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Influence of emulsifier type on gastrointestinal fate of oil-in-water emulsions containing anionic dietary fiber (pectin)

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ABSTRACT

The influence of emulsifier type and dietary fiber addition on the gastrointestinal fate of emulsified lipids was investigated using a simulated gastrointestinal tract (GIT): mouth; stomach; small intestine. The emulsions tested contained lipid droplets coated with different emulsifiers (sodium caseinate, Tween 80, or lactoferrin), as well as different initial levels of low methoxy pectin (0%, 0.025% and 0.5% w/w). In the absence of pectin, the initial rate of lipid digestion depended strongly on emulsifier type: being 20.5, 18.6, and 6.4 %FFA min^{-1} for Tween 80, lactoferrin, and caseinate, respectively. However, complete lipid digestion occurred for all these emulsions by the end of the small intestine phase. The slower initial rate of lipid digestion in the caseinate-stabilized emulsions was attributed to extensive droplet flocculation in the gastric phase, which would restrict the access of lipase to lipid droplet surfaces. Pectin addition increased the rate of lipid digestion in caseinate-stabilized emulsions (*e.g.*, by 100% for 0.025% pectin), which was attributed to its ability to suppress droplet flocculation. Conversely, high levels of pectin in the Tween 80- and lactoferrin-stabilized emulsions decreased the initial rate of lipid digestion (*e.g.*, by >35% for 0.5% pectin), possibly due to calcium binding or gel forming effects. These results indicate that the rate and extent of lipid digestion can be controlled by using different emulsifiers or by adding dietary fibers, which may be useful information for the rational design of functional foods and beverages.

1. Introduction

Oil-in-water emulsions are utilized in numerous commercial foods and food ingredients, including desserts, dips, ice cream, coffee, sauces, dressings, beverages, soups, and delivery systems (Cho et al., 2014; McClements, 2005, 2010; McClements & Li, 2010a). Different types of emulsifiers are often used to stabilize these products, including surface-active proteins, polysaccharides, surfactants, and phospholipids (Kralova & Sjoblom, 2009; Krog & Sparso, 2004). The physical, chemical, and biological characteristics of emulsion-based products depend strongly on the type of emulsifier used to stabilize them, and can therefore be tailored by selecting different emulsifiers (Fomuso, Corredig, & Akoh, 2002; Hur, Decker, & McClements, 2009; Mun, Decker, & McClements, 2007; Qian & McClements, 2011; Thanasukarn, Pongsawatmanit, & McClements, 2004). Traditionally, food scientists were primarily interested in the influence of emulsifiers on the stability and bulk physicochemical properties of emulsions prior to consumption, but more recently there has been increasing interest in the fate of emulsions after ingestion (Hur et al., 2009; Li, Hu, & McClements, 2011; Malaki Nik, Wright, & Corredig, 2011; McClements & Li, 2010a, 2010b; Mun et al., 2007). The main driving force for this research is to understand the role of emulsion composition and structure on their gastrointestinal fate, with the assumption that this knowledge is useful for the design of foods and beverages with improved nutritional quality.

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Commercial food products contain a variety of ingredients that can potentially alter the gastrointestinal fate of emulsions, including lipids, proteins, carbohydrates, and minerals, (Gidley, 2013; Golding & Wooster, 2010; Li, Hu, Du, Xiao, & McClements, 2011; Li et al., 2010; Li & McClements, 2014; Mackie & Macierzanka, 2010; Maldonado-Valderrama, Gunning, Ridout, Wilde, & Morris, 2009; Mun, Decker, Park, Weiss, & McClements, 2006; Singh & Sarkar, 2011; Singh, Ye, & Horne, 2009; Torcello-Gómez, Maldonado-Valderrama, Martín-Rodríguez, & McClements, 2011). Dietary fibers are indigestible polysaccharides that are widely used in emulsion-based food products due to their role as stabilizers, texturizers, and health-promoting ingredients (Dikeman & Fahey, 2006; Eastwood & Morris, 1992; Elleuch et al., 2011; Li & McClements, 2014). Previous researchers have focused on the impact of various dietary fibers on the potential gastrointestinal fate of food emulsions, such as alginate, pectin, and chitosan (Beysseriat, Decker, & McClements, 2006; David-Birman, Mackie, & Lesmes, 2013; Gidley, 2013; Klinkesorn & McClements, 2009; Li, Hu, Du, et al., 2011; Li et al., 2010; Li & McClements, 2014; Pasquier et al., 1996; Tokle, Lesmes, Decker, & McClements, 2012; Torcello-Gómez et al., 2011; Verrijssen et al., 2014). These studies have highlighted a number of potential physicochemical mechanisms by which dietary fibers could alter the lipid digestion process: formation of dietary fiber coatings around lipid droplets that inhibit adsorption of bile salts or lipase; promotion of droplet flocculation, which reduces the surface area of lipids easily assessable to lipase; binding of calcium ions or bile salts, which reduces their ability to remove free fatty acids from lipid droplet surfaces; inactivation of lipase through molecular complexation; alterations in mixing and mass transfer processes due to changes in solution rheology. Research is needed to identify the most important physicochemical mechanisms for specific systems.

Most of the previous studies mentioned above used simulated gastrointestinal tract (GIT) models that only mimicked the small intestine stage of lipid digestion. However, the mouth and stomach stages may also have an appreciable effect on the GIT fate of emulsions due to their influence on the structural organization and interfacial composition of lipid droplets (Hur et al., 2009). There is therefore a need to use more realistic *in vitro* models to study the influence of different factors on the gastrointestinal fate of ingested lipids (McClements & Li, 2010a; Minekus et al., 2014; Vors et al., 2012). These models should accurately simulate the most important aspects of *in vivo* GIT processing (such as chemical compositions, enzyme activities, temperature, and flow profiles), while being simple enough to routinely carry out in a research laboratory. Nevertheless, the results of *in vitro* digestion studies should always be validated using appropriate *in vivo* methods.

In the current study, we examined the influence of emulsifier type (sodium caseinate, Tween 80, and lactoferrin) and dietary fiber addition (low methoxy pectin) on the gastrointestinal fate of oil-in-water emulsions using a simulated GIT that includes the mouth, stomach, and small intestine phases. These emulsifiers were selected because they have distinctly different molecular characteristics: caseinate is a flexible protein with isoelectric point around pH 5 (pI $\approx$ 5); lactoferrin is a globular protein with pI $\approx$ 8; and Tween 80 is a non-ionic surfactant. We anticipate that lipid droplets coated by different emulsifiers would interact differently with anionic dietary fibers, as well as with other charged constituents in the GIT, such as digestive enzymes, calcium, and bile salts (Beysseriat et al., 2006). Our overall hypotheses are therefore that emulsifier type and dietary fiber addition will alter the GIT fate of emulsified lipids. An improved understanding of the role of emulsion composition and structure on their gastrointestinal fate could lead to the design of functional foods and beverages with improved health benefits.

2. Materials and methods

2.1. Materials

Powdered lactoferrin (Lot# 10404498) was obtained from Friesland Campina Domo (Delhi, NY). The manufacturer reported that the protein and ash content of this powder were 97.7.4% and 0.12%, respectively. Powdered sodium caseinate was purchased from the American Casein Company (Burlington, NJ). The manufacturer reported that the protein and moisture content of the powder were 91.4% and 5.0%, respectively. Tween 80 (Lot# MKBL8329V) was obtained from Sigma-Aldrich (Sigma Chemical Company, St. Louis, MO). Corn oil was purchased from a commercial food supplier (Mazola, ACH Food Companies, Memphis, TN). As stated by the manufacturer, the saturated, monounsaturated, and polyunsaturated fat content of this product were approximately 14, 29, and 57%, respectively. Low-methoxyl (LM) pectin was kindly donated by CP Kelco (Lille Skensved, Denmark). Pepsin from porcine gastric mucosa and lipase were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO) and as reported by the manufacturer their activity were 250 units/mg and 100–400 units/mg, respectively. Mucin from porcine stomach, porcine bile extract, sodium chloride, calcium chloride, monobasic phosphate and dibasic phosphate were obtained from either Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). All solvents and reagents were of analytical grade. Double distilled water from a water purification system (Nanopure Infinity, Barnstaeas International, Dubuque, IA) was used for preparation of all solutions.

2.2. Solution preparation

Emulsifier solutions were prepared by dispersing 1.0 wt% sodium caseinate, lactoferrin, or Tween 80 into 5 mM phosphate buffer (pH 7.0) solution and stirring for at least 2 h. The emulsifier solutions were then stored overnight at 4 °C to ensure complete hydration. The lactoferrin solution was filtered by qualitative filter (Fisher Scientific, PA) to remove any insoluble particles before further use. A LM-pectin solution (4.0 wt%) was prepared by dispersing weighed amounts of powdered pectin into 5 mM phosphate buffer solution (pH 7.0), and stirring for at least 3 h to ensure full dissolution. The pH of the solutions was then adjusted back to pH 7.0 using either NaOH or HCl if required.

2.3. Emulsion preparation

Stock emulsions were prepared by homogenizing 10 wt% oil phase (corn oil) with 90 wt% aqueous phase (1.0 wt% emulsifier solution, 5 mM phosphate buffer, pH 7.0) using a high-speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). The resulting coarse emulsions were then passed through a high pressure homogenizer (M110Y, Microfluidics, Newton, MA) with a 75 μm interaction chamber (F20Y) at an operational pressure of 11,000 psi for 3 passes. Some flocculation was observed in the lactoferrin-stabilized emulsions after one-night storage at 4 °C, and therefore these systems were passed through the high pressure homogenizer again using the same conditions to disrupt the flocs. Dietary fiber-free emulsions (controls) were formed by diluting the stock emulsions with 5 mM phosphate buffer (pH 7.0) to obtain a final corn oil concentration of 2.0 wt%.

Emulsions containing dietary fibers were formed by diluting the stock emulsions with LM-pectin solution and buffer solution followed by stirring for 30 min. The final compositions of these systems were 2% wt% oil and either 0.025 or 0.5 wt% LM-pectin.