

# Heat-induced aggregation and gelation of rapeseed protein



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## Introduction & Objective

In an effort to improve sustainability and minimize the impact on the climate there is a growing interest to replace animal protein by plant protein for human consumption. A promising source of plant protein is rapeseed that is already grown extensively to produce oil. Rapeseed proteins are not yet used for human consumption. In order to render them suitable for incorporation in food products different, gentler, methods of protein isolation need to be developed. Furthermore, the potential of modification of the proteins to improve their functionality needs to be explored. However, replacement of one protein with another generally leads to different textural properties in the formulated protein product compared to those of the original proteins. Therefore, the first step before introducing plant proteins into existing formulations is to acquire an understanding of their functionality. Moreover, the potential of rapeseed as a protein crop is untapped because the properties of rapeseed protein must be adapted to match consumer expectations for the texture and flavor of our food.

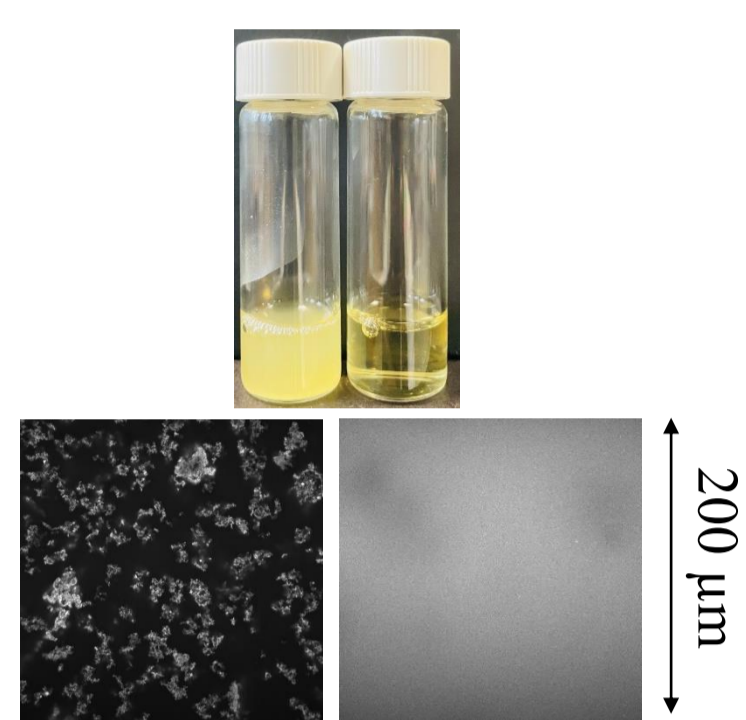
The objective of this study was to investigate the heat-induced aggregation and gelation of rapeseed protein isolate and the microstructure of the gels.

## Characterisation of the rapeseed protein isolate

Rapeseed protein isolate (RPI) was kindly provided by DSM. Samples of their principle protein components i.e. napin and cruciferin as well as their mixtures, were investigated. The RPI was extracted from a cold press cake using an innovative procedure. The powder contained 90% protein of which 80% was soluble over a broad pH range (4-10). Purified napin and cruciferin fractions were obtained by diafiltration through 100 kD pore size filters.

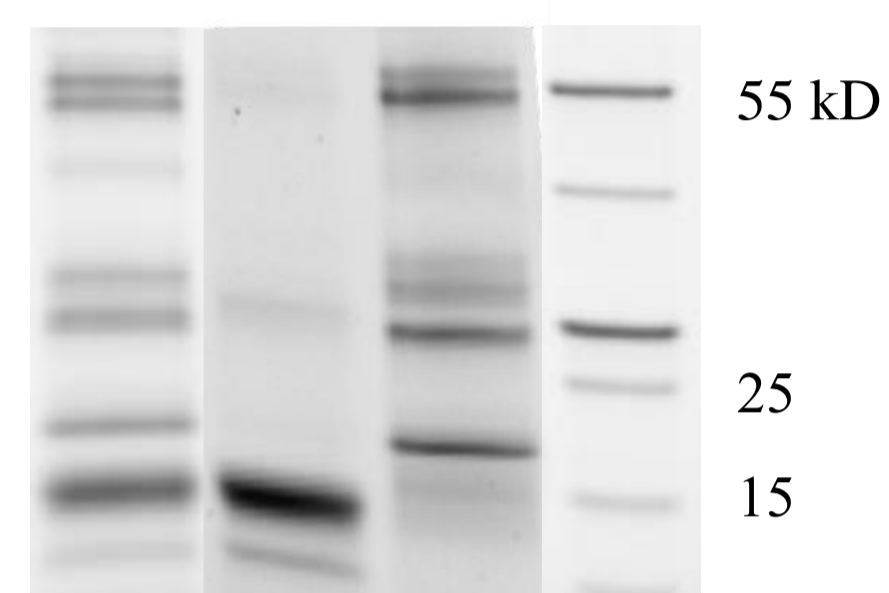
After visualization of rapeseed protein isolate (RPI) solutions, different characterizations were performed. Each suspension was adjusted to pH 10, centrifuged at  $5 \times 10^3$  g for 30 minutes (24 °C) the same day and then filtered using 0.2 µm filter. The filtered solutions were subsequently adjusted to different pH.

### Visualisation



After dispersion in water the system was turbid due to the presence of large aggregates, which could be removed by centrifugation and filtration.

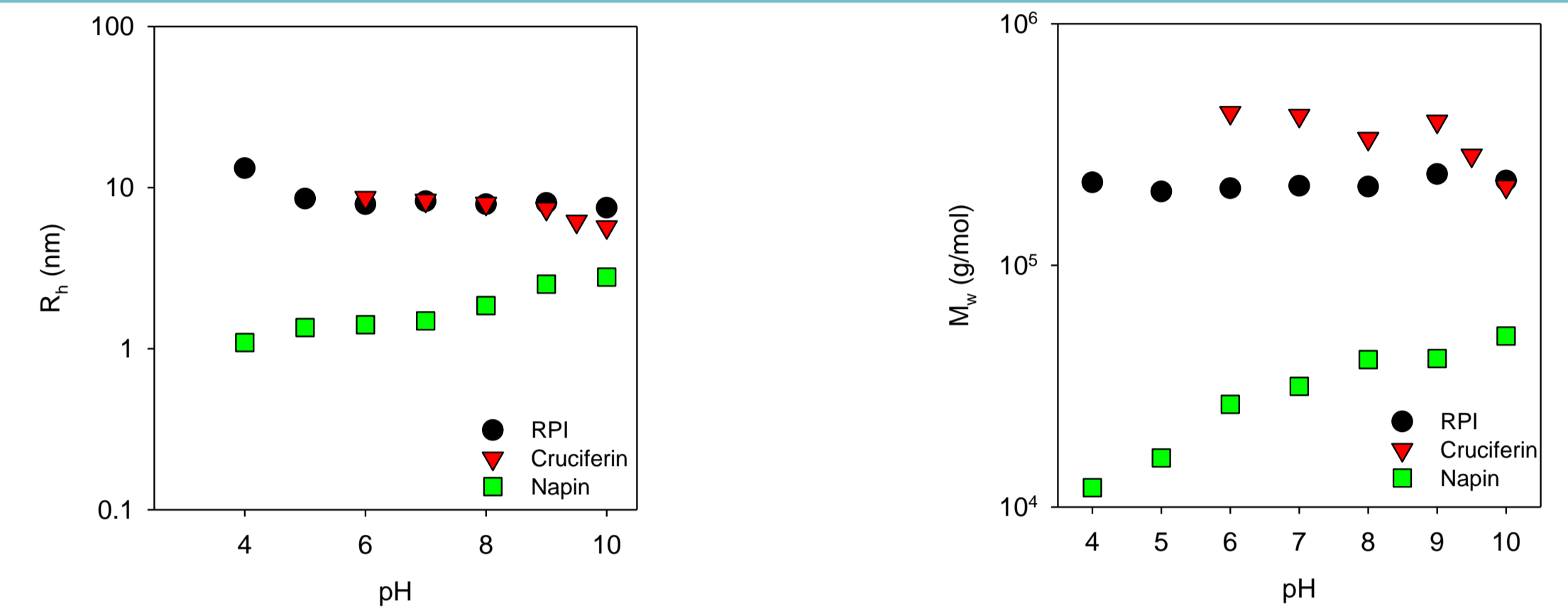
### Protein Composition



Marker, RPI, napin and cruciferin from left to right

The RPI contained the same amount of napin and cruciferin, but after removal of the aggregation the cruciferin content was only 33%.

### Hydrodynamic radius & molar mass as function of pH

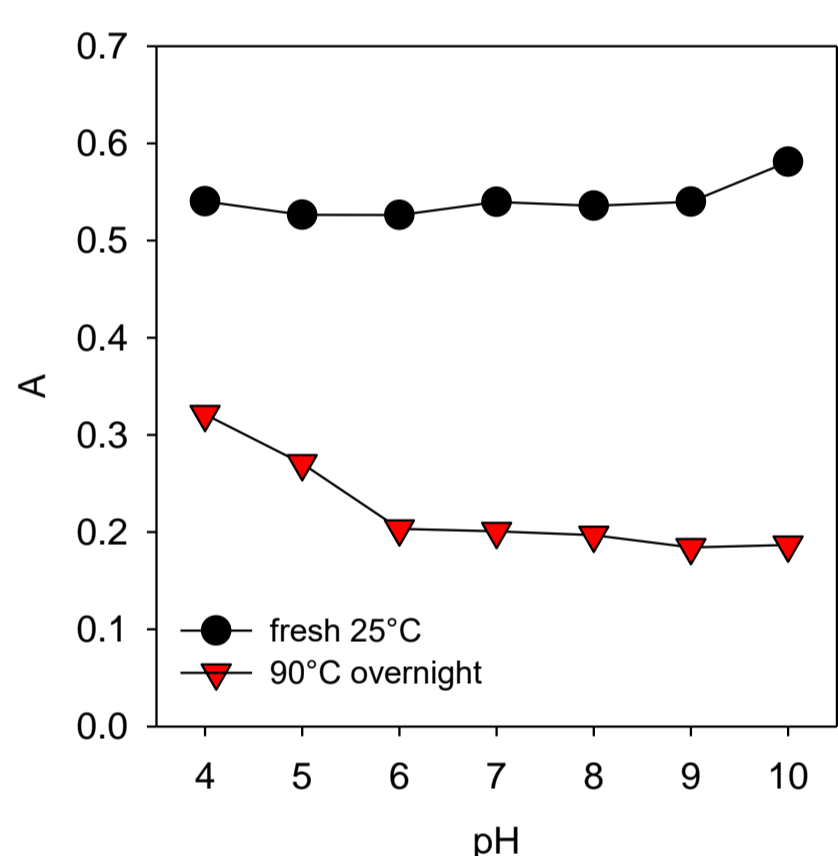


- RPI shows no effect of pH on the size and molar mass
- Cruciferin shows a weak decrease of the size and molar mass at pH 9 and 10
- The size & molar mass of napin increases with increasing pH

## Heat-induced Aggregation of rapeseed protein isolate

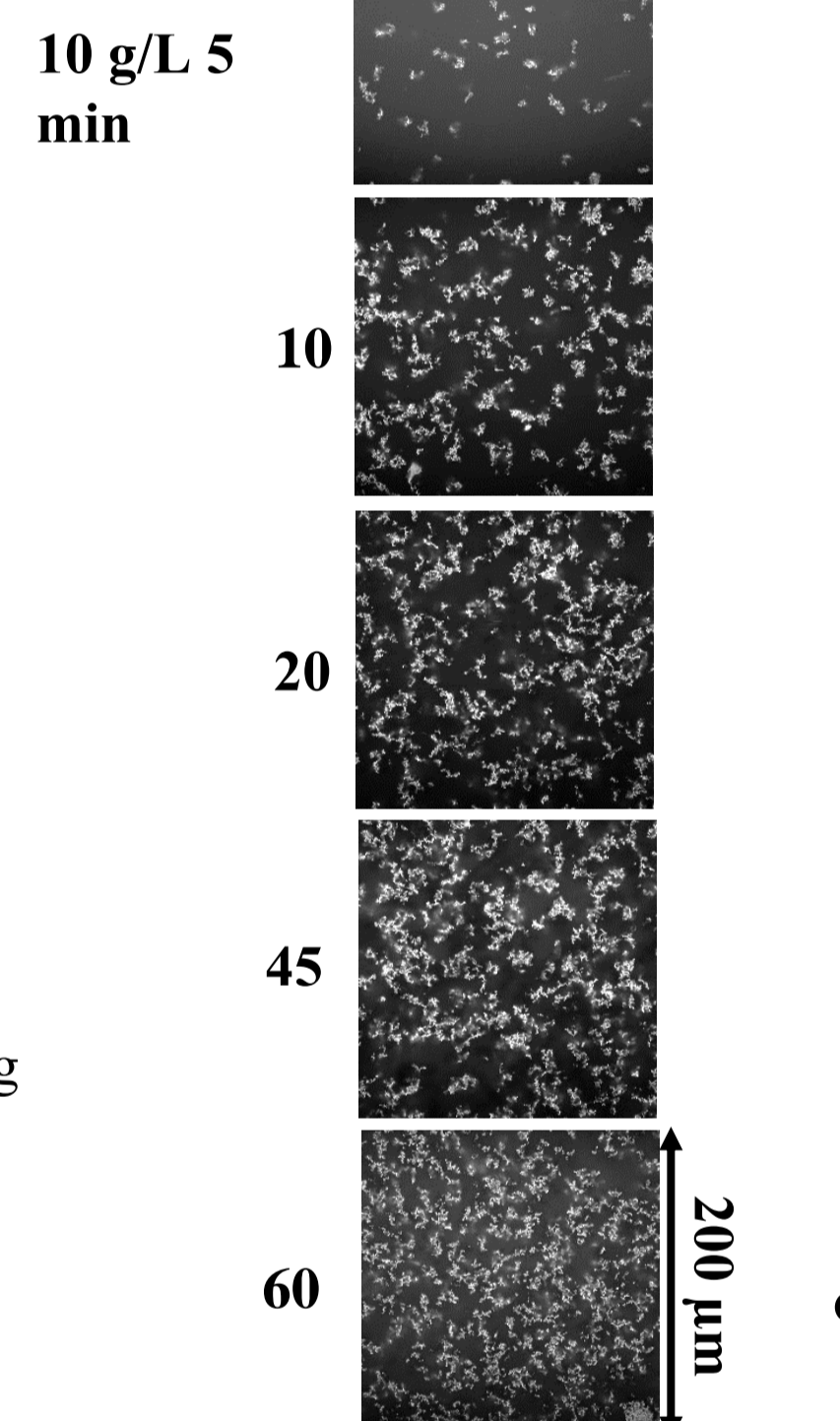
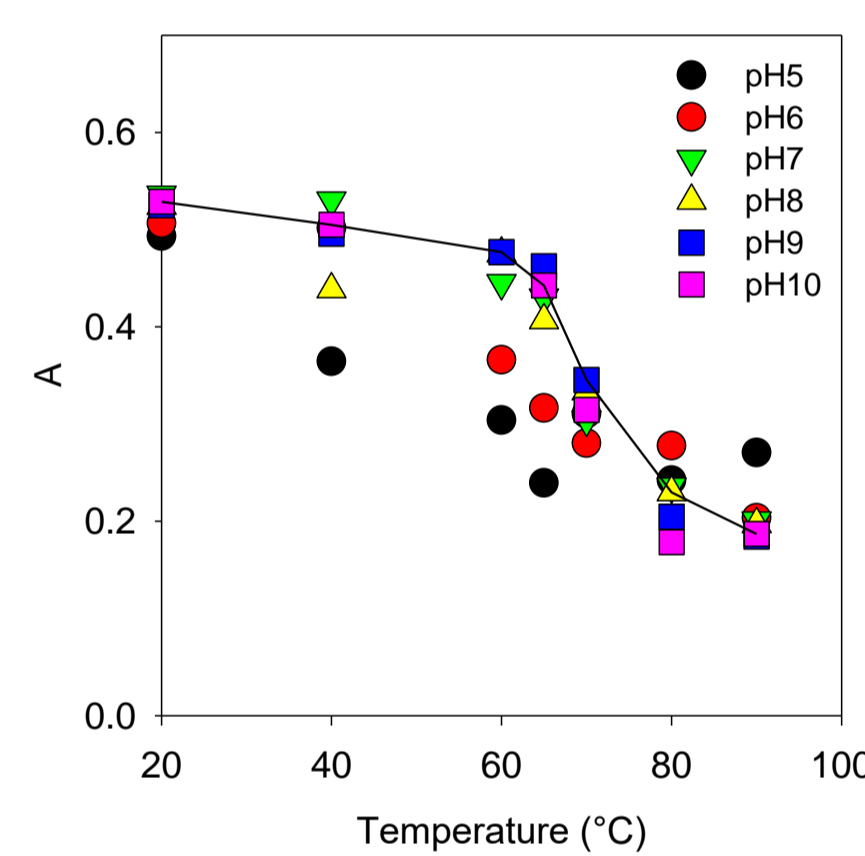
### Effect of T as function of pH

RPI solutions at 10 g/L were heated overnight. The absorbance was measured of the supernatant after centrifugation and filtration.

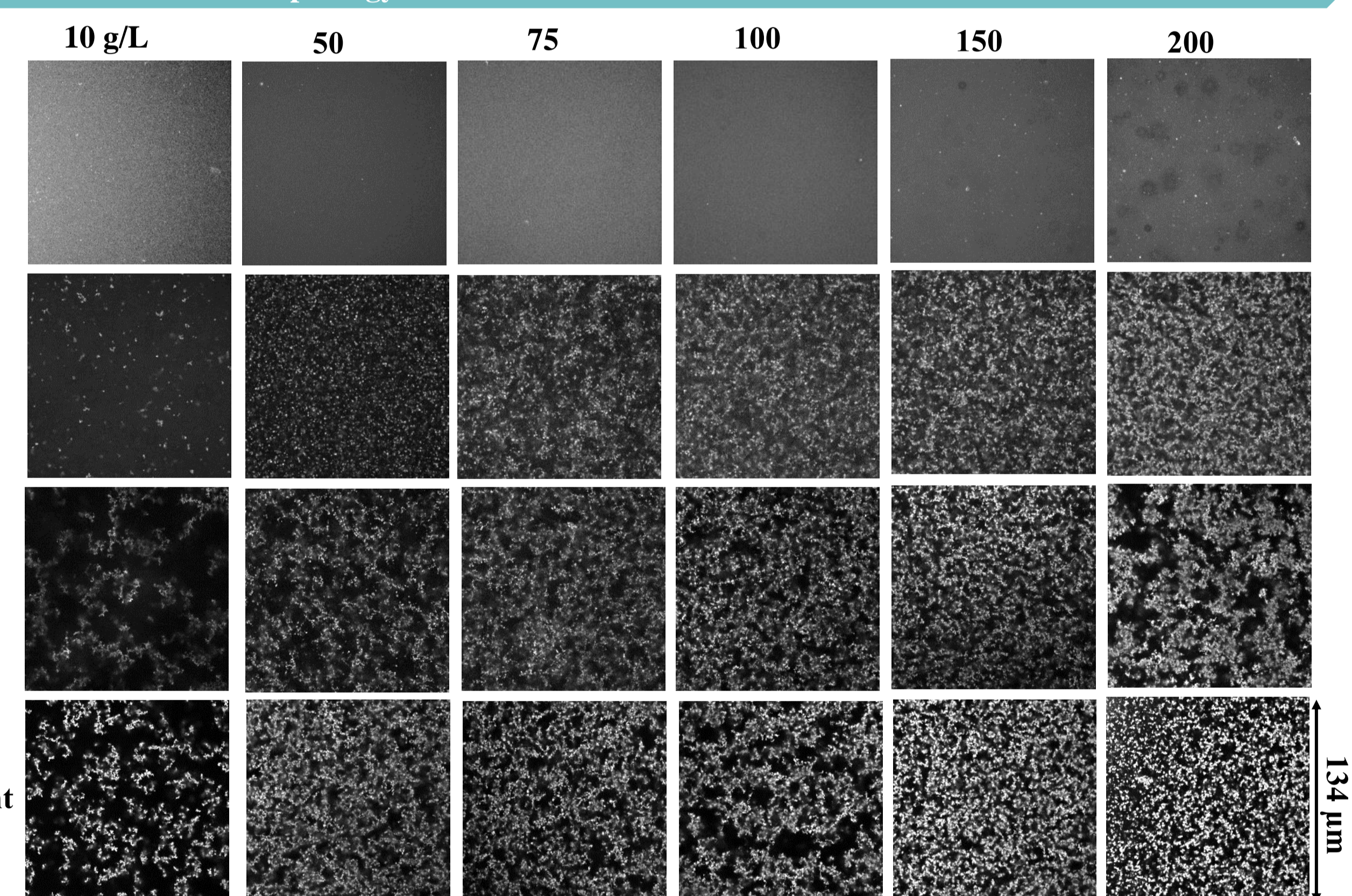


- An increasing fraction of protein in the supernatant decreased after heating with increasing temperature.
- At high temperatures only napin was left in the supernatant
- Napin by itself did not aggregate during heating, but was reduced at pH > 5

### Effect of pH



### Structural Morphology of RPI heated at 80°C



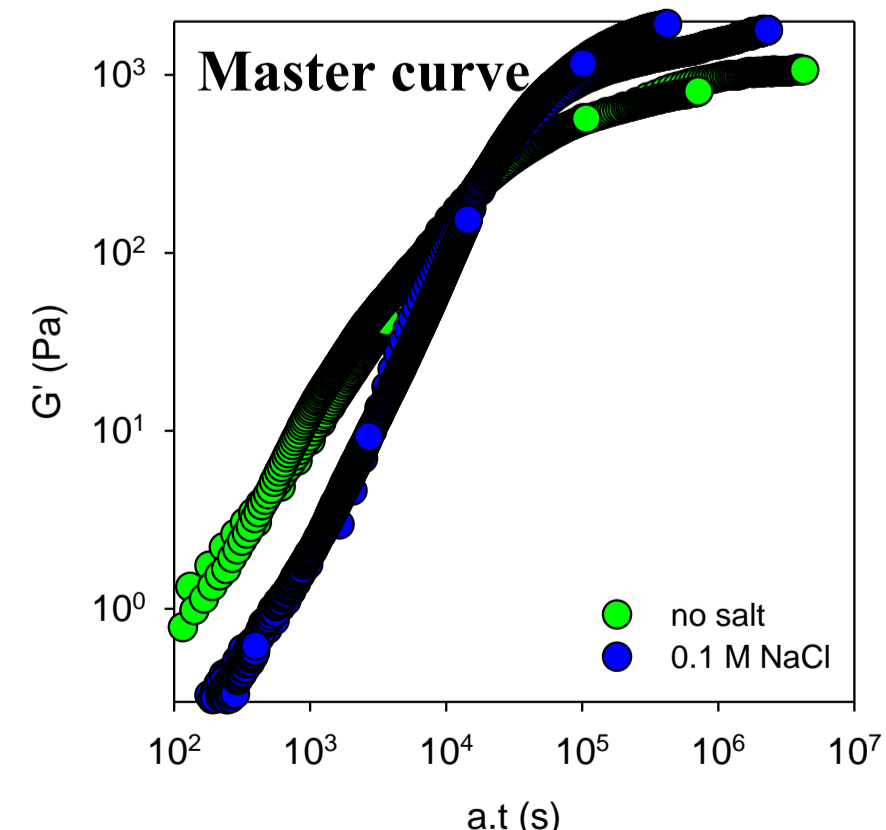
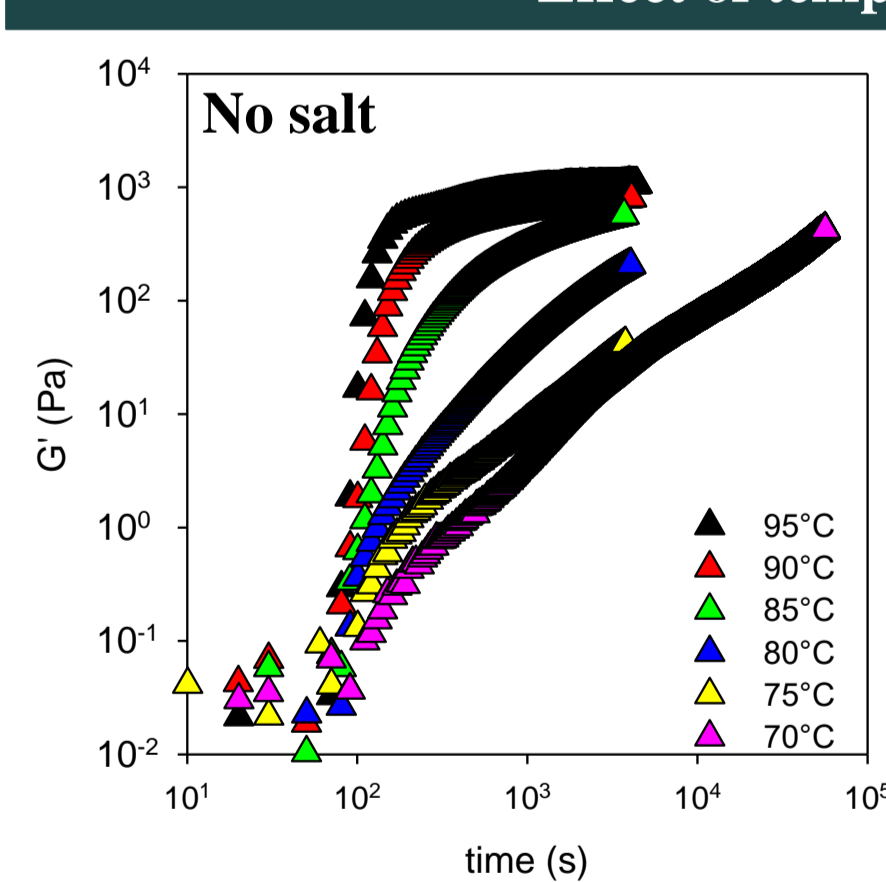
- Formation of particles after 5 min that subsequently aggregated and these aggregates can form a gel over time if the concentration is sufficiently large.
- The structure does not depend on the concentration, pH ≥ 6 or heating temperature ≥ 70°C

## Thermal Gelation

### Rapeseed protein isolate

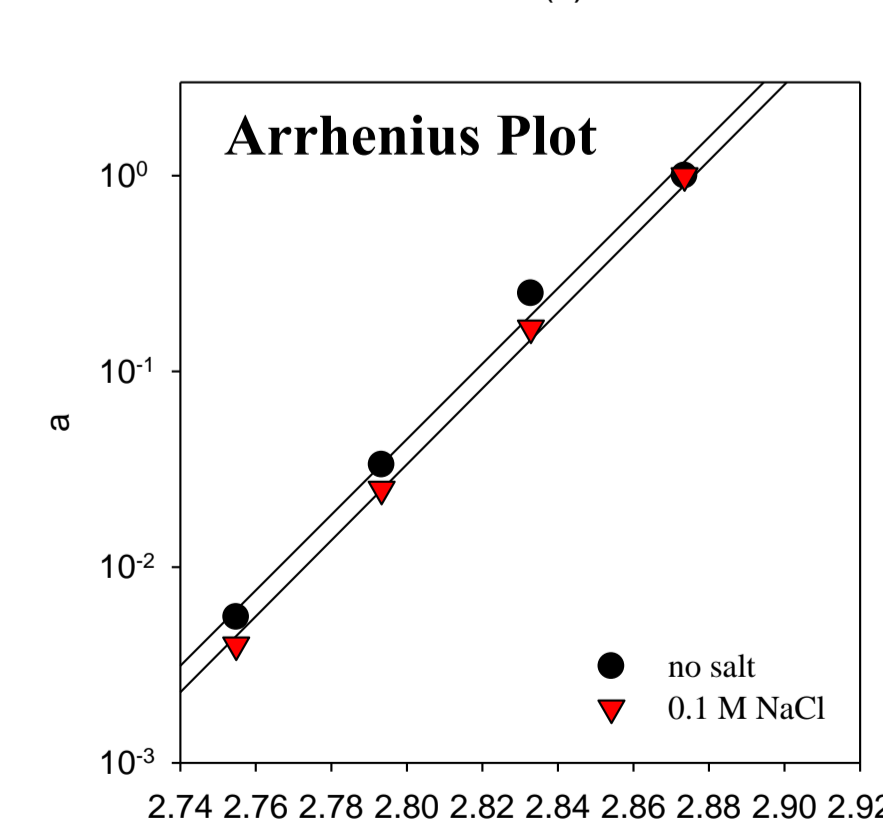
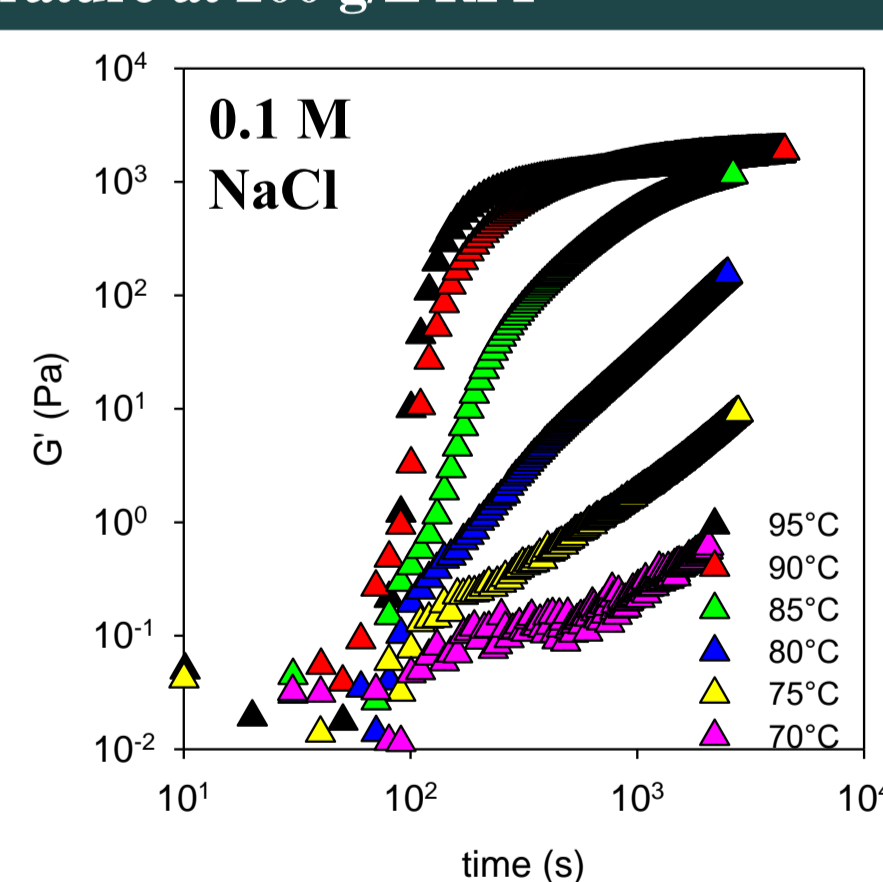
### Individual protein + mixtures

#### Effect of temperature at 200 g/L RPI

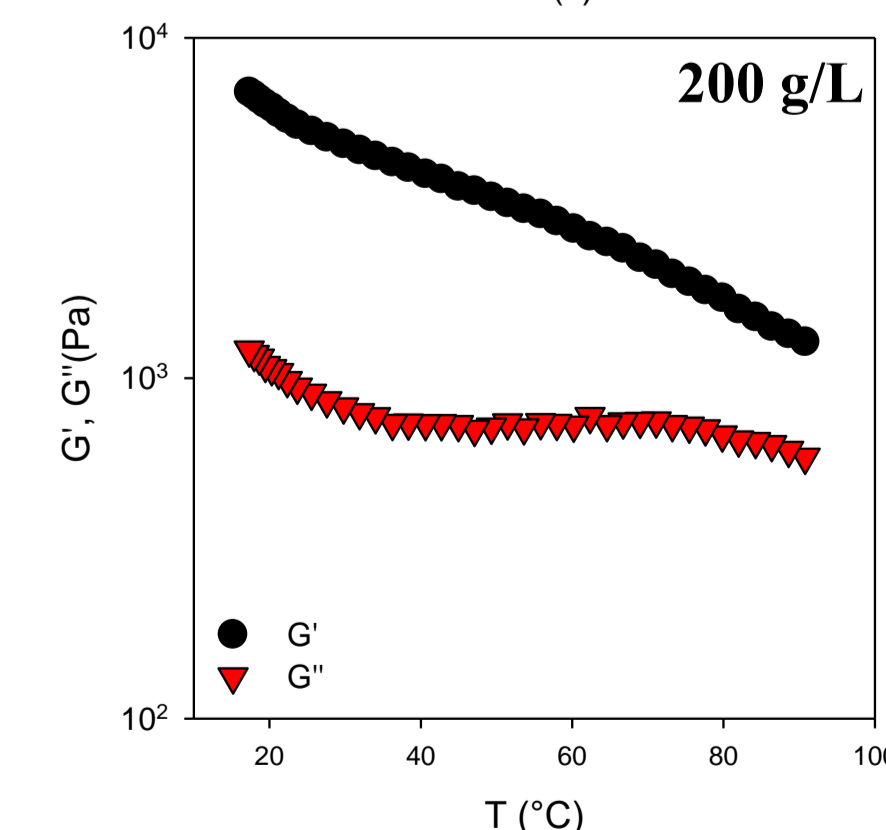
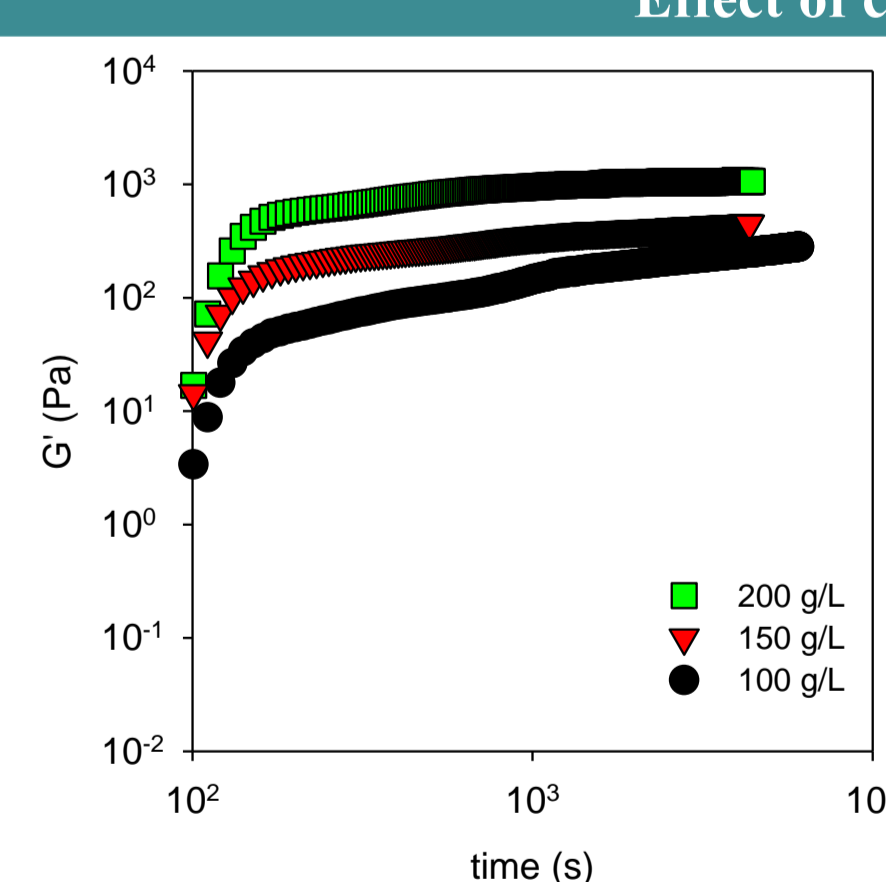


The gelation rate increased strongly with increasing temperature and was controlled by an activation energy of about 300 kJ/mol. Master curve could be obtained by time temperature superposition

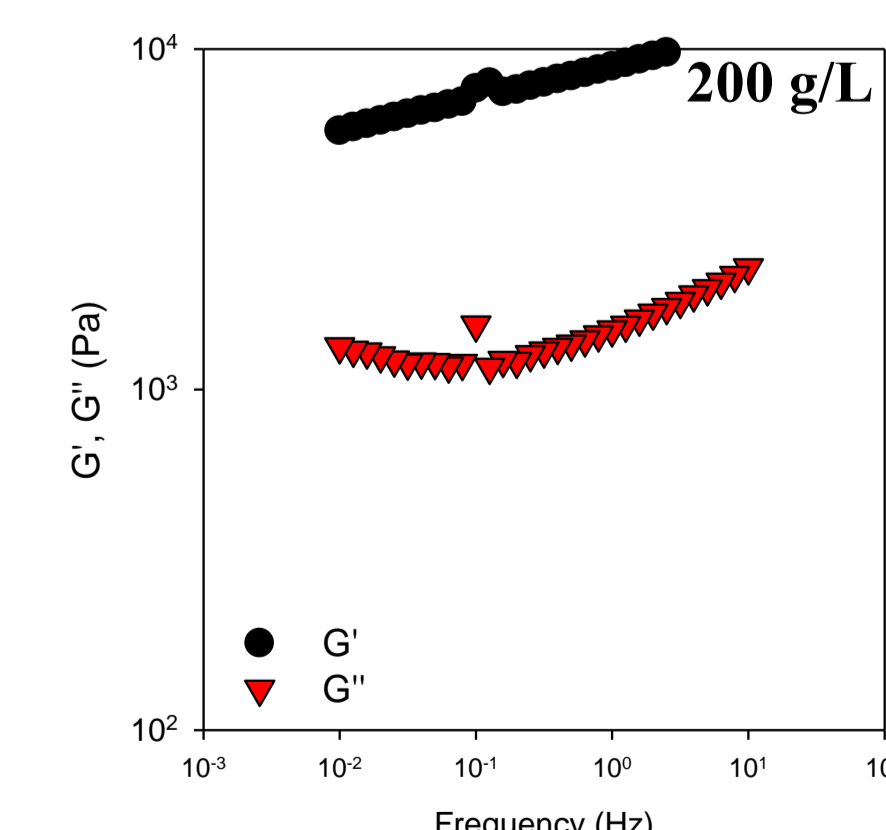
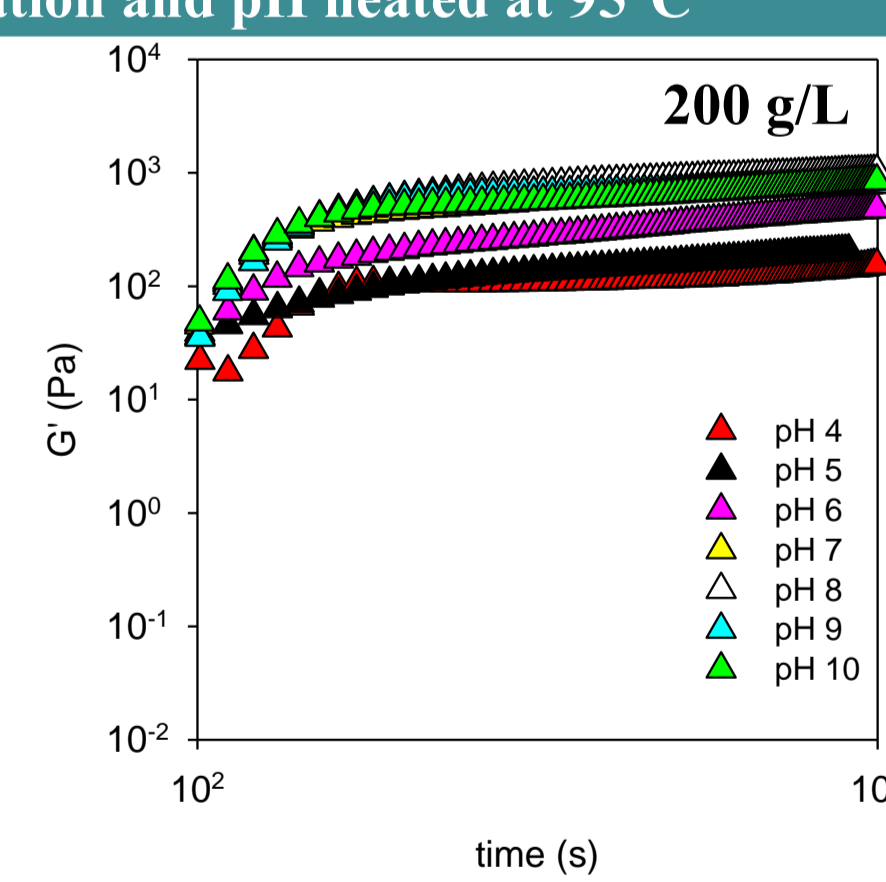
#### Effect of concentration and pH heated at 95°C



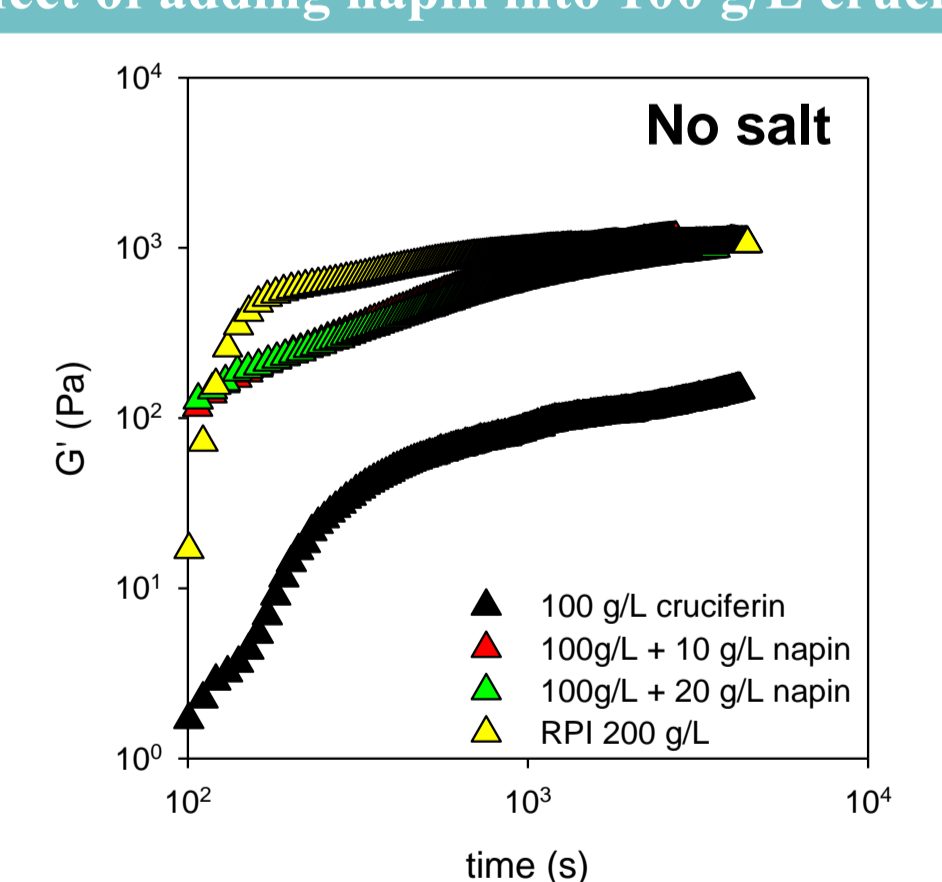
#### Effect of concentration and pH heated at 95°C



#### Effect of adding napin into 100 g/L cruciferin



#### Effect of adding napin into 100 g/L cruciferin



Cruciferin by itself forms a weak gel. However, the cruciferin gel is reinforced by addition of napin, which does not form a gel by itself.

- The elastic shear modulus increased with increasing protein concentration, but did not depend much on the pH between pH 6 and 10, nor on the addition of NaCl.
- During cooling the modulus further increased

## Conclusions

- RPI formed particles upon heating that aggregate and form gels at higher concentrations
- A large fraction of napin does not incorporate in the aggregates.
- The elastic modulus increases with increasing concentration, but here is no effect of pH (6-10) and NaCl (100 mM) on the gelation of RPI
- Napin does not gel by itself, but reinforces the cruciferin gel