Lactoferrin-based nanoemulsions to improve the physical and chemical stability of omega-3 fatty acids

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Omega-3 (ω-3) polyunsaturated fatty acids are highly susceptible to oxidation, have an intense odour and poor water solubility, which makes its direct application in foods extremely difficult. In order to reduce its oxidation process and improve the stability in an aqueous medium, protein-based nanoemulsions were produced and characterized. Lactoferrin (Lf) was used as an emulsifier at different concentrations (0.2% to 4% w/w). High energy methods (ultra turrax and high-pressure homogenizer) were applied to produce Lf-based nanoemulsions with ω-3 encapsulated. Nanoemulsions were characterized by physical and chemical stability at 4 and 25 °C. Results obtained revealed that Lf concentration influence the nanoemulsion size in which higher Lf concentrations decrease nanoemulsion size. It was also observed that nanoemulsions are physically stable when stored at 4 °C for 69 days while at 25 °C showed instability. The radical scavenging capacity of the nanoemulsions did not show significative changes over storage at 4 and 25 °C while a significative increase in oxidation was registered. Release profile at 37 °C showed that ω-3 had a slow release at pH 2 but was rapidly released at pH 7.4 from Lf nanoemulsions. Moreover, MTT assay revealed that 2% (w/w) Lf nanoemulsions with 12.5 µg/mL of ω-3 were not cytotoxic to Caco-2 cells. Nanoemulsions with high physical and chemical stability were selected and dried by two different methodologies: freeze-drying and nano spray-drying. FTIR-ATR, Raman spectroscopy and Circular Dichroism (CD) showed Lf structural changes after drying processes. This work provides important information regarding nanoemulsions' design and drying technologies aiming the encapsulation of lipophilic compounds for pharmaceutical and food applications.

1. Introduction

Omega-3 (ω-3) PUFA is an essential fatty acid (FA) composed majority of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are recognized for their relevant healthy benefits such as improve cardiovascular health, decrease inflammation, increase the cognitive function and enable a better neurological and visual development. They are also positively associated with prevention and/or treatment of some diseases such as diabetes, Alzheimer, allergies, arthritis, asthma and some types of cancer.[1,2] The Food and Agricultural Organization, World Health Organization and European Food Safety Authority (EFSA) recommended a dietary composed by EPA and DHA higher than 250 mg per day.[3] Taking this value into consideration, the food industry has increased its interest in enriching food with ω-3 PUFAs. However, one of the major problems associated with ω-3 PUFAs addition to food products is their high oxidation which affects food's quality and promotes the negative organoleptic properties associated to rancidity, features that contribute to a decreased acceptability by consumers.[1,4] In order to decrease ω-3 oxidation, encapsulation processes have been highly used. Recently, emulsions, especially oil-in-water (O/W) nanoemulsions, have taken an important role in the food industry mainly for the encapsulation of lipophilic bioactive compounds.[5]

Lactoferrin (Lf) is a glycoprotein from the transferrin family with a molecular weight of 80 kDa, which plays an important role in the defence system due to its antimicrobial, antiviral and antioxidant properties.[6] Previous studies indicate that Lf has excellent emulsifying capacity, forming O/W emulsions with small size and rather stable systems in the presence of divalent ions.[5–7] Lf, used either as an additive in the emulsion or deposited at the O/W interface, has been shown to increase the chemical stability of emulsions.[8] Encapsulation techniques, such as emulsification are usually applied in combination with drying techniques, in order to prevent chemical or biological degradation enabling the improvement of the suspension's stability as well as the extension of shelf-life of food products. There are several drying techniques that can be applied, such as spray drying and freeze drying.[9] Recently, a new generation of laboratory scale spray dryer system was developed to produce nanoparticles by nano spray-dryer, which is equipped with new technologies that enable the production of powders in the nano-size range by using an improved atomization process.[10] However, the impact of this process on the development of novel nanoparticles needs to be addressed.

Moreover, nanotechnology application to generate novel functional compounds also increase the human exposure to nanomaterials that besides their benefits, could also endanger the human health, which is one of the major concerns for using these systems in food and pharmaceutical industry.[5,11,12] Therefore, is extremely important to study possible toxicity problems involved in the oral administration of nanoemulsions. Caco-2 cells (derived from human colon adenocarcinoma) mimic the small intestine epithelium, which makes them highly promising to assess the cytotoxicity and predict the absorption in vitro.[12–15]

The main objective of this work was to develop and evaluate the formation of Lf-based nanoemulsions with ω-3 PUFA, assessing its physical and chemical stability under different production and storage conditions. Also, the effect of nanoemulsions with ω-3 on cell viability was assessed. Finally, nanoemulsions

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