Development and characterization of β-lactoglobulin nanohydrogels for bioactive compound delivery

Oscar Ramos[a,b], *Ricardo Pereira*[a], *Artur J. Martins*[a], *F. Xavier Malcata*[b] & *António Vicente*[a,*]

[a] CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal, [b] LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, University of Porto, Porto, Portugal

*avicente@deb.uminho.pt

Abstract

Whey proteins produced during bovine cheese manufacture, or membrane fractionation of bovine milk, account for increasingly valuable food ingredients – owing to their nutritionally-balanced composition in amino acid residues, coupled with their functional properties (e.g. solubility, gel-forming, emulsifying and foaming features). Whey protein-based gelling systems may indeed play several functional roles in food formulation by enhancing textural properties, acting as stabilizing agents or by being used as carrier of bioactive substances (e.g. nutraceuticals) [1]. The rates and pathways for manufacture of a protein gel system are controlled by heating conditions, protein concentration pH and ionic strength. The combination of different treatments can potentially interfere with unfolding and aggregation of whey proteins, and thus with protein-protein interactions.

β-Lactoglobulin (β-Lg) is the major protein fraction of bovine whey serum (i.e. 50 wt%), and a primary gelling agent; it is stable at low pH and highly resistant to proteolytic degradation in the stomach, further to its appropriateness as encapsulating agent [2].

The objective of this work was to understand the kinetics of aggregation during heat treatment, under narrow pH ranges, leading to formation of edible β-Lg nanohydrogels, as well as to rationalize the contribution and importance of covalent and noncovalent interactions to maintain structure thereof. Furthermore, the ability of said nanohydrogels to encapsulate bioactive compounds (e.g. vitamin B_2) was also assessed.

Aqueous dispersions of β-Lg (10 mg/mL) were accordingly produced, and formation of stable nanostructures was ascertained after heating at 80 ºC for 20 min, under different pH values (i.e. 4, 5, 6 and 7); particular emphasis was placed on pH 6.0. The protein aggregates formed were characterized for stability, role of covalent or non-covalent bonds, morphology, size, surface charge and content in accessible thiol groups. Stable dispersions of β-Lg nanohydrogels were obtained at pH 6, corresponding to aggregation yield of ca. 77% at 80 ºC, for a holding period of 20 min. Such structures were characterized by particle size between 100 and 150 nm, low degree of polydispersity (<0.2), and association efficiency of vitamin B_2 above 85%. Their ζ-potential varied from +20 to −40 mV, depending on pH. Nanohydrogels obtained at pH 5 displayed a lower content of accessible thiol groups than those obtained at pH values above or below. For pH between 5 and 6, large settling protein particulates were obtained, whereas soluble aggregates were formed at pH values off that range.

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