Poster 45 Design of micro- and nanostructures from β-lactoglobulin under selected environmental conditions

Lívia de Souza Simões[a], António A. Vicente[a] and Oscar L. Ramos[a,b]

[a]*CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*

[b]*LEPABE - Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia, University of Porto, Porto, Portugal*

Bovine β-lactoglobulin (β-Lg) is a globular protein and the major component of whey proteins (ca. 50 % of its protein content). Besides the high nutritional value, the biological properties and resistance to proteolytic degradation in the stomach, its gelation capacity is particularly important allowing the formation of bio-based micro- and nanostructures (e.g. particles and hydrogels). β-Lg when heated above a critical temperature (i.e. denaturation temperature: 76 ºC) undergoes conformational changes followed by subsequent protein-protein interactions. The order and rates of aggregation is highly dependent on the temperature, pH and protein concentration and can result in the formation of micro- and nanostructures with different properties and morphologies. The understanding of the kinetics of aggregation and of the combined effect of such environmental conditions is essential to design protein structures with the desired functionalities and applications. The objective of the present work was to understand the heat-induced aggregation of β-Lg, affected by combined environmental conditions (various pH, heating temperature and protein concentrations) that lead to the formation of β-Lg food-grade micro- and nanostructures.

In this study, β-Lg at various concentration (5, 10 and 15 $mg \cdot mL^{-1}$) was solubilized in 25 mM of sodium phosphate buffer at different pH values (3, 4, 6 and 7) and heated at different temperatures (60, 70 and 80 ºC) – below and above the denaturation temperature of β-Lg. Afterwards, the effect of aforementioned conditions on the β-Lg micro- and nanostructures formation was evaluated in terms of their particle size and polydispersity index (PDI) by dynamic light scattering.

β-Lg nanostructures showed particle sizes below 50 nm when formed at pH 3 and 7 for β-Lg concentrations of 5, 10 and 15 $mg \cdot mL^{-1}$ and heating temperatures of 60, 70 and 80 ºC, however displayed high PDI values (≥ 0.5). When the temperature of heating increased above the denaturation temperature of β-Lg (i.e. 80 °C), the PDI values of the structures at pH 6 showed the lowest values (≤ 0.2), independent of the β-Lg concentration used. At pH 4, it was possible to obtain structures at microscale (i.e. ≥ 3 µm) independent of the β-Lg concentration and heating temperature of 70 and 80 °C. At this pH, which is relatively close to the isoelectric point of β-Lg (i.e. 5.2), the net charge of proteins is ca. zero, so the protein structures tend to aggregate, thus showing higher size values.

Therefore, protein aggregation mechanisms appear to be controlled by the environmental conditions applied; therefore, an understanding of the quantitative effect of these conditions is crucial for rational design of protein structures at micro or nanoscale with tailor-made functionalities.