White Pan Bread and Sugar-Snap Cookies Containing Wheat Starch Phosphate, A Cross-Linked Resistant Starch[1]

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ABSTRACT

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Pup-loaf bread was made with 10, 30, and 50% substitution of flour with wheat starch phosphate, a cross-linked resistant starch (XL-RS4), while maintaining flour protein level at 11.0% (14% mb) by adding vital wheat gluten. Bread with 30% replacement of flour with laboratory-prepared XL-RS4 gave a specific volume of 5.9 cm^3/g compared with 6.3 g/cm^3 for negative control bread (no added wheat starch), and its crumb was 53% more firm than the control bread after 1 day at 25°C, but 13% more firm after 7 days. Total dietary fiber (TDF) in one-day-old bread made with commercial XL-RS4 at 30% flour substitution increased 3–4% (db) in the control to 19.2% (db) in the test bread, while the sum of slowly digestible starch (SDS) plus resistant starch (RS), determined by a modified Englyst method, increased from 24.3 to 41.8% (db). The reference amount (50 g, as-is) of that test bread would provide 5.5 g of dietary fiber with 10% fewer calories than control bread. Sugar-snap cookies were made at 30 and 50% flour replacement with laboratory-prepared XL-RS4, potato starch, high-amylose (70%) corn starch, and commercial heat-moisture-treated high-amylose (70%) corn starch. The shape of cookies was affected by the added starches except for XL-RS4. The reference amount (30 g, as-is) of cookies made with commercial XL-RS4 at 30% flour replacement contained 4.3 g (db) TDF and 3.4 g (db) RS, whereas the negative control contained 0.4 g TDF and 0.6 g RS. The retention of TDF in the baked foods containing added XL-RS4 was calculated to be >80% for bread and 100% for cookies, while the retention of RS was 35–54% for bread and 106–113% for cookies.

Starch is the major source of energy in the human diet, and its rate and extent of digestion appear to affect human health. Starch that is slowly but completely digested (SDS) in a food gives a low glycemic response in the blood (Englyst and Hudson 1997; Englyst et al 1992, 1999). Consumption of that type of starch is beneficial to the obese and to diabetics and prediabetics, who represent ≈20% of the population in developed countries. Moreover, low glycemic foods are probably healthful for all individuals (Saris et al 1998; Brand-Miller 2003; Englyst et al 2007; Wong and Jenkins 2007; Howlett and Ashwell 2008).

A portion of starch (RS) may resist digestion in a food, which reduces both glycemic response and caloric load and that, in turn, counters obesity. Bacterial fermentation of RS in the colon may sustain health by protecting against cancer, constipation, and diverticulitis, and by growth of beneficial microflora (Saris et al 1998; Champ et al 2003; Higgins 2004; Nugent 2005; Sharma et al 2008). RS has been included in the definition of dietary fiber by food and medical organizations, but not by a 2006 FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition (Englyst et al 2007; McCleary 2007). Epidemiological and diet-intervention data suggests that the consumption of whole grains and dietary fiber bestow health benefits to humans (Jones 2007). Whole grains are rich in nutrients and phytochemicals (Liu 2007), as well as in polysaccharides (Topping 2007) including SDS, RS, β-glucan, fructan, hemicellulose, and cellulose.

Four (Englyst et al 2007) and possibly five (Sajilata et al 2006; Regina et al 2007) classes of RS are recognized. They include physically entrained starch (RS1), granular starch with B-type crystallinity (RS2), retrograded amylose (RS3), substituted starch (RS4), and lipid-complexed amylose (RS5). Many cereal foods have been fortified with RS2 and RS3, including bread, pasta, noodles, tortillas, cakes, waffles, brownies, cookies, snacks, and breakfast cereals (Brown 2004; Nugent 2005; Sajilata et al 2006; Sharma et al 2008). Here we describe the preparation and qualities of white pan bread and sugar-snap cookies made with a wheat starch phosphate that is sufficiently cross-linked (XL-RS4) to give amylase-resistant starch (Woo and Seib 2002; Sang et al 2007). We also present the total dietary fiber (TDF) contents and in vitro starch digestibility profiles of those bakery foods and their starchy ingredients. Almost all the data were collected on a laboratory-prepared XL-RS4 made from wheat starch, but some was obtained at a later date on a commercial sample of wheat XL-RS4.

MATERIALS AND METHODS

Materials

All chemicals were reagent-grade unless otherwise stated and water was distilled. Sodium trimetaphosphate, sodium tripolyphosphate, 2-(*N*-morpholino)ethanesulfonic acid (M-8250) (MES), *tris*-(hydroxymethyl)aminomethane (TRIS) (T-1503), heat-stable α-amylase solution (A3306), protease (No. P3910), amyloglucosidase solution (*Aspergillus niger*) (A9913), amyloglucosidase powder (A7255, *Rhizopus mold*), pepsin (P7000), pancreatin (P7545), fungal α-amylase (A6211), guar gum (G4129), and D-glucose reference standard (G-7528) were purchased from Sigma Chemical (St. Louis, MO). Glass balls 6 mm in diameter (11-312D) were from Fisher Scientific (St. Louis, MO). Wheat starch (Midsol 50), vital wheat gluten (76.6% protein, db), and Fibersym RW (0.4% phosphorus) were obtained from MGP Ingredients (Atchison, KS), and bread flour and dry granular barley malt from Cargill (Wichita, KS). The flour contained no additives and had a protein content of 10.6% (14% mb). Potato starch (superior) was from Avebe America (Princeton, NJ). Shortening, sucrose, salt, yeast, and B-vitamin pills containing thiamin, riboflavin, and niacin were purchased locally. Sodium stearoyl 2-lactylate was donated by American Ingredients, now CSM Bakery Supplies North America (Kansas City, MO). Cookie flour (9.0% protein, 14% mb) was from Mennel Milling Company (Fostoria, OH); all-vegetable shortening from ADM (Decatur, IL), dextrose from A.E. Staley Manufacturing (Decatur, IL), and high-amylose (70%) corn starch (Hylon VII) and heat-moisture-treated (HMT) high-amylose (70%) corn starch (Novelose 240) from National Starch Food Innovation (Bridgewater, NJ).

General Methods

All analyses were conducted in duplicate unless otherwise noted. Moisture content was determined by Approved Method 44-15A, except Approved Method 62-05 was used for bread slices (AACC International 2000). Protein (%N × 5.7) in flour, wheat gluten, starch, and TDF was assayed by Dumas nitrogen (Leco,

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St. Joseph, MI). Glucose and starch assay kits were from Megazyme International Ireland Ltd. (Bray, Wicklow, Ireland). Swelling powers and solubilities of starch at 25 and 95°C were measured according to Crosbie (1991).

Gelatinization and retrogradation of starch were determined by differential scanning calorimetry. One part starch (≈10 mg, db) was sealed in an aluminum pan with three parts of water, and the sample was allowed to equilibrate overnight. The pan was heated from 4 to 140°C at a rate of 10°C/min in a Perkin-Elmer DSC Pyris-1 instrument (Norwalk, CT) equipped with cooler, temperature controller, and a thermal analysis data station. An empty sample pan was used as the reference. Onset (T_o), peak (T_p), and completion (T_c) temperatures, as well as transition enthalpy (ΔH) were determined by the system software. To determine retrogradation, a gelatinized sample was cooled in the DSC chamber to 5°C then stored at ≈5°C. After two weeks, the sample was reheated from 4 to 140°C at 10°C/min, and the extent of retrogradation was calculated as $(\Delta H_{\text{retrograded starch}}/\Delta H_{\text{gelatinized starch}}) \times 100$ (Jane et al 1999).

Phosphorylation of Wheat Starch; Preparation of Cross-Linked, Resistant Starch (XL-RS4)

Cross-linked resistant wheat starch (XL-RS4) was prepared in nearly quantitative yield (starch basis, sb) according to Woo and Seib (2002) with slight modification. Wheat starch (50 g, db), water (70 mL), sodium trimetaphosphate (5.9 g, 11.9%, sb), sodium sulfate (5 g, 10%, sb), and sodium tripolyphosphate (0.06 g, 0.12%, sb) were combined and mixed in a beaker. The reaction mixture was adjusted to pH 11.5 by adding 1*M* sodium hydroxide (≈25 mL) and it was stirred 3 hr at 45°C. After adjusting the slurry to pH 6.5 by adding 1*M* hydrochloric acid, the modified starch was collected by centrifugation, washed with water (7 × 150 mL), and dried at 40°C. The dried starch was milled in an impact (≈12,000 rpm) analytical mill (model A-10, 20–50 mL, Tekmar-Dohrmann, Cincinnati, OH; presently manufactured as MicroMill Grinder, IKA Works, Wilmington, NC) for 30 sec to give finely granulated XL-RS4.

Starch Digestion Profile: TS, RDS, SDS, and RS

Starch-digestion fractions were measured by a modification of the in vitro Englyst method given in Silvester et al (1995). The modifications included 1) use of *Rhizopus* amyloglucosidase from Sigma instead of AMG 400L, type LP from Novo-Nordisk; 2) 1.10 g vs. 1.35 g of pancreatin/g of sample (starch) in the amylolytic digest with a total volume of 25 mL; 3) ≈5× more amyloglucosidase activity (1,112 vs. 208 IU) in the amylolytic digest of 25 mL; 4) smaller (6 mm diameter vs. 15 mm) and more (30 vs. 5) glass balls in the digest; and 5) probably a lower shaking speed (90 vs. 160 strokes/min) with a shorter (25 mm vs. 35 mm) stroke-length during digestion (Englyst et al 1992). Wheat flours and potato starch served as reference standards to calibrate the shaking speed so that in vitro levels of RS matched those in vivo. Decreasing amyloglucosidase in the assay from 1,112 IU to 278 IU gave an erroneous level of RS (32 vs. 2%) in wheat flour.

Before assay, one-day-old bread was freeze-dried, and cookie was defatted and desugared. Both dried products were ground in the impact mill (model A-10 Analytical Mill, Tekmar-Dohrmann). In one experiment, one-day-old bread at 37% moisture content was assayed for RDS, SDS, and TS. Because of the insolubility of cross-linked starch, the bakery foods containing XL-RS4 as well as XL-RS4 starch itself, were not assayed directly for RS and TS. Instead, TS in the bakery foods fortified with XL-RS4 was calculated to be the sum of endogenous starch in flour plus the added modified starch (XL-RS4), and RS was the difference between calculated TS and the sum of experimentally determined RDS and SDS. Endogenous starch (TS) in flour and control bakery foods was determined with a kit from Megazyme. Endogenous glucose, sucrose and maltose in flour, which is ≤0.5% (D'Appolonia et al 1971), was ignored in all starch assays, as was the maltose (1.4–1.9%, dry solids basis) in bread. Sugars in control and test breads were determined by liquid chromatography at Medallion Labs (Minneapolis, MN). The levels of sugars on a dry weight basis were fructose 1.1%, glucose <0.1%, sucrose <0.1%, maltose 1.4–1.9%, and lactose 1.7–1.8%, all in agreement with Langemeier and Rogers (1995).

Starch digestion profiles were done in polypropylene centrifuge tubes (30 mL) submerged in a water bath (37°C), and shaken at 90 strokes/min with a stroke length of 25 mm on a table-top incubator shaker (model 50, Precision, Winchester, VA). To prepare the pancreatin/amyloglucosidase enzyme mixture, a magnetic stir bar was added to a test tube, followed by pancreatin enzyme (3.0 g) and water (20 mL) at 25°C. After agitation with a vortex mixer, the mixture was stirred for 10 min and then centrifuged at 1,500 × *g* for 10 min. An aliquot (15 mL) of the cloudy supernatant was removed from the tube and added to a solution of 60 mg of *Rhizopus* amyloglucosidase in 1.7 mL of water. Starch and XL-RS4 (≈600 mg, ds), freeze-dried, and ground bread (800 mg, ds), and fat- and sugar-extracted cookies that had been ground (800 mg, ds), were weighed each into centrifuge tubes. The lipid had been extracted from ground cookie (10 g) by petroleum ether (3 × 250 mL), and then the sugar was removed with 86% ethanol (3 × 100 mL) at 25°C. The extracted lipid amounted to 11.6% (db) and extracted sugar 38.0% (db), compared with the calculated values of 15 and 32%, respectively A gelatinized sample of XL-RS4 was prepared by boiling 1.0 part of that starch in 9.5 parts of water for 5 min. The product was isolated by centrifuging, oven-drying at 100°C, and grinding.

The standard, blank, and samples in the centrifuge (30 mL) tubes all contained guar gum (50 mg) as well as glass balls. An aliquot of 10 mL of pepsin solution (50 mg dissolved in 10 mL of 0.05*M* HC1) was added to the sample tubes only, and the mixture digested for 30 min at 37°C. Sodium acetate buffer (0.1*M*, pH 5.2, 10 mL) preconditioned to 37°C was then added to sample tubes, which were then vortexed. Glucose (25 mg/mL) standard solution in the acetate buffer was pipetted (20 mL) into the standard tubes, and the acetate buffer was pipetted (20 mL) into the blank tubes. Fifteen glass balls and 5 mL of the pancreatin/amyloglucosidase mixture were added to the standard and blank tubes, while 30 glass balls and 5 mL of the enzyme mixture were added to the sample tubes. The tubes were incubated in the shaking water bath, and after 20 min, a 0.5 mL aliquot of digest was pipetted into 20 mL of 66% ethanol, mixed, and centrifuged to obtain a clear supernatant called G_{20}. After 120 min of total digestion time, 0.25-0.50 mL was pippeted into 20 mL of 66% ethanol, mixed and centrifuged; this clear supernatant was G_{120}. The glucose concentrations in G_{20} and G_{120} were assayed using a glucose oxidase kit after correcting for the blank and used to determine the levels of rapidly digestible starch (RDS) and slowly digestible starch (SDS).

The sediment of the pancreatin/amyloglucosidase digest was treated to determine total starch (TS) (sample with no digestion time) and resistant starch (RS) (sample after 120 min of digestion) in flours and bakery foods. However, as stated above, TS for bread or cookies containing XL-RS4 was calculated as the sum of starch in flour plus added XL-RS4. The tubes were vortexed and boiled for 30 min, then vortexed again. The tubes were chilled in ice water to 4°C; 7*M* potassium hydroxide (10 mL) was added with mixing, and the tubes were placed on a horizontal shaker at room temperature for 30 min. A 1-mL aliquot of a sample was pipetted into 0.5*M* acetic acid (10 mL) and amyloglucosidase solution (200 μL) was added. The amyloglucosidase solution was prepared by dissolving the enzyme (50 mg) in water (1 mL). The tubes were incubated at 70°C for 30 min, then placed in a boiling water bath for 10 min. Water (20 mL) was added and the digest was centrifuged. An aliquot of the digest was assayed for total glucose (TG) using glucose oxidase. The various fractions of starch were calculated (dry solids basis) as TS = TG × 0.9; RDS = G_{20} × 0.9, SDS = (G_{120} – G_{20}) × 0.9, and RS = (TG – G_{120}) × 0.9, where 0.9 is the conversion factor of glucose to starch. The percentages of starch and starch-digestion fractions were reported on a dry solids basis.

TDF Analysis

TDF in starches, flours, and dry ground baked foods was determined by Method 991.43 (AOAC International 2000). TDF was expressed as the digestion residue on a dry solids basis after correcting for residual protein and ash.

White Bread

Bread was baked according to the standard pup-loaf procedure (Approved Method 10-10B, AACC 2000) except that fermentation time was 90 min instead of 180 min, and instant active dry yeast was used instead of compressed yeast. The 2% level (flour basis) of instant yeast is equivalent to ≈6% compressed yeast. In test loaves, bread flour (14% mb) was partially replaced by XL-RS4 to give 1:9, 3:7, and 1:1 blends (w/w, ds) of XL-RS4/flour. Positive control loaves were prepared by replacing flour with wheat starch instead of the XL-RS4, whereas negative control loaves contained no added starch. The wheat starch or XL-RS4 (≈10–50 g), which sometimes contained small gritty particles, was mixed with water (35 mL) before adding to the mixer. Protein contents of the control flours and test flours were adjusted to 11.0% (14% mb) by adding vital wheat gluten (0.5–6.5 g, as-is, to 100 g of flour on a 14% mb). The levels of ingredients in bread (and cookie) formulas were based on the weight of flour or flour blends (14% mb). Falling number (AACC Approved Method 56-81B) was used to determine the amount of malted barley flour to be added in the wheat flour. The falling number of flour that contains a normal level of amylolytic activity for breadmaking generally falls in the range of 220–250 sec (Pyler 1988a). A falling number of 235 sec was achieved when 0.09% malt was added to the flour compared with the blank of 510 sec. The bread (negative control) formula contained 100 g of malted flour (14% mb), water (optimum), 6.0 g of sucrose, 1.5 g of sodium chloride, 3.0 g of shortening (Crisco brand, Procter and Gamble, Cincinnati, OH), 4.0 g of nonfat dry milk, 2.0 g of instant yeast as-is, 0.5 g of sodium stearoyl 2-lactylate, and 10 mg of potassium bromate. Bread-dough absorption and mixing time were determined from the flour-water dough absorption estimated on the mixograph (AACC Approved Method 54-40A) and by the feel of the mixed dough.

Experimental doughs with both types of added wheat starch required a longer time to proof to height than the negative control. To reduce the proofing times of the test (XL-RS4 added) and the positive control (wheat starch added) doughs, B-vitamins and minerals were added before mixing. Minerals and B-vitamins are missing in starch, whereas wheat flour contains 0.10, 1.4, and 0.035 mg/100 g of thiamin, niacin, and riboflavin, respectively (Pyler 1988b) and 115 mg of nonprotein nitrogen/100 g (McMaster et al 1964). B-vitamin pills were ground in an equal weight of water using a mortar and pestle and the mixture diluted to 1 L with water and stirred overnight. The solution was calculated to contain 0.72, 5.0, and 0.5 mg/mL of thiamin, niacin and riboflavin, respectively.

Ammonium chloride (10 g), magnesium chloride (20 mg), potassium chloride (60 mg), monosodium phosphate (65 mg), and sodium sulfate (5 mg) were dissolved in water (1 L). Aliquots (1 mL each) of the vitamin and mineral solutions were added to the dry blended ingredients. Loaf volumes and weights were recorded immediately after baking. Loaves were cooled ≈1 hr at 25°C, sliced with a bread slicer (model 797, Oliver Products Company, Grand Rapids, MI), and stored in polyolefin bags at room temperature before determining crumb firmness, TDF, and starch-digestion fractions.

Firmness of Bread Crumb

A force-distance instrument (model TA-TX2, Stable Micro Systems, Texture Technologies, Scarsdale, NY), equipped with a 25.4 mm (1 in.) diameter acrylic probe, was used to determine the firmness of bread crumb. Two slices of bread from the center of a loaf were stacked to give a thickness of 25.4 mm (1 in.) for each test. The crumb was compressed a distance of 6.2 mm at a test speed of 1.7 mm/sec using a trigger of 10 g-force. Loaves were tested on 1, 3, and 7 days after baking, and two measurements were made on each loaf.

Sugar-Snap Cookies

Sugar-snap cookies were baked according to the standard baking Approved Method 10-50D (AACC International 2000) formula: shortening 64 g; sucrose 130 g; salt 2.1 g; bicarbonate of soda 2.5 g; glucose solution (8.9 g of glucose in 150 mL of water) 22.0; distilled water variable (≈35 mL); flour (14% mb) 225 g.

Cookie flour was partially replaced by high-amylose (70%) corn starch (Hylon VII), HMT high-amylose (70%) corn starch (Novelose 240), potato starch, and XL-RS4, to give blends with ratios of 3:7 and 1:1 (w/w, db) of starch to flour. The thickness of a cookie was determined by stacking six cookies, measuring the height, then restacking in different order and remeasuring. The mean value was reported as cookie thickness. The mean width of cookies was obtained by laying cookies edge to edge and measuring the width. Then the cookies were rotated 90° and the width remeasured and the mean width calculated. The spread factor was the mean diameter of the cookie divided by its mean thickness.

Snapping Force of Sugar-Snap Cookies

Cookies were baked, cooled to room temperature, and stored 1 day in polyolefin bags at 25°C. The force-distance instrument (TA-TX2) was used to measure the snapping force of cookies. A cookie was placed on top of a three-point bending rig with the base adjustable gap set at 50 mm. The acrylic probe with cross-sectional dimensions of 70 × 3.0 mm and height of 70 mm was advanced until the cookie broke. Six cookies were used for each testing. The instrument was operated in compression mode at a pretest speed of 2.5 mm/sec, test speed of 2.0 mm/sec, posttest speed of 10 mm/sec, and a trigger force of 20 g-force. Snapping force (kg-force) was calculated from this equation (Bourne 1982): Snapping force = $2\sigma bh^2/3L$, where σ is the failure stress (kg-force/cm^2), b is the width of the product (cm), h is the thickness of the product (cm), and L is the horizontal distance between the bottom supports (cm). The snapping force was reported as the mean of six replicates.

Statistical Analyses

Statistical analyses were performed using the Statistical Analysis System (SAS Institute, Cary NC). Means were compared by the least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Cross-Linked Resistant Wheat Starch (XL-RS4)

The XL-RS4 made from wheat starch in the laboratory had a phosphorus level of 0.38% and swelling powers at 25° and 95° of 1.9 g/g and 2.7 g/g, respectively, compared with unmodified wheat starch with 0.05% phosphorus and swelling powers of 2.1 g/g and 12.6 g/g (Table I). The XL-RS4 wheat starch showed 0.2% solubility at 95°C compared with 22.4% for the unmodified starch. The commercial sample of XL-RS4 wheat starch contained 0.40% phosphorus. Recent data (Sang et al 2007) indicates that two-thirds of the phosphorus in XL-RS4 wheat starch produced at pH 11.5 is present as a distarch monophosphate ester (cross-links) and one-third as a monostarch monophosphate ester (monosubstituents).

The TDF level (AOAC Prosky method) of the XL-RS4, which is the residue after digestion of a sample with heat-stable α-amylase at 95–100°C followed by digestions with protease and amyloglucosidase, amounted to 80% for the laboratory-prepared sample and 92% for the commercial XL-RS4 (Table I). In contrast, unmodified wheat starch contained <1% TDF. Laboratory-prepared samples of XL-RS4 contained RDS, SDS, and RS levels of 5, 33, and 63%, respectively. Commercial samples contained RDS, SDS, and RS levels of 2, 15, and 83%, respectively, according to a modified Englyst method. In contrast, wheat starch contained RDS, SDS and RS levels of 31, 63, and 3%, respectively. After heating the laboratory-prepared XL-RS4 in excess boiling water for 5 min, followed by oven-drying and mechanical grinding, TDF content was 39% and the digestion profile was 55, 26, and 29% of RDS, SDS and RS, respectively. The Englyst method to determine RS directly is not applicable to XL-RS4 because the cross-linked starch is insoluble in 1–4*M* sodium hydroxide at 25°C. Instead, an indirect method was used to calculate RS in XL-RS4 or in foods that contained XL-RS4. First, the total starch (TS) in a sample was calculated by summing the starch portions contributed by the ingredients. The TS levels in flours and untreated starches were determined experimentally, whereas TS levels in XL-RS4 was assumed to be 100%. Then RS in a sample was calculated by subtracting the sum of the experimentally determined RDS and SDS of that sample from the calculated TS.

The decreased TDF and RS contents of the gelatinized sample of XL-RS4 (Table I) can be attributed to several factors. The hydrothermal treatment and subsequent oven-drying of XL-RS4 increased swelling power ≈40% at 1.9–2.7 g/g. The increased swelling of the starch could increase enzyme accessibility to the inside of the granules. In addition, drying the gelatinized XL-RS4 resulted in adhesion of granules. Grinding the fused granules with the impact mill at ≈12,000 rpm apparently damaged the granules physically and probably created excessively fine particles that could reduce dietary fiber determined gravimetrically. When the gelatinized XL-RS4 was spray-dried, that product contained 71% TDF or ≈89% of its level (80%) before gelatinization, which agrees with results of a commercial spray-dried sample (Kyung S Woo, *personal communication*). Aparicio-Seguilan et al (2008) reported that autoclaving (121°C for 1 hr, 3×) a 22% slurry of phosphorylated (0.28% P) cross-linked banana starch, followed by freeze-drying and grinding through 149-mm openings, decreased TDF level by 11% from 95% down to 85%.

The XL-RS4 showed a ≈5°C increase in gelatinization temperature compared with wheat starch (T_{onset} 63° vs. 58°C) and a somewhat elevated enthalpy of gelatinization (12.8 vs. 11.6 J/g) (Table II). Chatakanonda et al (2000) found that cross-linked rice starch also prepared with sodium trimetaphosphate at alkaline pH, had a higher gelatinization temperature (≈5°C) and ≈10% decrease in enthalpy. Cross-linking of starch under conditions that do not induce gelatinization reduces the swelling of granules and inhibits the mobility of starch chains, which appears to compromise the disorganization of starch crystals by cooperative melting (Donald 2001). In contrast, cross-linked starches prepared under acidic (glutaric or citric acid) roasting (70–150°C) conditions largely eliminates the gelatinization endotherm, and any small endotherm that remains is shifted to a lower temperature compared with unmodified starch (Xie and Liu 2004; Kim et al 2008). XL-RS4 failed to exhibit a pasting curve in the RVA at 12% starch solids, and its pastes showed approximately one-half the extent of reassociation (ΔH of melting in its retrograded sample) compared with a 25% wheat starch gel after one to two weeks at 5°C (Table II). Retrogradation of the gelatinized cross-linked rice starch also was impaired compared with gelatinized normal rice starch (Chatakanonda et al 2000).

Breadmaking

Bread dough was mixed at optimum water absorption and optimum mixing time as determined by the formation of a smooth, extensible dough and by attaining maximum loaf volume.

TABLE I
Properties of Wheat Starch and Phosphorylated Cross-linked Resistant Wheat Starch (XL-RS4)

Wheat Starch	Phosphorus (%)	Swelling Power (SP) (g/g) 25°C	Swelling Power (SP) (g/g) 95°C	Solubility at 95°C (%)	AOAC Total Dietary Fiber (%)	In Vitro Digestibility[a] RDS	In Vitro Digestibility[a] SDS (%)	In Vitro Digestibility[a] RS
Unmodified								
Ungelatinized	0.05	2.1	12.6	22.4	0.7	31.1	62.7	3.2
XL-RS4								
Ungelatinized	0.38	1.9	2.7	0.2	80.0	4.6	32.5	(62.9)[b]
Ungelatinized-Com[c]	0.40	–	–	–	91.9	2.3	14.5	(83.3)[b]
Gelatinized	0.38	2.7	2.7	<0.1	38.7 (71)[d]	55.2	26.2	(28.6)[b]

[a] RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch. Levels are calculated based on dry weight of starch.

[b] Calculated by difference assuming dry solids in XL-RS4 is 100% starch, then subtracting the sum of RDS and SDS.

[c] Commercial sample of phosphorylated cross-linked wheat starch (Fibersym RW).

[d] Value in parenthesis for a sample boiled in ≈9 parts water followed by spray-drying (Nichols Engr. and Research Corp, Niro spray dryer, model 53). The other sample with 38.7% total dietary fiber was gelatinized, oven-dried, and ground with a small impact mill (Tekmar-Dohrmann, model A10).

TABLE II
Gelatinization and Retrogradation of Phosphorylated Cross-Linked Resistant Wheat Starch (XL-RS4) Determined by Differential Scanning Calorimetry at a 1:3 Starch-to-Water Ratio (w/w)[a]

Wheat Starch	Storage Weeks at 5°C	Onset T_o (°C)	Peak T_p (°C)	Completion T_c (°C)	Enthalpy (ΔH, J/g)	Retrogradation[b] (%)
Unmodified	0[c]	58.4b	63.5b	69.9b	11.6b	–
	1	40.4d	52.6c	60.2c	2.40d	20.8b
	2	42.0c	52.3c	61.0c	3.83c	33.1a
XL-RS4	0[c]	63.1a	68.0a	74.3a	12.8a	–
	1	37.2e	47.6d	54.0d	1.02f	8.8d
	2	36.9e	47.0d	55.2d	1.72e	14.8c
LSD ($P < 0.05$)		0.9	0.2	0.1	0.3	0.7

[a] Values followed by different letters in a row are significantly different at the 5% level.

[b] Retrogradation was determined by rescanning the sample after it had been heated to 140°C in the gelatinization scan then cooled and held at 5°C for 7 and 14 days. Retrogradation was calculated by dividing ΔH by 11.6 J/g, which is the gelatinization ΔH of unmodified wheat starch.

[c] Gelatinization of the starches in the first scan.

Compared with the positive control flour containing added wheat starch, plus added gluten to increase flour protein from 10.6 to 11.0% (14% mb), the test flour with added XL-RS4 plus gluten had 15–33% increased mixing time (Table III). The increased mixing time with added XL-RS4 was thought to be caused by relatively slow hydration compared with normal wheat starch. Mixograph studies showed little change in the absorptions of test versus control doughs, but mixing tolerance of dough was improved somewhat by adding XL-RS4 (data not given). However, doughs with increasing levels of added XL-RS4 or wheat starch caused increased handling stickiness, although stickiness declined at the time of second punch and even more at molding.

Bread doughs containing added starches, both XL-RS4 and wheat starch, required extended proof times. Starches at 30 and 50% substitution for flour caused proofing times to increase from 40 to 50 min, respectively, compared with 35 min for the negative control dough. Nutrients required for yeast fermentation include nitrogen, phosphorus, potassium, sulfur, and magnesium, and the B-vitamins, thiamin, pyridoxine, and nicotinic acid. Srisuthep (1974) reported that a mixture of ammonium chloride (0.16% of flour weight), monosodium phosphate (0.065%), magnesium chloride (0.021%), sodium sulfate (0.005%), and potassium chloride (0.058%) should be added in bread dough for optimum yeast fermentation. In the present work, adding thiamin (0.72 mg/100 g of flour), niacin (5.0 mg), and riboflavin (0.5 mg) plus a mixture of ammonium chloride (1.0 mg/100 g of flour), magnesium chloride (0.02 mg), potassium chloride (0.06 mg), sodium sulfate (0.005 mg), and monosodium phosphate (0.065 mg) decreased the proof time to 35–37 min in the experimental doughs, which was comparable to 35 min for the negative control dough.

TABLE III
Mixograph Data on Doughs Prepared from Blends of Wheat Flour and Wheat Starch or Phosphorylated Cross-Linked, Resistant Wheat Starch (XL-RS4)

Starch and Flour (w/w, db)	Absorption (%)	Mixing Time (min)
None (negative control)[a]	63	5.5
Wheat starch (positive control)[a]		
1:9	62	4.8
3:7	61	5.5
1:1	62	6.5
XL-RS4[b] (test)		
1:9	62	6.4
3:7	62	7.1
1:1	62	7.5

[a] Negative and positive control flours contained added vital wheat gluten (≈0.5 g) to give a protein level of 11.0% (14% mb) in the final flours.

[b] Laboratory-prepared XL-RS4 contained 80% total dietary fiber (Prosky method) and 63% resistant starch (Englyst method). Flours containing added XL-RS4 were adjusted to 11.0% protein (14% mb) by adding vital wheat gluten.

Loaf Volume, Moisture, and Crumb Firmness

Positive control breads made from wheat starch to flour blends of 1:9, 3:7, and 1:1 (w/w, db) gave decreased loaf volumes of 4, 6, and 12%, respectively, compared with the negative control bread. Test breads made with the same levels, but with XL-RS4 instead of wheat starch, depressed loaf volumes to almost the same extent (7–15%) (Table IV). Test doughs were proofed to the same height as the negative control but still the loaf volumes of the test breads were lower. The test doughs did not display sufficient oven-spring, probably because of the coalescence of small gas cells with large gas cells, in agreement with the more open crumb grain of the test loaves (Fig. 1). Baking losses were 1–2% higher for the test loaves compared with the negative control (Table IV), but moisture levels of bread 1–7 days old did not differ between test and control breads (Table V).

Generally, moisture in each type of bread decreased by 1–3% with a storage period of 1–7 days and the firmness of bread crumb increased (Table IV). The firmness of breads with added wheat starch and XL-RS4 were higher at 1 day of storage than the negative control, but the difference generally decreased after 3 and 7 days. Our crumb firmness data agrees with literature data showing that loss of moisture (Martin et al 1991) and loss of loaf volume (Eerlingen et al 1994; Every et al 1998) promote crumb firmness. The crusts of breads containing the added starches were visually lighter in color than the negative control bread, perhaps because the composite flours contained fewer LMW peptides than the negative control.

Yue and Waring (1998) tested HMT high-amylose (70%) corn starch (Novelose 240) in breadmaking and compared it to added wheat fiber, oat fiber, and cellulose at a level that provided 5 g of dietary fiber/50 g of bread (wb). High protein flour and vital wheat gluten (15% flour basis) also were used in the bread formulation. A blend of ≈3:7 (w/w) Novelose 240 (RS2) and flour was required to provide the desired level of dietary fiber. Quality scores for the dough and bread containing RS2 were higher than for the other fiber ingredients. The specific volume of 4.4 cm^3/g for the RS2 bread with 30% replacement of flour with Novelose 240 was one-third lower than the specific volume of 5.9 cm^3/g found in our work with 30% replacement of flour with the XL-RS4 (Table IV).

In another study by Eerlingen et al (1994), pup loaves were made by replacing 24% of the wheat flour (protein content 12.5%, 14% mb) with 4% vital wheat gluten plus 20% normal corn starch, high-amylose (70%) corn starch, or extruded and retrograded high-amylose (70%) corn starch (RS3 content 30%). One-day-old bread made with normal corn starch, high-amylose

TABLE IV
Bread Properties for Control Loaves[a,b] and for Loaves Containing Phosphorylated Cross-Linked, Resistant Wheat Starch (XL-RS4)[a,b]

Starch and Flour (w/w, db)	Weight (g)	Volume (cm^3)	Specific Volume (cm^3/g)	Baking Loss (%)	Crumb Firmness Compression Force (g-force) Day 1	Day 3	Day 7
None (negative control)	152b	951a	6.28a	15.3c	153.2e	236.6c	406.9d
Wheat starch (positive control)							
1:9	151c	915b	6.07b	16.0b	179.3d	271.7bc	427.8cd
3:7	151c	895bc	5.92c	16.1b	215.6c	296.7b	483.2b
1:1	153a	837d	5.47d	15.3c	289.9b	378.0a	605.4a
XL-RS4 (test)							
1:9	149d	887c	5.96bc	16.9a	222.2c	316.9b	475.2cb
3:7	151c	883c	5.86c	16.0b	233.9c	294.9bc	457.5cb
1:1	152b	816d	5.37d	16.0b	326.0a	374.7a	503.0b
LSD ($P < 0.05$)	0.8	22	0.1	0.4	30.7	41.1	50.1

[a] Values followed by different letters in a column are significantly different at 5% level.

[b] Breads made from blends of starch and flour with sufficient gluten to maintain 11.0% protein (14% flour or mb) in a blend. All bread contained sodium stearoyl 2-lactylate at 0.5 wt% based on flour and starch blends.

corn starch, and RS3 contained 0.4, 7.7, and 8.4% RS, respectively, by the Englyst method, which increased to 4.4, 10.2, and 11.0% after 7 days of storage. The control bread contained 0 and 4% RS on days 1 and 7. The corresponding specific loaf volumes were 4.6, 4.5, and 4.1 cm^3/g compared with 4.7 for the control bread. These literature data indicate that white pan bread with added RS2 and RS3 contains increased dietary fiber content, and that the loaf volume of the bread is depressed more than with added XL-RS4 wheat starch.

TDF and Starch Digestibility in Bread

Knowing the mass of ingredients, the fermentation loss of 5 g of the 6 g of sugar in the formula, and the TDF levels in flour and added starch, the pup loaves made with 0, 10, 30, and 50% substitution of flour with XL-RS4 had a calculated dry mass of 99–105 g and input levels of 2.9, 9.5, 22.4, and 35.6% (db) of TDF. The corresponding levels determined experimentally were 3.9, 4.2, 4.7, and 5.2% of TDF (Tables VI and VII). We conjectured that the low recovery of TDF in the test breads (Table VII) was caused by mechanical damage to the starch by high-speed grinding of the freeze-dried bread before assay and possibly by grinding the batch of laboratory-prepared XL-RS4 used in those breads.

A second batch of pup loaves was made with 30% flour replacement with a commercial sample of XL-RS4, along with a negative control. One-half of the test loaf and the negative control loaf was cut into small cubes, freeze-dried, and then ground gently with a mortar and pestle such that 98% of particles passed through a U.S. No. 18 sieve with openings of 1 mm. Assay of that

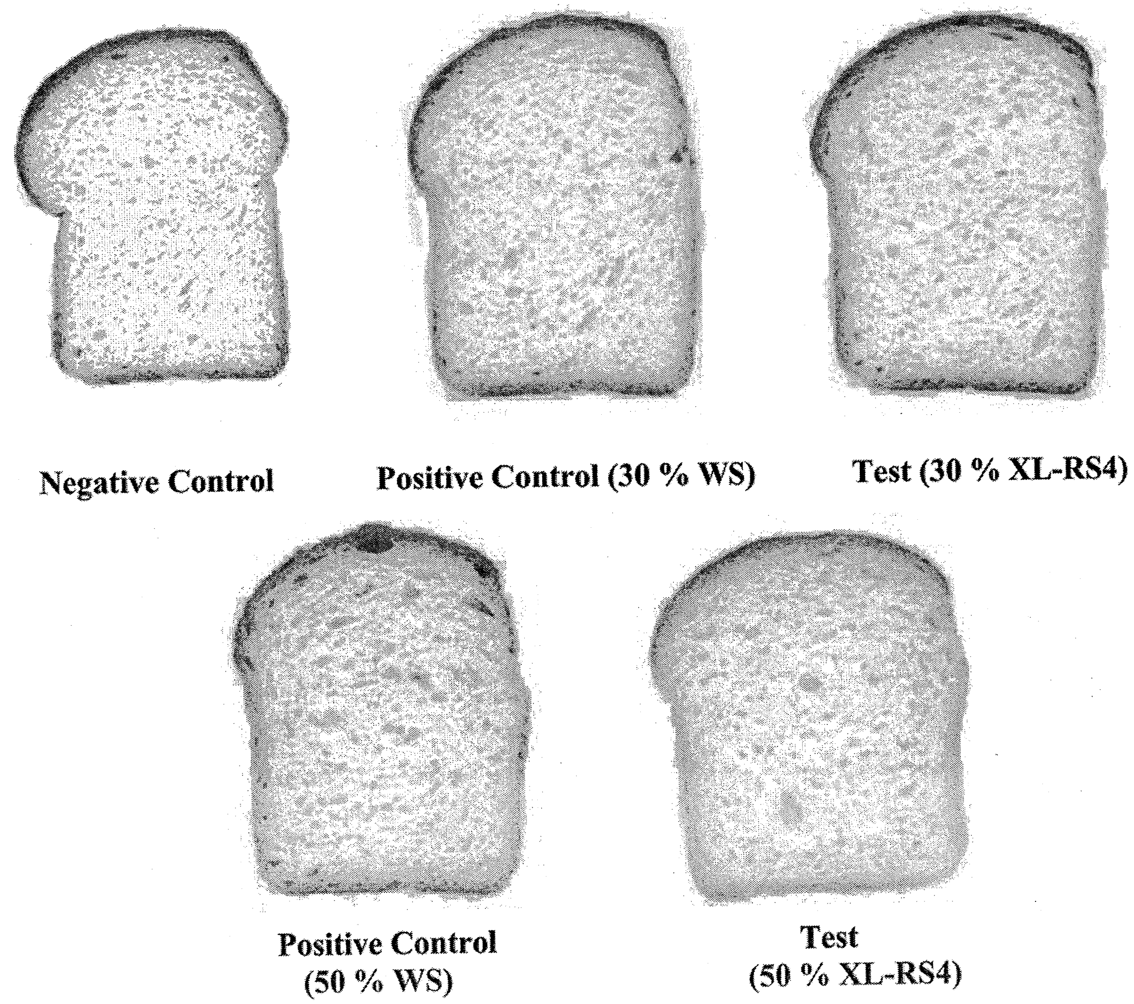

Fig. 1. Bread crumb of loaves made from blends (7:3 and 1:1, w/w) of bread flour and wheat starch (WS) and bread flour and cross-linked resistant wheat starch (XL-RS4) prepared in the laboratory. Bread flour and flour blends were adjusted to 11.0% protein (14% mb) by adding vital wheat gluten.

TABLE V
Moisture Contents (%wb ± SD) of Breads With and Without Added Wheat Starch or Phosphorylated Cross-Linked, Resistant Wheat Starch (XL-RS4)

Starch and Flour (w/w, db)	Day 1	Day 3	Day 7
Wheat flour (negative control)	34.7 (± 0.28)	34.3 (± 0.69)	33.2 (± 1.4)
Wheat starch (positive control)			
1:9	33.5 (± 0.52)	33.3 (± 0.09)	32.5 (± 0.13)
3:7	34.4 (± 0.07)	33.4 (± 0.11)	32.7 (± 0.04)
1:1	34.6 (± 0.18)	34.1 (± 0.65	33.3 (± 0.56)
XL-RS4 (test)			
1:9	34.0 (± 0.21)	33.1 (± 0.50)	30.9 (± 0.18)
3:7	34.4 (± 0.66)	33.3 (± 0.64)	33.3 (± 0.33)
1:1	34.9 (± 0.28)	34.1 (± 0.45)	32.1 (± 0.66)

one-day-old bread gave 21.2% (db) TDF (uncorrected for residual protein and ash) compared with 5.9% for the negative control (Table VI). Assaying the test bread (36% moisture content) directly without drying was done also on a 5-mm center slice; TDF was 20.4 ± 1.3% (db). The uncorrected TDF (5.9%) of the negative control bread was ≈2.0% higher than when corrected (3.9%). Applying a 2.0% correction to the TDF determined in the second batch of bread gives ≈19.2% TDF in the dry bread. Thus, recovery of TDF in the bread made with a 3:7 blend of commercial XL-RS4 and bread flour was 84.6% of the input level (22.7%) (Table VII). We presume that if all the test breads with 10–50% added XL-RS4 had been ground gently after drying, then all would have shown a high recovery of dietary fiber.

One serving size of bread is 50 g (wb) and the daily value of dietary fiber is 25 g. The bread made with a 7:3 blend of flour and XL-RS4 would provide 5.5 g (22%) of the daily value of dietary fiber. That same bread would contain ≈10% fewer calories than control bread, assuming XL-RS4 contains a food energy value of

TABLE VI
Total Dietary Fiber (TDF) and Starch Fractions (% of sample, ds)[a] in Ingredients and One-Day-Old Bread

		Fraction of Starch in Flour, Starch or Bread (%, db)			
Sample	TDF (%, db)	RDS	SDS	RS	TS
Flour and starch ingredients					
Wheat flour	3.3 ± 0.1	35.2	40.3	2.5	78.0
Wheat Starch	0.7 ± 0.1	31.1	62.7	3.2	97.0
XL-RS4	79.5 ± 0.1	4.6	32.5	(62.9)[b]	(100)[c]
XL-RS4 commercial	91.9 ± 0.0	2.3	14.5	(83.2)[b]	(100)[c]
One-day-old (25°C) bread[d]					
None added (negative control)	3.9 ± 0.2 (5.9 ± 0.0%)[e]	48.2b	21.3ed	3.0d	72.9
Wheat starch and flour[f] (positive control)					
1:9	3.9 ± 0.5	51.0a	20.0d	2.1e	72.8
3:7	3.3 ± 0.9	50.4a	21.1cd	2.3e	73.1
1:1	2.9 ± 0.6	47.7b	22.8c	3.0d	73.3
XL-RS4 and flour[f] (test)					
1:9	4.2 ± 0.8	42.7c	25.6b	(3.9)[g] c	(72.2)[h]
3:7	4.7 ± 0.5 (21.2 ± 0.0%)[e]	31.3d	35.6a	(6.2)[g] b	(73.1)[h]
1:1	5.2 ± 0.7	29.9e	35.4a	(9.4)[g] a	(74.7)[h]
LSD ($P < 0.05$)		1.4	2.0	0.5	–

[a] RDS, rapidly digestible starch; SDS, slowly digestible starch, RS, resistant starch; TS, total starch.

[b] Calculated by assuming XL-RS4 is 100% starch, then subtracting the sum of RDS plus SDS.

[c] Assumed dry solids to be 100% starch.

[d] Values followed by different letters in a column are significantly different at 5% level.

[e] Values in parenthesis were determined on second batch of pup loaves made with or without a commercial sample (Fibersym RW) of XL-RS4, and where the freeze-dried bread was ground with mortar and pestle. Other values are for loaves made with laboratory-prepared XL-RS4, and where the bread was freeze-dried and ground with a high-speed impact mill.

[f] Level of starch given in w/w ratio of starch to flour (db).

[g] Calculated by difference between the theoretical total starch (TS) level and the experimentally determined sum of RDS plus SDS.

[h] Theoretical total starch (TS) level calculated by sum of starch in flour and untreated starch, which was determined experimentally, plus starch in added XL-RS4, assumed to be 100%.

TABLE VII
Recovery of Total Dietary Fiber in White Pan Bread and Sugar-Snap Cookies

	Total Dietary Fiber (g/100 g dry solids)		
Sample	In	Out	Recovery (%)
Bread			
Control (negative)	2.9	3.9	135
Control (positive)	1.7–2.7	2.9–3.9	144–171
XL-RS4 and flour			
1:9	9.5	4.2	44.2
3:7	22.4 (22.7)[a]	4.7 (19.2)[b]	21.0 (84.6)[b]
1:1	35.6	5.2	15.3
Cookies			
Control (negative)	1.2	1.5	80.0
XL-RS4 and flour			
3:7	12.8 (14.6)[a]	1.5 (14.5)[c]	11.7 (99.3)[c]
1:1	20.5	1.8	8.8
Potato starch and flour			
3:7	2.2	1.0	45.5
1:1	2.7	0.9	33.3
High-amylose (70%) corn starch and flour			
3:7	3.2	2.7	84.4
1:1	4.3	3.0	69.8
Heat-moisture-treated high-amylose (70%) corn starch and flour			
3:7	5.6	3.9	70.0
1:1	8.4	6.0	71.4

[a] Second batch of bread and cookies made with commercial XL-RS4 (Fibersym RW).

[b] Uncorrected value of 21.2% was corrected for residual protein and ash by subtracting 2.0%. Correction of 2.0% was estimated from corrected dietary fiber (3.9%) of blank bread in the first bake compared with uncorrected dietary fiber (5.9%) of blank bread in the second bake.

[c] Uncorrected value of 15.8% was corrected for residual protein and ash by subtracting 1.3%. Correction of 1.3% was estimated from dietary fiber levels of blank cookies in the two bakes (2.5 vs. 1.2%).

8.2 J/g (2 kcal/g) (Mathers 1992). Unfortunately, in our study, the dietary fiber levels in the breads fortified with the high-amylose corn starches were not determined.

The bread flour (Table VI) in our work contained, on a dry-weight basis, 35.2% RDS, 40.3% SDS, and 2.5% RS with 78.0% TS. The cookie flour (Table VIII) contained 23.8% RDS, 54.7% SDS, and 2.5% RS with 81.0% TS. Englyst et al (1999) reported that a wheat flour contained 31.5% RDS, 31.5% SDS, and 1.8% RS with TS 64.8%. Presumably the wheat flour assayed by Englyst et al (1999) was a bread flour because it had a low starch content. When those starch-fraction percentages are expressed on a dry starch basis, our bread flour contained 45.1, 51.7, and 3.2% of RDS, SDS, and RS, respectively. Our cookie flour 29.4, 67.5, and 3.1%, respectively, whereas the Englyst et al (1999) flour had 48.6, 48.6, and 2.8% of RDS, SDS, and RS, respectively. The RDS, SDS, and RS fractions determined in our laboratory on another reference material (potato starch) were 4, 20, and 88%, respectively, compared with 4, 26, and 89% reported by Englyst et al (1999). The rate of agitation during the in vitro digestions of wheat flour and potato starch was chosen by Englyst et al (1999 and 1992) such that the in vitro levels of RS for those reference standards would equal the in vivo RS levels determined in ileostomy patients (Englyst et al 1996). The close agreement of our in vitro digestion results to those of Englyst et al (1999) on both bread flour and potato starch indicates a proper choice of digestion conditions. The higher level (54.7 vs. 40.3%) of SDS and lower level (23.8 vs. 35.2%) of RDS in the cookie flour (Table VIII) compared with bread flour (Table VI) may result from lower mechanical damage to starch during milling of a soft versus a hard wheat.

The pattern of starch digestibility in bread changed as the level of XL-RS4 was increased (Table VI). At 30% flour replacement with XL-RS4, the one-day-old bread contained 31.3% RDS, 35.6% SDS, and 6.2% RS based on the dry weight of bread compared with 48.2% RDS, 21.3% SDS, and 3.0% RS for the negative control. As expected, replacing bread flour with 30% wheat starch gave no change in the pattern of starch digestibility in the positive control bread compared with the negative control. The theoretical level of RS in the test bread containing 30% flour replacement with XL-RS4 was 17.4% (Table IX) based on 2.5% RS in the bread flour and 62.9% in XL-RS4 (Table VI). Theoretical and experimental values of RS level in dry bread differed significantly (6.2 vs. 17.4%), suggesting that mechanical grinding of dry bread or grinding of XL-RS4 reduced recovery of RS to 36%. On the other hand, the recovery of the sum of SDS plus RS in bread fortified with 10–50% (flour basis) of XL-RS4 was 70–85% (Table IX).

Cookie Fortified with SDS and RS

A preferred fiber additive in cookies would have a pleasant or bland flavor and cause no changes in dough consistency, cookie spread, color, and texture. Cookie doughs with added high-amylose (70%) corn starch (Hylon VII) and with HMT high-amylose (70%) corn starch (Novelose 240) showed increased dough consistency as sensed by feel, while cookie dough with added potato starch and XL-RS4 showed a somewhat decreased consistency. The spread factor of cookies decreased 28–38% with added high-amylose (70%) corn starch and 23–38% with HMT high-amylose (70%) corn starch, and it increased 6–7% with potato starch but remained almost constant with XL-RS4 (Table X, Fig. 2). The top grain of cookies, judged visually by the uniformity and size of the "islands", was inferior for the two corn starches in cookies, whereas the grain for the XL-RS4 and potato starch cookies matched the control. The force to break the cookie with

TABLE VIII

Total Dietary Fiber (TDF) and In Vitro Starch Digestibility (% of sample, ds)[a] in Sugar-Snap Cookie and Starch Ingredients

Sample	TDF % (db)	Flour %, RS or Cookie (db)			
		RDS	SDS	RS	TS
Flour and starch ingredients					
Cookie flour	3.06 ± 0.10	23.8	54.7	2.5	81.0
XL-RS4	79.5 ± 0.10	4.6	32.5	(62.9)[b]	(100)[c]
Potato starch	0.79 ± 0.11	2.4	8.0	83.5	93.9
High-amylose (70%) corn starch	14.3 ± 0.12	12.6	23.5	57.6	93.7
Heat-moisture treated high-amylose (70%) corn starch[d]	30.7 ± 0.10	11.1	24.1	58.9	94.1
Sugar-snap cookie[e]					
Control (no starch added)	1.2g (2.5)[f]	17.6a	19.8a	2.0f	39.4
XL-RS4					
3:7	1.5f (15.8)[f]	12.0cd	17.4b	(11.6)[g] de	(41.0)[h]
1:1	1.8e	10.6d	14.3c	(17.0)[g] b	(41.9)[h]
Potato starch and flour					
3:7	1.0hg	12.4cb	12.9dc	13.6dc	38.9
1:1	0.9h	10.4d	9.5e	19.8a	39.7
High-amylose (70%) corn starch and flour					
3:7	2.7d	13.8b	13.2dc	11.5e	38.5
1:1	3.0c	12.4cb	12.4d	14.2c	39.0
Heat-moisture treated high-amylose (70%) corn starch[d] and flour					
3:7	3.9b	13.9b	13.6dc	11.8de	39.3
1:1	6.0a	11.6cd	13.5dc	14.7c	39.8
LSD ($P < 0.05$)	0.2	1.7	1.6	2.0	–

[a] RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; TS, total starch. Levels based on dry weight of flour, starch, and cookies.

[b] Calculated by difference assuming XL-RS4 wheat starch is 100% starch, then subtracting the sum of RDS plus SDS.

[c] Assumed to be 100% starch.

[d] Novelose 240 from National Starch and Chemical Co.

[e] Values followed by different letters are significantly different at 5% level.

[f] Second batch of cookies made with and without a commercial sample (Fibersym RW) of XL-RS4. Before assay, cookies from the second batch were treated to remove fat and sugar, and the dried residue was ground gently with a mortar and pestle to pass through a U.S. No. 20 sieve (1-mm opening). Values given are uncorrected for residual protein and ash.

[g] Calculated by difference between theoretical total starch (TS) level and the sum of RDS plus SDS.

[h] Total starch determined by sum of starch in flour, which was determined experimentally, plus starch in XL-RS4, assumed to be 100%.

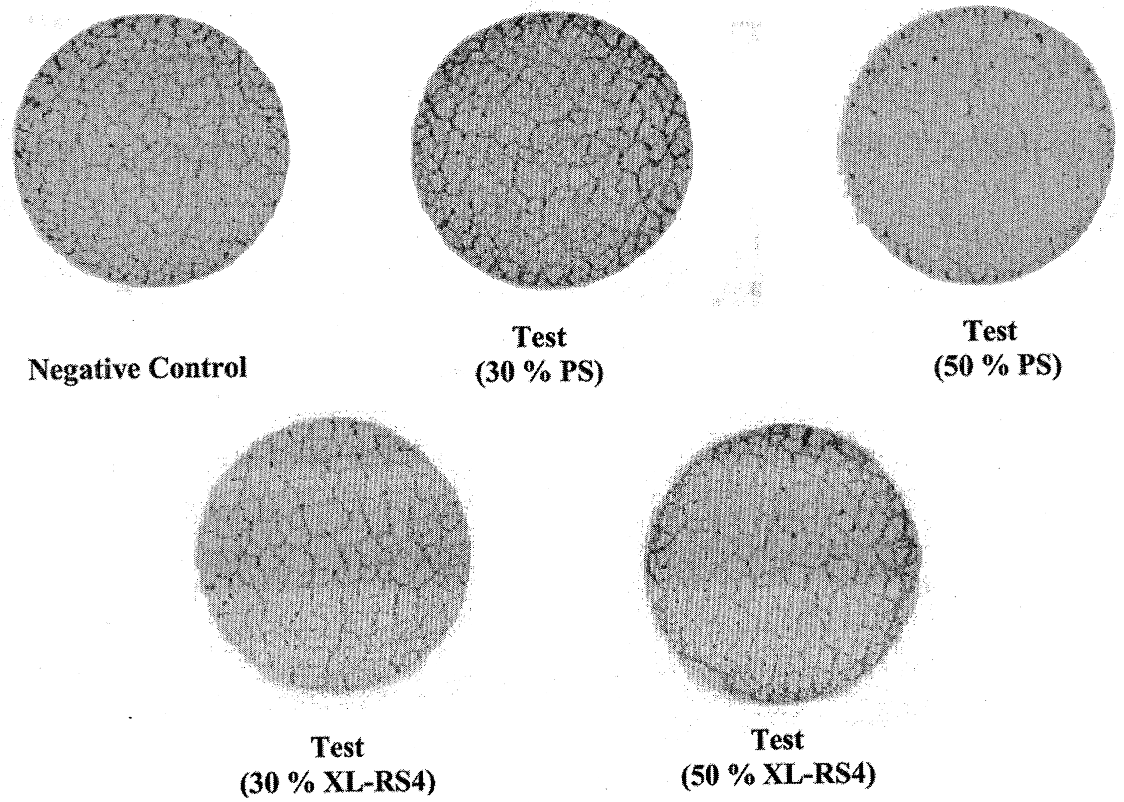

Fig. 2. Sugar-snap cookies made with blends (7:3 and 1:1, w/w) of cookie flour and potato starch (PS) and flour and cross-linked resistant wheat starch (XL-RS4) prepared in the laboratory.

either type of corn starch was 17–88% higher than for the control cookie and 20–26% lower with added potato starch as opposed to being equal with added XL-RS4. The increase in strength of the cookies with the added corn starches compared with the control may arise at least in part from ≈1% higher moisture contents (Table X). The increased moisture in the cookies containing the corn starches may explain reduced browning and pale appearance (data not shown). Sollars and Rubenthaler (1971) concluded that reconstituted cookie flour that had been fractionated and then reconstituted with wheat starch performed well in cookie making, and that potato starch gave cookies with fair quality. In addition, the reconstituted flour with wheat starch or potato starch gave cookies almost equal in diameter to the control.

Dietary Fiber and Pattern of Starch Digestibility in Cookies

Substitution of 50% of the cookie flour with XL-RS4, potato starch, high-amylose (70%) corn starch, and HMT high-amylose (70%) corn starch increased resistant starch (RS) from 2.0% in the control cookie to 17.0, 19.8, 14.2, and 14.7%, respectively, on a dry basis (Table VIII). The respective calculated values of RS in 394 g of dry cookie/225 g of flour (14% mb), where the RS originates from the flour and added starches were 16.1, 21.1, 14.8, and 15.1% (93–106% recovery) (Table IX). The low moisture content (≈25%, flour basis) of the cookie dough and high sucrose level (≈58%, flour basis) ensure that the RS fraction in cookie dough undergoes almost no change during baking, as determined in the past by both in vitro and in vivo testing (Englyst et al 1996). However, the SDS in all the cookies was generally ≈5% lower and the RDS ≈5% higher than predicted from ingredient inputs (data not given), even though starch gelatinization did not occur during cookie making. That difference indicates a small increase in digestibility of endogenous starch which was likely caused by sample preparation before assay (grinding, defatting, desugaring, and regrinding). The processing survival of the sum of SDS and RS in cookies was 76–85% (mean 80%) for both levels of RS fortification (Table IX).

With reference to the control cookies, the sum of SDS and RS increased ≈19–39% and 28–51% (Table IX), respectively, in cookies made with 30 and 50% replacement of flour with the added RS ingredients. However, the cookies contained, besides 10–14% RDS (Table VIII), another 17% rapidly available glucose in the form of sucrose. The food energy in the cookies was reduced ≈10% with 50% substitution of XL-RS4 or potato starch for flour, assuming the energy content of the RS is 8.2 J/g (2.1 kcal/g).

The TDF levels determined on the RS-fortified cookies are given in Tables VII and VIII. Assuming 100% survival of dietary fiber in the flour and added RS, the dietary fiber levels of cookies containing 30 and 50% replacement of flour with high-amylose (70%) corn starch were 3.2 and 4.3% TDF. The experimental values of 2.7 and 3.0%, respectively, were 84.4 and 69.8% of theory. The same calculations for Novelose 240 blended at 30 and 50% gave calculated levels of dietary fiber of 5.6 and 8.4%, whereas the actual levels were 3.9% (70% of theory) and 6.0% (71.4% of theory). However, the TDF levels in cookies fortified with potato starch were low (2–3%) because potato starch does not survive the hot (95–100°C) amylolytic digestion step.

The TDF levels in cookies fortified with 30 and 50% laboratory-prepared XL-RS4 were 1.5 and 1.8%, or only 11.7 and 8.8% of the theoretical levels of 12.8 and 20.5%, respectively (Table VII). Again, mechanical grinding during sample preparation was suspected of physically damaging the starch or creating excessively fine particles that were lost in the gravimetric assay. TDF assay on cookies made with 30% flour replacement with commercial XL-RS4 and ground with a mortar and pestle gave 15.8% TDF (Table VIII), which was corrected to 14.5%. The 14.5% TDF represents 99.3% recovery of dietary fiber added in the ingredients (Table VII). The loss of fine particles in the gravimetric assay of cookies containing XL-RS4 is consistent with the quantitative

TABLE IX
Recovery of Resistant Starch (RS) and Slowly Digestible Starch (SDS) in White Pan Bread and Sugar-Snap Cookies With and Without Additives

Sample	RS (g/100 g, ds) In	RS (g/100 g, ds) Out	Recovery (%)	RS + SDS (g/100 g, ds) In	RS + SDS (g/100 g, ds) Out	Recovery (%)
Bread						
Control (negative)	2.2	3.0	136	37.2	24.3	65
Control (positive)	2.2–2.4	2.1–3.0	96–125	38.2–44.1	22.1–25.8	57–59
XL-RS4 and flour						
1:9	7.2	3.9	54	41.2	29.5	72
3:7	17.4	6.2	36	49.4	41.8	85
1:1	27.0	9.4	35	57.0	39.4	70
Cookies						
Control (negative)	1.3	2.0	153	28.0	20.8	74
XL-RS4 and flour						
3:7	10.2	11.6	113	34.1	29.0	85
1:1	16.1	17.0	106	37.8	31.3	83
Potato starch and flour						
3:7	13.4	13.6	102	33.3	26.5	80
1:1	21.1	19.8	93	36.8	29.3	80
High-amylose (70%) corn starch and flour						
3:7	9.4	11.5	122	31.7	24.7	77
1:1	14.8	14.2	95	34.8	26.6	76
Heat-moisture-treated high-amylose (70%) corn starch and flour						
3:7	9.5	11.8	124	31.9	25.4	79
1:1	15.1	14.7	97	34.5	28.2	82

TABLE X
Quality of Sugar-Snap Cookies Made from Blends (14% mb) of Starches and Cookie Flour[a]

Flour-to-Starch (w/w)	Spread Factor[b]	Top Grain	Day-Old Cookies Moisture Content (%)	Day-Old Cookies Snapping Force (kg-force)
None (control)	107.9a	Good	2.7cd	9.22c
XL-RS4				
3:7	110.8a	Good	2.1ed	9.30c
1:1	107.8a	Good	2.2ed	9.04c
Potato starch				
3:7	113.8a	Good	2.0ef	7.35d
1:1	115.4a	Good	1.9ef	6.79d
High-amylose (70%) corn starch				
3:7	78.1bc	Poor	3.2cb	11.82b
1:1	66.5c	Poor	4.0a	16.61a
Heat-moisture-treated high-amylose (70%) corn starch[c]				
3:7	83.5b	Poor	3.2cb	10.80b
1:1	66.5c	Poor	3.5ab	17.32a
LSD ($P < 0.05$)	15	–	0.6	1.5

[a] Values followed by different letters in the same column are significantly different at 5% level.
[b] Spread factor = (width/thickness) × 0.981 (altitude correction factor).
[c] Novelose 240 from National Starch Co.

recovery (106 and 113% in Table IX) of RS from those cookies in the colorimetric, modified Englyst assay.

CONCLUSIONS

Cross-linked resistant wheat starch (XL-RS4) can be incorporated in white pan bread with <15% loss of loaf volume when added at 10–50% flour replacement, provided gluten and yeast nutrients are added to the bread formula. The firmness of bread containing XL-RS4 increases, especially at day one. Approximately 85% of TDF in a 7:3 blend of flour and XL-RS4 survives breadmaking, as well as 85% of the sum of SDS plus RS. XL-RS4 also can be incorporated in sugar-snap cookies at levels up to 50% flour replacement without significantly changing the texture and the appearance of the cookies. All of the resistant starch and dietary fiber in XL-RS4 survives the process to make sugar-snap cookies. High-speed impact milling of XL-RS4 and dried bakery foods containing XL-RS4 have the potential to cause low recovery of TDF and RS.

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