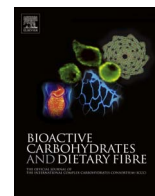




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Impact of dietary fibre on in vitro digestibility of modified tapioca starch: viscosity effect

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ABSTRACT

In the current work, amylolysis of modified tapioca starch in simulated small intestinal conditions was studied in the presence of each of four dietary fibre types including yellow mustard mucilage, soluble flaxseed gum, fenugreek gum or oat gum. Each fibre was used at concentrations that were matched across the four fibre types for post-digestion viscosity. Due to molecular heterogeneity these concentrations were different for each fibre. The progress of amylolysis was studied by measuring the decline of digesta apparent viscosity over time at constant shear rate 60 s^{-1} . Expressing these data as a percentage of initial viscosity, and plotting it as a natural logarithm against time transformed the data to a linear form with a slope denoted as a viscosity decay constant (k_v). Additionally, for some of the digesta samples progress of amylolysis was assessed by evaluating increases in reducing sugar concentration. Based on the k_v values, the progress of amylolysis was reduced as the concentration of each fibre and therefore viscosity of digesta increased. However, the influence to hinder amylolysis was diminishing with increase of digesta viscosity. The progress of amylolysis was similar when each fibre was present at a concentration to match for post-digestion viscosity. Measurements of changes in reducing sugar content also confirmed these findings. Therefore, it was concluded that to alter amylolysis to a similar extent fibres have to be present at amounts to result in similar post-digestion viscosity even though their concentrations may not match.

1. Introduction

Native starch is a polysaccharide produced by green plants as an energy store. In nature starch occurs in the form of granules with semi-crystalline structure. Most starch granules are composed of two polymers of D-glucose linked to one another through glycosidic bonds: the linear amylose and branched amylopectin. Starch consumed by humans has often undergone thermal treatment in the presence of water, up to an extent when irreversible loss of the granular structure occurs, referred to as gelatinization. Native granular starches are also often physically and chemically modified to obtain "modified food starches" that are used to formulate food products with specific textural qualities.

Starch is an important energy source for humans. Digestion of starch begins in the mouth by salivary α -amylase and continues in the stomach until gastric pH is lowered below ~ 4.0 to inactivate the enzyme. When chyme reaches the small intestine, its pH is elevated (~ 6.5) and starch digestion resumes by pancreatic α -amylase and possibly by a portion of salivary α -amylase that stays intact after passing through the stomach (Abramson, Fried & Meyer, 1987;

Kalantzi et al., 2006; Persson et al., 2005). α -amylases hydrolyze starch in a multiple attack action pattern when several glycosidic bonds are cleaved after the first random attack before dissociation of the enzyme-substrate complex (Bijttebier, Delcour & Goesaert, 2008; Gray, 1992). Studies have shown that human salivary and porcine pancreatic α -amylases hydrolyze starch mostly to maltose, maltotriose, maltotetraose and α -limit dextrins (Robyt, 2008). These products are further hydrolyzed at the intestinal brush border rather than in the lumen by specific glycosidases to produce single glucose units as a final product of starch digestion. Glucose is then transported across intestinal mucosal cells into the blood (Gray, 1992).

Several in vivo studies reported differences in post-prandial glycaemic and/or insulinemic responses after consumption of meals containing equal amounts of starch (Byrnes, Denyer, & Miller, 1995; Brand-Miller, Copeland, Ek & Wang, 2014; Bal, Ells, Kettlitz, Mathers & Seal, 2005; Cole, Cummings, Englyst, Englyst & Hudson, 1999; Goñi, Garcia-Alonso, & Saura-Calixto, 1997; Nestel, O'dea & Snow, 1981; Okadome, Sasaki & Sotome, 2015). These differences were related to the kinetics of amylolysis, as established by authors in in vitro experiments. Therefore, knowledge on digestion kinetics of various

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starches and methods of its manipulation can help to develop a dietary strategy to improve glycemic control, a factor that is believed to be important in the reduction of risk for Type 2 diabetes (Liu, Manson & Willett, 2002).

Several features such as the botanical nature of starch, the type and extent of physicochemical modification, and the presence of protein and lipids can affect the kinetics of amylolysis (Varatharajan et al., 2011; Copeland & Wang, 2013; Dhital, Gidley & Zhang, 2015b). Numerous *in vitro* studies revealed that addition of water-soluble dietary fibre (DF) can also alter the progress of amylolysis (Aravind, Sissons, & Fellows, 2012; Bordoloi, Singh, & Kaur, 2012; Dartois, Singh, Kaur, & Singh, 2010; Dhital, Dolan, Stokes, & Gidley, 2014; Fabek & Goff, 2015; Hardacre, Yap, Lentle, & Monro, 2015; Koh, Kasapis, Lim, & Foo, 2009; Okadome, Sasaki & Sotome, 2015; Slaughter, Ellis, Jackson, & Butterworth, 2002; Symons & Brennan, 2004 and Thondre, Monro, Mishra, & Henry, 2010). In fact, several *in vivo* studies showed that supplementation of starch-containing meals with DF can attenuate glycemic responses (Chillo, Ranawana, Pratt, & Henry, 2011; Ellis, Roberts, Low, & Morgan, 1995; Lan-Pidhainy, Brummer, Tosh, Wolever, & Wood, 2007; Lu, Walker, Muir, & O'Dea, 2004; Thakur, Mitra, Pal, & Rousseau, 2009; Thondre & Henry, 2009; Tosh, Brummer, Wolever, & Wood, 2008; Wood, Braaten, Scott, Riedel, & Poste, 1990). However, a correlation between the effect of DF on the kinetics of amylolysis and glycemic responses was not investigated in these studies. Moreover, in 2011 the European Food Safety Authority (EFSA) officially recognized the ability of cereal β -glucan (a type of DF) to reduce post-prandial glycemic responses.

Some DF including many polysaccharides such as arabinoxylans, galactomannans, pectins, and β -glucans, create thick solutions when mixed with water. The extent of thickening depends on molecular weight, concentration, chemical composition and conformation of a polysaccharide. Tosh et al. (2008) showed that there is a correlation between the ability of cereal β -glucan to enhance the viscosity of aqueous solutions and glycemic responses after consumption of a starch-containing meal. Particularly, there is evidence that DF results in attenuation of glycemic responses through its ability to alter luminal viscosity (Ellis et al., 1995). However, the exact mechanism by which DF causes this attenuation is unclear. Three most popular proposed mechanisms are: 1) delayed gastric emptying, 2) reduced digestive enzyme activity and 3) reduced rate of nutrient absorption (Dikeman & Fahey, 2006).

Several studies showed that DF can reduce the activity of pancreatic digestive enzymes including α -amylase (Hansen & Schulz, 1982; Ikegami et al., 1990; Isaksson, Lundquist, & Ihse, 1982). This reduction can be simply a result of reduced diffusion of the enzyme to starch substrate due to increased viscosity of lumen (Shelat et al., 2010). Therefore, if viscosity is the only factor that governs kinetics of amylolysis in DF-containing systems then DF of different biological nature would restrict amylolysis to a similar extent, as long as DF is present at concentrations to maintain a minimal level of viscosity.

Nevertheless, alternative mechanisms for the inhibition of amylolysis in the presence of DF have been proposed. Slaughter et al. (2002) observed that guar gum has an inhibitory effect on α -amylase. These authors concluded that the inhibition was due to the absorption of the enzyme to the galactomannan rather than due to enzyme diffusion impairment. Sasaki et al. (2015) established that interaction between xanthan gum and amylopectin occurs, perhaps through hydrogen bonding. These authors suggested that the interaction could result in inhibition of starch hydrolysis observed in the study.

The objective of the present study was to investigate the effect of four different DF types on digestion kinetics of modified tapioca starch in simulated small intestinal conditions at a shear rate equivalent to physiological. Each type of DF was chosen to produce equal viscosity at very different concentrations (due to the variances in botanical source of the DF). The progress of amylolysis was studied indirectly, by measuring the decline of digesta apparent viscosity over time.

Additionally, changes in digesta reducing sugar concentration for some of the digesta samples were determined.

2. Materials and methods

2.1. Extraction of dietary fibre

DF including yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) were extracted as described by Repin, Cui, and Goff (2016). Briefly, YMM and SFG were extracted from the yellow mustard bran (G.S. Dunn, Hamilton, Canada) and flaxseed hull (Natanola Health Inc., Winchester, Canada) respectively with water. Both types of DF were purified using steps including protein hydrolysis, dialysis against distilled water and ethanol precipitation. FG was extracted by suspending CANAFEN® Gum (Emerald Seed products Ltd., Avonlea, Canada) in water followed by centrifugation. Gum was precipitated with ethanol from the supernatant. OG was extracted from oat bran concentrate (Viterra, Portage la Prairie, Canada) using a dual-enzyme procedure. Chemical and mono-saccharide compositions, as well as the rheological behavior of these four DF types, are described in Repin et al. (2016).

2.2. Establishing dietary fibre concentrations for *in vitro* digestion

Hypothetically, if a meal of 500 mL contains 50 g of available carbohydrates, and if this meal is diluted 3 fold by the time it reaches the small intestine, the expected concentration of β -glucan in the small intestine after consumption of a meal that meets requirements of EFSA (2011) health claim on reduction of post-prandial glycemic responses would be 0.44% (w/v) (1 health claim equivalent (eq.)). Concentrations of YMM, SFG, and FG that under simulated small intestinal conditions represent from 0.5 to 3 apparent viscosity (at 60 s⁻¹) eq. of EFSA (2011) glycemia control health claims for cereal β -glucan were obtained as described in detail by Repin et al. (2016). Briefly, apparent viscosities (at 60 s⁻¹) of starch-free simulated small intestinal digesta samples supplemented with OG at several concentrations were measured and were used as benchmarks. Particularly, samples were supplemented with OG at amounts to result in concentrations of β -glucan expected in the small intestine after consumption of a meal that meets from 0.5 to 3 times the value of the EFSA (2011) health claim. Concentrations of YMM, SFG and FG that resulted in apparent viscosities (at 60 s⁻¹) close to the benchmark apparent viscosities in simulated small intestinal conditions were found (Table 1). Shear rate equal to 60 s⁻¹ was representing physiological shear rate experienced by digesta in the small intestine and lies in the range of shear rates used in literature where viscosities of small intestinal digesta were compared (Dikeman & Fahey, 2006; Fabek, Messerschmidt, Brulport, & Goff, 2014; Hardacre et al., 2015; Tharakan, 2009).

2.3. Preparation of simulated small intestinal digesta

Simulated small intestinal digesta was prepared similarly as

Table 1

Concentrations of yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) that represent 0.5, 1, 2 and 3 apparent viscosity (at 60 s⁻¹) equivalents of the European Food Safety Authority (2011) glycemia control health claim for cereal β -glucan.

Health Claim Equivalent	Concentration (% (w/v))			
	YMM	SFG	FG	OG
3.0	1.15	0.93	0.43	1.62
2.0	0.61	0.56	0.28	1.08
1.0	0.19	0.25	0.13	0.54
0.5	0.07	0.12	0.06	0.27

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