## NanoDairy Project: delivery systems of bioactive polyphenolic compounds to dairy matrices. Evaluation of stability, bioavailability and toxicity

**Ana Raquel Madureira<sup>1</sup>,** Débora Campos<sup>1</sup>, Vincenza Ferraro<sup>1</sup>, Sara Nunes<sup>2</sup>, Flávio Reis<sup>2</sup>, Bruno Sarmento<sup>3,4</sup>, Ana Maria Gomes<sup>1</sup>, Maria Manuela Pintado<sup>1</sup>

<sup>1</sup>CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital Apartado 2511 4202-401 Porto, Portugal; <sup>2</sup>IBILI – Laboratory of Pharmacology and Experimental Therapeutics, Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, Sub-Unit 1 (Pólo III), University of Coimbra, 3000-548 Coimbra, Portugal; <sup>3</sup>I3S -- Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal and INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Rua do Campo Alegre; <sup>4</sup>CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Rua Central de Gandra 1317, 4585-116, Gandra-PRD, Portugal

Formulation of new functional foods and ingredients has shown a considerable increase during the last two decades. The incorporation of phenolic compounds from fruit pulps and herbal extracts are being studied specially in dairy products, taking advantage of their richness in antioxidant properties, as other health benefits. Nevertheless, evidence exists on the occurrence of interactions between phenolic compounds and dairy proteins, which decrease the bioaccessibility and bioavailability of phenolic compounds and consequent biological activity. In addition, during storage and digestion of food products, the phenolic compounds can undergo degradation, leading to a decrease of bioactivity. Thus, the NANODAIRY project was thought to find an alternative for the incorporation of phenolic compounds in dairy matrices, by the formulation of phenolic compounds loaded nanoparticles (NP). As models, rosmarinic acid (RA) and rich RA rich herbal extracts – sage and savoury were chosen, owing to the several biological properties attributed to these ones. The first approach was to confirm the occurrence of interactions between phenolic compounds and dairy proteins analysed using spectrophotometry and other analytical techniques. Then, two types of NP were produced, solid lipid nanoparticles (SLN) and polymeric NP. Results from SLN studies and performance of the procedures described below will be presented. Solid lipid nanoparticles were produced using two types of waxes viz. witepsol and carnauba using hot homogenization and ultrasonication method. Characterization of the physical properties, thermal, chemical, morphological and antioxidant activities were performed. The lyophilisation process, the stability along storage time, as well the interfacial properties of the SLN were evaluated. Digestion simulation of the loaded SLN was performed, and the physical properties and release of phenolic compounds during the digestion stages were followed. Additionally, the impact of the SLN on the gut microbiota and on their metabolism were evaluated using human faeces and assessing fermentation processes. To achieve SLN toxicity, the cytotoxic, genotoxic and mutagenicity effects in blood cells were evaluated. Finally, *in vivo* studies were performed in Wistar rats, performing two different studies: acute and chronic administration of SLN, during 14 d and 6 wks. Body and tissue weights evolution, hematological and biochemical data, including glucose and lipid profile, renal and liver function markers, concentrations of RA and metabolites in serum, faeces, urine and selected tissues (heart, liver, kidney, stomach, adipose tissue, spleen, small intestine and cecum), as well as deposition of SLN on those tissues were followed. Quantification of gut microbiota groups in faeces, as well production of short chain fatty acids (SCFA) and quantification of fatty acids in faeces was also made. The incorporation of SLN in dairy matrices such as milk and yogurt was made, and the matrices were characterized in terms of the effects on lactic acid bacteria viability, pH, production of organic acids and textural properties.



