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# pH-dependent behaviour of soluble protein aggregates formed during heat-treatment of milk at pH 6.5 or 7.2

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## Introduction

Soluble (SA) and micelle-bound (MA) protein aggregates formed during the heat-treatment of milk are thought to increase the gelation pH and gel strength of acid milk gels. The ratio SA/MA increases as heat-treatment pH is increased and the resulting gels are stronger. The objective was to study the pH-dependent behaviour of SA produced by heat-treatment at pH 6.5 (SA<sub>6.5</sub>) and 7.2 (SA<sub>7.2</sub>) in order to get a better understanding of their role in acid gelation of heated skim milk.

## Materials & Methods

Milk was heated (90°C-10 min) at its natural pH (6.5) or at pH 7.2. The soluble phase (SPNT 1) was isolated by centrifugation (19000 g/4h) and acidified to pH values ranging from the initial pH (6.5 or 7.2) to 4.6 with HCl. Further centrifugation of these acidified samples yielded fractions (SPNTs 2) which contained the proteins still soluble at each pH value. SDS-PAGE and laser densitometry under dissociating (SDS) and reducing (SDS-DTT) conditions allowed the determination of protein composition of heat-induced SA<sub>6.5</sub> and SA<sub>7.2</sub>.

## Results & Discussion

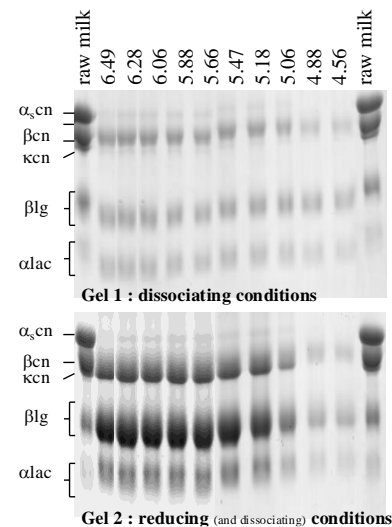


Fig.1. SDS-PAGE of fractions (SPNTs 2) adjusted to pH values between pH 6.49 and 4.56 from milk heat-treated at pH 6.5

- Gel 1: absence of proteins linked by disulfide bridges
- Gel 2: all proteins present
- Subtraction of the intensities of the bands : Gel 2 - Gel 1 yielded quantification of proteins linked by disulfide bridges

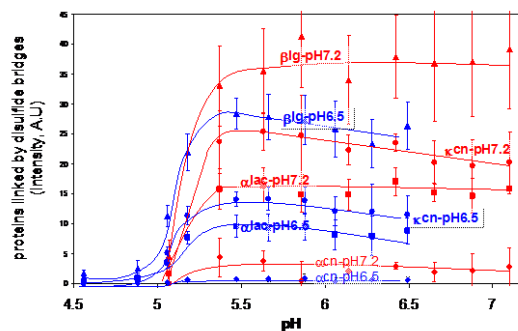


Fig.2. Proteins linked by disulfide bridges in SA<sub>6.5</sub> and SA<sub>7.2</sub> (from SDS-PAGE)

- SA =  $\beta 1g + \alpha 1lac + \kappa 1cn$  (+  $\alpha 5cn$ )
- More proteins included in covalent SA after heat-treatment at pH 7.2 than 6.5
- Same precipitation pH for soluble casein and SA (not shown)
- Same onset of precipitation for SA<sub>6.5</sub> and SA<sub>7.2</sub> ( $\approx 5.4$ ) but sharper decrease for SA<sub>7.2</sub>

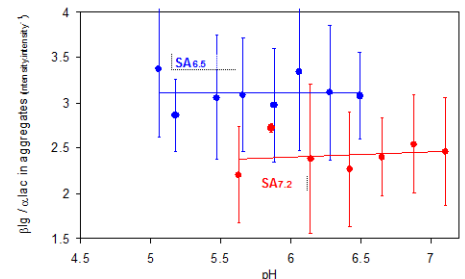
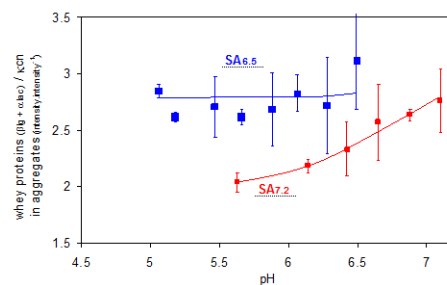


Fig.3. Ratio of proteins in covalent SA<sub>6.5</sub> and SA<sub>7.2</sub> (from SDS-PAGE)

- SA<sub>7.2</sub> : richer in  $\kappa 1cn$  and  $\alpha 1lac$  than SA<sub>6.5</sub>
- Variation of SA<sub>7.2</sub> composition during acidification : perhaps thiol / disulfide exchanges

## Conclusions

Soluble aggregates formed during heat-treatment of milk at pH 6.5 or 7.2 were composed of  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and  $\kappa$ -casein, however the ratios of whey proteins/ $\kappa$ -casein and  $\beta$ -lactoglobulin/ $\alpha$ -lactalbumin were higher in soluble aggregates formed at pH 6.5. Precipitation was initiated at the same pH value for both SA but occurred over a narrower pH range for soluble aggregates formed at pH 7.2. This, together with the fact that the aggregates prepared by heat-treatment of milk at pH 7.2 are more numerous in the soluble phase, may explain why gelation pH of skim milk increases with heat-treatment pH.