

## Design of $\beta$ -lactoglobulin nanostructures for encapsulation and controlled release of riboflavin in the gastrointestinal tract

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Bovine  $\beta$ -lactoglobulin ( $\beta$ -Lg) is a globular protein from milk and the major component of whey proteins (ca. 50 % of its protein content). It is a food-grade and Generally Recognized As Safe (GRAS) material, that have a high nutritional value and important biological and functional properties, particularly the capacity to form gels, which allows the formation of nanostructures that can be used to encapsulate nutraceuticals [1]. Besides,  $\beta$ -Lg is stable at low pH, and highly resistant to proteolytic degradation in the stomach. Riboflavin is an essential vitamin for the normal function of human brain and nervous system. However, this vitamin is poorly soluble in water and highly susceptible to light degradation, thus its encapsulation may represent a suitable solution to its protection, overcoming these issues [2]. This study aims at evaluating the ability of  $\beta$ -Lg food-grade nanostructures to encapsulate and control the release of riboflavin during the gastrointestinal (GI) passage.

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 in terms of size, surface charge and stability, morphology and association efficiency (AE) of riboflavin. Riboflavin-loaded nanostructures were then submitted to an *in vitro* GI model system, simulating the conditions of human GI tract (i.e. stomach, duodenum, jejunum and ileum) and their condition (e.g. temperature, pH, mixing, transit time, enzymes and other constituents such as bile). The experiments were carried out for 5 h, and the nanostructures were structurally characterized after each stage of digestion.

Stable  $\beta$ -Lg nanostructures were obtained at pH 6, showing a spherical shape, 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 26%.  $\beta$ -Lg nanostructures showed to be stable in the stomach being mostly degraded in the small intestine (which was determined by electrophoresis assay), where most of their riboflavin content was released i.e. 46%, 84% and 89% in the duodenum, jejunum and ileum, respectively. Hence,  $\beta$ -Lg nanostructures showed to be suitable carriers for riboflavin until reaching the intestine, where destabilization eventually occurs.

This study represent a significant contribute in the food science field by providing knowledge related to the applicability of food-grade nanomaterials for incorporation and controlled release of functional compounds, thus improving their bioavailability through protection from harsh conditions during GI digestion.

## References

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