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Effects of processing high amylose maize starches under controlled conditions on structural organisation and amylase digestibility

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ABSTRACT

The amylase digestibility of high-amylose maize starches has been compared before and after thermo-mechanical processing. Starches were analysed for enzyme-resistant starch yield, apparent amylose content, crystallinity (X-ray diffraction), and molecular order (NMR and FTIR), both before and after treatment with α-amylase. All samples had significant (>10%) enzyme-resistant starch levels irrespective of the type and extent of thermal or enzymic processing. Molecular or crystalline order was not a pre-requisite for enzyme resistance. Near-amorphous forms of high amylose maize starches are likely to undergo recrystallisation during the enzyme-digestion process. The mechanism of enzyme resistance of granular high-amylose starches is found to be qualitatively different to that for processed high-amylose starches. For all samples, measured levels of enzyme resistance are due to the interruption of a slow digestion process, rather than the presence of completely indigestible material.

1. Introduction

In plants, starch is synthesized in the form of water-insoluble semi-crystalline granules with a complex architecture which is specific to each particular plant. Unlike other dietary polysaccharides, starches contain only α-glucosidic linkages and, are potentially digestible by the amylolytic enzymes secreted by the human digestive tract (Englyst & Hudson, 1996). Various studies, however, have shown that structural conformation and other factors can influence the rate and extent of starch, *in vitro* and *in vivo*, and subsequent absorption in humans and animals (Botham et al., 1997; Cairns, Botham, Morris, & Ring, 1996; Englyst, Kingman, & Cummings, 1992; Faisant, Champ, Colonna, & Buleon, 1993b; Faisant et al., 1993a, 1995; Gidley et al., 1995). The sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals has been termed 'resistant starch (RS)' (Asp, 1992; Englyst & Cummings, 1990). RS plays important physiological roles and has the potential to improve human health and lower the risk of many diet-related diseases. RS is defined as the fraction of starch not digested in the small intestine that enters the colon where it is fermented by bacteria to short chain fatty acids (SCFA) and gases (Topping & Clifton, 2001). The SCFA, particularly butyrate, have been implicated in promoting good colonic health and preventing the incidence of colo-rectal cancer (Champ, 2004; Jacobasch, Schmiedl, Kruschewski, & Schmehl, 1999).

RS is classified into four major groups, namely RSI, RSII, RSIII, and RSIV (Topping et al., 2003). RSI arises from physically inaccessible starches, *e.g.*, within plant tissue structures, RSII is due to the condensed form and partial crystallinity of native (uncooked) starch granules, RSIII is derived from recrystallised (retrograded) starches, typically after food processing, and RSIV is from chemical modification of starches that inhibit amylase digestion. RSIII or retrograded starch is the form of resistant starch most often used as an ingredient in a range of foods and is therefore of both commercial and nutritional interest.

The molecular organisation of polymers within starch granules (sources of RSI and RSII) has been reviewed (Tester, Karkalas, & Qi, 2004). Many food processing methods reduce or eliminate RSI and RSII but have the potential to generate RSIII, particularly if high amylose starches are used. The currently accepted mechanism for the resistance of RSIII to amylase digestion is that linear amylose segments align themselves after gelatinization into condensed structures based on double helices (amylose retrogradation) that render the α-1,4 glucosidic linkages inaccessible to amylase. Generally speaking, RS III has been found to contain short and linear chains of thermally stable α-1,4 glucans of about

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10–100 chain lengths; significant double helix content and moderate crystallinity (mostly B-type) (Cairns et al., 1996; Eerlingen, Deceuninck, & Delcour, 1993b; Faisant et al., 1993a, 1993b, 1995; Gidley et al., 1995; Shamai, Shimoni, & Bianco-Peled, 2004). There are also a few reports of retrograded amylopectin contributing towards type III RS (Chung, Lim, & Lim, 2006; Eerlingen, Jacobs, & Delcour, 1994; Russell, Berry, & Greenwell, 1989), an issue that needs further investigation. Several studies have shown that high amylose starches and products made from them have high RS contents measured *in vivo* which correlate with enzyme-resistant values determined *in vitro* (Akerberg, Liljeberg, & Bjorck, 1998; Thompson, 2005; Leeman, Karlsson, Eliasson, & Bjorck, 2006).

Extrusion cooking is a common food processing method for foods such as breakfast cereals and pasta products. Extrusion of starch in the presence of sufficient water triggers a number of physico-chemical and functionality changes in starch granules, such as the loss of granular structure associated with melting of crystallites and underlying helices, and the generation of an amorphous structure. This structure may later reacquire ordered helical or crystalline order (=retrogradation) and become resistant to digestion by human α-amylase. Various investigators have reported that retrogradation following extrusion cooking results in formation of enzyme-resistant starch (Eerlingen, Crombez, & Delcour, 1993a; Faraj, Vasanthan, & Hoover, 2004; Kim, Tanhehco, & Ng, 2006; Unlu & Faller, 1998; Vasanthan & Bhatty, 1998). In these studies, the acquisition of double-helical or crystalline order was considered to be critical for the observed amylase resistance. The inability of a starch double helix to fit into the active site of α-amylase is a plausible logic for the ability of retrograded structures to avoid enzymatic hydrolysis.

In this study, two high amylose maize starches have been subjected to each of two model extrusion conditions in a capillary rheometer. Products have been characterised by X-ray diffraction, solid state ^{13}C NMR, infrared spectroscopy and apparent amylose analysis both before and after digestion with α-amylase. Characterisation of these samples by X-ray scattering and electron microscopy has been reported elsewhere (Lopez-Rubio, Htoon, & Gilbert, 2007). The results suggest that high amylose starches can have relatively high levels of enzyme resistance even after processing to a near-amorphous state.

2. Materials and methods

2.1. Materials

High-amylose maize starches, Hylon VII (National Starch and Chemicals Pty Ltd., Seven Hills, Sydney, Australia) and Gelose 80 (Penfords Australia, Lane Cove, Sydney, Australia) with approximately 70% and 80% amylose contents, respectively, were used as raw materials. The initial moisture content of starches was determined by oven drying at 135 °C for 2 h, and the amount of water required to give 35% or 50% w/v calculated. The calculated amount of water was added to starches and mixed in a kitchen mixer (Kenwood) for 5 min. The resulting dough was kept at 4 °C overnight in plastic sealed bags for moisture equilibration to 35% or 50% w/v prior to extrusion.

2.2. Extrusion by capillary rheometer

Extrusion has been chosen as a model process to study the formation of enzyme-resistant retrograded starch. In order to carry out processing under well-defined conditions, a bench top capillary rheometer (Rosand RH2000 Rheometers, Bohlin Instruments Ltd., England) was used. The capillary rheometer can deliver controlled shear and stress over a wide temperature range, with conditions monitored by a comprehensive data acquisition and analysis package, providing a high level of control over extrusion conditions. The rheometer was configured into two different settings to achieve different processing conditions (Table 1). Low shear processing was designated 'mild' (M) and high shear processing was designated 'extreme' (E). Both Gelose (G) and Hylon (H) samples were processed by each method to give GM, GE, HM, and HE samples.

2.3. In-vitro digestion and RS Isolation

The processed samples were ground in a mortar and pestle, passed through a 500 μM particle size sieve and a portion was dried at 135 °C for 2 h to determine the moisture content. Granular or finely ground processed sample (80 g) was mixed with artificial saliva [250 U of α-amylase (Sigma) at pH 7.0 in 1.2 L]. After 15–20 s, the mixture was incubated with acidified (0.02 M HCl) pepsin (1 mg/mL; Sigma) at 37 °C for 30 min. The solution was adjusted to pH 6.0 (NaOH) and the samples were treated with pancreatin (2 mg)/amyloglucosidase (AMG, 28 U: Sigma) enzyme mixture for 18 h at 37 °C in 0.2 M acetate buffer at pH 6.0 in a shaking water bath. Samples were inactivated by adding an equal volume of 95% ethanol and centrifuged (2000*g*, 10 min). The supernatant was discarded and the residue was washed twice, first with 0.2 M acetate buffer (pH 6.0) followed by water and then freeze-dried. A schematic diagram for high amylose starch processing is given in Fig. 1. The *in vitro* (enzyme-) RS residues are denoted by RS following the code denoting raw material and process conditions such as GRRS, GMRS, GERS, HRRS, HMRS and HERS (see Fig. 1).

The yield of enzyme-resistant starch was determined by subjecting accurately-weighed quantities of samples (ca. 500 mg) from the *in vitro* digestion process described above. The starch content of freeze-dried products was determined by dissolving in dimethyl sulphoxide, followed by complete hydrolysis to glucose using thermostable α-amylase and amyloglucosidase, and enzymatic determination of glucose (Megazyme, Ireland).

2.4. Apparent amylose content

The apparent amylose content of raw and processed starches was determined by an iodine colorimetric method as described by Hoover and Ratnayake (2005). Sample (20 ± 0.1 mg) in a tube was dispersed with 8 mL 90% DMSO and vortexed for 2 min. A series of potato amylose (A0512, Sigma) and maize amylopectin (S9679, Sigma) mixtures at various concentrations was prepared (to construct a standard curve) and treated in the same way as samples. The tubes were heated in a water bath at 80 °C for 15 min with mixing, cooled to room temperature and diluted to 25 mL. One millilitre of diluted solution was mixed with 40 mL distilled-deionized water and 5 mL iodine reagent (0.0025 M I_2/0.0065 M KI) in a 50 mL volumetric flask and volume was adjusted.

Table 1
Extrusion parameters for high amylose starches processed in a capillary rheometer

Processing conditions	Moisture (%)	Temperature (°C)	Shear rate (s^{-1})	Die setting
Low shear, high moisture and low temperature ('mild')	50	100	150	1 mm diameter, 8 mm length
High shear, low moisture and high temperature ('extreme')	35	140	750	1 mm diameter, 8 mm length