## Development of bio-based nanoemulsions to improve physical and chemical stability of omega-3 fatty acids

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- I. **Introduction** – Omega-3 polyunsaturated fatty acids ( $\omega$ -3 fatty acids) are known for their functional properties such as: improving cardiovascular health, decrease inflammation, increase cognitive function, and positively influence neurological and visual development. However,  $\omega$ -3 fatty acids are highly susceptible to oxidation, have an intense odour and present low water solubility, which makes their direct application in foods extremely difficult. In order to reduce these challenges, bio-based nanostructures have been developed to encapsulate bioactive compounds and thus enhance their physical and chemical stability during storage until consumption.
- П. Experimental - Lactoferrin (Lf), a protein derived from milk with a wide range of reported biological activities (e.g. antioxidant, antimicrobial and cancer prevention), was used as natural emulsifier for the development of oil-in-water nanoemulsions. Nanoemulsions were produced with a high-pressure homogenizer applied for 5 cycles at 20000 psi. Different Lf concentrations (0.2; 0.6; 1; 2; 3; 4 and 5% (w/w)) were tested. The nanoemulsions' physical properties were evaluated in terms of size and ζ-potential using dynamic light scattering (DLS). The morphology of nanoemulsions was analysed by transmission electron microscopy (TEM). The physical and chemical stability of these nanoemulsions was assessed during 69 days, at storage temperatures of 4 °C and 25 °C, being the chemical stability of nanoemulsions evaluated by antioxidant activity measurements using the DPPH radical scavenging assay and peroxide value.
- III. Results and Discussion - Results showed that according to the Lf concentration used, different properties were obtained. Nanoemulsions with Lf concentrations between 2 and 5 % (w/w) presented sizes around 160 nm and a  $\zeta$ -potential higher than +30 mV. For concentrations below 2 % (w/w), nanoemulsions presented sizes around 200 nm and a ζ-potential below +30 mV. It was noticed that higher Lf concentrations lead to smaller sizes and higher ζ-potential values. TEM measurements showed that nanoemulsions particles have a defined spherical shape. Results also showed that nanoemulsions with Lf concentration above 2 % (w/w) present better properties (smaller sizes and higher  $\zeta$ -potential) regarding their storage stability (Figure 1). Nanoemulsions stored at 4 °C did not exhibit significant variations in size and Z-potential Figure 1. Lactoferrin values, while at 25 °C the nanoemulsions suffered a size increase nanoemulsions 2 % and a reduction in  $\zeta$ -potential during storage. The antioxidant encapsulated capacity of the nanoemulsions did not show significative alterations over storage while a significative increase in oxidation was registered.

(w/w) with omega-3

IV. **Conclusions** - The concentration of Lf applied in the formulation of  $\omega$ -3 influenced its physical and morphological properties. The formulations for which a storage stability was assessed revealed that the formulations were physically stable. In terms of chemical stability, it was verified that the antioxidant capacity of the formulations did not suffer alterations during storage but the oxidation increased with the time. This work provides important information for the design of nanoemulsions aiming at the encapsulation of lipophilic compounds for pharmaceutical and food applications.

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