LWT - Food Science and Technology 65 (2016) 206–213

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Impact of encapsulation on the physicochemical properties and gastrointestinal stability of fish oil

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ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form
2 August 2015
Accepted 5 August 2015
Available online 7 August 2015

Keywords:
Chitosan
Functional food
Membrane emulsification
Microcapsule

ABSTRACT

Fish oil was successfully microencapsulated with specifically designed *N*-lauroyl chitosan shell material employing the membrane emulsification process. The behavior of the prepared microcapsules under the simulated digestive model and their physicochemical properties were studied. The experimental digestive model indicated the stability of the microencapsulated fish oil with no sign of undesired flocculi formation or disintegration. However, the un-encapsulated fish oil was trapped upon interaction with *N*-lauroyl chitosan and precipitated as unstable flocculi. The thermally stable, spherically-shaped fish oil microcapsules showed very high loading capacity (53.5%), encapsulation efficiency (62.6%) and maximum cumulative oil release (76.8%). The microcapsules showed sustained release of fish oil through diffusion rather than dissolution of the shell material. The results suggest that microencapsulation of fish oil is beneficial for its delivery, stability and bioavailability in the course of oral administration and *N*-lauroyl chitosan is a suitable shell material.

1. Introduction

The demand for functional food is growing due to its boosting health benefits (Lee, Yim, Choi, Ha, & Ko, 2012). For example, omega-3 (ω-3) fatty acids found in fish oil are reported to improve the cardiovascular activity, enhance long-term memory and normal brain function, reduce platelet aggregation and decrease cholesterol and triglycerides levels (Caterina, Madonna, Bertolotto, & Schmidt, 2007; Kralovec, Zhang, Zhang, & Barrow, 2012; Pang, Xie, Chen, & Hu, 2012). The gastrointestinal stability and bioavailability of orally administered fish oils are of major concerns. Additionally, ω-3 fatty acids are prone to degradation releasing unhealthy secondary oxidation products of polyunsaturated fatty acids, aldehydes, ketones, alcohols, hydrocarbons, organic acids and epoxy compounds (Fetterman & Zdanowicz, 2009; Shahidi & Zhong, 2010).

Microencapsulation of functional foods is an effective approach to achieve the desired attributes of stability, delivery and bioavailability (Gibbs, Kermasha, Alli, & Mulligan, 1999). For this purpose, microencapsulation of fish oil was achieved from its emulsions using various processes and shell materials (Garrett, 1965; McClements, 2012). Well-known microencapsulation processes include spray drying, coacervation and extrusion (Drusch & Berg, 2008; Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Rojas, 2015). Useful emulsification processes include membrane emulsification process (MEP), high speed blending, high pressure and ultrasonic homogenization (Charcosset, Limayem, & Fessi, 2004; Joscelyne & Trägårdh, 2000). In the MEP, which will be used in this work, the oil phase is pressurized through narrow pore size membranes (e.g. Shirasu Porous Glass (SPG)) into the aqueous phase to form the oil-in-water emulsion (Lambrich & Schubert, 2005; Matos, Suárez, Gutiérrez, Coca, & Pazos, 2013; Nazir, Schroën, & Boom, 2013). Fish oil microencapsulation has been reported using various shell materials such as chitosan, gelatin, maltodextrin, sugars, starch, milk, whey proteins and plant gums (Pourashouri et al., 2014). Chitosan and its derivatives function as emulsifiers and stabilizers to promote emulsion formation and stability of the oil droplets (Jayakumar, Menon, Manzoor Nair, & Tamura, 2010; Jumaa & Müller, 1999; Larsson et al., 2013; Pereda, Amica, & Marcovich, 2012). In the food industry, chitosans were used as antioxidants (Lin & Chou, 2004), antimicrobials, edible coatings (Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009) and for sustained release of vitamin D_3 (Li, Peng, et al., 2014; Li, Zhou, et al., 2014) as well as to increase the shelf-life of strawberries (Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011). However, chemical modification of chitosan is

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http://dx.doi.org/10.1016/j.lwt.2015.08.010
0023-6438/

practiced to overcome its shortcomings and enhance its desirable properties (Chou, Fu, Wu, & Yeh, 2003). For example, *N*-acylation of chitosan enhances its blood compatibility, biocompatibility, antithrombotic activity, anticoagulant activity, long term retention in systemic circulation and transfection efficiency (Lee, Ha, & Park, 1995; Lee, Powers, & Baney, 2004; Toh, Chen, Lo, Huang, & Wang, 2011). *N*-lauroylation of chitosan enhances its blood compatibility and suitability for oral and intravenous administration (Li, Peng, et al., 2014; Li, Zhou, et al., 2014). Sulfonation of *N*-lauroyl chitosan renders it amphiphilic leading to reduction in its protein adsorption (Shelma & Sharma, 2011). Amphiphilic chitosan derivatives enhance delivery and transport across the blood brain barrier (BBB) (Sharma, Lohan, & Murthy, 2012) which is known to be notorious for limiting delivery of a large number of drugs including anti-Alzheimer, antibiotics, neuroleptics to the brain (Aktaş et al., 2005). Nevertheless, many modified chitosans show instability in the gastrointestinal environment due to swelling behavior under such acidic environment (Xu et al., 2013). For better stability and bioavailability of ω-3 fatty acids, a suitable carrier system with the following characteristics is highly desirable. It should: (i) enhance the physicochemical stability of ω-3 fatty acids; (ii) impart optimum gastrointestinal stability and delivery; and (iii) show the desired loading, release and colloidal stability.

This study reports on the preparation of fish oil-loaded microcapsules using specifically designed *N*-lauroyl chitosan as shell material and compares the stability and behavior of microencapsulated and un-encapsulated fish oil under simulated gastrointestinal conditions. Also reported here are the physicochemical characteristics of the microcapsules focusing on stability, encapsulation efficiency, loading capacity and release properties.

2. Experimental

Materials, methods and characterizations techniques are detailed in the supporting information.

2.1. Synthesis of N-lauroyl chitosan as shell material

Chitosan (1.0 g, 5.80 mmol) was dissolved in a solution of acetic acid (60 mL, 1.0%, v/v) and methanol (40 mL). Lauroyl chloride (1.34 mL, 5.80 mmol) was slowly added to the stirred chitosan solution and the reaction mixture was further stirred at 30 °C for 5 h (Cho, Kim, & Park, 2012a). The reaction mixture was then added to a solvent mixture consisting of methanol and $NH_{3(aq)}$ (7:3, v/v, ~100 mL). The precipitates obtained were filtered, washed with de-ionized water (100 mL), methanol (25 mL) and diethyl ether (25 mL) then dried over P_2O_5 under vacuum for 24 h at 50 °C. *N*-lauroyl chitosan was obtained as an off-white powder (1.65 g, 73% yield).

2.2. Preparation of fish oil-loaded microcapsules using the MEP

The microcapsules were prepared from the oil-in-water emulsion using *N*-lauroyl chitosan as shell forming material. To create the emulsion using the MEP, the fish oil (2 mL) as the dispersed phase was passed over 2 h through the 0.5 μm hydrophilic SPG membrane into the continuous SDS aqueous phase (40 mL, 2.5 g/L SDS) under 150 kPa. To form the microcapsules, *N*-lauroyl chitosan solution (20 mL, 1%, w/v in 2%, v/v acetic acid) was added to the oil-in-water emulsion (40 mL) in round bottom flasks which were then sealed and stirred for 6 h at 30 °C. The weight ratio of fish oil to *N*-lauroyl chitosan was set at 9:1 (w/w) during the preparation of the microcapsules. After 6 h, the un-reacted *N*-lauroyl chitosan was removed by centrifugation and repeated washing with de-ionized water (5 × 25 mL). The off-white solid microcapsules were obtained by freeze drying using Virtis 25 EL Freezemobile lyophilizer (2.0 g, 93% yield).

3. Results and discussion

3.1. Synthesis and characterization of N-lauroyl chitosan

Chitosan was chemically modified through *N*-lauroylation to enhance its stability under gastric conditions during oral administration (Li, Peng, et al., 2014; Li, Zhou, et al., 2014). Lauroylation increases the hydrophobic character of chitosan and diminishes the nucleophilicity of its free amine groups. *N*-lauroyl chitosan was synthesized through one-step *N*-acylation of chitosan using lauroyl chloride. The product was obtained as an off-white powder in 73% yield and showed the following Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) spectra (Fig. 1). FTIR (KBr, υ_{max}, cm^{-1}): 3486 (O–H str); 2936, 2856 (C–H str of pyranose ring); 1743 (C=O str of lauroyl); 1643 (C–O str of amide I); 1176, 1110 (C–O–C str of glycosidic linkage). The absorption of C–H stretching of chitosan pyranose ring increased after *N*-acylation of chitosan. ^{1}H NMR (300 MHz; DMSO-*d*; δ[ppm]): 1.9 (three *N*-acetyl protons of *N*-acetyl glucosamine); 2.9 (H-2 proton of *N*-acetyl glucosamine or glucosamine); 3.2–3.6 (ring protons H-3, H-4, H-5 and H-6 considered to resonate); 4.6 (H-1 proton of glucosamine), 4.9 (H-1 proton of *N*-acetyl glucosamine) and 0.6, 1.0, 1.3, 1.6, 1.8, 2.3, 3.0, 3.2–3.6 ($CH_3(CH_2)_9CH_2$ protons of the lauryl moiety). The success of the lauroylation reaction is confirmed from the presence of the amide C=O (lauroyl amide) peak at 1743 cm^{-1} in the FTIR spectrum and the additional lauroyl aliphatic protons at 0.6, 1.0, 1.3, 1.6, 1.8, 2.3, 3.0, 3.2–3.6 ppm in the ^{1}H NMR. The Detailed FTIR ^{1}H NMR data are given in Table S1.

3.2. Preparation of the fish oil-loaded microcapsules

Only two reports described the use of membranes for the controlled preparation of fish oil-loaded microcapsules (Chatterjee & Judeh, 2015; Ramakrishnan, Ferrando, Aceña-Muñoz, Lamo-Castellví, & Güell, 2013). The lack of research in this area prompted us to specifically use the MEP to prepare the microcapsules. The fish oil (dispersed phase) was passed over 2 h at 150 kPa pressure through the 0.5 μm SPG membrane into the SDS aqueous solution (continuous phase) to form the fish-in-oil emulsion. Interestingly, any pressure lower than 150 kPa was not suitable for effective permeation due to the small pore size of the SPG membrane and high viscosity of fish oil. The microcapsules were formed through electrostatic interaction between the cationic *N*-lauroyl chitosan and the anionic SDS molecules found on the fish oil/water interface (Mun, Decker, & McClements, 2006). The fish oil to shell materials (*N*-lauroyl chitosan and SDS) were set at weight ratio of 6:1 (w:w) during the formation of the microcapsules.

3.3. Properties of the fish oil-loaded microcapsules

3.3.1. FTIR spectra

The fish oil-loaded microcapsules showed characteristic FTIR peaks of the fish oil, *N*-lauroyl chitosan and SDS components indicating successful microencapsulation (Fig. 2). Examples of the characteristic peaks include: 3464 cm^{-1} (O–H and N–H stretching of chitosan); 2957 cm^{-1} (C–H stretching of fish oil); 2931, 2858 cm^{-1} (C–H stretching of chitosan and SDS); 1743 cm^{-1} (C=O stretching of lauroyl amide); 1648 cm^{-1} (C=O stretching of amide I of chitosan and C=C ring stretching of fish oil); 1560 cm^{-1} (N–H bending of amide II of chitosan); 1464 cm^{-1} (C=C ring stretching of fish oil); 1105 cm^{-1} (P=O stretching of phosphate) and 1099 (S=O stretching of SDS).