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In vitro and *in vivo* study of fucoxanthin bioavailability from nanoemulsion-based delivery systems: Impact of lipid carrier type

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ABSTRACT

The impact of carrier oil type on the functionality of a lipophilic nutraceutical (fucoxanthin) encapsulated within nanoemulsions was investigated. Three carrier oils were investigated: long chain triacylglycerols (LCT); medium chain triacylglycerols (MCT); and indigestible oil (orange/mineral oil). Nanoemulsions containing LCT and MCT were completely digested under simulated gastrointestinal conditions, whereas those containing indigestible oil were not digested. Fucoxanthin solubility in mixed micelles formed by *in vitro* digestion decreased in the following order: LCT > MCT > indigestible oil. Animal feeding studies revealed that fucoxanthin was absorbed into the intestinal epithelial cells in the same order as observed for the *in vitro* solubility. However, the concentration of fucoxanthin in the serum of the rats was similar for all carrier oils. The present work highlights the importance of contrasting *in vitro* and *in vivo* experiments to assess the biological fate of functional ingredients incorporated in emulsion-based delivery systems.

1. Introduction

The isolation, purification, and utilization of functional ingredients derived from algae is gaining importance due to their bioactivity, sustainability, and low cultivation costs (Holdt & Kraan, 2011; Ugwu, Aoyagi, & Uchiyama, 2008). Fucoxanthin is a carotenoid belonging to the hydroxylated xanthophyll class that has an unusual allenic bond in the 5,6-monoepoxide (Kotake-Nara et al., 2001). It is found in specific types of brown algae where it is involved in the photosynthesis reaction (Maeda, Tsukui, Sashima, Hosokawa, & Miyashita, 2008; Takaichi, 2011). Fucoxanthin is responsible for the brown color of this type of algae due to the fact that it selectively absorbs light in the visible

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Chemical compounds: Fucoxanthin (PubChem CID: 5281239); Fucoxanthinol (PubChem CID: 11273547); Amarouciaxanthin A (PubChem CID: 16061220); Triheptadecanoin (PubChem CID: 3625612).
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region. It has also been reported to have antioxidant and anti-inflammatory properties (Peng, Yuan, Wu, & Wang, 2011; Sachindra et al., 2007) as well as anti-cancer activity (Kumar, Hosokawa, & Miyashita, 2013; Moghadamtousi et al., 2014). Moreover, recent studies have reported anti-obesity and anti-diabetic properties associated with the consumption of fucoxanthin (Awang et al., 2014; Maeda, Hosokawa, Sashima, Murakami-Funayama, & Miyashita, 2009). Fucoxanthin therefore has considerable potential as a nutraceutical ingredient that could be incorporated into functional food and beverage products. However, as with other carotenoids, there are a number of challenges that limit its potential application in foods, such as its low water-solubility, high melting point, and chemical instability (Hii, Choong, Woo, & Wong, 2010; Muthuirulappan & Francis, 2013).

Food grade nanoemulsions, which consist of small lipid droplets (<100 nm) dispersed in water, may be a suitable strategy for incorporating carotenoids into foods and of enhancing their bioactivity profiles (McClements, 2011; McClements & Xiao, 2012). Indeed, it has been reported that the administration of lipophilic bioactive compounds within a lipid matrix favors their incorporation into mixed micelles and therefore enhances their bioavailability in the gastrointestinal tract (Donhowe & Kong, 2014). When the lipase hydrolyses the triacylglycerols, the free fatty acids released form mixed micelles that solubilize and transport the lipophilic bioactive compounds (Yonekura & Nagao, 2007). Nevertheless, the micelle solubilization of lipophilic active compounds in the small intestine is driven by the intrinsic characteristics of the lipid nanoparticles that they are included in. For example, studies have shown that carotenoid (β-carotene) bioaccessibility increases with decreasing droplet size in emulsion-based delivery systems, which was attributed to faster and more complete digestion of the lipid phase leading to greater release and higher solubilization (Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013a). In addition, the nature of the lipid phase used to prepare the nanoemulsions has also been shown to have a major impact on the bioaccessibility of lipophilic bioactive compounds. Specifically, the triacylglycerol chain length determines the micelle structure and size and in turn the bioactive compounds' bioaccessibility in the gastrointestinal tract (Huo, Ferruzzi, Schwartz, & Failla, 2007). The use of long chain triacylglycerol oils for the formation of nanoemulsions led to a higher β-carotene bioaccessibility compared to medium or short chain triglycerides (Qian, Decker, Xiao, & McClements, 2012; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013b). Consequently, the selection of an appropriate lipid carrier and particle size is critical for designing emulsion-based delivery systems with optimized biological activities.

In vitro methods that simulate gastrointestinal tract (GIT) conditions are often used to assess the potential gastrointestinal fate of emulsion-based delivery systems (Fatouros & Mullertz, 2008; Hur, Lim, Decker, & McClements, 2011; McClements & Li, 2010a; Minekus et al., 2014). *In vitro* methods are particularly useful to rapidly and cheaply screen delivery systems with different characteristics, and therefore avoid the use of time-consuming, costly, and ethically challenging animal studies. Moreover, *in vitro* experiments enable one to identify critical physicochemical processes that may influence the performance of delivery systems under gastrointestinal conditions, such as the integrity, interactions, and release characteristics of colloidal particles in different regions of the GIT. However, *in vitro* models cannot mimic the complex physicochemical and physiological processes occurring in the digestive tracts of animals or humans (Park et al., 2007). To obtain more reliable and accurate information about the potential biological activity of functional ingredients encapsulated within delivery systems it is therefore important to compare the results of *in vitro* models with those of *in vivo* studies (Lee et al., 2008; Ostrowski & Baczek, 2010; Porter et al., 2004).

The purpose of the current work was therefore to study the influence of lipid carrier type on the biological activity of fucoxanthin-loaded nanoemulsions. Corn oil was used as an example of a digestible long chain triacylglycerol (LCT), Miglyol was used as an example of a digestible medium chain triacylglycerol (MCT), and a mixture of orange oil and mineral oil (80:20) was used as an example of a non-digestible oil. The results obtained using a well-established *in vitro* gastrointestinal model were compared with those obtained using an *in vivo* animal (rats) feeding model. Ultimately, the goal of this work was to provide important insights into the major factors influencing the design of nanoemulsion-based delivery systems to improve the oral bioavailability of lipophilic nutraceuticals.

2. Materials and methods

2.1. Materials

Corn oil (long chain triacylglycerol, LCT) was purchased from a local supermarket. Miglyol 812 (medium chain triacylglycerol, MCT) was purchased from SASOL (Houston, TX, USA). Orange flavor oil was obtained from The Chemistry Store (Cayce, SC, USA). Mineral oil, Tween 80, monobasic and dibasic phosphates, and Nile Red dye were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pepsin, bile salts and lipase were also obtained from Sigma. Fucoxanthin enriched MCT (1% w/w fucoxanthin) oil was bought from Restore Labs Co, (Gangneung, Korea). Triheptadecanoin ($C_{17:0}$) and tridecanoic acid ($C_{13:0}$) were purchased from Nu-Chek Prep Inc. (Elysian, MN, USA). All aqueous solutions were prepared using purified water from a Mili-Q filtration system.

2.2. Methods

2.2.1. Nanoemulsion formation

A lipid phase was prepared by mixing a 10% (v/v) of fucoxanthin-enriched MCT oil with 90% (v/v) of carrier oil (LCT, MCT, or non-digestible oil). The non-digestible oil consisted of a mixture of orange oil and mineral oil (80:20 v/v). Mineral oil was used as a ripening inhibitor to prevent Ostwald ripening from occurring in the nanoemulsion containing orange oil. Heptadecanoic ($C_{17:0}$) acid at 0.1% (w/w) was dissolved in the lipid phase by stirring until complete dissolution was achieved. We included heptadecanoic ($C_{17:0}$) acid in the nanoemulsions as a model fatty acid since it is not normally found in the animal's body, and therefore an increase in its concentration in small intestine tissues is a measure of its absorption. The final fucoxanthin concentration in the initial nanoemulsions was