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Corn protein hydrolysate as a novel nano-vehicle: Enhanced physicochemical stability and *in vitro* bioaccessibility of vitamin D_3

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ABSTRACT

The application of colloidal delivery systems for the encapsulation, protection and increased bioavailability of bioactive ingredients has recently gained increasing interest. The aim of this work was to prepare corn protein hydrolysate-based vitamin D_3 (CPH-VD_3) nanocomplexes. UV and FT-IR spectra indicated the existence of non-covalent interactions (i.e. hydrogen bonding) between CPH and VD_3. The result from X-ray diffraction further suggested the formation of amorphous structure of VD_3 after its complexation with CPH. Additionally, dynamic light scattering and transmission electron microscope showed that the CPH-VD_3 complexes exhibited a spherical structure with a size scale from 102 to 121 nm. The encapsulation and loading efficiency of VD_3 could reach 97% and 9%, respectively. Furthermore, the complexation obviously avoided spontaneous particle aggregation of VD_3 against ionic strength ([NaCl] $\leq$ 200 mmol/L). The remaining ratio of the encapsulated VD_3 after exposure to high levels of UV light was as high as 72%. More importantly, *in vitro* bioaccessibility of VD_3 could be up to 95% for the complexes. This study demonstrated that CPH could serve as a novel nano-vehicle for VD_3 and it opens up the possibility of using CPH to construct the colloidal delivery system.

1. Introduction

Over the past decade, there have been major advances in the development of food-grade colloidal delivery systems for improving dispersibility, stability, and bioavailability of lipophilic vitamins and nutraceuticals (McClements, 2015). Among these food-grade materials, food proteins are a versatile group of biopolymers, which have been made into various delivery platforms, such as micro- and nanoparticles, films and hydrogels, to delivery various hydrophobic bioactive substances (Israeli-Lev & Livney, 2014; Wan, Guo, & Yang, 2015). Structuring food-grade delivery system with protein fragments or hydrolysates is a new attempt. Some protein hydrolysates have been successfully applied in fabricating nano-vehicles for delivery of nutraceuticals, such as anchovy muscle protein hydrolysate for non-heme iron, zein hydrolysate for curcumin, and silver carp protein hydrolysate for zinc (Jiang, Wang, Li, Wang, & Luo, 2014; Wang, Wang, Yang, Guo, & Lin, 2015; Wu et al., 2014). In fact, protein hydrolysates may turn out to be good vehicles to deliver bioactive compounds. Compared to proteins, additional advantages of using protein hydrolysates as the vehicles include their low allergic characteristics and easy absorption properties (Guo, Hong, & Yi, 2015; Vandenplas, De-Greef, Hauser, & Paradice-Study, 2014). Additionally, protein hydrolysates themselves also offer some health benefits to human (e.g. antioxidant, antihypertensive and anticancer properties) which have not been found in native proteins (Chi, Hu, Wang, Li, & Ding, 2015; Jamdar et al., 2010; Zarei et al., 2015).

Corn gluten meal, a major by-product of corn wet milling, contains 60% (w/w) protein. However, its poor water solubility and severely imbalanced amino acid composition make it difficult to be used as a food ingredient. In recent years, due to the prominent antioxidant activity and anti ACE inhibitory capacity (Wang, Zheng, & Liu, 2013; Zhou et al., 2015), large amount of corn protein hydrolysate (CPH) were fabricated from the enzymatic hydrolysis of corn gluten meal. To our best knowledge, there haven't been any reports on the applications of CPH as the nano-vehicle so far. In fact, corn gluten meal contains plenty of zein, has the similar amino acid constitutions with zein (Lin et al., 2011). Additionally, comparing with zein hydrolysate, CPH may be more suitable serving as the nano-vehicle in the construction of colloidal delivery system. Since the corn gluten meal is one easily available and affordable food-

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grade material.

Vitamin D_3 (VD_3), as a model hydrophobic nutraceutical in present study, is an essential nutrient for human health. The enrichment of aqueous systems with VD_3 is highly challenging due to its insolubility in low or nonfat products. Even worse, VD_3 is sensitive to light, oxygen and high temperature, which rapidly induce isomerization or oxidation of VD_3 and adversely affect its chemical structure and physiological benefits (Ballard, Zhu, Nelson, & Seburg, 2007). In recent years, several colloidal delivery systems were developed to increase the physiochemical stability of VD_3, such as casein micelles, chitosan-zein complexes and chitosan–soy protein complexes (Haham et al., 2012; Luo, Teng, & Wang, 2012; Teng, Luo, & Wang, 2013). More recently, the microspheres formed by β-lactoglobulin and lysozyme were applied to increase the uptake of VD_3, and the bioavailability was substantially increased in the oral delivery (Diarrassouba et al., 2015). But, the protein hydrolysate-based colloidal delivery system for VD_3 has not been reported so far.

In this study, we attempted to utilize CPH as a novel nano-vehicle to construct the CPH-based VD_3 nanoparticles by the complexation of CPH with VD_3. The complexation of CPH and VD_3 was confirmed by the UV absorption spectroscopy, FT-IR and X-ray diffraction techniques. Encapsulation efficiency, colloidal properties, photochemical stability and *in vitro* bioaccessibility of the CPH-based VD_3 nanoparticles were also investigated.

2. Experimental

2.1. Materials

Corn gluten meal (CGM) was kindly granted by corn developing Co., Ltd. (Zhucheng, China). The protein content of CGM was 52.9 ± 1.1 g/100 g, determined by Kjeldahl method (N × 5.71, wet basis). α-Amylase (62 μkat per g) and Alcalase (endoproteinase from Bacillus licheniformis, 3.4 mkat per mL) were purchased from Novozymes North America Inc. (Franklinton, USA). Vitamin D_3 (>98%) was purchased from Aladdin (Shanghai, China). Pepsin powder (50–58 μkat per mg), pancreatin powder (from porcine pancreas - 4 × USP specifications) and bile extract were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade.

2.2. Preparation of corn protein concentrates (CPC)

CGM was obtained from the corn wet-milling process. The purification of CGM was performed using the following method. Briefly, defatting operation was carried out by subcritical extraction equipment (CBE-100L, Henan Province Subcritical Extraction Equipment Engineering Research Centre, China) with the butane as extraction agent at the solid-solvent ratio of 1:10 (g/mL). The defatted CGM was dispersed in deionized water (100 g/L), and shearing dispersion procedure was carried out by a high-shear dispersion homogenizer (IKA® LABOR PILOT 2000/4, Staufen, Germany) for 10 min. Then thermal stable α-amylase was added to the dispersion (5 g/L), incubating at pH 6.0 and 85 °C for 2 h. After neutralization with 1 mol/L NaOH, the suspension was subjected to filtration (F2.5-A-0.3M, Hyflux Ltd, Shanghai, China). The retentate was diluted with twofold volume water, and the filtration process was run again. Finally, the retentate was spray dried by a mini spray drier (B-29, BÜCHI Co Ltd, Flawil, Switzerland) equipped with a 0.5 mm (dia.) nozzle. Liquid flow rate and air flow rate were 3.5 mL/min and 35 L/h respectively. The inlet and outlet temperature were 150 and 100 °C. Finally, the pale yellow CPC (protein 85.0 ± 0.3 g/100 g) was obtained.

2.3. Preparation of CPH

The preparation of CPH was mainly according to the method described by Kong and Xiong (2006) with minor modifications. Briefly, CPC suspension in deionized water (50 g/L) was hydrolyzed with Alcalase in 50 °C water bath for 2.0 h. The ratio of enzyme to CPC suspension was 1.0 mL/L. The pH of the CPC suspension was adjusted to 9.0 (the optimal values for Alcalase) before hydrolysis was initiated and it was maintained at pH 9.0 by continuing dropwise adding 1 mol/L NaOH during hydrolysis. The process of hydrolysis was terminated when pH didn't change in 5 min. After hydrolysis, the pH of the broths was adjusted to 7.0 using 1 mol/L HCl, and the solution was heated at 95 °C for 5 min to inactivate the enzymes. Then the hydrolysate was centrifuged with 10, 800 g for 20 min at 25 °C. The supernatant was dialyzed (100 Da cutoff, Seccama, USA) for 24 h against deionized water and finally freeze-dried in a DELTA 1-24 LSC freeze-dryer (Christ, Germany). The lyophilized CPH powder (protein 88.0 ± 1.1 g/100 g) was placed in the ziplock bags and stored at 4 °C before use. In this experiment, degree of hydrolysis was 25% determined by pH-Stat method (Adler-Nissen, 1986). Additionally, the molecular weight of CPH was all below 1000 Da by RP-HPLC-MS (data not shown).

2.4. Preparation and colloidal characterization of CPH-VD_3 nanocomplexes

Accurately weighed amounts of VD_3 (10 mg) was dissolved in 1 mL of absolute ethanol to form stock solution. CPH powder (4.5, 9 and 15 mg) was dissolved in 2.94 mL deionized water. Then 60 μL VD_3 stock solutions were added dropwise into CPH solution under continuous stirring using a magnetic stirrer, and the obtained CPH/VD_3 mass ratios were 7.5:1, 15:1 and 25:1 respectively. These dispersions were subjected to centrifugation at 10, 800 g for 10 min, and the supernatant was stored at 4 °C before determination. Additionally, partial supernatant was subjected to freeze-drying for spectroscopical analysis, encapsulation efficiency and loading efficiency determination. As the contrast, VD_3 without CPH (free VD_3) with the same concentration was also prepared.

2.4.1. Analysis of particle size and zeta potential

The particle size and zeta potential of freshly prepared samples were measured using a Zetasizer Nano ZS (Malvern Instruments Co. Ltd., Malvern, UK). The size distributions were calculated from the scattered light intensity fluctuations by cumulant analysis of the autocorrelation function. The Zeta potential was obtained from the electrophoretic mobility using the Henry equation $UE = 2 \times \varepsilon \zeta f(K_a)/(3\eta)$, with the Smoluchowski approximation (i.e., $f(K_a) = 1.5$), where ε and η are the dielectric constant and viscosity of the dispersant, respectively. All measurements were carried out at 25 °C.

2.4.2. Transmission electron microscopy (TEM)

Morphologies of the samples were observed by TEM (XFlash 5030T, Bruker Instruments Co. Ltd., Karlsruhe, Gremany). Before determination, the freshly prepared dispersions were appropriately diluted with deionized water. Two microliter samples (10 μg/mL) were deposited onto a 200-mm carbon-coated copper grid, and the excess sample was sucked away carefully after 5 min. Then the samples were negatively stained with phosphotungstic acid solution (10 mg/mL), and the excess solution was sucked away carefully after 5 min. After that, the samples were dried overnight at room temperature in a desiccator and examined at 80 kV.