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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.
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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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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

assay of free L-ascorbic acid released, respectively, from 2, 2′-*bis*-(L-ascorbyl)-phosphate (sodium salt) and L-ascorbate 2-phosphate (sodium salt). A mixture of pancreatin and amyloglucosidase was prepared according to Englyst's procedure (1992), and the two substrate solutions were prepared by dissolving either sodium 2,2′-*bis*-(L-ascorbyl)-phosphate (0.1 mmol) or L-ascorbate 2-phosphate (0.1 mmol) in 20.0 ml of 0.1 M sodium acetate buffer (pH 5.4) containing dithiothreitol (0.04 g) and dry instant yeast (0.22 g). An aliquot (5.0 ml) of the enzyme mixture was added to a substrate solution (20 ml) and after incubation for 2 h at 37 °C, the reaction mixture was centrifuged for 10 min at 10,000 *g*. Free L-ascorbic acid in the supernatant was assayed by reverse-phase high performance liquid chromatography with electrochemical detection (Wang, Liao, Hung, & Seib, 1988). The pancreatin and amyloglucosidase used in the Englyst assay did not catalyze release of L-ascorbate from 2,2′-*bis*-(L-ascorbyl)-phosphate at 37 °C, indicating no broad-specificity phosphodiesterase activity in the mixture of enzymes. A small amount of L-ascorbate (0.075 μmol/min) was released at 37 °C from an excess of L-ascorbate 2-phosphate during a 2 h digestion by the mixture of enzymes indicating a low level of monophosphate monoesterase activity.

Differential scanning calorimetry was performed on a Perkin–Elmer Pyris 1 differential scanning calorimeter equipped with a thermal analysis data station (Norwalk, CT). The instrument was calibrated with indium. Starch (~10 mg, dry basis) was weighed to the nearest 0.01 mg into an aluminum pan, and water (40 mg) was added and the pan sealed. The mixture was allowed to equilibrate for 16 h at room temperature before analysis. Samples were heated from 20 to 180 °C at a heating rate of 10 °C/min with an empty pan as a reference. Onset (T_o), peak (T_p), and completion (T_c) temperatures were determined by the software, and the transition enthalpies (ΔH) were computed from peak areas and expressed as J/g of dry matter.

The swelling power of starches was determined according to Crosbie (1991) with minor modification. A mixture of starch (1.0 g, d.b.) in water (30 ml) was heated at 92 °C for 30 min, and during heating the starch slurry was inverted twice to prevent lump formation. The slurry was centrifuged at 3000 *g* for 20 min in a tarred centrifuge tube, and the weight of the swollen sediment determined. The supernatant was heated to dryness at 105 °C for 5 h and the weight of the residue recorded. Solubility was calculated as the ratio of dried supernatant to the initial weight of dry starch, and swelling power was the ratio of wet sediment over its dry weight.

2.3. In vitro starch digestion profile

Starch digestion profiles were determined by two modifications of the method of Englyst et al. (1992): (i) 30 glass beads ($d=5$ mm) were used in a digest instead of five glass balls ($d=1.5$ cm), and (ii) shaking a digest was done at 90 strokes/min with a displacement of 3.3 cm on a reciprocal shaking bath (Model 25 & 50, Precision, Winchester, VA) instead of 160 strokes/min at a displacement of 3.5 cm. RS was calculated by the difference between total starch and the sum of RDS plus SDS. The activity of the amyloglucosidase was not measured but was reported by Sigma to be 5000 Units/g solid, where one unit of activity is defined as liberating 1.0 mg of glucose from soluble starch in 3 min (1.85 μmol/min) at pH 4.5 at 55 °C. One supply of pancreatin from Sigma was found to contain 12.9×10^3 Ceralpha Units/g of solid where the Ceralpha Unit is defined as the amount of α-amylase that releases 1 μmol/min of *p*-nitrophenol at pH 5.4 and 40 °C from the non-reducing end-blocked *p*-nitrophenyl α-glycoside of maltohepatose in the presence of excess glucoamylase and α-glucosidase. The activity of the amyloglucosidase sample was calculated to be 154 nKat/mg of amyloglucosidase, and that of α-amylase was 215 nKat/mg of pancreatin, where nKat is defined to be the enzyme activity that converts one nanomole of substrate per second to product. The levels of amyloglucosidase and α-amylase used in the digestion step of the Englyst method were approximately 167 Units/g starch and 64.5×10^3 Ceralpha Units/g starch, respectively, or 5160 and 1077 nKat/g starch.

To determine the digestion profile on cooked starch, a raw sample (~0.6 g starch db) was digested 30 min at 37 °C with pepsin (50 mg) in 0.05 M hydrochloric acid (10 ml). After adding 0.25 M sodium acetate solution (10.0 ml), the mixture was heated in a boiling water bath for 30 min, cooled to room temperature with running tap water for 10 min, and then digested with pancreatin/amyloglucosidase (Englyst et al., 1992).

2.4. Determination of dietary fiber (TDF)

TDF in resistant starch was determined gravimetrically by a modification of Method 991.43 of the Association of Official Analytical Chemists (AOAC, 2000). RS contains almost no protein and ash, so the correction for those two contaminants in the fiber was omitted.

2.5. Cross-linking and simultaneous heat-moisture treatment of amylose mutants of corn starch-general procedure

Corn starches were phosphorylated/cross-linked by the procedure of Lim et al. (1993) except: a starch cake impregnated with STMP and other reagents was heated at a moisture level of 21–49% instead of 10–15%; heating was done at a temperature of 110 °C and an initial pH of 11.5 for 4 h in a sealed container instead of 130 °C in an open vessel at pH 6–11; and the phosphorylating agent was a 99/1 (w/w) mixture of STMP/STPP instead of other proportions.

In a typical reaction starch (50 g, dry basis) was stirred for 60 min at room temperature in water (70 ml) containing sodium sulfate (2.5 g, 5% starch basis, sb) plus different levels (2.8–11.2%, sb) of the phosphorylating agent

(STMP/STPP) at pH 11.5. The initial alkalinity in the starch slurry was adjusted by adding 1 M sodium hydroxide to pH 11.5, which was measured using a sodium-tolerant pH electrode (Model H-5510-022, Cole Palmer, Chicago, IL). The mixed slurry was placed in a forced-air drying oven at 40 °C and dried to a moisture level between 21 and 49% (wet basis of total mixture). The moist starch cake was sealed in a glass jar having volume 800 cm^3, and then heated to 110 °C for 4 h. Several reaction jars were reweighed after the heating period, and they did not lose weight. After cooling to 4 °C overnight, a reacted starch cake was dispersed in distilled water (100 ml), and the slurry was found to have pH 7–8, a decline of ~4 units from the initial pH 11.5. After adding 1 M hydrochloric acid to pH 6.5, the starch was collected by centrifugation (1500 *g* for 10 min) and was washed with distilled water (7×150 ml). The starch was dried at 40 °C and ground on a Wiley Mill through a No. 40 wire screen.

2.6. Preparation of resistant corn starches under optimum conditions

The experimental design for the response surface of the starch modification reaction was a central composite, rotatable, second-order design (Box and Wilson, 1951). Two independent variables were selected for the study: the moisture content of the impregnated starch cake between 21 and 49% (total mixture basis), and the level of phosphorylating (cross-linking) agent (STMP/STPP) at 2.8–11.2% (sb) in the moistened cake. The design had nine treatments with four replications at the center point. Three responses were measured: the phosphorus level in the modified starches, the percentage of phosphorus recovered in a modified starch, and the RS levels in the freshly cooked modified starch. Data were analyzed by the response surface regression procedure of the SAS software (version 8.0, SAS Institute, Cary, NC), and the final regression equations for the three responses were derived by using the backwards stepwise selection to drop terms that were insignificant ($P \geq 0.1$). Two response surfaces, one on phosphorus content and the other on RS, were overlaid to obtain the area giving maximum RS in the cooked starches with $P \leq 0.4\%$. Reaction conditions for Hylon VII chosen from that area were a moisture content of 45% and a phosphorylating agent (STMP/STPP) concentration of 10%. Those same conditions then were used to modify Hylon V. However, normal corn starch and waxy corn starch were reacted at a moisture content of 45% for 4 h at 110 °C with a phosphorylating agent (STMP/STPP) concentration of 6 and 5.5%, respectively.

3. Results and discussion

In the early 1990 s, a number of in vitro methods were developed to measure RS (McCleary & Monaghan, 2002; McCleary & Rossiter, 2004), as well as to measure RDS and SDS in addition to RS (Englyst et al., 1992). In vitro measurements of RS have been validated by in vivo measurements on ileostomy patients (Englyst et al., 1992; McCleary & Monaghan, 2002; Muir & O'Dea, 1993; Silvester, Englyst, & Cummings, 1995). Furthermore, RDS was shown to have a linear positive correlation with glycemic response (Englyst et al., 1999), and foods with RS levels of 3–50% showed a negative correlation with glycemic indices of 40–90 in 10 starchy foods (Bjorck, Liljeberg, & Ostman, 2000).

In this work we calibrated the modified Englyst assay procedure (Fig. 1) by setting the agitation speed for the α-amylase digestion step so that the RDS and RDS + SDS in two reference materials closely matched the values reported by Englyst et al. (1999). We used two sets of the same reference materials for calibration, and the digestion data for both sets were in close agreement (Table 1). The pancreatin and amyloglucosidase used in the digestion were shown to be devoid of monophosphate phosphodiesterase activity, and to have a trace of monophosphate monoesterase activity. It was assumed that either phosphoesterase was inactive at pH ~2 during the digestion step with pepsin. Hydrolysis of phosphate esters during α-amylase/amyloglucosidase digestion of the modified starch would have reduced the levels of SDS and RS.

RS in the modified starches also was estimated by the Prosky method for dietary fiber, since that method is the official test procedure used on foods in the USA (DeVries, 2004). It is well known that the Prosky assay is inappropriate for determining RS_2-type resistant starch, polydextrose, pyrodextrin, insulin and fructooligosaccharides, but it may be applicable to RS_3 and RS_4 (McCleary et al., 2004). AOAC International Method 991. 43 is a prominent version of the Prosky method, and that method specifies a water-bath temperature of 95–100 °C for the digestion of a sample with heat-stable α-amylase. We used a boiling water bath where the temperature of the digest was usually ~97 °C, and that temperature gave reproducible results of dietary fiber (RS) in the modified starches.

3.1. Simultaneous heat-moisture treatment and phosphorylation with cross-linking of Hylon VII to produce RS: a model study

The level of resistant starch in Hylon VII is known to increase upon heat-moisture treatment. In one example, Hylon VII with 37% moisture was heated 1 h at 100 °C, and RS (measured as TDF) increased from 12% in the blank to 38% in the treated sample (Shi et al., 1997). Prior work also showed that cross-linking of wheat starch was achieved by phosphorylation of semi-dry starch (initially 15% moisture content) by heating the starch above 100 °C with a mixture of 2% STMP and 5% sodium sulfate at pH >9.5 (Lim et al., 1993). Heating 2 h at 130 °C with those reagents at an initial pH 11 gave cross-linked starch with ~0.2% phosphorus.

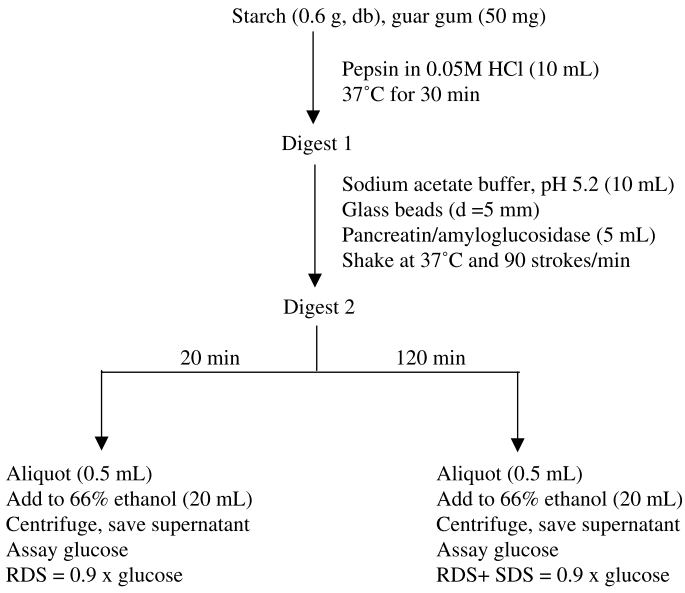

Fig. 1. Outline of modified Englyst's method to determine digestion profile of a starch without cooking. The level of resistant starch (RS) was calculated by difference (RS = 100 − RDS − SDS).

Phosphorylation especially with STMP rather than STPP gave cross-linking of starch at reaction pH's above pH ~9.5 (Lim et al., 1993; Woo and Seib, 1997).

After a series of preliminary experiments in this work simultaneous heat-moisture treatment and phosphorylation were done on Hylon VII at a moisture content between 21 and 49% (based on total reaction mixture) and a phosphorylating agent level between 2.8 and 11.2% (sb). The level of phosphorylation of starch increased with decreasing moisture content and increasing phosphorylating reagent (Fig. 2), because the hydroxyl groups of water compete for the phosphorylating agent with the hydroxyls of starch. The phosphorus in Hylon VII was increased from its endogenous level of 0.03% to a minimum of 0.16% upon heating for 4 h at 110 °C and 50% MC with 2.5% phosphorylating agent, and to a maximum of 0.47% phosphorus when heating with 10.5% phosphorylating agent at 20% MC (Fig. 2).

Phosphorus recovery in the starch product equals the percentage of phosphorus bound to starch based on that in the phosphorylating agent, in other words the reaction efficiency. Phosphorus recovery increased with decreasing moisture content and with a decreasing concentration of phosphorylating agent (Fig. 3). Ignoring phosphorus (~0.02%) endogenous to Hylon VII (Lim, Kasemsuwan, & Jane, 1994), the highest recovery of phosphorus esterified to Hylon VII accounted for 28% when the starch was

Table 1
Starch digestion profiles of reference samples measured by a modified Englyst's method

Reference sample	Starch fraction (%, sb)	
	RDS	RDS + SDS
Data of Englyst et al. (1999)[a]		
Englyst potato starch	4.5	30
Englyst wheat flour	49	97
Data from this work[b]		
Englyst potato starch	4.9 ± 1.3	23 ± 2.8
Englyst wheat flour	38 ± 0.5	97 ± 2.8
Avebe potato starch	2.0 ± 0.5	9.8 ± 1.0
Hard wheat flour[c]	36 ± 1.4	97 ± 1.4

Percentages of RDS and SDS are based on total starch. RDS, rapidly digestible starch; SDS, slowly digestible starch.
[a] Data from Englyst et al. (1999).
[b] Data are given as mean ± standard deviation of two measurements.
[c] Straight-grade four milled from the hard winter wheat variety Betty.

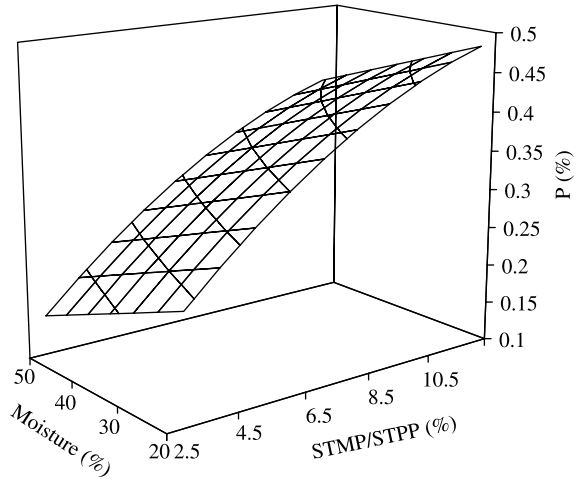

Fig. 2. Simultaneous heat-moisture treatment and phosphorylation of Hylon VII by heating at 110 °C for 4 h in a sealed container with varying levels of phosphorylating agent and moisture levels. The phosphorylating agent was a 99:1 (w/w) mixture of STMP/STPP. The second-order modeling equation was P (%, db) = $0.18 - 0.0029X + 0.050Y - 0.0016Y^2$ ($R^2 = 0.99$), where X = moisture content of mixture % (wet basis of reaction mixture) and Y = level of phosphorylating agent % (sb).

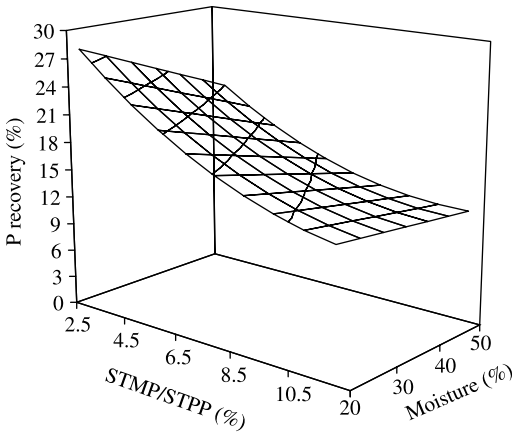

Fig. 3. Phosphorus recovery in simultaneous heat-moisture treatment and phosphorylation of Hylon VII. The phosphorus recovery rate was defined as the percentage of phosphorus bound to starch compared to that in the phosphorylating agent. The second-order model equation was phosphorus recovery (%) $=42-0.29X-3.7Y+0.12Y^2+0.020XY$ ($R^2=0.98$), where $X=$ moisture content of mixture % (wet basis of reaction mixture) and $Y=$ level of phosphorylating agent % (sb).

reacted with 2.5% STMP/STPP at 20% moisture content. The lowest recovery of 12% was observed under reaction conditions of 11.5% STMP/STPP at 50% moisture content.

The proportion of RS generated in the heat-moisture-treated and phosphorylated Hylon VII, after freshly cooking, increased as the level of phosphorylating agent (STMP/STPP) increased (Fig. 4). At each level of STMP/STPP, there was a different optimum moisture content to produce maximum RS. This was indicated by the significant interaction term of STMP/STPP and moisture content ($X\times Y$) and the second order term of moisture content (Y^2) in the

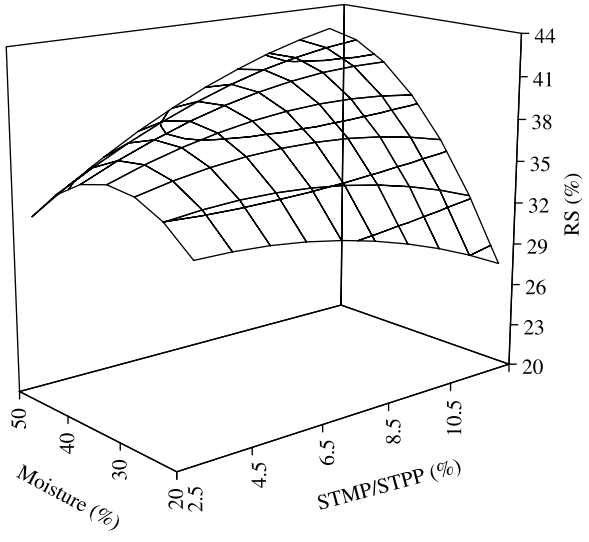

Fig. 4. RS (%) in freshly cooked modified starches prepared from Hylon VII. The second-order model equation for RS in the modified Hylon VII starch after cooking is RS (%, db) $=21+0.90X-0.74Y-0.015X^2+0.057XY-0.072Y^2$ ($R^2=0.86$), where $X=$ moisture content of mixture % (wet basis of reaction mixture) and $Y=$ level of phosphorylating agent % (sb).

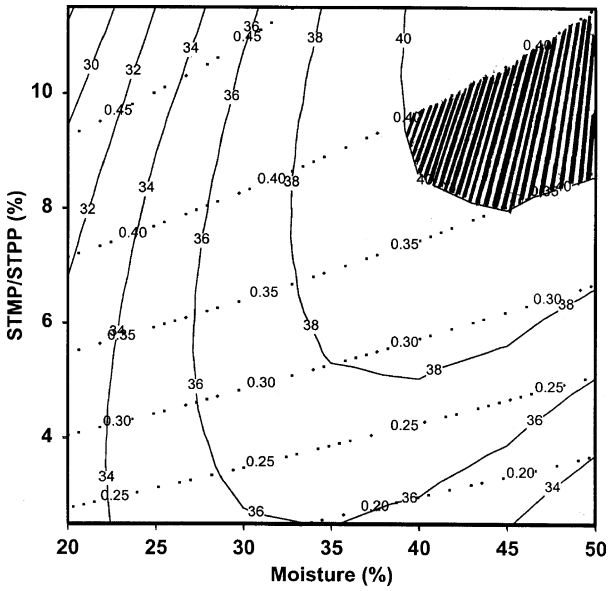

Fig. 5. Contour plot of % P overlaid on the contour plot of % RS for the cooked modified starch prepared by simultaneously phosphorylating and heat-moisture-treating Hylon VII. RS (%): — ; P (%): - - -.

regression equation (see Fig. 4). When the response surfaces for the levels of % RS and % P were overlaid in Fig. 5, the optimum operating zone (shaded area) for the reaction was located by the phosphorus level boundary of less than 0.4% P and the RS boundary at its highest level.

3.2. Starch-digestion profiles of simultaneously heat-moisture-treated and phosphorylated cross-linked Hylon VII

Within the operating zone of interest in Fig. 5, the variables of 45% moisture and 10% STMP/STPP were selected to produce a resistant starch from Hylon VII, and that modified starch was called Hylon VII45-10. The starch-digestion profiles of raw and cooked Hylon VII45-10 are given in Table 2. The phosphorus content (0.39%) and RS content (43%) of cooked Hylon VII45-10 were nearly equal to the values (0.39 and 41%) predicted by the two regression equations.

Cooked Hylon VII45-10 had a significantly higher level of RS than cooked Hylon VII, Hylon VII15-6, Hylon VII45-0, and Novelose 240 (Table 2). Novelose 240 is a commercial sample of RS made by heat-moisture-treatment of Hylon VII. Cooked Hylon VII45-10 had about 35% more RS than either Hylon VII45-0 or Hylon VII15-6, and 48% more RS than the commercial RS Novelose 240. Simultaneous heat-moisture-treatment and phosphorylation with cross-linking created higher levels of combined SDS and RS in cooked Hylon VII45-10 compared to cooked Hylon VII45-0, Hylon VII15-6, and Novelose 240. In the uncooked modified starches the

Table 2
Phosphorus, total dietary fiber, and starch fractions in modified Hylon VII

Sample	P (%, db)	Starch fraction (%, db)						TDF (%, db)
		Cooked starch			Raw starch			
		RDS	SDS	RS	RDS	SDS	RS	
Hylon VII45-10	0.39[b]	43[a]	14[b]	43[d]	26[d]	22[c]	52[b]	90[d]
Hylon VII15-6	0.38[b]	56[c]	12[a]	32[c]	6[a]	12[a]	82[d]	89[d]
Hylon VII45-0	0.03[a]	50[b]	18[c]	32[c]	42[e]	21[c]	37[a]	33[b]
Novelose 240	0.03[a]	49[b]	22[d]	29[b]	21[c]	25[d]	54[b]	44[c]
Hylon VII	0.03[a]	61[d]	14[a]	25[a]	10[b]	18[b]	72[c]	16[a]

Values followed by the same letters in the same column are not significantly different ($P < 0.05$). Hylon VII45-10 was prepared by heating a mixture of Hylon VII, 10% (sb) of STMP/STPP (99/1, w/w), 5% (sb) of sodium sulfate and 45% moisture content (total mixture basis) at initial pH 11.5 and 110 °C for 4 h in a sealed container. Hylon VII15-6 was prepared by heating a mixture of Hylon VII, 6% (sb) of STMP/STPP (99/1, w/w), 5% (sb) of sodium sulfate and 15% moisture content (total mixture basis) at initial pH 11.5 and 110 °C for 2 h in an open container (Lim and Seib, 1993). Hylon VII45-0 was prepared by heating Hylon VII containing 45% moisture content (total mixture basis) at 110 °C for 4 h in a sealed container.

combined levels of SDS plus RS were higher than ~75%, except for Hylon VII45-0.

RS levels determined as % dietary fiber (TDF) by hot enzymic digestion gave ~90% RS for Hylon VII45-10 and Hylon VII15-6, which was 2–5 times higher than TDF in the commercial resistant starch Novelose 240 and in Hylon VII (Table 2). However, RS levels for cooked Hylon VII45-10 and cooked Hylon VII15-6 determined by the Englyst procedure was not so highly elevated. In general, the data from the two methods moved in parallel, but the magnitudes of the levels differed because of many differences in the two assay methods, including reactants, temperature and time.

When one of the new modified starches is used in foods, the amounts of RS and SDS contributed by the starch would lie somewhere between the levels in the cooked and raw starches shown in Table 2. High-moisture foods fortified with the new modified starches and heated during food preparation could contain up to approximately one-fourth less combined SDS plus RS than those heated at low moisture, or at high sugar solids, for example. Starchy foods prepared at intermediate moisture would give intermediate levels of SDS plus RS. Hylon VII45-10 would provide the highest RS or combined RS plus SDS in food products prepared by heating at high moisture, whereas unmodified Hylon VII or Hylon VII15-6 would be suitable for foods prepared by heating at low moisture.

3.3. RS levels in simultaneously heat-moisture-treated and phosphorylated cross-linked amylose mutants of corn starch

Hylon V, normal corn starch, and waxy corn starch were heat-moisture treated and simultaneously phosphorylated by reacting at 45% moisture for 4 h and 110 °C with 10, 6, and 5.5% STMP/STPP, respectively. The three modified starches contained 0.39–0.40% *P* (Table 3). As the amylose content in corn starch increased from ~1 to 50%, the level of phosphorylating agent required to reach 0.4% phosphorus increased from 5.5 to 10% (sb). High-amylose starches may not imbibe as much phosphorylating agent during the soaking stage because amylose inhibits granule swelling.

After freshly cooking, only the phosphorylated and heat-moisture treated high-amylose starch (Hylon V45-10) contained more RS (32 vs. 13%) and less RDS (57 vs. 62%) compared to unmodified Hylon V. In the raw state the modified starches from either Hylon V or VII contained (Tables 2 and 3) 73–74% of their starch in SDS plus RS compared to 42–43% in the modified normal and waxy corn starches.

Table 3
Phosphorus, total dietary fiber, and starch fractions in modified amylose-mutants of corn starch

Samples	P (%, db)	Starch fraction (%, db)						TDF (%, db)
		Cooked starches			Raw starches			
		RDS	SDS	RS	RDS	SDS	RS	
Hylon V45-10	0.40[b]	56[b]	12[a]	32[b]	27[b]	21[a]	52[b]	89[d]
Corn45-6	0.39[b]	68[d]	17[b]	15[a]	58[c]	28[b]	14[a]	52[c]
Waxy45-5.5	0.39[b]	68[d]	18[b]	14[a]	57[c]	30[b]	13[a]	18[b]
Hylon V	0.03[a]	62[c]	24[c]	13[a]	8[a]	20[a]	72[c]	13[a]

Values followed by the same letters in the same column are not significantly different ($P < 0.05$). Hylon V45-10 was prepared by heating a mixture of Hylon V, 10% (sb) of STMP/STPP (99/1, w/w), 5% (sb) of sodium sulfate and 45% moisture content (total mixture basis) at pH 11.5 and 110 °C for 4 h in a sealed container. Corn45-6 was prepared by heating a mixture of normal corn starch, 6% (sb) of STMP/STPP (99/1, w/w), 5% (sb) of sodium sulfate and 45% moisture content (total mixture basis) at pH 11.5 and 110 °C for 4 h in a sealed container. Waxy45-5.5 was prepared by heating a mixture of waxy corn starch, 5.5% (sb) of STMP/STPP (99/1, w/w), 5% (sb) of sodium sulfate and 45% moisture content (total mixture basis) at pH 11.5 and 110 °C for 4 h in a sealed container.

Table 4
Physical properties of modified starches prepared from Hylon VII

Sample	P (%, db)	Gelatinization				Swelling power (g/g)	Solubility (%)
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)		
Hylon VII45-10	0.39[b]	96[b]	104[b] (P1) 120[c] (P2)	133[c]	5.4[a]	3.4[a]	1.1[a]
Hylon VII15-6	0.38[b]	69[a]	89[a]	109[a]	7.5[b]	3.2[a]	2.7[b]
Hylon VII45-0	0.03[a]	95[b]	106[b]	118[b]	5.5[a]	3.7[a]	4.1[c]
Hylon VII	0.03[a]	70[a]	88[a]	107[a]	13.4[c]	4.7[b]	5.0[d]

Values followed by the same letters in the same column are not significantly different ($P < 0.05$). Gelatinization: the weight ration of starch/water was 1/4. Hylon VII45-10, Hylon VII15-6, and Hylon VII45-0; see Table 2.

3.4. Physical properties of modified starches prepared from Hylon VII

Differential scanning calorimetry of Hylon VII45-0 showed a gelatinization endotherm in excess water that was 20–25 °C higher than unmodified Hylon VII (T_o and T_c of 95 and 118 °C vs. 70 and 107 °C) (Table 4). Those results were similar to the findings of Shi et al. (1997) who reported heat-moisture treatment of Hylon VII at 37% MC for 4 h at 100 °C increased T_o and T_c from 68 and 145 °C up to 92 and 150 °C. Cross-linking of Hylon VII by phosphorylation at a low MC of 15% gave Hylon VII15-6 with ~0.38% P but only a 1–2 °C rise in gelatinization temperature. The simultaneous heat-moisture-treatment and phosphorylation (cross-linking) of Hylon VII with 10% STMP/STPP at 45% MC gave Hylon VII45-10, and caused T_o to increase from 70 to 96 °C, T_p to increase from 88 °C and split into two peaks at 104 and 120 °C, and T_c to increase from 107 to 133 °C (Table 4).

The enthalpy of the gelatinization transition of Hylon VII45-10 decreased to 5.4 J/g from the 13.4 J/g for Hylon VII. That decline in enthalpy of gelatinization probably resulted from partial unwinding of double-helices during the modification reaction at 110 °C and 45% MC in the presence of sodium hydroxide (pH initial 11.5 → final 7.5). In addition, some of the phosphorus on Hylon VII45-10 is most likely monophosphate ester, which if substituted near the crystalline region may lower the enthalpy of gelatinization by enhancing swelling and cooperative melting of crystals. Unmodified Hylon VII had the largest enthalpy change during gelatinization, but most of the transition occurred below 100 °C, which indicated that much of its resistant fraction would be destroyed when boiled in excess water. The thermal transitions were in agreement with the RS contents of unmodified Hylon VII at 72% before and 25% after cooking (Table 3).

The swelling powers and solubilities of the phosphorylated starches Hylon VII45-10 and Hylon VII15-6 were reduced compared to the blank Hylon VII and the heat-moisture treated Hylon VII45-0 (Table 4).

4. Conclusions

Simultaneous heat-moisture treatment and phosphorylation (cross-linking) of high-amylose (~70%) corn starch gives a resistant fraction that survives cooking better than in the modified starches made by either treatment alone. In that type of modification the initial reaction pH yielding the highest proportions of resistant starch and slowly digestible starch remains to be determined.

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