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SCIENCE DIRECT
Carbohydrate Polymers 63 (2006) 167–175
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation ☆

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Received 20 January 2005; revised 13 July 2005; accepted 16 July 2005
Available online 15 December 2005

Abstract

Foods with increased levels of slowly digestible starch (SDS) and resistant starch (RS) are thought to provide nutritional benefits for humans. High-amylose (~70%) corn starch (Hylon VII) was simultaneously heat-moisture treated and phosphorylated/cross-linked with a 99/1 (w/w) mixture of sodium trimetaphosphate/sodium tripolyphosphate (STMP/STPP) at initial pH 11.5. Modeling was done to determine the effects of moisture (21–49% of total mixture) and STMP/STPP (2.8–11.2% of dry starch, sb) on the phosphorus (*P*) content of the modified starch and its level of RS after cooking. Reacting Hylon VII with 10% of STMP/STPP (sb) at 45% moisture for 4 h at pH 11.5 and 110 °C gave a product with 0.39% *P*, 14% SDS, and 43% RS in the freshly cooked starch compared to 0.03% *P*, 14% SDS, and 25% RS for untreated Hylon VII. The modified Hylon VII had 90% dietary fiber content and a higher gelatinization temperature of ~20 °C. Hylon V, waxy, and normal corn starches were modified similarly to ~0.4% *P*, and the raw and cooked modified starches contained, respectively, 73, 43, and 42% and 44, 32, and 32% of combined SDS and RS.

Keywords: Resistant starch; Heat-moisture treatment; Phosphorylation; Cross-linking

1. Introduction

Dietary starch has different physiological effects in humans depending on its rate and extent of digestion. The digestion of starch occurs predominantly in the small intestine where pancreatic α-amylase is released into the lumen and where amyloglucosidase, α-glucosidase, and maltase are embedded in the brush border of the intestinal wall. Approximately 10% digestion of starch is catalyzed by salivary α-amylase (Rendleman, 2000). The rate of starch digestion in food is altered by factors that are extrinsic and intrinsic to starch (Englyst, Kingman, & Cummings, 1992). The extrinsic factors include the food particle size, viscosity of the digest, α-amylase inhibitors, and the level of α-amylase in an individual, whereas the intrinsic factors include the swelling and solubilizing of starch granules (degree of cooking), extent of branching, and the physical association of starch chains and their degree of substitution.

Starch has been classified into rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS) (Englyst et al., 1992). RDS is rapidly and completely digested in the small intestine, while SDS is slowly but completely digested in the small intestine. RS was first observed in the development of methods to measure non-starch polysaccharides in foods (Englyst, Wiggins, & Cummings, 1982), and has been defined in the framework of EURESTA as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Delcour & Eerlingen, 1996).

Englyst et al. (1992) developed an in vitro method to evaluate RDS, SDS, and RS in food. Levels of RS determined in vitro on five food products by the Englyst method were similar to levels escaping in vivo digestion in ileostomy patients (Englyst et al., 1992). Moreover, values of rapidly available glucose or RDS in foods measured in vitro were highly correlated with their in vivo glycemic responses (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Food labels include RS within the dietary fiber level of a food. In most countries dietary fiber in food is

☆ Contribution No. 05-200-J form the Kansas Agricultural Experiment Station, Manhattan, KS 66506, USA.

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0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.07.022

determined gravimetrically after removal of lipid and enzyme-digestible protein and starch (Van der Kamp, 2004).

Processed foods rich in carbohydrates are often rapidly digested and are low in dietary fiber (Englyst et al., 1992; Englyst, Kingman, Hudson, & Cummings, 1996). In 70 grain-based foods, the levels of RS assayed as dietary fiber rarely exceeded 4% (Ranhotra, Gelroth, & Leinen, 1999). White and whole-wheat bread, rolled oats, and corn flakes, for example, were found to be 87–98% digested in ileostomy patients (Champ, 2004). At present, the average American ingests about 12–17 g of fiber each day, but the National Cancer Institute recommends a daily intake of 20–30 g. In addition, the American Diabetes Association recommends that diabetic patients consume as much as 35 g/day (Ohr, 2004). Starch ingredients with high levels of SDS and RS are needed to improve the nutritional profiles of grain-based foods.

1.1. Starch modifications to increase RS

Heat-moisture treatment and chemical modification have been used to produce RS. RS was increased by holding high-amylose starch at 30–40% moisture content for 1–4 h at 90–120 °C (Brumovsky & Thompson, 2001; Haralampu & Gross, 1998; Shi & Trzasko, 1997). Heat-moisture treatment may increase RS levels in starch by (i) growth or perfection of existing crystals (Hoover & Vasanthan, 1994); (ii) increased interaction between amylose and amylopectin (Kawabata et al., 1994); and/or (iii) transformation of single-chain amylose into its double helical crystals (Lorenz & Kulp, 1982).

Monosubstitution of starch with phosphoryl (Janzen, 1969), hydroxypropyl (Leegwater & Lutten, 1971), acetyl (Wootton & Chaudry, 1979), citryl (Wepner, Berghofwe, Miesenberger, Tiefenbacher, & Ng, 1999; Xie & Liu, 2004), and 1-octenylsuccinyl (Heacock, Hertzler, & Wolf, 2004) substituents have been shown to decrease starch digestibility with pancreatic α-amylase. The largest change in digestibility has been achieved through cross-linking of starch, although the usual level of cross-linking of thickener starches is too low to effect resistance to α-amylase (Woo & Seib, 2003). Mixtures of STMP/STPP can be used in the USA to phosphorylate and cross-link food-grade starch up to 0.4% add-on phosphorus. Cross-linked starches highly resistant to α-amylase have been prepared by reacting a slurry of starch (~35%) at alkaline pH 10–12 with a mixture of 5–15% STMP/STPP (99/1, w/w) (Kerr & Cleveland, 1957; Woo et al., 2003). In addition, starch can be cross-linked by impregnating starch with phosphate salts at pH 10–11, drying at 40 °C to less than ~20% moisture content, then roasting at 120–140 °C (Kerr & Cleveland, 1959; Lim & Seib, 1993; Solarek, 1986).

In contrast to a slurry reaction, cross-linking starch by phosphorylating with STMP in a semi-dry roasting process provides the possibility of simultaneous heat-moisture treatment of a starch. If the rate of phosphorylation is slower than the increase in association between starch chains, the dual modification could produce an increase in SDS and RS. The objective of this study was to produce food-grade resistant starch by simultaneous heat-moisture treatment and phosphorylation (cross-linking) of corn starch and its amylose mutants.

2. Materials and methods

2.1. Materials

High-amylose (~55 and ~70%) corn starches (Hylon V and Hylon VII), waxy corn starch (Amioca), and resistant starch (Novelose 240) were provided by National Starch & Chemical Company, Bridgewater, NJ. Normal corn starch, sodium trimetaphosphate (STMP), 2-(*N*-morpholino)ethanesulfonic acid (MES), *tris*(hydroxymethyl)aminomethane (TRIS), pepsin (Cat. No. P7000), pancreatin (Cat. No. P7545), amyloglucosidase (Cat. No. A7255), gum guar (Cat. No. G4129), acid-washed Celite, and L-ascorbate 2-phosphate (sodium salt) were purchased from Sigma Chemical Company, St Louis, MO. Pentasodium tripolyphosphate (STPP) was purchased from Fisher Scientific Company, Pittsburg, PA. Assay kits for glucose (Cat. No. K-SLUC), total dietary fiber (Cat. No. K-TDFR), and α-amylase (Cat. No. K-CERA) were purchased from Megazyme, Bray, Ireland. One set of reference materials, potato starch and wheat flour, intended for in vitro starch digestion profiling, was obtained from MRC Dunn Clinical Nutrition Center, Cambridge, England. The second set, potato starch and a soft wheat flour (15.0% protein content, db), was obtained, respectively, from Avebe America Inc. (Princeton, NJ) and the Flour-Milling Laboratory at Kansas State University. 2,2′-*Bis*-(L-ascorbyl)-phosphate was synthesized and purified as its crystalline barium salt, which was converted to the sodium salt using ion-exchange chromatography (Lee, Seib, Liang, Hoseney, & Deyoe, 1978). Instant dry yeast was purchased from Fleischmann's Yeast Inc. (Fenton, MO). All chemicals were reagent grade.

2.2. General methods

All assays were replicated at least twice unless otherwise stated. Moisture content was determined by drying at 130 °C for 1 h (AACC, 2000) and phosphorus content by the procedure of Smith and Caruso (1964). Assays of glucose, α-amylase, and total dietary fiber were done with Megazyme kits. Glucose concentration was measured by glucose oxidase/peroxidase assay (McCleary & Codd, 1991), and the activity of α-amylase by hydrolysis of a non-reducing-end blocked *p*-nitrophenyl α-maltohepatoside (Sheehan & McCleary, 1988).

Phosphodiesterase and phosphomonoesterase activities in pancreatin and amyloglucosidase were determined by