Stability of β-Lactoglobulin micro- and nanostructures under various environmental conditions

LS Simoes[1], AA Vicente[1] and OL Ramos[2]

[1] CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtas, 5710-057, Braga, Portugal
[2] CBQF – Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Porto, 4202-401, Portugal

β-Lactoglobulin (β-Lg) comprises about 50-55% of total proteins in bovine whey serum and it is its principal gelling agent. Heat-induced gelation of β-Lg above denaturation temperature (i.e. 76 ºC) leads to exposure of initially buried hydrophobic regions, followed by subsequent protein-protein interactions, which is a critical step to design micro- and nanostructures[1]. These bio-based structures can be a promising vehicle for encapsulation and controlled delivery of different bioactive compounds (e.g. antioxidants, vitamins). Nonetheless, developing successful delivery systems requires the assessment of the physicochemical stability of these structures under distinct environmental conditions[2]. In this sense, the present work aims at exploring the impact of several conditions such as pH, thermal processing, ionic strength and storage temperature on the physicochemical properties of β-Lg micro- and nanostructures. For this purpose, food-grade micro- and nanostructures, previously optimized, were prepared as follows: β-Lg powder (5 – 15 mg mL^{-1}) was solubilized in sodium phosphate buffer at pH 6 and then heated at 80 °C for 15 min. Subsequently, the effect of pH (from 2 to 10) using 0.1 mol L^{-1} NaOH and/or H_3PO_4; temperature (from 20 to 80 °C, with 5 ºC increments); ionic strength (from 0 to 200 mmol L^{-1}) by adding NaCl; and storage temperature (4 ºC and 25 °C) on β-Lg micro- and nanostructures stability were evaluated in terms of particle size, polydispersity index (PDI) and surface charge (S) changes by dynamic light scattering (DLS). For pH between 2 and 4, and 6 and 10, microstructures showed particle size values ranging from 202.4 nm to 252.6 nm ($p < 0.05$) and relatively low PDI (i.e. < 0.25). Nanostructures displayed particle sizes below 100 nm, except for pH 5. At this pH, β-Lg structures were heterogeneous, exhibiting a high PDI (i.e. > 0.8). The surface charge of β-Lg micro- and nanostructures changed from positive (ca. +18 mV) at pH 2, to negative (ca. – 20 mV) at pH 10, with net charge close to zero at pH 5 (i.e. near the isoelectric point of β-Lg). Regarding thermal stability, the particle size of micro- and nanostructures remained constant and homogenous (i.e. PDI < 0.25) until 70 ºC. Assessments of ionic strength showed that the addition of NaCl led to protein aggregation with consequent increase of particle size and PDI values ($p < 0.05$). In terms of storage stability, it was possible to observe that β-Lg structures remained stable over 36 days at 4 and 25 ºC, displaying particle sizes of 201.8 ± 8.6 nm at micro- and 64.7 ± 3.8 nm at nanoscale, PDI values below 0.25 and surface charge of ≈ - 20 mV ($p > 0.05$) between each condition tested. These findings provide new insights on which conditions the β-Lg micro- and nanostructures are more stable, and therefore more suitable to act as potential delivery systems of bioactive compounds.

References:
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