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Encapsulation of omega-3 fatty acids in nanoemulsions and microgels: Impact of delivery system type and protein addition on gastrointestinal fate

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ABSTRACT

Carefully designed delivery systems are required to encapsulate and protect omega-3 fatty acids in commercial food and beverage products, but then release them at the required site-of-action within the human gastrointestinal tract (GIT). Previously, we showed that the oxidative stability of flaxseed oil (a plant-based source of omega-3 fatty acids) encapsulated in nanoemulsion droplets or calcium alginate microgels (hydrogel beads) was improved using caseinate as a natural antioxidant. In this study, the impact of caseinate on the digestion of flaxseed oil encapsulated in these delivery systems was investigated using a simulated GIT. The flaxseed oil was incorporated into four delivery systems: nanoemulsions (*NE*); nanoemulsions mixed with caseinate (*NE* + *C*); hydrogel beads (*HB*); and, hydrogel beads containing caseinate (*HB* + *C*). The gastrointestinal fate of the flaxseed oil droplets depended on delivery system type and the presence of protein. The flaxseed oil in the nanoemulsions (*NE* and *NE* + *C*) was rapidly hydrolyzed within the simulated small intestine, with over 76% and 65% of free fatty acids (FFAs) being released in the first 5 minutes, respectively. Conversely, the flaxseed oil in the hydrogel beads (*HB* and *HB* + *C*) was digested much more slowly, with only around 37% and 22% being released in the same period. This knowledge may be useful for designing delivery systems to protect omega-3 fatty acids from oxidation in functional foods, while still allowing them to be released in the GIT.

1. Introduction

Nanoemulsion-based delivery systems (NEDS) are being developed to facilitate the incorporation of hydrophobic bioactives into foods, supplements and pharmaceuticals, to protect the bioactives from degradation during storage, and then to release the bioactives at an appropriate site within the gastrointestinal tract (GIT) after oral ingestion (McClements, Decker, & Park, 2009; Porter, Wasan, & Constantinides, 2008; Singh, Ye, & Horne, 2009). The structural complexity of NEDS can be designed to obtain specific functional attributes (Livney, 2015; McClements, 2012). Nanoemulsions are the basic building blocks of all NEDS, and typically consist of emulsifier-coated lipid droplets dispersed within an aqueous solution (Donsi, Sessa, Mediouni, Mgaidi, & Ferrari, 2011). The incorporation of hydrophobic bioactives into nanoemulsions is therefore one of the simplest and most cost-effective ways of controlling their stability and bioavailability (McClements & Xiao, 2012). However, for certain applications simple nanoemulsions are unable to provide the desired functional attributes, *e.g.*, the encapsulated bioactive may be released or degraded too rapidly during storage or within the GIT. There has therefore been growing interest in extending the functional attributes of nanoemulsions by trapping the small lipid droplets them inside biopolymer microgels so as to enhance the storage stability or modulate the gastrointestinal fate of the encapsulated bioactives (Dordevic et al., 2015; McClements, 2015; Torres, Murray, & Sarkar, 2016).

Biopolymer microgels are typically fabricated from proteins and/or polysaccharides using a variety of methods, including injection, phase separation, templating, and molding methods (Norton & Frith, 2001; Shewan & Stokes, 2013; Zhang, Zhang, Chen, Tong, & McClements, 2015). Many of these methods utilize two sequential processes: particle formation and then particle gelation (McClements, 2017). Typically, a mixture of lipid droplets and biopolymer solution is made to form a particle, and then the system conditions are altered to promote biopolymer gelation (Joye & McClements, 2014; Shewan & Stokes, 2013). Previously, nanoemulsion-loaded biopolymer microgels have been designed to protect bioactives during long-term storage (Chen et al.,

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2017), and then release them within the mouth (Zhang, Zhang, Decker, & McClements, 2015; Zhang, Zhang, Tong, Decker, & McClements, 2015), stomach (Liang, Line, Remondetto, & Subirade, 2010), small intestine (Zhang et al., 2016) or colon (Sookkasem, Chatpun, Yuenyongsawad, & Wiwattanapatapee, 2015). The release of the bioactives can be controlled by designing the composition and structure of the delivery system to respond to a specific environmental trigger, such as pH, ionic strength, temperature, or enzyme activity (Zhang, Zhang, Chen et al., 2015).

In a recent study, we showed that the stability of flaxseed oil to oxidation during storage could be greatly improved by encapsulating it within nanoemulsion-loaded alginate microgels (Chen et al., 2017). Moreover, it was shown that the addition of caseinate to these NEDS improved their oxidative stability during storage by acting as a natural antioxidant. In the current study, we therefore focused on the impact of microgel encapsulation and caseinate incorporation on the digestion of flaxseed oil using a simulated GIT. Our main objective was to determine whether encapsulation or caseinate would impact the digestion and release of omega-3 fatty acids from the delivery systems. This is important because a bioactive agent will only be able to demonstrate its beneficial effects if it is actually released within the GIT.

2. Materials and methods

2.1. Materials

Flaxseed oil was used as a plant-based source of omega-3 fatty acids, and was purchased from a local supermarket. The manufacturer reported that the flaxseed oil contained about 7.1%, 14.3%, and 71.4% (w/w) of saturated, monounsaturated, and polyunsaturated fats, respectively. Quillaja saponin (Q-Naturale 200, MW ~1650 g/mol) was used as a plant-based emulsifier, and was kindly donated by National Starch LLC (Bridgewater, N.J.). The form of quillaja saponin used was provided as an aqueous solution that contained approximately 78–80% water, with 65–75% of the dried material being saponin. The other components in the dried material were reported to be carbohydrates. Powdered sodium caseinate was used as a natural dairy-based antioxidant, and was purchased from Biomedicals LLC (Solon, OH). The following chemicals were obtained from Sigma Chemical Company (St. Louis, MO): sodium alginate (viscosity of 1% dissolved in water is 15–20 mPa s, MW 120–190 kDa); Nile red (N3013-100MG); fluorescein thiocyanate isomer I (FITC); mucin from porcine stomach (M2378-100G); pepsin from porcine gastric mucosa (P7000-25G); lipase from porcine pancreas pancreatin (L3126-100G); and porcine bile extract (B8631-100G). Double distilled water (DDW) from a water purification system (Nanopure Infinity, Barnstead International, Dubuque, IA) was used to prepare all aqueous solutions. All concentrations are expressed on a percentage weight-by-weight (%w/w) basis unless otherwise stated.

2.2. Fabrication of nanoemulsions

Initially, coarse oil-in-water emulsions were prepared by blending 10% oil phase (flaxseed oil) and 90% aqueous phase (7.1% Q-Naturale 200, 5 mM phosphate buffer, pH 7.0) using a high-shear mixer for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). This coarse emulsion was then homogenized further by passing it through a high-pressure microfluidizer (Microfluidics Microfluidizer M-110Y, Newton, MA) three times at an operating pressure of 12,000 psi. The omega-3 fatty acid-loaded nanoemulsions produced were then stored in a refrigerator at 4 °C prior to utilization. The composition and fabrication procedure used to fabricate the nanoemulsions in this study was based on recent experiments carried out in our laboratory (Chen et al., 2017). It should be noted that Q-Naturale 200 only contains about 14.5% of active ingredient (saponins), so that the actual emulsifier concentration in the aqueous phase was around 1.0 wt%.

2.3. Fabrication of nanoemulsion-loaded biopolymer microgels

Biopolymer microgels were fabricated using a mechanical encapsulation instrument (Encapsulator B-390, BUCHI, Switzerland) with a vibrating nozzle according to the method described in our previous paper (Chen et al., 2017). The operating parameters of the encapsulator used to prepare the microgels were as follows: the vibrating nozzle diameter was 120 μm; the electrode potential was 800 V; and, the operating frequency was 800 Hz.

An alginate solution (1.2%) was prepared by dispersing powdered sodium alginate in phosphate buffer solution (5 mM, pH 7.0) and then stirring overnight at ambient temperature. An alginate-caseinate solution was prepared by dispersing powdered sodium alginate (1.2%) and sodium caseinate (3%) into phosphate buffer solution and stirring for 12 h at ambient temperature. Nanoemulsions were then mixed with alginate or alginate-caseinate solutions so that the final system contained 2% flaxseed oil, 0.8% alginate, and either 0 or 2% caseinate. Any air bubbles in these mixtures were removed by placing then in an ultrasonic bath for 10 min. The encapsulator was then used to inject the nanoemulsion-biopolymer mixtures into a 10% calcium chloride solution that was being stirred continuously. The beads were kept in the calcium chloride solution for 30 min at ambient temperature to allow cross-linking to occur. The resulting hardened microgels were collected by filtration and then washed with distilled water to remove any excess Ca^{2+} from their surfaces. The flaxseed oil content within the microgels was calculated using the method described previously (Chen et al., 2017).

2.4. Simulated gastrointestinal tract

A GIT model that simulated the mouth, stomach, and small intestine stages of the human digestive tract was used to follow the potential gastrointestinal fate of the delivery systems. This model has been described in detail in previous studies carried out in our laboratory, and so only a brief summary is given below (Zhang, Zhang, Zhang, Decker, & McClements, 2015). It should be noted that this GIT model is closely related to the standardized international consensus method that has recently been described by Minekus et al. (2014).

All samples were preheated to 37 °C prior to mixing, and were maintained at this temperature with constant swirling (100 rpm) throughout the simulated GIT process using an incubator (Innova Incubator Shaker, Model 4080, New Brunswick Scientific, Edison, NJ, USA).

2.4.1. Initial system

Nanoemulsions and nanoemulsion-loaded biopolymer microgels with the same total flaxseed oil level (0.15 g) and same total mass (7.5 g) were prepared by diluting the initial samples with phosphate buffer solution (5 mM, pH 7.0). The samples were then placed in the stirring incubator at a rotation speed of 100 rpm for 15 min.

2.4.2. Mouth phase

The initial system (7.5 g) was mixed with simulated saliva fluid (7.5 g) containing 3% mucin. After being adjusted to pH 6.8, the mixture was placed in the incubator for 2 min to mimic oral conditions. It should be noted that mechanical mastication (simulated chewing) was not applied to the samples, which may be important in humans. However, the size of the beads was relatively small, and therefore they are more likely to be swallowed than chewed.

2.4.3. Stomach phase

The sample obtained from the mouth phase (15 g) was mixed with simulated gastric fluid (15 g) containing 0.32% pepsin. After being adjusted to pH 2.5, the mixture was placed in the incubator for 2 h to mimic gastric conditions. The samples were subjected to constant swirling throughout incubation in the stomach phase. In reality, they