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Influence of dietary fibers on lipid digestion: Comparison of single-stage and multiple-stage gastrointestinal models

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ABSTRACT

Simulated gastrointestinal tract (GIT) models are commonly used to establish the major factors influencing lipid digestion. In this study, two widely used static *in vitro* digestion models were compared for their ability to monitor the impact of dietary fibers on lipid digestion: a single-stage (small intestine) and a multiple-stage (mouth, stomach, small intestine) model. The impact of three dietary fibers with different electrical characteristics on the digestion of protein-coated lipid droplets was studied: cationic chitosan; anionic alginate; neutral locust bean gum (LBG). The particle size, particle charge, microstructure, rheology, and lipid digestion rate were measured. The GIT fate of the lipid droplets depended on dietary fiber type, with extensive droplet aggregation being induced upon fiber addition due to either depletion or bridging mechanisms. The microstructure and electrical characteristics of emulsions containing dietary fibers measured after the small intestine phase were fairly similar for the single- and multiple-stage GIT models, whereas the rate of lipid digestion was appreciably different. In the single-stage model the total amount of free fatty acids produced in the small intestine phase decreased in the following order: control (83%) ≈ LBG (87%) > chitosan (72%) > alginate (59%). However, in the multiple-state model the total amounts of free fatty acids produced were fairly similar for all fibers (83–94%). This study highlights the importance of selecting an appropriate simulated GIT model to examine the potential gastrointestinal fate of food, pharmaceutical, and feed systems.

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1. Introduction

Simulated gastrointestinal tract (GIT) models are particularly useful for rapidly screening the potential performance of different food and pharmaceutical formulations after ingestion, as well as for establishing the physicochemical phenomena that govern their behavior (Bornhorst, Ferrua, & Singh, 2015; Kostewicz et al., 2014; McClements & Li, 2010; Minekus et al., 2014). These GIT models vary greatly in their sophistication and their ability to accurately mimic gastrointestinal processes. Highly sophisticated dynamic methods, such as the TIM gastrointestinal systems from TNO (The Netherlands), provide detailed information about the performance of foods and pharmaceuticals under kinetic conditions that are designed to closely simulate human GIT conditions (Havenaar et al., 2013; Kostewicz et al., 2014; Yoo & Chen, 2006). However, these instruments are relatively expensive, time-consuming, and laborious to operate, and therefore it is difficult to rapidly screen many different samples. Conversely, simple static methods have also been developed to mimic certain aspects of the GIT, which are not as sophisticated, but that are relatively inexpensive, rapid, and easy to use. These static methods are particularly suitable for quickly screening many different samples and for analyzing changes in the properties of samples after exposure to each stage of the GIT model (Fu et al., 2015; McClements and Li, 2010; Minekus et al., 2014; Sarkar, Goh, & Singh, 2010; Williams et al., 2012). For these reasons, they have found widespread utilization within the food, nutrition, and pharmaceutical areas.

Even static methods can vary appreciably in their degree of

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sophistication. Some researchers have focused solely on the small intestine phase since this is where the majority of macronutrient digestion and absorption occurs, and therefore they have used static models that only simulate this region of the GIT (Ahmed, Li, McClements, & Xiao, 2012; Zangenberg, Mullertz, Kristensen, & Hovgaard, 2001). On the other hand, other researchers have used static methods that simulate the mouth, stomach, and small intestine phases, since it is postulated that passage of a sample through the mouth and stomach will appreciably alter its subsequent behavior in the small intestine (Mao & McClements, 2012; Minekus et al., 2014; Williams et al., 2012). As well as varying in the number of GIT stages included, static models may also vary in the incubation times and mechanical forces used in the different stages, as well as in the types and levels of gastrointestinal components included, such as enzymes, bile salts, phospholipids, minerals, buffers, acids and bases (McClements and Li, 2010; Minekus et al., 2014). It is therefore useful to compare the results obtained from the single-stage static model (small intestine) with the multiple-stage static model (mouth, stomach, and small intestine) under similar test conditions.

In this study, we examined the influence of three dietary fibers with different electrical characteristics on the GIT fate of protein-coated lipid droplets: cationic chitosan; anionic alginate; and neutral locust bean gum (LBG). Numerous previous studies have used static digestion models to show that dietary fibers influence the rate and extent of lipid digestion under simulated GIT conditions (Beysseriat, Decker, & McClements, 2006; Chang & McClements, 2016; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, Narvaez-Cuenca, & McClements, 2014; Espinal-Ruiz, Restrepo-Sanchez, Narvaez-Cuenca, & McClements, 2016; Hu, Li, Decker, & McClements, 2010a; Klinkesorn & McClements, 2009; Li & McClements, 2011b; Mao and McClements, 2012; Torcello-Gomez & Foster, 2016; Verrijssen, Verkempinck, Christiaens, Van Loey, & Hendrickx, 2015). The results from these studies have often been inconsistent depending on the nature of the dietary fibers and test methods used. Hu et al. (2010a) reported that anionic alginate greatly inhibited the digestion of protein-coated lipid droplets using a single-stage model (small intestine), whereas this phenomenon was not observed when a multiple-stage model (stomach and small intestine) was used (Tokle, Lesmes, Decker, & McClements, 2012). Conversely, cationic chitosan was found to inhibit lipid digestion in both single-stage and multiple-stage models, which was attributed to a variety of physicochemical mechanisms, such as lipid droplet coating (Beysseriat et al., 2006; Rodriguez & Albertengo, 2005; Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013), electrostatic binding of minerals, phospholipids, fatty acids or bile salts (Hu, Li, Decker, Xiao, & McClements, 2010b; Li, Hu, Du, & McClements, 2011a), enzyme inhibition (Mun, Decker, Park, Weiss, & McClements, 2006; Tzoumaki et al., 2013), and viscosity regulation (Kanauchi, Deuchi, Imasato, Shizukuishi, & Kobayashi, 1995).

In this paper, we compared single-stage (small intestine) and multiple-stage (mouth, stomach, and small intestine) GIT models for analyzing the impact of the three dietary fibers on the digestion of protein-coated lipid droplets. This comparison will help to identify any potential limitations of using the simple single-stage model for studying the impact of dietary fibers on lipid digestion. Three polysaccharides with different charge characteristics were used so that they might highlight any differences between the single- and multiple-stage models. In addition, we measured various structural and physicochemical properties (*e.g.,* microstructure, charge, and rheology) of the samples as they passed through the simulated GIT to obtain further insights into the key mechanisms by which different types of dietary fiber influence lipid digestion. The information obtained in this study may therefore be useful for designing functional foods that can control the gastrointestinal fate of lipids, as well as for selecting the most appropriate static GIT model to screen different formulations.

2. Materials and methods

2.1. Materials

All concentrations are expressed on a weight percentage basis (wt%), unless otherwise stated. Corn oil (containing 99 wt% triacylglycerols, with approximately 58.7 wt% polyunsaturated fatty acid, 28.7 wt% monounsaturated fatty acid, and 12.6 wt% saturated fatty acid) was purchased from a local commercial food supplier (Mazola, ACH Food Companies, Inc., Memphis, TN). Powdered β-lactoglobulin (97.4 wt% total protein, 92.5 wt% β-lactoglobulin, 2.4 wt% ash) was supplied by Davisco Food International (Lot #JE 001-0-415, Le Sueur, MN). Low molecular weight chitosan (≈50–190 kDa, 75–85% deacetylation), alginic acid (from brown algae), locust bean gum (≈310 kDa), and hydrochloric acid (HCl) were obtained from Sigma-Aldrich (St Louis, MO). Bile extract (porcine, B8613) and lipase from porcine pancreas (activity 2.0 USP units/mg, Type II, L3126) were also obtained from Sigma-Aldrich. The bile extract ($Ca^{2+} \leq 0.06$ wt%; critical micelle concentration of bile extract 0.07 ± 0.04 mM) has been reported to contain 49 wt% total bile salt (BS, 10–15 wt% glycodeoxycholic acid, 3–9 wt% taurodeoxycholic acid, 0.5–7 wt% deoxycholic acid, 1–5 wt% hydrodeoxycholic acid, and 0.5–2 wt% cholic acid) and 5 wt% phosphatidyl choline, with the mole ratio of BS to PC being about 15:1. Sodium hydroxide (NaOH) and calcium chloride ($CaCl_2 \cdot 2H_2O$) were purchased from Fisher Scientific. Double distilled water produced by a water purification system (Nanopure Infinity, Barnstead International, Dubuque, IA) was used for the preparation of all solutions.

2.2. Solution and emulsion preparation

2.2.1. Polysaccharide stock solutions

Chitosan stock solution (2 wt%) was prepared by dispersing 4 g of powdered chitosan into 196 g of 10 mM acetate buffer (pH 3). The solution was then stirred for 12 h at room temperature (850 rpm) and then sonicated for 2 min (frequency of 20 kHz, amplitude of 40%, and duty cycle of 0.5 s) using a commercial sonicator (Model 500, Sonic Disembrator, Fisher Scientific, Pittsburgh, PA) to ensure complete dissolution. Alginate and LBG stock solutions (2 wt%) were prepared by solubilizing 4 g of alginate and locust bean gum powders into 196 g of double distilled water; both of these solutions were then stirred overnight (850 rpm, 25 °C). Each solution was then stored at 4 °C and equilibrated at ambient temperature for 10 min before use.

2.2.2. Preparation of primary emulsion

A coarse emulsion was prepared by homogenizing 5 wt% corn oil with 95 wt% aqueous emulsifier solution (1 wt% β-lactoglobulin, 5 mM phosphate buffer, pH 7) using a high shear mixer (Speed 2, Model MW140/2009-5, Biospec Products Inc., ESGC, Switzerland). The coarse emulsion obtained was then passed 5 times through a high-pressure homogenizer (Microfluidizer M-110L processor, Microfluidics Inc., Newton, MA) operating at 9000 psi (≈62.1 MPa) to reduce the particle size further. The emulsion containing no dietary fibers was referred to as the “primary emulsion”.

2.2.3. Preparation of secondary emulsions

The mixture of the primary emulsion and dietary fiber was referred to as a “secondary emulsion”. For the multiple-stage model, aliquots of polysaccharide stock solution (2 wt% chitosan,

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