

Production of Boiling-Stable Granular Resistant Starch by Partial Acid Hydrolysis and Hydrothermal Treatments of High-Amylose Maize Starch

Jorge O. Brumovsky¹ and Donald B. Thompson^{1,2}

ABSTRACT

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The purpose of the present work was to examine whether partial acid hydrolysis (PAH) of a high-amylose maize starch (*ae*-VII) would enhance the effects of hydrothermal treatments to produce granular resistant starch (RS) that is stable to further heat treatment at atmospheric pressure. PAH *ae*-VII starches were prepared by heating 35% (w/w) suspensions with 1% (w/w) HCl at 25°C for 6, 30, and 78 hr. Native and PAH starches were then treated by annealing (ANN) or heat-moisture treatment (HMT). ANN was done at 70% moisture at 50, 60, or 70°C for 24 hr, and HMT was done at 30% moisture at 100, 120, or 140°C for 80

min. RS that survives boiling during analysis was determined by a modification of the AOAC method for determining total dietary fiber. RS was also determined by the Englyst method. Little change in the gelatinization enthalpy was found for *ae*-VII starch after PAH, ANN, or HMT as individual treatments. After PAH, either ANN or HMT led to decreased gelatinization enthalpy. HMT and ANN alone increased boiling-stable RS but decreased total RS. After PAH of *ae*-VII, either ANN or HMT tended to increase the yield of boiling-stable granular RS, with the greatest yield (≤63.2%) observed for HMT.

Resistant starch (RS) has been defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (EURESTA 1992). Even though RS escapes digestion in the small intestine, it may be fermented in the large intestine by colonic microflora (Cummings et al 1986; Englyst and MacFarlane 1986; Mathers 1992; Schulze 1992). In the last decade, there has been an increased interest in the nutritional implications of RS, not only because of its decreased caloric content, but also because RS may have a physiological effect similar to that of dietary fiber (Biliaderis 1991; Björck 1996; Brown 1996). Moreover, the fact that processing treatments may alter RS content in foods has gained the attention of food technologists (Annison and Topping 1994; Björck and Asp 1994).

RS has been classified as type I, resulting from physical inaccessibility in intact tissues or particulate materials; type II, resulting from the physical structure of the raw starch granules; and type III, resulting from the physical structure of associated starch molecules after cooking. Eerlingen and Delcour (1995) have recently added a fourth category, type IV, resulting from chemical modification that interferes with the enzyme digestion.

Although several *in vitro* analytical methods for RS determination have been developed (Berry 1986; Björck et al 1986; Champ 1992; Englyst et al 1992; Muir and O’Dea 1993), no *in vitro* method can determine RS according to the physiological definition above. Furthermore, the composition of isolated RS determined by *in vitro* analytical methods is different from the composition of the RS isolated by *in vivo* techniques (Faisant et al 1992, 1993). At the present time, there is no accepted analytical procedure for determining RS. The lack of an accepted method to determine RS represents an important limitation for evaluation of RS production processes. Care must be taken when RS production processes are compared using different analytical methods because the different methods detect different amounts of RS.

The method of Englyst et al (1992) determines the portion of starch and starch degradation products that remain undigested after 2 hr of hydrolysis with a mixture of pancreatin, amyloglucosidase, and invertase at 37°C. This method was developed to imitate the physiological conditions of starch digestion, and it has been quantitatively validated in humans, as the analyses give values in agreement with the average amount of starch escaping complete digestion and absorption in the human small intestine (Englyst et al 1996). Even though the Englyst method has some advantages for the deter-

mination of RS, it has not been commonly used for evaluating RS production processes, perhaps because of its complexity.

The most commonly used method for RS determination (AOAC 1985; Sievert and Pomeranz 1989, 1990; Szczodrak and Pomeranz 1991; Björck and Siljestrom 1992; Unlu and Faller 1998) is based on the AOAC method for measurement of total dietary fiber (TDF). The TDF method detects the undigested starch that remains after 35 min of boiling with thermally stable α -amylase. This method, which was not initially intended for RS determination, detects only the portion of RS that survives a boiling treatment. This is included in the TDF value that may be claimed on a food label as dietary fiber. The portion of RS isolated by the TDF method is important because it not only appears as dietary fiber but is also relatively thermally stable, remaining after thermal processing at 100°C. Although none of the analytical procedures are intended to evaluate the thermal stability of RS, thermally stable RS can be evaluated either by the Englyst method after a boiling step or by the TDF method.

Although the four types of RS would suggest at least four approaches to manufacturing RS, only the manufacture of type III RS has been extensively studied (Pomeranz 1992; Eerlingen and Delcour 1995; Gidley et al 1995). Manufacturing type IV RS is limited by the type and extent of derivatization that may be legally used in foods. Little information about strategies to improve the manufacture of types I and II RS exists in the literature.

A few varieties of starches are good sources of type II RS: banana (69–89% RS), potato (80–87% RS), and high-amylose maize starches (HAMS) (55–85% RS) (Sugimoto et al 1979; Dreher et al 1984). However, the enzyme resistance of these starches is highly reduced (HAMS) or completely lost (banana and potato) after moderate heat-processing conditions such as boiling in excess water. The lack of thermal stability of type II RS represents a limitation on use of food ingredients containing type II RS to increase dietary RS.

On the other hand, type III RS is considered to be very thermally stable (Gruchala and Pomeranz 1993; Eerlingen and Delcour 1995). The thermal stability of type III RS has made it suitable for uses in many foods. Several manufacturing processes have been developed to increase type III RS from starch (Iyengar et al 1991; Chiu et al 1994; Harris and Little 1994, 1995). In these processes, the preferred starch material was HAMS.

The thermal stability of granular RS has also been enhanced by hydrothermal treatments (Shi and Trzasko 1997; Haralampu and Gross 1998). Whether this RS should be considered type II is an open question. Annealing and heat-moisture treatments are two types of hydrothermal treatments that can modify the physicochemical properties of starch without destroying the granule structure (Jacobs and Delcour 1998). Both treatments involve incubation at specified moisture levels and temperatures above the glass transition

¹ Department of Food Science, Penn State University, University Park, PA 16802.

² Corresponding author: E-mail: dbt@psu.edu Phone: 814-863-0481. Fax: 814-863-6132.

temperature but below the gelatinization temperature. Heat treatments at high-moisture levels (>40% wet basis) have been termed annealing (ANN), and treatments performed at low-moisture levels (<35% wet basis) have been termed heat-moisture treatments (HMT) (Jacobs and Delcour 1998).

Several authors have stated that hydrothermal treatments reorganize the granule structure and increase its stability (Donovan et al 1983; Krueger et al 1987; Knutson 1990; Stute 1992; Kawabata et al 1994; Jacobs and Delcour 1998; Tester et al 1998). Native starch granules are composed of molecules in a metastable state. Although this metastable state represents an efficient ordering and packing of semicrystalline material inside the granule, hydrothermal treatments can improve the thermal stability of this material, producing granules that are more thermally stable. If manufacture of enhanced levels of granular RS is related to enhanced order of the granules, ANN or HMT should enhance the amount and the thermal stability of granular RS.

After thermal processing, acid or enzymatic hydrolysis has been used to increase the concentration of type II and III RS by removing

the nonenzyme-resistant material (Chiu et al 1994; Haralampu and Gross 1998). For manufacture of type III RS, hydrolysis treatments before subsequent thermal processing have been also applied to enhance retrogradation (Chiu et al 1994; Harris and Little 1994, 1995; Haralampu and Gross 1998). The structures responsible for type III RS are thought to be based on associated double helices principally from amylose regions (Gidley et al 1995). The length of these regions in retrograded amylose has been estimated as DP=24 (Jane and Robyt 1984). Eerlingen et al (1993) found that the chain length of the resistant regions (DP 19–26) was independent of the amylose average chain lengths originally used to form the type III RS, though the yield of RS increased with the average chain length of the amylose originally used to produce it. Gidley et al (1995) observed a broad distribution of chain lengths from DP 10–1,000 with an approximate relative maximum at DP 20–30.

The structures responsible for type II RS are not understood. If type II RS can also be accounted for at least partially by double helices that are associated with other double helices, a mild acid hydrolysis before a hydrothermal treatment should reduce the molecular

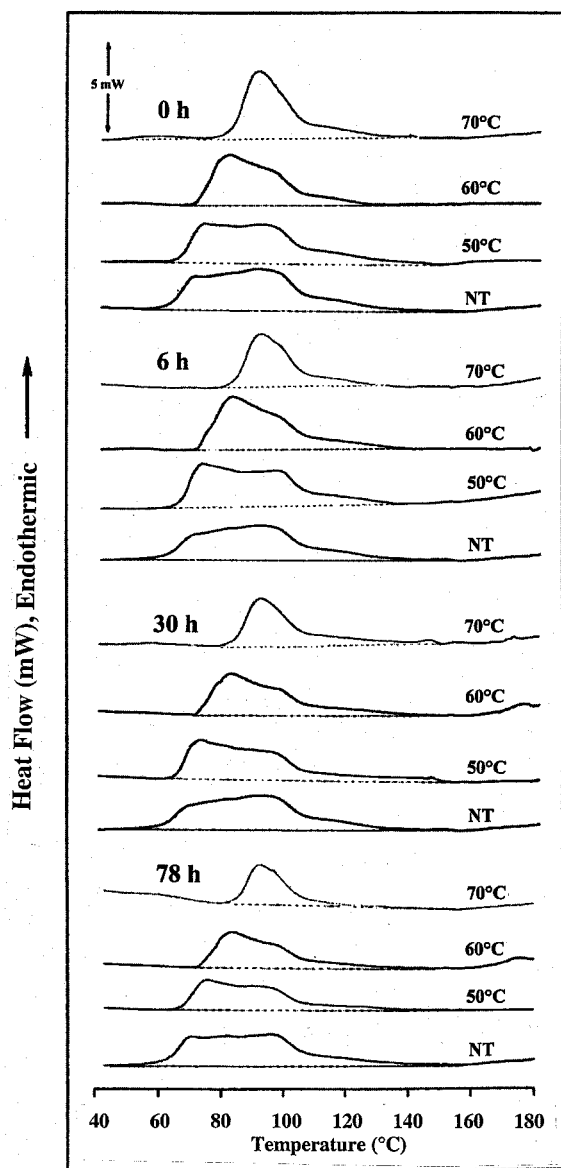


Fig. 1. Differential scanning calorimetry (DSC) thermograms for initial heating of native *ae*-VII starch, partial acid hydrolysis (PAH) of *ae*-VII starches, and PAH and annealing (ANN) of *ae*-VII starches. All samples contained ≈25 mg of starch at 30% solids; 0, 6, 30, and 78 hr refer to duration of PAH treatments; 50, 60, and 70°C refer to ANN temperature. NT = not treated by annealing.

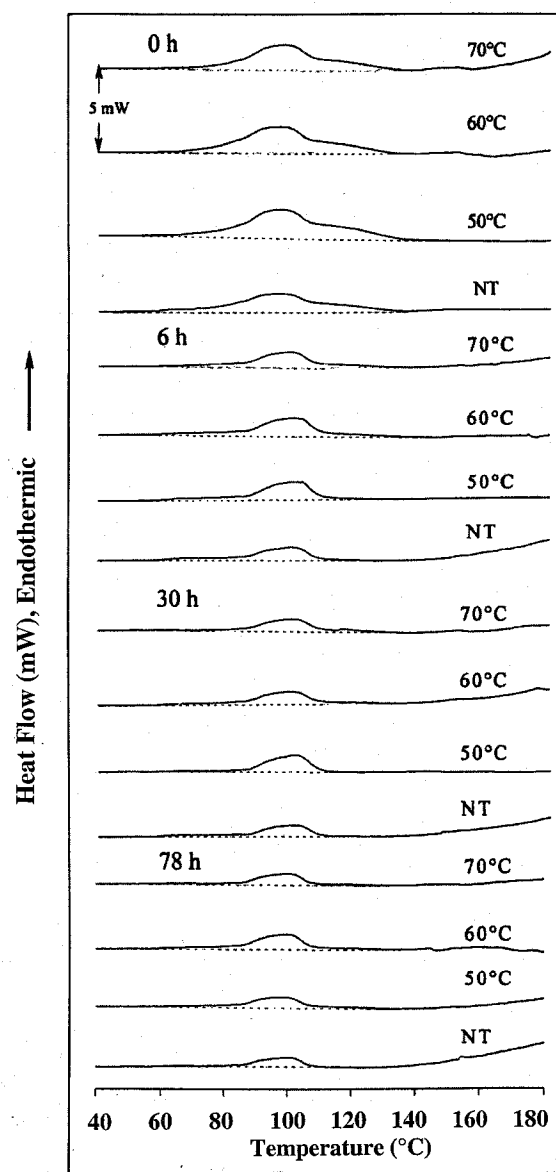


Fig. 2. Differential scanning calorimetry (DSC) thermograms for immediate reheating of native *ae*-VII starch, partial acid hydrolysis (PAH) of *ae*-VII starches, and PAH and annealing (ANN) of *ae*-VII starches. All samples contained ≈25 mg of starch at 30% solids; 0, 6, 30, and 78 hr refer to duration of PAH treatments; 50, 60, and 70°C refer to ANN temperature. NT = not treated by annealing.

weight and reduce kinetic constraints to molecular mobility necessary to improve the structural stability of the most resistant portions of the granule. Thus, we hypothesized that a partial acid hydrolysis (PAH) would enhance the effects of hydrothermal treatments (ANN or HMT) on the yield of thermally stable granular RS.

The first objective of this work was to enhance production of boiling-stable granular RS, as determined by the AOAC method, by a hydrothermal treatment (ANN or HMT) after PAH of HAMS. The second objective was to compare the RS values as determined by the Englyst and TDF methods, to see how the total RS and boiling-stable RS were related for the treated starches.

MATERIALS AND METHODS

Starch samples obtained were a commercial maize starch with 70% amylose (*ae*-VII, Hylon VII, National Starch and Chemical Co., Bridgewater, NJ), a commercial corn starch (common corn starch

[CCS] Melojel, National Starch and Chemical), potato starch (cat. no. 4251, Sigma-Aldrich, St. Louis, MO), and wheat starch (cat. no. 5127, Sigma-Aldrich). All reagents were ACS grade or better.

Amyloglucosidase solution (AMG 300 L, activity 300 AGU/mL) and heat-stable α -amylase (Termamyl 120 L, activity 120 KNU/mL) were obtained from Novo Nordisk BioChem North America, Inc., (Franklinton, NC). Pancreatin (cat. no. 7545, activity $8 \times$ USP/g) was obtained from Sigma-Aldrich. Invertase solution (cat. no. 390203D, 3,000 EU/mL) was obtained from BDH, Inc. (Carle Place, NY). A total dietary fiber assay kit (cat. no. K-TDFR) was obtained from Megazyme International Ireland Ltd. (Co. Wicklow, Ireland), which contained thermostable α -amylase (E-BLAAM) and amyloglucosidase (E-AMGDF). A glucose oxidase and peroxidase assay kit (cat. no. K-GLUC) was also obtained from Megazyme.

A glucose standard solution was prepared by weighing 20 g of glucose, 0.4 g of benzoic acid, and 13.61 g of sodium acetate trihydrate, and making up to 200 mL with deionized water.

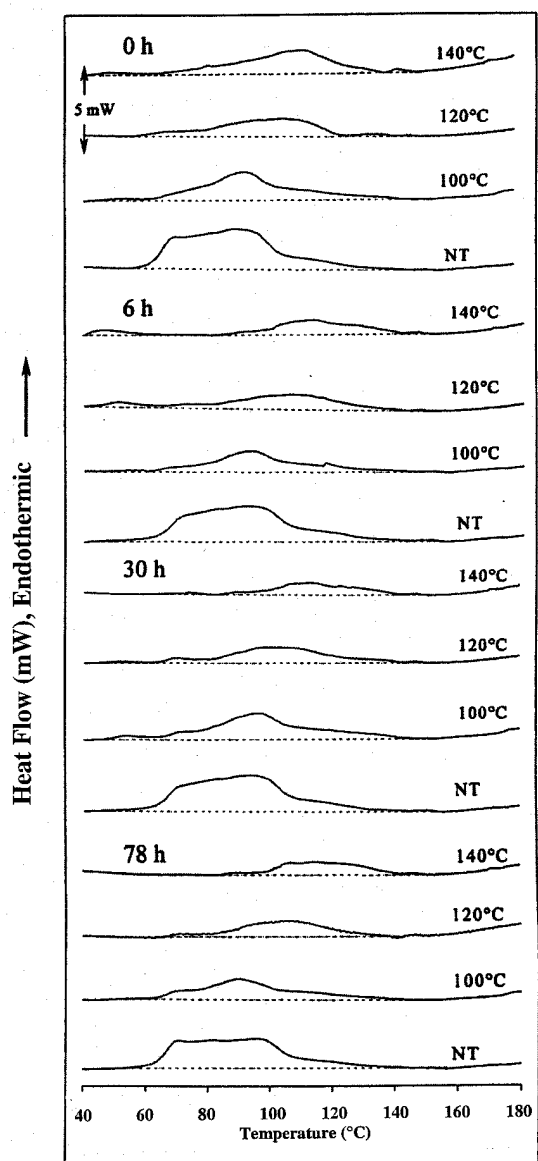


Fig. 3. Differential scanning calorimetry (DSC) thermograms for initial heating of native *ae*-VII starch, partial acid hydrolysis (PAH) of *ae*-VII starches, and PAH and heat-moisture treatment (HMT) of *ae*-VII starches. All samples were analyzed at 30% starch solids. HMT samples contained ≈ 15 mg of starch; 0, 6, 30, and 78 hr refer to duration of PAH treatments; 100, 120, and 140°C refer to HMT temperature. Starches not treated by annealing (NT) as shown in Fig. 1.

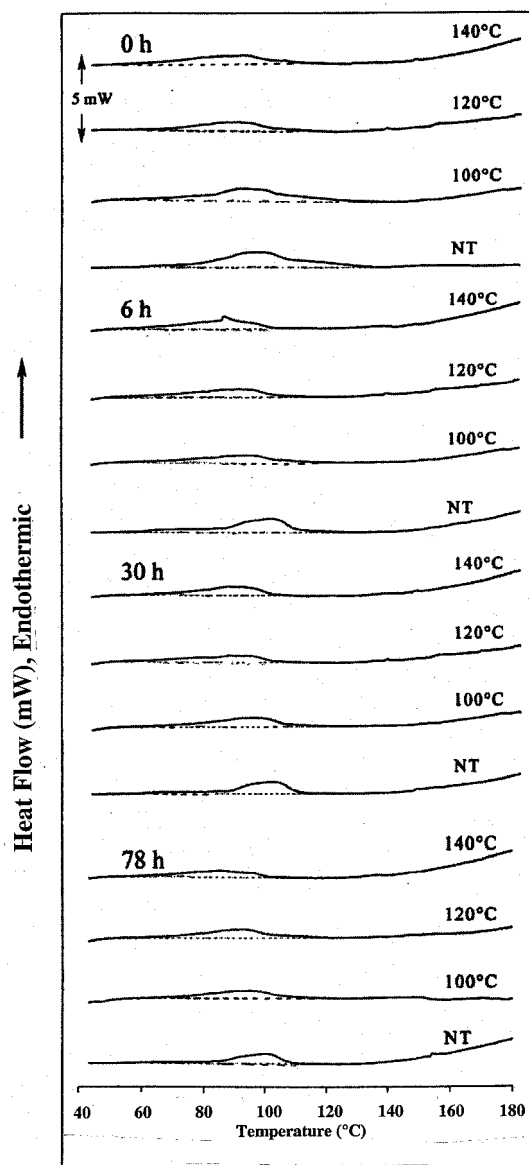


Fig. 4. Differential scanning calorimetry (DSC) thermograms for immediate reheating of native *ae*-VII starch, partial acid hydrolysis (PAH) of *ae*-VII starches, and PAH and heat-moisture treatment (HMT) of *ae*-VII starches. All samples were analyzed at 30% starch solids. HMT starch content was ≈ 15 mg; 0, 6, 30, and 78 hr refer to duration of PAH treatments; 100, 120, and 140°C refer to HMT temperature. Starches not treated by annealing (NT) as shown in Fig. 2.

Enzyme solution was prepared according to the Englyst method. Pancreatin (18 g) was added to 180 mL of water in a beaker and stirred by magnetic stirrer for 10 min. Half of the final volume was taken into each of two 225-mL centrifuge tubes and centrifuged at $1,500 \times g$ for 10 min. A portion of the cloudy supernatant (45 mL) was removed from each tube and transferred to a brown glass volumetric flask (90 mL total) containing 5.3 mL of amyloglucosidase solution, 6 mL of invertase solution, and made up to 200 mL with deionized water.

PAH

A 35% (w/v) suspension of *ae*-VII starch in water was prepared in a flask. While stirring, concentrated HCl was added to the flask to result in 1% HCl (w/w, HCl to dry starch) (Mussulman and Wagoner 1968). The flask was stoppered and put into an incubation chamber at 25°C. Equal fractions of the suspension were removed at 6, 30, and 78 hr of incubation. The fractions were immediately neutralized to pH 7 with a 3% (w/v) solution of NaOH, and vacuum-filtered. The samples were then suspended in deionized water and filtered again. The latter procedure was repeated three times, and a final wash was made with 95% ethanol. The samples were dried in a convection oven at 40°C and then ground using a mortar and pestle. The ground samples were screened through a 120-mesh sieve (125 μ m opening).

Native and hydrolyzed *ae*-VII starches were dispersed in 90% DMSO, dried, and examined by size-exclusion chromatography (SEC) on a Sepharose CL-2B column (Klucinec and Thompson 1998). SEC fractions were analyzed for total carbohydrate using the phenol-sulfuric acid assay (Dubois et al 1956).

ANN

Native starch and hydrolyzed *ae*-VII starches were annealed by incubating 30% (w/w) starch suspensions for 24 hr at 50, 60, or 70°C. The samples were filtered and then washed with water and ethanol. The samples were dried in a convection oven at 40°C, ground, and screened through a 120-mesh sieve (125 μ m opening).

HMT

Native starch and PAH *ae*-VII starches were heat-moisture treated by heating samples in a 25-mL stainless steel bomb placed in a thermostatically controlled convection oven ($\pm 1^\circ\text{C}$). Samples at 70% starch solids were heated at 100, 120, or 140°C for 80 min. Immediately after the treatments, the bomb was immersed in a water-ice bath for ≈ 5 min. The samples were removed and then dried in a convection oven at 40°C, ground, and screened through a 120-mesh sieve (125 μ m opening).

Experimental Design and Statistical Analysis

Two full-factorial designs with two factors at four levels were used. The first design studied the hydrolysis time and ANN temperature. The levels were no hydrolysis, or 6, 30, or 78 hr of hydrolysis, and no annealing, or 50, 60, or 70°C ANN temperature. The second design studied the hydrolysis time and HMT temperature. The levels for the second design were no hydrolysis, or 6, 30, or 78 hr of hydrolysis, and no HMT, or 100, 120, or 140°C for the HMT temperature. The starch samples of the experimental designs were studied by DSC, and the RS content was determined by the Englyst and TDF methods. Experimental data were analyzed based on a full model. Calculations were performed using the general linear model procedure provided by the software package Minitab v.12.23. All pairwise comparisons were determined by Tukey's test at a family error rate of $\alpha = 0.05$.

DSC Thermal Analysis

Thermal analysis was performed using differential scanning calorimetry (DSC 7, Perkin-Elmer, Norwalk, CT) equipped with a thermal analysis data station. Indium was used as a calibration standard. The reference cell contained a sealed, empty, stainless steel pan.

Starch samples were weighed into preweighed stainless steel pans. For untreated and ANN samples, starch mass was ≈ 25.0 mg, dwb. For HMT samples, starch mass was lower (≈ 15.0 mg, dwb) due to difficulty in sealing larger quantities after adding water. Deionized water was added to make $\approx 30\%$ (w/w) starch suspensions. The samples were stirred with a needle. The pans were sealed, total weights were determined, and the suspensions were stored overnight at room temperature. Samples were heated from 20 to 180°C at 10°C/min. Samples were then quench-cooled from 180 to 20°C and were immediately reheated to 180°C at 10°C/min. Gelatinization enthalpy and onset temperatures were calculated for each thermal event using thermal analysis software (7 Series Software, Perkin-Elmer). Thermal analyses were performed at least in duplicate.

TABLE I
Thermal Analysis After Partial Acid Hydrolysis (PAH) and Annealing (ANN) of *ae*-VII Starch on Heating and Immediate Reheating^a

PAH (hr)	ANN ^b (°C)	Heating		Reheating
		T_o^c (°C)	ΔH^d (J/g)	ΔH (J/g)
0	NT ^e	63.0 \pm 0.34a ^f	17.8 \pm 0.44f	8.4 \pm 0.62e
0	50	67.4 \pm 0.15b	20.0 \pm 0.19g	10.0 \pm 0.08e
0	60	73.2 \pm 0.12c	19.1 \pm 0.12f	8.9 \pm 0.16e
0	70	83.7 \pm 0.08d	18.2 \pm 0.15f	9.7 \pm 0.21e
6	NT	61.4 \pm 0.39a	18.5 \pm 0.25f	5.0 \pm 0.31d
6	50	67.3 \pm 0.23b	20.3 \pm 0.30g	5.1 \pm 0.14d,c
6	60	74.2 \pm 0.31c	18.4 \pm 0.16f	6.4 \pm 0.11d
6	70	84.5 \pm 0.42d	14.5 \pm 0.31c	5.8 \pm 0.23d
30	NT	61.3 \pm 0.32a	18.7 \pm 0.14f	3.7 \pm 0.18b
30	50	67.0 \pm 0.18b	17.0 \pm 0.21e	4.2 \pm 0.13b
30	60	74.2 \pm 0.11c	17.3 \pm 0.24e	5.1 \pm 0.13c
30	70	85.7 \pm 0.24d	12.5 \pm 0.18b	5.3 \pm 0.13c
78	NT	61.7 \pm 0.32a	19.6 \pm 0.20f	3.1 \pm 0.57a
78	50	67.4 \pm 0.40b	15.2 \pm 0.08d	3.2 \pm 0.13a
78	60	74.0 \pm 0.14c	16.1 \pm 0.25d	4.1 \pm 0.16b
78	70	85.4 \pm 0.29d	11.0 \pm 0.07a	3.5 \pm 0.21a

^a Initial heating and immediate reheating at 20–180°C at 10°C/min. Mean \pm standard deviation of two to three replicates.

^b Annealing temperature of 30% solids for 24 hr.

^c Onset temperature.

^d Enthalpy.

^e Not treated by annealing.

^f Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE II
Thermal Analysis After Partial Acid Hydrolysis (PAH) and Heat-Moisture Treatment (HMT) of *ae*-VII Starch on Heating and Immediate Reheating^a

PAH (hr)	HMT ^b (°C)	Heating		Reheating
		T_o^c (°C)	ΔH^d (J/g)	ΔH (J/g)
0	NT ^e	63.0 \pm 0.34a ^f	17.8 \pm 0.44d	8.4 \pm 0.62d
0	100	76.2 \pm 0.45d	16.4 \pm 0.41c	7.3 \pm 0.47d,c
0	120	76.3 \pm 0.61d	17.0 \pm 0.47d	6.1 \pm 0.39c
0	140	79.0 \pm 0.47e	17.5 \pm 0.52d	6.4 \pm 0.29c
6	NT	61.4 \pm 0.39a	18.5 \pm 0.25d	5.0 \pm 0.31b
6	100	75.0 \pm 0.53d	16.2 \pm 0.39c	4.8 \pm 0.45b
6	120	79.7 \pm 0.45e	14.9 \pm 0.37b	3.5 \pm 0.47a
6	140	88.2 \pm 0.37f	12.3 \pm 0.51a	3.0 \pm 0.50a
30	NT	61.3 \pm 0.32a	18.7 \pm 0.14d	3.7 \pm 0.18a
30	100	72.8 \pm 0.62c	16.3 \pm 0.55c	3.6 \pm 0.34a
30	120	77.3 \pm 0.55e	15.4 \pm 0.49b	3.0 \pm 0.41a
30	140	94.0 \pm 0.47g	11.3 \pm 0.37a	3.1 \pm 0.37a
78	NT	61.7 \pm 0.32a	19.6 \pm 0.20d	3.1 \pm 0.57a
78	100	67.2 \pm 0.43b	16.6 \pm 0.51c	2.9 \pm 0.29a
78	120	79.9 \pm 0.61e	15.3 \pm 0.47b	3.2 \pm 0.35a
78	140	95.2 \pm 0.59g	10.9 \pm 0.35a	2.8 \pm 0.28a

^a Initial heating and immediate reheating at 20–180°C at 10°C/min. Mean \pm standard deviation of two to three replicates.

^b HMT temperature of 70% solids for 80 min.

^c Onset temperature.

^d Enthalpy.

^e Not treated by HMT.

^f Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

RS Determination

The method used here was modified from Englyst et al (1992) for analysis of essentially protein-free starch samples. By this procedure, RS is defined as the starch not hydrolyzed after incubation with pancreatic amylase and amyloglucosidase at 37°C after 120 min. RS was expressed as the percentage of total dry starch:

$$RS\% = ([TS - DS]/TS) \times 100$$

where TS = mass of total starch (g) and DS = mass of digested starch (g).

DS Determination

Starch samples (≈ 0.5 g) were weighed into 50-mL polypropylene centrifuge tubes. A 5-mL portion of sodium acetate solution (0.5 mol/L) and 10-mL portion of guar gum solution (5 g/L, in HC1 0.05 mol/L) were added to the sample tubes and the blank tube. A volume of 5 mL of glucose standard solution and 10 mL of guar gum solution (5 g/L, in HC1 0.05 mol/L) were added to the standard tube. Five glass balls (≈ 15 mm diameter) were added to each tube and the contents mixed using a vortex mixer. The tubes were completely immersed in a shaking water bath at 37°C to equilibrate for ≈ 5 min. Samples were removed from the water bath and 10 mL of the prepared enzyme solution were added to each tube. The tubes were immediately returned to the water-bath and the shaking action was initiated. The water bath was adjusted to a stroke speed of ≈ 150 strokes/min and a stroke length of 35 mm to ensure mixing action of the glass balls. After 120 min, the shaking action of the water-bath was interrupted and 50 μ L was removed from each tube and transferred to 2-mL microcentrifuge tubes containing 1 mL of absolute ethanol. The microcentrifuge tubes containing the samples were mixed using a vortex mixer and analyzed for glucose by the glucose oxidase and peroxidase assay. The remaining material in the 50-mL tube was analyzed for total starch.

Glucose Oxidase and Peroxidase Assay

Samples were centrifuged for 5 min at $1,500 \times g$. A 100- μ L aliquot of supernatant was added to a microcentrifuge tube containing 1.5 mL of glucose oxidase and peroxidase reagent. The tube was incubated in a water bath at 45°C for 20 min. The sample contents were transferred to spectrophotometer cuvetts. Absorbances of samples and standard were read against a reagent blank.

TS Determination

After the removal of 50 μ L at 120 min, the remaining contents of the 50-mL sample tubes were placed in a boiling water bath for 30 min. After vortex mixing, the sample tubes were cooled in an ice water bath for 15 min. A 10-mL volume of KOH solution (7 mol/L) was added, and the mixture was vortex-mixed again. The tubes were returned to the ice water bath and shaken for 30 min. Tubes were removed from the ice water bath and 200 μ L of the contents was added to 2-mL microcentrifuge tubes containing 1 mL of 1 mol/L acetic acid. A 40- μ L aliquot of AMG solution (diluted 1:5.25 with water from the original AMG solution) was added to the tubes and vortex-mixed. The tubes were placed in a 70°C water bath for 30 min followed by 10 min in a boiling water bath. A 100- μ L aliquot of the contents was added to a fresh 2-mL tube containing 1 mL of absolute ethanol, vortex-mixed, and analyzed for glucose by the glucose oxidase and peroxidase assay.

Determining Boiling-Stable RS by the TDF Method

The method used for determining the boiling-stable RS portion followed the pattern of the AOAC method for determining total dietary fiber (AOAC 1985). The method was adapted for essentially protein-free starch analysis. Starch samples (0.5 g) were suspended in 20 mL of MES/TRIS buffer (pH 8.2) and incubated with 25 μ L of thermostable α -amylase (Megazyme E-BLAAM) at 95–100°C for 35 min. The samples were then cooled at 60°C and adjusted to pH 4.1–4.8 with 5% NaOH or 5% HC1. The samples

were next incubated with 100- μ L of amyloglucosidase (Megazyme E-AMGDF) at 60°C for 30 min. Mixtures were vacuum filtered with #42 filter paper and washed with 10 mL of deionized water, 95% ethanol, and acetone. The boiling-stable portion of RS was determined as the residue remaining after drying the samples in a convection oven at 103°C overnight.

Determining Boiling-Stable RS by the Englyst Method

Before RS analysis as determined by Englyst et al (1992), starch samples in a 50-mL centrifuge tube were placed in a boiling water bath for 35 min, and immediately cooled in an ice water bath for 5 min.

Time Course of Starch Digestion

The digestion of native *ae*-VII starch, PAH and ANN *ae*-VII starches, and PAH and HMT *ae*-VII starches were followed at intervals through 4 hr. The extent of the digestion was determined by the Englyst method. Samples were boiled for either 0 or 35 min before digestion. The digestion of corn, wheat, and potato starches, with or without a 35-min boiling treatment, was monitored as well.

RESULTS

DSC Thermal Analysis

The native *ae*-VII starch showed a typical broad endotherm characteristic of HAMS during initial heating (Fig. 1). This broad thermogram has been interpreted as resulting primarily from two overlapping endothermic phase transitions. The enthalpy of the lower portion of the temperature range represents an irreversible transition associated with amylopectin gelatinization. This transition is not observed after cooling and reheating (Fig. 2). Most of the enthalpy of the higher portion of the temperature range represents a reversible transition associated with melting of amylose-lipid complexes. This transition is also observed after cooling and reheating (Fig. 2), and is not observed in lipid-free starches (Biliaderis et al 1985; Sievert and Würsch 1993; Boltz and Thompson 1999). A portion of the endotherm is observed at $>120^\circ\text{C}$ (Fig. 1) and it is not observed on reheating (Fig. 2). This portion may be related to melting of amylose (Klucinec and Thompson 1999).

PAH of *ae*-VII starch for ≤ 30 hr did not produce major changes in the DSC endotherms (Fig. 1). After 78 hr of acid hydrolysis, the low-temperature region appeared to be somewhat more pronounced. Hydrolysis treatment had no effect on the onset temperatures and the transition enthalpies during the initial heating (Table I). However, hydrolysis treatments significantly decreased the enthalpy associated with the amylose-lipid complexes as observed during immediate reheating. These transition enthalpies were lower, at least partially, because of a loss of peak area at $>105^\circ\text{C}$ (Fig. 2).

ANN *ae*-VII starch without PAH influenced the shape of the endotherms that occurred during heating (Fig. 1). With increasing ANN temperature, sharper and narrower transition peaks were observed. The transition enthalpy of the starch annealed at 50°C increased slightly, but the enthalpies of starches annealed at 60 or 70°C were not different from the enthalpy of the nonannealed starch (Table I). In the absence of PAH treatments, ANN *ae*-VII starch did not significantly affect the shape or the transition enthalpies of the endotherms ascribed to amylose-lipid complexes as observed on reheating (Fig. 2 and Table I).

A significant ($\alpha < 0.001$) interaction between hydrolysis time and annealing temperature was observed in the ANOVA of the transition enthalpies obtained either by heating or by reheating of PAH *ae*-VII starches and followed by ANN. PAH followed by ANN affected the onset temperature and the transition enthalpy of *ae*-VII starch not only during heating but also during reheating (Table I). When the treatments combining PAH and ANN are compared at the same level of ANN, it is apparent that longer hydrolysis times reduced transition enthalpies during heating and on reheating.

HMT of *ae*-VII starch without hydrolysis influenced the shape of the endotherm obtained during heating (Fig. 3). Broader and

more symmetrical endotherms extending to higher temperatures were observed for HMT samples. As the HMT temperature increased, the peaks and the onset temperatures shifted to higher temperatures. The gelatinization enthalpies of HMT *ae*-VII starches were similar to those of the untreated starch (Table II). During reheating, the transition enthalpies of all HMT samples were lower than the transition enthalpy of the non-HMT starch (Fig. 4 and Table II).

A significant ($\alpha < 0.001$) interaction between hydrolysis time and HMT temperature was observed in the ANOVA of the transition enthalpies obtained either by heating or by reheating of PAH *ae*-VII starches and followed by HMT. The endotherms of the PAH and HMT samples presented a shape and temperature range similar to the endotherms of corresponding HMT samples alone. In contrast to the almost unchanged enthalpies obtained for the three HMT samples without PAH, the combination of hydrolysis and HMT led to a significant decrease in gelatinization enthalpies relative to samples with the same PAH treatment but without HMT. In general, for combinations of PAH and HMT, higher HMT temperatures resulted in lower gelatinization enthalpies (Table II).

PAH decreased the proportion of high molecular weight to low molecular weight material in *ae*-VII starch, as shown by the size-exclusion chromatograms of dispersed and dried native and hydrolyzed *ae*-VII starches on the Sepharose CL-2B column (Fig. 5).

RS Analysis

Table III shows the RS content of native *ae*-VII starch obtained by the TDF method and the Englyst method. For native *ae*-VII starch, the TDF method determined far less RS ($\approx 18.4\%$) than did the Englyst method ($\approx 78.7\%$). PAH treatments alone did not influence RS content of *ae*-VII starch as detected by the TDF method nor by the Englyst method. As determined by the TDF method for ANN without PAH, only the 70°C treatment altered the RS content of *ae*-VII starch, increasing it to a value of $\approx 28\%$.

In contrast, as determined by the Englyst method, the 50°C ANN treatment led to a significant decrease in RS content. Even greater decreases in RS were observed for the 60 and 70°C ANN treatments, respectively.

A significant ($\alpha < 0.001$) interaction between hydrolysis time and annealing was observed in the ANOVA of RS obtained by the TDF method and the Englyst method. As determined by the TDF method, PAH before ANN at 60 or 70°C of *ae*-VII starch tended to yield more RS than ANN alone. As determined by the Englyst method, PAH before ANN at 70°C of *ae*-VII starch yielded less RS than ANN at 70°C alone (Table III), but no effect was observed for different PAH times for the other ANN treatments.

As detected by the TDF method for the HMT alone, RS content increased ($\leq 53\%$) to a greater extent than for ANN (Tables III and IV). However, as determined by the Englyst method, the HMT at 120 or 140°C produced samples with a lower RS content ($\approx 55\%$), similar to that for ANN at 70°C ($\approx 53\%$).

A significant ($\alpha < 0.001$) interaction between hydrolysis time and HMT was observed in ANOVA of RS obtained by the TDF and Englyst methods. As determined by the TDF method, certain combinations of HMT and PAH produced higher RS values (for several combinations, $\text{RS} \approx 60\%$) than for PAH alone or for HMT alone (Table IV). As determined by the Englyst method, HMT at 100 or 120°C after PAH tended to yield more RS than the HMT alone, while the opposite effect was observed for HMT at 140°C .

Stability of Granular RS to Boiling

Figure 6 shows the time course of starch digestion of corn, wheat, and potato starch by the Englyst method before and after 35 min of boiling in excess water. The data are expressed as the percentage of undigested starch over time. The linear decrease in the percentage of undigested potato starch was in agreement with Englyst et al (1992), as was the value of $\approx 75\%$ RS. Furthermore,

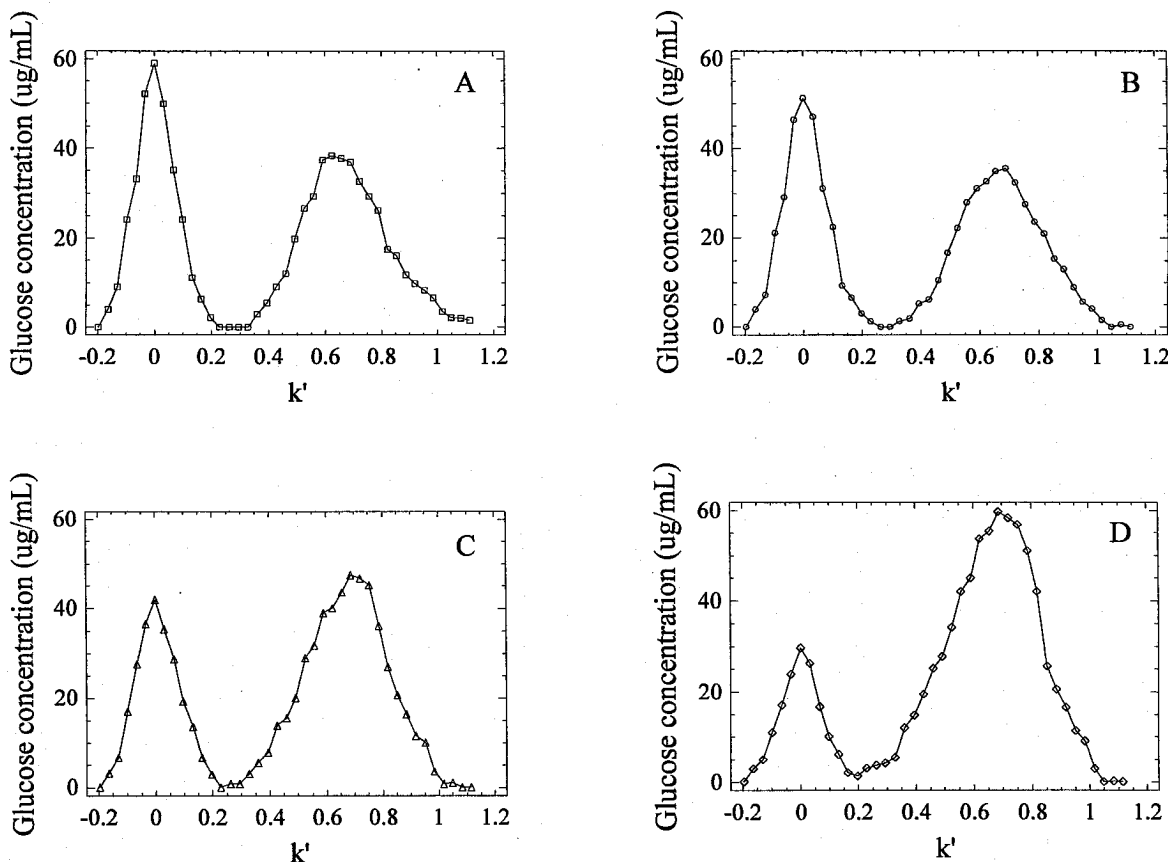


Fig. 5. Chromatograms of dispersed and dried native and partial acid hydrolysis (PAH) *ae*-VII starch. A, native *ae*-VII starch. B, *ae*-VII starch with 6 hr of PAH. C, *ae*-VII starch with 30 hr of PAH. D, *ae*-VII starch with 78 hr of PAH.

there was complete digestion of boiled potato starch after 1 hr of enzymatic hydrolysis. Although boiled wheat and corn starches were rapidly digested well before 2 hr of enzymatic hydrolysis, a considerable amount of undigested raw wheat and corn starches remained after 2 hr of enzymatic hydrolysis (≈ 25 and 35% , respectively).

As determined by the Englyst method, boiling of *ae*-VII starch before analysis reduced the RS content from 78.7 to $\approx 20\%$ (Fig. 7). In neither case was the decrease with time linear, as observed for native potato starch. Total RS for starch annealing at 70°C after 6 hr of PAH was less than for the native *ae*-VII starch. However, after boiling, this combination treatment increased the boiling-stable RS content when compared with the native *ae*-VII starch. Total RS for HMT at 120°C after 30 hr of PAH was also less than that of the

native *ae*-VII starch. After boiling, this PAH and HMT combination produced the highest value for boiling-stable RS.

DISCUSSION

DSC Thermal Analysis

PAH of *ae*-VII starch without a subsequent hydrothermal treatment did not show an important effect on the gelatinization enthalpies (Table I). Mild acid hydrolysis treatments are known to preferentially attack the less organized regions of the granule (Jenkins and Donald 1997); thus, the regions responsible for the enthalpic transition may not have been affected. Klucinec and Thompson (1998) suggested that the enthalpy at $>105^\circ\text{C}$ on an immediate rescan (Fig. 2) could be due to longer amylopectin chains of *ae*-VII starch or to interaction between longer chains of amylopectin and amylose. Hydrolysis treatments appear to preclude these interactions, which otherwise occur on quench-cooling after the initial heating in DSC. Hydrolysis likely occurred in regions between ordered regions in such a way as to retain the ordered structure but to preclude reformation of that structure after heating to 180°C .

ANN with or without PAH led to sharper and narrower peaks with increasing annealing temperature (Fig. 1 and Table I). Although the combination of PAH and ANN led to decreased gelatinization enthalpy, ANN without PAH could be considered true annealing, as no decrease in gelatinization enthalpy was observed, indicating that no partial gelatinization occurred (Stute 1992; Jacobs and Delcour 1998). The decreased enthalpy observed for samples with ANN after PAH suggests that PAH may make the *ae*-VII starch granules more susceptible to gelatinization.

HMT with or without PAH led to considerable loss of enthalpy for the low-temperature region and enhanced enthalpy at $>100^\circ\text{C}$, resulting in lower and broader endotherms. Although HMT alone had little effect on the gelatinization enthalpy, the combination of PAH and HMT led to a decrease in the gelatinization enthalpy. For combination treatments, the effect of HMT temperature was apparently more important than the time of PAH (Table III). Combination treatments also led to the greatest decreases in enthalpy observed on immediate reheating. The loss of some gelatinization enthalpy reveals that some melting had occurred during HMT (Stute 1992).

Several authors have subjected HAMS to treatments above the gelatinization temperature in excess water to enhance formation of type III RS (Sievert and Pomeranz 1990; Sievert et al 1991; Pomeranz 1992; Gruchala and Pomeranz 1993; Escarpa et al 1996). These authors related the formation of type III RS, as determined by the TDF method, to the high-temperature endotherm ($\approx 150^\circ\text{C}$) observed. Thermal characteristics of retrograded *ae*-VII starch isolated by the TDF method (Sievert and Pomeranz 1989, 1990; Eerlingen and Delcour 1995) and of ANN and HMT *ae*-VII starches studied in this work were fundamentally different in that no high-temperature endotherm at $\approx 150^\circ\text{C}$ was observed here. To the extent that these different thermal characteristics are both in some way related to enzyme resistance, the nature of enzyme-resistant structures in the two cases would likely be different as well.

Starch Digestion

The digestion of raw potato starch was followed over time using the digestion procedure of the Englyst method. Both raw and boiled raw potato starches were used, as positive and negative controls. The high percentage of undigested raw potato starch and the complete digestion of boiled potato starch after 120 min of α -amylase hydrolysis agreed with the results obtained by Englyst et al (1992) (Fig. 6), confirming that the method was correctly employed. Just as for the boiled potato starch, boiled corn and wheat starches were completely digested after 2 hr of amylolysis. However, the native corn and wheat starches remained 30–40% undigested, indicating a substantial amount of type II RS in these starches. Although the literature abounds with descriptions of raw potato or banana starches as good sources of type II RS, there is little information in the literature addressing the question of type II RS of corn and wheat.

TABLE III
Resistant Starch (RS) Content from Two Methods for *ae*-VII Starch Annealed (ANN) After Partial Acid Hydrolysis (PAH)

PAH (hr)	ANN ($^\circ\text{C}$) ^a	RS% ^{b-d}	
		Boiling-Stable RS ^e	Total RS ^f
0	NT ^g	18.4 \pm 0.4a	78.7 \pm 0.7e
0	50	17.2 \pm 1.0a	74.6 \pm 0.6d
0	60	17.5 \pm 0.5a	65.8 \pm 1.2c
0	70	28.1 \pm 0.6c	52.6 \pm 3.0b
6	NT	17.2 \pm 1.3a	74.2 \pm 1.8e
6	50	18.5 \pm 0.5a	74.0 \pm 1.0d,e
6	60	22.3 \pm 1.5b	64.8 \pm 1.9c
6	70	32.7 \pm 0.6d	46.6 \pm 2.1a
30	NT	17.5 \pm 1.5a	77.8 \pm 0.7e
30	50	21.0 \pm 1.1b	72.5 \pm 0.8d
30	60	22.7 \pm 1.1b	65.0 \pm 1.6c
30	70	30.6 \pm 1.2d	47.0 \pm 2.6a
78	NT	16.5 \pm 0.7a	79.2 \pm 0.2e
78	50	17.6 \pm 1.0a	72.6 \pm 1.1d
78	60	23.2 \pm 0.9b	64.0 \pm 0.9c
78	70	27.3 \pm 1.7c	42.8 \pm 1.8a

^a Annealed at 30% solids for 24 hr at temperatures indicated.

^b % of dry matter.

^c Mean \pm standard deviation of RS determinations for four to five replicates.

^d Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^e Detected as TDF according to AOAC (1985).

^f Detected according to Englyst et al (1992).

^g Not treated by annealing.

TABLE IV
Resistant Starch (RS) Content from Two Methods for *ae*-VII Starch Heat-Moisture-Treated (HMT) After Partial Acid Hydrolysis (PAH)

PAH (hr)	HMT ($^\circ\text{C}$) ^a	RS% ^{b-d}	
		Boiling-Stable RS ^e	Total RS ^f
0	NT ^g	18.4 \pm 0.4a	78.7 \pm 0.7g
0	100	31.0 \pm 0.6b	66.8 \pm 0.1e
0	120	43.9 \pm 0.3c	55.9 \pm 1.4c
0	140	52.7 \pm 0.6d	55.3 \pm 1.9c
6	NT	17.2 \pm 1.3a	74.2 \pm 1.8g
6	100	37.8 \pm 0.4c	70.3 \pm 1.0e
6	120	57.7 \pm 0.7f	59.2 \pm 2.1d
6	140	48.8 \pm 0.5d	44.1 \pm 1.6a
30	NT	17.5 \pm 1.6a	77.8 \pm 0.7g
30	100	41.2 \pm 1.1c	73.7 \pm 1.7f
30	120	60.7 \pm 1.2f	66.0 \pm 1.4c
30	140	59.6 \pm 2.2f	48.7 \pm 1.1b
78	NT	16.5 \pm 0.7a	79.2 \pm 0.2g
78	100	57.3 \pm 1.2f	78.1 \pm 0.8g
78	120	63.2 \pm 1.4g	60.6 \pm 0.6d
78	140	55.2 \pm 2.2e	48.2 \pm 1.1b

^a HMT at 70% solids for 80 min at the temperatures indicated.

^b % of dry matter.

^c Mean \pm standard deviation of RS determinations for four to five replicates.

^d Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^e Detected as TDF according to AOAC (1985).

^f Detected according to Englyst et al (1992).

^g Not treated by heat-moisture treatment.

It would appear that while raw corn and raw wheat starches are better digested than raw potato starch, they both contain important amounts of type II RS. It is clear that boiling eliminates the type II RS for potato, wheat, or corn starch.

RS Analysis

One of the problems in evaluating hydrothermal treatments as a means of producing RS is that analytical procedures for determination of RS often differ among laboratories. Because different analytical procedures may involve variable thermal treatments in the analyses, the physical changes produced by hydrothermal treatments may not be equally detected among methods.

Many investigators assume that since boiling eliminates the enzyme resistance of potato and banana starches, two common sources of type II RS, a boiling treatment will eliminate type II RS in general, and thus a method including boiling is commonly used to quantify type III RS exclusive of type II RS. However, it is clear that for HAMS a portion of the native starch remains resistant after boiling (Berry 1986). When the Englyst method is applied to native HAMS, a relatively high level of RS is observed (Fig. 7), and this material is by definition type II RS. When the TDF method is applied to native HAMS, the starch recovered may represent type II RS, but the amount recovered is much less. We assume that the difference is primarily due to the boiling treatment, since work with the Englyst method measured after boiling also gives decreased values for RS. Thus we conclude that only a portion of type II RS from HAMS is stable to the boiling treatment. We call this material boiling-stable granular RS rather than boiling-stable type II RS because we cannot preclude the possibility that some intragranular retrogradation occurred. In the present work, we show how the proportion of boiling-stable granular RS may be manipulated. When this proportion is increased, the RS in the starch ingredient has an improved ability to survive a thermal treatment commonly encountered in food processing.

Annealing of *ae*-VII starch at 70°C increased the content of the boiling-stable granular RS, as determined by the TDF method

(Table III). Even more boiling-stable granular RS was obtained when certain ANN treatments followed PAH of *ae*-VII starch. The Englyst method does not determine boiling-stable granular RS because the enzyme hydrolysis conditions are conducted at 37°C. However, if the starch is boiled immediately before the pancreatic hydrolysis, the Englyst method can be used to estimate RS that survives boiling. When native *ae*-VII starch is analyzed by the Englyst method, the result ($\approx 79\%$ RS) includes not only a boiling-stable component ($\approx 22\%$) but also a nonboiling-stable component ($\approx 57\%$) (Fig. 7).

As determined by the Englyst method without boiling, total RS decreased as the annealing temperature increased. RS decreased even more when ANN treatments were applied after PAH (Table III). This behavior is in contrast to changes in boiling-stable RS as determined by the TDF method. However, when the total RS content of 70°C ANN treatment after 6 hr of PAH was determined by the Englyst method after boiling ($\approx 22\%$, Fig. 7) better agreement with the boiling-stable RS, as determined by the TDF method ($\approx 33\%$, Table III) was obtained. HMT at 120°C after PAH for 30 hr also increased the boiling-stable type II RS content ($\approx 43\%$, Fig. 7), even as total RS decreased ($\approx 66\%$).

The increases in the boiling-stable granular RS content were observed despite the gelatinization enthalpy remaining constant after ANN. This observation may appear to be at variance with the idea that enzyme resistance is due to the formation of crystalline material (Eerlingen et al 1993; Eerlingen and Delcour 1995) or to the formation of more double helices (Gidley et al 1995). This apparent paradox can be resolved if the material normally able to gelatinize at boiling temperatures became more thermally stable due to ANN or HMT. As noted above, most of the gelatinization enthalpy of native *ae*-VII starch occurs below the boiling temperature. Figure 1 shows that ANN treatments shifted the gelatinization enthalpy to higher temperature ranges. Figure 3 shows that HMT shifted the gelatinization enthalpy to even higher temperature ranges. Thus, the amount of boiling-stable granular RS may be related to the increased enthalpy at >95 – 100°C in DSC endotherms. In this way, these reordered structures may have equal or less total enthalpy than native starches,

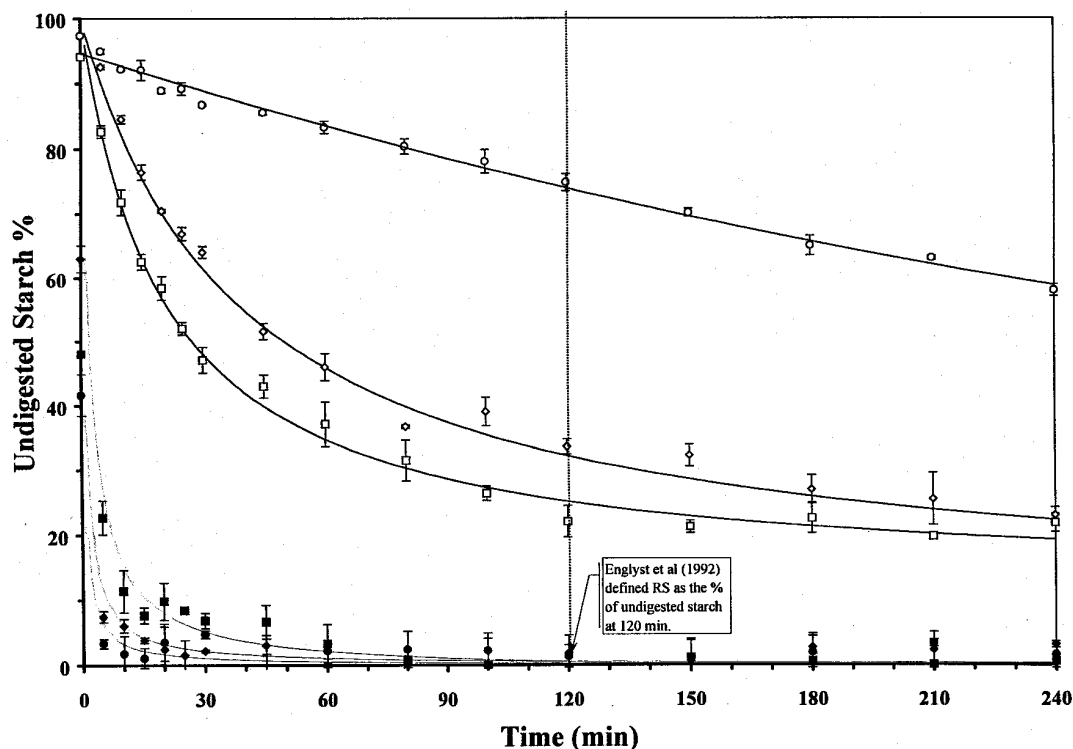


Fig. 6. Digestion of corn, wheat, and potato starch (\blacklozenge , \blacksquare , \bullet) determined by the Englyst method before (open symbols) and after (filled symbols) a boiling step. Error bars represent standard deviations. Lines are provided to guide the eye, with no attempt to imply a mechanism. Lines obtained by linear or nonlinear regression of experimental data.

and yet a greater proportion of the enthalpy >100°C, accounting for the increased proportion of boiling-stable granular RS.

The DSC results suggest that some of the starch responsible for the enzyme resistance evaluated at 37°C (Englyst et al 1992) may be related to the enthalpy component located at temperatures below boiling because ANN and HMT reduced the proportion of the overall enthalpy observed at <95–100°C as well as the RS content determined by the Englyst method.

Both ANN and HMT may be considered processes that result in more perfect structures. Figures 1 and 3 show how the area of the DSC thermograms tends to be located at higher temperatures for either treatment. By definition, annealing should not reduce the gelatinization enthalpy. However, there is no restriction that HMT retain the initial enthalpy (Stute 1992; Jacobs and Delcour 1998), even though that was the case for the HMT without prior PAH.

The decrease of the molecular weight caused by PAH would allow a greater freedom of polymer motion. For this reason, we anticipated an enhanced ability to form more stable structures on subsequent hydrothermal treatments. DSC evidence suggests that the effect of PAH on enthalpy after ANN was about the same as the effect of PAH on HMT. By the Englyst method, the additional effect of PAH on ANN was about the same as for HMT. However, by the TDF method, the highest value for a combination of PAH and HMT (≈63%) was about double that for the highest combination of PAH and ANN (≈33%). This outcome might be surprising because the total enthalpy is similar for PAH–ANN and PAH–HMT combinations. Our best explanation is that certain PAH–HMT combinations caused greater proportions of structures stable to treatment at 100°C in excess water. Thus, these combinations generated a higher proportion of boiling-stable granular RS from *ae*-VII starch.

A few combinations of PAH–HMT led to higher values of boiling-stable RS by the TDF method than for total RS by the Englyst method (Table IV). We did not compare these two measures statistically because the determination of boiling-stable RS and total RS were by fundamentally different methods. When methodology

was more directly comparable (see Fig. 7 for analysis of the 30 hr PAH and 120°C HMT combination), the proportion of RS after a boiling treatment was lower (≈40%) than when using the TDF method (Table IV) to determine boiling-stable RS. Thus, the apparent incongruity seems related to the differences in methodology for the TDF and the Englyst methods.

Gidley (1995) has pointed out that type III RS isolated at room temperatures includes crystalline and helical material along with considerable noncrystalline material protected by being in an amorphous network linking the crystalline regions. Although the resistance of type II RS is probably not due to an identical relationship between amorphous and crystalline regions as observed for type III RS, it may be due to a similar structural theme. It is interesting that PAH alone has little effect on either total RS or boiling-stable RS of *ae*-VII.

PAH would preferentially attack the amorphous portions of the granule, providing potential freedom for chain ends to form double helices and for double helices to associate. The hydrothermal treatments would allow the potential mobility of the chains to be realized and form more highly ordered structures. HMT appears to be better suited than ANN for increasing the boiling-stable granular RS from PAH *ae*-VII starch.

CONCLUSIONS

RS values determined by the TDF method (AOAC 1985) and the Englyst method (Englyst et al 1992) may be influenced much differently by a particular processing treatment. These values likely result from different features responsible for type II RS. Care must be taken when RS production processes are compared using different analytical methods. For determination of type II RS, we suggest the determination of boiling-stable RS in addition to total RS. HMT may produce a reduction in total RS similar to ANN, but it is more effective than ANN for generating boiling-stable granular RS. The yield of boiling-stable granular RS by HMT is increased even more after some PAH treatments.

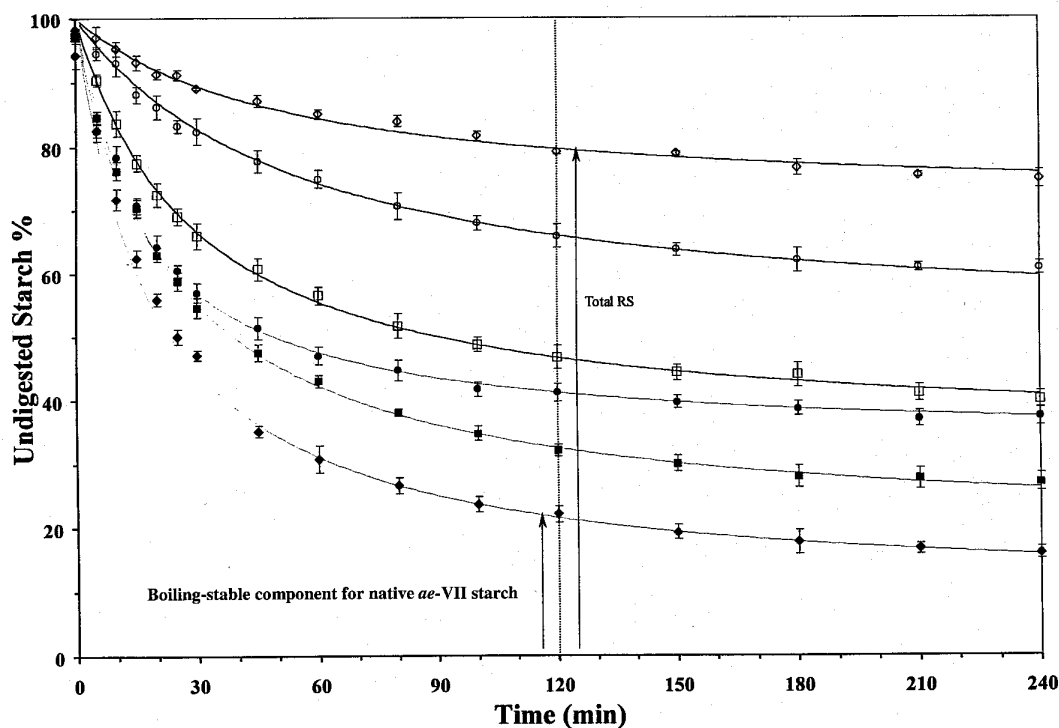


Fig. 7. Digestion of *ae*-VII starch (◆), *ae*-VII starch hydrolyzed 6 hr and annealed at 70°C (■), and *ae*-VII starch hydrolyzed 30 hr and heat-moisture treated at 120°C (●) determined by the Englyst method before (open symbols) and after (filled symbols) a boiling step. Error bars represent standard deviations. Lines are provided to guide the eye, with no attempt to imply a mechanism. Lines obtained by linear or nonlinear regression of experimental data. Arrows indicate total resistant starch (RS) and boiling-stable component for native *ae*-VII starch.

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