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Physiochemical properties of highly cross-linked maize starches and their enzymatic digestibilities by three analytical methods

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ABSTRACT

Waxy maize, normal maize and high amylose maize starches were highly cross-linked (CL) with phosphate groups. The CL starches were characterized by phosphorus content, settling volume, gelatinization temperature, pasting curve, X-ray diffraction and microscopy. Their digestibilities were determined by the Englyst, Available Carbohydrate Dietary Fiber (ACDF), and AOAC 991.43 methods where starch digestion with α-amylase was done, respectively, at 37, 80 and 95 °C. The CL waxy maize starch had ~10% more phosphorus content (0.36% vs 0.32%) than the CL normal and high-amylose maize starches. Total dietary fiber (TDF) levels of the unmodified and CL starches determined by the AOAC and ACDF methods increased with increasing amylose content. The resistant starch (RS) contents of the three CL starches decreased in the order, CL high amylose (85%) > CL waxy (82%) > CL normal (61%) maize starch. The digestibilities of the unmodified and CL maize starches at 80 and 95 °C were positively correlated with their settling volumes at those temperatures, indicating that increased granule swelling decreased TDF levels. Photomicrographs of the starch granules after digestion in the TDF and RS assays showed less internal erosion of granules in CL high-amylose starch compared to CL normal and waxy maize starches.

1. Introduction

Starch consists of amylose and amylopectin molecules, occurs in plants in granular form, and is commercially extracted from maize, wheat, potato, rice, and tapioca. In humans, starch is digested predominantly by amylases in the small intestine. Based on *in vitro* digestibility, starch may be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). *In vivo* RS is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy humans (Birt et al., 2013). RS occurs because of its inaccessibility to amylase. Depending on the nature of inaccessibility, there are five classes of RS- RS1, RS2, RS3, RS4 and RS5 (Birt et al., 2013). RS1 is found in whole or coarsely ground grain where starch may be encased inside cells or in a strong protein matrix; RS2 consists of raw starch granules that resist amylase digestion, and RS3 is retrograded or recrystallized starch. RS4 is chemically modified starch with non-native chemical bonds formed, and RS5 is amylose complexed with lipid, usually a fatty acid (Zhao et al., 2011). RS is one component of dietary fiber because of its indigestibility and its potential to promote health. Dietary fiber is identified as carbohydrate polymers and oligomers that escape digestion in the small intestine and pass into the large intestine, where they are slightly or nearly completely fermented. *In vitro* RS assay and total dietary fiber (TDF) test are conducted using different sources of enzyme at different enzymatic digestion conditions (AOAC, 2000; Maningat et al., 2013a, b; McCleary, 2010). An RS assay is normally performed at 37 °C using porcine pancreatic amylase and amyloglucosidase, whereas a TDF test involves treatment with a heat stable α-amylase at high temperatures (e.g. 95–100 °C).

Chemical modification of starch affects its digestion in the small intestine (Woo and Seib, 2002; Haub et al., 2010; Maningat and Seib, 2013), and the degree to which depends on starch source, type and degree of modification, extent of starch gelatinization, and the source of enzyme used. In the case of starch modified by cross-linking with phosphorylating agents, food-grade starches are regulated in the United States to contain up to 0.4% add-on

Abbreviations: ACDF, Available Carbohydrate Dietary Fiber; CL, cross-linked; DSC, differential scanning calorimetry; P, phosphorus; RDS, rapidly digestible starch; RS, resistant starch; SDS, slowly digestible starch; STMP, sodium trimetaphosphate; STPP, sodium tripolyphosphate; TDF, Total dietary fiber.

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phosphorus (P). Most commercial cross-linked (CL) starches are produced to thicken food. CL starch thickeners have a low degree of phosphorylation (<0.04% P) and can be readily and practically completely digested by α-amylase (Ostergard et al., 1988). Conversely, highly CL starches with a phosphorus add-on of up to 0.4%, which can be produced with a combination of sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP), contain a high total dietary fiber (TDF) content (58–76%) (Woo and Seib, 2002). A high level of cross-linking reduces digestibility probably by strongly inhibiting the swelling of starch granules, which restricts access of amylase to starch molecules. The level of RS and TDF in a food or starch ingredient is most commonly determined by an *in vitro* method because an *in vivo* assay in humans is costly and associated with ethical issues. However, the *in vitro* levels of RS are method dependent and there is debate about which *in vitro* method should be used (Maningat et al., 2013a, b).

To gain a better understanding of how the RS content is affected by the analytical method, three *in vitro* methods were selected to determine RS and TDF content in CL maize starches in this study. Two methods chosen are the popular and classical assays by Englyst et al. (1992) for RS and by Prosky assay for TDF as represented by AOAC 991.43. The third method chosen is that titled, the "available carbohydrates and dietary fiber" (ACDF) assay procedure. The ACDF assay is similar to AOAC 991.43 except that the digestion of starch with heat-stable α-amylase is done at 80 °C instead of 95–100 °C and acetic acid is used to adjust the pH before amyloglucosidase digestion. Removal of digestible starch from a food sample in the Englyst assay is done in a single digestion step of 37 °C and pH 5.2 using a mixture of pancreatic α-amylase and glucoamylase. In contrast, digestible starch is removed in the Prosky and ACDF assays in two stages; the first is with heat-stable bacterial α-amylase at 95 °C or 80 °C at pH 8.0 and the second with fungal glucoamylase at 60 °C and pH 4.5. For certain samples, the ACDF method is claimed to produce results similar to the AOAC 991.43 method (Available Carbohydrates and Dietary Fibre assay procedure, Megazyme International Ireland, Ltd., 2006). However, RS2 and RS3 are less hydrolyzed in the ACDF method due to a decreased digestion temperature of 80 °C rather than 95 °C, which results in a higher content of RS reported as TDF for starch (McCleary and Rossiter, 2006). The digestibilities of unmodified maize starch have been reported (Brewer et al., 2012; McCleary, 2007) but the digestibilities of CL maize starches by different *in vitro* methods are not well documented. The objectives of this study were to determine the physical properties of highly CL maize starches with different amylose contents and to compare their digestibilities by the Englyst, ACDF, and AOAC 991.43 methods.

2. Materials and methods

2.1. Materials

Waxy (Amioca), normal (Melojel), and high-amylose (HYLON VII) maize starches were obtained from National Starch LLC (Bridgewater, NJ), which is now Ingredion Inc. Those starches contained 0.5, 25, and 71% amylose, respectively, as determined by the potentiometric iodine method (Shi et al., 1998). STMP was purchased from MP Biomedicals, LLC (Solon, CA). STPP, sodium sulfate, sodium hydroxide, and hydrochloric acid were obtained from Fisher Scientific (Pittsburgh, PA). TDF assay kit (catalogue no. K-TDFR 05/12) and ACDF assay kit (catalogue no. K-ACHDF 09/11) were obtained from Megazyme International Ireland, Ltd. (Wicklow, Ireland). To conduct the Englyst assay, porcine pancreatin (catalogue no. P7545) with α-amylase activity of 200 United States Pharmacopeia (USP) units/mg and amyloglucosidase (catalogue no. A7255) with an enzyme activity of 20,300 units/g were purchased from Sigma–Aldrich, Inc. (St. Louis, MO). One unit (U) of α-amylase activity is equivalent to 0.9 mg of glucose released from soluble starch in 3 min at 37 °C and pH 5.8 and 1U of amyloglucosidase is equivalent to 0.7 mg of glucose released from soluble starch in 3 min at 37 °C and pH 5.8. All chemicals were reagent grade.

2.2. General methods

P content was assayed using the procedure of Smith and Caruso (1964). Moisture content was determined by AACC Air Oven Method 44-15 (AACC, 2000), which entails heating a sample at 135 °C for 2 h.

2.3. Preparation of CL phosphorylated starch

CL waxy, normal, and high-amylose maize starches were prepared by the method of Woo and Seib (2002) using a combination of 12% (starch basis) STMP and STPP (99:1/w:w) as phosphorylating agents. The reaction was carried out at pH 11.5 and 45 °C for 3 h. The CL starches were isolated by neutralization, washing thoroughly with water and drying at 40 °C in an oven to about 10% moisture.

2.4. Settling volume measurement and microscopic observation

The settling volume was determined using the method of Tayal (2004) with a slight modification. Starch (5.0 g, db) was constantly stirred in 100 mL distilled water within a water bath at 37 °C for 30 min. After the slurry was cooled to room temperature with constant stirring, 20.0 g of the slurry was transferred to a 100 mL graduated cylinder containing distilled water (80 g). The settling volume of the starch in the final mixture, which approximately equaled 1.0 g of starch on a dry basis, was taken after 24 h. The same steps were repeated with the temperature of the water bath adjusted to 80 °C and 95 °C. The slurries heated to 80 °C and 95 °C were placed on microscope slides and viewed under an Olympus BX-51 microscope (Olympus, Tokyo, Japan) fitted with a 40× objective. Slurries of the three unmodified and the three CL starches were also prepared at room temperature (24 °C) for microscopic observation as blank samples to compare with those in the heated slurries.

2.5. Pasting properties

The pasting properties of the CL starches suspended in distilled water were determined using a Micro Visco-Amylograph (C.W. Brabender Instruments, Inc, South Hackensack, NJ). CL starch was added to distilled water to prepare a 20% suspension (dry weight basis, w/w). The heating and cooling cycles were programmed to raise the temperature of the suspension from 50 °C to 90 °C at a rate of 10 °C/min, to hold it at 90 °C for 6 min, and to cool it to 50 °C at a rate of 10 °C/min. The viscosity of a starch suspension was expressed in Brabender Units (BU).

2.6. Thermal properties

The gelatinization temperatures and enthalpies of the unmodified and CL starches were measured by a differential scanning calorimeter (DSC) (TA Instruments Q100, TA Instruments, New Castle, DE) at a solid content of 33.3% (w/w, dry basis). A starch/water mixture was stirred well and allowed to equilibrate in a closed vessel for 1 h at 25 °C. Then, 30 mg of a mixture was weighed into a 40 μl aluminum crucible, which was immediately hermetically sealed. The heating scan was performed from 10 °C to 130 °C at a rate of 10 °C/min. A sealed empty crucible was used as a reference, and the DSC instrument was calibrated using indium. The