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Thermal Properties of Starch from Selected Maize (Zea mays L.) Mutants During Development

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Abstract

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ABSTRACT

The changes in thermal properties of maize starches during five stages of kernel maturity, (12, 18, 24, 30, and 36 days after pollination [DAP]), from three mutant genotypes, *amylose extender* (*ae*), *sugary*-2 (su_2), and *waxy* (*wx*) in an OH43 background, and the OH43 genotype were studied using differential scanning calorimetry (DSC). Within a genotype, DSC values of starches at 24, 30, and 36 DAP were similar to each other and often were significantly different ($P < 0.05$) from the values at 12 DAP, indicating possible differences in the fine structure of starch during endosperm development. For su_2 starches, the gelatinization onset temperature (T_{oG}) significantly decreased after 12 DAP and remained low throughout the study. The gelatinization range (R_G) had a similar pattern. For *wx* starches, T_{oG} at 18 DAP was significantly lower than at 12 DAP but tended to increase after 18 DAP. The R_G increased significantly after 12 DAP and significantly decreased after 30 DAP. Thus, thermal properties of starches during early development were different from those of their mature counterparts, and differences among the mutant genotypes and the normal starch originated from the earliest endosperm development stage studied (12 DAP).

The food industry currently uses chemically modified starches from many sources to suit their specific needs. Chemical modification of starch is utilized because there are few native starches that provide specific desirable functional properties. Consumer demand for "clean-label" products containing "natural" ingredients has increased the demand for natural sources of starch with desirable functional characteristics in many food applications. These natural starches require less processing and might also reduce production costs. A number of patents have been issued on the use of single-, double-, and triple-mutant maize starches as thickening agents that, for example, are stable in an acidic environment (White et al 1994), exhibit superior freeze-thaw stability (Friedman et al 1988), and act as fat substitutes (Pearlstein et al 1994). There are many other patents for uses of other natural starches.

Many studies have characterized thermal properties of different maize genotypes by using differential scanning calorimetry (DSC) (Sanders et al 1990, White et al 1990, Inouchi et al 1991a, Wang et al 1992). Extensive variations in the DSC values among various maize genotypes were reported in these studies, suggesting differences in starch structure and functional properties. Inouchi et al (1983) studied the change in fine structure of starches of normal maize and five endosperm mutants of maize (*amylose-extender* [*ae*], *waxy* [*wx*], *dull* [*du*], *sugary*-1 [su_1] and *sugary*-2 [su_2]) at three stages of growth (21, 28, and 35 days after pollination [DAP]) by gel filtration after debranching by isoamylase. Starches of normal, *ae*, *du*, su_1, and su_2 maize had increasing amylose contents during the three developmental stages studied. In normal, *ae*, su_1, and su_2 starches, the content of amylopectin (with 11–20 DP after debranching by isoamylase) decreased and contents of amylose plus the intermediate materials increased from 21 to 28 DAP. From 28 to 35 DAP, the amylose content increased. Inouchi et al (1984) studied the DSC properties of these same starches (except for su_1) at the same three stages of growth (21, 28, and 35 DAP). Within a starch genotype, the onset temperatures (T_o), peak temperatures (T_p) and heat of gelatinization (ΔH) were nearly the same for all stages of development. No relationship was established between the DSC values and the change in fine structure of the mutant starches they studied during kernel development. In addition, no data were available regarding the changes in thermal properties after refrigerated-storage retrogradation at the different developmental stages. Retrogradation is related to the stability of a starch paste during storage.

The purpose of this investigation was to study the changes in thermal properties of starch from several maize (*Zea mays* L.) genotypes at earlier stages of maturity than previously reported. The DSC was used to determine the differences in thermal properties of starch from three maize mutants (*ae*, su_2, and *wx*) and normal maize from the same genetic background (OH43). The su_2 maize used in this study was a unique line of su_2 mutant in an OH43 background that has been patented for use in acidic foodstuffs (White et al 1994). The DSC properties of the su_2 starch was compared with the properties of the regular su_2 maize mutant during development reported by Inouchi et al (1983).

MATERIALS AND METHODS

Materials

Kernels of OH43 and its single mutants *ae*, su_2, and *wx* used in this study were grown in a summer nursery near Ames, IA, in 1991. The materials were harvested at five different stages of maturity: 12, 18, 24, 30, and 36 DAP and were freeze dried at –80°C for 72 hr (mc <1%) immediately after harvesting. The corn was planted in a randomized complete-block design with at least seven replicates for each DAP of each mutant. The average growing temperature in the summer nursery near Ames between May and October was about 66.4°F with 3.41 in. of precipitation (Hallauer et al 1991). Three ears of each mutant at each DAP were randomly selected for DSC analysis. All samples were stored at 4°C at 45% rh until analyzed.

Starch Isolation

Starches were isolated according to a small-scale wet-milling procedure described by White et al (1990). The starch was dried at room temperature (22–24°C) with a fan circulating air over the starch. Two separate starch extractions, ≈0.5 g of kernels each, were made from each of three ears per stage of growth per genotype for the determination of DSC thermal properties. The starch

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yield after isolation was ≈40–50% of total starch content. For each starch extraction, two DSC runs were performed, and the 12 values were averaged except for su_2 at 12 DAP. From the available nine ears of su_2 at 12 DAP, only one contained enough starch for DSC analysis because of inadequate endosperm development. Therefore, only two DSC runs were performed for su_2 starch at 12 DAP, and the average values are reported.

DSC

For DSC analysis, a Perkin-Elmer DSC 7 analyzer equipped with a thermal-analysis data station (Perkin-Elmer Corp., Norwalk, CT) was used. Analysis of starch gelatinization was conducted as described by White et al (1990). All starch samples (≈4.0 mg, dwb) were weighed in aluminum sample pans with 8 mg of distilled water and were heated from 30–120°C at a rate of 10°C/min, except for *ae* starch. Because gelatinization of *ae* starch extends beyond 100°C (Inouchi et al 1991a, Wang et al 1992), stainless steel pans and a heating range of 30–150°C were used. For all the DSC analyses, an empty pan with 8 mg of distilled water was used as a reference. DSC parameters recorded were onset (T_{oG}), range (R_G), enthalpy (ΔH_G), and peak height index (PHI). Onset (T_{oG}), peak (T_{pG}), and enthalpy (ΔH_G) were computed directly by the DSC software. At the water level used, the DSC endotherms were essentially symmetrical, which allowed the total gelatinization range to be computed as $2(T_{pG} - T_{oG})$ as described by Krueger et al (1987). The PHI, which is the ratio $\Delta H_G / (T_{pG} - T_{oG})$, was calculated to allow quantitative evaluation of variations in peak shape (Krueger et al 1987). To determine retrogradation characteristics, starch samples used for gelatinization were stored for seven days at 4°C and then rescanned on the DSC to determine onset temperature (T_{oR}), range (R_R), enthalpy(ΔH_R), and starch gel retrogradation (%*R*) as described by White et al (1989). The %*R* is a measure of the tendency of the gelatinized starch to retrograde after low-temperature storage. The lower the %*R*, the less tendency there is to retrograde, and the more stable is the gelatinized starch paste. All DSC values reported are the average of two scans each for three extractions from each stage of growth for each genotype except for su_2 at 12 DAP. Enthalpies were calculated on a starch dry weight basis.

Statistical Analysis

A completely randomized design was used to determine significance of kernel maturity on DSC parameters. Analysis of variance (ANOVA) and least significant difference (LSD) analyses (α = 0.05) were computed by using the Statistical Analysis System (SAS 1990).

RESULTS AND DISCUSSION

The DSC properties of starches of OH43 and its mutants at five different stages of maturity are summarized in Table I and Fig. 1. The DSC values of starches from 12 and 18 DAP were generally more variable among replicates than were those at later stages of maturity. In addition, starches at 24, 30, and 36 DAP were similar to each other in DSC values and often were significantly different from the values at 12 DAP.

Normal Starch

For normal starch, no significant differences were found for the DSC values among DAP except for R_G and %*R* (Table I). The R_G at

TABLE I
Mean Differential Scanning Calorimetry (DSC) Parameters for Maize Genotypes[a] at Five Stages of Kernel Maturity[b]

		Gelatinization[c]				Refrigerated-Storage Retrogradation[d]			
Genotype	DAP	T_{oG} (°C)	R_G (°C)	ΔH_G (cal/g)	PHI	T_{oR} (°C)	R_R (°C)	ΔH_R (cal/g)	%*R*
Normal	12	68.8a[e]	7.5b	2.98a	0.81a	45.1a	18.6a	1.70a	56.7a
	18	69.8a	9.8a	3.59a	0.78a	44.8a	19.1a	1.81a	50.6b
	24	68.9a	7.9ab	3.41a	0.87a	43.4a	19.1a	1.73a	51.0b
	30	69.5a	7.0b	3.54a	1.02a	44.4a	18.1a	1.65a	46.6b
	36	68.4a	9.1ab	3.48a	0.77a	43.7a	18.5a	1.74a	50.1b
		(0.56)	(1.15)	(0.24)	(0.10)	(0.72)	(0.43)	(0.06)	(3.63)
ae	12	69.1a	12.7b	2.79a	0.45a	53.7a	24.4b	0.80a	31.6a
	18	71.0a	30.1a	3.95a	0.29ab	62.5a	42.4ab	2.02a	46.4a
	24	68.1a	33.7a	4.17a	0.26b	63.9a	55.1a	1.67a	40.7a
	30	68.3a	32.7a	3.74a	0.23b	57.7a	60.4a	2.29a	59.1a
	36	68.0a	28.8a	3.39a	0.24b	59.3a	46.4ab	2.16a	66.7a
		(1.25)	(8.56)	(0.54)	(0.09)	(4.04)	(13.9)	(0.60)	(14.1)
su_2	12	70.2a[f]	11.7a	1.27a	0.22b	47.0ab	16.3ab	0.66a	55.3a
	18	59.4b	9.2ab	1.60a	0.38a	43.2b	16.8a	0.60ab	38.5ab
	24	57.2b	8.5b	1.71a	0.40a	45.5ab	13.5cd	0.44bc	25.6b
	30	57.4b	8.3b	1.65a	0.40a	45.9ab	14.6bc	0.42c	25.4b
	36	56.3b	8.6b	1.56a	0.36a	49.2a	12.2d	0.39c	25.2b
		(5.76)	(1.40)	(0.17)	(0.08)	(2.19)	(1.91)	(0.12)	(13.2)
wx	12	70.8a	6.6c	2.74b	0.85ab	45.1a	17.6a	1.73c	63.2a
	18	68.6b	8.4b	3.87a	0.92ab	44.3a	18.3a	2.36b	61.1a
	24	69.2ab	10.1a	3.95a	0.78b	44.7a	18.0a	2.51ab	63.5a
	30	69.6ab	9.7ab	4.25a	0.88ab	44.7a	18.3a	2.66a	62.8a
	36	70.1ab	8.5b	3.94a	0.93a	42.9a	19.6a	2.48ab	63.1a
		(0.84)	(1.37)	(0.58)	(0.06)	(0.85)	(0.75)	(0.36)	(0.95)

[a] Normal, *amylose extender* (*ae*), *sugary*-2 (su_2) and *waxy* (*wx*).
[b] Values at each day after pollination (DAP) are the means of three replicates from three separate ears. Average standard deviations of data for all DAP for each genotype are in parentheses.
[c] T_{oG} = gelatinization onset temperature; R_G = gelatinization range calculated as $2(T_{pG} - T_{oG})$, as described by Krueger et al (1987); ΔH_G = enthalpy of gelatinization; PHI = peak height index calculated as $\Delta H_G/(T_{pG} - T_{oG})$ as described by Krueger et al (1987).
[d] T_{oR} = retrogradation onset temperature; R_R = retrogradation range; ΔH_R = enthalpy of retrogradation; %*R* = ratio of enthalpy of retrogradation to enthalpy of gelatinization.
[e] Means followed by different letters are significantly different ($P < 0.05$) among DAP within a genotype.
[f] Values reported for 12 DAP are the average of two DSC runs.

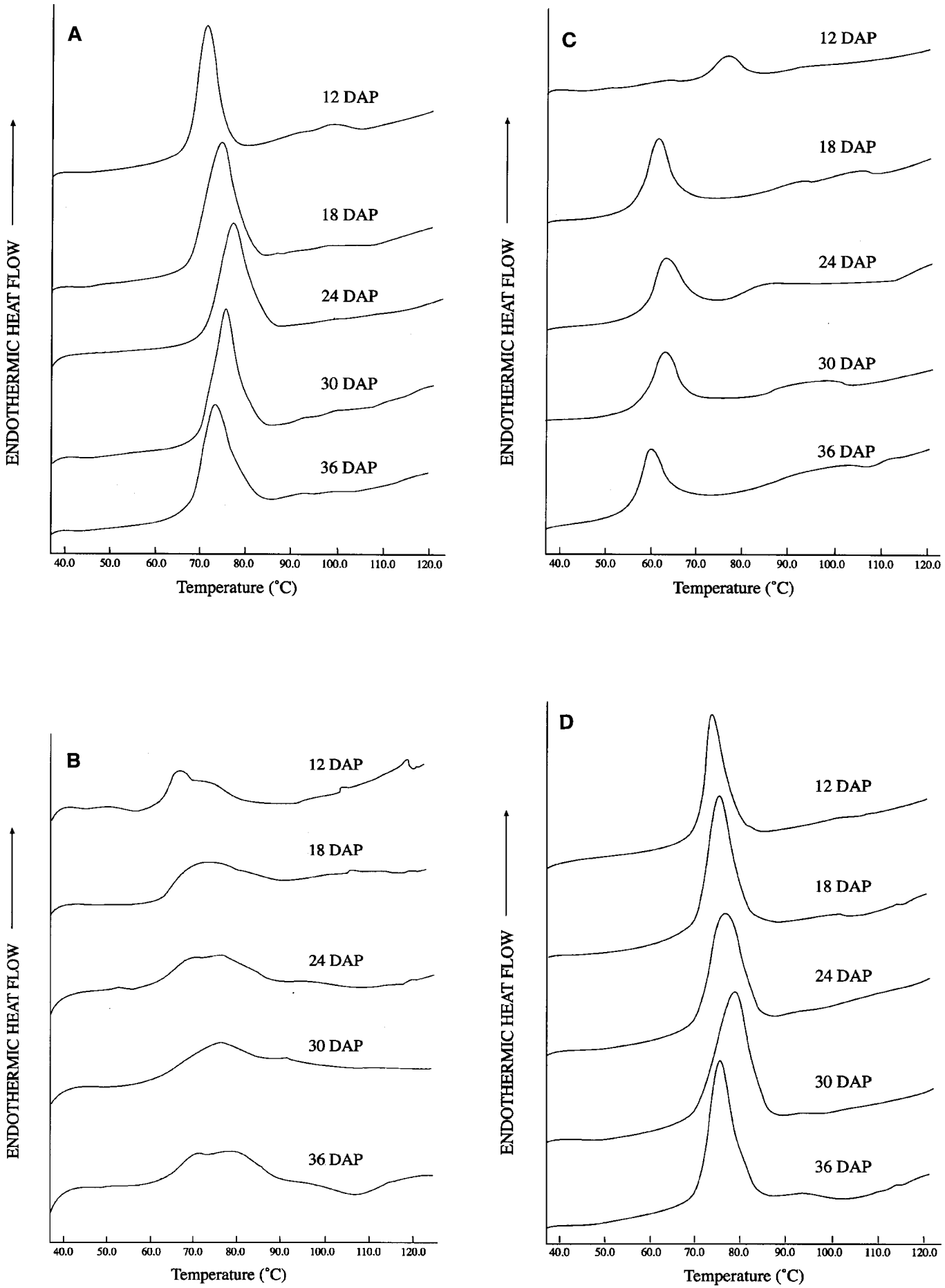

Fig. 1. Representative differential scanning calorimetry (DSC) gelatinization thermograms of starches at various days after pollination (DAP): **A,** normal; **B,** *ae* ; **C,** *su*$_2$; **D,** *wx*.

12 and 30 DAP were significantly narrower ($P < 0.05$) than the R_G at 18 DAP, and %R at 12 DAP was significantly greater than %*R* at other DAP. The %*R* value is relative to the original ΔH_G. Because the latter value tended to be lower at 12 DAP, and the ΔH_R value was similar to values at other DAP, the %*R* for starch at 12 DAP was greater than the %*R* at other DAP. Previous researchers showed that starch granules were observed in corn endosperm as early as 4 DAP (Whistler and Thornburg 1957), but the most rapid increase in starch content occurred in the period from 12 to 20 DAP (Wolf et al 1948). As a result, the starch extracted from the maize kernels at 12 and 18 DAP might be composed of a wide distribution of components of starches with different chain lengths and of greater heterogeneity than those at later stages of maturity and, thus, exhibit different DSC values. Peaks resulting from the melting of amylose-lipid complexes were not apparent at 12 DAP (Fig. 1A). One out of three replicates showed the peaks resulting from melting of amylose-lipid complexes at 18 DAP (peak beginning at ≈85°C), whereas two out of three replicates showed those peaks at 24 DAP. Starting at 30 DAP, the peaks representing melting of amylose-lipid complexes were apparent in all replicates. Lipids in normal maize are thought to begin depositing in the kernel at ≈21 days after fertilization (DAF) (Watson 1987). The DAF is equivalent to DAP. Therefore, the peaks caused by melting of amylose-lipid complexes from 24 to 36 DAP were expected. The inconsistencies in melting of the peaks we observed at 18 DAP might be a result of biological variation during development of that particular plant.

The T_{oG}, T_{oR}, ΔH_G, ΔH_R, PHI, and R_R of normal starch were slightly greater in this study at all stages than those reported by Inouchi et al (1984) and Wang et al (1992), where mature kernels were analyzed. For example, the T_{oG} and ΔH_G in Inouchi's study (1984) were 68°C and 3.3 cal/g, respectively, whereas in the current study T_{oG} ranged from 68.4 to 69.8°C and ΔH_G ranged from 2.98 to 3.59 cal/g. The R_G was narrower at 12, 24, and 30 DAP (7.5, 7.9, and 7.0°C, respectively) in this study than in the study reported by Wang et al (1992) (8.8°C), whereas the R_G at 18 and 36 DAP (9.8 and 9.1°C, respectively) were wider in the current study. These differences in DSC values between studies may be attributable to environmental effects on the source of maize used (White et al 1991).

ae Starch

For *ae* starches, DSC values among DAP were not significantly different from each other except for R_G, R_R, and PHI (Table I). Representative DSC gelatinization thermograms of *ae* starches at various DAP are shown in Fig. 1B. The *ae* starch peaks were low and broad, and the peak temperature value was ill-defined as also noted by others (Inouchi et al 1984, Inouchi et al 1991a, Wang et al 1992). As a result, the R_G, R_R, PHI, ΔH_G, and ΔH_R for *ae* starches varied greatly among replicates and DAP. The R_G and R_R of *ae* starches increased significantly from 12 to 24 DAP and tended to decrease from 24 to 36 DAP. There were no significant differences among DAP for T_{oG}, T_{oR}, ΔH_G, and ΔH_R. These changes might be explained by the changes in amount of intermediate materials for *ae* starches during development, which Inouchi et al (1983) reported to increase from 21 to 28 DAP and then decrease again from 28 to 36 DAP. Yuan et al (1993) suggested that the lack of homogeneity of ordered structure inside the granules might cause a broader gelatinization range. With an increase in intermediate materials, a broader R_G and R_R might be expected. The PHI of starches at 12 DAP were almost twice as great as those at later developmental stages, indicating taller and sharper endothermic peaks (i.e., narrower R_G at 12 DAP), possibly reflecting the effect of the increase in the amount of intermediate materials on the R_G and R_R during endosperm development.

Biliaderis et al (1980) suggested that the melting temperature range is affected by the degree of branching of amylopectin (the greater the degree of branching, the wider the melting temperature range). Inouchi et al (1983) reported that the ratio of amylopectin "with shorter chains" to amylopectin "with longer chains" can be used as an index of the extent of branching of amylopectin (the greater the ratio, the greater the degree of branching). Although Inouchi et al (1983) found no significant differences in this ratio for *ae* starch from 21 DAP to 35 DAP, the increase of R_G and R_R from 12 DAP to 24 DAP found in the current study might be attributed to the increase in the extent of branching of amylopectin in *ae* starch from 12 to 24 DAP. Another possible reason for the increase of R_G and R_R from 12 DAP to 24 DAP might be the formation of peaks from the melting of amylose-lipid complexes, beginning at ≈85°C (Fig. 1B). Perhaps the chain lengths of starches were not long enough at 12 DAP for proper complex formation, or perhaps there was not enough lipid present. Inouchi et al (1983) reported that amylose chains of *ae* starch at ≈20 DAP appeared to be relatively shorter than those at later stages of development. Watson (1987) reported that lipid deposition in maize starts at ≈21 DAF and continues at a steady state to maturity.

Except for data at 12 DAP, the values for T_{oG}, R_G, ΔH_G, and PHI found in this study were similar to those reported by Sanders et al (1990) and Wang et al (1992) but greater than those reported by Inouchi et al (1991a). The reported differences of T_{oG}, R_G, ΔH_G, and PHI at 12 DAP might be because of insufficient starch for analysis and variations in distribution of chain length materials in immature kernels. The DSC values found in this study, which are greater than the values found by Inouchi et al (1991a), might be attributable to environmental effects as suggested by White et al (1991).

su$_2$ Starches

For *su*$_2$ starches, the T_{oG} significantly decreased from 12 to 18 DAP. Representative DSC gelatinization thermograms of *su*$_2$ starches at various DAP are shown in Fig. 1C. The standard deviation among DAP was high for T_{oG} because the DSC thermogram for starch from 12 DAP was distinct from those at other DAP and had a significantly greater T_{oG} (Fig. 1C). Because only two DSC runs were performed due to limited material availability, the difference for T_{oG} might be attributed to the biological variation of that individual plant. More material is needed to verify the differences. The melting of amylose-lipid complexes was observed at all DAPs, with peaks beginning at ≈85°C except at 12 DAP (Fig. 1C). This observation might be because lipid was not deposited in significant amounts in the maize before 21 DAF as reported by Watson (1987) or because chain lengths of starches were not long enough for complex formation. In work by Inouchi et al (1983), amylose chains of *su*$_2$ starches at ≈20 DAP were shorter than those at later stages of development. The R_G tended to decrease from 12 to 24 DAP and remained the same from 24 to 36 DAP, but the ΔH_G tended to increase from 12 to 18 DAP. Inouchi et al (1983) reported that for *su*$_2$ starch, contents of amylopectin "with shorter chains" decreased and contents of amylose and intermediate materials increased from 21 to 28 DAP, and contents of amylose increased from 28 to 35 DAP. The decrease of T_{oG} and R_G, and the increase of ΔH_G found during maturation of the kernels in the current study might be explained by an increase in amylose and intermediate materials, and amylopectin with long external chains as a result of elongation of the shorter chains. The changes in the T_{oG}, R_G, and ΔH_G might also be attributable to the changes in native alignment and hydrogen bonding of the starch molecules during development (White et al 1990).

Some significant differences also were observed for the DSC values of retrograded *su*$_2$ starch. These differences did not follow any specific pattern among DAP or DSC characteristics. The differences were likely a result of biological variations in the plants. The T_{oG} and R_G at all stages, were greater than those in earlier studies by Inouchi et al (1984), Inouchi et al (1991a), and Campbell et al (1995). The T_{oR} found in the current study were greater than those reported by Inouchi et al (1991b) at all stages, whereas

the ΔH_R were greater at 12 and 18 DAP but lower at 24, 30, and 36 DAP than those reported by Inouchi et al (1991b). These data suggest that the DSC properties, and perhaps the distribution of carbohydrate fractions and hydrogen bonding among starch molecules of the su_2 line in the OH43 background, evaluated in the current study differed from the regular su_2 mutant evaluated in Inouchi's study (1984).

wx Starch

The T_{oG} of *wx* starches decreased significantly from 12 to 18 DAP, and tended to increase slightly from 18 to 36 DAP. The R_G and ΔH_G of *wx* starches increased significantly from 12 to ≈24 DAP, and decreased slightly from 24 to 36 DAP. The DSC gelatinization thermograms of *wx* starches at various DAP are shown in Fig. 1D. Little to no melting of amylose-lipid complexes was apparent, which was expected because the *wx* starch was essentially free of amylose at 36 DAP; however, a slight peak beginning at 85°C was noted. Possibly, some long branch chains of amylopectin complexed with the lipid. In work by Inouchi et al (1983), the intermediate fraction of *wx* starches tended to increase from 22 to 35 DAP (i.e., 3.9–4.9%). Also, the ratio of amylopectin with "shorter chains" to amylopectin "with longer chains" tended to increase from 22 to 28 DAP (i.e., 3.0–3.2) and decrease from 28 to 35 DAP (i.e., 3.2–2.9), although the differences were not significant. Yuan et al (1993) suggested that higher T_{oG} might be because of the greater proportion of longer chains in the amylopectin of a starch genotype, which might result from elongation of the shorter chains of amylopectin during endosperm development. The type of crystalline packing might also affect the T_{oG} of starch granules. The increase in R_G of our *wx* starches from 12 to 24 DAP might indicate a lack of homogeneity of ordered structure inside the starch granules (Yuan et al 1993). This observation is supported by the increase in the intermediate materials reported by Inouchi et al (1983). Some significant differences were observed for the PHI of gelatinization among DAP but no specific pattern was observed among DAP, which suggested these differences may have been a result of biological differences in individual plants. The T_{oG} and ΔH_G at 36 DAP of *wx* starches were slightly greater than those reported by Inouchi et al (1984), who obtained values of 67°C and 3.7 cal/g for the T_{oG} and ΔH_G (stage of growth not specified), respectively. But the R_G and ΔH_G at 36 DAP in the current study were much lower than those reported in another study by Inouchi et al (1991a) using mature kernels (19°C and 4.6 cal/g for the R_G and ΔH_G, respectively).

The ΔH_R of *wx* starch increased significantly from 12 to 18 DAP and remained high in the current study, whereas no significant differences were observed for T_{oR}, R_R, and %*R* at any DAP. Changes in amylopectin structure might account for the changes in ΔH_R of *wx* starches among DAP (Inouchi et al 1991b). The DSC values of retrograded *wx* starches, except R_R, in this study were greater than those reported by Wang et al (1992), who got values of 39.9°C, 21.2°C, 1.9 cal/g and 52.8% for T_{oR}, R_R, ΔH_R and %*R*, respectively, using mature kernels. The T_{oR} at all stages were much greater and the ΔH_R were lower in the current study than those reported by Inouchi et al (1991b), who obtained values of 28°C and 3.0 cal/g for T_{oR} and ΔH_R, respectively, using mature kernels. These differences might, again, be a result of environmental effects.

CONCLUSION

The DSC properties of starches vary with stages of corn endosperm development, and likely with genetic background. The present results suggest that a starch with desired thermal properties might be obtained by appropriate choice of endosperm genotype and stage of maturity during harvesting. Further work might be necessary, however, to study how other functional properties change with endosperm maturity. Also, starch yield, especially at early stages of endosperm development, would be lowered so the feasibility of industrial application would need evaluation.

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