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Fabrication, characterization and antimicrobial activities of thymol-loaded zein nanoparticles stabilized by sodium caseinate–chitosan hydrochloride double layers

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ABSTRACT

Thymol-loaded zein nanoparticles stabilized with sodium caseinate (SC) and chitosan hydrochloride (CHC) were prepared and characterized. The SC stabilized nanoparticles had well-defined size range and negatively charged surface. Due to the presence of SC, the stabilized zein nanoparticles showed a shift of isoelectric point from 6.18 to 5.05, and had a desirable redispersibility in water at neutral pH after lyophilization. Coating with CHC onto the SC stabilized zein nanoparticles resulted in increased particle size, reversal of zeta potential value from negative to positive, and improved encapsulation efficiency. Both thymol-loaded zein nanoparticles and SC stabilized zein nanoparticles had a spherical shape and smooth surface, while the surfaces of CHC-SC stabilized zein nanoparticles seemed rough and had some clumps. Encapsulated thymol was more effective in suppressing gram-positive bacterium than un-encapsulated thymol for a longer time period.

1. Introduction

Foodborne pathogens are the leading cause of foodborne illness in the United States. They are responsible for a number of multi-state and expensive outbreaks related to food products including fresh produces (e.g. cantaloupe, tomato and lettuce) and meat products (e.g. beef jerky and ham). These outbreaks have negative impacts on consumer confidence on food safety. On the other hand, consumers more appeal to fresh and minimally processed products, and the products with natural ingredients and improved microbiological safety.

Many essential oils (EOs) have shown a broad spectrum of biological activities, including growth inhibition against bacteria, fungi and yeasts (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Thymol (5-methyl-2-iso-propylphenol), a natural antimicrobial agent from thyme and thyme oil, showed significant antimicrobial activity against both gram-positive and gram-negative bacteria (Wattanasatcha, Rengpipat, & Wanichwecharungruang, 2012). Although the exact mechanism is not completely understood, essential oils containing phenolics are thought to disrupt the membrane of microorganisms (Di Pasqua, Hoskins, Betts, & Mauriello, 2006). Moreover, thymol is a generally-recognized-as-safe (GRAS) food additive according to the United States Food and Drug Administration (FDA). However, thymol has relatively poor water solubility, strong impact on food flavor, and may interact with various food constituents, such as protein and fat. These properties may alter its antimicrobial efficacy and limit the application of thymol as a food antimicrobial agent (Marques, 2010).

Nano-/micro-encapsulation technology has been recently applied to improve the physicochemical properties of food ingredients (Lai & Guo, 2011; Luo, Teng, & Wang, 2012; Luo, Zhang, Whent, Yu, & Wang, 2011; Patel, Bouwens, & Velikov, 2010; Shah, Ikeda, Michael Davidson, & Zhong, 2012; Wattanasatcha et al., 2012; Wu, Luo, & Wang, 2012; Zhong, Tian, & Zivanovic, 2009). Shah et al. (2012) incorporated thymol into whey protein isolate-maltodextrin conjugate capsules, and demonstrated that the efficiency and stability of the nanodispersions were affected by the emulsion composition. Recently, thymol was encapsulated into water dispersible submicron-sized ethylcellulose/methylcellulose spheres, attaining better antimicrobial activity than methylparaben, a conventional food preservative (Wattanasatcha et al.,

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2012). Zein, a corn prolamine protein, is a GRAS food-grade ingredient. It has three quarters of lipophilic and one quarter of hydrophilic amino acid residues. Because of its high hydrophobicity, zein has been investigated in food and pharmaceutical industries for encapsulation and sustained release of hydrophobic bioactives, such as fish oil (Zhong et al., 2009), α-tocopherol (Luo et al., 2011), vitamin D (Luo et al., 2012), curcumin (Patel, Hu, Tiwari, & Velikov, 2010) and 5-fluorouracil (Lai & Guo, 2011). Our group has successfully encapsulated thymol into zein nanoparticles using a liquid–liquid dispersion method (Wu et al., 2012). However, zein, as a protein, has an isoelectric point at around 6.2 (Patel et al., 2010), which may result in poor physical stability and redispersibility of freeze-dried zein nanoparticles at a neutral pH in aqueous systems, limiting its application in delivery systems in food and pharmaceutical industries.

The synthetic and natural polymeric materials, including small-molecule surfactants (Surh, Gu, Decker, & McClements, 2005), phospholipids (Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005), proteins (Hong & McClements, 2007) and polysaccharides (Calero, Muñoz, Cox, Heuer, & Guerrero, 2013) are used to stabilize nanoparticles in different systems. Sodium caseinate (SC), a protein isolated from milk, is particularly attractive as an emulsifier because it is nontoxic, natural, bland taste and widely available (McClements, 2005). SC acted as an effective stabilizer to prevent the aggregation of zein nanoparticles in solutions at neutral pH under high ionic strength conditions (Patel et al., 2010; Patel et al., 2010). Li et al. fabricated antimicrobial films based on zein colloidal nanoparticles coated with SC as a stabilizer (Li, Yin, Yang, Tang, & Wei, 2012). The results showed that SC costing was able to improve the zein nanoparticle stability and avoid the flocculation during film formation.

In addition, layer-by-layer electrostatic deposition of polyelectrolytes onto oppositely charged surfaces was reported to effectively generate oil-in-water emulsions with multi-component interfacial coatings (Calero et al., 2013; Mun, Decker, & McClements, 2006). The formed relatively thick and highly charged double-layer interfaces may increase the electrostatic and steric repulsion between emulsion droplets, which may increase the particle stability against the environmental stresses. However, high energy processing, such as high speed or high pressure homogenization, was required for preparing the emulsions (Hong & McClements, 2007; Hu, Li, Decker, Xiao, & McClements, 2011). To the best of our knowledge, few studies have been conducted to investigate the utilization of layer-by-layer electrostatic deposition technique to coat another polyelectrolyte layer onto SC stabilized zein nanoparticles and the investigation of compositions of double layers of stabilizers on its physical stability and encapsulation efficiency.

Chitosan coating has shown potential in food preservation due to the inherent antimicrobial properties (Alvarez, Ponce, & Moreira, 2013). Recently, water-soluble chitosan hydrochloride (CHC) has been reported as a model positively charged polyelectrolyte (Seyfarth, Schliemann, Elsner, & Hipler, 2008), which may overcome the low water solubility of chitosan at neutral pH. As a continuation of our research in nano-encapsulation to develop novel applications of food ingredients, this study was conducted to investigate the possible application of CHC coating to improve the antimicrobial and physicochemical properties of SC stabilized thymol-loaded zein nanoparticles.

2. Materials and methods

2.1. Materials

Zein with a minimum protein content of 97% was provided by Showa Sangyo (Tokyo, Japan). Thymol and sodium caseinate were obtained from Sigma–Aldrich Chemical Co., Ltd (St. Louis, MO, USA). Chitosan hydrochloride (deacetylation degree of 80–90% and molecular weight of 100 KDa, Batch Code: HK120808061) was purchased from Jinke Biochemical Co. Ltd. (Wenzhou, Zhejiang, China). All other reagents were of analytical grade and used without further purification. Water purified with a Milli-Q system was used for all experiments.

2.2. Preparation of SC stabilized and CHC-SC stabilized thymol-loaded zein nanoparticles

Zein and thymol were dissolved in 80 ml/100 ml aqueous ethanol solutions to obtain a stock solution with final concentrations of 20 and 4 mg/ml, respectively. The nanoparticles were prepared using a liquid–liquid dispersion method with some modifications following a laboratory procedure previously reported (Wu et al., 2012). Briefly, 2 ml of stock solution was added to 10 ml of Milli-Q water without and with (0.4–5.0 mg/ml) SC, under continuous stirring (750 rpm) using a magnetic stirrer (IKA®C-MAG HS 7, IKA WORKS Inc., Wilmington, NC, USA) for 30 min. N_2 was flowed through the solution to remove the ethanol from the system using a nitrogen evaporator (N-EVAPTM 112, Organomation Associates Inc., Berlin, MA, USA).

For preparation of CHC-SC stabilized thymol-loaded zein nanoparticles, the CHC aqueous solutions with concentrations ranging from 0.25 to 1 mg/ml were added slowly to the same volume of the SC stabilized zein nanoparticle dispersions (0.5 mg/ml) with a mild stirring (750 rpm) for 30 min at ambient temperature. The zein to SC mass ratio used throughout this study was 1:1 (w/w). The freshly prepared nanoparticle dispersions were measured for particle size, zeta potential and encapsulation efficiency.

2.3. Analysis of particle size and zeta potential

Samples (including the freshly prepared and re-dispersed ones after lyophilization for 48 h) were measured for dynamic light scattering (DLS) capacity using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK) after appropriate dilutions to avoid multiple scattering effects. The DLS measurements were done with a wavelength of 633 nm and an angle detection of 90°. Reflective index and viscosity of water were 1.590 and 0.8904 cP, respectively, which were used for calculating effective diameter from autocorrelation. The polydispersity index (PDI) reflecting the particle size distribution of nanoparticles was also determined. To study the effect of pH on zeta potential, an MPT-2 multipurpose autotitrator (Serial No. MAL511780) was used in combination with the Zetasizer (0.1 M HCl and 0.1 M NaOH were used as titrates). All measurements were carried out at 25 °C and the results reported were the average of three readings.

2.4. Encapsulation efficiency of nanoparticles

The encapsulation efficiency (EE) of the nanoparticles was defined as the material content that was encapsulated into the nanoparticles:

$$\mathrm{EE}(\%) = \frac{\text{Total thymol amount} - \text{Free thymol amount}}{\text{Total thymol amount}} \times 100$$

The total thymol amount was the thymol used in nanoparticle preparation. The free thymol was measured using a membrane separation method with an Amico® Ultra-4 centrifugal filter device (Millipore Ireland Ltd., Lot: RIEA70245) with 10 KDa molecular weight cut off, according to a laboratory procedure (Luo et al., 2011). After being centrifuged at 3900 *g* for 30 min (Allegra™ X-22R Centrifuge, Beckman Coulter Inc., Brea, CA, USA), free thymol penetrated into the filter receiver, and thymol encapsulated in the nanoparticles stayed in the filter unit. The free thymol content