



Enhancing oxidative stability of encapsulated fish oil by incorporation of ferulic acid into electrospun zein mat



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ABSTRACT

A composite zein nanofibrous mat containing fish oil and ferulic acid was successfully fabricated by electrospinning. The process became more fluent and continuous by adding 30 g/L glycerol into the polymer solutions and using modified coaxial electrospinning. The average diameter of nanofibers was 440 nm. The loading capacity and encapsulation efficiency of fish oil were 20% and 94%, respectively. FTIR data demonstrated that fish oil and ferulic acid were successfully embedded into the nanofibers and there were interactions among the molecules of zein, fish oil and ferulic acid. Addition of ferulic acid into the nanofibers significantly improved the oxidative stability of encapsulated fish oil; moreover, it did not change the release behavior of fish oil. The release of encapsulated fish oil was controlled by a combination of diffusion and macromolecular chain relaxation. This composite nanofibrous mat with favorable oxidation stability and release property is potential in application as nutrition additive.

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1. Introduction

Omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA; 20:6), have shown to be beneficial to human health, but the high degree of unsaturated carbon-carbon double bonds render them sensitive to light, heat and oxygen, resulting in the poor oxidation stability which limits their application as nutrition additive (Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010). Fish oil is one of the major sources of PUFAs, thus it is essential to protect it and improve its oxidation stability. For this purpose, encapsulation techniques have been considered for protection of fish oil, such as spray drying, liposome entrapment, coacervation, bubbles, porous inorganic capsules and layered nanocapsules, etc (Azmin, Harfield, Ahmad, Edirisinghe, & Stride, 2012; Mehta et al., 2017; Nangrejo et al., 2009). These methods typically produce micro and nano particles, which may limit the application and development of fish oil-loaded products.

Electrospinning is a simple and highly versatile method that produces nanometer sized fibers, which have many structural and functional advantages (Wen et al., 2016b, 2016a). Nowadays,

electrospinning is a promising method for encapsulation of bioactive compounds. The coaxial electrospinning, as a developed electrospinning technique, has emerged as an alternative to encapsulate bioactive compounds. Other volatile and sensitive materials have been encapsulated by co-axial system (Yao, Chang, Ahmad, & Li, 2016; Yao et al., 2017), however, in this study, a modified coaxial electrospinning technology was used to produce a single nanofibrous mat for encapsulation of fish oil.

Zein is a protein from corn, which provides many advantages such as biocompatible and biodegradable, thus is considered as a potential candidate for encapsulation of functional ingredients. Generally, the most common approaches for embedding fish oil with zein were evaporation-induced self-assembly and liquid-liquid dispersion. However, the fish oil product encapsulated by these methods is powder-like, and the oxidative stability of which needs to be improved.

Ferulic acid (FA) is a polyphenolic compound that exhibits a wide range of therapeutic effects which is attributed to its anti-inflammatory, antimicrobial, and anticancer properties (Panwar, Sharma, Kaloti, Dutt, & Pruthi, 2016). It was reported that ferulic acid can enhance the oxidative stability of fish oil by adding it as an antioxidant into the fish oil enriched milk (Sørensen, Lyneborg, Villeneuve, & Jacobsen, 2015). To date, there are only a few reports referring to the encapsulation of fish oil in zein fibers and beads via electrospinning (Moomand & Lim, 2014a, 2014b, 2015). It

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was found that the electrospun zein fibers provided a greater oxidative stability of encapsulated fish oil in comparison to non-encapsulated fish oil. To further improve the oxidative stability of fish oil, we previously encapsulated fish oil in the coaxial electrospun nanofibrous mat (Yang et al., 2017). The oxidative stability of encapsulated fish oil in the coaxial nanofibers was obviously enhanced compared to that in single nanofibers. However, the release behavior of encapsulated fish oil changed since less amount of fish oil released from the coaxial electrospun nanofibrous mat. Therefore, in order to enhance the oxidative stability of encapsulated fish oil while not to change its release property, ferulic acid was added into the electrospinning solution containing zein and fish oil to fabricate composite single nanofibrous mat in this study. The addition of ferulic acid into the nanofibrous mat can not only improve the oxidative stability of encapsulated fish oil, but also enhance the nutritional value of the composite nanofibrous mat due to the synergic effect of fish oil and ferulic acid.

2. Materials and methods

2.1. Materials

Zein from corn (grade Z0001) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Fish oil was a kind gift from Sinomega Biotech Engineering Co., Ltd (Zhejiang, China). Pepsin, trypsin and ferulic acid were supplied by Aladdin Chemistry Co., Ltd (Shanghai, China). Anhydrous ethanol, glycerol and hexane are analytical grade (99.0%). Potassium bromide (KBr) is spectrum pure grade (99.5%), and xylenol orange sodium salt is indicator grade. All the above reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Guangzhou, China).

2.2. Electrospinning

2.2.1. Preparation of electrospinning solutions

Ferulic acid solution was prepared by dissolving 3 g ferulic acid in 100 mL aqueous ethanol (ethanol:water 800:200 mL/mL) under constant magnetic stirring (RT5, IKA Group, Staufen, German) for 10 min. Then, 25 g zein was dissolved in ferulic acid solution by constant stirring for 0.5 h. After that, 7.5 g fish oil was added and mixed for 1 h. To investigate the effect of glycerol, 3 g glycerol was additionally added to the above-mentioned solution and mixed for 10 min. Before loading into syringes, all solutions were kept for half an hour to make sure no air bubble in the liquid phase was observed.

2.2.2. Electrospinning process

For single electrospinning, the prepared solution was loaded in a 10 mL syringe equipped with a 22 gauge steel needle (outer diameter of 0.71 mm and inner diameter of 0.41 mm). A syringe pump (NE-300, New Era Pump Systems Inc., Farmingdale, New York, USA) was used and the feeding rate was fixed at 0.6 mL/h. The applied voltage was 16 kV, and the distance from the needle tip to the collector was 14 cm.

For modified coaxial electrospinning, the polymer solution used as core solution was loaded in a 10 mL syringe. Anhydrous ethanol used as shell solution was loaded in a 10 mL syringe fitted with an 18 gauge steel needle (outer diameter of 1.27 mm and inner diameter of 0.84 mm) (Fig. 1). The electrospinning conditions were: shell flow rate 0.2 mL/h, core flow rate 0.6 mL/h, applied voltage 16 kV, and the distance 14 cm.

All the experiments were carried out at $24 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ under $45\% \pm 2\%$ relative humidity.

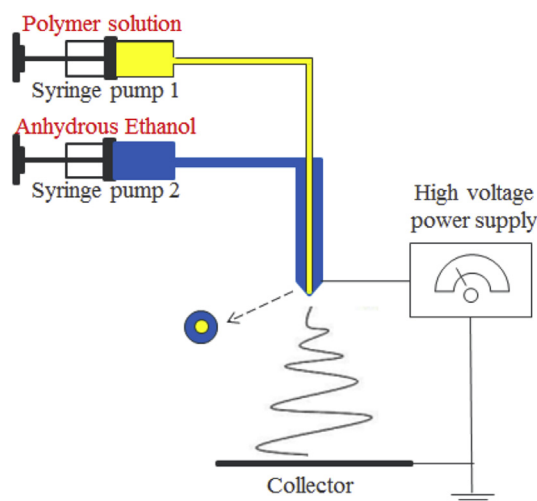


Fig. 1. Schematic diagram of the modified coaxial electrospinning setup.

2.3. Characterization and measurement

The mats obtained were left at ambient temperature ($24 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$) overnight before being investigated for their morphology using a scanning electron microscope (SEM) (EVO18, Carl Zeiss, Oberkochen, Germany). Prior to examination, the samples were coated with Pt for 40 s using a sputter coater (K550, Emitech Co., London, UK) under vacuum to render them electrically conductive. Images were recorded at an accelerating voltage of 10 kV. The fiber diameter distribution was calculated by analysis of around 100 fibers from the SEM image.

Infrared spectra of samples were recorded using a Bruker Model Equinox 55 Fourier transform infrared (FTIR) spectrometer (Bruker Co., Karlsruhe, Germany) in a range of $4000\text{--}500 \text{ cm}^{-1}$. For electrospun nanofibrous mat, Attenuated total reflectance (ATR) was used for FTIR measurement. While for fish oil, ferulic acid and zein, KBr disks were adopted for FTIR measurement. Each measurement was an average of 16 scans at 4 cm^{-1} resolution.

2.4. Loading capacity and encapsulation efficiency

Loading capacity (LC) and encapsulation efficiency (EE) were determined by measuring the non-entrapped fish oil or ferulic acid according to Moomand and Lim (2014a) with some modifications. Mat (90 mg) was submerged in hexane or water (8 mL) for 1 min to remove the unencapsulated fish oil or ferulic acid, respectively, from the surface. The mixture was filtered, and the absorbance of the fish oil and ferulic acid was then determined using a UV-vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) at 260 nm and 320 nm, respectively. The EE and LC values were calculated as Eq. (1) and Eq. (2):

$$LC = (A - B)/C * 100 \quad (1)$$

$$EE = (A - B)/A * 100 \quad (2)$$

Where A was the total theoretical mass of fish oil or ferulic acid, B was the free mass of fish oil or ferulic acid in the collection solution, and C was the mass of the mat.

2.5. Oxidative stability analysis

The oxidative stability of the encapsulated fish oil was tested at 25, 45 and 60 $^\circ\text{C}$ under aerobic and anaerobic conditions. The

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