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

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## Introduction

In the manufacture of yoghurts, the extensive heat-treatment of milk at 80-95°C for several minutes has been shown to increase the pH of gelation and the final elastic modulus of the gel. These changes correlate well with the formation of micelle-bound (MC) and serum (SC) heat-induced whey protein/ $\kappa$ -casein complexes. The objective of this study was to investigate the specific contributions of the heat-induced complexes and the casein micelles to the formation of acid milk gels using a highly relevant labelling procedure for CLSM, and rheology.

## Results & Discussion

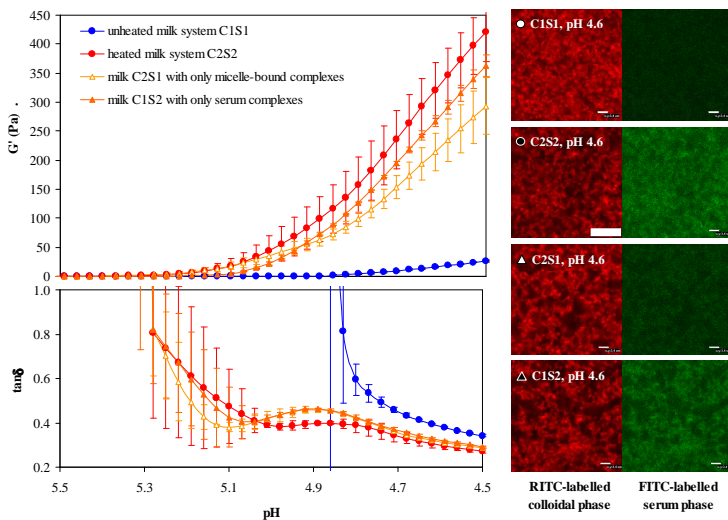
- Comparison C1S1/C2S2 systems confirms the literature on the effect of heat-treatment on acid gelation of milk :  $\nearrow$  pH<sub>gel</sub>;  $\nearrow$  dG'/dpH;  $\nearrow$  G';  $\nearrow$  solid-like behaviour;  $\nearrow$  tan $\delta$ <sub>max</sub> present;  $\nearrow$  pore diameter;  $\nearrow$  visible connectivity (Fig1 and Table1)
- When MC or SC was present (C1S2 and C2S1) behaviour similar to heated milk system (C2S2): same pH<sub>gel</sub>, G', tan $\delta$  and microstructure
- FITC-labelled SC visible in C1S2 and C2S2. super-imposable with RITC-labelled casein micelles  $\rightarrow$  co-location of SC and micelles

Table 1. Mean values for pH of gelation, pH at the local maximum of tan $\delta$  and G' and tan $\delta$  at pH 4.5

	pH <sub>gel</sub>	pH <sub>max tan<math>\delta</math></sub>	G' <sub>pH 4.5</sub> (Pa)	tan $\delta$ <sub>pH 4.5</sub>
C1S1	4.86 <sup>a</sup>	-	26 <sup>a</sup>	0.343 <sup>a</sup>
C1S2	5.11 <sup>b</sup>	4.90 <sup>a</sup>	363 <sup>b</sup>	0.294 <sup>b</sup>
C2S1	5.24 <sup>b</sup>	4.93 <sup>a</sup>	293 <sup>b</sup>	0.287 <sup>b</sup>
C2S2	5.22 <sup>b</sup>	4.92 <sup>a</sup>	421 <sup>b</sup>	0.270 <sup>b</sup>

Means with different superscripts in column differ significantly ( $P < 0.05$ )

Fig.1: Development of the elastic modulus and tan $\delta$  of milk systems during acidification. Right hand side images show the final microstructure of systems at pH 4.6. Scale bar is 10  $\mu$ m.



## Conclusions

Confirmation that the heat-induced complexes were responsible for the earlier destabilisation of milk system at pH  $\sim$ 5.2 and for enhanced G'

Heat-induced complexes located in the serum or the colloidal phase or both  
 $\rightarrow$  same rheological and microstructural changes of acid gels  
 + co-location of SC and casein micelles in the gels

Casein micelles = the building material of the gel with SC attached to their surface

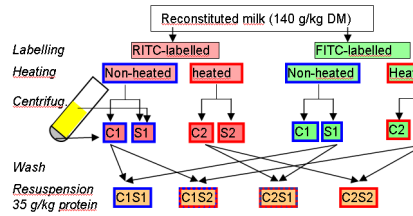


**suggested that SC interacted with the casein micelles before gelation**  
**Possibly C1S2 = C2S1 = C2S2 at the gel time**



**SC provide casein micelles with new gelation functionality**

## Materials & Methods



Systems acidified by GDL addition at 35°C

Monitored on an inverted microscope (Nikon) with a 60x oil-immersion objective  
 - FITC  $\lambda_{exc}$  = 488 nm -  $\lambda_{em}$  = 515  $\pm$  15 nm  
 - RITC  $\lambda_{exc}$  = 543 nm -  $\lambda_{em}$  = 590  $\pm$  25 nm  
 - Observations at 30  $\mu$ m depth

Monitored by low amplitude dynamic oscillation method (AR2000, TA Instruments)

- $t_{gel}$  defined as the time when  $G' > 1$  Pa.
- Neither the presence nor the nature of the fluorescent probes nor the labeled phase modified the acid gelation as measured by rheology (preliminary results)
- 2 repetitions of the complete sample preparation and gelation performed

- Values of pH<sub>gel</sub> confirmed by CLSM: 5.0 (C1S2-C2S1-C2S2) - 4.8 (C1S1) (Fig.2)
- No evidence of any particular sequence in the aggregation of casein micelles or heat-induced SC, meaning simultaneous aggregation (Fig.2)
- When FITC-labelled SC were present (C2S2 and C1S2), resulting overlay images are orange (red+green) as compared to C1S1 - C2S1 without SC (red)

Fig.2: FITC/RITC overlay images of the systems (RITC on colloidal components in red and FITC fixed on soluble components in green)

