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Amylolysis of wheat starches. II. Degradation patterns of native starch granules with varying functional properties

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ABSTRACT

Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were employed to investigate degradation patterns of native starch granules from wheat (*Triticum aestivum* L.) by different starch-degrading enzymes. The starches examined were from a waxy wheat and four varieties with slightly elevated amylose content, but with different functional properties. Differences in the digestion patterns after partial α-amylolysis of starch granules were noted between the starches. The waxy starch seemed to be degraded by endocorrosion, whereas the amylose-rich starches followed a slower mode of hydrolysis starting from the granular surface. X-ray diffractograms of the amylose-rich starches were not significantly altered by 2 h of α-amylolysis, whereas partial hydrolysis of the waxy starch decreased scattering intensity at higher 2θ angles, consistent with a different mode of attack by α-amylase in the initial digestion stages of granules of waxy and amylose-rich starches. We propose these differences are due to the combined effects of the change in packing density and partial preference for hydrolysis of amorphous material. The native starch granules were also attacked by beta-amylase, isoamylase and amyloglucosidase, which indicates that α-amylase is not the only starch-degrading enzyme that is able to initiate starch hydrolysis of native granules.

1. Introduction

Starch is the main digestible carbohydrate in the human diet and it contributes a substantial amount of energy for human metabolism. Most of the starch in the diet is digested to glucose, which is readily absorbed into the blood stream. Starch not digested in the upper gut is referred to as resistant starch, which is associated with nutritional benefits through moderating the glycemic response and prebiotic effects in the large intestine. The enzymic breakdown of amylose and amylopectin by human digestive enzymes involves salivary and pancreatic α-amylases (α-1, 4-glucan-4-glucanohydrolases; EC 3.2.1.1), which are calcium-containing enzymes that belong to a family of endo-amylases that catalyse the cleavage of α-D-(1,4) glycosidic bonds. These enzymes occur ubiquitously and play a dominant role in starch breakdown in microorganisms, plants and animals (Kandra, 2003). The α-D-(1,6) glycosidic bonds are broken by amyloglucosidase (EC 3.2.1.2). Other enzymes that may also play a role in the breakdown of starch by various plant and microbial species are β-amylase (EC 3.2.1.2), an exo-amylase that cleaves maltose units from the non-reducing end of α-D-(1,4) glucan chains; isoamylase (EC 3.2.1.68), which acts as a debranching enzyme; and amyloglucosidase (EC 3.2.1.3), which hydrolyses α-D-(1,4) and α-D-(1,6) glucosidic bonds.

The susceptibility of native starch granules to degradation by amylases depends on the source of the starch and of the amylase (Planchot et al., 1995). Starches from different botanical sources have been shown to have different digestion kinetics and degradation patterns with α-amylase (Fuwa et al., 1980; Slaughter et al., 2001). Much less information is available on the variability of α-amylolysis of starch from different varieties of the same plant species. The rate and extent of α-amylolysis of granular starch are influenced by many factors that can affect access of the enzyme to its substrate and the release of reaction products. These include granule integrity, size and relative surface area, crystallinity and porosity of granules, amylose to amylopectin ratio, and structural inhomogeneities (Buleon et al., 1998; Franco et al., 1998; Jacobs et al., 1998; Kong et al., 2003; Planchot et al., 1995). Proteins and lipids on the surface of starch granules and hydrolysis products such as malto-oligosaccharides have been shown to inhibit amylolysis (Colonna et al., 1988; Greenwell et al., 1985; Shin et al., 2004).

Various microscopic techniques have been used to reveal different patterns of granule disruption after partial digestion *in vitro* of starch by α-amylases (reviewed by Svihus et al., 2005). Digestion patterns observed include surface pitting and erosion,

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formation of pores, and concentric layered shell structures (Gallant et al., 1973; Lynn and Cochrane, 1997; Planchot et al., 1997). Although partial enzymic and acid hydrolysis of starch granules were shown in some studies to produce a concentric layered shell structure (Buleon et al., 1998), evidence of enrichment of crystallinity in partially degraded granules is limited, leaving questions about whether the amorphous domains of starch granules are preferentially attacked by starch-degrading enzymes. In the present study we used scanning electron microscopy (SEM) and X-ray diffraction (XRD) to investigate varietal effects on the degradation patterns of native starch granules using α- and β-amylases, amyloglucosidase and isoamylase. The starches were among those that were examined for their digestion kinetics with α-amylase, as described in a previous paper (Blazek and Copeland, 2010).

2. Materials and methods

2.1. Materials

Four wheat varieties (Diamondbird, Batavia, V306 and SM1046) were selected from a group of varieties produced by a breeding program of the Value Added Wheat CRC Ltd (VAWCRC) aimed at producing wheat lines with elevated amylose content. These four varieties were selected for differences in their swelling and pasting properties (Blazek and Copeland, 2008). A waxy variety was also included in the study. Starch granules were extracted from flour using a procedure based on the method of Akerberg et al. (1998) as described by Blazek and Copeland (2008). In this procedure, proteins and lipids adhering to the granule surface are removed by treatment with pepsin and ethanol, respectively. Total amylose content (T-AM) of the starches, as determined by iodine binding according to Chrastil (1987), was 2% for the waxy, 36% for Diamondbird, 37% for Batavia, 39% for V306 and 43% for SM1046.

2.2. Enzymic digestion of granular starch

Digestion of starch granules was based on the simplified protocol by Muir and O'Dea (1992) with increased enzyme-to-starch ratio to ensure excess enzyme activity so that substrate availability is the limiting factor during digestion. Starch (20 mg, 10% moisture) was digested at 37 °C with 30 International Units (IU) of porcine pancreatic α-amylase type VI-B (EC 3.2.1.1; P3176, Sigma Chemical Co., St Louis, MO) in 5 ml of Tris–maleate buffer (50 mM, pH 6.9). The starches (20 mg) were also exposed to other starch-degrading enzymes: β-amylase (EC 3.2.1.2) from *Bacillus cereus* (100 IU, E-BCBAM, Megazyme International Ireland, Ltd., County Wicklaw, Ireland) in sodium–acetate buffer (100 mM, pH 7.2) at 40 °C; isoamylase (EC 3.2.1.68) from *Pseudomonas* sp. (10 IU, E-ISAMY, Megazyme International Ireland, Ltd., County Wicklaw, Ireland) in sodium–acetate buffer (50 mM, pH 4.5) at 50 °C; and amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* (5 IU, A7095, Sigma Chemical Co., St Louis, MO) in sodium–acetate buffer (50 mM, pH 4.5) at 37 °C. The contamination levels by α-amylase were reported to be less than 0.0001% for β-amylase and less than 0.001% for isoamylase (Megazyme). The incubation mixtures were agitated on an orbital shaking platform rotating at 100 orbits/min. Enzymic reactions were stopped by centrifugation at 2000g for 10 min, washing the pellet twice with acetone, drying the starch residues at room temperature and allowing them to equilibrate to the same moisture content in a desiccator prior to imaging.

2.3. Scanning electron microscopy

The dried starch powder was thinly spread onto circular metal stubs coated with double-sided adhesive carbon tape. The powder on the stubs was gold coated in an Eiko IB-5 Sputter Coater at 6 mA, 5 min in an argon gas environment, yielding a coating thickness of approximately 15 nm. Images of the starch granules were acquired with a Philips XL30 scanning electron microscope. Representative micrographs of each sample were taken at low magnification of 4000× under an accelerating voltage of 12 kV, spot size of 3 μm and a working distance of 10 mm, followed by higher magnification up to 20,000× to visualize features of interest.

2.4. X-ray diffraction

X-ray diffraction (XRD) measurements of starch samples were performed with a Difftech Mini Materials Analyser X-ray diffractometer (GBC Scientific Equipment Pty. Ltd.). The X-ray generator was equipped with a cobalt anode ($\lambda = 1.78897$ Å) operating at 1 kW and 3.36 mA. X-ray diffractograms were acquired at room temperature (20 ± 1 °C) over the 2θ range of 5–35 degrees at a rate of 0.50 degrees 2θ per minute and a step size of 0.05 degrees 2θ. Starch crystallinity was calculated using the peak-fitting approach as described by Lopez-Rubio et al. (2008) using the Igor software package (Wavemetrics, Lake Oswego, Oregon).

3. Results

The amounts of reducing malto-oligosaccharides released after 60 min of digestion did not vary significantly amongst wheat varieties. However, from 60 min onwards, the hydrolysis of the waxy starch continued to increase linearly, whereas the digestion of the remaining varieties started to level off. After 2 h of digestion, the difference between the waxy and amylose-rich starches was significant and it continued to increase with time (Fig. 1). After 2 h of digestion, the extent of digestion was 21% for Diamondbird, 17% for Batavia, 19% for V306 and 16% for SM1046 as compared to 27% for waxy wheat (Fig. 1). It should be noted that extent of digestion was measured from the release of reducing sugars, which is likely to give a value that is less than the number of bond cleavages. Percentage of digestion was calculated as a proportion of the maximum reducing equivalents based on the theoretical amount of maltose that can be released from the starch.

3.1. Degradation patterns of starch granules by pancreatic α-amylase

The α-amylolysis of waxy wheat starch was characterized by the formation of holes on the granular surface and disruption of the

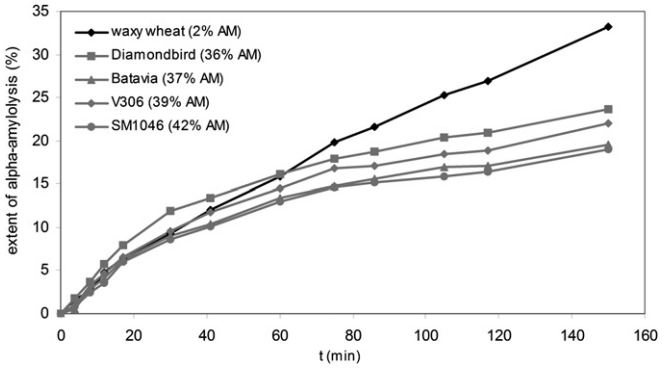

Fig. 1. Degradation kinetics of five starch varieties by pancreatic alpha-amylase. Starch (20 mg) was subjected to alpha-amylase (30 IU) and reducing malto-oligosaccharides were measured as described in the text.