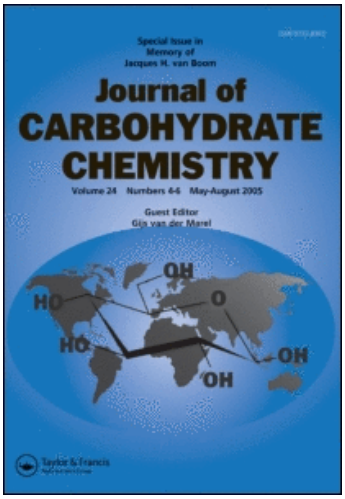

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BEHAVIOR OF STARCHES DERIVED FROM VARIETIES OF MAIZE CONTAINING DIFFERENT GENETIC MUTATIONS. III. EFFECTS OF BIOPOLYMER SOURCE ON STARCH CHARACTERISTICS INCLUDING PASTE CLARITY[1]

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ABSTRACT

Starch was extracted from three mutant maize genotypes (*waxy*, (*wx*), *waxy shrunken1* (*wxsh1*), and *dull waxy* (*duwx*). Each starch was found to have distinct physical and functional properties. Starch from *wxsh1* was found to have a slower rate of retrogradation, enhanced freeze-thaw stability, and greater clarity when compared to either *wx* or *duwx*. This clarity was stable over time. Neither the degree of clarity of the *wxsh1* paste nor its stability was affected by the presence of salt or sugar. Starch from several different backgrounds containing *duwx* produced during several growing seasons, had a smaller particle size distribution than either *wx* or *wxsh1*. Starch especially from *duwx*, had a distinct Brabender Visco-Amylograph profile.

INTRODUCTION

Behavioral and functional properties of starch biopolymers reflect their structural composition. The selection of native starch biopolymer structures from different genetic varieties of maize which have some of the functional properties of chemically modified starches is a main objective of this laboratory's project. Recognition of these properties can lead to successful utilization of such materials in specific applications. Variance in starch structure reflects the unique biosynthetic complement of each plant source. Maize is especially amenable to study on biochemistry-structure-function relationships, because of the availability of a rich array of different mutations. A spectrum of starches has been characterized[2] and strategies for their commercial uses have been discussed.[3] General differences in amylopectin and amylose content have been presented[4] while their impact on granule morphology has been discussed in the second paper of this series.[5] The effects of chemical modification on starch from one double mutant of maize have also been described.[6] This report will compare several novel highly branched starches whose behavior is substantially different from one another despite the fact that they all conform to the old definition of "amylopectin". Among these behavioral characteristics will be the effects on the optical properties of starch pastes both in the absence and in the presence of low molecular weight materials, as well as thermal properties, rheological properties, particle size and morphology, and size exclusion chromatography profile.

RESULTS AND DISCUSSION

Starch is the major storage biopolymer in the plant kingdom. It is comprised of glucose molecules linked together through glucosidic bonds. The majority of these linkages are in the α 1→4 configuration; the remaining linkages are α 1→6. The α 1→6 bonds serve as branch points. More highly branched starch biopolymers are commonly called "amylopectin" while the less highly branched polymers are called "amylose". There is no convenient analytical

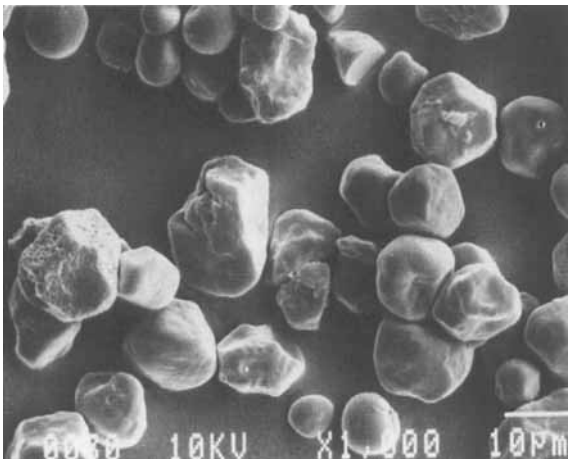

Fig. 1. micrograph of *Wx* starch

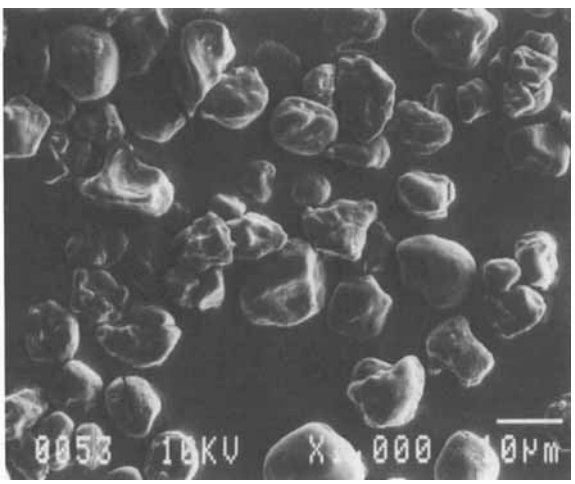

Fig 2. micrograph of *Duwx* starch

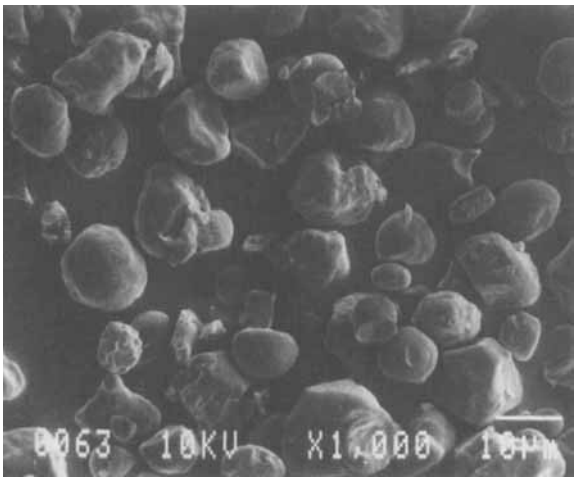

Fig. 3. micrograph of *WxSh1*

method for measuring "amylopectin", and it is usually determined by the absence of "amylose". This latter value is usually determined by methods based on iodine binding. "Amylopectin" values, alone, are not necessarily predictors of starch behavior. Indeed, considerable variability was observed among physical properties of the three all-amylopectin-type starches in this study which are independent of the amylopectin content. Some characteristics are similar while others are different. The size and morphology of granules in each population reflect the nature of their component biopolymers, the genetic background of the maize plant, and the particular environmental conditions of that growing year.[5] **Figures 1, 2,** and **3** are copies of scanning electron micrographs of commercial all-amylopectin-type starches from maize containing the *waxy* (*wx*), *dull waxy* (*duwx*), and *waxy shrunken1* (*wxsh1*) genotypes, respectively. These granules also fall into the same general categories previously described: angular, dimpled, irregular, and nearly round.[7] Differences are seen between the mutants: *duwx* has smaller granules with fewer angular and more irregular granules, while *wxsh1* granules are roughly similar to those of *wx*.

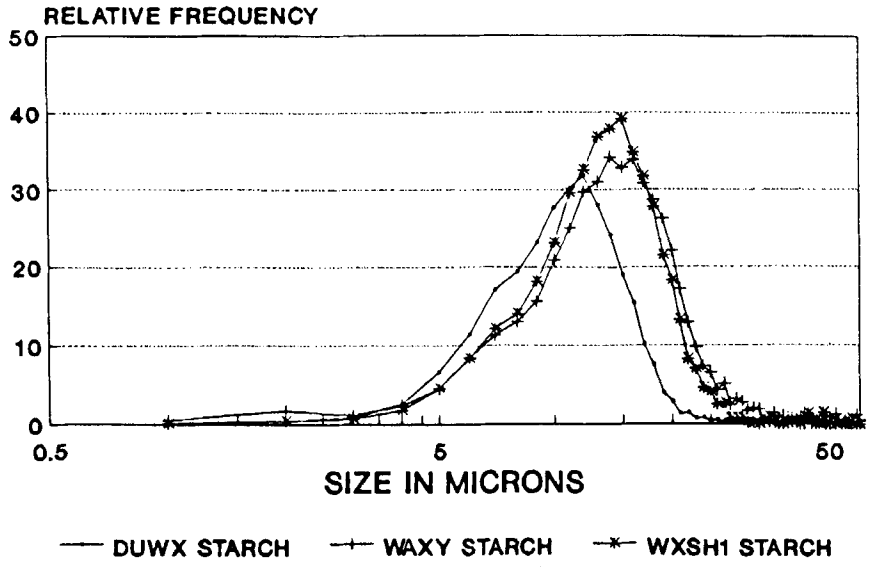

Fig. 4. Size distribution of high-amylopectin-type starches.

There were also differences in particle size distribution among the mutants (**Figure 4**). The size distribution (volume mode), for *wx* maize and *wxsh1* is similar. Granules of *duwx* had both a smaller volume median and mode, confirming observations made from scanning electron micrographs.

Differential scanning calorimetry (DSC) showed little difference between the starches in either the onset, peak temperatures of gelatinization, or range (Table 1).

Size exclusion chromatography (SEC) showed little difference in the elution profiles of these all-amylopectin-type starches (**Figure 5**). Some starch polymers from different maize mutations have distinctive elution patterns with SEC. This, perhaps, results from varying levels of compactness of the molecules, different fracture patterns resulting from sample preparation or chromatography, or different attainable molecular sizes. For some mutations, however, the relationship between biochemistry and structure is becoming increasingly clear. For example, the presence of the *wx* gene has been reported to correspond to a decrease in bound starch granule synthase and a

Table 1. DSC Value of Starches

Starch	Peak Temp °C	Enthalpy Joules/gm	Onset Temp °C	Endset Temp °C
duwx	73.6	15.3	66.5	86.4
waxy	72.4	15.0	66.1	82.6
wxsh1	73.4	15.2	67.1	83.9

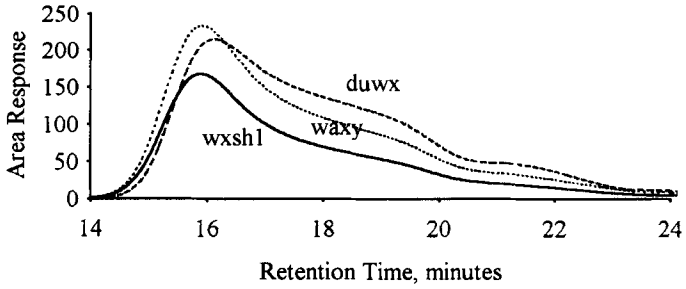

Fig. 5. Size exclusion chromatography of high-amylopectin-type starches. Detection is by refractive index. Peak area is determined by integrating the area under the peak.

corresponding decrease in the amount of amylose, as measured by iodine binding and column chromatography.[8] Amylopectin from the *wx* mutant was also reported to have reduced average chain length when compared to amylopectin isolated from common, or normal, maize.[9,10] Amylopectin from *duwx* maize has been shown by size exclusion chromatography to have a decreased ratio of long B chains to short B chains.[11] Using a commercial pulsed amperometric detection system, *duwx* has been shown to have a greater area percent in the DP 11-14 range[12] than *wx*. Taken together, these data suggest that the amylopectin from *duwx* may have a more compact, more highly branched, structure[12] than *wx*.

Despite the apparent similarities between these all-amylopectin-type starches, we have observed major differences in functional properties between the starches. Those discussed here include, the Brabender Visco-Amylograph profiles, gel clarity and stability, and freeze-thaw stability.

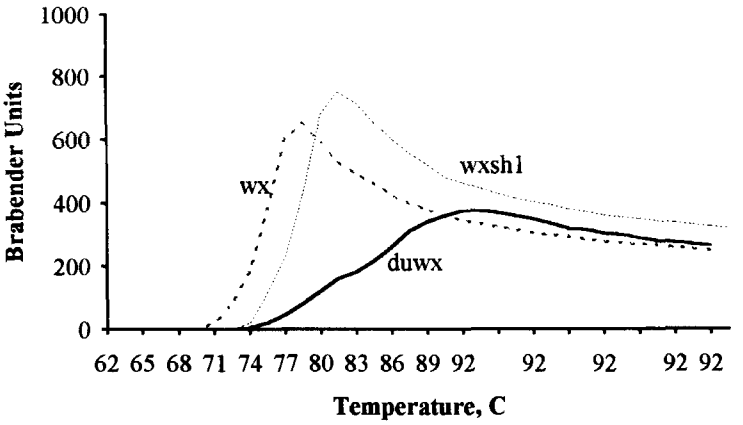

Fig. 6. Brabender Visco-Amylograph Profile of all-amylopectin-type starches.

Starch with the *wx* gene gave a high peak viscosity during gelatinization, followed by a rapid viscosity drop on the Brabender Visco-Amylograph (**Figure 6**). The heating peak viscosity of *duwx* starch was both reduced in intensity and inhibited in the rate of viscosity development. On the other hand, the visco-amylograph profile of *wxsh1* was not appreciably different from that of *wx*.

The swelling power of *duwx* was found to be lower than that of *wx* starch.[13] This corresponds with studies which demonstrated that swelling power, or phase-volume, is an important parameter governing the viscosity of a starch paste.[14] Perhaps the tighter, more highly branched structure of *duwx* starch acted to restrict the swelling of the granule during gelatinization and thus inhibited peak viscosity development.

Clarity of starch pastes is an important consideration in some food applications. We have found commercial white waxy (*whwx*) maize starch pastes clearer than those derived from commercial *wx* maize or tapioca starches (**Figure 7**). No structural differences have been reported between amylopectin of *wx* and *whwx*. It may be that the reduced carotenoid level and reduced protein content of the isolated *whwx* granules account for their greater clarity.[15] Pastes of *wxsh1* starch were considerably clearer than those of *wx*

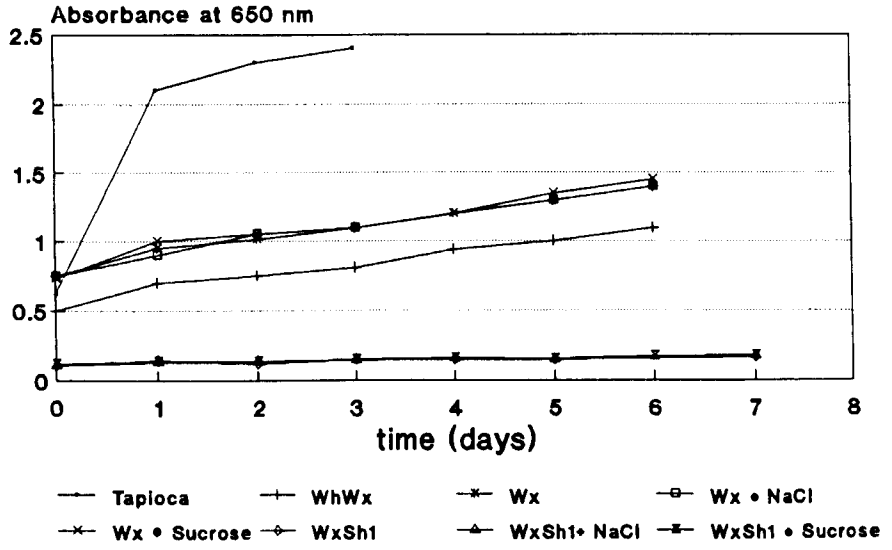

Fig. 7. Opacity (O.D.) Brabender Pastes

or *whwx* (**Figure 7**). The clarity of the *wxsh1* paste was also more stable at 4°C over time than the others (**Figure 7**). Clarity of the starch paste was not affected by the presence of salts or sucrose (**Figure 7**).

Starch characteristics responsible for the clarity of waxy shrunken are not currently understood. It has been found, however, that the rate of retrogradation of *wxsh1* is slower than that of *wx* when measured by mechanical spectroscopy (**Figure 8**). One possible speculation is that the slower reassociation of the molecules increased the time needed to form junction zones which are large enough to diffract light in the *wxsh1* paste.

Major differences appeared when freeze-thaw stabilities of unmodified all-amylopectin-type starches were compared to those of a commercial *wx* starch (PG-10) which had been modified with both phosphorous oxychloride and propylene oxide. In the presence of sugar, unmodified *wxsh1* starch had good freeze-thaw stability through at least ten cycles of freezing and thawing (**Figure 9**). In the presence of salt, seven cycles were observed. Sucrose retards the rate of retrogradation as measured by the rate of storage modulus increase. The rate of increase of storage modulus for *wxsh1* starch is much

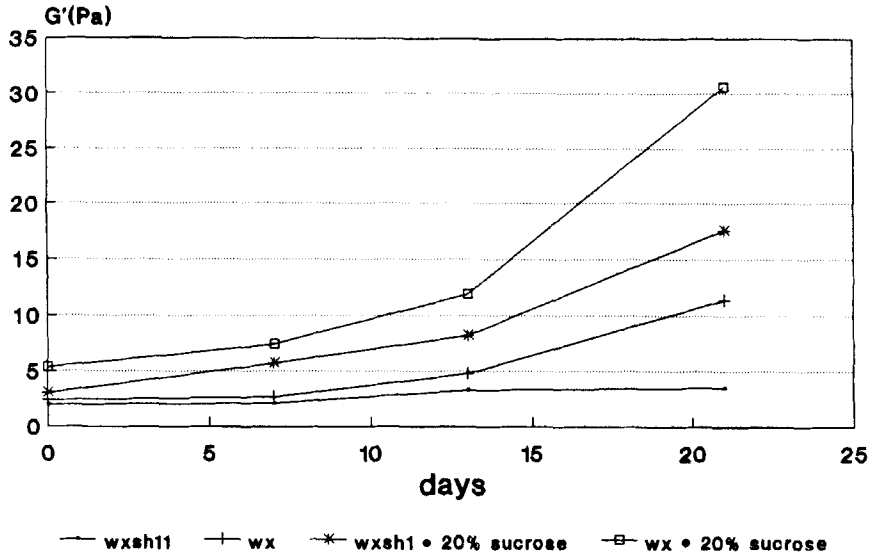

Fig. 8. Storage modulus development of all-amylopectin pastes. The storage modulus (G') is an indication of paste firmness. Increased G' is associated with retrogradation.[16]

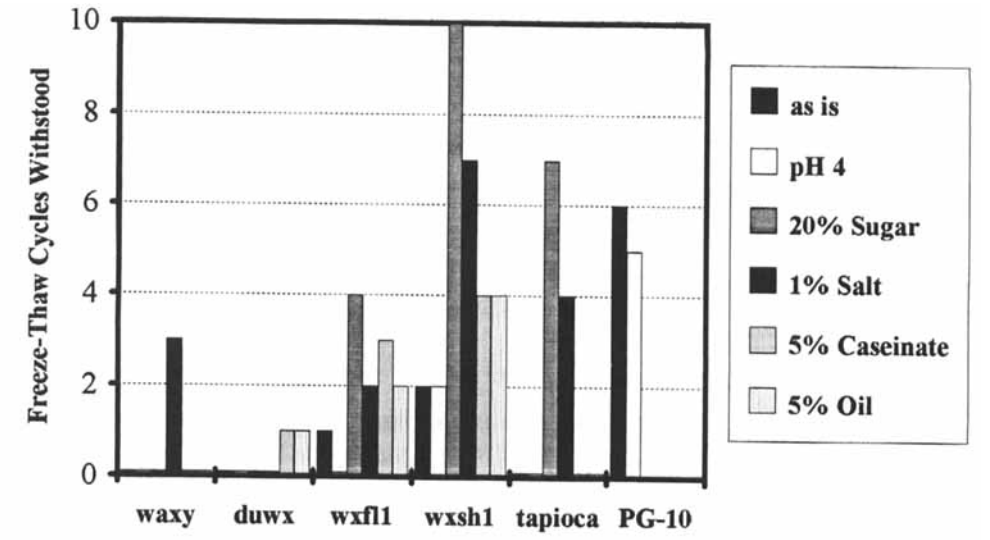

Fig. 9. Freeze-Thaw Stability

slower than that of *wx* (**Figure 8**). This suggests differences in molecular structure which slow the rate of association of the dispersed polymers. The slower retrogradation of cereal amylopectins, when compared to pea, potato, and canna amylopectins, has been partially attributed to their shorter average chain length.[16] A decrease in the mole fraction of chains between DP 16-24, and an increase in the mole fraction between DP 6-9, were found to correspond to both a decreased tendency to retrograde and an increased freeze-thaw stability for the waxy maize, waxy barley, and two waxy rice starched studied.[17] Despite recent advances in knowledge of starch structure, much remains to be determined to understand fully the relationship between structure and functional properties of starches, such as paste clarity and rate of retrogradation.

CONCLUSION

Distinctive functional properties are obtained from maize biopolymers by altering the balance of starch biosynthetic enzymes through genetic breeding. Starches from maize containing the *duwx, wxsh1,* and *wx* mutations were found to have distinctive Brabender Visco-Amylograph profiles, optical properties, and freeze-thaw stabilities, despite the fact that they can be classified as all-amylopectin-type starches by iodine binding assays. Relationships between polymer structure and functionality are not yet clear.

EXPERIMENTAL

Starches. Where possible, starches were extracted from hybrid maize plants. Selection was made from maize plants with a broad variety of backgrounds, including, but not limited to, OH43 and W64A. Emphasis was placed on determining stability of starch structures and functions which were independent of the maize background. Wet-milling was performed in a commercial process or by laboratory steeping in a manner analogous to plant scale steeping based on the procedure of Anderson.[18]

Paste Preparation. Pastes used for clarity, freeze-thaw stability, and mechanical spectroscopy experiments were all prepared in the following manner. A 5.5% (dry basis) slurry of starch was prepared in double deionized water and gelatinized in a Brabender Visco-Amylograph. After the slurry reached 62 °C, it was heated at a rate of 1.5 °C per minute to 92 °C and held for 15 minutes. Amylograms were recorded using the 700 gram centimeter cartridge. The hot paste was then poured quickly into a vacuum flask, maintained at about 70 °C on a steam bath, and degassed under vacuum for about 10 min. If the paste was to be stored, 0.01% sodium azide was added to the slurry water. If sugar or salt was necessary, it was dissolved at appropriate concentrations in the double deionized water before slurrying the starch.

Paste Clarity. This was determined using a modification of the procedure of Craig et al.[19] The paste was degassed, pipetted into cuvettes, and allowed to cool to room temperature (about 40 min). Cuvettes were stored at 5 °C, and visable light absorbance (650 nm) of samples in 5 mL cuvettes was measured daily for 6 to 7 days. Starch from *wxsh1* maize from different maize backgrounds was analyzed. Analyses were repeated twice and averages reported here. The standard deviation was consistently less than ± .010 absorbance units below readings of 2.00.

Mechanical Spectrometry. The degassed warm paste was carefully divided into several samples which were placed in plastic containers. The samples were allowed to cool to room temperature (about 40 min), and sealed before storing at 5 °C. At 7, 14, and 21 day intervals, samples were removed, allowed to come to room temperature, and the storage modulus of at least 3 aliquots was measured at 25 °C. Measurements were performed using a Rheometrics RFSII Spectrometer; 50 mm parallel plates, 1.00 mm gap, and 2% strain (linear viscoelastic range extended to at least 6% strain). Analyses were repeated at least twice. Storage modules values reported here are averages with the standard deviation being less than 12 percent.

Freeze-thaw Stability. Stability of starch pastes was assessed by storing aliquots, 10 mL, of the cooled Brabender pastes in covered plastic centrifuge

tubes (15 mL) at 0 °C for at least 12 h. These samples were allowed to thaw for 4 to 5 h at room temperature, several samples were retained, and the remainder re-frozen for subsequent cycles. The thawed samples were centrifuged at 1500 x g for 10 min. Any water which separated to the top of a tube was immediately decanted and weighed. At the point in the test, when the water loss exceeded 30% of the total initial paste weight, the paste was deemed no longer freeze-thaw stable (Amaizo Method). Analyses were repeated twice; values reported here are averages.

Particle Size Analysis. Analysis was performed using a Brinkmann particle size analyzer model 2010. Data presented here were the results of volume mode calculation. Two hundred mg of starch were dispersed in 100 mL of deionized water. Aliquots,(2 mL), were measured in polystyrene cuvettes (1 cm x 1 cm) for 5 min intervals. Analysis was to a 95% confidence level. Data presented for *duwx* maize were typical from more than twenty independent analyses. Samples were from four different maize backgrounds and from four different growing seasons.

Differential Scanning Calorimetry. Calorimetry was performed on starch samples at 30% solids using a Mettler model 30 calorimeter. Slurries were heated at a rate of 10 °C per minute from 10 to 130 °C, in sealed 40 *u*L aluminum crucibles. An empty crucible was used as the reference cell. Experiments were performed in triplicate; average analyses reported had a standard deviation less than 0.1 °C and 0.4 joule/g.

Scanning Electron Microscopy. Starches were examined using a JOEL JSM-840 electron microscope. Starch was sprinkled on double sided tape and coated with approximately 200 angstroms of gold before viewing. At least four representative samples were photographed for each starch.

Size Exclusion Chromatography. Chromatography was performed using the microwave bomb method of Delgado et al.[20] Each analysis was performed in triplicate.

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