Structure and surface properties of soluble protein aggregates isolated from heated skim milk

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Introduction

Heat-induced denaturation and aggregation of milk whey proteins has long been correlated with higher gelation pH and higher gel strength of acid gels. Soluble and micelle-bound aggregates are formed during heating, which interact with casein micelles on gel formation. The objective of this study was to characterise the aggregates' properties to get a better understanding of their role during acid gelation of heated skim milk.

Materials & Methods

Soluble aggregates that are formed in skim milk heated to 90°C for 10 min were isolated by ultracentrifugation, UF-concentration and FPLC separation of the supernatant. Composition and purity were assayed by RP-HPLC and SDS-PAGE. Size and shape were determined by light scattering techniques and TEM. Surface charge at various pH values was measured by zetametry, and surface hydrophobicity was estimated through ANS binding.

Results & Discussion

RP-HPLC analysis and SDS-PAGE in reducing or non-reducing conditions showed that isolated soluble heat-induced protein aggregates were composed of whey proteins and κ casein, mainly linked by disulfide bridges. Purity was >82% with some contamination due to other caseins.

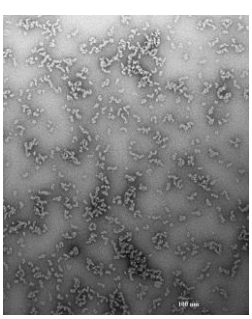

Fig.1. TEM micrograph of isolated aggregates. Clustering was artefactual.

The diameter of aggregates was ~25 nm by TEM (Fig.1), for ~70 nm hydrodynamic diameter by DLS and MALLS. The observed size difference may be due to the occurrence of an hydration layer, especially if κ casein is located on the aggregate surface as in micelle (hairy layer ~12 nm). Processing of MALLS data gave a MW of ~10^7 g.mol^{-1}.

The zeta potential of aggregates in milk ultrafiltrate was -17 mV at pH 6.7, and nil at pH 4.5 (isoelectric point - Fig.2). Visible precipitation occured at pH 4 and 5 (not shown). Under similar ANS/protein conditions, the surface hydrophobicity of aggregates was high, compared to standard β-lg and micellar casein (Fig.3).

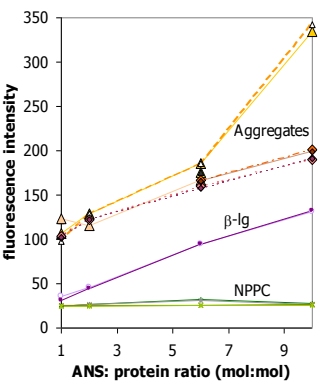

Fig.3. Fluorescence intensity of ANS/protein binding of aggregates, micellar casein (NPPC) and β-lg.

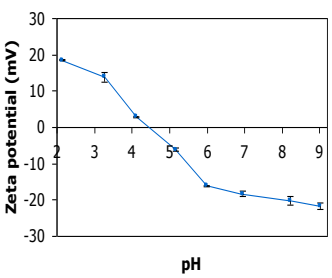

Fig.2. Zeta potential of the agreggates as a function of pH

The results indicate that the high gelation pH of heated milk (~5.4) could be due to heat-induced aggrega-tes having a high hydrophobicity/ charge balance compared to micelles.

Conclusions

The heating of milk leads to the formation of whey protein/κ casein aggregates. These aggregates are acid-precipitable, range from 10 to 100 nm in size and have a high MW. They probably contribute to destabilisation of the milk system on acidification by precipitating at higher pH than the micelles due to the difference in the balance of their surface hydrophobicity and charge, rather than by increasing the apparent isoelectric point of the aggregate-coated micelles.

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