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

Marie Renan, Omar Mekmene, Marie-Hélène Famelart, V Arnoult-Delest, D Paquet, F Guyomarc'H, Gérard Brule

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

Renan M.[1*], Mekmene O.[1], Famelart M.H.[1*], Arnoult-Delest V.[2], Pâquet D.[2], Guyomarc'h F.[1], Brulé G.[1]

([1]) INRA - STLO Science et Technologie du Lait et de l'Œuf, 65 route de St Brieuc, 35 042 Rennes cedex, France
([2]) Danone Vitapole, RD128, 91767 Palaiseau cedex, France

(*) Tel. +33.(0)2.23.48.53.43, Fax. +33.(0)2.23.48.53.50, @ : marie.renan@rennes.inra.fr / marie-helene.famelart@rennes.inra.fr

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 (**SA6.5**) and 7.2 (**SA7.2**) 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 SA6.5 and SA7.2.

Results & Discussion

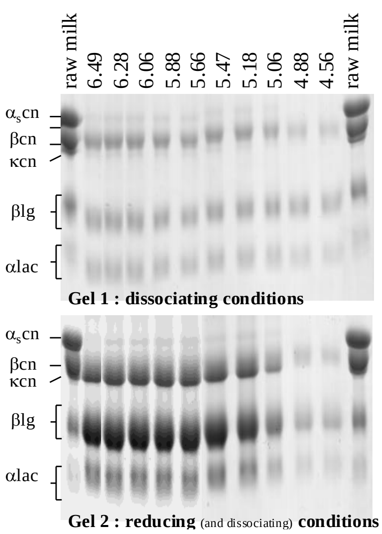

Fig.1. SDS-PAGE of fractions (SPNTs 2) adjusted to pH values between pH 6.49 and 4.56 from milk heat-treated at pH 6.5

- 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

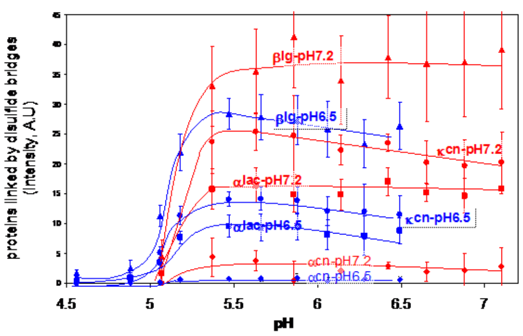

Fig.2. Proteins linked by disukfide bridges in SA6.5 and SA7.2 (from SDS-PAGE)

- SA = βlg + αlac + κcn (+ αscn)
- 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 SA6.5 and SA7.2 (≈ 5.4) but sharper decrease for SA7.2

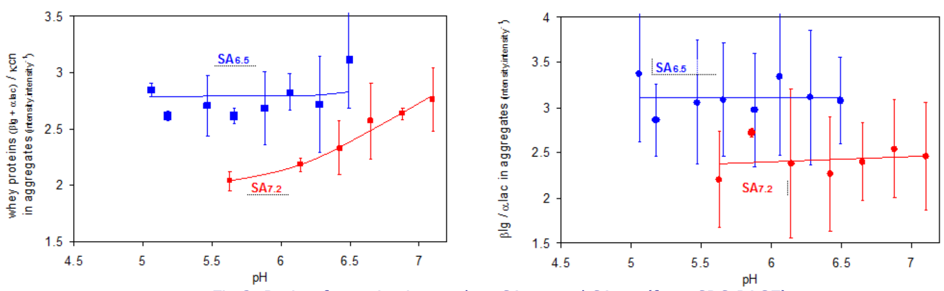

Fig.3. Ratio of proteins in covalent SA 6.5 and SA 7.2 (from SDS-PAGE)

- SA7.2 : richer in κcn and αlac than SA6.5
- Variation of SA7.2 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 β-lactoglobulin, α-lactalbumin and κ–casein, however the ratios of whey proteins/κ–casein and β-lactoglobulin/α-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.