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Improvement of physicochemical properties of encapsulated echium oil using nanostructured lipid carriers

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ABSTRACT

Implementing ω-3 polyunsaturated fatty acids (ω-3 PUFA), naturally found in echium oil (EO), can highly improve the nutritional value of fortified foods. However, PUFA is prone to oxidation. In this study, the role of nanostructured lipid carriers incorporated into whey protein isolate (WPI)-stabilized EO droplets in oil-in-water emulsions was analyzed. Lipid carriers such as lauric (LA), palmitic (PA), and stearic (SA) acids were used. The results reveal that lipid carriers, especially LA, improve the physical stability of these droplets by decreasing their particle size by decreasing the number of surface pores; shown by SEM images and XRD data. Rheological data further show that the emulsions incorporated with LA had higher viscosity and there was also a crossover shift to lower strains in the G′-G″ curve of the emulsions incorporating LA. TBARS assay indicated that LA was more effective in protecting EO against oxidation than both palmitic and stearic acids.

1. Introduction

Long-chain ω-3 polyunsaturated fatty acids (PUFAs) are well-known for their health benefits in the body. It has been shown that PUFAs lower the risk of different types of cancer and cardiovascular diseases, improve brain functions, and alleviate inflammatory diseases (Bakhshabadi et al., 2017; Ghorbanzade, Jafari, Akhavan, & Hadavi, 2017; Karim et al., 2017). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two significant PUFAs which are found in food sources such as marine or vegetable oils (Liao, Luo, Zhao, & Wang, 2012). Fish and krill oils are marine-based sources of EPA and DHA, while echium and flaxseed oils are two main vegetable-based oils (Binsi et al., 2017b; Comunian et al., 2016, 2017). Echium oil contains 33% α-linolenic acid and 9–16% stearidonic acid, an intermediate in the biosynthesis of EPA and DHA (Comunian et al., 2017). However, echium oil has low oxidation stability, which accelerates rancidity and results in undesirable primary and secondary oxidation products.

Different forms of colloidal delivery systems, have been proposed to protect ω-3 PUFAs and guarantee their safe delivery into the body (Ravanfar, Comunian, Dando, & Abbaspourrad, 2018). Colloidal systems are categorized into different groups, such as liposomes, multiple emulsions, solid lipid nanoparticles, nanocrystal suspensions, and multilayer emulsions (Choi et al., 2016; McClements, 2012). Emulsions are colloidal systems that are widely applied as a template in encapsulating food ingredients, preventing oxidation, enhancing controlled release for *in vivo* digestion, and improving storage stability (Liao et al., 2012). Oil-in-water (O/W) emulsion delivery systems have been utilized to prevent oxidation and improve the shelf life of PUFA-containing oils. These delivery systems improve resistance against adverse environmental conditions or harsh human gastrointestinal tract conditions such as enzymatic activities and acidic conditions, as well (Faraji, McClements, & Decker, 2004; Komaiko, Sastrosubroto, & McClements, 2016; Wang, Liu, Chen, & Selomulya, 2016). In addition to these emulsion delivery systems, some other compounds can be co-encapsulated into the emulsion droplets to physicochemically postpone oxidation, compounds such as antioxidants or lipid carriers (Comunian, Abbaspourrad, Favaro-Trindade, & Weitz, 2014; Comunian et al., 2016; Faraji et al., 2004; Helgason et al., 2009; Katouzian, Faridi Esfanjani, Jafari, & Akhavan, 2017; McClements, 2012; Weiss et al., 2008). These lipid carriers can partially or fully solidify O/W emulsion encapsulates (Berton-Carabin, Coupland, & Elias, 2013). Researchers have studied the effects of different lipid carriers on food ingredients and drugs in emulsion particles and in which ways they can improve the shelf life of lipophilic food and drug compounds (Berton-Carabin et al., 2013; Elias et al., 2006; McClements, 2012; Weiss et al., 2008). However, to the best of our knowledge, no research has been done to investigate the effects of different lipid carriers of different chain lengths on echium oil encapsulation.

The purpose of this study was to investigate how different lipid carriers delay the oxidation of echium oil and enhance its physicochemical properties. Lipid carriers such as LA, PA, and SA were employed to make partial or fully solidified colloidal particles entrapping

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echium oil. The physicochemical properties of these emulsions were analyzed by z-sizer, morphological assays (scanning electron microscopy (SEM) and transmission electron microscopy (TEM)), X-ray diffraction (XRD), thermogravimetric analysis (TGA), rheometry, and thiobarbituric acid reactive substances (TBARS) assay.

2. Materials and methods

Echium oil was provided by NEWmega™ Echium Oil (Ref. 15,200, De Wit Specialty Oils, De Waal, Tescel, The Netherlands). Lauric acid, palmitic acid, stearic acid, and petroleum ether were purchased from Sigma-Aldrich (St. Louis, MO). Food grade WPI was obtained from Davisco Foods International Inc. (Le Sueur, MN, US). Hexane was purchased from VWR International (Houston, TX). All other chemicals used were of analytical grade. Milli-Q water was used to prepare all solutions and emulsions.

2.1. Preparation of echium oil encapsulated in solid lipid nanoparticle

The oil phase contained echium oil and different types of lipid carriers including LA, PA, and SA. The oil phase was prepared by dissolving 25 wt% of lipid carriers into 75 wt% echium oil. To dissolve the lipid carriers, the echium oil was heated and stirred at 75 °C for 5 min to ensure a homogeneous mixture obtained. The water phase was prepared by dissolving 2 wt% WPI in water at room temperature (20 ± 2 °C) and then preheated at 75 °C for 2 min. The pre-heating step avoids solid lipid pre-crystallization which can interrupt the emulsion preparation process before the formation of any particles. The oil phase was then added to the water phase at a 1:9 (w/w) ratio, and homogenized by a high-speed homogenizer at 15,000 rpm for 3 min. After preparing the emulsion, it was allocated into two falcon tubes. One of the tubes was instantly stored in an ice bath (fastcooling process) and the other one was cooled down at room temperature (20 ± 2 °C) (slowcooling process). The samples were labelled as WPI/EQO/LA, WPI/EQO/PA, and WPI/EQO/SA, corresponding to the different lipid carriers used in the preparation of the emulsions. In addition, a control was prepared without any lipid carriers and labelled as WPI/EQO.

2.2. Characterization

2.2.1. Encapsulation efficiency

Encapsulation efficiency shows the percentage of initial echium oil encapsulated in the core of the emulsion droplets. For the investigation of echium oil encapsulation efficiency (EE), two volumes of hexane were added to one volume of emulsion. Then, the free oil in the continuous phase was washed out. Using centrifugation at 5000 rpm, the water and oil phases (hexane + extracted free oil) were separated. Then, a filter paper (No. 4, Whatman, Maidstone, UK) was used to separate the solid phase (powder) from the liquid phase (solvent and extracted oil). To ensure that all surface oil was fully washed, the aforementioned process was repeated using 40 ml of petroleum ether and the separated oil phase was added to the previously extracted oil (Jafari, Assadpoor, Bhandari, & He, 2008). The amount of initial and extracted oil allows to EE to be calculate using the following equation:

$$\textit{Echium oil encapsulation efficiency } \%(EE\%) = \frac{\textit{Total echium oil} - \textit{Surface echium oil}}{\textit{Total echium oil}} \times 100 \quad (1)$$

2.2.2. Particle sizes and z-potential measurements

The average size, size distribution, and z-potential of the particles were measured by dynamic light scattering technique (Nano ZS90, Malvern Instruments, Worcester, UK) at 25 °C. The analysis of the samples was done 24 h after the preparation of each sample. The emulsion samples were diluted 100-fold and three independent measurements were carried out for each prepared sample. Then, the averages of the measurements were used to determine the final particle sizes.

2.2.3. Scanning electron microscopy (SEM)

The microstructural properties of the prepared emulsion and spray-dried powders were evaluated by SEM. Samples were mounted on a small aluminum platform with double-sided carbon tape and then sputter-coated with gold under argon for 30 s. Micrographs were acquired with a JCM-6000 NeoScope (JEOL, Japan) set at 15 kV.

2.2.4. Transmission electron microscopy (TEM) of powders

To study the emulsion micro- and nano-structure, the emulsion samples were observed by TEM. TEM was performed using Hitachi FEI T12 Spirit TEM STEM (TEM, Hitachi, Tokyo, Japan) at 120 kV field emission voltage. To prepare the samples, the original emulsion samples were diluted 100-fold with double distilled water and then one small (4 µl) droplet was taken and placed on a copper grid and lyophilized.

2.2.5. X-ray diffraction

Bruker D8 Advance ECO powder diffractometer (MA, USA) provided by the Cornell Center of Materials Science (CCMR) was used to analyze the X-ray diffraction (XRD) of the powder prepared by spray-drying the emulsions. The XRD analysis was carried out by running the powder samples from 2 to 45° under a continuous scan at a step size of 0.026 with 2θ $\min^{-1}$.

2.2.6. Rheology of emulsions

A cone and plate geometry (40 mm diameter stainless steel cone-and-plate geometry and 5 µm gap) was used to measure the rheological properties of the prepared emulsions (using AR1000-N rheometer; TA Instrument, UK). Duplicate measurements were performed for each emulsion.

2.2.7. Thermogravimetric analysis (TGA)

The thermal behavior of the prepared samples was analyzed using a Q500 Thermogravimetric Analyzer (TA Instruments/New Castle, DE). The samples were put on a platinum support under a nitrogen atmosphere at a flow rate of 60 ml/min. The furnace was heated from 25 °C to 600 °C at a rate of 10 °C/min.

2.2.8. Oxidative properties of microcapsules

The thiobarbituric acid reactive substances assay (TBARS) was used to evaluate the formation of secondary products resulting from the echium oil oxidation process. The oxidation of the prepared emulsion was measured using a protocol from McDonald and Hultin that was slightly modified (McDonald & Hultin, 1987). To investigate the oxidation of a prepared emulsion, 500 µl of the emulsion was mixed with 3.0 ml thiobarbituric acid (TBA) reagent which included 15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid, and 0.25 M HCl in a glass test tube. The sample was then placed in a boiling water bath for 15 min followed by the tube being cooled down in an ice bath for 10 min and centrifuged at 1000*g* for 15 min. After centrifugation, 3 ml of butanol was added and the tube was stirred and vortexed again. After 10 min the absorbance was measured at 532 nm using UV–Vis spectrometer. Concentrations of TBARS were determined using a standard curve prepared by using 1133-tetraethoxypropane.

2.2.9. Interfacial tension measurement

The interfacial tension between 2% (w/w) WPI water phase solution and different oil phases containing different lipid carriers such as 2.5% (w/w) LA, PA, and SA oil phase mixture was measured using the pendant drop method and a standard Ramé-Hart Contact Angle Goniometer (model 190 CA, Ramé-Hart Instrument Co., Netcong, NJ, USA). The measurement of the interfacial tension was carried out by filling a