Vitamin E-loaded hydroxyapatite Pickering emulsions as new product design for fortified food applications

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Lipophilic vitamin E is a vital component of human health; however, it degrades quickly and loses bioactivity due to oxidative instability. In this study, vitamin E-loaded Pickering emulsions stabilised by nano-hydroxyapatite (n-HAp) were produced using a static mixer (NETmix), a technique enabling continuous production and droplet size tailoring. Thus, oil-in-water (O/W) emulsions containing vitamin E at a content of 1mg/mL were produced with different droplet sizes using an O/W ratio of 20/80 (*v/v*). Their stability during *in vitro* gastrointestinal digestion and vitamin E bioaccessibility was studied. It was observed that n-HAp particles break in the stomach and subsequently aggregate as random calcium phosphates in the small intestine, leading to low vitamin E bioaccessibility due to oil entrapment. The emulsion showing the highest vitamin E bioaccessibility (3.29±0.57%, sample with the larger average droplet size) was used to produce fortified gelatine and milk, resulting in an increased bioaccessibility (10.87±1.04% and 18.07±2.90%, respectively), associated to the presence of macronutrients. Overall, n-HAp Pickering emulsions offer advantages for vitamin E encapsulation directed to fortified foods development, a process that can be extended to other lipophilic vitamins.

Introduction

Emulsions are widely used in various fields (food, pharmaceuticals, and agrochemical), playing an important role in traditional and novel emulsion-based products [1]. Emulsions are bi-phasic systems where a third component is needed to stabilise the two immiscible phases. In Pickering emulsions, the third component, acting as the stabiliser, corresponds to solid particles. These emulsions provide high physical and chemical stability due to their irreversible solid particles' adsorption at the oil-water interface [2]. Thus, Pickering emulsion stabilisation arose as an alternative to traditional emulsifiers, contributing to the development of clean-label products [3] and promising encapsulation systems for bioactive compounds such as vitamins [4], receiving high interest, both from academic and industrial perspectives.

Vitamin E is essential in human health, avoiding cellular ageing and reducing diseases such as dementia, cancer, and cardiovascular disorders [5]. However, vitamin E has a high lipophilic character needing to be encapsulated to increase the stability and compatibility with hydrophilic food matrices. Hydroxyapatite (HAp) has been described as a suitable Pickering stabiliser, stabilising O/W emulsions due to its hydrophilicity [6]. Thus, HAp is appropriate for developing Pickering emulsions systems to protect and deliver lipophilic compounds and further develop functional foods.

Pickering emulsions are usually produced through batch processes, which makes it difficult for large-scale production (industrial implementation). Aiming at achieving a more feasible industrial process, in this work, the NETmix technology was tested to produce vitamin E-loaded Pickering emulsions in continuous mode with high reproducibility. NETmix is a structured mixer and reactor consisting of a network of static mixing chambers interconnected by transport channels (Figure 1A).

Following reported results pointing out Pickering emulsions as promising systems to improve vitamins bioaccessibility, n-HAp Pickering emulsions were firstly tested as vitamin E carriers, then used to produce fortified milk and gelatine, and their behaviour in the gastrointestinal tract (GIT), and vitamin bioaccessibility evaluated.

Materials and methods

To produce the vitamin E-loaded Pickering emulsions, sunflower oil and vitamin E were used as the oil phase, and water containing dispersed n-HAp particles as the aqueous phase. Firstly, the two phases were fed into NETmix in the pre-mixed mode (Figure 1A), with the total flow rate adjusted according to the desired Reynolds number. Next, to reduce the droplet size, 17 cycles were performed.

Results

Figure 1B shows the optical microscope image of the obtained vitamin E-loaded Pickering emulsion, where it is possible to observe an emulsion with a spherical shape and reduced-size droplets. The Cryo-SEM image shows a Pickering droplet evidencing the n-HAp layer around the oil core, and EDS analysis (data not shown) confirmed the presence of this material at the oil surface. The results are promising and show the feasibility of using the NETmix technology to produce vitamin E-loaded Pickering emulsions. This fact is an advantage from an energy point of view (NETmix is a low-energy device) and allows continuous production that supports a high-volume scale compatible with industrial production, facilitating the development of end-user applications, namely food applications loaded with lipophilic vitamins.

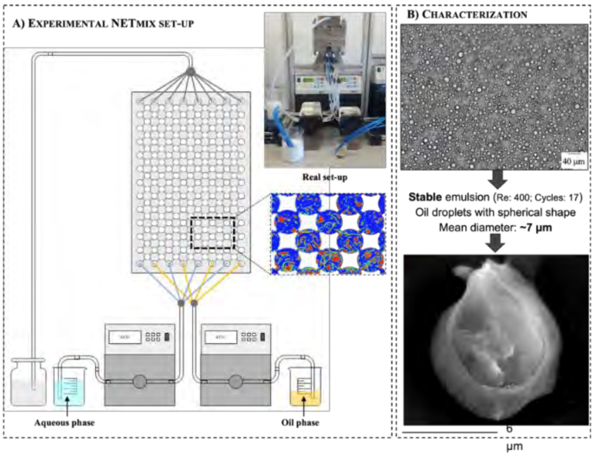

Figure 1. A – Experimental NETmix set-up and B – Optical and cryo-SEM Pickering emulsion characterisation.

The vitamin E-loaded Pickering emulsions were digested in vitro through a simulated GIT: mouth, gastric, and small intestine. Regarding digestion, the tested vitamin E-loaded Pickering emulsions presented similar behaviour along GIT, with n-HAp particles being dissolved in gastric conditions with subsequent formation of aggregates under the intestinal environment. When the vitamin E-loaded PE NET-high was incorporated in food matrices (gelatine and milk, Figure 2 B), vitamin E bioaccessibility increased significantly (10.87 ± 1.04% for gelatine and 18.07 ± 2.90% for milk), putting in evidence the positive effect of the food matrix in the bioaccessibility (Figure 2 A).

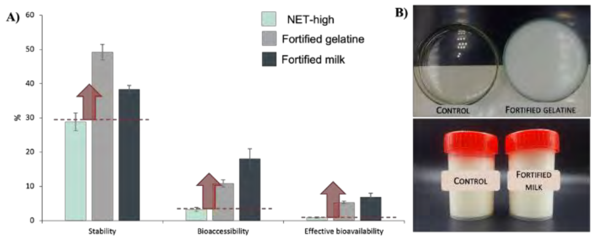

Figure 2. A – Stability, bioaccessibility, and effective bioavailability of vitamin E loaded Pickering emulsions after in vitro digestion; and B – Photographic register of control samples and fortified foods.

Conclusions

Overall, n-HAp Pickering emulsions offer advantages for vitamin E encapsulation directed to fortified foods development, a process that can be extended to other lipophilic vitamins and other Pickering stabilisers. The obtained results also pointed out the interest in proceeding with further studies to understand the effect of the food matrix composition on the achieved bioaccessibility. Moreover, it also highlights the importance of combining the study of PEs with their final applications to evaluate more accurately the real potential of these innovative solutions. More information on the developed work can be consulted in Ribeiro et al. [4].

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