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Formulation and characterization of chitosan hydrochloride and carboxymethyl chitosan encapsulated quercetin nanoparticles for controlled applications in foods system and simulated gastrointestinal condition

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ABSTRACT

Quercetin (QUE) has attracted widespread attention in food industries because of its potential bioactive functions. However, the application of QUE is quite limited due to its poor water solubility, stability and bioavailability. In this work, we constructed QUE-loaded chitosan hydrochloride (CHC) and carboxymethyl chitosan (CMCN) nanoparticles through electrostatic interaction, in order to enhance the bioavailability of QUE in functional foods and dietary supplements. At the optimal ratio (CMCN:CHC = 2.0 mg/mL:1.0 mg/mL), QUE-loaded CHC-CMCN nanoparticles (QUE-CDNPs) exhibited an average size of 386.3 ± 10.1 nm, zeta potential of −21.5 ± 1.0 mV, polydispersity index of 0.122 ± 0.03 and encapsulation efficiency of 70.0% ± 5.3%. The efficacy of successful QUE delivery of the prepared nanoparticles was examined by SEM, FT-IR and XRD. The QUE-CDNPs were found capable of controlled release of QUE for ten successive days in 50% ethanol, water-oil (50:50) simulants or 95% ethanol and whisky. QUE release was relatively higher in 50% ethanol, water-oil (50:50) simulants or whisky with higher DPPH scavenging activity than that of QUE-CDNPs in 95% ethanol. These results indicated that the enclosure of QUE in CDNPs improved its chemical stability and solubility, and had higher biological activity as assessed by antioxidant properties in 50% ethanol, water-oil (50:50) simulants or whisky systems. Furthermore, we confirmed that only a partial release of QUE from CDNPs was provoked in gastric fluid condition, whereas in intestine fluids, QUE showed a release as high as ca. 86%. Our study suggests that the CDNPs may be utilized to control the release of QUE in the gastro-intestinal condition and three food systems (i.e. 50% ethanol, water-oil (50:50) simulants or whisky systems), and this simple approach can be applied to other bioactive compounds with low aqueous solubility.

1. Introduction

Quercetin ($C_{15}H_{10}O_7$, molecular weight of 302.2 g/mol, QUE), the major representative of the flavonoid compounds, is found as one of the most potent dietary polyphenol in fruits, vegetables, teas and beverages (Hertzog, Hollman, & Katan, 1992; Jiang, Engelhardt, Thräne, Maiwald, & Stark, 2015; Justesen, Knuthsen, & Leth, 1998; Miean & Mohamed, 2001). QUE has a wide spectrum of bioactive activities, including anti-inflammatory (Hisanaga, Mukai, Sakao, Terao, & Hou, 2016), anti-oxidative (Miura, Muraoka, & Fujimoto, 2003), anti-microbial and anti-proliferative (Haghi, Azimi, & Rahimi, 2017) functions. Consequently, it has attracted considerable interest as an important component in human diet for functional foods in food industry. This may be achieved by consuming more QUE-rich products or functional foods. However, simply increasing the consumed amount of QUE is not sufficient to increase its potential health benefits due to its low aqueous solubility (Rich, Buchweitz, Winterbone, Kroon, & Wilde, 2017) and poor chemical stability, imposing a great challenge for incorporating it into food products (Bilia, Bergonzi, Morgenni, Mazzi, & Vincieri, 2001). Therefore, encapsulation technologies (Khalid et al., 2016; Kim, Ng, Dong, Das, & Tan, 2012; Yegin, Perez-Lewis, Zhang, Akbulut, & Taylor, 2016) have been developed as a promising approach in order to enhance the

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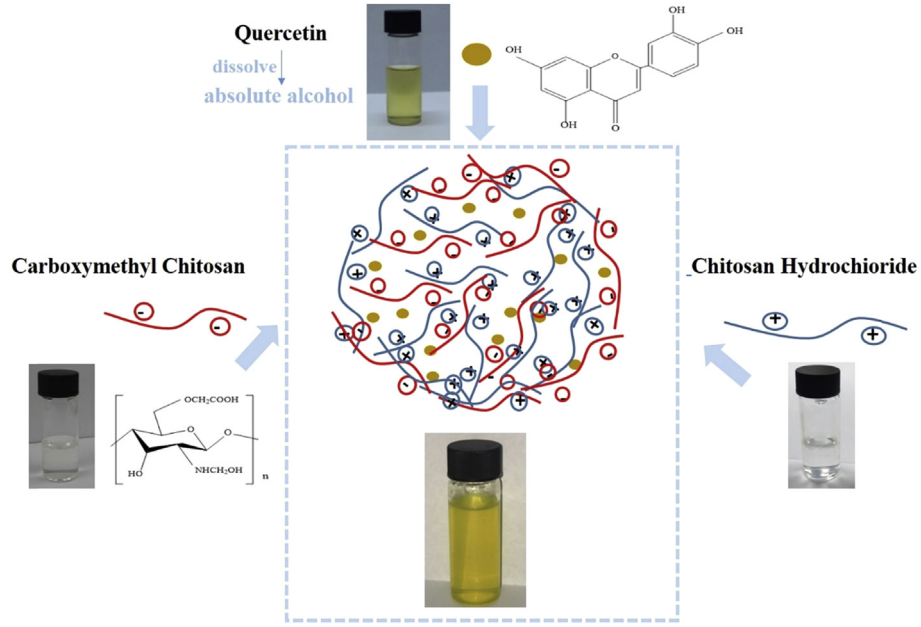

Fig. 1. Schematic representation of the formation of quercetin encapsulated chitosan derivative nanoparticles (QUE-CDNPs).

stability and aqueous solubility of QUE to extend its application in food products.

Compared to many common encapsulation materials like alginate, maltodextrin and protein, etc. (Alba, Sagis, & Kontogiorgos, 2016; Li, Roos, & Miao, 2017; Li, Woo, Patel, & Selomulya, 2017; Morales et al., 2017; Oymaci & Altinkaya, 2016), chitosan has many unique characteristics such as nontoxicity, biocompatibility and biodegradability (Younes & Rinaudo, 2015). It is a renewable natural biopolymer acquired from crustacean shells and has been intensively studied in a variety of biomedical and food applications. The negatively charged carboxymethyl chitosan (CMCN) and positively charged chitosan hydrochloride (CHC) are two different water-soluble chitosan derivatives, and they could form self-assembled CMCN-CHC nanoparticles (CDNPs) by ionic gelation based on intermolecular electrostatic interaction (Ge, Yue, Chi, Liang, & Gao, 2018). The nanoparticles of chitosan derivatives could transiently trigger disintegration of the tight junctions (TJs) between intestinal epithelium, thus enhancing the paracellular permeability for facilitating oral bioavailability (Hsu et al., 2012; Wang et al., 2017). Meanwhile, nanoparticles of chitosan derivatives are soluble in water, resulting in an enhanced nutraceutical stability (i.e. flavonoids, vitamins, proteins, and probiotics) (Carlan, Estevinho, & Rocha, 2017; Liang et al., 2017; Sari et al., 2016; Singh et al., 2017). More importantly, it has been demonstrated that they have the function of controlled release of bioactive materials in food models and intestinal environment (He et al., 2017; Liang et al., 2017) for multiple functional purposes. However, there has still been very few studies done on the development and application of QUE loaded CMCN-CHC nanoparticles (QUE-CDNPs) based on self-aggregates. Therefore, it is of great interest to explore the technique for encapsulating QUE into CDNPs effectively.

In this study, we successfully constructed water soluble QUE loaded nanoparticles of chitosan derivatives (CHC/CMCN) for improved solubility and protection that can be well applied as an efficient QUE delivery system suitable for functional foods. Moreover, the application of CDNPs as carriers of QUE was evaluated through *in vitro* gastro-intestinal (GI) digestion. For better loading of QUE, the nanoparticles were optimized to small particle size, high zeta potential, and high encapsulation efficiency. Then the morphology of the CDNPs and QUE-CDNPs was observed by scanning electron microscope (SEM) and the encapsulation of QUE was confirmed based on molecular interactions by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Finally, the release profile of the QUE in nanoparticles and DPPH radical scavenging power were investigated in simulant and real food samples, and the controlling release rate of QUE was evaluated through *in vitro* GI digestion.

2. Materials and methods

2.1. Materials

CMCN with a degree of deacetylation of 80% and CHC with 90% deacetylation were acquired from Meilunbio Co. Ltd. (Dalian, China). Quercetin (QUE, > 95%) and DPPH (2, 2-diphenyl-1-picrylhydrazyl) were obtained from Sigma (St. Louis, MO, USA). Tween 65 and soybean oil were supplied by Shanghai Yuanye biotechnology Co., Ltd. (China) and Soybean Oil (COFCO Corporation, China), respectively. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) with pancreatin and pepsin were purchased from Meilunbio Co. Ltd. (Dalian, China). Acetic acid and acetonitrile of chromatography grade were purchased from Tedia Co Ltd (USA). The whisky (Talisker distillery, Britain), as an alcoholic (45.8% vol.) beverage, was purchased from Metro supermarket in Hefei (China). Other chemicals and solvents used were analytical grade. Solutions were filtered through syringe filters (diameter 0.22 μm). All the solutions used in the experiments were prepared with ultrapure water, which was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2. Preparation of QUE loaded chitosan derivative nanoparticles

The QUE loaded CHC-CMCN nanoparticles were prepared by a modified ionic gelation method described in Liang et al. (2017) and He et al. (2017). The schematic representation of QUE loaded nanoparticles is shown in Fig. 1. Initially, the CMCN and CHC solutions were prepared in different volumes with distilled water. The QUE (3.75 mg) was subsequently dissolved in absolute ethanol. The syringe was filled with the above-mentioned prepared solution and secured onto a syringe pump. QUE solution was injected into CHC solution and then CMCN solution was added into the resultant solution through a peristaltic pump with plastic needle tubing (internal diameter 0.75 mm, injection rate 150 μL/minute) under magnetic stirring at 900 rpm. After centrifugation, the nanoparticle suspension was sonicated for 10 min to