Thermal and Gelling Properties of Maize Mutants from the OH43 Inbred Line[1]

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Cereal Chem. 69(3):328-334

ABSTRACT

Starches were isolated from the maize (*Zea mays*) inbred line Oh43, from its single mutants (amylose extender [*ae*], brittle-1 [*bt1*], brittle-2 [*bt2*], dull-1 [*du1*], floury-2 [*fl2*], horny [*h*], shrunken-2 [*sh2*], sugary-1 [*su1*], and waxy [*wx*]), and from the double-mutant combinations within Oh43. Differential scanning calorimetry was used to determine the onset temperature (T_o), range, and enthalpy (ΔH) of gelatinization and retrogradation, and percentage of retrogradation. The gel strength was measured by using a Voland-Stevens texture analyzer. For gelatinization, the starches of *wx du1* and *sh2 du1* had the highest T_o. Double-mutants *ae bt2* and *ae du1* had the highest T_o of retrogradation. The highest ΔH of gelatinization was observed for *h wx*. The gelatinization enthalpy peak for *bt1* starch had a characteristic low temperature shoulder and wide range. Compared with the respective single mutants, most double-mutant combinations had higher T_o and ΔH for gelatinization and lower T_o for retrogradation. For gel strength, the *du1* starch gave the lowest values for firmness and stickiness among the samples. Double mutants generally had gel strength measurements lower than those of the single mutants *bt1, bt2, fl2, h,* and *sh2* but higher than those of *du1*.

Several endosperm mutants that are genetically recessive have their primary effect on the synthesis of starch or on a particular protein in maize (*Zea mays* L.) (Ikawa et al 1981; Yeh et al 1981; Inouchi et al 1983, 1987; Fuwa et al 1987; Sanders et al 1990). Identified recessive mutant genes include amylose extender (*ae*), brittle (*bt*), dull (*du*), floury (*fl*), horny (*h*), opaque (*o*), shrunken (*sh*), sugary (*su*), and waxy (*wx*). These mutants cause variations in amylose percentage or the total amount of starch accumulation. The nomenclature of these mutants is, in part, based on the effect that these mutant genes exert on the appearance or phenotype of the kernel. Some genotypes that cause the same effect but are controlled by different genes on different chromosomes are given a number after the named genotype (for example, *sugary-1* [*su1*] and *sugary-2* [*su2*]).

Because of the diverse applications of starch in industries, chemical and/or physical modifications often are made to the starches to meet the needs of the users. However, with the increasing difficulty in achieving the regulatory approval of chemically modified starches in the food industry (Sanders et al 1990), there is a great potential for novel starches from mutant genotypes that bear desired properties. Furthermore, such novel starches might replace chemically modified starches, thereby providing economic advantages by reducing the cost of processing.

The mutant genes can influence the total starch content and the amylose-amylopectin ratio. The *ae* mutant is associated with a high amylose content of the endosperm starch, whereas the *wx* starch has essentially no amylose (Shannon and Garwood 1984). In differential scanning calorimetry (DSC) analyses, the *wx* starch showed thermal behavior similar to that of normal corn starch. The *ae* starch, however, did not exhibit a clear peak, and the endotherm extended beyond 100°C (Stevens and Elton 1971). The special properties of different mutants, such as gelatinization characteristics and susceptibility to enzymes, have been described elsewhere (Inouchi et al 1984, Boyer and Liu 1985, Krueger et al 1987b, Brockett et al 1988, Ninomya et al 1989, Sanders et al 1990).

The double-mutant combinations create additional modifications in the structure and properties of starch granules (Ikawa et al 1981, Yeh et al 1981, Fuwa et al 1987, Brockett et al 1988, Ninomya et al 1989, Sanders et al 1990). For example, when the *ae* gene was introduced as a double mutant, amylose content increased and an intermediate fraction and amylopectin with longer branches were found (Ikawa et al 1981). The DSC thermograms of double-mutant starches with the *wx* gene shifted to a narrower temperature range (R) compared with those of their respective single mutants (Sanders et al 1990).

Important physical properties of starches include the thermal requirements for gelatinization, the susceptibility of gelatinized starch to retrogradation, and the shear modulus of the starch gel. The temperature of gelatinization can be studied by using DSC or by loss of birefringence under a polarized light microscope equipped with a hot stage. DSC has been widely used to study the thermal behavior of starch because it requires only a small sample size, both gelatinization temperature and enthalpy can be obtained, and it is easy to operate (Nakazawa et al 1985). DSC also can be applied to retrograded starches to measure transition temperature and enthalpy.

The objective of the present work was to examine the thermal properties of native and retrograded starches and gelling properties using single and double mutants of Oh43.

MATERIALS AND METHODS

Materials

Mature kernels of Oh43 and its single and double mutants (Table I) were used in this study and were identified according to their kernel phenotypes (Garwood and Creech 1972). Single mutants were obtained from the Maize Genetics Cooperation Stock Center at Urbana, IL. Single mutants were crossed in all combinations and self-pollinated. The double mutants were selected on the basis of having kernel phenotypes different from those of Oh43 and their respective single mutants. They were grown either in a winter nursery in Puerto Rico during 1989-1990 or near Ames, IA, in 1990. Plants were self-pollinated or crossed as appropriate, and ears were harvested at full maturity. After harvest, corn ears were dried at 38°C for five days to 13% moisture content. The samples were stored in a cold room at 4°C and 45% relative humidity until analyzed.

Single-Kernel Starch Isolation

Starches were isolated as described by White et al (1990) except that a 30-μm sieve was used and starch from two kernels was extracted at a time. Two separate extractions per starch type were run, and starch from a single isolation was used to determine both thermal and gel properties.

Differential Scanning Calorimetry

The DSC studies were performed by using a Perkin-Elmer DSC 7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). The gelatinization of starch was accomplished as previously described by White et al (1990), and refrigerated-storage retrogradation was done by the procedure of White et al (1989). Approximately 3.5 mg (dry-weight basis [dwb]) of starch was weighed accurately into an aluminum pan,

[1]This is a joint contribution of journal paper J-14604 of the Iowa Agriculture and Home Economics Experiment Station at Iowa State University, Ames, IA, and the Field Crops Research Unit, USDA-ARS. Projects 2568 and 2778.
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and 8 mg of distilled water was added. The pan was hermetically sealed and allowed to equilibrate at least 1 hr before analysis. Samples were heated from 30 to 110°C at a rate of 10°C/min. Enthalpy (ΔH), onset (T_o), and peak (T_p) temperatures were computed automatically. At the water level used, the endotherms were essentially symmetrical, which allowed the total gelatinization range to be computed as $2(T_p - T_o)$ as described by Krueger et al (1987a). The results are the average of three scans each for two extractions from one sample. Enthalpies were calculated on a starch dry-weight basis. The peak height index

TABLE I
Differential Scanning Calorimetry Properties of Starches[a]

	Gelatinization				Refrigerated-Storage Retrogradation			
Starch	T_o[b] (°C)	R[c] (°C)	ΔH_g[d] (cal/g)	PHI[e]	T_o (°C)	R (°C)	ΔH_r[f] (cal/g)	$r\%$[g] ($\Delta H_r/\Delta H_g$)
Oh43	67.2	8.8	2.9	0.67	42.6	16.9	1.5	49.5
Single mutants								
ae[h]	68.7	31.3	3.7	0.24	...[i]	...	...	...
bt1	63.4	14.9	2.5	0.33	42.7	15.4	1.2	46.8
bt2	66.8	10.6	2.9	0.55	42.7	16.6	1.3	45.1
du1	67.2	9.1	2.1	0.46	42.9	17.7	1.5	73.5
fl2	66.2	9.1	2.9	0.64	44.2	15.1	1.4	49.7
h	65.6	9.4	2.7	0.57	44.1	15.8	1.4	52.7
sh2	64.3	8.5	2.4	0.57	42.1	16.5	1.1	46.7
su1	64.6	7.1	2.1	0.59	43.4	14.1	0.9	45.1
wx[h]	68.6	9.0	3.6	0.80	39.9	21.2	1.9	52.8
Double mutants								
ae bt2[h]	67.1	10.3	2.9	0.57	44.4	14.0	1.3	44.2
ae h[h]	69.6	9.5	2.7	0.58	40.7	20.7	1.4	49.4
ae sh2[h]	68.2	9.7	2.9	0.60	42.2	17.5	1.3	44.6
ae su1[h]	65.4	10.7	2.6	0.49	43.3	15.5	1.3	47.7
ae wx[h]	70.1	9.2	2.8	0.61	43.7	14.9	1.5	51.7
bt1 du1[h]	67.3	8.5	2.8	0.66	44.0	14.6	1.3	47.1
bt1 su1[h]	67.8	9.4	3.0	0.63	43.4	15.7	1.3	44.4
bt1 wx[h]	68.2	10.3	3.1	0.60	41.6	19.3	1.6	51.1
bt2 du1[h]	66.9	9.5	2.8	0.59	44.2	14.9	1.4	50.0
bt2 sh2[h]	67.8	9.8	2.9	0.59	42.6	17.1	1.4	49.3
bt2 wx[h]	69.4	9.4	3.0	0.64	42.9	16.5	1.5	50.9
fl2 ae	67.1	10.9	3.2	0.59	42.6	18.1	1.3	42.1
fl2 bt1	68.1	7.0	3.0	0.87	39.6	18.3	1.5	49.7
fl2 bt2	68.1	7.7	3.3	0.84	40.7	20.8	1.5	46.5
fl2 du1	67.8	6.9	3.3	0.94	41.8	18.2	1.5	45.3
fl2 h	68.3	6.9	3.1	0.89	42.3	18.2	1.4	46.0
fl2 su1	67.1	7.8	3.2	0.81	41.3	19.4	1.5	48.2
fl2 wx	67.4	8.1	3.1	0.77	41.4	19.0	1.6	53.9
h bt1	68.1	5.8	3.2	1.09	41.3	16.9	1.4	45.4
h bt2	68.5	8.7	3.0	0.69	38.2	19.9	1.6	51.7
h du1	67.2	7.8	2.9	0.74	41.1	18.6	1.6	53.6
h fl2	67.7	7.9	3.2	0.80	41.4	19.4	1.5	47.0
h sh2	68.4	6.7	3.1	0.93	42.0	18.2	1.4	45.6
h su1	67.5	7.5	2.8	0.75	42.5	17.8	1.4	50.3
h wx	69.7	4.9	3.6	1.45	42.2	17.0	1.5	42.7
sh2 bt1	67.7	9.0	2.8	0.63	41.5	19.1	1.6	58.1
sh2 du1	70.3	5.1	3.3	1.31	40.3	18.7	1.6	48.3
sh2 fl2	69.3	7.1	3.0	0.85	41.5	18.5	1.4	47.0
sh2 h	65.0	10.1	3.0	0.58	42.4	18.6	1.4	48.0
sh2 su1	68.9	9.0	3.0	0.66	40.5	20.5	1.6	52.5
sh2 wx[h]	68.1	10.9	3.0	0.55	42.0	18.0	1.5	51.0
su1 bt2	67.0	8.6	3.1	0.71	43.4	16.9	1.6	51.1
su1 du1	68.2	8.1	2.5	0.62	39.7	16.6	1.4	57.9
su1 h	68.7	6.6	2.7	0.82	42.2	20.2	1.4	53.9
su1 sh2	67.5	7.5	3.0	0.79	41.3	18.3	1.4	47.0
su1 wx	67.4	7.9	3.1	0.78	42.6	17.1	1.4	47.0
wx du1	70.9	7.5	3.3	0.87	42.4	18.9	1.5	44.9
wx su1	68.3	5.4	3.3	1.23	42.2	17.3	1.5	45.3
$LSD_{0.05}$ Means	0.70	0.72	0.2		1.06	1.78	0.1	4.58

[a] Values are the average of three determinations each from two separate extractions. *ae* = Amylose extender, *bt* = brittle, *du* = dull, *fl* = floury, *h* = horny, *sh* = shrunken, *su* = sugary, and *wx* = waxy.
[b] Onset temperature.
[c] Gelatinization range calculated as 2 ($T_p - T_o$), as described by Krueger et al (1987a).
[d] Enthalpy of gelatinization.
[e] Peak height index = $\Delta H/(T_p - T_o)$ as described by Krueger et al (1987a).
[f] Enthalpy of retrogradation.
[g] Ratio of enthalpy of retrogradation to enthalpy of gelatinization.
[h] Mutants grown in Ames, IA. Other mutants were grown in Puerto Rico.
[i] Data are omitted because its broad thermogram extended beyond 100°C.

(PHI), which is the ratio $\Delta H/(T_p - T_o)$, was calculated to allow a quantitative evaluation of variations in peak shape (Krueger et al 1987a).

Gel Properties

Limited quantities of starches were available, so the preparation of starch gels was adapted to a small size as follows. Starch (60.0 ± 0.1 mg dwb) was put in a vial (4.7 cm high and 1.5 cm diameter), and distilled water was added to a total weight of 1.00 g to make a starch gel of 6% (w/w). A half-inch stirring bar was inserted into the vial, and the vial was placed on a cold hot plate stirrer and stirred slowly until the starch was dispersed. The sample then was heated to boiling with stirring, held for 20 sec, and removed from the hot plate stirrer. High amylose starches were boiled for 2 min to ensure complete gelatinization. The stirring bar was carefully removed, and the vial was tapped gently on a hard surface to redistribute the gel to the bottom of the vial. The vial was covered with Parafilm and placed at 25°C for 4 hr to allow the gel to set and cool before analysis.

The resistance to penetration of the gel was determined with a model TA-100 Voland-Stevens texture analyzer (Voland Corp., Hawthrone, NY) fitted with an L6512 series flat-bed recorder. The gel was compressed at a speed of 0.2 mm/sec to a distance of 3 mm with a punch probe (TA53, 3 mm diameter) with the chart recorder speed at 10 cm/min. The peak height at 3-mm compression was termed firmness, and the negative peak height during retraction of the probe was termed stickiness (Fig. 1), according to Takahashi and Seib (1988). One gel was measured for each starch extraction.

Statistical Analyses

Analysis of variance and data and starch group comparisons were computed with the general linear models program (SAS Institute 1989). Multiple comparisons were done by least significant difference (LSD) after a preliminary F test (Steel and Torrie 1960). Correlation analyses were done on the enthalpy data of DSC and on the gel strength data.

RESULTS AND DISCUSSION

Gelatinization Properties

The DSC properties of starches of Oh43 and its single and double mutants are summarized in Table I, and LSDs are listed for each property. A summary of significant differences among DSC properties of single- and double-mutant starches is presented in Table II, and some representative thermograms are shown in Figures 2 and 3. Mutants that did not grow in Puerto Rico during 1989–1990 were grown in Ames, IA, in 1990. This environmental effect may have affected their DSC properties (White et al 1991). Among the single mutants, the onset temperature of gelatinization (T_o) was highest for *ae*, at 68.7°C, and lowest for *bt1*, at 63.4°C. The R and enthalpy of gelatinization (ΔH_g) of *ae* were larger in this study than in previous studies (Krueger et al 1987b, Brockett et al 1988, Sanders et al 1990) but smaller than in other studies (Wootton and Bamunuarachchi 1979, Biliaderis et al 1980). The reported differences may be attributable to environmental effects (White et al 1991). The *wx* genotype produced higher T_o and ΔH_g for gelatinization than did other single mutants, which was similar to previous reports (Inouchi et al 1984, Fuwa et al 1987).

The PHI ($\Delta H/[T_p - T_o]$) was developed by Krueger et al (1987a) to differentiate raw and annealed starches. The PHI provides a numerical value that describes the relative shape of the endotherm; e.g., a tall, narrow endotherm has a higher PHI than does a short, broad endotherm. The thermogram of *bt1* exhibited an unusual low-temperature shoulder that gave *bt1* starch the lowest T_o, the broadest R, and the lowest PHI (excluding *ae*) among single mutants (Fig. 2). The *du1* starch had the lowest ΔH_g (2.1 cal/g), which was lower than that of the same genotype (2.9 cal/g) reported in earlier studies performed at the same starch-water ratio (Inouchi et al 1984, Fuwa et al 1987). The ΔH_g values of *bt1, du1, sh2,* and *su1* were lower than that of the normal starch ($P < 0.05$). The normal, *ae, sh2,* and *wx* starches had higher PHI values than those reported by Krueger et al (1987a,b). The PHI values for normal starch (Oh43) varied from 0.32 to 0.43 in their study (1987a), compared with 0.67 in our study.

Starches from the double mutants had T_o values for gelatinization that ranged from 65.0°C for *sh2 h* to 70.9°C for *wx du1*. The R ranged from 4.9°C for *h wx* to 10.9°C for *sh2 wx*. The *h wx* starch showed a very sharp and well-defined endotherm, giving it the narrowest R, the highest ΔH_g, and the highest PHI among double mutants (Fig. 3). The *wx su1* also exhibited a sharp endothermic peak and a high ΔH_g similar to that of the *h wx* (Fig. 3). The double-mutant combinations containing the *wx* gene (*h wx, wx su1,* and *wx du1*) had higher ΔH_g values

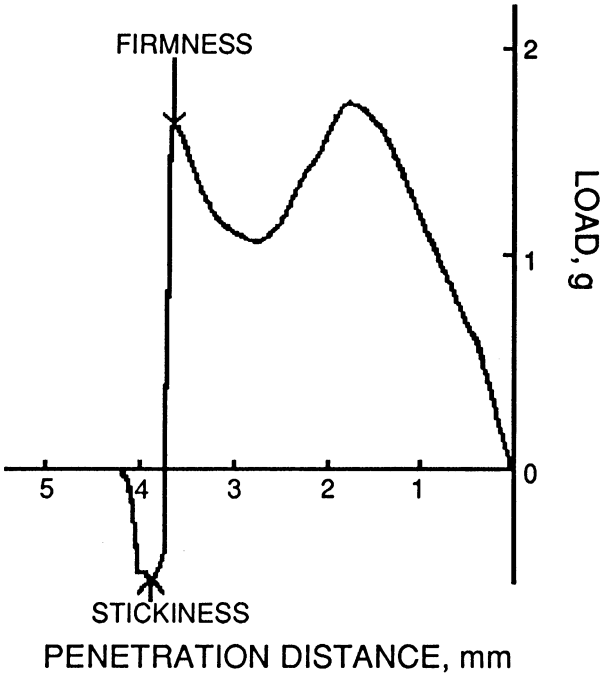

Fig. 1. Load penetration curve of 6% (w/w) commercial corn starch gel measured by the Voland-Stevens texture analyzer. The gel was aged for 4 hr at 25°C before measurement.

TABLE II
Summary of Significant Differences Among Differential Scanning Calorimetry Properties of Single and Double Mutant Starches[a]

	Gelatinization			Retrogradation			
Starch Group Comparison	T_o[b]	R[c]	ΔH_g[d]	T_o	R	ΔH_r[e]	r%[f]
Oh43 vs. all mutants	NS[g]	NS	NS	NS	NS	NS	*[h]
Oh43 vs. single mutants	NS	NS	NS	NS	NS	NS	NS
Oh43 vs. double mutants	NS	NS	*	NS	NS	NS	NS
Single vs. double mutants	**	**	**	**	**	NS	NS
ae[i] vs. other mutants	NS	**	NS	NS	NS	NS	NS
bt1 vs. other mutants	**	**	NS	**	NS	NS	NS
bt2 vs. other mutants	NS	**	NS	NS	NS	NS	NS
du1 vs. other mutants	**	**	NS	NS	NS	NS	**
fl2 vs. other mutants	NS	**	**	**	NS	NS	**
h vs. other mutants	*	**	NS	**	*	NS	NS
sh2 vs. other mutants	**	NS	NS	**	*	NS	NS
su1 vs. other mutants	NS	**	*	NS	NS	NS	NS
wx vs. other mutants	**	*	**	NS	NS	NS	NS

[a] *ae* = Amylose extender, *bt* = brittle, *du* = dull, *fl* = floury, *h* = horny, *sh* = shrunken, *su* = sugary, and *wx* = waxy.
[b] Onset temperature.
[c] Gelatinization range calculated as 2 ($T_p - T_o$) as described by Krueger et al (1987a).
[d] Enthalpy of gelatinization.
[e] Enthalpy of retrogradation.
[f] Ratio of enthalpy of retrogradation to enthalpy of gelatinization.
[g] Not significant at $P < 0.05$.
[h] * and ** = Significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.
[i] All single and double mutants containing this recessive mutant gene.

than that of the normal starch ($P < 0.05$), which was in agreement with the results of Sanders et al (1990). The PHI values for single and double mutants were higher than reported previously (Krueger et al 1987a,b), with *h bt1*, *h wx*, and *wx su1* having PHI values larger than one.

When the mutants containing the same recessive mutant gene were grouped and compared with other mutants, some trends were noted (Table II). For gelatinization, the double-mutant combinations had significantly higher T_o and ΔH_g and lower R values than the single mutants ($P < 0.01$). No significant difference was found, except ΔH_g for the Oh43 versus double mutants comparison, when Oh43 was compared with either single or double mutants. When the *ae* gene was introduced, a broad R for the gelatinization peak was seen. The mutants containing the *bt1* or the *du1* gene exhibited significantly lower T_o and higher R than other mutants. In contrast, the *h* or *wx* gene produced mutants with high T_o and low R values. As indicated earlier, the mutants with the *wx* gene produced significantly higher ΔH_g ($P < 0.01$) than did the other mutants. Most mutants containing the same recessive mutant gene possessed distinctive thermoproperties, which may be useful as an index or reference in the mutant screening process.

Correlation coefficients (r values) were determined among all DSC parameters; however, few r values were greater than 0.5. The r value between T_o and ΔH_g for single mutants was 0.72, indicating some correlation between T_o and ΔH_g. But the r value between these same parameters was only 0.29 for the double mutants. These different r values for the same parameters support the idea that the influence of a particular gene on thermal properties varies according to the presence of other mutant genes (Sanders et al 1990). Furthermore, the thermal properties are influenced by structural characteristics of the starch, such as the amylose-amylopectin ratio, differences in fine structure, and degree of crystallinity.

Gelatinization is a semicooperative process (Donovan 1979, French 1983) in which the amorphous regions take up water and swell to a gel phase, generating strain on the crystalline regions. This action stresses the crystallites so that they cooperatively melt at a lower temperature than when not associated with the gel phase. The structural relationship between amorphous regions and crystallites in a starch granule is responsible for the shape and T_o of the endotherm (Krueger et al 1987b). The *wx* starch, being primarily amylopectin, possesses a different amorphous-crystalline structural relationship than does the normal starch granule. Mutant combinations with the *wx* gene produce endosperm starch with no amylose (Boyer et al 1976, Ikawa et al 1981, Yeh et al 1981, Boyer and Liu 1985, Fuwa et al 1987, Sanders et al 1990). Both Stevens and Elton (1971) and Inouchi et al (1984) reported higher ΔH_g and R values for *wx* starch than for normal starch and concluded that there is a more important contribution from amylopectin than from amylose in gelatinization.

In our study, the *wx* starch showed a sharper endotherm and narrower R than did the normal starch and, therefore, a higher PHI value. The narrowed R for gelatinization of *wx* starch might suggest that the melting of starch is highly cooperative and that more energy is needed for initiation in the absence of the amylose-rich amorphous regions (Krueger et al 1987b). Some double mutants containing the *wx* gene (*fl2 wx*, *h wx*, *su1 wx*, *wx du1*, and *wx su1*) had higher PHI than that of normal starch, but *ae wx*, *bt1 wx*, and *bt2 wx* had lower PHI values. Although the *su1 wx* and *wx su1* starches contained the same recessive mutant genes, they exhibited different thermograms (Fig. 3), which may be attributed to the different contributions originating from the female (pistil) or male (pollen) (Yamada et al 1978). These

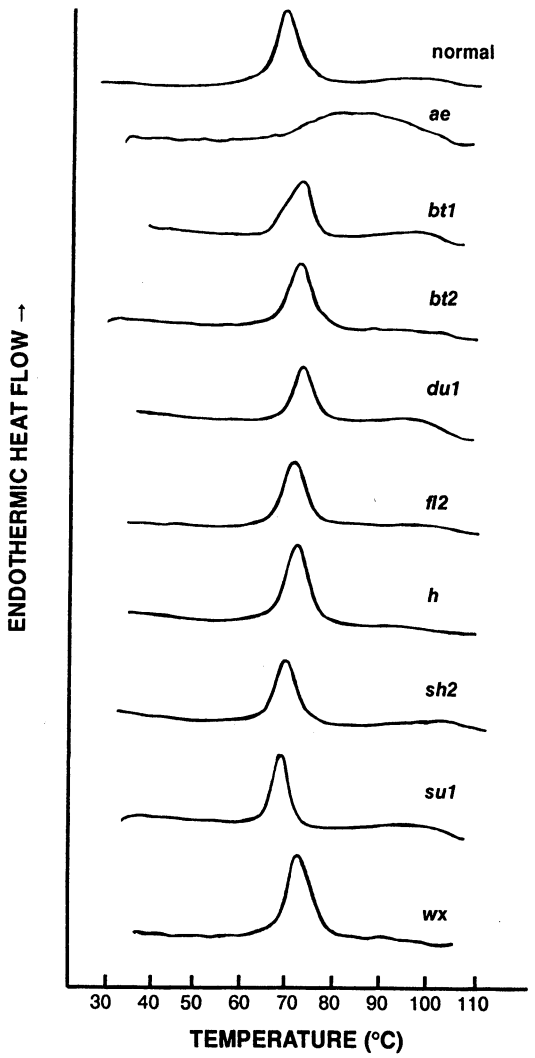

Fig. 2. Differential scanning calorimetry thermograms of single-mutant starches within the Oh43 inbred line.

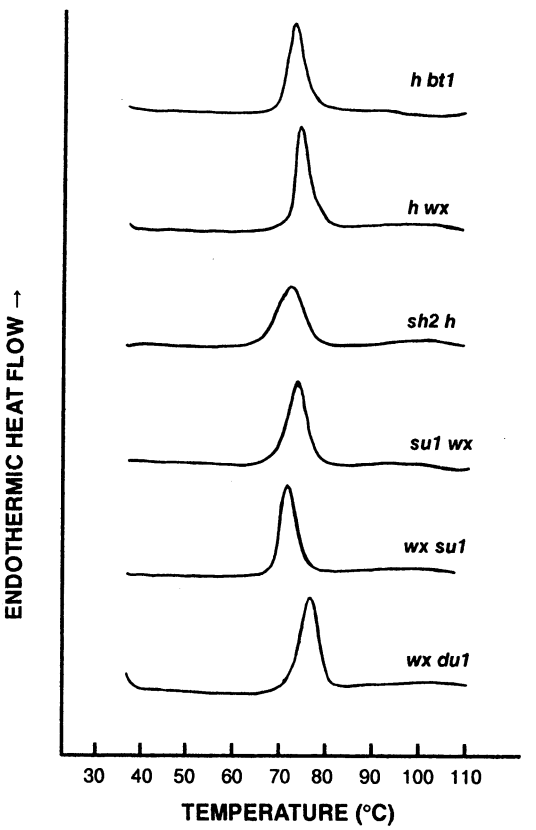

Fig. 3. Differential scanning calorimetry thermograms of selected double-mutant starches within the Oh43 inbred line.

observations suggest that the fine structure of amylopectin among different double mutants containing the *wx* gene may differ and that amylopectin plays a complex role in determining the thermal properties of starch, as suggested by Sanders et al (1990).

The *ae, dul*, and *sul* genotypes are reported to increase amylose content of starch (Ikawa et al 1981, Yeh et al 1981, Inouchi et al 1983, Boyer and Liu 1985). This increase in amylose may dilute the crystalline regions. Consequently, the crystallites may be so far apart that cooperative melting is not possible. Low ΔH and PHI were observed in the *ae, dul*, and *sul* starches, perhaps because of this dilution theory. Inouchi et al (1984) also reported that the ΔH values of the starches increased with decreasing apparent amylose contents. Boyer et al (1976) showed that the *ae* starch possessed longer outer chains than did the *wx* starch. In the present study, the longer exterior chains of *ae wx* starch may be responsible for a broader R, lower ΔH, and lower PHI than those for *wx* starch. In similar work by Yeh et al (1981), most of the mutant combinations containing the *ae* gene produced long exterior chains of amylopectin and, thus, relatively broad endotherms as indicated by their low PHI values. The results suggest that the ratio of amylose to amylopectin in the starch granule, the distribution of amorphous and crystalline regions, and the fine structure of amylopectin are all important in determining the gelatinization properties of the starch.

Refrigerated-Storage Retrogradation

The DSC properties of the starch samples stored at 4°C for seven days (retrogradation) are reported in Table I, and summarized group comparisons are listed in Table II. The endothermic transition for all recrystallized starches occurred at a lower temperature than that of gelatinization ($P < 0.01$), with values ranging from 38.2°C for *h bt2* to 44.4°C for *ae bt2*. Also, the R for the enthalpy peak of retrogradation was broader than that of the native starch ($P < 0.01$). When the gelatinized starch molecules reassociated during storage at 4°C, they formed a weaker structure than in the native molecules, as indicated by the smaller enthalpy values of retrogradation (ΔH_r). The ΔH_r for all samples ranged from 0.9 for *sul* to 1.9 cal/g for *wx*. The ΔH_r of *ae* was difficult to determine because its broad range extended beyond 100°C, so these data were omitted. For most samples, the ratio of ΔH_r to ΔH_g ($r\%$) was close to 50%, meaning that the energy required to regelatinize the starches after seven days of storage at 4°C was about half of its original value. The $r\%$ for the *dul* starch was far higher than the others, at 73.5, which simply reflected its low ΔH_g value.

The *wx* starch displayed the highest retrogradation tendency, as shown by its highest ΔH_r, which supports the idea that amylopectin is responsible for the retrogradation as measured by using DSC (Russell 1983, Eliasson 1985, Eliasson and Ljunger 1988). All of the double mutants containing the *wx* gene had lower ΔH_r than did the *wx* starch ($P < 0.05$). Although the *wx* gene is epistatic in its ability to produce amylopectin, these molecules may vary in structure once the *wx* gene is combined with another mutant gene. Thus, although amylopectin plays an important role in the retrogradation of starch during storage (White et al 1989), the fine structure of amylopectin may play an even more important role in determining the thermal behaviors of starch.

There were no significant differences between Oh43 and single mutants or between Oh43 and double mutants for the retrogradation properties (Table II). The double mutants had significantly ($P < 0.05$) lower T_o and broader R than those of the single mutants for retrogradation. The mutants containing the *bt1* gene had higher T_o and mutants containing the *fl2* or *h* or *sh2* gene had lower T_o than other mutants ($P < 0.01$). No significant difference in ΔH_r was found for all comparisons.

The major variations in the fine structure of amylopectin are the chain length, the distribution of chain lengths, and the ratio of short to long chains (Kalichevsky et al 1990). The branching chain length of amylopectin may have an important effect on the rate of aggregation. As mentioned earlier, starches containing the *ae* gene have longer exterior chains, which may result in a steric effect that decreases the association of starch molecules and lowers ΔH_r compared with that of the *wx* starch ($P < 0.05$). On the other hand, the double-mutant combinations containing the *wx* gene did not exhibit higher ΔH_r than the double mutants not containing the *wx* gene in the present study (Table II). These results suggest that although DSC evidently is sensitive to the amylopectin fraction of the retrograded starches, another type of molecular interaction also may be involved, such as an interaction between amylose and amylopectin (Miles et al 1985a).

Gel Properties

Table III lists the gel properties of all samples as measured by the texture analyzer. A typical load-penetration curve of commercial corn starch at 6% (w/w) solid is shown in Figure 1. Because of the limited sample size available, it was not possible to make large gels in which a freshly cut surface could be exposed, as

TABLE III
Firmness and Stickiness of Starch Gels

Mutant[a]	Firmness[b] (g)	Stickiness[b] (g)
Oh43	2.6	0.8
Single mutants		
ae	2.4	1.1
bt1	2.1	0.6
bt2	2.3	0.9
du1	0.8	0.4
fl2	2.6	0.8
h	2.3	0.7
sh2	3.3	0.8
su1[c]	...	...
wx[c]	...	...
Double mutants		
ae bt2	3.0	1.0
ae h	2.3	0.8
ae sh2	3.3	1.2
ae su1	2.4	0.8
ae wx[c]	...	...
bt1 du1	2.1	0.8
bt1 su1	2.9	0.8
bt1 wx[c]	...	...
bt2 du1	2.1	0.6
bt2 sh2[c]	...	...
bt2 wx[c]	...	...
fl2 ae	2.7	0.7
fl2 bt1	1.8	0.4
fl2 bt2	1.6	0.6
fl2 du1	2.4	0.5
fl2 h	1.4	0.5
fl2 su1	1.6	0.6
fl2 wx[c]	...	...
h bt1	2.0	0.7
h bt2	1.4	0.7
h du1	1.4	0.5
h fl2	1.3	0.5
h sh2	2.0	0.5
h su1[d]	...	...
h wx[c]	...	...
sh2 bt1[d]	...	...
sh2 du1[c]	...	...
sh2 fl2	1.3	0.5
sh2 h	1.8	0.5
sh2 su1	0.9	0.4
sh2 wx[c]	...	...
su1 bt2	1.6	0.6
su1 du1	2.4	0.7
su1 h	1.5	0.4
su1 sh2	2.1	0.5
su1 wx[c]	...	...
wx du1[d]	...	...
wx su1[d]	...	...

[a] *ae* = Amylose extender, *bt* = brittle, *du* = dull, *fl* = floury, *h* = horny, *sh* = shrunken, *su* = sugary, and *wx* = waxy.
[b] Gram-force recorded by the Voland-Stevens instrument. Values are the average of two determinations from two separate extractions.
[c] Gel too weak to support the probe.
[d] Insufficient sample.

described by Takahashi and Seib (1988). Therefore, all the load-penetration curves in this study showed a drop in force after the probe penetrated the gel surface (noted at about the 1.8-mm distance on Fig. 1) likely resulting from the break through the "skin" on the gel surface. Nonetheless, the peak height at 3-mm compression (noted at about the 3.8-mm distance on Fig. 1) was an accurate measure of inside gel firmness as measured by Takahashi and Seib (1988). All of the *wx*-containing starches and a few others formed weak gels not measurable under the test conditions because the gels were too soft. The present conditions required a force of 0.5 g to be reached before the probe traveled its 3 mm through the gel. Thus, the probe hit the bottom of the vial before traveling the required distance through the soft gels. The firmness of the starch gels ranged from 0.8-g force for the *du1* starch to 3.3-g force for the *sh2* and *ae sh2* starch. The *ae sh2* exhibited the highest stickiness at 1.2-g force, whereas *du1, fl2 bt1, sh2 su1*, and *su1 h* had the lowest stickiness scores of 0.4-g force.

Correlations between gel strength parameters and all DSC thermal behavior parameters were run, with most correlation values being less than 0.6, so the data are not shown. Firmness and stickiness values correlated somewhat with ΔH_r with r values of -0.74 and -0.62, respectively. These negative correlations suggest that starches with greater tendency to retrograde produced less firm and less sticky gels. Much of this behavior could be explained by the effects of *wx* versus *ae* starch.

Initial gel formation has been reported to correlate with the amylose fraction, which, being linear, has the ability to quickly form junction zones, reassociate, and reestablish intermolecular hydrogen bonds (Howling 1980). The increase in the firmness of a starch gel after the initial cool down is related to the crystallization of amylopectin within the gelatinized starch granule (Ring et al 1987). Because it is a branched molecule, amylopectin cannot form junction zones and, thus, maintains a poor resistance to penetration. Some researchers propose that gelation of an amylose dispersion occurs only after exceeding a certain concentration (C^*) (Miles et al 1985a, Ring et al 1987). The gel formation arises as a result of a phase separation that produces polymer-rich and polymer-deficient regions. If the amylose concentration is sufficiently high, the polymer-rich regions form an interconnected gel network (Miles et al 1985a). The C^* for amylose of molecular weight 5×10^5 was ~1.5% (Miles et al 1985a). At a fixed molecular weight, the branching of amylopectin reduced the hydrodynamic volume, resulting in a C^* that was shifted toward a higher value than for a linear chain.

By studying the gelation of amylose and amylopectin, Miles et al (1985a) and Ring et al (1987) found that the formation of a network, as measured by the shear modulus, lagged behind the development of crystallinity, as detected by X-ray diffraction and DSC. The low correlations between texture analyzer and DSC results in the current study support these results. Miles et al (1985b) also showed that the formation of a starch gel could be separated into two processes, short term and long term. The short-term process was dominated by irreversible gelation within the amylose matrix, and the long-term one was linked to a reversible crystallization involving amylopectin. The negative correlations between ΔH_r and firmness and stickiness measurements in the current work supports their observations. Increased formation of a retrograded gel (ΔH_r) did not mean increased firmness and stickiness, suggesting more than one development process.

CONCLUSIONS

Amylose and amylopectin both are important to the thermal properties and firmness of starch gels; however, the various responses of the samples to DSC analyses suggest that structural differences beyond those of amylose and amylopectin also influence these characteristics. The data should be verified by studying the mutant effect in other varieties and under different growing conditions. Other work has shown an environmental effect on DSC properties of starches grown in two environments (White et al 1991). The T_o values were higher and the R values were lower in starches grown in a tropical rather than temperate environment; however, there was a cultivar by location interaction. These observations should be considered when evaluating the few samples in our work that were grown near Ames, IA, rather than in Puerto Rico. But, for the most part, the starches grown near Ames were *ae* single and double mutants that can be compared within one environment. Also, averaged over all samples, the double mutants had higher T_o and ΔH and lower R values than did the single mutants. To understand these relationships, future work will involve studying the effect of single and double mutants of Oh43 on the structures of starch components. In some cases, the fine structures of amylose and amylopectin will be determined to relate the physical properties to the chemical structures.

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[Received August 5, 1991. Accepted December 13, 1991.]