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Controlling the potential gastrointestinal fate of β-carotene emulsions using interfacial engineering: Impact of coating lipid droplets with polyphenol-protein-carbohydrate conjugate

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ABSTRACT

The impact of interfacial coatings comprised of polyphenol-protein-carbohydrate conjugates on the properties of nutraceutical-fortified lipid droplets during digestion was investigated. Surface-active chlorogenic acid-lactoferrin–polydextrose (CA-LF-PD) conjugate was synthesized as emulsifier to stabilize lipid droplets in β-carotene-enriched oil-in-water emulsions. Changes in droplet size, charge, and microstructure were monitored as β-carotene emulsions were passed through a simulated gastrointestinal tract model (mouth, stomach, small intestine). LF-coated droplets were unstable to flocculation at pH 8.0–9.0, due to the reduction in electrostatic repulsion, but CA-LF-PD conjugate-coated droplets were stable. Emulsions stabilized by ternary conjugate had better stability to droplet aggregation under simulated GIT conditions than other systems, which increased β-carotene bioaccessibility. The importance of including an oral phase in the simulated GIT model was also demonstrated. The ternary conjugate-stabilized emulsions developed in this study have potential applications as protectors and carriers of hydrophobic drugs, supplements and nutraceuticals.

1. Introduction

During the past few decades, there have been notable improvements in the development of emulsion-based delivery systems to control the uptake of lipids, and to encapsulate, protect, and release lipid-soluble components (Singh & Sarkar, 2011). However, emulsions are thermodynamically unfavourable systems that have a propensity to break down due to numerous physicochemical mechanisms, including gravitational separation, flocculation, coalescence, Ostwald ripening and phase separation (McClements, Decker, & Weiss, 2007). As a consequence, there is considerable interest in understanding the relationship between the composition and structure of food emulsions and their physicochemical stability. Engineering the properties of the interfacial layer surrounding the lipid droplets in oil-in-water emulsions can be used to enhance emulsion stability and performance (Evans, Ratcliffe, & Williams, 2013). A variety of approaches can be used to engineer interfacial properties, including co-adsorption of emulsifiers, layer-by-layer deposition, physical complexation, and covalent conjugation (Dickinson, 2003). In this study, the utilization of ternary conjugates formed by covalent cross-linking of proteins, carbohydrates and polyphenols was focused on. Multifunctional properties can be engineered into these conjugates by careful selection of the components and reaction conditions used to assemble them. For example, in an emulsion system containing protein, carbohydrate and polyphenol, the protein provides surface-activity thereby enabling the conjugates to adsorb to the lipid droplet surfaces, the carbohydrate improves droplet stability by generating a strong steric repulsion, and the polyphenol improves chemical stability by acting as an interfacial antioxidant. Thus, protein-carbohydrate conjugates or polyphenol-protein-carbohydrate conjugates can enhance the physicochemical stability of emulsions under various food-processing conditions (Liu, Ma, McClements, & Gao, 2016; Liu, Wang, Sun, McClements, & Gao, 2016; Yang et al., 2015).

Recently, there has been considerable interest in controlling the gastrointestinal fate of emulsions so as to create functional foods and beverages that can improve human health and wellness (Golding & Wooster, 2010; Singh, Ye, & Horne, 2009). Consequently, there is a need to understand how lipid droplets coated

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by conjugates behave within the human gastrointestinal tract (Lesmes & McClements, 2012; Xu et al., 2014). Improved knowledge of the gastrointestinal fate of these emulsions would facilitate the design of functional foods with tailored physiological attributes, such as satiety enhancement, bioavailability improvement, or reduced fat absorption (Golding & Wooster, 2010; Singh et al., 2009).

Carotenoids are lipid-soluble pigments found in many vegetable crops, whose increased consumption may benefit human health by reducing incidences of cancer and heart disease, as well as by improving eye health (Krinsky & Johnson, 2005; Kopsell & Kopsell, 2006; Rao & Rao, 2007). β-carotene is one of the most commonly used carotenoids in functional foods, supplements, and pharmaceutical products because of its high pro-vitamin A activity and strong antioxidant capacity. However, it is highly prone to chemical degradation through an oxidation mechanism, especially at elevated temperatures, upon light exposure, at high oxygen levels, under acidic conditions, and in the presence of pro-oxidants, such as transition metals and free radicals (Boon, McClements, Weiss, & Decker, 2010). Numerous studies have shown that the stability and/or bioaccessibility of β-carotene can be increased when it is incorporated into oil-in-water emulsions (Ribeiro et al., 2006; Qian, Decker, Xiao, & McClements, 2012b; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013). Consequently, well-designed emulsion-based delivery systems may be utilized to improve carotenoid bioactivity by increasing the amount that reaches the systemic circulation in an active form.

In our recent study, we conjugated polydextrose (PD) molecules to chlorogenic acid (CA)–lactoferrin complex and used them to stabilize β-carotene emulsions (Liu, Wang, Xu, Sun, & Gao, 2016). The constituents used to assemble these conjugates were selected to provide multiple functional attributes. The protein molecules helped anchor the conjugates to the lipid droplet surfaces and have some antioxidant activity. The carbohydrate molecules help prevent droplet aggregation by generating a strong steric repulsion. The polyphenols have strong antioxidant activity thereby retarding carotenoid oxidation. The objective of the current study was to understand the impact of these ternary conjugates on the potential gastrointestinal fate of the β-carotene emulsions using simulated gastrointestinal tract (GIT) conditions. In particular, we examined the impact of the adsorbed ternary conjugates on lipid droplet stability, lipid digestion, and β-carotene bioaccessibility using an *in vitro* GIT model.

2. Material and methods

2.1. Materials

CA (purity > 99%) was purchased from BSZH Science Company (Beijing, China). LF (purity > 96.1%) was attained from Hilmar Ingredients (Hilmar, CA, USA). PD (type III, E12003) was supplied by Henan Tailijie Bioceth Co., Ltd. (Henan, China). β-Carotene suspension (30 wt% β-carotene in sunflower oil) was purchased by Xinchang Pharmaceutical Co., Ltd. (Zhejiang, China). Medium-chain triglyceride (MCT) oil was provided by Lonza Inc. (Allendale, NJ, USA). Dialysis bags with 12–14 kDa molecular weight cut-offs were obtained from Biodee Biotechnology Co., Ltd (Beijing, China). All other chemicals were of analytical grade, unless otherwise stated. All concentrations are expressed as weight percent, *i.e.*, g/100 g of solution.

2.2. Preparation of CA-LF-PD conjugate

The CA-LF-PD conjugate was synthesized according to our previous method (Liu, Wang, Xu, et al., 2016). Briefly, 2% LF was dissolved in distilled water at pH 9.0 and stirred overnight to ensure complete dispersion and dissolution, and 0.4% CA was dissolved in distilled water and its pH was adjusted to 9.0. These two solutions were then mixed together equally under continuous stirring (120 rpm) and 0.02% sodium azide was added to prevent microbial growth. The mixture was maintained at 25 °C for 24 h under continuous stirring with free exposure to air, and then dialyzed to remove free CA for 48 h against deionized water and then lyophilized using freeze-drying apparatus (Alpha 1-2 D Plus, Marin Christ, Germany) to obtain the CA-LF conjugate.

To prepare CA-LF-PD conjugate, CA-LF conjugate and PD were respectively dissolved in distilled water at a concentration of 20 mg/ml and stirred overnight at 25 °C. Then CA-LF conjugate solution was mixed with PD solution (1:1, w/w) and the mixture were adjusted to pH 7.0 and then lyophilized. The resultant powders were incubated at 60 °C and 79% relative humidity in the presence of saturated KBr solution for a period of 24 h. The ternary conjugates obtained were kept in a desiccator in the freezer until used. Pure LF and a physical mixture of CA-LF conjugate and PD were also prepared under the same conditions, and used as controls.

2.3. Preparation of β-carotene emulsions

Aqueous phases were prepared by dispersing 0.7% LF, CA-LF conjugate, CA-LF-PD conjugate, or CA-LF-PD physical mixture in double distilled water and then stirring overnight at 4 °C to ensure complete hydration. The oil phases were prepared by dispersing β-carotene oil suspension in MCT, heating to 140 °C for 30 s, and stirring until the carotenoid was completely dissolved. Coarse β-carotene emulsions stabilized by different emulsifiers were prepared by homogenizing 5 wt% oil phase and 95 wt% aqueous phase at room temperature, using a high-speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). Fine emulsions were then formed by passing the coarse emulsion through a high pressure homogenizer (Microfluidizer, M110Y, Microfluidics, Newton, MA) at an operational pressure of 900 psi for 3 passes. The resulting emulsions were immediately cooled down to 25 °C and stored in a refrigerator at 4 °C before use. The final amount of β-carotene in the fine emulsions was 0.1%.

2.4. Particle charge and size measurements

A micro-electrophoresis/dynamic light scattering instrument was used to measure the ζ-potential and mean droplet diameter of diluted emulsions (Zetasizer Nano ZS, Malvern Instruments, Malvern, England). Samples were diluted 100 times in double distilled water or 5 mM phosphate buffer (pH 7.0) prior to analysis to avoid multiple scattering. This instrument was only used to determine the droplet size of emulsions containing relatively small droplets (d < 1 μm) because it is based on the Brownian motion of the droplets.

The mean droplet diameter of emulsions containing relatively large particles (d > 1 μm) was measured using a static light scattering analyzer (LS 13 320, Beckman Coulter, Brea, CA, USA). Samples were added into a measurement chamber containing buffer until the instrument gave an optimum obscuration rate between 8% and 12%. A refractive index of 1.330 was used for the aqueous phase, and 1.445 for the oil phase. All measurements were performed at 25 °C.

2.5. Stability study at different pH values

The pH stability of the β-carotene emulsions was evaluated by measuring changes in particle size and charge at different pH values (pH 2.0–9.0). pH values were adjusted to the desired values