**Structural characterization of heat-induced β-lactoglobulin nanohydrogels under the effects of selected physical conditions**

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β-Lactoglobulin (β-Lg) is the major protein fraction in bovine whey serum (ca. 50% of its protein content). It is a bio-based and a Generally Recognized As Safe (GRAS) material, with a high nutritional value, that can be used to encapsulate nutraceuticals essentially due to its gelation capacity, which allows the formation of nanohydrogels. Furthermore, β-Lg displays a high binding capacity, under specific environmental conditions and it is resistant to proteolytic degradation in the stomach. These features make of β-Lg an excellent bio-based material to be used as carrier of nutraceuticals.

The objective of this work was to understand the impact of different conditions (β-Lg concentration and heating times) in the physical properties of β-Lg nanohydrogels. In this study, β-Lg at various concentration (5, 10 and 15 mg·mL-1), were solubilized in 25 mM of sodium phosphate buffer (at pH 6) and heated at 80 °C for several holding time periods (5, 15 and 25 min). The protein structures formed were then characterized in terms of their stability, morphology, polydispersity index, size and surface charge.

Stable nanohydrogels were obtained at pH 6, when treated at 80 ºC for heating periods longer than 15 min, characterized by a low polydispersity (< 0.2). At these conditions, nanohydrogels showed increasing particle size values, ranging from 50 nm to 110 nm, and surface charge from -15 mV to -20 mV, as β-Lg concentration increased. Nanohydrogels prepared at the same conditions, but treated for shorter periods of time, showed to be unstable, characterized by higher polydispersity (≥ 0.5) and surface charges of - 7 mV, independently of the β-Lg concentration used.

The results obtained in this study represent a significant contribute to enrich the knowledge about the impact of several environmental conditions on β-Lg nanohydrogel characteristics and thus in the desired properties intended for their final application.