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Simulated intestinal hydrolysis of native tapioca starch: Understanding the effect of soluble fibre

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ABSTRACT

Soluble fibre has been shown to have a reducing effect on plasma glucose levels, which is of particular importance due to the rapidly increasing rates in type 2 diabetes. Researchers speculate that an increase in digesta viscosity is responsible for lowering glucose in the blood; however, the exact mechanism remains to be elucidated. In the present study we examined the effect that adding viscous soluble fibres would have on starch digestibility during simulated intestinal digestion. Tapioca starch was formulated with skim milk powder to act as a control. The treatments consisted of xanthan gum, guar gum, soluble flaxseed gum and soy soluble polysaccharide (SSPS) at constant viscosity. Subsequently, all solutions were exposed to a 3-stage *in vitro* digestion, mimicking the salivary, gastric, and small intestinal steps. Light scattering results showed that the particle size of starch granules decreased as digestion proceeded. Microscopy showed evidence of substantial degradation along the surface of the granules extracted from the control, flax and SSPS solutions upon completion of simulated small intestinal digestion. The progression of these changes was attenuated for granules that were extracted from the guar gum and xanthan gum solutions, which we believe is linked to their rheological behaviour as both solutions had greater viscosities inside the digesta in comparison to the other treatments. The results of this study suggest that the glucose-lowering ability of viscous fibres may be related to their ability to reduce the rate at which starch granules are hydrolysed inside the lumen.

1. Introduction

Starch is a major component of our everyday diet as it is the main energy source in human food. Starch is found encapsulated inside granules in a variety of plant tissues and organs, including roots, leaves, shoots, fruits, grains, and stems (Eliasson, 2004). In grains, it is abundant in rice, maize, barley, wheat, sorghum, pulses and tubers (Dona, Pages, Gilbert, & Kuchel, 2010). Most naturally occurring starch granules consist of two types of polysaccharides, amylose and amylopectin. Both are polymers consisting of α-(1–4)-D-glucose, where amylose is the smaller of the two, with a molecular weight (MW) of approximately 10^6 Da, and is a primarily linear polymer with few branch points along the backbone. Amylopectin on the other hand consists of a large number of short chains bound at the reducing ends via α-1,6-linkages, creating a much larger (MW is approximately 10^8 Da) branched molecule and it makes up approximately 70% of most starch varieties (Takeda, Hizukuri, & Juliano, 1986; Dona et al., 2010). Because both polymers are α-glucans, starch is readily digestible in humans. It is for that reason that starch digestibility has a tremendous impact on human health, where studies have shown that rapidly digestible starch may promote the metabolic syndrome, including insulin resistance and diabetes, due to sugars (glucose) being released and absorbed more quickly (Byrnes, Miller, & Denyer, 1995). Subsequently, many researchers have focused their studies on analysing *in vitro* starch digestibility in order to better understand the link between food consumption and chronic disease.

Although starch digestion is initiated inside the mouth through the action of ptyalin (salivary amylase), the majority of it is carried out in the small intestine where pancreatic α-amylase hydrolyses the polymer into smaller fragments, mainly maltose, maltotriose and α-limit dextrins that are subsequently hydrolysed to glucose through the action of brush-border enzymes, which are exo-glucosidases that act on the non-reducing end of the oligomers, prior to glucose being absorbed (Gray, 1992; Boron & Boulpaep, 2009). In addition to enzymatic hydrolysis being affected by the physicochemical properties of the starch granules themselves, such as amylose/amylopectin ratio, surface porosity, degree of crystallinity and difference in polymorphic form (Varatharajan et al., 2011), incorporation of non-starch polysaccharides, specifically soluble fibres, have been associated with glycemic reductions.

Soluble fibres thicken when they come in contact with

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digestive secretions and it is through this increase in viscosity that soluble fibres are believed to have an effect on glycemic reductions, as shown through both *in vitro* and *in vivo* studies (Dikeman, Murphy, & Fahey Jr., 2006; Brownlee, 2011). Despite accumulating research studies indicating the ability of soluble fibres to decrease the rate of starch degradation, the exact mechanism(s) by which they are able to do so remain unclear. Although studies have been done that have examined how rheological properties might impact starch hydrolysis (Fabek, Messerschmidt, Brulport, & Goff, 2014; Sasaki & Kohyama, 2012), the researchers used sugar release as an indicator of starch digestibility.

Recently, we reported on the ability of different fibres to not only reduce the amount of glucose that is being liberated and diffused throughout simulated digestion, but also the impact they have on lowering reducing sugar release (Fabek & Goff, 2015). The findings of our study indicated that soluble fibre functionality, as it pertains to *in vitro* glycemic reductions, may be multi-faceted through reducing both luminal digestion of starch and the subsequent release/mobility of glucose. However, the study was conducted using quantitative analyses of starch digestion. Brennan (2005) suggested a potential effect of soluble fibre-induced viscosity on altering starch granule digestion. Moreover, interactions of starch with dietary fibre in pasta and cereal products have been shown to reduce starch digestion thereby lowering the glycemic response (Granfeldt & Björck, 1991; Brennan & Tudorica, 2008; Aravind, Sissons, Egan, & Fellows, 2012). However, few studies have examined the rheological properties of fibres during simulated digestion and how they relate to starch degradation and even fewer studies examine the effects of different types of soluble fibres. Although most human foods that contain starch are cooked (gelatinized), cooking can greatly modify, degrade and even destroy granular structure (Dona et al., 2010). Subsequently, most studies using fibre-enriched foods such as cereals, pastas, and breads allow for starch gelatinization, which makes it difficult to visualize the likely changes occurring to the microstructure of starch granules. Despite numerous research studies looking at the effects of dietary fibres on starch digestion, it remains unclear as to how different types of fibre are able to impact the hydrolysis of starch granules and therefore moderate the *in vitro* glycemic response.

This study was undertaken as a follow up to our earlier work, which focused on the effects of soluble fibre inclusion on reducing sugar release (RSR) and glucose liberation (Fabek & Goff, 2015). The aim of the present study is to investigate the effects that viscous fibres, xanthan gum (XG), guar gum (GG), soluble flaxseed gum (SFG), and soy soluble polysaccharide (SSPS) have on native (uncooked) starch granules during *in vitro* digestion. Granules were extracted and purified from digesta at different times of simulated small intestinal digestion to observe for changes in particle size using light scattering. Microscopy analyses were also performed on isolated starch granules to visualize changes occurring in surface topography using scanning electron microscopy and light microscopy.

2. Materials and methods

2.1. Materials

Four hydrocolloids were employed in this study, which included guar gum, (Danisco Canada Inc., Toronto, ON, Canada), xanthan gum (Sigma Chemical Co., St. Louis, MO, USA), soluble flaxseed gum extracted from flaxseed hulls (Fabek et al., 2014), and DA-100 variety soy soluble polysaccharides (Fuji Oil Co. Ltd., Osaka, Japan). Tapioca starch (batch number H88 80008) was purchased from Ingredion (Bridgewater, NJ, USA). Skim milk powder was purchased from Gay Lea Food Corp. (Mississauga, ON, Canada). Study-specific enzymes and chemicals were purchased from the following distributors: simulated gastric fluid (SGF) from Ricca Chemical Company (Arlington, TX, USA), purified pepsin, sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, potassium chloride, potassium citrate, potassium phosphate, and sodium chloride from Fisher Scientific (Fair Lawn, NJ, USA), pancreatin from MP Biomedicals (Solon, OH, USA), α-amylase, amyloglucosidase (*Aspergillius niger*), mucin from porcine stomach, sodium L-lactate, ammonium nitrate, urea, and uric acid sodium salt from Sigma-Aldrich (St. Louis, MO, USA), hydrochloric acid 2N solution and glycerol from Fisher Scientific (Nepean, ON, Canada), bile salts from Fisher Science Education (Hanover Park, IL, USA) anhydrous ethyl alcohol from Commercial Alcohols, the industrial and beverage alcohol division of Green-Field Ethanol Inc. (Brampton, ON, Canada).

2.2. Sample preparation

Solutions of commercial (native) tapioca starch and skim milk powder (SMP) were prepared by dissolving weighed amounts of each in deionised water. Tapioca starch was chosen as it is widely used in the food industry due to its low lipid concentration and bland flavour, which allows it to be utilized in a wide range of food products (Eliasson, 2004; Muadklay and Sanguansri, 2008). Skim milk powder, which is a commonly utilized food ingredient due to desirable functional and nutritional properties (Guyomarc'h, Warin, Muir, & Leaver, 2000), was used in order to create a food-like matrix by providing a protein source to the different treatments. To investigate the effect of soluble fibre inclusion, XG, GG, SFG and SSPS were added, according to their matched apparent viscosities at 20 s^{-1}. The final concentrations of XG, GG, SFG, and SSPS were 4% w/w, 3% w/w, 7% w/w and 20% w/w, respectively, and all solutions contained the same concentration of starch (4% w/w) and SMP (8.65% w/w). The solutions were left to stir until fully dissolved and the control sample (no fibre) was left to stir at room temperature until experimentation to avoid sedimentation of starch granules. All solutions were prepared at room temperature to avoid granule swelling and amylose leaching in order to preserve the granular structure of starch.

2.3. In vitro digestion

All treatments were digested using a three-stage *in vitro* digestion procedure, mimicking salivary, gastric, and small intestinal digestion, adapted from our earlier studies (Fabek et al., 2014; Fabek and Goff, 2015). In short, 15 g samples with 4 glass balls (to induce churning) were preincubated with artificial salivary fluid (5.0 mL) containing alpha amylase (75 U mL^{-1}) for 5 min inside a shaking water bath (Thermo Scientific, Marietta, OH, USA) at 37 °C, at a speed of 60 rpm, mimicking the agitation speed during swallowing (Borwankar, 1992). The ensuing digest was then exposed to 7 mL simulated gastric fluid, [0.2%NaCl (w/w) in 0.7% HCl (w/v)], containing 3.2 mg mL^{-1} of pepsin (pH = 1.8 ± 0.1). The mixture was incubated at 37 °C, at a speed of 175 rpm, for 1 h. Following simulated gastric digestion, 4.6 mL simulated bile fluid (SBF), containing 8 mg mL^{-1} bile salts, 14 mL simulated intestinal fluid (SIF), pH 7.6 ± 0.1, containing 5 mg mL^{-1} pancreatin dissolved in 0.5 M sodium phosphate buffer and calcium chloride solution, and 2.9 mL of amyloglucosidase (112 U/mL) were added to each solution. The mixture was placed back in the shaking water bath and simulated intestinal digestion proceeded at 37 °C for 4 h. All digestions were performed in triplicate.