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Simulated gastrointestinal fate of lipids encapsulated in starch hydrogels: Impact of normal and high amylose corn starch

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ABSTRACT

The influence of starch type (resistant starch (RS) *versus* native (NS) starch) and concentration (10 and 35 wt.%) on the potential gastrointestinal fate of digestible lipid (corn oil) droplets encapsulated within starch hydrogels was studied using a simulated gastrointestinal tract (GIT). The NS used was a normal corn starch, whereas the RS used was a high amylose corn starch. Changes in morphology, organization, size, and charge of the particles in the delivery systems were measured as they passed through each stage of the GIT model: mouth, stomach, and small intestine. The GIT fates of three types of delivery system were compared: free lipid droplets; lipid droplets in RS-hydrogels; and, lipid droplets in NS-hydrogels. Encapsulation of the lipid droplets in the hydrogels had a pronounced influence on their GIT behavior, with the effect depending strongly on starch type. The starch granules in the RS-hydrogels remained intact throughout the simulated GIT because their compact structure makes them resistant to enzyme digestion. The initial rate of lipid digestion in the small intestine phase also depended on delivery system type: emulsion > RS-hydrogels > NS-hydrogels. However, the lipid phase appeared to be fully digested at the end of the digestion period for all samples. These results provide useful information for designing functional foods for improved health. For example, food matrices could be developed that slowdown the rate of lipid digestion, and therefore prevent a spike in serum triacylglycerols in the blood, which may be advantageous for developing functional foods to tackle diabetes.

1. Introduction

An increased awareness by consumers about the relationship between a nutritious diet and human health has been one of the major reasons for the increasing popularity of foods enriched with dietary fiber (Aleixandre & Miguel, 2008; Sajilata, Singhal, & Kulkarni, 2006; Viuda-Martos et al., 2010; Warrand, 2006). Dietary fibers are defined as those polysaccharides that are indigestible within the upper part of the human gastrointestinal tract (GIT), such as cellulose, pectin, gums, and resistant starch (Biliaderis & Izydorczyk, 2007; Cui, 2005). The incorporation of dietary fibers into foods also influences their physicochemical properties and sensory attributes due to their water holding, oil holding, lightening, emulsifying, thickening and/or gelling properties (Elleuch et al., 2011; Santipanichwong & Suphantharika, 2009; Winuprasith & Suphantharika, 2015). Thus, these effects must be taken into account when formulating functional food products enriched with dietary fibers otherwise consumers will reject them.

Starch has been divided into three major categories depending on its susceptibility to digestion within the human GIT: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Consumption of RDS typically leads to a rapid increase in blood glucose concentration after ingestion because it is rapidly digested by amylase within the GIT. The increase in blood glucose levels is more gradual for SDS because it is digested at a slower rate, however it is still fully digested within the small intestine. RS is not completely hydrolyzed by enzymes in the small intestine and therefore does not cause a pronounced increase in blood glucose levels, but it will subject to bacterial fermentation in the colon (Englyst et al., 1992; Singh, Dartois, & Kaur, 2010). The hydrolysis of digestible starch begins in the mouth due to the presence of salivary α-amylase, but the majority of hydrolysis takes place within the small intestine due to the presence of pancreatic amylase (Lehmann & Robin, 2007). Many factors have been reported to influence the rate of starch hydrolysis, including botanical source, food processing, particle size and amylose/amylopectin ratio (Benmoussa, Moldenhauer, & Hamaker, 2007; Chung, Liu, Lee, & Wei, 2011; Lehmann & Robin, 2007; Wang & Copeland, 2013). In addition, the ability of amylase to physically interact with the starch molecules in foods is an important factor affecting the rate of starch hydrolysis, which may be impacted by the presence of other food components that can inhibit diffusion or adsorption of the enzyme, such as proteins, lipids, and dietary fibers (Colonna, Leloup, & Buléon, 1992; Singh et al., 2010).

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RS has received much attention due to its potential health benefits, such as inhibiting the onset of colon cancer, diabetes, and obesity, as well as acting as a prebiotic that promotes a healthy colonic microflora (Brouns, Kettlitz, & Arrigoni, 2002; Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Nugent, 2005; Sharma, Yadav, & Ritika, 2008). Moreover, resistant starches containing non-gelatinized granules are often easier to incorporate into functional foods than other sources of dietary fibers because they do not cause such a large increase in viscosity due to their relatively low effective volumes (Baixauli, Salvador, Martinez-Cervera, & Fiszman, 2008; Charalampopoulos, Wang, Pandiella, & Webb, 2002). Resistant starches have been classified into five sub-types according to the major factors limiting their enzymatic degradation: *RS1* — starch granules trapped in non-digestible food matrices (such as in grains, seeds, or tubers); *RS2* — starch granules that have not been gelatinized (such as raw potatoes, unripe bananas, and high amylose starches); *RS3* — retrograded starch formed when foods are cooked and then cooled; *RS4* — chemically modified starches (Nugent, 2005; Sajilata et al., 2006; Sharma et al., 2008); *RS5* — amylose-lipid complexes that inhibit amylase access to starch (Brown, Yotsuzuka, Birkett, & Henriksson, 2006; Jiang, Lio, Blanco, Campbell, & Jane, 2010; Thompson, Maningat, Woo, & Seib, 2011).

There has been increasing interest in using food matrix effects to control the behavior of lipids within the human gastrointestinal tract (Golding & Wooster, 2010; McClements, Decker, & Park, 2009; McClements & Xiao, 2014; Singh & Ye, 2013). This knowledge is being utilized to design delivery systems to encapsulate, protect, and control the release of nutrients and nutraceuticals within the GIT (Kosaraju, 2005; McClements & Li, 2010), as well as to design functional foods to control satiety, satiation, and serum lipid levels (Keogh et al., 2011; Lundin, Golding, & Wooster, 2008; Steingoetter et al., 2015). Studies have reported that dietary fibers may influence the GIT fate of lipids through numerous physicochemical and physiological mechanisms: (i) they may interact with lipase and/or co-lipase, thereby reducing its enzyme activity; (ii) they may adsorb to lipid droplet surfaces and form a protective coating that prevents lipase access; (iii) they may promote or hinder lipid droplet aggregation, thereby altering the amount of lipid surface exposed to lipase; (iv) they may increase the viscosity of the aqueous solution surrounding the lipid droplets, thereby altering mass transport processes; (v) they may trap the lipids within an indigestible food matrix, thereby limiting lipase access (Beysseriat, Decker, & McClements, 2006; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, Narvaez-Cuenca, & McClements, 2014; McClements et al., 2009; Pasquier et al., 1996; Torcello-Gomez & Foster, 2014; Zhang, Zhang, Zhang, Decker, & McClements, 2015). However, the impact of dietary fibers on lipid digestion depends on their molecular structure and physicochemical properties, and therefore has to be established for each type of dietary fiber.

The aim of the current study was to determine the influence of starch type (native *versus* resistant) and concentration on the gastrointestinal fate of lipids encapsulated within starch hydrogels using a simulated GIT that included mouth, stomach and small intestine phases. In this study, normal corn starch was used as an example of a native starch, whereas high amylose corn starch was used as an example of a resistant starch (Jiang et al., 2010). We hypothesized that hydrogels formed from resistant starch would inhibit the digestion of the lipid droplets in the GIT by preventing the lipase molecules from coming into close proximity to the lipid droplet surfaces. To test this hypothesis, changes in the microstructure and physicochemical properties (particle size and charge) of the hydrogel-based delivery systems were measured as they passed through the various phases of the GIT model, and the impact of delivery system properties on the rate and extent of lipid digestion was determined. The information obtained in this study provides knowledge about the influence of starch type on the gastrointestinal fate of starch-based hydrogel delivery systems, which may be useful in the development of functional food products with enhanced health benefits.

2. Materials and methods

2.1. Materials

The starches used in this study were a resistant corn starch (RS) and a native corn starch (NS). The native corn starch was provided by Tate & Lyle (Decatur, IL, USA), while the resistant corn starch (Hi-Maize® 260) was purchased from Ingredion (Bridgewater, NJ, USA). The manufacturer reported that the resistant starch ingredient was derived from high amylose corn starch. Mucin (from porcine stomach), α-amylase (1254 units/mg protein from porcine pancreas), pepsin (from porcine gastric mucosa), pancreatin (from porcine pancreas 8× USP specifications), porcine bile extract, Tween 80 and Nile Red dye were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Corn oil was purchased from a local supermarket. All other chemicals were of analytical grade. Double distilled water was used for the preparation of all solutions and emulsions which was obtained using a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Methods

2.2.1. Emulsion preparation

A stock oil-in-water emulsion was prepared by homogenizing 10 wt.% lipid phase (corn oil) with 90 wt.% aqueous phase (1 wt.% Tween 80 in 10 mM phosphate buffer, pH 7.0) using a high shear blender (M133/1281-0, Biospec Products, Inc., ESGC, Basle, Switzerland) for 2 min. The coarse emulsion was then passed through a microfluidizer (M110Y, Microfluidics, Newton, MA, USA) four times at a homogenization pressure of 12,000 psi.

2.2.2. Starch-hydrogel preparation

An emulsion containing 2 wt.% lipid was prepared by diluting the stock emulsion (10 wt.% lipid) with 10 mM phosphate buffer solution (pH 7.0), and then weighed amounts of starch (10 or 35 wt.% RS or NS) were dispersed into the diluted emulsion and stirred at 400 rpm for at least 10 min to ensure they were homogenous. The emulsion-starch dispersions were then heated at 100 °C for 10 min with continuous stirring at 400 rpm. After heating, the samples were cooled in an ice water bath, and then the samples were stored at 4 °C overnight.

2.2.3. Simulated gastrointestinal tract model

Each sample was passed through a three-step simulated gastrointestinal tract (GIT) model, which included mouth, gastric, and small intestinal phases, which was slightly modified from that used in a previous study (Mun, Kim, & McClements, 2015): the starch-hydrogel samples were crushed into small pieces before samples were exposed to the simulated mouth system to simulate fragmentation during mastication.

2.2.3.1. Mouth phase. Simulated saliva fluid (SSF), containing mucin and various salts, was prepared according to a previous study (Sarkar, Goh, Singh, & Singh, 2009). The digestive enzyme α-amylase was added to the SSF before mixing with the samples. Amylase was added at an activity level of 100 units/mL, which is the average activity reported during mastication (Yamaguchi et al., 2004). The samples (emulsion or starch-hydrogels) were mixed with SSF at a 50:50 mass ratio and the mixture was adjusted to pH 6.8. The mixture was incubated at 37 °C for 10 min with continuous agitation at 100 rpm in a temperature controlled incubator (Innova Incubator Shaker, Model 4080, New Brunswick Scientific, New Jersey, USA).

2.2.3.2. Gastric phase. Simulated gastric fluid (SGF) was prepared using a method reported previously (Sarkar et al., 2009) by dissolving 2 g of NaCl, and 7 mL of HCl (37%) in 1 L of water, adjusting the pH to 1.2 using 1.0 M HCl, and then adding 3.2 g of pepsin. The sample from the mouth phase was mixed with SGF at a 50:50 mass ratio and the pH of the sample was adjusted to 2.5. The sample was then incubated at 37 °C for 2 h with continuous agitation at 100 rpm.