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Effects of chitosan coating on curcumin loaded nano-emulsion: Study on stability and *in vitro* digestibility

Jinglei Li [a], In-Cheon Hwang [b], Xiguang Chen [c], Hyun Jin Park [a,*]

[a] School of Life Sciences and Biotechnology, Korea University, 5-Ka, Anam-Dong, Sungbuk-Ku, Seoul 136-701, Republic of Korea
[b] Central Research Institute, Kyung-Nong Co. Ltd., Kyungju 780-110, South Korea
[c] College of Marine Life Science, Ocean University of China, Qingdao, 266003, Shandong, PR China

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ABSTRACT

Nano-emulsion (NE) composed of MCT oil, Tween 80 and lecithin was fabricated by ultrasonication method to encapsulate curcumin. Loading ability and efficiency of curcumin were 0.548 mg/mL and 95.10% respectively which indicated its water dispersibility was increased by 1400 fold. Chitosan with low, middle and high molecular weight (3 kDa, 30 kDa and 190–310 kDa respectively) was applied for coating the prepared NE. After chitosan coating, zeta potential value of NE was changed from negative to positive. At the same time, chitosan coating prevented NE phase separation in ionic strength test and inhibited degradation of curcumin during thermal and UV irradiation treatment. Using pH-stat method, it was found that middle and high molecular weight chitosan coating may interfere with lipolysis of NE during the *in vitro* digestion which also slightly decreased curcumin bio-accessibility. Therefore, NE coated with chitosan is a promising delivery system to promote the applications of curcumin in functional food and beverage system.

1. Introduction

Nutraceuticals are bioactive compounds contained in food at relatively low level that may be beneficial to maintain human health and prevent certain chronic diseases (McClements, 2015). Curcumin is one of the most widely investigated nutraceuticals. It is the primary active ingredient of the perennial herb *Curcuma Longa* (turmeric) which has been traditionally used as nutritional supplement and herbal medicine in many Asian countries for thousands of years (Aggarwal, Sundaram, Malani, & Ichikawa, 2007). Recent studies have demonstrated that curcumin has a wide spectrum of therapeutic activities, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, wound healing, and potential prevention ability to neurodegenerative diseases (Aditya et al., 2013; Basnet & Skalko-Basnet, 2011; Begum et al., 2008; Takahashi, Uechi, Takara, Asikin, & Wada, 2009). Moreover, curcumin has very good safety profile as examined by thousands of years' usage and several clinical trials: as high as 8 g/day dosage would not cause any adverse effects (Cheng et al., 2001).

However, the utilization of curcumin in functional food and related products is restrained by several of its drawbacks. Curcumin has extremely low solubility and dissolution rate in aqueous media due to strong inter and intra-molecular hydrogen bonds (Heger, van Golen, Broekgaarden, & Michel, 2014). Meanwhile, curcumin is sensitive to several environmental factors like heating, UV irradiation, and higher pH value (Li, Lee, Shin, Chen, & Park, 2015). To effectively exert its beneficial activities, curcumin is favored to be dispersed in the aqueous medium which creates a demand for a suitable and reliable delivery system. In the past decades, several approaches such as nano-delivery system, chemical modification and physical dispersion have been exploited by many researchers to increase its solubility, stability and bioavailability (Li, Lee, Shin, Chen, & Park, 2015, Li, Shin, Chen, & Park, 2015; Paradkar, Ambike, Jadhav, & Mahadik, 2004; Shin, Chung, Kim, Joung, & Park, 2013; Wang et al., 2015).

In order to better integrate curcumin into functional food and

* Corresponding author.
E-mail address: hjpark@korea.ac.kr (H.J. Park).

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beverage system, nano-delivery systems fabricated by food grade materials have attracted much attention in recent years (Li, Shin, Lee, Chen, & Park, 2016). Nano-delivery systems may confer curcumin with rapid dissolution speed, higher stability, tailored release pattern, higher permeation rate, higher bioavailability and many other advantages compared with other similar structured but micro-size or larger delivery systems (McClements, 2015; Yu & Huang, 2012). As a consequence, various kinds of nano-delivery systems have been evaluated to encapsulate curcumin in order to better incorporate it into food and beverage system (Aditya et al., 2013; Li, Lee et al., 2015; Li, Shin et al., 2015).

Oil in water (O/W) nanoemulsion (NE) is an colloidal dispersion system composed of small lipid droplets in the range of 50–200 nm which are dispersed within an aqueous medium (Tadros, Izquierdo, Esquena, & Solans, 2004). NE is thermodynamically unstable and need certain emulsifiers on the oil-water interface to stabilize the colloidal system. Compared with conventional emulsion which has droplets size of several micrometers, NE is particularly attractive to encapsulate, protect and deliver lipophilic nutraceuticals for food and related applications. Wang et al. reported that NE with the size of 79.5 nm was more effective than conventional emulsion to increase the anti-inflammation activity of curcumin (Wang et al., 2008). Setthacheewakul et al. formulated curcumin loaded NE with the size about 30 nm and demonstrated that it could increase curcumin absorption by 14 fold in the animal study (Setthacheewakul, Mahattanadul, Phadoongsombut, Pichayakorn, & Wiwattanapatapee, 2010).

Biopolymer is usually used for coating nano-delivery system to increase its stability, absorption rate as well as modulate the payload release pattern (Abbas, Bashari, Akhtar, Li, & Zhang, 2014; Ozturk, Argin, Ozilgen, & McClements, 2015; Sood, Jain, & Gowthamarajan, 2014). Chitosan is a natural polysaccharide widely applied for the functional food, drug delivery and tissue engineering (Kean & Thanou, 2010). Our previous study has demonstrated that nanoliposomes coated with chitosan have prolonged absorption in the gastrointestinal tract which could be contributed to its mucoadhesive properties (Shin et al., 2013). Klinkesorn and McClements studied the influence of chitosan coating on the lipase digestibility of tuna oil emulsion (Klinkesorn & McClements, 2009). They found that chitosan coated emulsion could be digested by enzyme and chitosan coating may be useful for delivering lipophilic bioactive compounds.

In the present study, we formulated curcumin loaded NE via ultrasonication treatment. Tween 80 was used as surfactant to stabilize the oil droplets due to its steric effect. Lecithin was added as co-surfactant to offer strong negative charge value to NE and facilitate polymer coating. Chitosan with various molecular weight was employed to coat the prepared NE. We attempted to assess the effects of chitosan coating on the colloidal stability and storage properties of NE. Moreover, we also investigated the effects of chitosan coating on the stability of NE under environmental stresses that are common in food processing including various ionic strengths, thermal treatment and UV irradiation. At last, the *in vitro* digestion and *in vitro* bio-accessibility of curcumin loaded in NE was quantified by the pH-stat method. Our results indicate that chitosan coating is effective to increase the stability of emulsion but slightly decrease its digestibility. This study will be helpful to solve the problems associated with curcumin and better incorporate it in functional food and beverage system.

2. Materials and methods

2.1. Materials

Curcumin with 98% purity was purchased from Acros Orangnics (NJ, USA). Medium chain triglycerides (MCT) was purchased from Now Foods Co. (IL, USA). Lecithin from soybean was obtained from Junsei Chemical Co. (Tokyo, Japan). Tween 80 was provided by Samchun Chemical Co. (Pyeongtaek, Korea). Mucin from porcine stomach, bile extract from porcine, lipase from porcine pancreas and chitosan with molecular weight of 190,000–310,000 and degree of deacetylation (DD) of 85% was obtained from Sigma–Aldrich (MO, USA). Chitosan with molecular weight of 30,000 and DD of 89.2% was acquired from Biotech Co. (Mokpo, Korea). Oligo-chitosan with molecular weight of 3000 and DD of 90% was acquired from Chitolife Co. (Pyeongtaek, Korea). All the chitosan samples were used as received with no further purification. All other reagents and solvents were of analytical grade and used as received.

2.2. Preparation of nanoemulsion (NE)

Ultrasonication treatment was applied to prepare curcumin loaded NE according to previous report (Abbas et al., 2014). About 70 mg of curcumin was dispersed into 10 mL MCT oil and was fully dissolved by heating and stirring overnight. After filtration through syringe filter (0.45 μm) to remove undissolved crystals if any, MCT oil was fully mixed with lecithin. Tween 80 was dissolved into distilled water which was added to the mixture of MCT oil and lecithin. Based on our previous studies and preliminary tests, the weight ratio of MCT oil, lecithin, Tween 80 and distilled water was kept at 10:6:4:80. The mixture of oil, surfactants and water was stirred for about 10 min before subjected to a high speed blender (Ultra-Turrax T25 IKA Works Inc., Wilmington, NC, USA) at 16,000 rpm for 5 min at room temperature to prepare the coarse emulsion. After that, 100 mL of the coarse emulsion solution was treated by ultrasonication (Sonics & Materials, Inc., Newtown, CT, USA) at the power output of 150 W for 20 min in the ice bath using the continuous working model. Prepared NE sample solution was transferred to glass vials for following tests.

2.3. Preparation of chitosan coated nanoemulsion (CNE)

Chitosan with various molecular weight was added into 2% acetic acid solution at the weight ratio of 1%. All the solution was stirred overnight and filtered through paper filter to remove undissolved large particles. Chitosan coating was conducted by adding 10 mL NE into 10 mL chitosan solution under stirring for overnight. Ultrasonication treatment at the power output of 75 W for 5 min was applied to decrease the size as well as break chitosan bridging that may be formed by emulsion droplets and chitosan.

2.4. Curcumin concentration and loading efficiency determination

Curcumin concentration was calculated according to a calibration formula listed below:

$$Y = 0.1483^{*}X + 0.0487,\ r^2 = 0.995\ ; \qquad (1)$$

where Y was the absorption at 419 nm and X was the concentration of curcumin (μg/mL).

The calibration curve was generated by the absorption readings at 419 nm of several standard curcumin ethanol solutions. NE sample was dissolved in ethanol and the absorption at 419 nm was recorded.

Loading efficiency was calculated by the weight ratio of curcumin determined in the NE solution and curcumin initially added.