Enzyme-Resistant Starch: Studies Using Differential Scanning Calorimetry

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ABSTRACT

Factors governing the formation of enzyme-resistant starch (RS) from high-amylose maize starch under laboratory conditions were investigated. Those factors studied included sample size, gel dimensions, number of heating-cooling cycles, and cooling conditions. Cooling to about 20°C produced RS with higher enthalpies than cooling to 4°C or freezing at about −20°C. Freeze-drying was not superior to oven-drying. Heating of resuspended RS increased enthalpies. RS could be stored at −20°C odor-free for at least eight months. A rancid odor developed within several weeks of storage at room temperature . Thermal characteristics of RS, as measured by differential scanning calorimetry (DSC), were sample-size-dependent. To determine the stability of RS, samples were preheated under conditions similar to those in baking bread: temperatures of 100 and 200°C, both dry and with water. Preheating RS, especially with water, at 200°C resulted in a disintegration of the RS. No significant changes in DSC characteristics took place during preheating at 100°C. The presence of gluten or wheat starch in RS blends (as they might exist in bread) reduced measurable DSC enthalpies.

Resistant starch (RS), fundamentally an α-glucan, is a product of starch retrogradation. Due to its specific physical properties, crystallized amylose, the main component of RS, is resistant to α-amylolysis (Berry 1986; Englyst and MacFarlane 1986; Ring et al 1988; Russell et al 1989; Sievert and Pomeranz 1989, 1990; Siljeström et al 1989). RS is not absorbed in the small intestine; it reaches the large intestine together with the nonstarch polysaccharides and lignin (Rexen 1990). There is presently considerable interest in the nutritional implications of RS in foods. The relatively slow rate of hydrolysis in the gastrointestinal tract of humans is associated with low-glycemic responses and may have physiological effects similar to those of dietary fiber (Biliaderis 1991). However, the precise and meaningful implications (from physiological and analytical standpoints) of RS content cannot be determined until detailed studies of the chemical and physiological properties of RS are completed. The methodology of RS determination should also be improved. At present, the quantitative assay of RS in food is based on the isolation of total dietary fiber using a gravimetric-enzymatic method (Prosky et al 1988). The isolated RS, after treatment with dimethyl sulfoxide or 2*N* KOH, which changes the RS structure and makes it available to amylolysis, can be determined by several methods for specific carbohydrates (Englyst et al 1982, Berry 1986). However, these methods do not take into account the crystallinity level of RS, which is an important factor in its digestibility. Differential scanning calorimetry (DSC) gives an endothermic transition of ~155°C for retrograded and recrystallized amylose (Biliaderis et al 1985; Sievert and Pomeranz 1989, 1990; Szczodrak and Pomeranz 1991).

The objective of this study was to determine factors affecting the analysis of RS from amylomaize VII (a high-amylose maize starch).

This study had four parts: 1) the process for preparing RS from amylomaize VII starch; 2) RS-water interactions (various amounts and various ratios); 3) thermostability of RS and effect of heat pretreatment (in the presence or absence of water) on thermoanalytical characteristics of RS as determined by DSC; and 4) the effect of gluten and wheat starch on the thermoanalytical characteristics of RS.

MATERIALS AND METHODS

Amylomaize VII

Commercially available starch, amylomaize VII, was obtained from American Maize Products Co. (Hammond, IN). Amylomaize VII contained 55.9% amylose, 1.4% protein, 0.12% ash, and 1.7% free lipids on a moisture-free basis.

Gluten

Commercial dry gluten was obtained from Kröner Stärke Co., Ibbenbueren, Germany. The gluten contained 77.2% protein and 1.30% ash on a 14% moisture basis.

Wheat Starch

Commercial dry wheat starch was obtained from Centennial Mills Co. (Spokane, WA). The wheat starch contained 0.4% protein and 0.24% ash on a 14% moisture basis.

Chemical Analyses

Protein was determined by Kjeldahl nitrogen (N × 5.7) (AACC Method 46-11A); ash by dry combustion (AACC Method 08-01); and free lipids by exhaustive extraction with petroleum ether, followed by evaporation to constant weight under vacuum (AACC Method 30-25) (AACC 1983). RS was determined as described for dietary fiber (Prosky et al 1988) by using three enzymes: heat-stable α-amylase (Takalite L-340 from *Bacillus licheniformis*, Miles Laboratories, Inc., Elkhart, IN); amyloglucosidase (A-3042 from *Aspergillus niger*, Sigma Chemical Co., St. Louis, MO); and protease (P-5380 type VIII from *B. licheniformis*, Sigma Chemical Co.). Quantification of amylose (as percent of starch) was done according to Hovenkemp-Hermelink et al (1988). All analyses were done at least in duplicate. Average results of RS analyses are given on a dry matter basis.

DSC

The DSC thermograms were recorded with a Perkin-Elmer DSC-4 instrument fitted with a 3,600 thermal analysis data station and a Perkin-Elmer graphics plotter 2 (Perkin-Elmer Corp. Instrument Div., Norwalk, CT). An indium standard was used for temperature and energy calibration. Samples (10 mg dmb) were weighed into large-volume stainless steel capsules (Perkin-Elmer no. 0319-0218). Then 20 μl of distilled water was added and the capsules were sealed by a quick press and allowed to equilibrate for 2 hr at ambient temperature before they were analyzed by DSC.

The samples were heated from 20 to 180°C at a scanning rate of 10°C/min. For each endotherm, initial (T_i), peak (T_p), and completion (T_c) transition temperatures were determined by a computerized system developed by the Perkin-Elmer Corp. The transition enthalpy (ΔH) was calculated from the peak area and expressed as joules per gram of dry matter. When the samples contained a mixture of RS and other components (wheat starch or gluten), the results were recalculated and reported as RS in joules per gram.

To study the interactions of RS with water, 10.0-mg samples of RS were tested with distilled water in 10.0-, 15.0-, 20.0-, 30.0-, and 40.0-μl volumes. Capsules with 10.0 mg of Al_2O_3 and appropriate amounts of water were used as reference samples.

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DSC studies were made in two series. First, samples of 20.0, 10.0, 7.5, and 5.0 mg were tested with RS-to-water ratios of 1:2. Second, 10.0-mg samples containing 5.0, 2.5, 1.0, and 0.5 mg of RS and a balance of Al_2O_3 were analyzed with 20.0 μl of water. References for the first series were equivalent weights of Al_2O_3 and volumes of water. For the second series, the uniform reference sample was used: 10.0 mg of Al_2O_3 and 20.0 μl of water.

Preparation of RS Samples for Estimating Thermostability

This part of the study involved preheating samples at 100 and 200°C, both with and without water, under conditions that resemble the production of the crumb and crust of baked dough.

Heat Treatment of RS in the Absence of Water

One set of 3-g RS samples was placed in crucibles and heated at 100°C in the oven (Thelco model 18, Precision Scientific Co., Chicago, IL) for 1, 16, 24, and 48 hr. Another set of 3-g RS samples was heated at 200°C in a Sybron furnace (Thermolyne Co., Dubuque, IA) for 15, 30, 60, and 120 min. The heated samples were kept sealed in jars until analyzed.

Heat Treatment of RS in the Presence of Water

Samples of 100.0 or 200.0 mg of RS were weighed into PYREX 5-ml test tubes, supplemented with water, and sealed. To obtain a 1:2 ratio of RS to water, 200 μl of water was added to the 100.0-mg samples. The 200.0-mg RS samples were mixed with water in 1:1 and 5:2 ratios. One set of samples was placed in the oven at 100°C for 1 hr and one at 100°C for 12 hr. Another set was heated in the furnace at 200°C for 30 min. Immediately after heating, the samples were freeze-dried and kept closed in jars until analyzed.

Preparation and Heat Treatment of RS Samples with Wheat Starch and Gluten

The RS was tested for thermal characteristics in the presence of wheat starch and gluten, in blends that might exist in bread. The ratios of RS to gluten or wheat starch were 1:9, 1:3, 1:1, 3:1, and 9:1. In addition, RS was mixed with both gluten and wheat starch. The ratio for the three-component (RS, gluten, and wheat starch) blend was 4:2:3. The dry mixture was analyzed by DSC immediately after heating at 100 or 200°C for 25 min. In a second experiment, water was added to the RS-gluten-wheat starch blend in a 3:1 ratio of dry material to water. The wet blends were sealed in PYREX test tubes and heated at 100 or 200°C for 25 min. After heat treatment, the samples (total 10 mg dry matter plus 20 μl of water) were cooled, freeze-dried, and kept closed in jars at room temperature until analyzed by DSC. Differences of 1°C in transition temperatures and 10% in transition enthalpies were significant at the 5% level.

RESULTS AND DISCUSSION

Amylomaize RS Production

The process for preparing RS from amylomaize VII starch, as depicted in Figure 1, was based on the procedure of Sievert and Pomeranz (1989). Several modifications were tested and introduced.

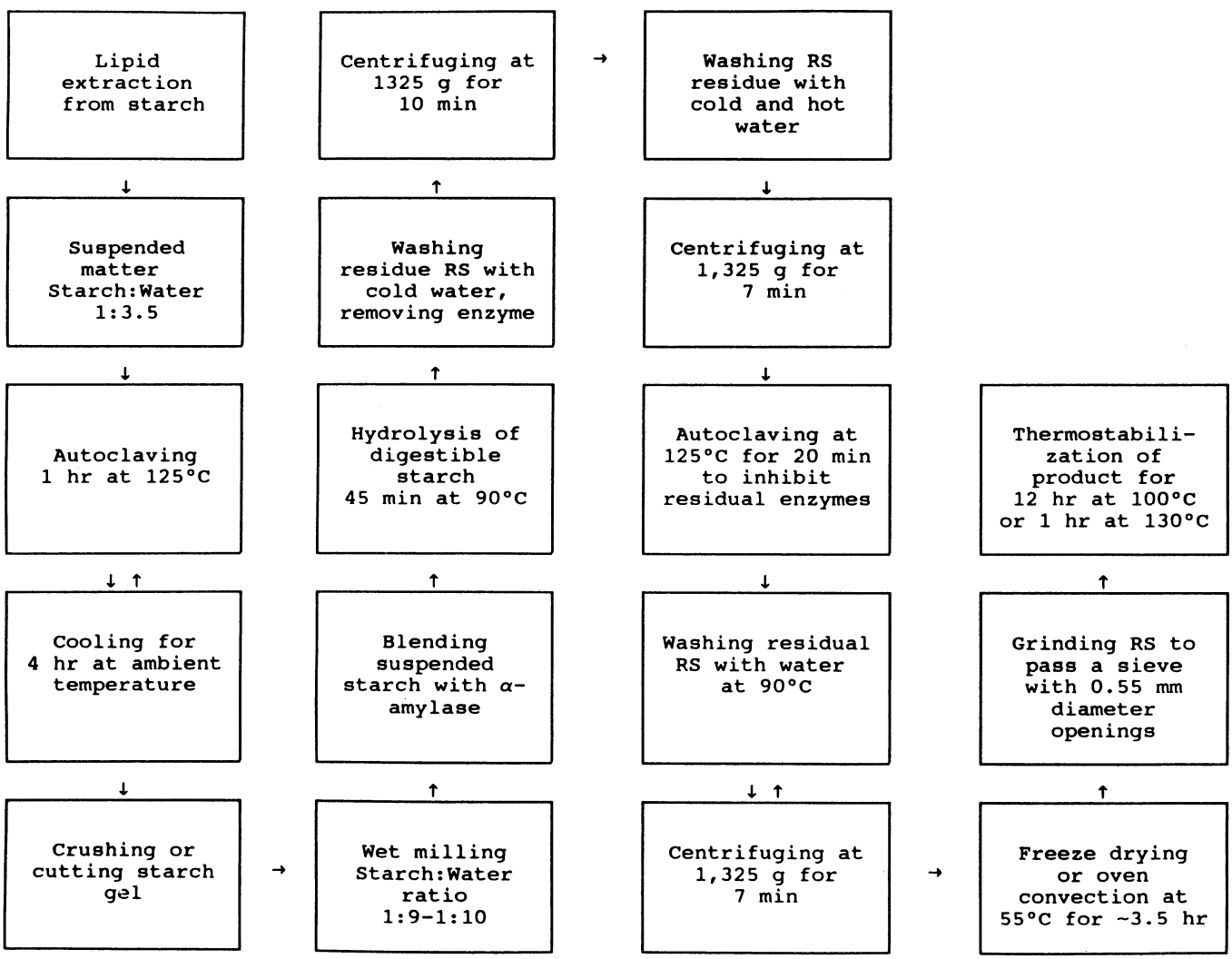

Fig. 1. Process for preparing enzyme-resistant starch (RS) from amylomaize VII starch.

Formation of RS

The RS is formed during retrogradation or crystallization of amylose. A maximum yield of high-enthalpy RS is obtained by heat treatment (autoclaving) of a high-amylose starch in the presence of water and subsequent cooling. Highest yields and enthalpies were obtained by autoclaving for 1 hr at 125°C with a starch-to-water ratio of 1:3.5. Up to four autoclaving-cooling cycles of 100- and 200-g amylomaize samples were used to determine the RS yield and its thermal transition characteristics.

The results are summarized in Table I. The lowest yield and thermal enthalpy were registered for RS isolated from amylomaize VII after one autoclaving-cooling cycle. Additional autoclaving-cooling cycles increased the RS yield but did not fundamentally change its transition characteristics. In all subsequent work, three cycles were used for RS formation.

The transfer of heat under pressure into the starch-water mixture during autoclaving was important. Yields and thermal characteristics of RS produced in containers of various shapes and sizes are listed in Table II. The isolated RS from a gel prepared from 400 g of amylomaize had a mean transition enthalpy of 43 J/g. There was, however, an enthalpy gradient from ~50 J/g in the external layer to ~37 J/g in the central part.

The geometrical form, primarily the total volume and the surface area under pressure, can affect the thermal characteristics of RS, as shown in Table II for RS made from 600-g amylomaize VII samples. One sample was produced in a cylinder with internal diameter of 160 mm, height of 140 mm, and exposed surface area of 200.9 cm^2. A second cylinder had a diameter of 220 mm, height of 74 mm, and a surface area of 379.9 cm^2. Transition enthalpies of RS were 26 J/g in the first cylinder and 43 J/g in the second cylinder.

TABLE I
Effect of Autoclaving-Cooling Cycles used for Treatment of Amylomaize VII Starch on Yield and Differential Scanning Calorimetry Characteristics of Isolated Enzyme-Resistant Starch (RS)

Number of Autoclaving-Cooling Cycles	RS Yield (%)	Transition Temperatures[a,b] (T, °C) and Transition Enthalpies[c] (ΔH, J/g)			
		T_i	T_p	T_c	ΔH
100 g of Amylomaize					
1	20.7	107.1	151.9	169.8	36.5
2	25.4	115.3	154.2	169.7	39.7
3	30.0	112.1	154.4	169.7	40.8
4	31.7	110.2	154.8	168.5	42.1
200 g of Amylomaize					
1	21.9	104.6	152.4	170.4	33.4
2	26.4	116.8	152.8	168.4	40.4
3	28.8	106.4	153.4	169.5	40.7
4	31.4	112.2	154.2	167.7	40.5

[a] T_i, T_p, T_c = initial, peak, and completion transition temperatures, respectively.
[b] SD < 1.0°C; $n = 3$.
[c] SD < 10% of the mean; $n = 3$.

Crystallinity of RS is governed by cooling between successive autoclaving cycles. Originally, autoclaved samples were allowed to cool at ambient temperature about 1 hr and stored overnight in the refrigerator at 4°C. The effect of gel-cooling conditions on RS transition enthalpy is summarized in Table III. Some samples were frozen (at about −20°C) between autoclaving cycles. The cooling-freezing regime did not consistently affect the RS transition temperatures or enthalpy. No changes in thermal characteristics of RS resulted from 4-hr cooling periods between successive autoclaving cycles. Each 4-hr cycle included 1 hr at 20°C (ambient temperature) and 3 hr at 4°C. A 25% increase in enthalpy to ~50 J/g was registered for RS isolated from a gel kept for 4 hr between autoclaving cycles at 20°C, rather than at 4°C. This is in accord with the classical polymer crystallization theory of Wunderlich (Biliaderis 1991) that predicts a growth of less-perfect crystals as the degree of supercooling ($T_m - T$) increases, where T_m = lower melting temperature and T = cooling temperature. The sample stored at ambient temperature after autoclaving had a higher enthalpy than the sample stored in a refrigerator (49.7 J/g vs. 40.9 J/g [Fig. 2]). The sample stored at ambient temperature showed enthalpy peaks, which were absent or difficult to discern in the sample stored in low temperature. Those differences in shape and magnitude of the enthalpy peaks were consistent during repeated testing for at least four months. Gels kept at room and refrigerator temperature were smooth; frozen gels were crumbly.

Isolation of RS

Before enzymatic treatment, the starch gels were cut into pieces up to 1 cm^3 in size and were subjected to a two-step wet-milling process. The first step used a Hamilton Beach blender and the second step used a Polytron stirrer (Brinkman Instruments, Westbury, NY). Omitting the second step did not affect the RS yield or thermal characteristics. The comminuted suspensions were treated with one part thermostable α-amylase per 20 parts amylomaize at 90°C for 45 min (Fig. 1). During the enzymatic digestion, the samples were mixed by a magnetic stirrer. After hydrolysis, the suspension was diluted with 1,000 ml of distilled water at 18°C and centrifuged at 1,325 × g for 10 min. The insoluble residue (crude RS) was washed twice with 2,000 ml of water at 18°C and twice with 2,000 ml of hot water at 90°C. The insoluble RS was suspended in 750 ml of hot water at 90°C and autoclaved for 20 min at 125°C to inactivate residual α-amylase. To remove digested, nonresistant starch and other by-products of the enzymatic-digestion process, four washings with 2,000 ml of hot water at 90°C were performed. Between washings, RS was centrifuged at 1,325 × g for 10 min.

Autoclaving is important for improving RS crystallinity. Additional autoclaving of freeze-dried, isolated RS suspended in four volumes of water improved its crystallinity as indicated by an increased enthalpy (about 10%) and increased initial transition temperature.

TABLE II
Effect of Gel Dimensions on Yield and Transition Enthalpy of Enzyme-Resistant Starch (RS) Isolated from Amylomaize VII

Amylomaize VII Weight (g)	Gel Dimensions				RS Yield (%)	Transition Enthalpy (ΔH, J/g)[a]
	Diameter (mm)	Height (mm)	Surface (cm^2)	Volume (cm^3)		
100	100	60	78.5	4,700	30	40.8
200	160	47	200.9	9,400	31	36.4
200	120	83	130.0	9,400	28	40.7
400	160	94	200.9	18,800	25	43
600	160	140	200.9	28,200	16	External part 50 Central part 37 26
600	220	74	379.9	28,200	28	External part 28 Central part 24 43

[a] SD < 10% of the mean; $n = 3$.

Drying RS

The purified RS was either freeze-dried or oven-dried at 55°C. Freeze drying required three days; conventional drying required 3–5 hr. The dried material was ground to pass a screen with 0.5-mm round openings.

There were no significant differences in transition temperatures and enthalpies of the samples dried by these two methods; the enthalpies were 40.9 and 39.2 J/g for the freeze-dried and oven-dried samples, respectively. The freeze-dried sample was, however, fluffier and had a lower packing density (40 g/100 cm^3) than the oven-dried sample (76 g/100 cm^3) when used to fill a graduated volumetric cylinder. The RS from amylomaize VII contained 5.7% moisture, 0.15% ash, 2.2% protein, 2.3% free lipids, and 69.0% insoluble dietary fiber.

TABLE III
Effect of Gel-Cooling Conditions on Thermal Characteristics of Enzyme-Resistant Starch (RS) Isolated from Heat-Treated Amylomaize VII in the Presence of Water

Cooling Conditions		Transition Temperatures[a] (T,°C)[b] and Transition Enthalpies (ΔH, J/g)[c]			
Temperature (°C)	Time (hr)	T_i	T_p	T_c	ΔH
20	1	101.8	154.4	170.8	41.3
4	16				
20	1	114.6	153.8	169.7	39.8
−20	16				
20	1	110.3	154.7	167.8	37.8
4	3				
20	4	111.3	155.6	169.9	50.8

[a] T_i, T_p, T_c = initial, peak, and completion transition temperatures, respectively.
[b] SD < 1.0°C; $n = 3$.
[c] SD < 10% of the mean; $n = 3$.

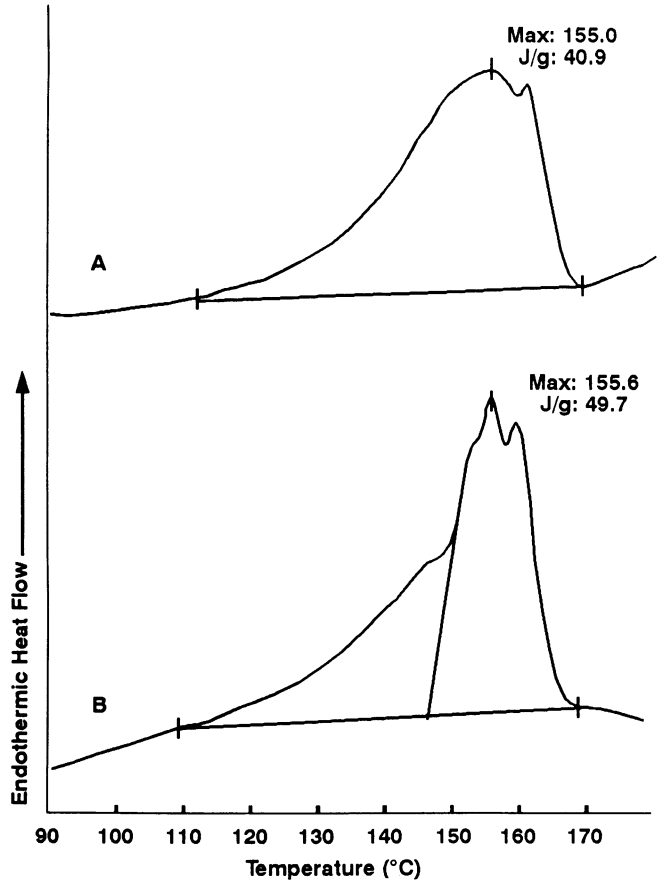

Fig. 2. Differential scanning calorimetry thermograms of enzyme-resistant starch isolated from a gel cooled in a refrigerator at 4°C (**A**) and from a gel cooled at ambient temperature (**B**).

Off-flavor of RS

Freshly produced RS had the flavor of a sound flour. It developed a rancid odor within a few weeks, which is typical of lipid changes and decomposition. Autoclaved and cooled amylomaize VII (without isolation of RS by α-amylase hydrolysis) and freeze-dried samples also had a rancid odor when stored several weeks at ambient temperature. Thus, apparently, lipids in amylomaize VII were responsible for the off-flavor in RS kept at room temperature. RS stored at −20°C had no off-flavor for at least eight months. RS prepared from partially defatted amylomaize VII (by petroleum ether or supercritical CO_2) had a higher enthalpy (increase from 44 to 48 J/g) and was free of rancid odor longer (for at least seven months) than RS produced from nondefatted amylomaize. RS from defatted amylomaize VII contained less than 1% free lipids, whereas RS produced from nondefatted amylomaize VII contained 2.3% free lipids. RS from ethanol- or acetone-extracted amylomaize VII retained the solvent odor, which was practically impossible to eliminate at room temperature. Thermal oxidation of lipids through air-convection heating of RS at 100°C for 12 hr or at 130°C for 1 hr eliminated, or at least reduced, the off-flavor of RS stored for up to four months. Isolation and identification of the off-flavor compounds is being investigated (L. Gruchala and Y. Pomeranz *unpublished results*).

DSC of RS with Various Amounts of Water

Thermal characteristics of RS analyzed with different volumes of water are presented in Figure 3. The 10.0-mg samples were

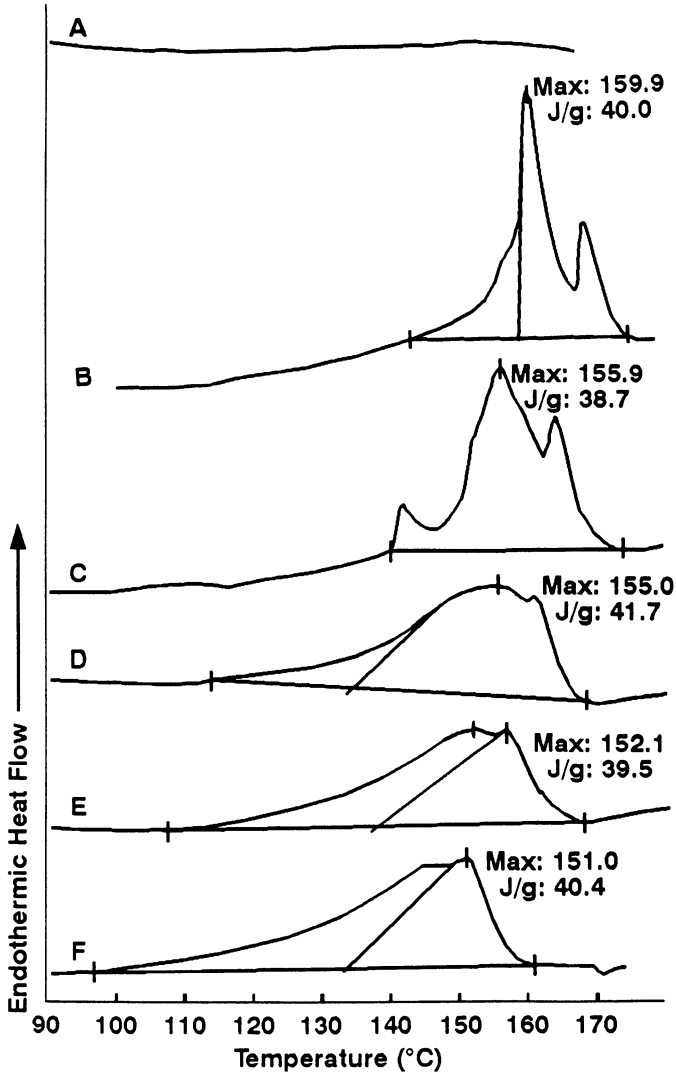

Fig. 3. Differential scanning calorimetry thermograms of 10-mg enzyme-resistant starch interacted with water. **A**, 0.0 μl; **B**, 10 μl; **C**, 15 μl; **D**, 20 μl; **E**, 30 μl; and **F**, 40 μl.

scanned with water-to-RS ratios of 0:1, 1:1, 1:1.5, 2:1, 3:1, and 4:1. Thermal transitions were recorded only for RS samples that interacted with water. The nature of the transition varied with the volume of water added to the 10-mg RS samples. Transitions for the samples with low RS-to-water ratios (1:1 and 1:1.5) were composed of several contiguous peaks resulting from the contribution of a number of components in the RS. The transition temperatures T_i, T_p, and T_c were inconsistent and not reproducible and varied for each analyzed sample. The results suggest that RS is composed of variable forms due to intermolecular forces among α-glucan chains that are characterized by miscellaneous affinities to water. Czuchajowska et al (1991) suggested that RS comprises crystalline regions and less-ordered domains, the proportions of which depend on the heat treatment of the starch sample. Indeed, RS might be composed of the parallel-stranded double helices with different tertiary organizations. Also, the poor B-type pattern shown for the RS fraction by the X-ray diffraction method (Russell et al 1989, Sievert et al 1991) confirms the presence of small or imperfect crystallites.

Increasing the water-to-RS ratio to 2:1 resulted in a thermal transition with a distinct main peak at 155°C (Fig. 3). The shoulder of that main peak indicated the presence of a second peak around 160°C. Increasing the water-to-RS ratio decreased the T_{p1} to 152 and 151°C for 3:1 and 4:1 ratios, respectively.

Though transition peaks of different shapes were recorded for RS with different amounts of water, there were no consistent changes in enthalpy values. It is suggested that RS samples with the same weight mass contain the same number of bonds, which react in forming endothermal transitions. Biliaderis (1991), as cited by Sievert et al (1991), suggested that DSC thermal responses are directly related to the helices present, regardless of their tertiary organization. Accordingly, helices with no crystallographic character also require energy for melting and are, therefore, thermally detectable.

Quantitative Determination of RS Using DSC Analysis

Variations in RS sample size in the presence of water were analyzed by DSC. Figures 4 and 5 summarize the thermal characteristics of RS samples in the 20.0–0.5 mg range mixed with water and Al_2O_3 at different ratios. A 20-mg RS sample with an RS-to-water ratio of 1:2 produced an enthalpy at least 20% lower than a smaller sample (Fig. 4). Note that results in Figures 4 and 5 were recalculated to a constant RS weight. Apparently, the reaction of 20-mg RS samples with 40 μl of water was not complete (Fig. 4A), which may be due to limited penetration of steam into the big sample. Some of the capsules used to test the 20.0-mg RS samples exploded. The 2.5-mg RS samples with 5 μl of water were too small. The results were highly erratic (data not presented here).

The thermal characteristics of small samples in the presence of Al_2O_3 differed from those of the bigger samples (Fig. 5). Increasing the water-to-RS ratio resulted in lower peak temperatures. Those decreases were not due to the presence of Al_2O_3. Adding Al_2O_3 to 5.0-mg RS samples did not influence the thermal transition characteristics (results not shown). Although the transition characteristics for 2.5-mg RS samples were reproducible, those for 1.0- and 0.5-mg RS samples analyzed in mixtures with Al_2O_3 were erratic, especially the transition enthalpies (Fig. 5).

Establishing the effect of sample size on DSC characteristics is critical to quantitative analyses of small amounts of RS in blends. DSC analysis of RS must also take into account the peak character. Single, distinct peaks with a temperature around 155°C were recorded for 20-mg RS samples (Fig. 4). This was governed (or at least influenced) by the effect of the large sample weight.

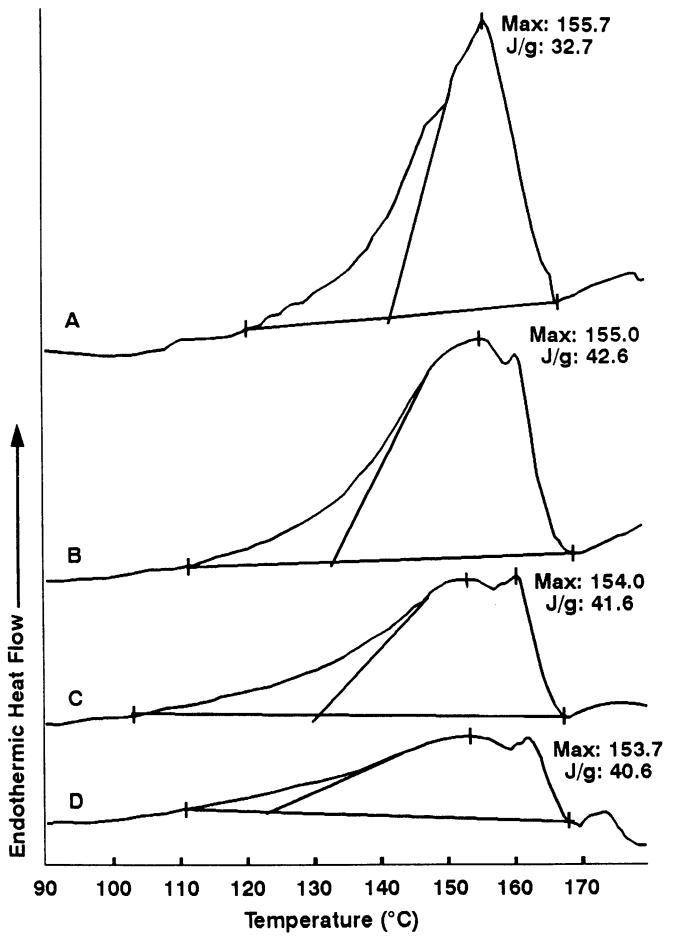

Fig. 4. Differential scanning calorimetry thermograms of samples all with enzyme-resistant starch (RS) to water ratios of 1:2. **A**, 20 mg RS; **B**, 10 mg RS; **C**, 7.5 mg RS; and **D**, 5 mg RS.

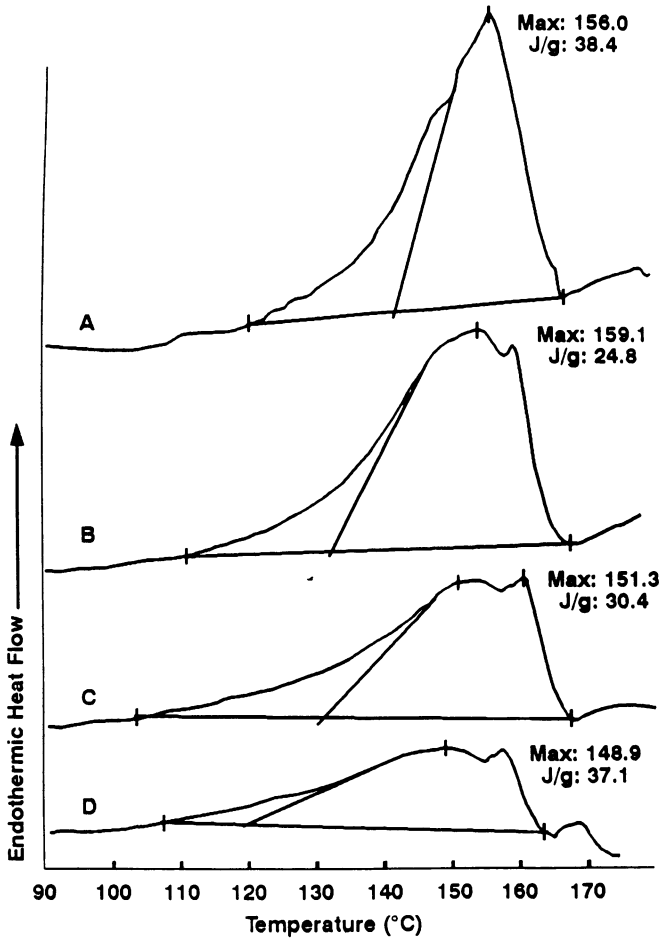

Fig. 5. Differential scanning calorimetry thermograms of enzyme-resistant starch (RS) and Al_2O_3 blends (total 10 mg dry matter plus 20 μl of water). **A**, 5.0 mg RS; **B**, 2.5 mg RS; **C**, 1 mg RS; and **D**, 0.5 mg RS.

A main peak with one or more less-resolved contiguous peaks was recorded for smaller samples. Obviously this affected peak temperatures. The more detailed resolution of analyzed compounds effected by the use of small samples is specific to the DSC method, as discussed in the Instruction Manual of DSC-4 Perkin-Elmer Analyzer (Anonymous 1982).

Thermostability of Dry RS

Table IV presents thermoanalytical characteristics of RS isolated from amylomaize VII starch that was preheated at 100 and 200°C without water and tested by DSC (total 10 mg dry matter plus 20 μl of water). The transition temperatures and enthalpies for RS heated at 100°C for 48 hr remained almost constant and comparable to those of RS before the heat treatment. Dry heating RS for 1 hr increased the enthalpy by 3 J/g; the increase was consistent but not statistically significant. More extensive changes were recorded for RS heated at 200°C: peak temperature and enthalpy decreased over time accompanied by RS darkening and a burning smell.

Effect of Heat Pretreatment in the Presence of Water on Thermoanalytical Characteristics of RS

The transition enthalpies and transition temperatures of RS analyzed by DSC after preheating at 100 or 200°C with different amounts of water are presented in Table V.

For RS samples heated for 1 or 12 hr at 100°C with water, no major changes for characteristic transitions were observed. For RS samples heated for 30 min at 200°C with water, no transition at the temperature range was recorded. RS is not stable at 200°C in the presence of water and probably not under the pressure created by steam. However, the RS heated with a small amount of water (5:2 ratio) showed a weak transition, with the peak temperature at 161.2°C and a low enthalpy of 1.9 J/g. Discoloration of RS heated at 200°C with and without water indicated that destruction of RS at that temperature involves not only decomposing crystallinity but also nonenzymatic browning.

TABLE IV
Differential Scanning Calorimetry (DSC) of Enzyme-Resistant Starch (RS)[a] Isolated from Amylomaize VII Preheated Without Water at 100 and 200°C for Different Times

	Transition Temperatures[b] (T,°C)[c] and Transition Enthalpies (ΔH, J/g)[d]			
Time of Heating	T_i	T_p	T_c	ΔH
At 100°C, hr				
0	101.9	154.0	170.7	40.9
1	105.0	154.0	169.3	43.9
16	105.0	153.1	171.1	40.3
24	101.8	153.4	167.8	39.4
48	101.8	152.8	166.8	38.9
At 200°C, min				
1	101.9	154.0	170.0	40.9
15	101.8	153.2	168.9	40.3
30	102.8	149.8	164.9	42.3
60	102.8	149.1	165.7	39.1
120	95.2	147.9	163.6	32.2

[a]10 mg of total dry matter plus 20 μl of water.
[b]T_i, T_p, T_c = initial, peak, and completion temperatures, respectively.
[c]SD < 1.0°C, $n = 3$.
[d]SD < 10% of the mean; $n = 3$.

TABLE V
Effect of Heat Pretreatment of Enzyme-Resistant Starch (RS) in the Presence of Water on Thermoanalytical Characteristics of RS[a] Tested by Differential Scanning Calorimetry

	Transition Temperatures[b] (T,°C)[c] and Transition Enthalpies (ΔH, J/g)[d]			
Ratio of RS to H_2O	T_i	T_p	T_c	ΔH
1:0	131.4	154.0	170.7	40.9
At 100°C, 1 hr				
5:2	107.1	153.2	167.8	41.3
1:1	109.1	153.4	167.3	40.3
1:2	107.4	153.6	167.3	40.1
At 100°C, 12 hr				
5:2	109.4	153.4	167.3	41.3
1:1	108.2	153.2	167.8	40.9
1:2	109.8	153.2	167.8	43.2
At 200°C, 0.5 hr				
5:2				
1:1	No characteristic transition for RS			
1:2				

[a]10 mg of total dry matter plus 20 μl of water.
[b]T_i, T_p, T_c = initial, peak, and completion temperatures, respectively.
[c]SD < 1.0°C; $n = 3$.
[d]SD < 10.0% of the mean; $n = 3$.

The Effect of Gluten and Wheat Starch on Thermoanalytical Characteristics

The thermoanalytical characteristics of RS in the presence of wheat starch are given in Figure 6. The thermal characteristics

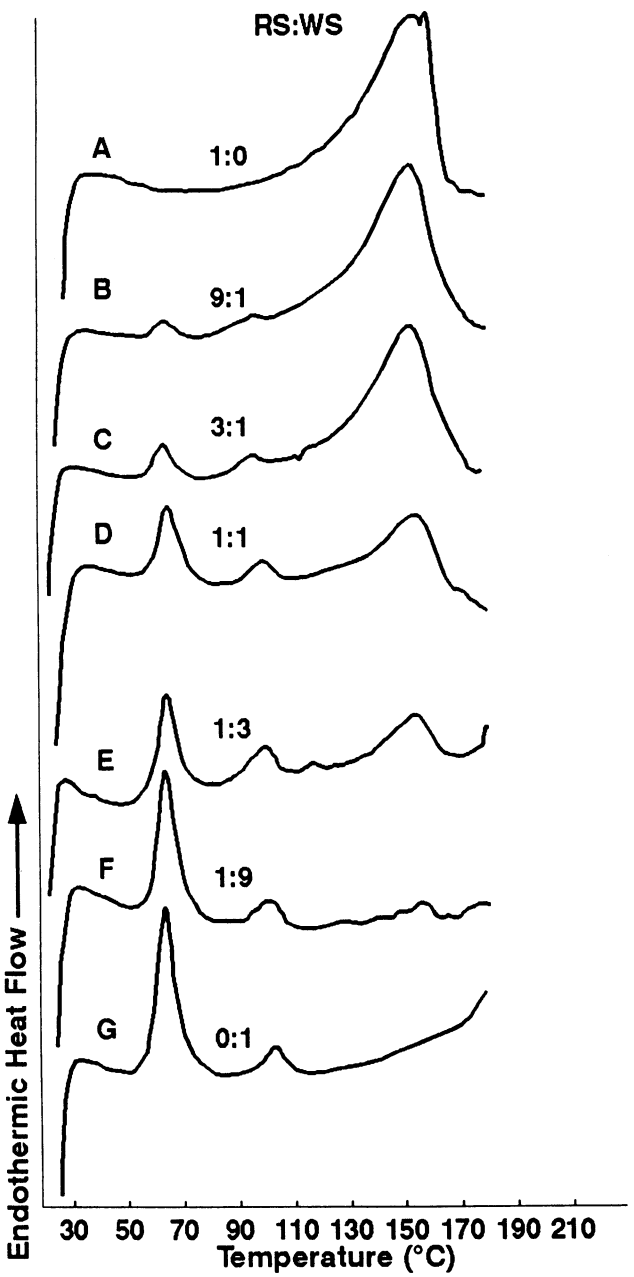

Fig. 6. Differential scanning calorimetry thermograms of enzyme-resistant starch (RS) in the presence of wheat starch (WS) at different ratios. **A**, 1:0; **B**, 9:1; **C**, 3:1; **D**, 1:1; **E**, 1:3; **F**, 1:9; and **G**, 0:1. Total 10 mg dry matter plus 20 μl of water.

of RS in the presence of gluten or wheat starch are summarized in Table VI. Adding gluten or wheat starch decreased transition enthalpies of RS, calculated as joules per gram. The amount of decrease depended on the level of gluten or starch in the RS blends. RS blends with either gluten or starch in ratios of 1:1 or 1:3 yielded similar 40% reductions of enthalpies. An RS content of about 10% in the analyzed samples (1:9 ratio) yielded a marginal transition that could not be measured precisely. The lower enthalpies of RS in blends cannot be attributed only to an interaction with either gluten or starch. The components of the analyzed mixtures in the RS transition temperature range have a rubberlike mobility and seem to interact easily. RS is stable and does not interact below 100°C, where it retains its gelatinization temperature (T_g) hard, glassy state and where molecular motions are significantly restricted (results to be reported elsewhere). Gelatinization characteristics of raw wheat starch in RS mixtures (Table VII) show that transition enthalpies and temperatures of wheat starch gelatinization in the 50–80°C range were not significantly affected by wide ranges in RS-to-wheat starch ratios.

Thermal characteristics of RS-gluten blends plus wheat starch (preheated with or without water) are given in Table VIII. The enthalpy of the 4:2:3 blend preheated without water and tested by DSC (total 10 mg of dry matter plus 20 μl of water) was 10.5 J/g; recalculated for the RS alone, it was 23.6 J/g. The value of 23.6 J/g was substantially lower than the enthalpy for RS alone (40.9 J/g) (Table VIII). When the blend of RS and gluten plus wheat starch was mixed in a water-to-total solids ratio of 1:3 and was freeze-dried before testing by DSC, the calculated enthalpy for RS increased to 26.3 J/g (Table VIII). Preheating dry blends without water at 100 and 200°C for 25 min did not essentially change the RS thermal transition characteristics. Preheating a mixture of RS, gluten, and wheat starch with water at 100°C did not affect RS thermal transition characteristics. Heat treatment of RS at 200°C for 25 min in the presence of water and gluten plus wheat starch eliminated the transition enthalpy around 155°C.

TABLE VI
Effect of Gluten or Wheat Starch on Thermoanalytical Characteristics of Enzyme-Resistant Starch (RS)[a] Tested by Differential Scanning Calorimetry

Ratio of RS to Gluten or Starch	Transition Temperatures[b] (T,°C)[c] and Transition Enthalpies (ΔH, J/g)[d]			
	T_i	T_p	T_c	ΔH
RS to Gluten				
9:1	113.5	153.0	167.8	30.0
3:1	114.9	151.8	168.0	28.3
1:1	118.9	151.4	165.3	25.9
1:3	126.6	151.2	166.0	22.3
1:9		Weak transition for RS		
0:1		No transition for RS		
RS to Starch				
9:1	114.8	153.0	167.1	31.6
3:1	116.5	153.9	168.3	27.4
1:1	120.1	153.9	168.2	23.0
1:3	128.2	153.3	168.1	22.9
1:9		Weak transition for RS		
0:1		No transition for RS		
RS alone	101.9	153.9	170.7	40.9

[a] 10 mg of total dry matter plus 20 μl of water.
[b] T_i, T_p, T_c = initial, peak, and completion transition temperatures, respectively.
[c] SD < 1.0°C; $n = 3$.
[d] SD < 10% of the mean; $n = 3$.

TABLE VII
Characteristics of Wheat Starch Gelatinization Transition in the Presence of Enzyme-Resistant Starch (RS)[a] Tested by Differential Scanning Calorimetry

Ratio of Wheat Starch to RS	Transition Temperatures[b] (T,°C)[c] and Transition Enthalpies (ΔH, J/g)[d]			
	T_i	T_p	T_c	ΔH
Wheat starch control	52.9	63.9	80.9	11.3
9:1	51.9	63.7	79.5	10.9
3:1	50.9	63.6	79.2	10.1
1:1	54.2	64.1	78.8	10.0
1:3	54.6	63.9	76.1	8.3
1:9	55.4	63.1	71.7	9.5

[a] 10 mg of total dry matter plus 20 μl of water.
[b] T_i, T_p, T_c = initial, peak, and completion transition temperatures, respectively.
[c] SD < 1.0°C; $n = 3$.
[d] SD < 10% of the mean; $n = 3$.

CONCLUSIONS

High yields (about 30%) of RS with an enthalpy around 40 J/g were produced from a high-amylose maize starch subjected to three autoclaving-cooling cycles, purification after treatment with thermostable α-amylase, and thermostabilization of the product at 100–130°C. Thermal characteristics (transition temperatures, shapes of curves, and enthalpies calculated as joules of RS per gram) as determined by DSC were affected by sample size and RS-to-water ratio. Enthalpies were reduced in the presence of wheat starch or wheat gluten. Preheating RS at 100°C in the presence or absence of water had no effect on its thermal characteristics; preheating at 200°C (especially in the presence of water) resulted in RS decomposition. Consequently, most added RS would likely be retained in the crumb of a bread where the temperature during baking seldom reaches, and does not exceed, 100°C (Pomeranz 1986, 1991). Most of the RS in the crust would probably be destroyed. The extent of destruction would depend on the rate of heating during baking (and rate of water evaporation) and final crust temperature. At least 15–20% RS would have to be included in the bread formula to establish

TABLE VIII
Effect of Preheating Mixtures of Enzyme-Resistant Starch (RS),[a] Wheat Starch (WS), Gluten (G), and Water (W) on RS Thermal Transition Characteristics Tested by Differential Scanning Calorimetry

Ratio of RS:G:WS:W in Preheated and Tested Sample	Treatment of Sample	Transition Temperatures[b] (T,°C)[c] and Transition Enthalpies (ΔH, J/g)[d]			
		T_i	T_p	T_c	ΔH
4:2:3:0	Control	124.6	153.9	171.0	23.6
4:2:3:3	Freeze drying	121.6	153.9	167.9	26.3
4:2:3:0	Heating at 100°C 25 min	120.1	153.9	170.0	25.6
4:2:3:0	Heating at 200°C 25 min	114.1	152.1	170.0	26.7
4:2:3:3	Heating at 100°C 25 min and freeze drying	118.9	152.9	166.3	24.4
4:2:3:3	Heating at 200°C 25 min and freeze drying		No characteristic transition for RS		
RS control		111.3	153.4	169.5	40.9

[a] 10 mg of total dry matter plus 20 μl of water.
[b] T_i, T_p, T_c = initial, peak, completion transition temperatures, respectively.
[c] SD < 1.0°C; $n = 3$.
[d] SD < 10% of the mean; $n = 3$.

the presence of RS in bread crumb by DSC alone. Smaller amounts of RS in baked products can probably be determined by a combination of methods such as gravimetric-enzymatic isolation combined with DSC. Studies of such methods are being conducted in our laboratories.

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