



# Characterization of chitosan-ferulic acid conjugates and their application in the design of $\beta$ -carotene bilayer emulsions with propylene glycol alginate

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## ABSTRACT

The current study investigated the formation of chitosan-ferulic acid (FA) conjugates (CFC), and the application of CFC and propylene glycol alginate (PGA) in designing  $\beta$ -carotene bilayer emulsions. CFC was synthesized based on the carbodiimide-mediated coupling reaction, which was confirmed by FTIR and  $^1\text{H}$  NMR analysis. The resulting CFC could have a high ratio of FA grafted onto chitosan ( $83.20 \pm 0.81$  mg/g) and exhibited stronger antioxidant activity than FA, and better water solubility than chitosan alone. Being oppositely charged, PGA and CFC formed bilayer interface covering the oil droplets of  $\beta$ -carotene emulsions through electrostatic attraction. The results showed that the concentration of CFC affected the interfacial content of FA, particle size, and surface charge of the droplets in  $\beta$ -carotene emulsions. Compared to chitosan or chitosan-FA mixture (CFM) stabilized bilayer emulsions with PGA, CFC stabilized bilayer emulsions showed better creaming stability against different environmental stresses (i.e., pH, heat and ionic strength) and higher  $\beta$ -carotene stability when exposed to thermal treatment and UV light. The knowledge obtained in the present study could be useful in designing bilayer emulsions for bioactive compounds with improved performance.

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## 1. Introduction

Different from conventional emulsions with simple interfacial structures, a multilayer emulsion is characterized by its two or more layers covering oil droplets, and formed through a layer-by-layer technique using oppositely charged emulsifiers or biopolymers based on electrostatic adsorption. With a thicker interface, a multilayer emulsion usually has a good stability against flocculation, coalescence and creaming (Mao & Miao, 2015). Moreover, the multilayered interface can be tailor made to have desirable structures, e.g., thickness, compactness, porousness, charge, and controlled responsiveness to environmental stresses, e.g., pH, ionic strength, temperature (Guzey & McClements, 2006; Hou et al., 2010; Mao, Roos, O'Callaghan, & Miao, 2013). Therefore, multilayer emulsions are favorable for the design of delivery systems for bioactive compounds, and they have been applied to

improve the stability of  $\beta$ -carotene, fish oil and vitamins, inhibit the digestion of lipid, and control the release of flavors (Chang & McClements, 2015; Mao, Wang, Liu, & Gao, 2017; Mao et al., 2013).

Bilayer emulsions are usually produced with two proteins (e.g.,  $\beta$ -lactoglobulin and lactoferrin) or a protein and a polysaccharide (e.g.,  $\beta$ -lactoglobulin and pectin), as biopolymers are effective in stabilizing food emulsions (Guzey & McClements, 2007; Moreau, Kim, Decker, & McClements, 2003; Tokle, Mao, & McClements, 2013). The problem associated with bilayered interface containing a protein is first that the outer layer might desorb from the interface at pH close to pI of the protein, as electrostatic adsorption is not applicable at this pH. Second, the sensitivity of proteins to heat, ionic strength, and pressure also limits the applications of protein-stabilized bilayer emulsions (Xiang, Liu, Fan, & Gao, 2015). In this context, interests are turned to bilayer emulsions stabilized by two polysaccharides, whose properties are less affected by pH. Currently, most food-grade polysaccharides are neutral or negatively charged (e.g., pectin, carrageenan, xanthan), while only very few cationic polysaccharides are available when dispersed in water. As a kind of cationic polysaccharide with excellent emulsifying property, chitosan has been used to form bilayer emulsions with

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other polysaccharides. Hou et al. (2010, 2012) prepared  $\beta$ -carotene emulsions stabilized by soybean polysaccharide-chitosan bilayers, and they found that the adsorption of a chitosan layer onto the pre-adsorbed polysaccharide layer could significantly inhibit  $\beta$ -carotene degradation during storage at different temperatures. The percentage of  $\beta$ -carotene remained after storage was dependent on the concentration and molecular weight of the chitosan, and the emulsion stabilized by chitosan of medium molecular weight at a concentration over 0.25% (w/w) exhibited the best  $\beta$ -carotene stability. However, natural chitosan is only soluble at acidic solutions, and has poor solubility in water. Therefore, different types of modified chitosan have been developed, among which polyphenol-grafted chitosan is more attracted, as the inclusion of polyphenols may bring new functions for chitosan in designing delivery systems, e.g., antioxidant property (Lei, Liu, Yuan, & Gao, 2014; Wei & Gao, 2016a; Xie, Hu, Wang, & Zeng, 2014). Several studies revealed that when ferulic acid (FA) was grafted onto chitosan, the newly formed complex had a good solubility in water, while maintaining a good emulsifying property as chitosan (Kumar et al., 2016; Yang & Song, 2015). Chitosan-ferulic acid conjugates (CFC) have been synthesized via free radical grafting, chemical and enzymatic approaches (Liu, Ma, Gao, & McClements, 2017; Aljawish et al., 2014; Liu, Wen, Lu, Kan, & Jin, 2014). During free radical grafting and enzymatic modification, the reaction between chitosan and the phenol hydroxyl groups of FA might result in the loss of antioxidant property of FA (Huber et al., 2017; Moreno-Vázquez et al., 2017). And it was difficult to improve the grafting ratio via chemical modification, and the adverse conditions during chemical reaction could lead to the degradation of FA (Woranuch & Yoksan, 2013). The carboxyl group of ferulic acid could react with the amino and primary hydroxyl groups of chitosan via carbodiimide-mediated coupling, consequently, the phenolic hydroxyl group of ferulic acid could be retained. Thus, a novel reaction approach based on the carbodiimide method was investigated, in order to improve the grafting ratio and maintain the functional property of FA in this study.

Propylene glycol alginate (PGA) is an alginate derivative formed via esterification reaction between alginic acid and propylene oxide. It is composed of 1, 4 linked-D-mannuronic acid (31–65%) and L-guluronic acid (69–35%), and is regarded as one distinct group of surface-active food-grade polysaccharides. PGA is largely used to stabilize food emulsions, e.g., yogurt, acidified milk drinks, creams, dressings (Cheong et al., 2014; Paraskevopoulou, Boskou, & Kiosseoglou, 2005). To the best of our knowledge, there was no research report on PGA based bilayer emulsions. PGA is negatively charged when dispersed in water, and it can interact with chitosan to form a bilayer at the oil-water interface to stabilize emulsions. Therefore, the purposes of the current study were to synthesize CFC with a high grafting ratio, and apply CFC and PGA to form bilayer emulsions as delivery systems for bioactive compounds. As a well-known bioactive compound,  $\beta$ -carotene has been widely investigated, and it was also chosen as a model bioactive in the present study to evaluate the properties of CFC-PGA bilayer emulsions in protecting  $\beta$ -carotene from degradation during thermal treatment and UV light exposure.

## 2. Materials and methods

### 2.1. Materials

Chitosan (50–190 kDa, 75–85% deacetylation degree) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Ferulic acid (FA) (99% purity) was purchased from Beijing BSZH Science Company (Beijing, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 99% purity) was purchased

from Yuanyebio Co., Ltd. (Shanghai, China). *N*-Hydroxy succinimide (NHS, 99% purity) was purchased from Xiya Reagent Co., Ltd. (Shandong, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich. Propylene glycol alginate (PGA,  $\geq 70\%$  degree of esterification) was supplied by Hanjun sugar industry Co., Ltd. (Shanghai, China).  $\beta$ -Carotene suspension (30% w/w  $\beta$ -carotene in sunflower oil) was supplied by Xinchang Pharmaceutical Co., Ltd. (Zhejiang, China). Medium-chain triglyceride (MCT) oil was obtained from Lonza Inc. (Allendale, NJ, USA). All other chemicals used were of analytical grade, unless otherwise stated.

### 2.2. Synthesis of CFC

Chitosan (0.5 g) was dissolved in 70 mL distilled water (pH 4.0), and pH of the solution was then adjusted to 6.0 using 1M NaOH. The solution was stirred overnight at 25 °C. FA (mole ratios to chitosan: 0.5:1.0, 1.0:1.0, 1.5:1.0, 2.0:1.0, 2.5:1.0), EDC·HCl (2 mol equivalent to FA) and NHS (2 mol equivalent to FA) were dissolved in ethanol (30 mL) at 4 °C while being stirred for 1 h. The solutions were then added to the chitosan solution. The reaction was performed at 4 °C for 1 h and at 25 °C for 24 h. Following a dialysis for 24 h with a 12 kDa membrane, the mixture was centrifuged at 4500 rpm at 25 °C for 1 h using an Anke TDL-5A centrifuger (Shanghai, China), and the supernatant was dialyzed for 48 h with a 12 kDa membrane. The final solution was lyophilized to acquire CFC powder in a lyophilizer (LGJ-12, Beijing Songyuan Huaxing Technology Development Co. Ltd., China). Control chitosan without FA was prepared following the same method. Native chitosan referred to the chitosan without any treatment. Chitosan and FA mixture (CFM) was prepared by mixing chitosan solution and FA solution (same chitosan to FA ratios as those of CFC) and incubated at room temperature for 24 h followed by freeze drying.

### 2.3. Characterization of CFC

#### 2.3.1. Determination of FA content in CFC

The total FA content was measured using the Folin-Ciocalteu method with appropriate modifications, with FA as a standard (Gong et al., 2012). Briefly, 2.5 mL Folin-Ciocalteu reagent (0.2 N) was added to the 1 mg/mL CFC solution (0.5 mL) at 25 °C and incubated at dark for 5 min. Then, 2 mL Na<sub>2</sub>CO<sub>3</sub> solution (7.5%, w/w) was added and the mixture was kept at dark for 2 h to finish the reaction. The absorbance of the final solution at 760 nm was measured using a Shimadzu UV-1800 UV-vis spectrophotometer (Tokyo, Japan).

#### 2.3.2. Structural characterization of CFC

FT-IR spectra of CFC was recorded by a Spectrum 100 FT-IR spectrophotometer (PerkinElmer, Waltham, USA) over a wavenumber range of 4000–450 cm<sup>-1</sup> with 16 scans at a resolution of 4 cm<sup>-1</sup>. The sample (2 mg) and desiccated KBr (198 mg) were mixed and ground into fine powder and then pressed into pellet for FTIR measurement. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra was taken at 25 °C on an Agilent DD2 (600 MHz) spectrometer (Agilent, Santa Clara, CA, USA). CD<sub>3</sub>COOD/D<sub>2</sub>O was used as a solvent for native chitosan and CFC.

#### 2.3.3. Determination of antioxidant activity

The measurement of radical scavenging ability of samples (CFC, CFM and FA aqueous solutions) was carried out using the method reported in previous studies (Aksoy, Gulcin, & Kufrevioglu, 2016; Mishra, Ojha, & Chaudhury, 2012). DPPH scavenging ability: The

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