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Nanoemulsion-based delivery systems for nutraceuticals: Influence of carrier oil type on bioavailability of pterostilbene

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ABSTRACT

Nanoemulsion-based delivery systems can be utilized in functional foods and beverages to improve the bioavailability of nutraceuticals. We determined the influence of carrier oil type on the bioavailability of pterostilbene encapsulated in nanoemulsions containing either flaxseed or olive oil as the carrier oil. The nanoemulsions were then subjected to a simulated gastrointestinal tract (mouth, stomach, small intestine), and the resulting micelle phases were used to establish pterostilbene bioavailability using a Caco-2 cell model. Both nanoemulsions significantly enhanced the bioaccessibility of pterostilbene within the micelle phase. However, olive oil nanoemulsions increased trans-enterocyte transport of pterostilbene more effectively than flaxseed oil ones. Moreover, the patterns of metabolism of pterostilbene during its trans-enterocyte transport were dramatically different when pterostilbene was delivered using nanoemulsions with different carrier oil types.

1. Introduction

Nanoemulsion-based-delivery systems have been used to encapsulate, protect and deliver various kinds of hydrophobic bioactive agents to improve their bioaccessibility and bioavailability (Ahmed, Li, McClements, & Xiao, 2012; Pool, Mendoza, Xiao, & McClements, 2013; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013; Yang & McClements, 2013; Zheng et al., 2014). Nanoemulsions have a number of poten-

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Abbreviations: PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; UFAs, unsaturated fatty acids; SFAs, saturated fatty acids; FFAs, free fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SSF, simulated saliva fluid; SGF, simulated gastric fluid; SSIF, simulated small intestinal fluid; TEER, transepithelial electrical resistance; PBS, phosphate buffered saline; GIT, gastrointestinal tract

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tial advantages as nutraceutical and pharmaceutical delivery systems. Their small particle size and large surface area may lead to enhanced digestion rates, faster formation of mixed micelles, higher release of bioactive agents, more rapid diffusion across the mucus layer, and enhanced epithelium cell permeability (Sivakumar, Tang, & Tan, 2014; Ting, Jiang, Ho, & Huang, 2014; Yu & Huang, 2013). Moreover, nanoemulsions may inhibit the oxidation of chemically labile bioactive compounds, thereby increasing their shelf life and reducing their degradation in the gastrointestinal tract (GIT) (Augustin et al., 2011; Frede et al., 2014). Recently, increasing attention has been focused on the utilization of food-grade nanoemulsions to encapsulate and deliver lipophilic bioactive agents, such as nutraceuticals (carotenoids, curcumin, flavonoids) (Ahmed et al., 2012; Augustin et al., 2011; Gulseren, Guri, & Corredig, 2014; Svelander, Lopez-Sanchez, Pudney, Schumm, & Alminger, 2011; Yu & Huang, 2012; Zheng et al., 2014) and oil-soluble vitamins (Mayer, Weiss, & McClements, 2013b; Sy et al., 2012; Yang & McClements, 2013).

The carrier oil used to formulate food-grade nanoemulsions plays an important role in determining the bioavailability of encapsulated components (Qian, Decker, Xiao, & McClements, 2012; Zheng et al., 2014). The carrier oil should rapidly and fully digest, as well as form mixed micelles that have a high solubilization capacity for the bioactive component (Li, Xiao, & McClements, 2012). Some polyphenols have poor bioavailability due to their low solubility and susceptibility to metabolism in GIT fluids. There have been some attempts to utilize emulsion-based delivery systems to improve the bioavailability of bioactive agents, such as resveratrol. However, the low solubility of resveratrol in both water and oil phases impeded the successful application of emulsions for this purpose.

Many resveratrol derivatives are available with different molecular characteristics, physicochemical properties and biological activities (Mulakayala et al., 2013; Ruan et al., 2006). Pterostilbene (trans-3,5-dimethoxy-4-hydroxystilbene) is a naturally-derived compound predominantly found in blueberries, several types of grapes, and some tree woods (Adrian, Jeandet, Douillet-Breuil, Tesson, & Bessis, 2000; Manickam, Ramanathan, Jahromi, Chansouria, & Ray, 1997; Rimando, Kalt, Magee, Dewey, & Ballington, 2004). It is a methoxylated analog of resveratrol that exhibits various biological activities, including anticancer and anti-inflammation (Kapetanovic, Muzzio, Huang, Thompson, & McCormick, 2011). Furthermore, it has been shown that pterostilbene has stronger inhibitory effects than resveratrol on colon cancer cells (Nutakul et al., 2011).

Compared with resveratrol, pterostilbene has some potential advantages for incorporation into nanoemulsion-based delivery systems, such as improved oil solubility. The purpose of the current research was to examine the influence of carrier oil type on the potential gastrointestinal fate of oil-in-water pterostilbene-enriched nanoemulsions using human epithelial adenocarcinoma (Caco-2) enterocytes. These nanoemulsions could be utilized as delivery systems in functional foods, dietary supplements or pharmaceutical products. The knowledge gained from this will be helpful for developing efficient and reliable delivery system for hydrophobic bioactive compounds.

2. Materials and methods

2.1. *Materials*

Pterostilbene was synthesized and purified using a published method (McNulty & McLeod, 2013). Refined flaxseed oil and refined olive oil were purchased from Soapgoods Inc (Norcross, GA, USA). Lipase (from porcine pancreas pancreatin), bile extract (porcine) and sulfatase (from Helix pomatia) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. *Pterostilbene-enriched nanoemulsion preparation*

Oil phases were prepared by dispersing 160 mg of powdered pterostilbene into the carrier oils (8 mL), followed by stirring (stirring bar at 100 rpm) at room temperature for 30 min to ensure full dissolution. For the control ("non-encapsulated") group, phosphate buffered saline (PBS) was used instead of oil. Aqueous phases were prepared by adding Tween 20 (1 mL) into PBS (200 mL), followed by stirring (stirring bar at 100 rpm) at room temperature for 10 min. Coarse emulsions were formed by mixing oil phase (8 mL) and aqueous phase (192 mL), and then blending them for 2 min at ambient temperature using a high-speed mixer (TissueTearor, Biospec Products, Bartlesville, OK, USA). Coarse emulsions were then passed through a high-pressure homogenizer for three passes at 9 Kpsi (Model M-110Y Microfluidizer processor, Microfluidics, Newton, MA, USA) to form an oil-in-water nanoemulsion.

2.3. In vitro *digestion model*

Each nanoemulsion was passed through a three-step *in vitro* digestion model that simulates mouth, gastric and small intestine digestion. The particle size and charge of the samples were measured after incubation in each stage. Simulated saliva fluid (SSF) was prepared according to a previous study (Pool et al., 2013). Simulated gastric fluid (SGF) was prepared by dissolving NaCl (2 g) and HCl (7 mL) in water (total 1 L volume), then the pH was adjusted to 1.2 (Sarkar, Goh, Singh, & Singh, 2009). The simulated small intestinal fluid (SSIF) contained 2.5 mL pancreatic lipase solution (60 mg, PBS), 3.5 mL bile extract solution (187.5 mg, PBS, pH 7.0, 37 °C) and 1.5 mL salt solution (0.5 M $CaCl_2$ and 5.6 M NaCl in water).

2.3.1. *Mouth phase*

Simulated saliva fluid (SSF, 30 mL) was added to 10 mL of initial sample (control or nanoemulsion), the mixture was then