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Extrusion induced low-order starch matrices: Enzymic hydrolysis and structure

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ABSTRACT

Waxy, normal and highwaymen maize starches were extruded with water as sole plasticizer to achieve low-order starch matrices. Of the three starches, we found that only high-amylose extrudate showed lower digestion rate/extent than starches cooked in excess water. The ordered structure of high-amylose starches in cooked and extruded forms was similar, as judged by NMR, XRD and DSC techniques, but enzyme resistance was much greater for extruded forms. Size exclusion chromatography suggested that longer chains were involved in enzyme resistance. We propose that the local molecular density of packing of amylose chains can control the digestion kinetics rather than just crystallinity, with the principle being that density sufficient to either prevent/limit binding and/or slow down catalysis can be achieved by dense amorphous packing.

1. Introduction

As a major macronutrient in human diets, starch is converted to glucose by the mammalian enzyme system (i.e., α-amylases and mucosal α-glucosidases) and absorbed in the small intestine, and often provides more than 50% of total caloric intake (Nishida, Uauy, Kumanyika, & Shetty, 2004). Fast digestion of starch-containing foods may contribute to general chronic diseases in people such as type II diabetes, obesity, and cardiovascular disease. In contrast, starch with slow digestion rate has been proposed to control glycemic response and insulin secretion, and (partially) passes to the large intestine as resistant starch where it functions as a carbon source to stimulate bacterial fermentation, producing metabolites such as short-chain fatty acids (Englyst & Cummings, 1985). In order to eliminate complex intrinsic host factors and individual diversity, resistant starch is most commonly measured by in vitro methods that simulate in vivo conditions of starch digestion and referred to as 'enzyme-resistant starch (ERS)' (to distinguish it from true RS which is defined as the amount of starch that escapes digestion in the small intestine and therefore passes to the large intestine) (Chanvrier et al., 2007), particularly to elucidate structure-digestibility relationships for starch-containing food.

While rapidly, slowly digestible and resistant starch fractions in the current classification suggested by Englyst and Cummings (1985) have been widely used, recent evidence suggests that ERS can be better expressed as a kinetic phenomenon rather than a thermodynamically defined entity (Butterworth, Warren, Grassby, Patel, & Ellis, 2012; Htoon et al., 2009; Zhang, Dhital, & Gidley, 2013). For example, potato starch granules (a 'resistant' starch) are not completely resistant to hydrolysis when subjected to higher enzyme concentrations, although the digestion rate is slow (Warren, Zhang, Waltzer, Gidley, & Dhital, 2015). The presence of amorphous material in enzyme-resistant fractions also confirms that the resistance is not simply based on a specific crystalline structure that is completely undigested (Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008a). Kinetic analysis of digestion is a powerful tool to understand heterogeneous reactions between complex starch substrates and enzymes. There are two types of

Abbreviations: CP/MAS, cross-polarized magic angle spinning; DSC, differential scanning calorimetry/calorimeter; G50, gelose 50; NMR, nuclear magnetic resonance; LOS, log of slope; NMS, normal maize starch; SEC, size exclusion chromatography; SEM, scanning electron microscope; WMS, waxy maize starch; XRD, X-ray diffractometry/diffractometer.

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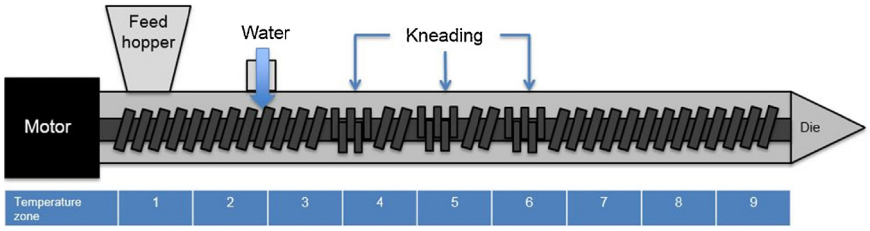

Fig. 1. Scheme of the extrusion system used in this study. (The barrel temperature profile for WMS and NMS: 105, 115, 125, 130, 130, 130, 130, 125, 120 (last barrel), and 105 (die block) °C; the temperature profile for the G50 starch: 105, 120, 135, 150, 150, 150, 150, 135, 120, and 105 °C).

rate-limiting steps which can determine enzymic digestion kinetics: (i) enzyme access/binding limited by physical barriers (e.g., intact plant tissues, whole grains and complex food products); (ii) enzyme catalysis limited by starch structural features, such as chemically modified starch, and crystalline/ordered forms such as retrograded starch and starch-lipid complexes. The ERS classification based on mechanisms to achieve lower digestion rate/extent has been recently reviewed (Dhital, Warren, Butterworth, Ellis, & Gidley, 2015; Zhang, Dhital, & Gidley, 2015). Although, it has been generally accepted that crystallinity plays a major role in determining ERS in the absence of non-starch physical barriers, recent evidence has shown that apparent crystallinity of native starches is not directly linked with the percentage of ERS obtained after extrusion (Chanvrier et al., 2007; Htoon et al., 2009; Shrestha et al., 2010). Htoon et al. (2009) reported that highly amorphous extruded high-amylose maize starches could deliver high ERS contents in vitro. Even for native starch granules, crystallinity alone cannot explain their relative resistance to digestion (Zhang, Ao, & Hamaker, 2006). Therefore, there should be additional mechanisms involved in the formation of enzyme-resistant fractions apart from crystallinity. We hypothesize that the local molecular density of starch chains, in both native and processed starches, can control the digestion rate and extent. Although, crystallinty is one way to achieve local molecular density, it appears that non- or weakly-crystalline chains can also pack in an equally enzyme-resistant form, the details of which are currently poorly understood.

Extrusion is a common commercial processing technique for starch-based foods such as pasta and breakfast cereals. The main advantages of extrusion processing include the ability to handle viscous polymers in the presence of plasticizer (normally water in food use). Similarly, the combination of a high temperature with a large amount of mechanical energy input during a short time period can be used to promote structural changes of starch such as gelatinization, melting, degradation and fragmentation (Lai & Kokini, 1991). Generally, molecular, supramolecular and granular structures are disrupted by thermal (barrel temperature), humidity (plasticizer content) and energy input (e.g., screw speed, feeding rate, die size and screw configuration) during extrusion cooking, each of which could be expected to increase the accessibility of degrading enzymes to starch polymers in extruded products. The intense shear regime within the extruder can cleave α-(1 → 4), α-(1 → 6)-bonds as well as starch ordered structures such as crystallites and double helices. Amylopectin (highly branched large molecule) is degraded to a larger extent than the essentially linear and lower molecular weight amylose, and the degradation of amylopectin mainly occurs in the outer branch chains (Liu, Halley, & Gilbert, 2010). The larger molecules of amylopectin together with high branching density and short branch length are associated with higher susceptibility to shear degradation (Liu et al., 2010). Fragmentation of starch during extrusion depends on the operating conditions of the extruder such as screw speed, temperature, and moisture content as well as the type of starch used.

In the current study, we aim to understand the structural origins of enzyme resistance, especially from (near) amorphous conformations using starch extrudates and cooked starches as model systems. For this purpose, three maize starches with different amylose contents were extruded with water as a sole plasticizer, and in vitro digestion kinetic profiles of starch extrudates were examined. On the basis of the molecular and microscopic structures of initial extrudates and digestion remnants, mechanisms of enzyme resistance from starch matrices with non- or low-order conformations are discussed.

2. Materials and methods

2.1. Materials

Three commercial starches, i.e., waxy (WMS), normal (NMS), and high-amylose (Gelose 50, G50) maize starches, were used in this study. NMS was from New Zealand Starch Ltd. (Auckland, New Zealand), and the other two starches were purchased from Ingredion Pty., Ltd. (Lane Cove, NSW, Australia). The apparent amylose contents of WMS, NMS, and G50 were found to be 0.1%, 27.5%, and 56.8%, respectively, using an iodine colorimetric method (Hoover & Ratnayake, 2001). Porcine pancreatic α-amylase (A3176, activity 23 units/mg) and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extrusion processing

The extrusion processing was performed on a Haake Polylab co-rotating twin-screw extruder (Thermo Fisher Scientific, Karlsruhe, Germany) equipped with a 3 mm diameter cylindrical die at a constant feed rate of 0.4 kg/h. The screw diameter was 16 mm, and the length/ diameter ratio was 42:1. The extruder configuration, temperature profile and interval assignment of the extruder barrel are shown in Fig. 1. For WMS and NMS, the barrel temperature profile was set at 105, 115, 125, 130, 130, 130, 130, 125, 120 (last barrel), and 105 (die block) °C, and the screw speed was set at 60 rpm, and plasticizer (water) content was 35 wt%. In order to achieve gelatinization for the more thermally-stable G50 starch, higher temperature profiles (105, 120, 135, 150, 150, 150, 150, 135, 120, and 105 °C), water content and screw speeds were used (45 wt% and 80 rpm for batch 1; 50 wt% and 60 rpm for batch 2). All process parameters were automatically recorded by Haake Polysoft software (Thermo Fisher Scientific, Karlsruhe, Germany). Samples were collected when a steady motor torque was reached, then immediately frozen in a liquid nitrogen bath, freeze-dried to avoid any further retrogradation, and ground using a cryogenic mill (Freezer/Miller 6850, Metuchen, NJ, USA) for further digestion and structural analysis. In order to elucidate the particle size effect on digestion properties, the NMS and G50 extrudates were segregated by size using seven screen sieves (size: 20, 32, 53, 75, 90, 125 and 150 μm, Labtechnics, Kilkenny, Australia) under gravity with mechanical agitation using a sieve shaker (Labtechnics, Kilkenny, Australia).