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Improved stability and controlled release of lutein-loaded micelles based on glycosylated casein via Maillard reaction

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ABSTRACT

Lutein was encapsulated in glycosylated micelles and the controlled delivery property was investigated. The particle size at different pH and $CaCl_2$ concentration showed that the stability of micelles based on glycosylated casein was significantly superior to that of casein/dextran mixture. The bigger molecular weight of the dextran moieties attached to the casein molecules improved the steric hindrance effect. The cumulative release rate showed that lutein could be bound with glycosylated casein in the micelles and uniformly scattered in simulated gastric fluid, while micelles composed of casein and dextran mixtures rapidly disintegrated and released the embedding lutein within 0.5 h. Additionally, micelles based on glycosylated casein significantly increased the bioaccessibility of lutein from 14.63% to 62.45%. Therefore, glycosylated casein via Maillard reaction could be used as the carrier of lutein, which might effectively control its release behavior in the simulated gastrointestinal tract.

1. Introduction

Lutein is a hydrophobic, oxygenated carotenoid belonging to xanthophyll family (Bone, Landrum, Guerra, & Ruiz, 2003). It is responsible for the intense yellow to red color found in different fruits, vegetables and egg yolks. Research findings indicate that lutein has anti-cancer and antioxidant properties making it a possible candidate for reducing age-related macular degeneration (Peng et al., 2016; Sharavana & Baskaran, 2017). A daily intake of 5–12 mg of lutein is recommended for positive health effects (Shegokar Ranjita, 2012). However, lutein is highly sensitive to light, pH, high temperature and other prooxidants due to its high degree of unsaturation (Burri, 2013). Additionally, poor solubility in aqueous media and low bioavailability are some factors affecting lutein nutraceutical functions and application in food.

With advancement of science and technology, the structural design principles could be used to create delivery systems with increased stability, solubility, enhanced bioactivity and controlled release (Matalanis, Jones, & McClements, 2011). Several delivery carriers have been applied to encapsulate lutein, including liposomes (Tan et al., 2014), lipid nanoparticle (Lacatusu et al., 2013), emulsion (Lim & Roos, 2017) and protein stabilized nanoparticle (Yi, Fan, Yokoyama, Zhang, & Zhao, 2016). Among this carriers, protein delivery system was previously reported as far as the easiness of preparation and delivery of hydrophobic nutraceutical compounds are concerned (Li et al., 2017). However, bioactive loaded in protein nanoemulsions is prone to degradation during in vitro digestion due to the sensitivity of proteins to pH variation, ionic strength, pepsin proteolysis effect and thin interfacial layer (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011).

Protein glycosylation is frequently used to improve protein functionality in order to obtain new functional food ingredients with improved biological and technological properties (Chen et al., 2013; Meng, Kang, Wang, Zhao, & Lu, 2017). Ovalbumin and dextran nanogel prepared by Maillard reaction was successfully used to deliver curcumin (Feng, Wu, Wang, & Liu, 2016). Also, oat protein and dextran conjugate prepared by Maillard reaction improved the stability of oil in water O/W emulsion (Zhang, Guo, Zhu, Peng, & Zhou, 2015). Soy protein isolate and soy soluble polysaccharides conjugates prepared through Maillard reaction were reported to enhance physical stability of citral in oil in water (O/W) emulsion (Yang et al., 2015). Conjugate-based formulations are promising materials for controlled delivery of lipophilic bioactive compounds in simulated gastrointestinal conditions. Lutein was encapsulated in nanoemulsion stabilized with casein

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and dextran conjugates prepared by Maillard reaction. However, free casein and dextran were still existing in the system, and a large amount of oil was used to obtain nanoemulsions (Gumus, Davidov-Pardo, & McClements, 2016). Furthermore, free casein might lead to system instability due to aggregation and precipitation in the gastrointestinal tract. Although many studies have been conducted to improve the stability of protein, clear description of the effect of polysaccharide molecular weight on the improvement of physiochemical stability and gastrointestinal fate are limited. It is challenging to address the influence of pH, ionic strength, pepsin, trypsin, and bile salt on the property of glycosylated casein as delivery system of lutein. These investigations further inspire us to prepare glycosylated casein and search for a promising lutein carrier with good stability and release properties.

To present a clear view on the effect of conjugate on the gastrointestinal stability and controlled release, the glycosylated casein with dextran were obtained by Maillard reaction followed by the removal of free casein and dextran through ultrafiltration techniques. Micelles were preferably used since they could withstand temperature, high pressure and UV light during processing and storage (Ranadheera, Liyanaarachchi, Chandrapala, Dissanayake, & Vasiljevic, 2016). Lutein was loaded in glycosylated casein micelles by ethanol injection and high pressure homogenization method. The microstructure of lutein-loaded casein and glycosylated casein micelles were compared by using transmission electron microscopy. To get further insights on the influence of environmental factors on the stability of casein/dextran micelles and glycosylated casein micelles, the size dependence on the pH and ionic strength were compared by means of dynamic light scattering technique. The physicochemical characterization of lutein-loaded micelles and their release profiles in simulated digestion fluid containing pepsin, pancreatic complex enzyme and bile salt were also compared.

2. Materials and methods

2.1. Materials

Casein (molecular mass 25–30 kDa, 99% purity) (technical grade) and standard lutein (99% purity) were from Sigma Chemical Co. (St. Louis MO, USA). Dextran (D; molecular mass 20 kDa and 40 kDa, 97% purity). Pepsin (activity 3000–3500 U mg^{-1}) and pancreatin consisting of protease (activity 285 U mg^{-1}), lipase (activity 56 U mg^{-1}) and amylase (activity 288 Umg^{-1}) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Other solvents/chemicals used were of analytical grade and were obtained from China Medicine (Group) Shanghai Chemical Reagent Co. (Shanghai, China).

2.2. Preparation of casein-dextran conjugates by Maillard reaction and ultrafiltration

A model Maillard reaction system was modified from the procedure by (Clark and Tannenbaum, 1970). Appropriate amounts of casein (5.0 g) and dextran (35 g) with different molecular weights of 20 kDa (D20) and 40 kDa (D40) were dissolved in 0.667 M phosphate buffer (pH 7) to give a final concentration of 8 mg/mL of casein. The solution was lyophilized with Stoppering Tray Dryer (Labconoco, USA) for (48 h). Then the frozen-dried powder was passed through 120 mesh sieve and placed in petri dishes sealed with puncture foil in dark. In order to obtain the Maillard reaction products, the powder composed of casein and dextran mixture was reacted at 60 °C for 20 h and relative humidity of 78.9% in a desiccator containing saturated KBr solution (Muhoza et al., 2017). The samples were collected after 20 h, placed in an ice-bath to cool before storage at 4 °C.

Ultrafiltration was used to remove free protein and dextran. The Maillard reaction products of casein and dextran were dissolved in distilled water to a concentration of 0.25% (w/v) and prefiltered through a 0.45 μm pore membrane filter (Millipore Corp., Bedford, MA, USA). The solid phase was then conducted with a 20 mL Microsep Advance Centrifugal Device (molecular weight-cut off of 100,000; Pall Corporation, MI, USA) at 1000 rpm for 30 min until 1/8 of the initial volume remained, followed by the addition of deionized water to the original volume. This procedure was repeated three times, and then the retentates were freeze-dried and then stored at 4 °C. The glycosylated casein prepared with different dextran were referred as C-D20 and C-D40.

2.3. Preparation of lutein-loaded micelles

The lutein-loaded micelles were prepared by ethanol injection method combined with ultra-high pressure homogenization as previously prepared with slight modification (Dai et al., 2015). Glycosylated casein was dissolved in deionized water (at 55 °C) and the concentration of casein was adjusted to 1 mg/mL. Then, 2 mL of lutein-ethanol solution containing 5 wt% lutein was rapidly injected using a syringe as a pump into 20 mL of protein solution (55 °C) with magnetic stirring. After agitation in the dark for 30 min, the ethanol was removed by rotary evaporation (55 °C, 0.1 MPa) to form an aqueous dispersion of micelles. The micelles were placed in an ice bath to cool down. The samples were treated by ultra-high pressure homogenization (a pressure of 1050 bar, first cycle; and increased the pressure to 1400 bar, second cycle) by using NS1001-L2K mechanical homogenizer (Niro-Soavi, S.p.A., Parma, Italy), and then stored at 4 °C.

2.4. Determination of encapsulation efficiency

The encapsulation efficiency (EE) was calculated according to the following formulas:

$$Encapsulation\ Efficiency(\%) = \frac{Total\ lutein - Free\ lutein}{Total\ lutein} \times 100 \quad (1)$$

Free lutein content was determined by organic solvent washing method. Three mL *n*-hexane and 0.5 mL of lutein-loaded micelles were mixed by vortexing vigorously for 3 min at ambient temperature. The mixed sample was centrifuged at 2000 rpm for 5 min for collecting the upper organic phase. The extraction was repeated twice by adding 2 mL *n*-hexane. Finally, the collected organic phase was combined together in a tube and diluted to 10 mL with n-hexane. The free amount of lutein was quantified spectrophotometrically (UV-1600 spectrophotometer; Mapada Instruments Co., Ltd., China) at 450 nm, with *n*-hexane as a blank.

The total lutein content in micelles was determined by sodium dodecyl sulfate (SDS) solubilization method. Two milliliter of SDS (1.5 M) was added to micelles (0.5 mL) and mixed by vortex to disrupt the micelle structure. The total volume for each flask was made up to 10 mL with deionized water to give a final SDS concentration of 0.3 M and then incubated at 30 °C for 30 min, followed by quantified spectrophotometrically (UV-1600 spectrophotometer; Mapada Instruments Co., Ltd., China) at 450 nm.

2.5. Z-average diameter and PDI analysis of micelles

Dynamic laser light scattering (DLS) was used to determine the particle size distribution, including z-average diameter (Dz) and polydispersity index (PDI) using Zetasizer Nano ZS 90 (Malvern Instruments, Worcestershire, UK) equipped with a He/Ne laser (λ = 633 nm) and scattering angle 90°. Aliquots of 1 mL micelles were diluted to 10 mL with the same buffer solution to avoid multiple scattering phenomena due to interparticle interaction. Immediately, the diluted sample was transferred into the polystyrene cuvette for size determination at 25 ± 0.1 °C, and then the z-average diameter (Dz) and particle size distribution (polydispersity index, PDI) were recorded. The measurements were performed on three individual samples, and the results given were average.

Zeta potential measurements of micelles were also performed by