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Nanoparticulation of enzymatically cross-linked whey proteins to encapsulate caffeine via microemulsification/heat gelation procedure

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ABSTRACT

A co-surfactant free microemulsion was formulated with sunflower oil, span 80 and whey protein solution and used as nanoreactor to generate caffeine-enveloping capsules through heat gelation of protein. Transglutaminase-induced cross-linking of proteins prior to microemulsification decreased the mean diameter from 478 to 318 nm for core-free particles and from 232 to 118 nm for capsules. As well, the lower limit of capsules size decreased from 78 nm to 45 nm due to enzymatic cross-linking. Scanning electron microscopy showed that morphology of particles and capsules was not completely spherical which was attributed to the protrusion of protein molecules out of aqueous droplets during gelation. The enzymatic treatment yielded in particles with higher glass transition temperature due to the reinforced structure of particulate gel. Fourier transform infrared spectroscopy confirmed the structural changes in proteins by heat and establishment of covalent cross-linkages by the enzyme action manifested by a band at 1078 cm^{-1}.

1. Introduction

There is an increasing demand of consumers for functional foods enriched with bioactive compounds. It is not however, simply possible to add nutraceuticals to numerous foods due to their inherently low solubility in some foods, as well as, negative affect on flavor and texture of product (Aghbashlo, Mobli, Madadlou, & Rafiee, 2013). Nutraceuticals may be as well, degraded by sunlight, oxygen, food processing operations and/or during gastrointestinal digestion putting limits to simple incorporation of those in foods. Encapsulation provides an alternative to protect the sensitive ingredients (Giroux, Houde, & Britten, 2010) during storage and a vehicle to carry those within the foods and finally deliver to the target organ i.e., small intestine in the body (Weiss, Takhistov, & McClements, 2006). Although, microencapsulation technology has been widely developed in the last decades, and is used extensively in food industry (Lee & Rosenberg, 2000), nanoencapsulation is new especially in the food sector. Due to their subcellular size, nanoparticles may improve the bioavailability of bioactive compounds. They can significantly prolong the components residence time in the gastrointestinal tract via decreasing the influence of intestinal clearance mechanism and increasing the surface available to interact with biological tissues. They can also penetrate deeply into tissues through fine capillaries, cross the epithelial lining fenestration and are generally taken up efficiently by cells, thus allowing efficient delivery of bioactive components to target sites in the body (Chen, Remondetto, & Subirade, 2006; Zimet & Liveney, 2009).

Microemulsions are thermodynamically stable colloidal systems with clear/transparent appearance because of nanoscalar (5–100 nm) diameter of dispersed phase droplets (Paul & Moulik, 2001). A water-in-oil (W/O) microemulsion can act as a nanoreactor for synthesis of biopolymeric particles in a controlled size manner (Zhang & Zhong, 2010) and architecture. There is however, minor reports regarding the preparation of fully food-grade microemulsions in the literature. The type of organic phase in this case is limited to triglycerides; as well as, surfactants allowed are solely those approved for alimentary applications e.g. sorbitans or polysorbates. Another challenge encountered is the constraint of using short chain alcohols (mainly ethanol, propanol and butanol) as co-surfactants in microemulsion preparation. Co-surfactants contribute in reduction of interfacial tension between the oil and water phases (Karamustafa & Çhelebi, 2008). Since alcohols are toxic, several investigations have been carried out to formulate co-surfactant free microemulsions. Constantinides and Scalart (1997) compared two co-surfactant free microemulsions prepared with

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different oils for encapsulating various water-soluble drugs. Recently, Rukmini, Raharjo, Hastuti, and Supriyadi (2012) prepared a micoemulsion consisted of virgin coconut oil, different mixtures of food-grade nonionic surfactants (Span 20, Span 60 and Tween 20) in order to obtain different hydrophilic–liphophilic balance values, and deionized water as the aqueous phase.

Whey proteins are highly nutritious and posses unique functional properties such as surface activity, gelation, shielding and protective properties, biocompatibility and biodegradability (Livney, 2010). Therefore, they have been considered for preparation of nanoparticles and capsules in food industries (Chen et al., 2006; Gunasekaran, Ko, & Xiao, 2007). Zhang and Zhong (2010) produced nanoparticles through thermal aggregation of whey proteins inside the aqueous droplets of W/O microemulsions. Heat stability of generated particles was enhanced via cross-linking of protein molecules by enzyme transglutaminase (EC 2.3.2.13) before microemulsification and heat treatment (Zhang & Zhong, 2009). To the best of authors' knowledge, there is no report in the literature on synthesis of nutraceutical-containing capsules from whey proteins by using microemulsion systems. The objective of the present study was therefore to enclose caffeine as a model drug (Gunasekaran et al., 2007; Luo, Zhang, & Xu, 2012) within nanoparticles obtained via heat gelation of enzymatically reinforced whey proteins inside the aqueous droplets of a co-surfactant free W/O microemulsion.

2. Materials and methods

2.1. Material

Whey protein isolate (WPI) was a kind gift from Arla Food Ingredients (Vibyj, Denmark). Caffeine powder was purchased from FTZ JC YUJIE International Inc. (Qingdao, China). Transglutaminase was purchased from Sigma–Aldrich (St. Louis, MO, USA). The enzyme activity was 3.5 U/mg. Pepsin, pancreatin and sodium azide were also purchased from Sigma–Aldrich (St. Louis, MO, USA). Sunflower oil was commercially supplied by FRICO (Sirjan, Iran). Sorbitan monooleate (Span 80) and other chemicals were supplied by Merck (Darmstadt, Germany).

2.2. Preparation of microemulsion

A co-surfactant free W/O microemulsion was prepared by using sunflower oil and span 80 as the oil phase mixture and bi-distilled water as the aqueous phase. The ternary phase diagram was prepared and areas with clear/transparent appearance of system considered as the microemulsion formation zone in the diagram.

The WPI solution was prepared by dissolving 5 g protein powder in 60 mL bi-distilled water, stirring it at 500 rpm for 30 min at room temperature and making it up to the final volume of 100 mL. Sodium azide (100 mg/L) was added to prevent the microbial growth. The solution was stored at 4 °C for 10 h to warrant complete hydration. Phase diagram for WPI solution was prepared similarly with that for water and the borders of microemulsion formation zone were determined. The final formula of 48:48:4% (w/w/w) was chosen for sunflower oil: span 80: WPI solution, respectively, for preparing the W/O miroemulsion as the nanoreactor for synthesis of nanoparticles and capsules.

2.3. Synthesis of particles and capsules

Sunflower oil and span 80 were mixed in equal masses and rested overnight to obtain an equilibrated mixture with clear appearance. The hydrated WPI solution (5 g/100 g) was either supplemented with trasnglutaminase (625 μL/100 g protein solution) or equal volume of bi-distilled water and incubated at 50 °C overnight while shaken at 120 rpm (Gallenkamp Orbital shaker incubator, Rankin Biomedical Corporation, MI, USA). The solution was then added drop wise to the oil/span phase while stirring continuously at 70 rpm at ambient temperature (30 °C) until the microemulsion with transparent appearance was obtained. The emulsion was heated at 90 °C for 15 min, cooled down rapidly and centrifuged (refrigerated centrifuge model 2K 15, SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany) at 5000 × *g* for 5 min. After decanting the supernatant, the pellet was washed with fresh chloroform twice and then with absolute ethanol, followed by bi-distilled water. The suspended nanoparticles were precipitated by centrifuging at 18,300 × *g* for 5 min. The pellet was vacuum dried (VTS 70 vacuum oven, Ehret, Germany) for 1 h at 130 mmHg and 60 °C and kept at −80 °C till analysis.

For preparation of caffeine-loaded nanocapsules, the hydrated WPI solution was supplemented with 0.25 g caffeine to obtain 1:20 mass ratio of caffeine to WPI and either enzymatically treated or not before incubation at 50 °C as described above. The whole procedures of preparation, separation, washing and drying of generated capsules were performed the same as preparing particles.

Accordingly, four treatments were prepared: particles from noncross-linked protein, caffeine-loaded capsules from noncross-linked protein, particles from enzymatically cross-linked protein, and caffeine-loaded capsules from enzymatically cross-linked protein.

2.4. Characterization of generated particles and capsules

2.4.1. Particle size distribution

Size distribution and polydispersity of particles and capsules were determined by photon correlation spectroscopy using a dynamic light scattering (DLS) instrument (ZetaPlus, Brookhaven Instrument Corporation, NY, USA). For this purpose, 5 mg of prepared particles and capsules was dispersed in 1.5 mL phosphate buffer solution (0.05 mol/L, pH 7.0) and shaken continuously at room temperature for 1 h before size measurements.

To investigate the capability of microemulsion system for particle size control, a WPI solution (5 g/100 g) incubated at 50 °C overnight was heat treated at 90 °C for 15 min as done for microemulsions and subjected to size measurement. The hydrodynamic size of freshly hydrated WPI, as well, that of WPI solution incubated overnight at 50 °C but not heat-treated was also measured in order to monitor the influence of each step on the particle size of protein assemblies. All samples were read 5 times to obtain the mean size.

2.4.2. Morphology

The morphology of particles and capsules was observed by a scanning electron microscope (SEM, MIRA//LMU microscope, TESCAN, Brno, Czech Republic). Samples were mounted on aluminum stubs and sputter-coated with gold before imaging under SEM.

2.4.3. Thermal behavior

Two mg of each dry sample was weighed and analyzed by a temperature ramp from −20 to 350 °C at 10 °C/min, and nitrogen flow of 80 mL/min. Samples were heated by a differential scanning calorimeter (Star System DSC1, Mettler Toledo, OH, USA).

2.4.4. Fourier transform infrared (FTIR) spectroscopy

Samples were grinded together with potassium bromide and then pressed into a disc which was scanned with a FTIR spectrometer (Perkin Elmer Spectrum one, MA, USA) in 500–4000 cm^{-1}.