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Enhancing the bioaccessibility of hydrophobic bioactive agents using mixed colloidal dispersions: Curcumin-loaded zein nanoparticles plus digestible lipid nanoparticles

Liqiang Zou [a], Bingjing Zheng [b], Ruojie Zhang [b], Zipei Zhang [b], Wei Liu [a,*,1], Chengmei Liu [a], Hang Xiao [b], David Julian McClements [b,c,**,1]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, No. 235 Nanjing East Road, Nanchang 330047, Jiangxi, China
[b] Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA
[c] Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia

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ABSTRACT

The potential of mixed colloidal dispersions for increasing the bioaccessibility of a hydrophobic bioactive agent (curcumin) was examined. Curcumin was encapsulated within zein nanoparticles (fabricated by antisolvent precipitation) to obtain a high loading capacity and good chemical stability. These protein nanoparticles were then mixed with digestible lipid nanoparticles (fabricated by microfluidization) designed to increase curcumin bioaccessibility by forming mixed micelles in the small intestine. Changes in particle properties (size, charge, and organization) were measured as the mixed colloidal dispersions were passed through a simulated gastrointestinal tract: mouth; stomach; and small intestine. Curcumin bioaccessibility increased with increasing lipid nanoparticle concentration in the mixed colloidal dispersions, which was attributed to an increase in the solubilization capacity of the mixed micelle phase. This study suggests that delivery systems containing mixed colloidal particles (protein and lipid nanoparticles) may be designed to increase the bioaccessibility of lipophilic bioactive agents.

1. Introduction

Turmeric is a natural extract traditionally used as a spice and dye in foods because of its characteristic flavor profile and yellow color (Prasad, Gupta, Tyagi, & Aggarwal, 2014; Syed, Liew, Loh, & Peh, 2015). Turmeric may also be utilized as a bioactive agent because it contains appreciable levels of curcumin, which has been shown to have strong anti-inflammatory, antioxidant, and anti-cancer activities (Aggarwal, Kumar, & Bharti, 2003; Heger, van Golen, Broekgaarden, & Michel, 2014). In nature, curcumin typically exists in three different molecular forms that have varying levels of bioactivity: curcumin, demethoxycurcumin, and bis-demethoxycurcumin (Heger et al., 2014). Clinical trials have shown that curcumin is highly effective against colorectal cancer (Carroll et al., 2011) and pancreatic cancer (Dhillon et al., 2008). Curcumin is believed to exhibit these bioactive effects due to its ability to interfere with various biochemical pathways (Kunnumakkara, Anand, & Aggarwal, 2008). There has therefore been considerable interest in the utilization of curcumin as a bioactive agent in functional foods, supplements, and pharmaceuticals (Schneider, Gordon, Edwards, & Luis, 2015). Nevertheless, there are a number of challenges that currently limit the incorporation of curcumin into commercial products designed to improve human health associated with its low water-solubility, chemical instability, and poor oral bioavailability (Ahmed, Li, McClements, & Xiao, 2012; Fu et al., 2014; Heger et al., 2014).

The challenges associated with utilizing curcumin in commercial products may be overcome by encapsulating it within colloidal delivery systems (McClements, 2012; Patel & Velikov, 2011). Various types of colloidal delivery systems have been studied as a means to enhance the water-dispersibility, chemical stability, and oral bioavailability of curcumin, including casein micelles (Esmaili et al., 2011; Pan, Luo, Gan, Baek, & Zhong, 2014), zein nanoparticles (Gomez-Estaca, Balaguer, Gavara, & Hernandez-Munoz, 2012; Patel, Hu, Tiwari, & Velikov, 2010), curcumin nanoparticles (Margulis, Magdassi, Lee, & Macosko, 2014), colloidosomes (Zhao, Pan, Nitin, & Tikekar, 2014), liposomes (Chen et al., 2015; Niu et al., 2012), nanoemulsions (Ahmed et al.,

* Correspondence to: W. Liu, State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, 330047, Jiangxi, China.
** Correspondence to: D.J. McClements, Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA.
E-mail addresses: liuwei@ncu.edu.cn (W. Liu), mcclements@foodsci.umass.edu (D.J. McClements).
[1] These authors contributed equally to this manuscript.

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2012; Yu, Shi, Liu, & Huang, 2012), and emulsions (Tikekar, Pan, & Nitin, 2013). Each of these colloidal delivery systems has certain advantages and disadvantages in terms of its ease of preparation, cost, robustness, and functional performance. An alternative approach developed to increase curcumin bioavailability is to mix a curcumin-rich material (such as a powder) with an *excipient nanoemulsion* that consists of digestible lipid droplets dispersed within an aqueous medium (Zou, Liu, Liu, Xiao and McClements, 2015a,b; Zou, Zheng, et al., 2015). An excipient nanoemulsion may have no health benefits itself, but it increases the health benefits of any bioactive agents consumed with it by increasing their bioavailability. An excipient nanoemulsion may improve the oral bioavailability of co-ingested bioactive agents through numerous mechanisms, such as enhancing the solubility in gastrointestinal fluids, retarding chemical degradation, or increasing absorption (McClements & Xiao, 2014).

In the current study, we investigate the possibility of using mixed colloidal dispersions containing two different types of nanoparticles to increase curcumin bioaccessibility. The main advantage of this kind of delivery system is that one type of nanoparticle can be designed to overcome one problem limiting the bioavailability of a bioactive agent (such as poor chemical stability), while another type of nanoparticle can be designed to overcome another problem (such as low bioaccessibility). In this study, curcumin was encapsulated within protein nanoparticles prepared using an antisolvent precipitation method, and then these nanoparticles were mixed with a dispersion of digestible lipid nanoparticles prepared using high-pressure homogenization. The protein nanoparticles are designed to have a high loading capacity and to protect the curcumin from chemical degradation during storage. On the other hand, the lipid nanoparticles are designed to rapidly digest within the gastrointestinal tract and form mixed micelles that can solubilize and transport the hydrophobic curcumin molecules. Previous studies using curcumin have shown that zein nanoparticles give better stability against chemical degradation than lipid nanoparticles since the diffusion of reactants is more limited by a semi-solid zein matrix than by a fluid lipid matrix (Pan, Tikekar, Wang, Avena-Bustillos, & Nitin, 2015). Other studies have shown that lipid nanoparticles are highly effective at increasing the bioaccessibility of curcumin by forming mixed micelles after they are digested (Ahmed et al., 2012). Thus, mixed colloidal dispersions can be designed to make use of the relative advantages of these two different types of colloidal system. This study therefore provides useful information for the optimization of delivery systems designed to increase the oral bioaccessibility of hydrophobic bioactive agents.

2. Materials and methods

2.1. Materials

Corn oil purchased from a local supermarket was used as a digestible long chain triglyceride (LCT). The following chemicals were purchased from the Sigma Chemical Company (St. Louis, MO): zein (Lot SLBD5665V), curcumin (SLBH2403V), mucin from porcine stomach (SLBH9969V), pepsin from porcine gastric mucosa (SLBL1993V), lipase from porcine pancreas pancreatin (SLBH6427V), porcine bile extract (SLBK9078), Tween 80 (BCBG4438V), and Nile Red (063K3730V). Sodium caseinate was purchased from the American Casein Company (Burlington, NJ). Whey protein isolate (WPI) was kind donated by Davisco Foods International Inc. (Le Sueur, MN, USA). All other chemicals were of analytical grade. Double distilled water was used to prepare all solutions and emulsions.

2.2. Preparation of digestible lipid nanoparticles

Initially, an aqueous phase was prepared by dispersing 1% (*w*/*w*) Tween 80 into an aqueous buffer solution (5.0 mM phosphate buffer saline (PBS), pH 4.0) and then stirring for at least 2 h. A coarse oil-in-water emulsion was then formed by blending 10% (*w*/*w*) corn oil and 90% (*w*/*w*) aqueous phase using a high-shear mixer for 2 min (M133/1281–0, Biospec Products, Inc., ESGC, Switzerland). Nanoemulsions containing digestible lipid nanoparticles were then formed by passing the coarse emulsion three times through a microfluidizer (M110Y, Microfluidics, Newton, MA) with a 75 μm interaction chamber (F20Y) at an operational pressure of 12,000 psi.

2.3. Preparation of curcumin-loaded protein nanoparticles

Curcumin-loaded protein nanoparticles were formed from a hydrophobic protein (zein) using an antisolvent precipitation method. Initially, curcumin (1.32 mg/mL) and zein (26.4 mg/mL) were dissolved in 80% (*w*/*w*) ethanol solution at a mass ratio of 1:20. Then, 25 mL of aqueous ethanol solution was rapidly injected into 75 ml of Tween 80 solution (PBS, pH = 4.0) that was stirred at 1200 rpm using a magnetic stirring (IKA R05, Werke, GmbH). The resulting colloidal dispersion was then continually stirred for another 30 min at the same speed. The ethanol remaining in the final colloidal dispersions was evaporated using a rotary evaporator (Rotavapor R110, Büchi Crop., Switzerland), and the same volume of pH 4.0 PBS was added to compensate for the lost ethanol.

2.4. Preparation of mixed nanoparticle colloidal dispersions

The 10% corn oil-in-water nanoemulsions prepared by microfluidization were diluted with different amounts of PBS (5 mM, pH = 4.0) to form nanoemulsions containing 2.5%, 5.0%, or 10% corn oil. Nanoemulsions (20 mL) containing different concentrations of lipid nanoparticles were then mixed with dispersions of curcumin-loaded zein nanoparticles (20 mL). The resulting mixtures were then incubated at room temperature while being stirred at 400 rpm for 30 min. Zein-loaded curcumin nanoparticles (20 mL) mixed with PBS (5 mM, pH = 4.0) (20 mL) were as a lipid-free control.

2.5. Color analysis of mixtures

The color of the nanoparticle mixtures was measured using an instrumental colorimeter (ColorFlex EZ 45/0-LAV, Hunter Associates Laboratory Inc., Virginia, USA). Color was expressed in CIE units: L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness). An aliquot of sample (15 mL) was placed in a 64-mm path length glass sample cup and then illuminated with D65-artificial daylight (10° standard angle). Three replicate measurements were performed and the results were averaged.

2.6. Particle characterization

The mean particle diameter and particle size distribution of excipient nanoparticle mixtures before and after exposure to gastrointestinal conditions were monitored using static light scattering (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). Samples were diluted with appropriate buffer solutions (same pH as sample) and stirred in the dispersion unit at a speed of 1200 rpm. The particle size is reported as the surface-weighted (d_{32}) or volume-weighted (d_{43}) mean diameter. The electrical charges (ζ -potential) of the particles in the samples were measured using a micro-electrophoresis instrument (Nano-ZS, Malvern Instruments, Worcestershire, UK). Samples were diluted with appropriate buffer solutions prior to measurements to avoid multiple scattering effects.

The mean particle diameters (Z-average), particle size, and electrical charges (ζ -potential) of micelles collected by centrifuging the raw digesta were measured by a dynamic light scattering instrument and micro-electrophoresis instrument, respectively (Nano-ZS, Malvern Instruments, Worcestershire, UK). Micelles were diluted with buffer solution (5 mM PBS, pH 7.0) prior to measurements to avoid multiple