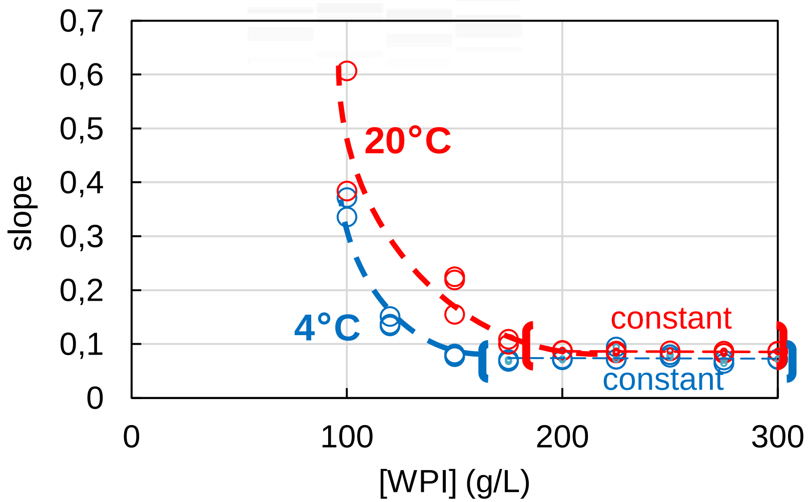
Results

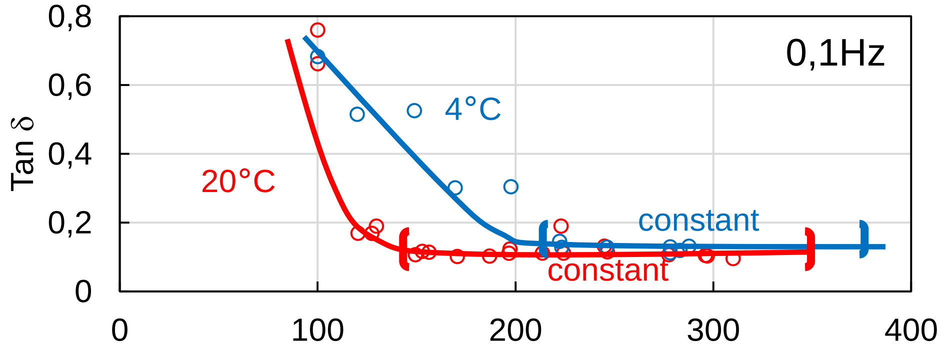
Gelation at 4 and 20°C: slope of log_{10}(G'),log_{10}(G") = f(log(freq))

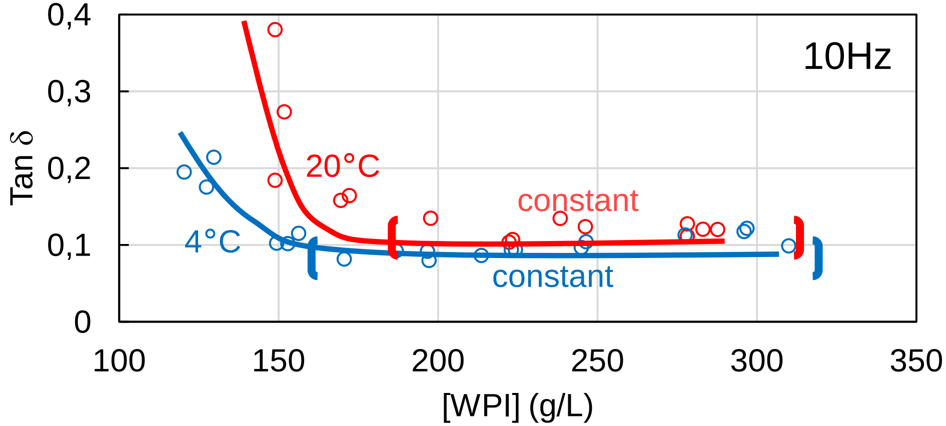


Results

Gelation at 4 and 20°C: slope of log(G',G") = f(log(freq))







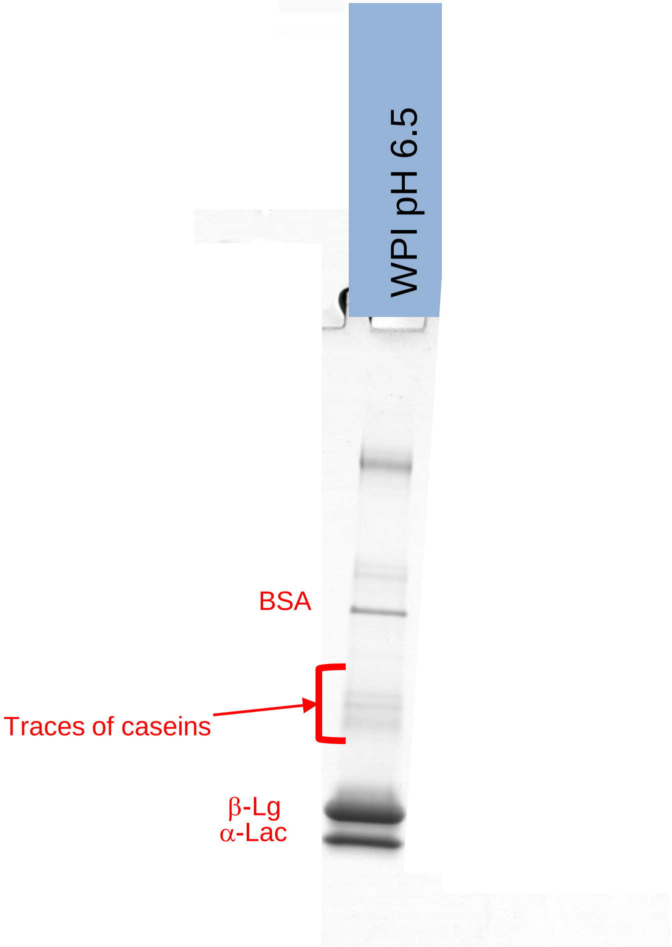
- ✓ [WPI] ~180 to 300 g/L ⇒ same slope for mechanical spectra

- ✓ At high [WPI]⇒ tanδ is constant
- ✓ Only the number of interactions increases with [WPI], but the nature of interactions is the same

Results

Type of interactions (SDS-PAGE)

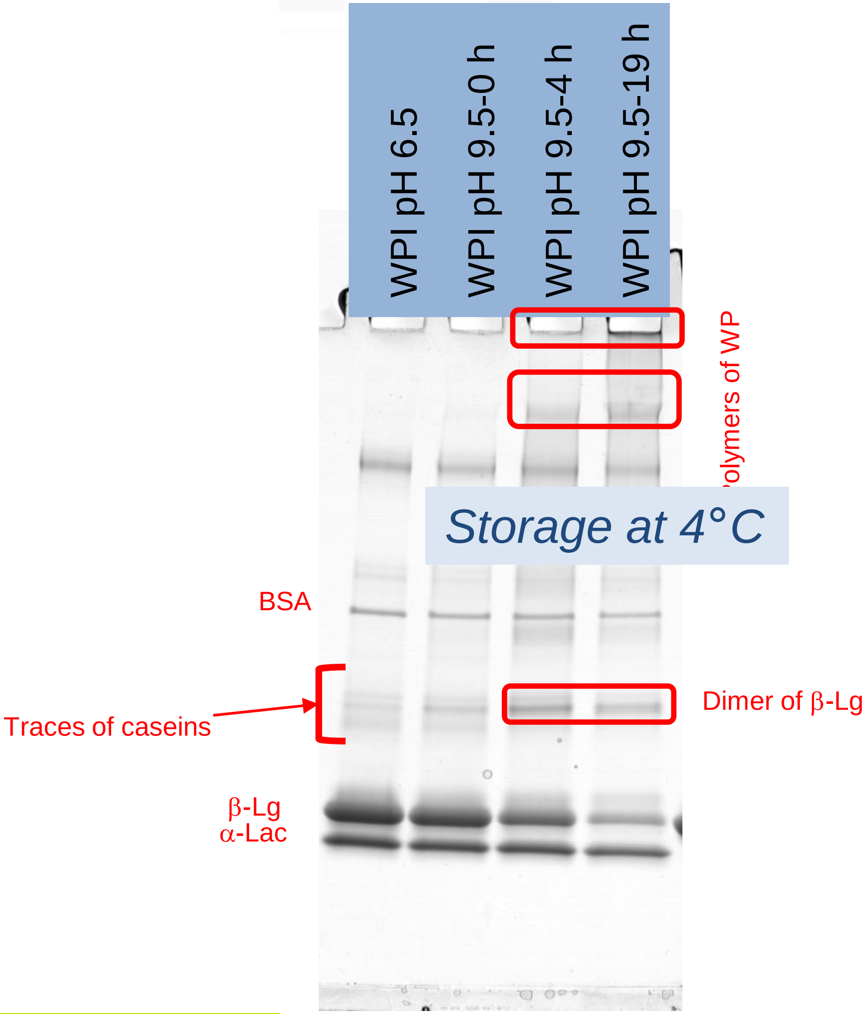
Without reduction of SS



Results:

Type of interactions (SDS-PAGE)

Without reduction of SS

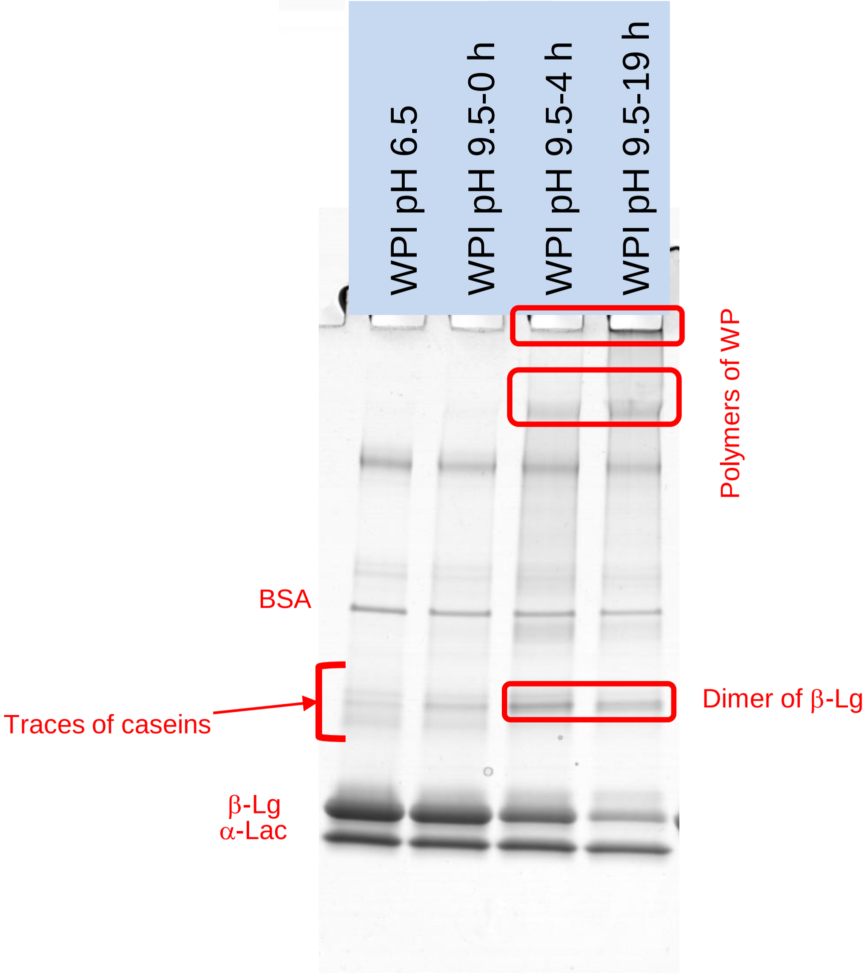


Dimers and polymers of whey proteins form during storage at 4°C at pH 9.5,
β-Lactoglobulin is mainly involved in polymers

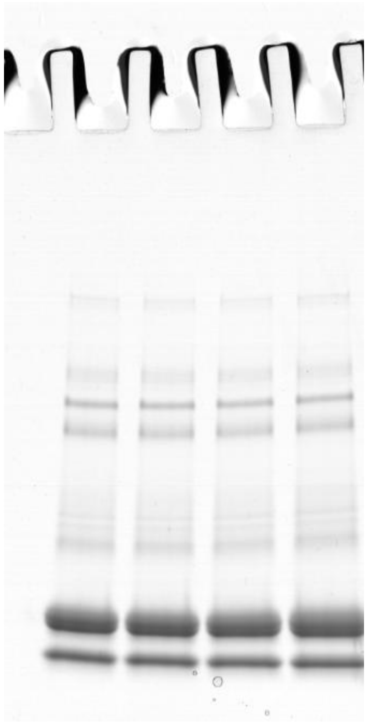
Results

Type of interactions (SDS-PAGE)

Without reduction of SS



After reduction of SS

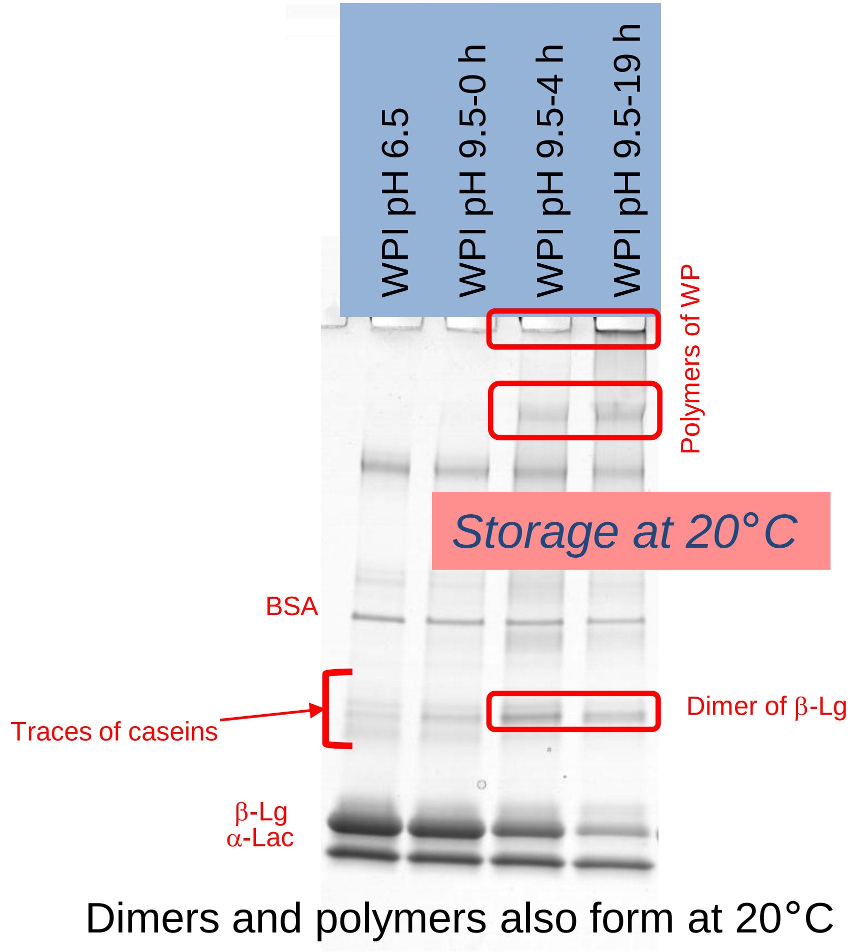


No dimers and polymers of whey proteins

Results

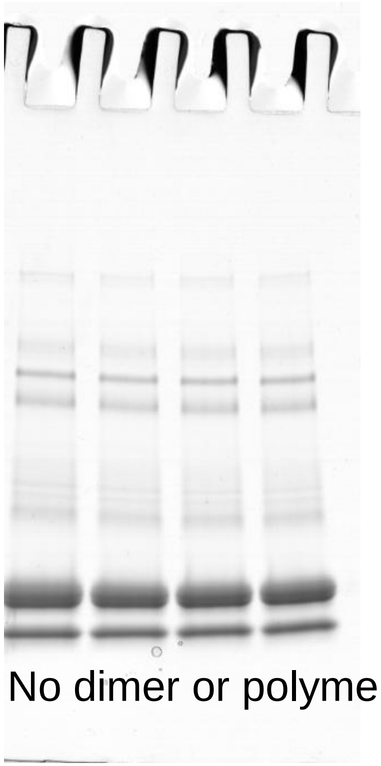
Type of interactions (SDS-PAGE)

Without reduction of SS



Dimers and polymers also form at 20°C

After reduction of SS



No dimer or polymers

- ✓ Polymers of whey proteins form by SS bonds during storage at pH 9.5 at 4 and 20°C
- ✓ Exposure of SH due to a slow denaturation of whey proteins at pH 9.5

Conclusions

The results improve our understanding of β-Lg polymerisation at alkaline pH values.

This understanding is useful for applications in food processes:

- To produce aggregates or gels to increase the texture of food matrices,
- To induce denaturation of whey proteins to increase their functional properties such as foaming or emulsifying properties,
- To prevent denaturation/aggregation of whey proteins, for instance during the production of native whey proteins (for nutritional ingredient production) or polymer-free whey protein powders (for production of dairy beverages…),
- For optimisation of the alkaline cleaning of membranes used in tangential filtration processes

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Thanks to my colleagues: Florence Rousseau

and Pascaline Hamon for her help in electrophoresis

MERCI

THANK YOU FOR YOUR ATTENTION