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Niosome-loaded cold-set whey protein hydrogels

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ABSTRACT

The α-tocopherol-carrying niosomes with mean diameter of 5.7 μm were fabricated and charged into a transglutaminase-cross-linked whey protein solution that was subsequently gelled with glucono delta-lactone. Encapsulation efficiency of α-tocopherol within niosomes was ≈80% and encapsulation did not influence the radical scavenging activity of α-tocopherol. Fourier transform infrared (FTIR) spectroscopy suggested formation of ε-(γ-glutamyl) lysine cross-linkages by transglutaminase and that enzymatic cross-linking increased proteins hydrophobicity. FTIR also proposed hydrogen bonding between niosomes and proteins. Dynamic rheometry indicated that transglutaminase cross-linking and niosomes charging of the protein solution enhanced the gelation process. However, charging the cross-linked protein solution with niosomal suspension resulted in lower elastic modulus (G') of the subsequently formed gel compared with both non-cross-linked niosome-loaded and cross-linked niosome-free counterparts. Electron microscopy indicated a discontinuous network for the niosome-loaded cross-linked sample. Niosome loading into the protein gel matrix increased its swelling extent in the enzyme-free simulated gastric fluid.

1. Introduction

Most nutraceuticals are highly susceptible to degradation by food processing operations, adverse environmental conditions, and/or gastrointestinal digestion. Thus, encapsulation within appropriately designed and fabricated delivery systems is required to protect them from destructive circumstances (McClements, Decker, Park, & Weiss, 2009). Alpha-tocopherol, an oil-soluble nutraceutical, is the most abundant and biologically active form of vitamin E which acts as antioxidant and might prevent cardiovascular diseases, atherosclerosis and cancer (Liang, Line, Remondetto, & Subirade, 2010). The utilization of α-tocopherol has been hampered due to its light, heat and oxygen sensitivity and lipophilic nature. Therefore, various delivery systems such as emulsions (Yang, Decker, Xiao, & McClements, 2015), solid-lipid nanoparticles (Trombino et al., 2009), and protein and polysaccharide matrices (Duclairoir, Orecchioni, Depraetere, & Nakache, 2002; Pierucci, Andrade, Farina, Pedrosa, & Rocha-Leão, 2007; Somchue, Sermsri, Shiowatana, & Siripinyanond, 2009; Song, Lee, & Lee, 2009) have been employed to encapsulate α-tocopherol.

Surfactants self-assemble spontaneously into vesicular structures which can be used as model for cell membranes and, furthermore, as vehicles for drugs and bioactive materials (Liu & Guo, 2005). Non-ionic surfactant vesicles, the so-called niosomes, have found great applicability in pharmaceutical, nutraceutical and cosmetic sectors since the late 70's when they were patented by the company L'Oreal (Handjani-Vila, Ribier, Rondot, & Vanlerberghie, 1979). Niosomes are capable of encapsulating both hydrophilic and lipophilic materials and exhibit some advantages with respect to liposomes including less production costs and higher chemical stability. Niosomes formed from sorbitan monoesters are amongst the most widely investigated vesicles (Hao & Li, 2011; Yoshioka, Sternberg, & Florence, 1994). Despite being a superb vehicle for delivery purposes, niosomes might suffer from several drawbacks including aggregation, fusion, and drug leakage (Moghassemi & Hadjizadeh, 2014). Complementary mechanisms are, therefore, required to modulate the characteristics of drug-conveying niosomes. Proniosomes were presented as powdery alternatives to circumvent the problems related to handling and storage-allowed aggregation of niosomes. In essence, proniosomes are surfactant-coated water-soluble carriers that can be hydrated by brief agitation

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in hot aqueous media immediately before use (Hu & Rhodes, 2000). Introduction of other forms of niosomal products that are edible and demonstrate stability against sedimentation, aggregation and core leakage is, however, required to widen the application of niosomes in food and animal feed sectors in addition to pharmaceutics.

Whey proteins confer beneficial effects on human health and possess privileged functional properties such as surface activity, biocompatibility, biodegradability and the ability to form cold- and heat-set hydrogels. A two-step procedure that is heat denaturation of proteins followed by changing the ionic strength or acidification in the direction of the isoelectric pH is required for fabrication of whey protein cold-set gels (Ju & Kilara, 1998). These types of hydrogels are appropriate for protecting heat sensitive nutraceuticals and show good potential for designing functional food commodities (Chen, Remondetto, & Subirade, 2006). Cold-set whey protein hydrogels are, however, prone to syneresis and sensitive to mechanical abrasion and enzymatic degradation. These might limit the applicability of whey protein hydrogels for conveying and delivery of their cargo to final consumer. Eissa, Bisram, and Khan (2004) examined the rheological characteristics of transglutaminase-cross-linked cold-set whey protein gels. They observed that the elastic modulus and yield/fracture stress and strain of the enzymatically cross-linked gel was higher than those of the non-cross-linked counterparts. The enzyme transglutaminase catalyzes acyl transfer reaction between peptide-bound glutamine residues as acyl donor and primary amines as acceptor. This enzyme has been widely used for protein cross-linking in food industry (Aboumahmoud & Savello, 1990).

It was of interest for the authors of the present communication to fabricate α-tocopherol-carrying niosome-loaded cold-set whey protein hydrogels reinforced by transglutaminase and characterize their mechanical and techno-functional properties. The protein gel provides an efficient protecting, orally administrable and conventionally edible matrix for niosomes whilst, the niosomal particles convey α-tocopherol within the hydrogel and can improve its bioavailability by solubilizing within the intestinal tract and promoting its permeation through epithelial cells (Song et al., 2014). The bioavailability of lipophilic nutraceuticals depends on formation of sufficient amount of mixed micelles in the upper small intestine that solubilize the core and boost their intake by enterocytes (Joye, Davidov-Pardo, & McClements, 2014). The delivery system introduced in the present article can point to a new avenue for development of functional dairy foods.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) with at least 90% protein content was kindly gifted by Arla Food Ingredients (Vibyj, Denmark). Sorbitan monostearate (span 60), α-tocopherol, pepsin, and transglutaminase (activity 1500 u g^{-1}) were purchased from Sigma–Aldrich (Wicklow, Ireland). Sodium hydroxide, glucono deltalactone (GDL), phosphate buffered saline, sodium azide, hydrochloric acid, and other chemicals were procured from Merck (Darmstadt, Germany).

2.2. Niosome preparation

Multilamellar niosomes were prepared using thin film hydration method. Accurately weighed quantities of sorbitan monostearate, cholesterol (molar ratio of 7:3), and α-tocopherol were dissolved in 10 mL chloroform in a 100 mL round-bottom flask. Total amount of surfactants and α-tocopherol were 200 and 11.6 μM, respectively. Organic solvent was then evaporated under vacuum and constant rotation at 60 °C using a rotary evaporator (Heidolph, Germany). The dried thin film was hydrated with de-ionized water in the rotary evaporator at 60 °C for 1 h under atmospheric pressure. This was followed by centrifugation (Sigma 8 k centrifuge, Germany) at 18,000×*g* for 30 min to remove non-trapped α-tocopherol and rehydrating the niosome pellet with de-ionized water.

2.3. Niosomes characterization

2.3.1. Vesicle size measurement

The size of niosomes was measured by using static light diffraction method by a Cilas 1090 particle size analyzer (Orleans, France) equipped with a 5 mw He/Ne (635 nm) laser beam. The measurement carried out 24 h after preparation at 25 °C. The particle size measurements are presented as d(0.1) μm, d(0.5) μm and d(0.9) μm on a surface weighted basis that is the size of 10%, 50% and 90% of the particles below these values. The surface area mean diameter and the volume mean diameter, known as D [3,2] and D[4,3], respectively were calculated using the software provided with the apparatus. The span, which is the distribution width of the particles in the niosome suspension was measured as follows:

$$\text{Span} = \frac{d(0.9) - d(0.1)}{d(0.5)} \quad (1)$$

2.3.2. Optical microscopy

Niosomes were imaged by a camera (SSC-DC388, SONY, Japan) attached onto an optical microscope (BX51, OLYMPUS, Japan) at 10 × 40 magnification.

2.3.3. Encapsulation efficiency

Encapsulation efficiency of niosomes was determined by using freshly prepared samples. Samples were centrifuged at 18,000×*g* for 30 min at 4 °C. Pellet was diluted in 50 mL methanol so as to break niosomal membrane. The concentration of released α-tocopherol was determined spectrophotometrically (UNICO spectrophotometer, New Jersey, USA) in methanol at 285 nm. Each experiment was carried out in triplicate and results are expressed as mean ± standard deviation. The entrapment efficiency was defined as follows:

$$\text{Encapsulation efficiency } (\%) = \frac{\text{released tocopherol content from niosomes}}{\text{initial tocopherol content used in niosomes preparation}} \times 100 \quad (2)$$

2.3.4. DPPH radical scavenging activity assay

Radical scavenging activity was determined according to the technique reported by Tan et al. (2014) with a slight modification. Briefly, 1 mL of free (unencapsulated) α-tocopherol ethanolic solution or α-tocopherol-loaded niosomes with same concentration of