

Cross-Linked Resistant Starch: Preparation and Properties¹

K. S. Woo² and P. A. Seib^{3,4}

ABSTRACT

Cereal Chem. 79(6):819–825

Resistant starches (RS) were prepared by phosphorylation of wheat, waxy wheat, corn, waxy corn, high-amylose corn, oat, rice, tapioca, mung bean, banana, and potato starches in aqueous slurry ($\approx 33\%$ starch solids, w/w) with 1–19% (starch basis) of a 99:1 (w/w) mixture of sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) at pH 10.5–12.3 and 25–70°C for 0.5–24 hr with sodium sulfate or sodium chloride at 0–20% (starch basis). The RS₄ products contain $\leq 100\%$ dietary fiber when assayed with the total dietary fiber method of the Association of Official Analytical Chemists (AOAC). In vitro digestion

of four RS₄ wheat starches showed they contained 13–22% slowly digestible starch (SDS) and 36–66% RS. However after gelatinization, RS levels fell by 7–25% of ungelatinized levels, while SDS levels remained nearly the same. The cross-linked RS₄ starches were distinguished from native starches by elevated phosphorus levels, low swelling powers (≈ 3 g/g) at 95°C, insolubilities ($< 1\%$) in 1M potassium hydroxide or 95% dimethyl sulfoxide, and increased temperatures and decreased enthalpies of gelatinization measured by differential scanning calorimetry.

Starch is consumed in a variety of foods and serves as a major source of energy for humans. The digestion of starch is mediated by salivary and pancreatic α -amylases that release oligosaccharides and dextrans into the lumen of the small intestine. The latter are further hydrolyzed to glucose by glucoamylase and α -glucosidase in the brush border region of the small intestine.

Starch sometimes resists digestion by α -amylase. Englyst et al (1992) classified ingested starch based on its probable digestive fate in vivo. They proposed three classes of dietary starch: 1) rapidly digestible starch (RDS), which is likely to be digested in the human intestine; 2) slowly digestible starch (SDS), which is likely to be slowly yet completely digested in the small intestine; and 3) resistant starch (RS), which is likely to resist digestion in the small intestine.

RS is defined as the sum of starch and starch degradation products not digested in the small intestine of healthy individuals. It is subdivided into four categories depending on the cause of resistance (Englyst et al 1992; Eerlingen et al 1993): RS₁, physically inaccessible starch due to entrapment in a nondigestible matrix; RS₂, raw starch granules with crystallinity; RS₃, retrograded amylose; and RS₄, chemically modified starch.

SDS and RS are of particular interest because of possible potential health benefits for humans. A high proportion of SDS relative to RDS in a starchy food indicates a food with a low glycemic index. Foods with a low glycemic load are thought to be beneficial for all individuals, especially for type II diabetics (Englyst et al 1999; Björck et al 2000; Roberts 2000; Roberts et al 2000; Jenkins et al 2001).

RS is recognized as one component of nondigestible carbohydrates in humans, where it has been shown to be a mild laxative, to be almost totally fermented in the colon to short chain fatty acids, and to reduce fecal pH and secondary bile acids. The short chain fatty acids, mainly acetate, propionate, and butyrate, stimulate colonic blood flow and fluid and electrolyte absorption. Butyrate is a preferred nutrient for colonocytes and, moreover, it inhibits the development of colonic cancer cells (Brown et al 2001; Topping and Clifton 2001). Taken together, these data suggest it may be desirable to increase the level of SDS and RS in foods.

Chemical modification has long been known to inhibit in vitro digestibility of starch (Janzen 1969; Leegwater and Luten 1971; Conway and Hood 1976; Hood and Arneson 1976; Wepner et al 1999).

The extent of in vitro resistance is related to the degree and type of modification, the extent of granule gelatinization, and the choice of enzyme (Filer 1971). Cross-linking of starch is practiced industrially to stabilize granule structure and to restrict swelling. However, the level of cross-linking of starches used as thickeners in food is too low to elicit resistance to α -amylase (Wurzberg and Vogel 1984; Ostergard et al 1988). Increasing the degree of cross-linking of starch granules may be expected to inhibit the entrance of α -amylase molecules (formula weight 50–60 kDa) into granules (Colonna et al 1992). Modified food starch is prepared by reaction with polyfunctional reagents such as phosphoryl chloride (POCl₃), sodium trimetaphosphate (STMP), and the mixed anhydride of acetic and adipic acid.

The objectives of this study were to prepare food-grade cross-linked starches with increased resistance to α -amylase digestion, and to characterize the cross-linked starches. Feeding studies with hamsters and mice, yet to be published, indicate that a distarch phosphate with 0.32% phosphorus and 76% AOAC resistant starch prepared from wheat contained increased levels of SDS and RS.

MATERIALS AND METHODS

Materials

Corn, potato, and rice starches, STMP, sodium tripolyphosphate (STPP), epichlorohydrin, 2-(*N*-morpholino) ethanesulfonic acid (MES), *tris*(hydroxymethyl)aminomethane (TRIS), amyloglucosidase solution, and pancreatin (P7545) were obtained from Sigma Chemical (St. Louis, MO). Wheat starch (Midsol 50) was obtained from Midwest Grain Products (Atchison, KS). Mung bean starch was obtained from a local oriental market. Tapioca starch, waxy corn starch (Amioca), high-amylose corn starch (Hylon VII), and a retrograded RS₃ resistant starch (Novelose 330) were obtained from National Starch and Chemical (Bridgewater, NJ). A second RS₃ product (CrystaLean) was obtained from Opta Food Ingredients (Bedford, MA). Oat starch was obtained from POS Pilot Plant Corporation (Saskatoon, SK, Canada). Waxy wheat starch was isolated and purified from a waxy wheat (donated by R.A. Graybosch, University of Nebraska, Lincoln, NE) by protease digestion of cracked kernels (Reddy and Seib 2000). Thermostable α -amylase (Termamyl) solution was obtained from Novozymes (Franklinton, NC). Phosphoryl chloride was obtained from Aldrich Chemical (Milwaukee, WI). A glucose enzyme-assay kit was obtained from Boehringer Mannheim (Indianapolis, IN).

Methods

Protein, ash, and moisture contents, respectively, were determined by Approved Methods 46-13, 08-01, and 44-15 (AACC 2000). Phosphorus levels in starch samples (5–10 g) were determined by the procedure of Smith and Caruso (1964).

The pasting behavior of starch was examined in a Brabender Visco-graph-E (C.W. Brabender, Hackensack, NJ) at 75 rpm with a

¹ Contribution No. 02-198-J, Department of Grain Science and Industry, Kansas State University, Manhattan, KS.

² Research associate, Graduate School of Biotechnology, Laboratory of Food and Biomaterial Chemistry, Korea University, Seoul, S. Korea.

³ Professor, Department of Grain Science and Industry, Kansas State University, Manhattan, KS, 66506.

⁴ Corresponding author. Fax: 785-532-7010. E-mail: pas@wheat.ksu.edu.

torque attachment of 700 g·cm at full scale. An aqueous slurry of starch (8.0%, db) was heated at the rate of 1.5°C/min from 30 to 95°C, maintained at 95°C for 30 min, cooled to 50°C, and held at 50°C for 30 min (Mazurs et al 1957).

Starch solubility in dimethyl sulfoxide was examined by mixing starch (0.5 g, as-is basis) with 90% DMSO (100 mL) while shaking in a water bath. After 30 min, insoluble starch was recovered by centrifuging at 8,000 × g for 15 min. The supernatant was decanted, and the sedimented starch washed with ethanol (100 mL, 2×) followed by drying at 40°C. The dried residue was weighed to determine insolubility in DMSO.

X-ray diffractograms were obtained with an X-ray diffractometer (model 42273, Philips, Mahwah, NJ) operated at 35kV and 20 mA. X-ray diffraction patterns of starch were taken with Cu/Ni foil-filtered, K_α radiation. The samples were scanned through 2θ (diffraction angle) range of 5–35° at 2° of 2θ/min. A step interval of 0.01° and a count time of 1 sec were used.

Scanning electron micrographs (SEM) (Etec-Autoscan, Hayward, CA) were taken at an accelerating potential of 20 kV. The starch samples were sprinkled onto double-sided adhesive tape on top of specimen stubs and then coated with gold under vacuum.

Differential scanning calorimetry equipment (Pyris 1, Perkin-Elmer Norwalk, CT) included a thermal analysis data station. Starch (2 mg, db) was weighed into a small volume aluminum pan (Perkin-Elmer); distilled water (≈8 μL) was added and the pans were sealed. The DSC run was performed at 20–120°C at a scanning rate of 10°C/min. The instrument was calibrated with indium.

Unless otherwise stated, assays were performed in triplicate.

Determination of Total Dietary Fiber by the AOAC Method

RS was determined using the Sigma kit TDF-100A for Method 991.43 of the Association of Official Analytical Chemists (AOAC 2000), except no corrections were made for protein and ash in the undigested residue. Starch (1.00 g, db) was dispersed in 0.05M MES-TRIS buffer solution (40 mL, pH 8.2) in a 400-mL tall-form beaker, and heat-stable α-amylase solution (50 μL) was added. The beaker was covered with aluminum foil and placed in a water bath at 95–100°C for 35 min, during which time the contents were gently swirled every 5 min by hand. After cooling to 60°C, protease (100 μL) was added and the mixture was incubated for 30 min and continuously stirred at low speed. The solution was adjusted to pH 4.0–4.7 by adding 0.56M hydrochloric acid solution (4.0–4.5 mL), and amyloglucosidase solution (300 μL) was added. After incubating for 30 min, 4 volumes of 95% ethanol (≈200 mL, preheated to 60°C) were added, and the mixture was allowed to stand for 1 hr at

room temperature. The precipitate was collected on a bed of diatomaceous earth (1.0 g as a filter aid) on a sintered glass crucible (porosity no. 2) that had been preheated at 105°C to constant weight. The insoluble residue was washed with 78% ethanol (15 mL, 2×), absolute ethanol, and acetone. The crucible with the residue was dried overnight in a forced-draft oven at 105°C, and weighed after cooling to room temperature in a desiccator over anhydrous calcium sulfate. Total dietary fiber was the insoluble residue expressed as the percentage of starch on a dry basis.

α-Amylase Digestibility of Cross-Linked Resistant Starch

The digestion profile of starch was determined by a modification of the procedure of Englyst et al (1992). To prepare enzyme solution I, amyloglucosidase solution (0.14 mL) was diluted to 6.0 mL with water. Enzyme solution II was prepared by suspending porcine pancreatin (12 g) in water (80.0 mL), centrifuging the mixture for 10 min at 1,500 × g, then transferring a portion (54.0 mL) of the supernatant into a flask. Enzyme III was prepared immediately before use by mixing water (4.0 mL), enzyme solution I (6.0 mL), and enzyme solution II (54.0 mL).

A starch sample (200 mg, db) was mixed with sodium acetate buffer (pH 5.2, 15.0 mL) and enzyme solution III (5.0 mL) in a polypropylene centrifuge tube (30.0 mL). The tube was incubated in a shaking water bath (90 strokes/min) at 37°C. After 20 min, an aliquot (0.5 mL) of the digest was added to 66% ethanol (20.0 mL), mixed, and centrifuged. The glucose concentration in the supernatant was determined by glucose oxidase, and RDS was calculated by multiplying glucose (mg) released by 0.9 and dividing by 200 mg. The glucose concentration at a digestion time of 120 min was determined in the same manner, and SDS was obtained by the difference of

TABLE II
Levels of Phosphorus and AOAC Dietary Fiber in Cross-Linked Low-Swelling Resistant Starch (RS₄) Prepared from Wheat in Aqueous Slurry with Different Ratios of STMP/STPP^a

STMP/STPP (w/w)	Phosphorus (%)	RS ₄ (%)
1:99	0.03	<1.0
25:75	0.13	21.6 ± 2.1
50:50	0.21	56.6 ± 1.0
75:25	0.29	63.7 ± 1.0
99:1	0.32	75.6 ± 1.8
100:0	0.32	75.7 ± 2.1

^a All reactions on wheat starch in aqueous slurry (33% starch) with 10% (sb) of different mixtures of sodium trimetaphosphate (STMP)/sodium tripolyphosphate (STPP) at pH 11.5 and 45°C for 3 hr in the presence of 10% (sb) sodium sulfate.

TABLE I
Dietary Fiber Levels of Cross-Linked Resistant Starch (RS₄) Prepared from Wheat^a

Reagent (% , sb)	Cross-Linking Reaction Conditions				Product	
	pH	°C	hr	Na ₂ SO ₄ (% , sb)	Phosphorus (%)	RS ₄ Level (%)
Blank	11.5	45	3	10	<0.01	<1.0
0						
STMP/STPP ^b	11.5	45	3	10	0.32	75.7 ± 1.6
12						
POCl ₃ ^c						
0.1	11.0	25	1	2	<0.1	<1.0
0.1	12.0	25	1	15	<0.1	<1.0
1.0	11.5	25	1	15	0.17	52.7 ± 6.6
2.0	11.5	25	1	15	0.28	85.6 ± 4.5
Epichlorohydrin						
0.3	11.5	25	1	15	nd ^d	1.8 ± 1.0
1.0	11.5	25	1	15	nd	57.4 ± 3.9
2.0	11.5	25	1	15	nd	75.8 ± 0.3

^a Samples prepared with different reagents in aqueous slurry with ≈33% starch. Resistant starch (RS) level measured by AOAC method using a thermally stable α-amylase at 100°C for 30 min.

^b Sodium trimetaphosphate and sodium tripolyphosphate (11.9:0.1, w/w).

^c Phosphoryl chloride.

^d Not determined.

digested starch between 20 and 120 min. Undigested starch was the RS fraction, calculated by the difference between starch minus the sum of RDS and SDS.

Cross-Linking Reactions of Wheat Starch

Phosphoryl chloride (POCl₃) and epichlorohydrin. In all alkaline cross-linking reactions on starch slurries, a combination pH electrode (model H-5510-022, Cole-Palmer, Chicago, IL) suited to high-sodium ion concentration was used. Cross-linking of starch with POCl₃ was done essentially by the method of Felton and Schopmeyer (1943). Wheat starch (50.0 g, db) was slurried for 1 hr at 25°C in water (70.0 mL) and sodium sulfate (1.0 g) was added, followed by ≈4 mL of 1.0M sodium hydroxide until the slurry reached pH 11.0. POCl₃ (0.1%, w/w, starch basis [sb]) was injected with a microliter syringe into the starch slurry, and after 1 hr, the slurry was adjusted to pH 5.5 with 1M hydrochloric acid. The starch was recovered by centrifuging (15,000 × g, 10 min), washing with water (100.0 mL, 4×), and drying at 40°C. Another cross-linking reaction was done with POCl₃ (0.1%, w/w, sb) in the same manner, except at pH 12.0 and 15% (sb) sodium sulfate.

In a second series of cross-linking reactions, 1.0 or 2.0% (sb) POCl₃ was added dropwise to starch (50 g, db) being slurried in water (70 mL) at 25°C and pH 11.5 containing sodium hydroxide and sodium sulfate (15%, sb). The pH of the reaction mixture was kept constant at pH 11.5 by adding 1.0M sodium hydroxide. The mixture was neutralized and the cross-linked starch was isolated as before.

Cross-linking of starch with epichlorohydrin was done according to the procedure of Caldwell et al (1953). Cross-linking reactions were in slurry (starch 50 g, water 70 mL) with 0.3, 1.0, and 2.0% (sb) epichlorohydrin in the presence of 15.0% (sb) sodium sulfate at 25°C and pH 11.5 for 14 hr, and the cross-linked starch was isolated as before.

Semi-moist reaction with STMP/STPP. Wheat starch (50.0 g, db) was stirred in water (70.0 mL) at 25°C containing 2.5 g of sodium sulfate (5.0%, sb) and 2.0 or 4.0% of 99:1 (w/w) mixture of STMP/STPP (Lim and Seib 1993). The slurry was adjusted to pH 11.0 by adding 1.0M sodium hydroxide and then stirred 1 hr. The entire slurry was dried to <15% moisture content in a dish (d = 96.8 mm, h = 11.1 mm) before heating 2 hr at 130°C in a forced convection oven. The reacted solids were cooled to room temperature and dispersed in distilled water (100.0 mL). The cross-linked starch was isolated as before.

In aqueous slurry with STMP/STPP. Reference Method: 12% (sb) STMP/STPP, pH 11.5, 45°C, 10% (sb) sodium sulfate. Wheat starch (50.0 g, db), water (70.0 mL), STMP (5.9 g, 11.9%, sb), STPP (0.06 g, 0.12%, sb) and sodium sulfate (5.0 g, 10%, sb) were placed in a round-bottom flask and the mixture was adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25.0 mL, 2.0%, sb). The slurry was stirred continuously, warmed to 45°C, and held at 45°C during a 3-hr reaction

period. After the reaction period, the pH of the slurry declined by ≈0.2–0.3 pH units. The slurry was adjusted to pH 6.5 by adding 1.0M hydrochloric acid, usually <20 mL, and the starch was collected by centrifugation, washed with water (150 mL, 7×), and dried at 40°C. Inadequate washing of the reacted starch results in contamination of phosphorylated starches with inorganic phosphorus. The phosphorylated starch contained 0.38% phosphorus, and its yield and that of all other phosphorylated starches was >99%. A blank sample was prepared by stirring wheat starch at pH 11.5 for 3 hr with sodium sulfate (10%, sb), but with no STMP or STPP.

RS₄ samples from waxy wheat, corn, waxy corn (Amioca), high-amylose corn (Hylon VII), rice, potato, mung bean, tapioca, and oat starches were also prepared according to the same procedure. In waxy corn, high-amylose corn, and rice starches, the solids content of the starch slurry was adjusted to ≈29% (w/w) by adding extra water (20 mL), which facilitated dispersion of the starch granules.

Variations of Phosphorylation Conditions

With a temperature gradient in the presence of sodium sulfate (10%, sb). Wheat starch (50 g, db), water (70 mL), STMP (6.93 g), STPP (0.07 g), and sodium sulfate (5.0 g) were placed in a round-bottom flask and the mixture was adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25 mL). The slurry was stirred continuously and heated at an average rate of 1.1°C/min from 25 to 70°C, then held at 75°C for 20 min. After cooling to room temperature, the slurry was adjusted to pH 6.5, and the modified starch was washed and isolated as before.

With a pH gradient. Wheat starch (50 g, db), water (70 mL), and sodium sulfate (5.0 g) were placed in a round-bottom flask with a mixture of STMP (8.91 g) and STPP (0.09 g), and the mixture was heated to 45°C and adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25 mL). The slurry was stirred for 5 min, and 10 mL of sodium hydroxide solution was added. The addition of sodium hydroxide solution was repeated twice more with 5-min intervals to give pH 12.3, and the slurry was continuously stirred another 15 min. After a total reaction time of 30 min, the slurry was adjusted to pH 6.5 by adding 1.0M hydrochloric acid (usually <45 mL), and the modified starch was isolated as before.

At high temperature (70°C) in the presence of 20% (sb) sodium sulfate. Wheat starch (50 g, db), water (70 mL), and a high level of sodium sulfate (10.0 g) were placed in a round-bottom flask at 25°C, and the mixture was adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25 mL). The slurry was stirred continuously, heated to 70°C, and then a mixture of STMP (4.96 g) and STPP (0.05 g) was added. After stirring at 70°C for 30 min, the slurry was neutralized, cooled, and the modified starch was isolated as before.

At room temperature for 5 hr in the presence of 10% (sb) sodium sulfate. Starch was phosphorylated in a slurry reaction at room temperature for a somewhat longer reaction time (5 hr vs. 3 hr), reduced reaction temperature (25°C vs. 45°C), and increased level of

TABLE III
Preparation of Cross-Linked, Low-Swelling RS₄ Prepared from Wheat Starch with STMP/STPP Under Various Reaction Conditions

Reaction No.	Reaction Condition ^a					Product	
	pH	Temperature (°C)	Na ₂ SO ₄ (% sb)	STMP+STPP (% sb)	Time (hr)	Phosphorus (%)	AOAC RS (%)
1	11.5	45	10	12	3	0.32	75.7 ± 2.1
1a ^b	11.5	45	10 (NaCl)	12	3	0.62	99.6 ± 1.0
2	11.5	25–70 ^c	10	14	1	0.33	85.9 ± 1.9
3	11.5–12.3 ^d	45	10	18	0.5	0.35	88.1 ± 3.1
4	11.5	70	20	12	0.5	0.32	81.2 ± 3.3
5	11.5	25	10	14	5	0.32	83.3 ± 0.3
6	12.0	25	0	10	12	0.33	85.7 ± 1.9
7	11.5	45	10	19	1	0.33	85.8 ± 2.3

^a All reactions done on wheat starch in aqueous slurry (≈33% starch solids) with different levels of a 99:1(w/w) mixture of STMP/STPP. Concentrations (wt%) of sodium sulfate and total STMP/STPP are on a starch basis (sb).

^b NaCl was used instead of Na₂SO₄.

^c Temperature during reaction began at 25°C and was ramped to a final temperature of 70°C at a rate of 1.1°C/min. Total reaction time was 1 hr.

^d Reaction began at pH 11.5 and was ramped to a final pH of 12.3 by incremental additions (10 mL, 3×) of 1.0M sodium hydroxide to the reaction mixture with 5-min intervals and a total reaction time of 0.5 hr.

STMP/STPP mixture (14% vs. 12%, sb). Wheat starch (50 g, db), water (70 mL), STMP (6.94 g), STPP (0.06 g), and sodium sulfate (5.0 g) were placed in a round-bottom flask, and the mixture was adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25 mL). The slurry was continuously stirred and held at room temperature for 5 hr, after which time the reaction mixture was neutralized, and the modified starch was isolated as before.

With 19% (sb) STMP/STPP at medium temperature (45°C) with 10% (sb) sodium sulfate. Wheat starch (50 g, db), water (70 mL), STMP (9.4 g), STPP (0.1 g), and sodium sulfate (5.0 g) were placed in a round-bottom flask and the mixture was adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25 mL). The slurry was stirred continuously, warmed to 45°C, then held at 45°C for 1 hr, after which time the reaction mixture was neutralized and the modified starch was isolated as before.

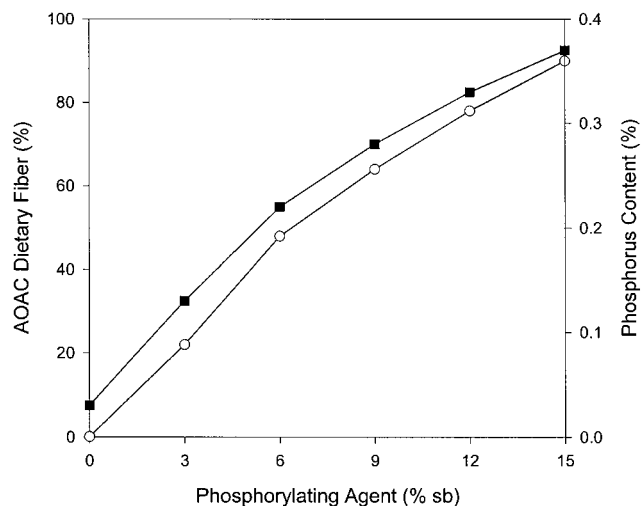


Fig. 1. Dietary fiber (○) (resistant starch, RS₄) and phosphorus level (■) of cross-linked, low-swelling RS₄ prepared by reaction of wheat starch (≈33%, w/w) in aqueous alkali with phosphorylating reagent (99:1, w/w, mixture of sodium trimetaphosphate [STMP] and sodium tripolyphosphate [STPP]) at pH 11.5 and 45°C for 3 hr in the presence of 10% (sb) sodium sulfate.

At room temperature and high pH without the presence of sodium sulfate. Wheat starch (50 g, db), water (70 mL), STMP (6.94 g), and STPP (0.06 g) were placed in a round-bottom flask at 25°C and stirred for 5 min. Then the mixture was adjusted to pH 12.0 by adding 1.0M sodium hydroxide (45 mL) and stirred continuously at room temperature for 12 hr. After the reaction, the starch slurry was neutralized to pH 6.5, and the modified starch was isolated as before.

With 12% (sb) of various proportions of STMP and STPP at pH 11.5, 45°C, and 10% (sb) sodium sulfate. A series of phosphorylated starches were prepared from wheat starch with mixtures of STMP and STPP in different ratios while maintaining the total level of phosphorylating agents (STMP+STPP) at 12% (sb). All other reaction conditions were identical to those used in the reference method.

With 12% (sb) STMP/STPP in the presence of 2–6% (sb) sodium chloride. Wheat starch (50 g, db), water (70 mL), STMP (6.94 g), STPP (0.06 g), and sodium chloride (1.0, 2.0, and 3.0 g) were placed in a round-bottom flask and the mixture was adjusted to pH 11.5 by adding 1.0M sodium hydroxide (25 mL). The slurry was stirred continuously, warmed to 45°C, then held at 45°C for 1 hr, after which time the reaction mixture was neutralized, and the modified starch was isolated as before.

Statistical Analysis

Data were statistically analyzed with the SAS software system (v. 6.10, SAS Institute, Cary, NC). Means and least significant differences (LSD) were calculated by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Cross-Linking with POCl₃, Epichlorohydrin, or STMP/STPP

Cross-linking of wheat starch in aqueous slurry (≈33% starch) with the maximum permissible levels (CFR 2001) of POCl₃ (0.1%, sb) and epichlorohydrin (0.3%, sb) gave no AOAC dietary fiber (RS) when digested at 100°C for 30 min with a heat-stable α-amylase (Table I). The same result would be expected for normal starches cross-linked with the maximum allowable level of adipic anhydride. Cross-linking with 1–2% (sb) of POCl₃ and epichlorohydrin, however, gave products with 53–76% of AOAC dietary fiber (RS, Table I).

Reaction of wheat starch in aqueous slurry with 12.0% (sb) of a 99:1 (w/w) mixture of STMP/STPP in 10.0% (sb) sodium sulfate for

TABLE IV
Dietary Fiber (RS) Levels, Solubility in Dimethyl Sulfoxide (DMSO), and Phosphorus Contents of Commercial RS₃ and Distarch Phosphates (RS₄)

Sample	AOAC RS (%)	DMSO Solubility (%)	Phosphorus (%)	Granule Appearance
RS ₃ type resistant starches				
Novelose 330 ^a	39.9 ± 0.6	100 ± 1	0.03	Shrunken and fused
CrystalLean ^b	19.2 ± 0.7	100 ± 1	0.04	Shrunken ellipsoids
Cross-linked, RS ₄ type resistant starches ^c				
Wheat starch				
Prime	<1.0	100 ± 1	0.06	Smooth disks and spheres
Alkali-treated	<1.0	100 ± 1	0	Smooth disks and spheres
RS ₄	75.7 ± 2.1	0 ± 1	0.32	Smooth disks and spheres
Corn starch				
Prime	<1.0	100 ± 1	0.02	Smooth spheres and polyhedrons
Alkali-treated	<1.0	100 ± 1	0	Smooth spheres and polyhedrons
RS ₄	57.8 ± 1.9	1 ± 1	0.33	Smooth spheres and polyhedrons
Potato starch				
Prime	<1.0	100 ± 1	0.07	Smooth ellipsoids
Alkali-treated	<1.0	100 ± 1	0.06	Smooth ellipsoids
RS ₄	72.8 ± 0.8	1 ± 2	0.32	Smooth ellipsoids
Rice starch				
Prime rice	<1.0	100 ± 1	0.07	Smooth tiny polygons
Alkali-treated	<1.0	100 ± 1	0	Smooth tiny polygons
RS ₄	5.4 ± 0.8	1 ± 2	0.32	Smooth tiny polygons

^a National Starch and Food (Bridgewater, NJ).

^b Opta Food Ingredients (Bedford, MA).

^c Distarch phosphates were prepared in aqueous slurry (40% starch solids) with 12% (sb) of a mixture (99:1, w/w) of STMP/STPP at pH 11.5 and 45°C for 3 hr in the presence of 10% (sb) sodium sulfate.

3 hr at 45°C and pH 11.5 gave a modified wheat starch with 0.32% phosphorus that showed highly restricted swelling (swelling power ≈3) and gave ≈76% dietary fiber (resistant starch) by the AOAC method (Table I). Those results indicate that distarch phosphate containing a high level of phosphorus would be counted as dietary fiber when assaying a food by the AOAC procedure.

Preparation of RS₄ Samples

Distarch phosphate RS₄ was prepared by reaction of granular starch either in a semi-moist condition or in an aqueous slurry with 28–33% starch solids (Tables II and III). Cross-linked wheat starches with 0.22 and 0.43% phosphorus were prepared by roasting starch with a 2 and 4% (sb) 99:1 (w/w) mixture of STMP/STPP. The two starches gave, respectively, 40 and 88% RS₄. Distarch phosphate RS₄ was produced in a slurry reaction either with a short or a long reaction time, depending on other variables. Short reaction times of <1 hr are sufficient if the pH and temperatures are increased to just below the point of starch gelatinization (Table III). Reactions 3 and 4 in Table III illustrate a short reaction time used to prepare modified wheat starch with 81–88% RS₄.

STMP is a more effective phosphorylating agent than STPP in alkaline pH, as shown in Table II. Increasing proportions of STMP increased the incorporation of phosphorus and the level of RS₄. Most phosphorylating reactions in this investigation were done at a 99:1 (w/w) ratio of STMP/STPP. The level of phosphorylating reagent and other conditions in a given reaction mixture were chosen to maintain the residual phosphorus in the product at 0.3–0.5%.

Sodium sulfate or sodium chloride is usually needed in a short-time modification process to inhibit gelatinization of starch granules and to accelerate the phosphorylation reaction. Granular wheat starch (37%) in water requires 68, 88, and 119% more sodium hydroxide to reach pH 12.0 in the presence of 4, 8, and 16% sodium sulfate than with no added sodium sulfate (Matsunaga and Seib 1997). The extra hydroxide and sodium ions were present almost entirely in the starch granules. High concentrations of STMP/STPP also accelerated the reaction, as illustrated for wheat starch in Fig. 1. General reaction conditions for rapid preparation of cross-linked RS₄ starches are an alkaline (sodium hydroxide) pH 11–12, a temperature of 35–45°C, 5–20% (sb) sodium sulfate or chloride, and 5–19% (sb) STMP/STPP (99:1, w/w) (Table III).

Rapid cross-linking with STMP/STPP can be achieved not only by a combination of elevated pH, elevated temperature, and elevated levels of sodium sulfate and STMP, but also by ramping up the temperature or the pH as the phosphorylation reaction proceeds (Table III). With ramping of the pH or temperature, reaction times for phosphorylation may be <0.5 hr. However, control of the level of phosphorylation becomes more difficult as the reaction is accelerated.

It is possible to produce distarch phosphate RS₄ without adding sodium sulfate or chloride. In that case, the temperature and pH must be reduced to <50°C to avoid gelatinization, and reaction times on wheat starch become many hours instead of ≤1 hr (reaction 6, Table III). If the starting starch has a higher gelatinization temper-

ature than wheat starch, for example corn starch, then phosphorylating reactions with no sodium sulfate may be conducted at ≈60°C with concomitant shorter reaction times (data not shown).

The phosphorylation reaction with STMP/STPP is rather inefficient. For example, of the phosphorus added to a reaction mixture when reacting starch with 10 and 19% (sb) of a 99:1 (w/w) mixture of STMP/STPP (reactions 6 and 7, Table III), ≈5.8 and ≈11.0% of the phosphorus added became substituted on starch, while the remainder was present in the discarded aqueous phase.

Table III shows seven different slurry reaction conditions used on wheat starch to produce distarch phosphate RS₄, all with residual phosphorus levels of 0.32–0.35% and AOAC RS levels of 76–89%. The level of residual phosphorus on the modified starch is easily monitored throughout a reaction period. The level of RS in the distarch phosphate produced at alkaline pH is directly associated with the level of phosphorus added to the starch (Fig. 1).

In Vitro Digestibility of RS₄ Samples

When the AOAC dietary fiber method was applied to measure RS₄ levels in various starches, the prime starches from wheat, waxy wheat, corn, waxy corn, potato, rice, tapioca, and oat gave <1% RS₄ (some results given in Table IV). Distarch phosphate samples with ≈0.3–0.4% residual phosphorus prepared from wheat, corn, and potato starches by a moderately rapid reaction gave 58–75% RS₄, but for unknown reasons, the sample from rice gave only 5% RS₄. Two commercial RS products, Novelose 330 and CrystaLean which

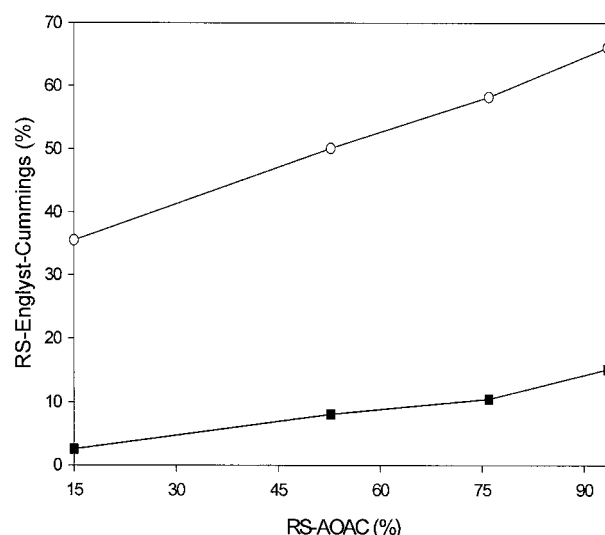


Fig. 2. Correlation between AOAC dietary fiber (RS) level and Englyst et al (1992) RS levels for four cross-linked wheat starches with 0.13–0.38% phosphorus. Modified starches were assayed in ungelatinized (○) and pregelatinized (■) state as in Englyst et al (1992), but they were added in the ungelatinized state only to the hot (100°C) digest of the AOAC procedure.

TABLE V
AOAC Dietary Fiber (RS) Levels and Digestion Profiles of Cross-Linked Wheat Starches^a

Reaction Time (hr)	Phosphorus (%)	AOAC Dietary Fiber (%)	Digestion Profile (Englyst et al 1992) ^b					
			Before Gelatinization (%)			After Gelatinization (%) ^c		
			RDS	SDS	RS	RDS	SDS	RS
1	0.13	14.0	10.6	53.9	35.5	84.2	13.2	2.6
2	0.21	52.7	1.4	48.5	50.1	75.0	16.9	8.1
5	0.32	76.0	0	41.8	58.2	74.2	15.3	10.5
7	0.38	93.4	0	33.9	66.1	71.3	13.5	15.2

^a Samples prepared in aqueous slurry (33% starch solids) with 12% (sb) of a mixture of 99:1 (w/w) STMP/STPP at pH 11.5 and 45°C in the presence of 10% (sb) sodium sulfate.

^b RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch. RS (Englyst et al 1992) method calculated as 100 – (RDS+SDS), assuming sample was 100% starch.

^c Samples heated to boiling in the digestion buffer, cooled, and assayed.

TABLE VI
Gelatinization^{a,b} of Cross-Linked Low-Swelling RS₄ Starches^c in Excess Water Determined by Differential Scanning Calorimetry

Starch Products	Phosphorus (%)	RS ₄ (AOAC) (%)	T _o	T _p	T _c	ΔH (J/g)
Wheat starch						
Prime	0.06	<1.0	56.6	61.4	66.9	9.8
RS ₄	0.32	76	60.9	69.7	77.4	8.9
Waxy wheat starch						
Prime	0.03	<1.0	60.0	64.6	72.4	13.1
RS ₄ -waxy	0.32	80	66.9	71.8	76.0	12.2
Corn starch						
Prime	0.02	<1.0	64.3	69.4	73.7	11.9
RS ₄ -corn	0.33	58	69.6	74.8	81.6	10.5
Waxy corn starch						
Prime	0.02	<1.0	65.2	71.4	77.1	15.7
RS ₄ -waxy	0.34	35	73.6	78.3	82.0	12.4
Potato						
Prime	0.07	<1.0	57.3	63.6	70.5	11.6
RS ₄ -potato	0.32	73	59.4	65.3	71.2	12.3

^a Results are the average of at least two duplicates. Standard deviation was <5%.

^b T_o, T_p, and T_c = onset, peak, and completion of endothermic peak temperatures; ΔH = enthalpy of gelatinization (J/g of dry matter).

^c Cross-linked low-swelling starches were prepared in aqueous slurry (33%, starch solids) with 12% (sb) mixture of STMP/STPP (99:1, w/w) at pH 11.5 and 45°C for 3 hr in the presence of 10% (sb) sodium sulfate.

contain retrograded amylose, are the so-called RS₃ type. The levels of RS₃ in Novelose and CrystaLean measured by the AOAC assay were 40 and 19%, respectively.

The Englyst et al (1992) profiling of the digestibility of a starch or a starchy food into RDS, SDS, and RS fractions was applied to a set of four cross-linked wheat starches with phosphorus levels of 0.13–0.32%. The resulting RS levels, before and after gelatinization, correlated with the RS levels determined using the AOAC method (Table V and Fig. 2). Before the Englyst et al (1992) assay, gelatinization of cross-linked starches was done by boiling in an excess of pH 5.2 acetate buffer. But in the AOAC method, RS gelatinization of starch took place during the assay. The RS level of Englyst et al (1992) in gelatinized samples declined by 7–25% of that in the raw product, and the greatest decrease was in the lightest cross-linked starch (Table V).

It may be that cross-linking of starch somehow restricts the entrance of α-amylase through porous channels (Huber and BeMiller 2000) that lead into the interior of cereal starches, or the cross-links interfere with formation of the α-amylase and starch complex. α-Amylase digestion of cereal and cassava starches occurs predominantly from the inside of the granules out (Gallant et al 1972, 1997), but potato starch is eroded at the granule surface, perhaps because pores do not protrude through the surface. The distribution of the phosphate cross-links in the modified starch granules is not known. Reaction of potato starch with a cationic epoxide at pH 11 for 20 hr at 42°C gave a uniform distribution of ether groups in the granule with substitution near the nonreducing end and the branch points in amylopectin (Manelius et al 2000).

The application of the total dietary fiber method (AOAC 2000) to measure RS₄ requires strict control of the procedure. In contrast, α-amylolysis of the two commercial RS₃ starches (Novelose 330 and CrystaLean) were not affected by the level of heat-stable α-amylase (50 μL vs. 100 μL) or the method of shaking (intermittent hand shaking vs. magnetic stir plate) or the digestion time period of 30 min to 2 hr (data not shown). However, in the AOAC assay of the RS₄ starches prepared here, the RS measured was affected by the method of agitation. Gentle shaking (by hand) prescribed in the AOAC method was used throughout this work. However, vigorous stirring by a magnetic stir bar decreased RS by ≤25%.

Properties of Cross-Linked RS₄

SEM showed that the distarch phosphate RS₄ samples had the same shapes and smooth surfaces as their parent starches. Descriptions of shapes of the resistant starches are given in Table IV.

The distarch phosphate RS₄ samples obviously had elevated levels of phosphorus, and they were practically insoluble in DMSO. The

X-ray diffraction data (wide angle) showed distarch phosphate RS₄ made from cereal and potato starches gave, respectively, the A- and B-type polymorphic crystal patterns with corresponding reflections (Colonna et al 1987). The pasting curves of all RS₄ starches prepared in this work did not rise above the baseline at 8% starch solids in a slurry.

The gelatinization characteristics of RS₄ starches in 4 parts water are summarized in Table VI. The RS₄ resistant starches showed higher transition temperatures (T_o, T_p, and T_c) but lower transition enthalpies (ΔH) than their respective parent starches. These changes in gelatinization behavior are consistent with annealing of the starch in the warm excess aqueous environment (Jacobs et al 1995). However, cross-linking could inhibit cooperative melting of crystals in starch granules, which could account for some of the increase in gelatinization temperature.

CONCLUSIONS

A general method has been demonstrated to increase the level of α-amylase resistance (AOAC dietary fiber) in almost any starch. Cross-linking of starch with mixtures of mostly STMP and some STPP under alkaline (sodium hydroxide) conditions restricts swelling of starch and imparts increasing resistance to α-amylase digestion as phosphorus incorporation increases to 0.4–0.5%. A range of phosphorylation conditions can be used to produce the distarch phosphates with RS₄ properties.

The distarch phosphates are identical to their parent starches in appearance as seen by SEM, and they give the same diffraction pattern in wide-angle X-ray crystallography. Distarch phosphates with >5% AOAC (Prosky) dietary fiber are characterized by insolubility in DMSO, low swelling power, no pasting curve in the amylograph, and increased gelatinization temperature and decreased gelatinization enthalpy in the DSC endotherm peak.

ACKNOWLEDGMENTS

We would like to thank Mal-Shick Shin, Chonnam National University for RS assay by the Englyst et al (1992) method.

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[Received December 10, 2001. Accepted July 17, 2002.]