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Effects of pectin on molecular structural changes in starch during digestion

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ABSTRACT

Starch digestion rate is strongly related to metabolic diseases such as obesity and diabetes. Starchy foods always contain non-starch components, which can affect starch digestibility. Mixtures of ungelatinized corn starch with a common non-starch component, pectin, were used to investigate pectin's effect on starch digestibility rate and evolution of starch molecular structure during digestion using amyloglucosidase and pancreatin. The whole-molecule size distribution and the chain-length distribution of chains were measured by size-exclusion chromatography and fluorophore-assisted carbohydrate electrophoresis. Digestion profiles and changes in molecular size distributions of whole and debranched digesta during digestion show that addition of pectin significantly decreased starch digestion rates. While pectin did not change the amylose/amylopectin ratio during most of the digestion, it decreased the digestion rate of short amylopectin chains compared to long ones. UV–visible spectral data suggested that a major contributor to this digestion rate change is from substantial pectin/amyloglucosidase interaction. This suggests an approach to designing nutritionally more beneficial starch-based foods by taking account of interactions between pectin and digestive enzymes.

1. Introduction

Starch provides ~50% of the average human energy intake in developed countries and the percentage is even higher in many developing countries (Hoang et al., 2008). It is a branched homopolymer made up of glucose units extended with (1→4)-α linear glycosidic linkages and branched with (1→6)-α glycosidic linkages, comprising two main molecules: amylose (AM) and amylopectin (AP). AM is largely linear with a few long-chain branches and molecular weight ~10^{5-6}, and AP is hyperbranched with numerous short chains and molecular weight ~10^{7-8}. The structural features of AM and AP (including amylose fraction) in starch affect its cooking, eating, nutritional and other physiochemical properties.

Rapid digestion of starch by humans can cause a sharp increase of plasma glucose and results in very little starch reaching the lower bowel, which may lead to increased risk of obesity, type 2 diabetes and colorectal cancers (Dona, Pages, Gilbert, & Kuchel, 2010). The digestibility of starch can be affected by starch structure, including its molecular and granular structure, crystal type, and granule size (Zhang, Venkatachalam, & Hamaker, 2006), and by other types of causes such as food structure, texture, viscosity and interactions with other components (Singh, Dartois, & Kaur, 2010) in a food matrix.

Components such as non-starch polysaccharides are always present in starch-containing foods, and also can be added to starch-containing products during food processing to improve the texture, water mobility, stability and viscosity. It has been suggested that additives which strongly increase the viscosity of starchy foods would decrease the hydrolysis rate of amylase, contributing to nutritional benefits (Brennan, 2005; Dhital, Warren, Butterworth,

Abbreviations: AM, amylose; AMG, amyloglucosidase; AP, amylopectin; AUC, area under the curve; CLD, chain length distribution; DMSO, dimethyl sulfoxide; DP, degree of polymerization; SEC, size-exclusion chromatography; FACE, fluorophore-assisted carbohydrate electrophoresis; RID, refractive index detector.

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Ellis, & Gidley, 2017). One such additive is pectin, a cell-wall material mainly composed of galacturonic acid (Yapo, 2011) and commonly used as gelling agent and stabilizer (Willats, Knox, & Mikkelsen, 2006). Previous studies (Sasaki & Kohyama, 2012; Sasaki, Sotome, & Okadome, 2015) have reported that the addition of pectin increased the viscosity of starch suspensions and decreased the starch *in vitro* digestibility. They also suggested the pectin's suppressive effect on starch digestibility is related to a number of factors and is not only due to the rheological properties. It is noted that what matters for diffusion control of a reaction is the relative rate of diffusion of the reacting entities, which may or may not be related to the bulk viscosity. While a pectin solution has a high *bulk* viscosity, the enzymes involved are relatively small (~10 nm); at all except very high pectin concentration, the average space between pectin molecules is much larger than 10 nm: that is, the *local* viscosity for the enzyme is close to that of pure solvent. The hypothesis of the present paper is that a major effect in this slowed digestion rate will be from some sort of interaction between starch and pectin in the food matrix.

There have as yet been no studies of starch molecular structural changes during digestion in the presence of pectin. Since such studies have yielded useful mechanistic information for starch without additives, e.g. (Zhang, Sofyan, & Hamaker, 2008), it is useful to do the same to understand any effects of pectin addition. This is implemented here using size-exclusion chromatography (SEC, also known as GPC and HPLC-SEC) and fluorophore-assisted carbohydrate electrophoresis (FACE) to characterize both the molecular size distributions of whole starch molecules, using SEC, and the chain-length distributions (CLDs), using both SEC for the amylose chains and FACE (which gives a very accurate CLD, but currently is confined to chains shorter than ~ 180 glucose units (Wu, Li, & Gilbert, 2014)) for the amylopectin chains.

In the present work, a suspension of ungelatinized corn starch is used as substrate, and *in vitro* digestion uses pancreatin and amyloglucosidase. Interactions between pectin and amyloglucosidase are investigated by UV/visible absorption spectroscopy. Only one starch and one pectin concentration are considered here. However, the possible mechanism whereby pectin affects the digestion rate would most likely be the same for different starch and pectin concentrations. Sasaki and co-workers (Sasaki & Kohyama, 2012) showed that the same concentration of pectin as used here significantly affected the digestion rate of starch, but did not examine specific reasons for this effect. The present paper is based on this previous work and elucidates the underlying mechanisms by measuring molecular structural evolution.

2. Materials and methods

2.1. Materials

Corn starch (S4126), pectin from citrus peel (P9135) and porcine pancreatic pancreatin (P1750) were purchased from Sigma-Aldrich Co., US. Amyloglucosidase, isoamylase from *Pseudomonas* sp. and a glucose content assay kit were from Megazyme International Ireland Ltd., Ireland. Pullulan standards for SEC analysis were from Polymer Standards Service (PSS) GmbH, Germany, and cover the molecular weight range 342 to 2.35×10^6. GR grade dimethyl sulfoxide (DMSO) was from Merck Co. Other chemicals were reagent grade.

2.2. In vitro digestibility and fitting to first-order kinetics

The preparation of starch suspension and starch suspension mixed with pectin were carried out following a method modified from the literature (Sasaki & Kohyama, 2012). Pectin (125 mg on dry basis per group) was dispersed with 7.5 mL of distilled water in six 50 mL screw-capped tubes and stirred at 500 rpm for 35 min using a magnetic stirrer. Six tubes without pectin were also prepared. After complete dispersion (judged by a clear appearance), the pectin solution was heated at 50 °C for another 30 min with continuous stirring. Then 200 mg of corn starch was added to each tube and thoroughly dispersed by vortex mixing, and 2 mL of 0.125 M HCl solution was then added. The starch suspensions were incubated at 37 °C with vigorous magnetic stirring for 30 min. The pH of the starch suspensions was adjusted to 5.2 by adding ~0.5 mL of 2.5 M sodium acetate solution. To prepare the enzyme solution for digestion, 20 mg of pancreatin (4 × USP specifications) and 1 mL of amyloglucosidase (3260 U/mL) were mixed and thoroughly dispersed in 50 mL of water. After centrifuging at 2000*g* for 10 min, the supernatant of the enzyme solution was heated at 37 °C in a water bath before use. The digestion of starch suspensions was then performed by adding 2.5 mL of enzyme solution in each tube with magnetic stirring. After 0, 2, 4, 6, 8, 24 and 48 h of incubation, absolute ethanol was added to each tube to stop the hydrolysis of starch. The glucose concentration in the supernatant was determined by using the Megazyme glucose assay content kit after centrifugation at 1500*g* for 10 min.

Digestibility curves were then fitted to a first-order equation, which in integrated form is:

$$C_t = C_f\left(1 - e^{-kt}\right) \tag{1}$$

C_t is the percentage of starch digested at time t (min), C_f (where the subscript f is for "final") is the estimated percentage of starch digested by the end of reaction time and k (min^{-1}) is the starch digestion rate coefficient.

In practice, C_f and k are measured using a logarithm-of-slope (LOS) analysis described in detail elsewhere (Butterworth, Warren, Grassby, Patel, & Ellis, 2012) through a transformed equation:

$$\ln\frac{dC_t}{dt} = \ln\left(C_f\, k\right) - kt \tag{2}$$

The derivative dC_t/dt is obtained using second-order finite difference. From eq (2), a plot of the logarithm of this derivative against time yields C_f and k, if this plot is linear.

2.3. Size-exclusion chromatography

The whole-molecule SEC used an Agilent 1260 Infinity SEC system (Agilent, Santa Clara, CA, USA) with a refractive index detector (RID, Optilab UT-rEX, Wyatt, Santa Barbara, CA, USA) following a published method (Cave, Seabrook, Gidley, & Gilbert, 2009; Vilaplana & Gilbert, 2010). Starch samples were dissolved in dimethyl sulfoxide (DMSO) with 0.5% (w/w) LiBr at a concentration of 2 mg/mL. DMSO/LiBr is used as a solvent combination both to dissolve starch completely and to minimize degradation (Schmitz, Dona, Castignolles, Gilbert, & Gaborieau, 2009), and it is also used as the mobile phase for SEC analysis after being filtered through a 0.45 μm hydrophilic Teflon membrane filter. Starch samples were injected into a series of PSS separation columns (Polymer Standards Service, Mainz, Germany): SUPREMA precolumn, Gram 30 and Gram 3000. The injection volume was 100 μL, the flow rate 0.3 mL/min, and the column oven temperature 80 °C. A series of pullulan standards with different peak molecular weights ranging from 342 to 2.35×10^6 was used to convert elution volume to molecular size (the SEC hydrodynamic volume, V_h, or the equivalent hydrodynamic radius, R_h, which is the separation parameter for SEC) using the Mark-Houwink equation. The Mark-