

## DEVELOPMENT OF B-LACTOGLOBULIN NANOSTRUCTURES FOR ENCAPSULATION AND CONTROLLED RELEASE OF NUTRACEUTICALS

Daniel A. Madalena<sup>1</sup>, Óscar L. Ramos<sup>1,2</sup>, Ricardo N. Pereira<sup>1</sup>, Ana C. Pinheiro<sup>1</sup>, José A. Teixeira<sup>1</sup>, F. Xavier Malcata<sup>2</sup> and António A. Vicente<sup>1,\*</sup>

<sup>1</sup>CEB- Centre of Biological Engineering, University of Minho, 4710-057, Braga, Portugal

<sup>2</sup>LEPABE - Department of Chemical Engineering, University of Porto, Porto, Portugal

\*avicente@deb.uminho.pt

$\beta$ -Lactoglobulin ( $\beta$ -Lg) is the major protein fraction in bovine whey serum (ca. 50% of its protein content), and a primary gelling agent.  $\beta$ -Lg has a high nutritional value, is stable at low pH and highly resistant to proteolytic degradation in the stomach, besides being able to act as encapsulating agent. These features make it an excellent bio-based material to be used as carrier of nutraceuticals. This study aimed at assessing the ability of  $\beta$ -Lg nanostructures to both encapsulate and control release of nutraceuticals (e.g. vitamin B<sub>2</sub>) throughout their course in an *in vitro* gastrointestinal (GI) system. The size and stability of said nanostructures during passage through the GI model was also evaluated. At nano-scale, these structures exhibit a large surface area-to-volume ratio, thus contributing to improve nutraceutical solubility and bioavailability, as well as preventing undesirable chemical reactions – while maintaining nutraceutical activity until their release. This is particularly important for vitamin B<sub>2</sub> that is poorly soluble in water and susceptible to degradation by light. Vitamin B<sub>2</sub> is essential for growth, development and regular maintenance of the human body, and plays a relevant role in the normal function of human brain and nervous system.

In this study, aqueous dispersions of  $\beta$ -Lg (1%) were accordingly produced, and formation of stable  $\beta$ -Lg nanostructures was ascertained at pH 6.0, after heating at 80 °C for 10 min. The nanostructures formed were characterized for size, surface charge and stability, as well as for association efficiency (AE) of vitamin B<sub>2</sub>. Nanostructures containing vitamin B<sub>2</sub> were then submitted to an *in vitro* GI model system, which simulates the conditions prevailing in the human GI tract – i.e. main digestive compartments (stomach, duodenum, jejunum and ileum) and their condition (temperature, pH, mixing, transit time, enzymes and other constituents such as bile). The experiments were carried out for 5 h, and samples were analysed after each stage of digestion (i.e. stomach, duodenum, jejunum and ileum) for size, surface charge and stability.

Stable  $\beta$ -Lg nanostructures were obtained at pH 6, showing particle sizes of 170.2±0.85 nm, low degree of polydispersity (i.e. PDI = 0.074±0.027),  $\zeta$ -potential of -34.9±0.49 mV, and AE of 74%.  $\beta$ -Lg nanostructures exhibited a metastable structure upon passage throughout stomach (i.e. particle size, PDI and  $\zeta$  potential of 247±9.33 nm 0.18±0.03 and 18.4±2.85 mV, respectively). Concerning their passage throughout the intestine, such nanostructures formed higher size aggregates with an increased heterogeneity and loss of stability. This could be concluded from the significant increase in their particle size and PDI, as well as decrease of  $\zeta$  potential, i.e. duodenum (696.4±206.8 nm, 0.733±0.129 and -28.9±0.943), jejunum (830.1±163.4 nm, 0.707±0.08 and -25.8±0.991) and ileum (1526±639.9 nm, 0.841±0.133 and -31±6.31). Regarding vitamin B<sub>2</sub>, a release of ca. 11% was observed after their passage through the stomach, while 46, 84 and 89% release was observed after passage through the duodenum, jejunum and ileum, respectively. Hence,  $\beta$ -Lg nanostructures showed to be suitable carriers for vitamin B<sub>2</sub> until reaching the intestine, where destabilization eventually occurs.

Topic: Biopolymer processing technologies and polymer stability