Food Research International xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Bioaccessibility and antioxidant activity of curcumin after encapsulated by nano and Pickering emulsion based on chitosan-tripolyphosphate nanoparticles

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ARTICLE INFO

Article history:
Received 5 June 2016
Received in revised form 16 August 2016
Accepted 20 August 2016
Available online xxxx

Keywords:
Nano and Pickering emulsions
Curcumin encapsulation
Digestion
Bioaccessibility
Antioxidant activity

ABSTRACT

In the current study the influence of different lipid-based formulations (Pickering and nanoemulsions) and their droplet size on curcumin encapsulation and bioaccessibility, as well as on its anti-oxidant activity was investigated. Oil-in-water Pickering emulsion stabilized by chitosan-tripolyphosphate (CS-TPP) nanoparticles and nanoemulsions containing an organic phase (Span80:Tween80), were prepared with either medium chain triglyceride (MCT) or corn oil as long chain triglyceride (LCT). An in vitro gastrointestinal (GIT) model consisting of mouth, gastric and intestinal phases was used to characterize the rate and extent of lipid phase digestion of the ingested samples. A centrifugation method determined fraction of curcumin released into mixed micelles after digestion (bioaccessibility). These findings showed that after subjecting to simulated GIT model, all the emulsion systems experienced a progressive increase in mean particle size, due to droplet flocculation and coalescence after digestion. Electrical charge (ζ) of particles was observed to become highly negative as they passed through GIT due to accumulation of anionic bile salts, phospholipids and free fatty acids at their interfaces. The rate and extent of lipid digestion and bioaccessibility of curcumin increased with decreasing mean droplet diameter (NMCT > NCO > PMCT > PCO). Finally, we showed that as compared to free curcumin, the encapsulated curcumin showed higher radical scavenging activity (RSA), which confirmed the protective effect of the emulsion systems on the antioxidant activity of curcumin.

1. Introduction

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Fig. 1) is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*, and is widely used in traditional Indian and Chinese medicines. Curcumin has been found to exert a wide range of beneficial biological and pharmacological activities including antioxidant (Sharma, 1976), anti-inflammatory (Chainani-Wu, 2003), antimicrobial (De et al., 2009) and anticancer (Aggarwal, Kumar, & Bharti, 2003). Furthermore, the hepato- and nephro-protective (Venkatesan, Punithavathi, & Arumugam, 2000), thrombosis suppressing (Srivastava, Dikshit, Srimal, & Dhawan, 1985), myocardial infarction protective (Nirmala & Puvanakrishnan, 1996), hypoglycemic (Arun & Nalini, 2002), and antirheumatic (Deodhar, Sethi, & Srimal, 1980) effects of curcumin are also well established. However, the clinical advancement of this promising natural compound is hindered by its poor water solubility and short biological half-life, resulting in low bioavailability in both plasma and tissues. The main reasons attributing to the low bioavailability are supposed to be the poor solubility of curcumin in aqueous media, rapid hydrolysis followed by molecular fragmentation at physiological pH and inactivity of its metabolic products (Lin, Pan, & Lin-Shiau, 2000). Facing this problem, a number of attempts have been made to increase the aqueous solubility and stability and hence the bioavailability of curcumin through encapsulation of curcumin in surfactant micelles (Iwunze, 2004), phospholipids (Semalty, Semalty, Rawat, & Franceschi, 2010), cyclodextrine (Baglole, Boland, & Wagner, 2005), hydrogel (Shah, Mishra, Kumar, Priyadarsini, & Bajaj, 2008), liposomes (Letchford, Liggins, & Burt, 2008), polymeric micelles (Takahashi, Uechi, Takara, Asikin, & Wada, 2009), nanoparticles (Das, Kasoju, & Bora, 2010).

In this context emulsion based delivery systems are being used increasingly for encapsulating lipophilic bioactive compounds in food, pharmaceutical and cosmetic industry (Cheng, Decker, Xiao, &

Abbreviations: CS, chitosan; TPP, tripolyphosphate; NPs, nanoparticles; MCT, medium chain triglyceride; LCT, long chain triglyceride; NMCT, nanoemulsion with MCT oil; PMCT, Pickering emulsion with MCT oil; NCO, nanoemulsion with corn oil; PCO, pickering emulsion with corn oil; RSA, radical scavenging activity; FFAs, free fatty acids; DPPH, (1.2, and 2,2-diphenyl-1-picrylhydrazyl); SOR, surfactant oil ratio; ANOVA, analysis of variance; LSD, least significant difference.

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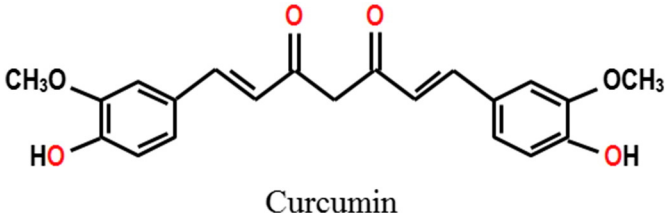
http://dx.doi.org/10.1016/j.foodres.2016.08.022
0963-9969/



Curcumin

Fig. 1. Structure of curcumin.

McClements, 2012). The design of these emulsions which are known to allow controlled release of the encapsulated bioactive compounds throughout gastrointestinal tract has attracted much attention in recent years (Shani-Levi, Levi-Tal, & Lesmes, 2013). The mostly used emulsion systems are either Pickering emulsions or nanoemulsion which can be prepared by homogenizing the oil phase (having dissolved lipophilic bioactive compound), with aqueous phase containing emulsifier. The two types of emulsions can be easily distinguished from each other based on their particle sizes as the nanoemulsions have smaller mean droplet radius (nm) than Pickering emulsion, having larger mean droplet radius (μm). As a matter of fact, after ingestion, food product is exposed to a wide range of physical and biochemical (e.g. dilution effect, pH, enzymes, bile salts, etc.) conditions as it passes through the mouth into stomach and intestines. Starting in mouth, food goes through the processing of mixing with saliva and air, heating or cooling to body temperature, changes in ionic strength and pH, distortion during mastication, and to salivary enzymes and biopolymers such as mucin. After mouth, food will travel to stomach through esophagus and will be mixed with gastric fluid that contains different enzymes (pepsin and gastric lipase) and electrolytes (Na^+, Cl^-, Ca^{2+} etc.) (Pal, Brasseur, & Abrahamsson, 2007).

Digestion of lipids within the gastrointestinal tract is a complex process and to date the impact on release and uptake of any encapsulated lipophilic components is not fully understood. Therefore, the in vitro digestion models have recently gained much attention as a tool for understanding basic physicochemical processes that occur during lipid digestion and release of encapsulated components (McClements & Li, 2010). In particular, in vitro lipolysis models ("pH-stat methods") have been developed as a powerful means of quantifying lipid digestion process and release of lipophilic substances into various colloid phases formed during lipid digestion, e.g., mixed micelles (Pouton, 2006).

Due to smaller droplet sizes, nanoemulsions are considered to be more stable to particle aggregation and gravitation separation. These systems are generally employed for encapsulation of hydrophobic bioactive compounds to improve their dispersion into food products, to protect them against degradation or interaction with other ingredients, to reduce the impact on organoleptic properties of food and to improve their bioavailability (Donsi, Sessa, Mediounic, Mgaidic, & Ferrari, 2011). On the other hand, Pickering emulsions which are stabilized by solid particles have also been focused as important formulations for encapsulation of bioactive compounds in food, cosmetic and pharmaceutical industries. However, despite extensive understanding of unique physical properties of these emulsions, there is limited characterization of the in vitro digestion of bioactive compounds encapsulated in emulsions stabilized by chitosan-tripolyphosphate (CS-TPP) nanoparticles. These CS-TPP nanoparticles are formed by the ionic gelation between the positively charged primary amino groups of CS and the negatively charged groups of polyanion such as TPP, the most extensively used ion cross linking agent (Shu & Zhu, 2002). The interaction between CS and TPP needs mild conditions of temperature and pH (Zhang, Oh, Allen, & Kumacheva, 2004) and the size of the obtained NPs can be easily controlled by varying CS: TPP ratio, pH and molar mass of the CS (Tsai, Chen, Bai, & Chen, 2011). This physical cross-linking between CS and TPP avoids chemical cross linking which is not only toxic to the organism but also can damage the drugs (Berger et al., 2004).

The current study was aimed to evaluate the influence of carrier oil and emulsion type on the bioaccessibility of encapsulated curcumin using a simulated gastrointestinal model comprising mouth, stomach and small intestine. These results will be of great importance for designing effective delivery systems to encapsulate curcumin for application within food and pharmaceutical products.

2. Experimental section

2.1. Materials

Chitosan (CS, Mw 5×10^5–7×10^5 Da and degree of deacetylation about 90.5%) was purchased from Qingdao Yunzhou Biochemistry Co., Ltd. (Shandong, China). Medium chain triglyceride (MCT) was purchased from Boxing 145 Chemical Reagent Co., Ltd. (Wuhan, China). Corn oil (LCT) was purchased from local supermarket. Curcumin (95.0% purity), was purchased from National Medicine Group Chemal Reagent Co., Ltd. Sodium Tripolyphosphate (TPP), glacial acetic acid, Porcine bile extracts, Tween 80, Span 80, sodium azide, ethanol, NaCl, $CaCl_2$, NaOH and HCl were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Pancreatic lipase and DPPH (1.2, and 2,2-diphenyl-1-picrylhydrazyl) were purchased from Aladdin Chemistry Co., Ltd. All the reagents were of analytical grade and used without further purification. Water used in all experiments was purified by deionization and filtration with a Millipore purification apparatus (Millipore, MA, USA) to a resistivity higher than 18.0 MΩ cm.

2.2. Synthesis of CS-TPP NPs

CS-TPP NPs were prepared using the ionic gelling techniques as described in our previous work (Shah et al., 2016). Briefly, CS solution was prepared by dissolving CS (0.5 wt%) powder in 0.5% acetic acid solution, which was further diluted with de-ionized water to make a total volume of 200 mL. The mass ratio of CS to glacial acetic acid was kept at 2:3. The resultant mixture was stirred for overnight at room temperature (around 25 °C). A solution of TPP at concentration of 1.0 mg/mL (0.1 wt%) was prepared by dissolving TPP powder in ultrapure water. Nanoparticles were then prepared by adding TPP solution drop-wise to CS solution (at CS:TPP mass ratio of 5:5) under constant stirring till the formation of an opalescent suspension that indicated particles formation.

2.3. Preparation of the curcumin encapsulated Pickering emulsion

Curcumin was added to the oil phase (MCT or LCT) at the concentration of 0.1 wt% and the mixture was stirred overnight with magnetic stirring to ensure maximum dissolution to curcumin in the oil. The mixture was then centrifuged at 14,000*g* for 10 min to sediment the un-dissolved curcumin. The curcumin encapsulated Pickering emulsion was then achieved as described in our previous work (Shah et al., 2016). Briefly, curcumin containing oil phase (MCT or LCT) and aqueous phase (CS-TPP NPs) were taken in a glass vial. The mixture was then homogenized with an UltraTurrax® T25 device equipped with a S25N-18G shaft (IKA, Germany) rotating at a speed of 10,000 rpm for 3 min. The emulsion so formed was transferred to glass bottles and was stored at room temperature (around 25 °C) for further studies. The oil fractions in the Pickering emulsion were kept at 5 wt%.

2.4. Preparation of the curcumin encapsulated nanoemulsion

Curcumin encapsulated nanoemulsion was prepared by spontaneous emulsification using the method as described by Saberi, Fang, and McClements (2013). Briefly, an organic phase was prepared by mixing nonionic surfactants (Span80:Tween80 = 1.5:8.5) and oil phase (5 wt% MCT or LCT containing 0.1 wt% curcumin) for 10 min at SOR2:1 (Surfactant Oil Ratio). Then this mixed organic phase was

Please cite this article as: Shah, B.R., et al., Bioaccessibility and antioxidant activity of curcumin after encapsulated by nano and Pickering emulsion based on chitosan-tripolyp..., *Food Research International* (2016), http://dx.doi.org/10.1016/j.foodres.2016.08.022