Structural and functional stabilization of glycomacropeptide via encapsulation within multiple emulsions

Carla G. Moutinho\textsuperscript{1,2}, José A. Teixeira\textsuperscript{2}, Victor M. Balcão\textsuperscript{1,2}

\textsuperscript{1}Bioengineering and Biopharmaceutical Chemistry Research Group, Fernando Pessoa University, Porto, Portugal; \textsuperscript{2}IBB - Institute for Biotechnology & Bioengineering, Centre Biological Engineering, University of Minho, Braga, Portugal

Bovine glycomacropeptide (GMP), derived from whey proteins, has been demonstrated to possess an interesting bioactivity that has attracted a lot of attention over the last few years. In particular, its ability to bind \textit{Vibrio cholerae} and \textit{Escherichia coli} enterotoxins, inhibit bacterial and viral adhesion, suppress gastric secretions, promote bifidobacterial growth and modulate immune system responses. Of these, protection against toxins, bacteria and viruses, and modulation of the immune system, are the most promising applications for this bioactive dairy macrop peptide. The development of strategies that may allow its structural and functional stabilization via nanoencapsulation within multiple emulsions may increase its food and biopharmaceutical applicabilities. In this research effort, bovine GMP was (thermodynamically) stabilized via entrapment within water-in-oil-in-water (W/O/W) multiple emulsions aiming at mimicking the multifunctional design of biology, with several lipid matrices, and stabilizing layer compositions. Due to their compartmentalized internal structure, multiple emulsions are ideal for encapsulation since they can carry both polar and non-polar (bio)molecules. The composition of the stabilizing layer of the nanosystem was changed by using different poloxamers and proportions of lecithin. Physicochemical characterization of the optimized GMP-encasing nanovesicle formulations encompassed determination of Zeta potential and particle hydrodynamic size over storage time, surface morphology via CRYO-SEM, and microcalorimetric analysis via DSC.