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Physical and antimicrobial properties of spray-dried zein–casein nanocapsules with co-encapsulated eugenol and thymol

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ABSTRACT

Encapsulation of essential oil components in zein/casein complex nanoparticles using an anti-solvent precipitation method is well-established, but the properties of nanocapsules after spray-drying have not been studied. In the present work, eugenol and thymol were co-encapsulated in zein/casein nanoparticles at pH 6.0–8.0, and the properties of resulting spray-dried powders were characterized. The spray-dried zein/casein complexes were hydrated easily and the resulting dispersions with particles smaller than 200 nm were stable. The encapsulated EOCs showed the controlled release in 24 h, with the encapsulated eugenol showing a higher release rate than thymol. The encapsulated eugenol and thymol were present in milk whey at a concentration much lower than their overall concentrations (2.5 mg/mL each) and solubility but bactericidal and bacteriostatic effects were observed for *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A, respectively. Therefore, the spray-dried capsules may have the potential to be used as antimicrobial preservatives in food products.

1. Introduction

Outbreaks of foodborne illnesses due to consumption of ready-to-eat meals contaminated with pathogens cause negative social and economic impacts. Consequently, the use of efficient antimicrobial preservatives in combination with other intervention strategies is increasingly being recognized, especially the naturally derived antimicrobials that provide label friendliness. Essential oils (EOs) or their components (EOCs) extracted from various parts of edible, medicinal, and herbal plants have been frequently studied due to their excellent activities against bacteria, viruses, fungi, parasites, and insects (Burt, 2004). Many EOs and EOCs are classified by the U.S. Food and Drug Administration as generally recognized as safe (GRAS) (Burt, 2004; Shaaban et al., 2012). Thymol, carvacrol, and eugenol, major components in EOs derived from thyme, oregano, and clove bud, are amongst the most-studied EOCs (Castillo et al., 2014; Chinou et al., 2009; Johny et al., 2010; Pan et al., 2014). The hydrophobic and volatile nature of EOCs requires technologies such as delivery systems to achieve uniform distribution and controlled release in aqueous food systems.

It is also well known that there is synergistic antimicrobial activity when some EOCs are used in combination. For example, the minimum bactericidal concentration (MBC) of carvacrol, thymol, and eugenol against *Listeria innocua* was 150, 250, and 450 mg kg^{-1}, respectively (García-García et al., 2011). When their binary mixtures were studied, the combinations of 62.5 mg kg^{-1} thymol and 75 mg kg^{-1} carvacrol, or 56.25 mg kg^{-1} thymol and 125 mg kg^{-1} eugenol completely inhibited the growth of *L. innocua*. The ternary mixture of carvacrol–thymol–eugenol at respective concentrations of 75, 31.25, and 56.25 mg kg^{-1} was also effective in completely inhibiting *L. innocua*. Therefore, these combinations could reduce their doses as antimicrobial preservatives to lower the cost and potential impacts on sensory quality. However, studies on co-encapsulating EOCs in one delivery system are scarce.

Nano-/microencapsulation of EOCs in food biopolymers is a group of technologies that can possibly solve challenges facing their applications. This has been studied recently for thymol encapsulated in sodium caseinate (NaCas) (Pan et al., 2014) or whey protein–maltodextrin conjugates (Shah et al., 2012). The encapsulated thymol was more effective than unencapsulated thymol in inhibiting foodborne pathogens in milk, resulting from the enhanced distribution and solubility. Much work however is needed before delivery systems of EOCs can be used in the food industry. For example, these delivery systems are preferably produced as spray-dried powder for convenience of storage, transportation, and utilization.

In the present work, zein, a group of prolamins (alcohol-soluble proteins) from corn, was studied as a GRAS biopolymer to produce spray-dried powder with co-nanoencapsulated thymol and

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eugenol. Zein has more than 50% hydrophobic amino acid residues on the surface and is not water soluble (Chen et al., 2013, 2014b; Luo and Wang, 2014). Zein is only soluble in 55–90% aqueous alcohol and easily precipitates as nanoparticles after mixing with water to an overall alcohol concentration incapable of dissolving zein (Zhong and Jin, 2009). This anti-solvent precipitation property has been used to encapsulate fish oil (Zhong et al., 2009), α-tocopherol (Luo et al., 2011), vitamin D3 (Luo et al., 2012), daidzin (Zou and Gu, 2013), and curcumin (Gomez-Estaca et al., 2012; Patel et al., 2010b) in zein nanoparticles. A challenge of utilizing hydrophobic zein nanoparticles is their poor dispersibility in aqueous systems with acidity close to the isoelectric point (*pI*) of zein, *ca.* pH 6.2 (Patel et al., 2010b). It is particularly more problematic for freeze-dried or spray-dried zein nanoparticles that are difficult to be redispersed in water. To solve this problem, water soluble and amphiphilic biopolymers can be used to form complexes with zein nanoparticles to create repulsive steric and electrostatic forces. In our previous study (Chen and Zhong, 2014), zein and NaCas were co-dissolved in hot 50% aqueous ethanol adjusted to pH 8.0 before anti-solvent precipitation to form nanoparticles. We observed that nanoparticles contained κ-casein and zein and the spray-dried powder was easily re-dispersed in water with good stability during storage. In the present study, we are interested in applying the complex nanoparticles to encapsulate volatile EOCs to prepare spray-dried powder.

The first objective of the present work was to study the properties of zein/casein complexes in co-encapsulating thymol and eugenol. The second objective was to study encapsulation properties after preparing spray-dried powder from nanocomplexes. The third objective was to characterize release properties of thymol and eugenol from spray-dried powder and their solubility in milk. The fourth objective was to evaluate the antimicrobial activity of spray-dried capsules against bacteria in milk.

2. Materials and methods

2.1. Materials

Purified α-zein was purchased from Acros Organics (Morris Plains, NJ). NaCas was a product from American Casein Company (Burlington, NI). Anhydrous ethanol was purchased from Decon Laboratories Inc. (King of Prussia, PA). HPLC grade methanol and water were purchased from Fisher Scientific (Pittsburgh, PA). Tryptic soy broth (TSB, Remel®™, Fisher Scientific) medium was prepared by dissolving 30 g powder in 1000 mL water. Tryptic soy agar (TSA) was prepared by adding 12 g agar (Fisher Scientific) into the TSB medium. Ultra-high-temperature (UHT) processed 2% reduced-fat milk (Simple Truth Organic™, Kroger Co., San Diego, CA) was purchased from a local grocery store.

2.2. Sample preparation

The previous method of preparing zein/casein nanoparticles was adopted to co-encapsulate eugenol and thymol (Chen and Zhong, 2014), with modification. Firstly, 50 mL of 50% v/v aqueous ethanol with 5 mM sodium phosphate was adjusted to pH 6.0, 7.0, or 8.0, followed by mixing with 2 g zein, 2 g NaCas, 1 g thymol, and 1 g eugenol, and the pH was readjusted if needed. After heating the mixture at 90 °C for 30 min, the hot solution was sheared into 150 mL of room temperature (21 °C) buffer with 5 mM sodium phosphate adjusted to the same pH as the mixture solution by homogenization at 10,000 rpm for 2 min (IKA® 25 digital ULTRA TURRAX®, IKA® Works, Inc., Wilmington, NC). The dispersion was then spray-dried (mini spray dryer B-290, BÜCHI Corporation, Flawil, St. Gallen, Switzerland) using the following parameters: inlet temperature of 105 °C, outlet temperature of 60 °C, a feed rate of 15%, and an aspirator setting of 100%. Spray-dried powder was collected and stored at −20 °C before use. Another set of samples with only one EOC was prepared by dissolving 2 g thymol or eugenol in the aqueous ethanol with same amounts of zein (2 g) and NaCas (2 g) adjusted to pH 8.0. Two replicates of spray-dried samples were prepared for each treatment.

2.3. Particle size and morphology

Dimensions of particles in fresh dispersions or those reconstituted with spray-dried powder were measured using a dynamic light scattering (DLS) instrument (Delsa Nano C particle size/zeta potential analyzer, Beckman Coulter, Fullerton, CA). The volume fraction-length ($d_{4,3}$) mean diameters were calculated based on number (n_i) of particles with diameter d_i using Eq. (1).

$$d_{4,3} = \frac{\sum_{i=1} n_i d_i^4}{\sum_{i=1} n_i d_i^3} \quad (1)$$

Atomic force microscopy (AFM, model Multimode 8, Bruker Corp., Santa Barbara, CA) was used to characterize the morphology of particles. Spray-dried powder was hydrated in deionized water at 1 mg/mL and diluted to 40 ppm solids in deionized water. Four μL of each diluted sample was spread evenly onto freshly cleaved mica sheets that were mounted on sample disks and dried overnight. The samples were scanned using a rectangular cantilever probe (FESPA, Bruker Corp.) with aluminum reflective coating on the backside and a quoted force constant of 2.80 N/m. Images were generated with a preset scan area of 5.0 × 5.0 μm at a scanning speed of 1 Hz. By using the instrument software, two-dimensional images were used to estimate average particle size. Three-dimensional topographic images were used to generate the mean heights of detected particles.

Scanning electron microscopy (SEM) was used to observe the morphology of fresh dispersions and spray-dried powder. A fresh dispersion was diluted to 0.3 mg/mL total solids and 5 μL of each diluted sample was spread on a silica chip substrate (5 × 5 mm, SPI Supplies, West Chester, PA). The samples were dried in a vacuum oven (TempCon®, Baxter Scientific Products, McGaw Park, IL) at 3333 Pa and room temperature (21 °C) overnight before imaging using a LEO 1525 SEM microscope (SEM/FIB Zeiss Auriga, Oberkochen, Germany). The powdered sample was glued directly onto an adhesive tape mounted on the specimen stub and sputter-coated with a gold layer of *ca.* 5 nm thickness before imaging using the above SEM microscope.

2.4. Encapsulation performance

To determine the total thymol/eugenol content in spray-dried powder, 10 mL of 60% aqueous ethanol was used to dissolve 10 mg powder by mixing for 3 h on an end-to-end shaker. After centrifugation at 4545*g* for 10 min (Sorvall RC-5B plus; Sorvall, Newtown, CT) at 21 °C, the supernatant was filtered through a 0.45 μm polyvinylidene fluoride (PVDF) syringe filter (Fisher Scientific, Co., Pittsburgh, PA) to collect the permeate for HPLC analysis. To determine the free oil content, 10 mg powder was mixed with 2 mL distilled water and vortexed for 30 s, the dispersion was centrifuged at 13150*g* for 5 min (model MiniSpin® plus, Eppendorf, Westbury, NY), and the supernatant was filtered as above to obtain the permeate for HPLC analysis.

To quantify eugenol and thymol, a reversed phase HPLC system (1200 series, Agilent Technologies, Waldbronn, Germany) was used. The system consisted of a quaternary pump module, a degasser, an auto-sampler, a temperature-controlled column chamber, and an Agilent diode array detector. Chromatograms were