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

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Disciplines
Food Biotechnology | Food Chemistry | Food Processing | Food Science | Human and Clinical Nutrition

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

Y.-J. WANG, P. WHITE, and L. POLLAK

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 [dul1], 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 (To), 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 dul1 and sh2 dul1 had the highest To. Double-mutants ae bt2 and ae dul1 had the highest To 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 To and ΔH for gelatinization and lower To for retrogradation. For gel strength, the dul1 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 dul1.

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, for example, opaque-2 (O2), brittle (bt), dull (dul), 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,
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 ($\Delta H$), 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 calculated 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

<table>
<thead>
<tr>
<th>Starch</th>
<th>$T_o$ (°C)</th>
<th>$R$ (°C)</th>
<th>$\Delta H$ (cal/g)</th>
<th>PHII</th>
<th>$T_p$ (°C)</th>
<th>$R$ (°C)</th>
<th>$\Delta H_f$ (cal/g)</th>
<th>$r%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oh43</td>
<td>67.2</td>
<td>8.8</td>
<td>2.9</td>
<td>0.67</td>
<td>42.6</td>
<td>16.9</td>
<td>1.5</td>
<td>49.5</td>
</tr>
</tbody>
</table>

**Single mutants**

- $ae^{b}$: 68.7, 31.3, 3.7, 0.24
- $bt^{l}$: 63.4, 14.9, 2.5, 0.33
- $bl^{2}$: 66.8, 10.6, 2.9, 0.37
- $du^{l}$: 62.7, 9.1, 2.1, 0.46
- $fl^{2}$: 66.2, 9.1, 2.9, 0.64
- $h$: 65.6, 9.4, 2.7, 0.57
- $sh^{2}$: 64.3, 8.5, 2.4, 0.57
- $su^{l}$: 66.4, 7.1, 2.1, 0.80
- $wx^{b}$: 68.6, 9.0, 3.6, 0.80

**Double mutants**

- $ae$ $bt^{2b}$: 67.1, 10.3, 2.9, 0.57
- $ae$ $h^{b}$: 69.6, 9.5, 2.7, 0.58
- $ae$ $sh^{2b}$: 68.2, 9.7, 2.9, 0.60
- $ae$ $su^{b}$: 65.4, 10.7, 2.6, 0.49
- $ae$ $wx^{b}$: 70.1, 9.2, 2.8, 0.61
- $bt^{l}$ $dl^{b}$: 67.3, 8.5, 2.8, 0.66
- $bt^{l}$ $su^{l}$: 67.8, 9.4, 3.0, 0.63
- $bt^{l}$ $wx^{b}$: 68.2, 10.3, 3.1, 0.60
- $bt^{l}$ $dl^{b}$: 66.9, 9.5, 2.8, 0.59
- $bt^{l}$ $sh^{2b}$: 67.8, 9.8, 2.9, 0.59
- $bt^{l}$ $wx^{b}$: 69.4, 9.4, 3.0, 0.64
- $fl^{2}$ $ae$: 67.1, 10.9, 3.2, 0.59
- $fl^{2}$ $bt^{l}$: 68.1, 7.0, 3.0, 0.87
- $fl^{2}$ $bi^{2}$: 68.1, 7.7, 3.3, 0.84
- $fl^{2}$ $dl^{b}$: 67.8, 6.9, 3.3, 0.94
- $fl^{2}$ $h$: 68.3, 6.9, 3.1, 0.89
- $fl^{2}$ $su^{l}$: 67.1, 7.8, 3.2, 0.61
- $fl^{2}$ $wx$: 70.4, 8.1, 3.1, 0.77
- $h^{bt}$: 61.8, 5.8, 3.2, 1.09
- $h^{bt^{2}}$: 68.5, 8.7, 3.0, 0.69
- $h^{dl}$: 67.2, 7.8, 2.9, 0.74
- $h^{fl^{2}}$: 67.7, 7.9, 3.2, 0.80
- $h^{sh^{2}}$: 68.4, 6.7, 3.1, 0.93
- $h^{su^{l}}$: 67.5, 7.5, 2.8, 0.75
- $h^{wx}$: 69.7, 4.9, 3.0, 1.74
- $sh^{2}$ $bt^{l}$: 67.7, 9.0, 2.8, 0.63
- $sh^{2}$ $dl^{b}$: 70.3, 5.1, 3.3, 1.31
- $sh^{2}$ $fl^{2}$: 69.3, 7.1, 3.0, 0.85
- $sh^{2}$ $h$: 65.0, 10.1, 3.0, 0.58
- $sh^{2}$ $su^{l}$: 68.9, 9.0, 3.0, 0.66
- $sh^{2}$ $wx^{b}$: 68.1, 10.9, 3.0, 0.55
- $su^{l}$ $bt^{2}$: 67.0, 8.6, 3.1, 0.71
- $su^{l}$ $dl^{b}$: 68.2, 8.1, 2.5, 0.62
- $su^{l}$ $h$: 68.7, 6.6, 2.7, 0.82
- $su^{l}$ $sh^{2}$: 67.5, 7.5, 3.0, 0.79
- $su^{l}$ $wx$: 67.4, 7.9, 3.1, 0.78
- $wx^{l}$ $dl^{b}$: 70.9, 7.5, 3.3, 0.87
- $wx^{l}$ $su^{l}$: 68.3, 5.4, 3.3, 1.23

**Means**

- LSD$_{0.05}$: 0.70, 0.72, 0.2
- $R$ (°C) = 1.06, 1.78, 0.1
- $r%$: 4.58

---

1. Values are the average of three determinations each from two separate extractions. $ae$ = Amylose extender, $bt$ = brittle, $du$ = dull, $fl$ = floury, $h$ = horny, $sh$ = shrunked, $su$ = sugary, and $wx$ = waxy.
2. Onset temperature.
3. Gelatinization range calculated as $2(T_p - T_o)$, as described by Krueger et al. (1987a).
4. Enthalpy of gelatinization.
5. Peak height index = $\Delta H/(T_p - T_o)$ as described by Krueger et al. (1987a).
7. Ratio of enthalpy of retrogradation to enthalpy of gelatinization.
8. Mutants grown in Ames, IA. Other mutants were grown in Puerto Rico.
9. Data are omitted because its broad thermogram extended beyond 100°C.
(PHI), which is the ratio $\Delta H/(T_p - T_c)$, was calculated to allow a quantitative evaluation of variations in peak shape (Krueger et al. 1987a).

**Results and Discussion**

**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 dw) 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., Hawthorne, 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.

**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 ($\Delta H_g$) 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 (Wootten and Bamunuarachchi 1979, Biladeris et al. 1980). The reported differences may be attributable to environmental effects (White et al. 1991). The $wx$ genotype produced higher $T_o$ and $\Delta H_g$ for gelatinization than did the single mutants, which was similar to previous reports (Inouchi et al. 1984, Fuwa et al. 1987).

The PHI ($\Delta H/[T_p - T_c]$) 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, broad 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 $dul$ starch had the lowest $\Delta H_g$ (2.1 cal/g), which was lower than that of the same genotype ($P < 0.05$). The $R$ and $\Delta H_g$ of $bt1$, $dul$, $sh2$, and $sul$ 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 $sh2$ to $70.9°C$ for $wx$ $dul$. 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 $\Delta H_g$, and the highest PHI among double mutants (Fig. 3). The $wx$ $sul$ also exhibited a sharp endothermic peak and a high $\Delta H_g$ similar to that of the $h wx$ (Fig. 3). The double-mutant combinations containing the $wx$ gene ($h wx$, $wx$ $sul$, and $wx$ $dul$) had higher $\Delta H_g$ values

**Figure 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

<table>
<thead>
<tr>
<th>Starch Group Comparison</th>
<th>Gelatinization</th>
<th>Retrogradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_o$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Oh43 vs. all mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Oh43 vs. single mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Oh43 vs. double mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Single vs. double mutants</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>ae vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$bt1$ vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$bt2$ vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>dul vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>fl2 vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>h vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>sh2 vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>sul vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>wx vs. other mutants</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$ae$ = Amylose extender, $bt$ = brittle, $dul$ = dull, $fl$ = floury, $h$ = horny, $sh$ = shrunken, $su$ = sugary, and $wx$ = waxy.

$*P < 0.05$.

$*P < 0.01$ levels of probability, respectively.

$\Delta H_g$ for gelatinization than did other single mutants, which was similar to previous reports (Inouchi et al. 1984, Fuwa et al. 1987).
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 $wx$ $sul$ 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 $T_0$ and $\Delta H_g$ and lower $R$ values than the single mutants ($P < 0.01$). No significant difference was found, except $\Delta H_g$ 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 $dul$ gene exhibited significantly lower $T_0$ and higher $R$ than other mutants. In contrast, the $h$ or $wx$ gene produced mutants with high $T_0$ and low $R$ values. As indicated earlier, the mutants with the $wx$ gene produced significantly higher $\Delta H_g$ ($P < 0.01$) than did the other mutants. Most mutants containing the same recessive mutant gene possessed distinctive thermophysical properties, 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 $T_0$ and $\Delta H_g$ for single mutants was 0.72, indicating some correlation between $T_0$ and $\Delta H_g$. 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 $T_0$ 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 $\Delta H_g$ 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 ($fl2$ $wx$, $h$ $wx$, $sul$ $wx$, $wx$ $dul$, and $wx$ $sul$) had higher PHI than that of normal starch, but $ae$ $wx$, $bt1$ $wx$, and $bt2$ $wx$ had lower PHI values. Although the $sul$ $wx$ and $wx$ $sul$ 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.](image1)

![Fig. 3. Differential scanning calorimetry thermograms of selected double-mutant starches within the Oh43 inbred line.](image2)
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 reassocciated during storage at 4°C, they formed a weaker structure than in the native molecules, as indicated by the smaller enthalpy values of retrogradation (ΔHr). The ΔHr for all samples ranged from 0.9 for sul to 1.9 cal/g for wx. The ΔHr 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 ΔHr to ΔHg (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 ΔHg value.

The wx starch displayed the highest retrogradation tendency, as shown by its highest ΔHr, 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 ΔHr 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 Tg and broader R than those of the single mutants for retrogradation. The mutants containing the bt1 gene had higher Tg and mutants containing the fl2 or h or sh2 gene had lower Tg than other mutants (P<0.01). No significant difference in ΔHr 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 ΔHr 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 ΔHr 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).

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Firmness&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Stickiness&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> = Amylose extender, <sup>b</sup> = Amylose extender, <sup>d</sup> = Amylose extender, <sup>f</sup> = Amylose extender, <sup>g</sup> = Amylose extender, <sup>h</sup> = Amylose extender, <sup>s</sup> = Amylose extender, <sup>u</sup> = Amylose extender, <sup>w</sup> = Amylose extender, <sup>x</sup> = Amylose extender, <sup>y</sup> = Amylose extender, <sup>z</sup> = Amylose extender.
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, f2 bt1, sh2 sul, and sul 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 $\Delta H_f$ 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 amylopectin 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 X 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 $\Delta H_f$ and firmness and stickiness measurements in the current work supports their observations. Increased formation of a retrograded gel ($\Delta H_f$) 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 $T_c$ 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 $T_c$ and $\Delta H_f$ 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.

LITERATURE CITED


MILES, M. J., MORRIS, V. J., and RING, S. G. 1985a. Gelation of


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