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Development of cress seed mucilage/PVA nanofibers as a novel carrier for vitamin A delivery

Arezoo Fahami, Milad Fathi*

Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, 84156-83111, Iran

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ABSTRACT

Nowadays carbohydrate nanofibers have distinct advantages in delivery of bioactive components because of their biocompatibility and large specific surface area. In this work encapsulation of vitamin A in different concentrations with cress seed mucilage/poly vinyl alcohol (CSM/PVA) solutions was investigated. The optimum nanofibers had diameters in the range of 90.25–169.95 nm. FTIR analysis of vitamin-loaded nanofibers indicated characteristic peaks of CSM, PVA and vitamin A. The amorphous structure of the nanofibers changed to crystalline form after encapsulation of vitamin A. Thermal stability of vitamin A increased by nanoencapsulation. Release behavior was studied in simulated gastric and intestinal fluids and the release kinetic was evaluated by different mathematical models (i.e. zero order, first order, Higuchi, Ritger and Peppas, Peppas and Sahlin, Hixon-Crowell and Kopcha). The coefficients of Ritger-Peppas, Peppas-Sahlin and Kopcha models were determined which confirmed that diffusion transport was the main mechanism for release of the vitamin A through the nanofibers. The lower release rate of vitamin A in simulated gastric fluids indicated that produced nanofibers were appropriate carriers for increasing stability of food bioactives against acidic conditions.

1. Introduction

Nowadays, there is a growing interest for enhancing the quality of foods. Enrichment of foods by adding desirable nutrients or bioactive products such as minerals, plant extracts (essential oils) or vitamins is favorable to development of functional foods. Vitamin A is a fat-soluble bioactive which includes retinol, retinal, retinoic acid, and several provitamin A carotenoids, plays a vital biological role in human body such as vision health, embryonic development, cellular growth and differentiation, participating in the collagen synthesis process, maintenance of epithelial cellular integrity and immune function (Carlotti, Rossatto, Gallarate, Trotta, & Debernardi, 2004; Gonçalves, Estevinho, & Rocha, 2017). Recent studies showed that vitamin A could prevent different diseases, such as Alzheimer's disease, plurimetabolic syndrome and obesity (Mingaud et al., 2008; Sauvant, Cansell, Sassi, & Atgié, 2012). It exhibits poor solubility in aqueous solutions and has unfavorable effects on the flavor, odor and transparency of beverages. Furthermore, vitamin A is unstable in the presence of lights, oxidants, metals and free-radical (Arayachukeat, Wanichwecharungruang, & Tree-Udom, 2011; Sauvant et al., 2012).

Encapsulation technique increases vitamin A solubility in aqueous media by entrapment into a hydrophilic structure, and slows down the degradation processes until the product is delivered to the sites where absorption is desired (Sauvant et al., 2012). One way to protect sensitive food bioactives against environmental conditions is incorporating through nanofiber by electrospinning process.

Electrospinning as a new nanoencapsulation method uses electrostatic forces to generate polymer fibers. This relatively simple technique produces fibers with diameter ranging from ten to hundreds nanometers (Rezaei, Tavanai, & Nasirpour, 2016). The large specific surface, high porosity, high loading capacity, high encapsulation efficiency, simultaneous delivery of different compounds and easy to operate make nanofiber a good candidate as an entrapment system (Fathi, 2015). Furthermore, since electrospinning is generally operating under moderate temperatures, entrapment of thermally unstable additives such as vitamins and antioxidants in fiber matrix becomes convenient (Mascheroni et al., 2013). For food applications, these carriers are generally achieved by carbohydrates and/or proteins due to their food grade natures, biodegradability, non-toxicity and cost effectiveness.

* Corresponding author.
E-mail address: mfathi@cc.iut.ac.ir (M. Fathi).

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Cress (*Lepidium sativum*) as a member of *Brassicaceae* family usually grows in Iran, India, North America and some parts of Europe. Cress seeds as a good source of hydrocolloids, contain large amount of mucilaginous substances with molecular weight of about 540 kDa and anionic nature, which make them a good candidate for electrospinning (Naji & Razavi, 2014). The cress seed mucilage possesses a high mannose to galactose ratio (8.2) (Taheri & Razavi, 2015). However, due to different chain conformations and repulsive forces exist among the poly anions in the solution, application of mucilage for electrospinning is limited (Carla Santos et al., 2014). Therefore, blending these bio-polyelectrolytes with a non-toxic, water soluble, synthetic polymers such as poly vinyl alcohol (PVA) or polyethylene oxide (PEO), reduces repulsive forces within the charged biopolymer solutions and allows spinning of the fibers (Bonino et al., 2011).

The aims of this work were encapsulation of vitamin A using cress seed mucilage nanofibers and characterization of physico-chemical properties of produced nanocarriers.

2. Materials and method

2.1. Materials

Cress seed was purchased from local market in Isfahan, Iran. Vitamin A palmitate was obtained from Amin Pharmaceutical Company (ATA Pharma Sarl, Luxembourg). Other used reagents were, fully hydrolyzed PVA with molecular mass of 145 kDa (Merck, Germany), hydrochloric acid (37%, Merck, Germany), potassium chlorite (Merck, Germany), sodium hydroxide (Merck, Germany), potassium dihydrogen phosphate (Merck, Germany), extra pure n-hexane (Merck, Germany) and tween 80 (Merck, Germany).

2.2. Cress seed mucilage extraction

Extraction of mucilage was performed based on Taheri and Razavi (2015) method. The optimal conditions were, pH of 10, temperature of 35 °C and water to seed ratio (v/w) of 30:1. In order to segregate the slurry from cress seeds, the mixture was passed through a blender after 30 min. To eliminate any kind of impurity from the slurry, the solution was passed through a cheese paper. Finally, the mucilage was dried by freeze dryer and kept in a dry and cool place before future applications. Cress seed mucilage contained moisture (7.17%), ash (11.5%) (including calcium (0.17%), potassium (0.062%), sodium (0.032%), magnesium (0.0076%)), protein (2.45%), fat (1.85%) and sugar (77.03%) (mostly consists mannose (38.9%), arabinose (19.4%), galacturonic acid (8%), fructose (6.8%), glucuronic acid (6.7%), galactose (4.7%), rhamnose (1.9%), and glucose (1.0%)) (Karazhiyan et al., 2009).

2.3. Solution preparation

To prepare cress seed mucilage solution (CSM, 1% W/V), specific amount of the mucilage (0.05 g) was homogenized in water/acetone solution (70:30) (5 mL) and hydrated overnight in a refrigerator. PVA solution (5% w/v) was obtained by dissolving 0.25 g PVA in 5 mL deionized water at 70 °C, stirring for 2 h at an ambient temperature and finally was kept in refrigerator overnight. According to previous results, cress seed mucilage and PVA with the ratio of 60:40 were mixed together to produce CSM/PVA solution, which was recognized as the optimal ratio for electrospinning due to their lower diameter with uniform shapes of fibers and lower amount of PVA (Fahami & Fathi, 2017). For the next step, different concentrations of vitamin A (10, 20 and 30% w:w) with constant amount of tween 80 (1% w/v) were added to CSM/PVA solution. Electrical conductivities of CSM/PVA and CSM/PVA/vitamin A solutions were characterized by a digital conductivity meter (model 3540, Jenwey) at room temperature.

2.4. Electrospinning procedure

Electrospinning was operated at voltage of 18 kV and flow rate of 0.2 mL/h with a constant distance of 17 cm between needle (needle inner diameter of 0.6 mm) and collector at 25 °C.

2.5. Characterization of vitamin A loaded nanofibers

2.5.1. Encapsulation efficiency and loading capacity

In order to calculate encapsulation efficiency (EE) and loading capacity (LC) of the fibers with different amounts of vitamin A, nanocarriers (10 mg) were submerged in 30 mL hexane and were shaken for 15 min. The mixture was passed through a syringe microfilter (cut off pore size of 0.45 μm). The absorbance of the liquid was evaluated using an UV-visible spectrophotometer at wavelength of at 360 nm (Amdidouche, Darrouzet, Duchene, and Poelman (1989)). The encapsulation efficiency and loading capacity of the vitamin A were determined by the following expressions:

$$EE\% = \frac{W_T - W_F}{W_T} \times 100 \quad (1)$$

$$LC\% = \frac{W_T - W_F}{W_L} \times 100 \quad (2)$$

where, W_T represents the total amount of vitamin A, W_F shows the free amount of vitamin A in the solution and W_L indicates the total weight of fibers. The measurements were performed in triplicate. The sample with highest amount of encapsulation efficiency and loading capacity was selected for further analysis.

2.5.2. Scanning electron microscopy (SEM)

The morphology and the mean diameter of the fibers, before and after encapsulation, were investigated using scanning electron microscopy (Philips XL30). Before imaging, the samples were sputtered with a layer of gold. The mean diameter of more than 50 fibers was measured from different SEM images by image analysis (ImageJ, National Institutes of Health).

2.5.3. Fourier transform infrared spectroscopy (FTIR)

To evaluate chemical interactions of functional groups, FTIR spectra of CSM powder, PVA, vitamin A, CSM/PVA nanofiber and vitamin A loaded nanofiber were obtained by Fourier transform spectrophotometer (Jasco FT/IR-680 plus) with a resolution of 4 cm^{-1} and a frequency range of 400–4000 cm^{-1}.

2.5.4. X-ray diffraction (XRD)

To analyze crystallinity of CSM powder, PVA powder, CSM/PVA nanofiber and vitamin A loaded nanofiber, X-ray diffraction measurement was performed by a Philips X'Pert diffractometer using CuK α radiation ($\lambda = 1.5406$ Å). The samples were scanned within a 2θ range of 10 to 100° and a scan rate of 0.5° min^{-1} by a power of 40 kV with filament current of 30 mA. Crystallinity index of the samples were calculated by the following expression:

$$\text{Crystalline index} = \frac{\text{crystalline area}}{\text{crystaliine area} + \text{amorphous arrea}} \times 100 \quad (3)$$