

## **Characterization of $\beta$ -lactoglobulin nano-hydrogels formed by heat treatment under selective pH range conditions**

Óscar L Ramos, Ricardo N Pereira, X. Malcata, António A. Vicente

Institute for Biotechnology and Bioengineering, Centre of Biological Engineering,  
University of Minho, Braga, Portugal

Institute of Chemical and Biological Technology, New University of Lisbon, Oeiras,  
Portugal

Bovine  $\beta$ -lactoglobulin ( $\beta$ -Lg) is a globular protein from milk that has considerable potential as a functional ingredient in food, cosmetic, and pharmaceutical applications. It is, indeed, the major component (i.e. 50 wt%) and the primary gelling agent of whey proteins.  $\beta$ -Lg is stable at low pH and highly resistant to proteolytic degradation in the stomach. Another interesting feature is its ability to act as an encapsulating agent. The objective of this work was to understand the kinetics of aggregation during heat treatment, under narrow pH ranges, that leads to the formation of food-grade  $\beta$ -Lg nano-hydrogels, and to postulate a model for its mechanism of aggregation. In this study, aqueous dispersions of  $\beta$ -Lg were accordingly generated, and formation of stable  $\beta$ -Lg nano-aggregates were evaluated after heating under different temperatures (75, 80, and 85 °C), holding periods (5, 10, 15, and 20 min), and pH values (4, 5, 6, and 7); particular emphasis was put at pH ca. 6.0. The protein aggregates formed were characterized for stability (turbidity), morphology, size, surface charge, and content in accessible thiol groups. Stable dispersions of  $\beta$ -Lg nano-hydrogels were obtained at pH 6, corresponding to an aggregation yield of ca. 65, 77, and 92% at 75, 80, and 85 °C, respectively, for a holding period of 20 min. Nano-hydrogels at pH 6 were characterized by a particle size between 160 and 250 nm and a low degree of polydispersity (<0.2). Their  $\zeta$ -potential was +20 or -40 mV, depending on pH. Nano-hydrogels obtained at pH 5 displayed a lower content of accessible thiol groups as compared to those obtained at pH above or below. For pH between 5 and 6, large sedimenting protein particulates were obtained, whereas soluble aggregates were formed at pH outside that range.