Rapid Screening to Identify Unusual Thermal Starch Traits from Bulked Corn Kernels

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ABSTRACT

Differential scanning calorimetry (DSC) is used routinely to screen for starch thermal properties. In early generations of line development, the established analysis separately evaluates starch extracted from five, single corn kernels. A thermal property trait carried by a recessive gene would appear 25% of the time; thus, if five separate kernels were evaluated, the likelihood of detecting an unusual thermal trait is high. The objective of the current work was to expedite selection by examining five kernels at a time, instead of one, hypothesizing that we would be able to detect different thermal properties in this blend. Corn lines, all from the same genetic background (ExSeed68 or Oh43), with known thermal functions (*amylose-extender*, *dull*, *sugary-1*, *sugary-2*, and *waxy*) were blended with normal starch (control) in ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0, and analyzed with DSC. The values for each ratio within a mutant type were unique ($\alpha < 0.01$) for most DSC measurements, especially for gelatinization onset temperature, change in enthalpy of gelatinization, and range of gelatinization. These results support the five-kernel method for rapidly screening large amounts of corn germplasm to identify kernels with unusual starch traits.

Differential scanning calorimetry (DSC) was first utilized by Stevens and Elton (1971) to study starch gelatinization. DSC is an excellent method for researching starch gelatinization because it allows the use of a wide range of starch-to-water ratios, is not limited to temperatures <100°C, and estimates transition enthalpies (Biliaderis et al 1980). Also, DSC requires only a small amount of sample, is easy to operate, and is relatively rapid compared with other methods (Sanders et al 1990; Campbell et al 1995). These factors make it conducive for breeding programs in which large numbers of corn genotypes are screened for desirable starch properties such as low gelatinization onset temperature (T_{oG}) or low change in enthalpy of gelatinization (ΔH_G).

Variations in DSC measurements have been demonstrated for a variety of maize mutants including *amylose-extender* (*ae*), *dull* (*du*), *sugary-1* (*su1*), *sugary-2* (*su2*), and *waxy* (*wx*) (Inouchi et al 1984; Brockett et al 1988; Ninomya et al 1989; Sanders et al 1990; Inouchi et al 1991; Wang et al 1992; Campbell et al 1995; Perera et al 2001; Tziotis 2001). These particular mutants cause changes from normal corn starch in amylose percentage and phytoglycogen accumulation (Shannon and Garwood 1984). For example, the *ae* mutation results in starch with 50–70% apparent amylose content (Ikawa et al 1981; Yeh et al 1981; Shannon and Garwood 1984), which may dilute the crystalline regions, thus causing a loss of cooperative melting (Wang et al 1992). The *ae* mutation also was reported to increase the chain length of amylopectin (Ikawa et al 1981), which would then require a higher temperature to gelatinize (Wang et al 1992). Therefore, the *ae* starch typically has a broad gelatinization peak that is not complete until up to 120°C, and a high ΔH_G (Brockett et al 1988; Inouchi et al 1991). The *du* and *su1* genotypes also are reported to increase apparent amylose percentage (Ikawa et al 1981; Yeh et al 1981) but do not have broad gelatinization peaks typical of *ae* starch (Inouchi et al 1984; Brockett et al 1988; Wang et al 1992). They both, however, typically possess a lower ΔH_G value and a T_{oG} a few degrees below that of normal starch, which may be a result of slightly lower and less perfect crystallinity in the starch (Inouchi et al 1984). Starch from the *su2* genotype also has a higher apparent amylose content than normal starch but gelatinizes at a much lower temperature and ΔH_G, which may be a result of the very low percentage of crystallinity and higher amount of short branch-chains of amylopectin in *su2* starches than in normal starches (Inouchi et al 1984; Perera et al 2001). The *wx* genotype causes an elimination of amylose content, unlike the other mutants presented here (Inouchi et al 1984). This mutant results in starch with ≈100% amylopectin, the crystalline component of starch, which requires more energy to gelatinize (Inouchi et al 1984).

Recently, there has been interest in developing corn starches that naturally possess properties similar to those of chemically modified corn starches. The Germplasm Enhancement of Maize (GEM) project has developed and identified exotic by adapted lines that are partially from germplasm foreign to corn races grown in the United States and which may be useful for agronomic, nutritional, and industrial reasons (Pollak 2002). Our laboratory routinely screens corn sources for starch traits that may be useful to the starch industry such as low T_{oG} or low percentage of retrogradation (%R), as well as other criteria (Seetharaman et al 2001). As described earlier, DSC is one of the most rapid methods available for such screening. Earlier methods, however, typically involved extracting starch from single corn kernels such as utilized by Ji et al (2003), for a total of up to 10 kernels from one source. Obanni and BeMiller (1995) described a technique of screening corn through ghost structures, which are the remnants of starch granules after autoclaving small amounts of starch. The greatest value for starch, however, is in the thermal properties. It would be advantageous to expedite the procedure by bulk-extracting starch from a pool of kernels instead of only one kernel, while still being able to recognize the presence of starch with different properties through DSC analysis. Obanni and BeMiller (1997) studied properties of blends of different types of starches such as normal corn, *waxy* corn, *amylose-extender* corn, potato, and wheat. They reported that the DSC output did not resemble either of the components in the mixture. However, Liu and Lelievre (1992) reported that the DSC endotherms for blends of wheat and rice starch were the sum of the outputs for each of the components when the starch concentration was <30%. Preliminary data in our laboratory indicated similar results when two starch types were blended together. For example, we used a mixture of normal and *su2* starch in different ratios in the DSC pan, which resulted in independent peaks on the DSC, both similar to their respective starch type.

The objectives of this study were to investigate the use of DSC as a screening method for detecting unique thermal properties in a blend of two starch types, and to determine whether the starches

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gelatinized independently when mixed in different ratios in the DSC pan in excess water. The practical purpose for this work would to be able to detect the presence of a recessive gene affecting starch gelatinization within a segregating corn type. We created model systems with different ratios of mutant (*ae*, *du*, *su1*, *su2*, and *wx*) to normal starches to determine the number of kernels containing starch with different functional properties needed in a bulk extraction to be detectable by DSC.

MATERIALS AND METHODS

Materials

Corn (*Zea mays* L.) lines, all from the same genetic background (ExSeed68), with known thermal functions (*amylose-extender 25* [*ae*], *dull 39* [*du*], *sugary-1* [*su1*], *sugary-2* [*su2*], and *waxy 55* [*wx*]) were obtained from ExSeed Genetics (Ames, IA) along with the wild type of the ExSeed68 background (WT), with normal corn starch. The genes of corn lines from the same background were identical, except for those modified by the genetic mutation. The *ae*, *du*, *wx*, and WT lines were grown and harvested in Ames, IA, during the summer of 1999. The *wx*, *su1*, and a second WT control were grown in the summer of 2000. Another *su2* line in the Oh43 background, Oh43*su2*, was obtained from the USDA-ARS (Ames, IA) along with the Oh43 parent line. These lines were grown and harvested in Ames, IA, during the summer of 1989. All ears were harvested at physiological maturity and dried at 35°C until the moisture content reached 12%. All seeds were stored at 4°C and 45% rh until analyzed.

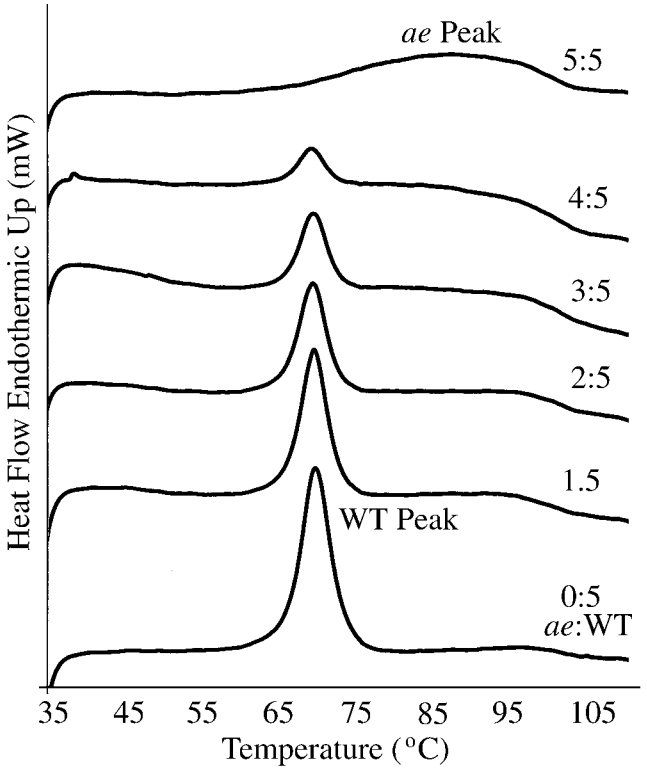

Fig. 1. Differential scanning calorimetry (DSC) thermogram of ratios of *ae* and wild type (WT) corn starch.

Starch Extraction

Corn starch was extracted as bulked five-kernel samples from each line, according to the method of White et al (1990) with modifications by Krieger et al (1997) and Ji et al (2004). Each starch type was extracted in triplicate with replicates analyzed separately with DSC.

Differential Scanning Calorimetry

Gelatinization characteristics of starch samples were determined by using differential scanning calorimetry (DSC) (Perkin Elmer DSC 7, Norwalk, CT) equipped with thermal analysis software (Perkin Elmer).

Samples were weighed on the same balance (Mettler AE 104, Toledo, OH). The starches with known thermal properties (*ae*, *du*, *wx*, *su1*, *su2*, Oh43*su2*) were blended in an aluminum DSC pan with their respective background starch, either WT or Oh43 grown in the same season as the respective mutant, in ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0 (mutant starch [dry weight] to background starch [dry weight]) to give a total starch weight of 3.50 mg. These ratios are reported here as mutant starch ratios 0, 1, 2, 3, 4, and 5, respectively. This plan allowed visualization of the effect of one kernel out of five being unique, two out of five, and so on, up to five out of five kernels. Water was added to the blended starch sample in a water-to-starch ratio of 2:1 and the sample was allowed to equilibrate for at least 30 min before DSC analysis. All samples were analyzed by DSC from 30 to 110°C at 10°C/min. Starch from the *ae* mutation typically does not fully gelatinize until ≈120°C, but preliminary results showed that unique properties were still detectable up to 110°C. Data parameters collected from the computer included gelatinization onset temperature (T_{oG}), gelatinization peak temperature (T_{pG}), gelatinization conclusion temperature (T_{cG}), and change in enthalpy of gelatinization (ΔH_G). The ΔH_G was calculated on a starch dry weight basis. Also calculated were the range of gelatinization (R_G) (T_{cG} minus T_{oG}) and peak height index (PHI) [ΔH_G (dry basis)/(1/2 × R_G)].

TABLE I
Thermal Properties[a] of Corn Starch Blends with Ratios of *ae* Starch and Wild Type (WT) Starch

	Peak 1a[b]						Peak 1b[b]					
Ratio[c]	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H_{1/2R_G}$)	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H_{1/2R_G}$)
0	65.1ab	69.1a	73.1a	8.0a	11.6a	2.9a	87.1a	95.9a	102.4ab	15.3d	1.6d	0.21c
1	65.0a	69.1a	72.8b	7.7ab	8.9b	2.3b	83.7a	96.0a	102.7ab	19.0cd	1.9cd	0.20c
2	65.0a	69.1a	72.6bc	7.6b	6.5c	1.7c	84.0a	95.9a	103.2a	19.2cd	2.5c	0.27bc
3	65.0a	69.1a	72.5c	7.4b	4.3d	1.2d	82.8a	96.0a	103.1a	20.2c	3.4b	0.34b
4	65.4b	69.1a	72.0d	6.6c	1.7e	0.5e	76.5b	93.8b	102.6ab	26.1b	4.2b	0.32b
5	...	...	...	...	...	...	68.2c	84.9c	101.9b	33.8a	11.3a	0.67a
r[d]	0.17	0.04	–0.87	–0.65	–0.99	–0.99	–0.86	–0.70	–0.19	0.86	0.82	0.82
P	0.3746	0.838	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.27	<0.0001	<0.0001	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).
[b] Peak 1a is the WT peak (normal). Peak 1b is the *ae* peak (unusual starch), except for ratio 0. Peak 1b shows the amylose-lipid complex thermal properties.
[c] Each ratio is a proportion of unusual starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).
[d] Pearson's correlation coefficient of means within a column; *P* value tests Ho: $r = 0$.

Statistical Analysis

Calculations were performed with SAS statistical software (v. 8.02) (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was used to test the hypothesis that means were not different within each DSC parameter for each ratio within a mutant type. Tukey's multiple range test was used to test for differences between ratios within a DSC parameter ($\alpha = 0.01$). Pearson correlation coefficients also were calculated for each DSC parameter within each mutant starch.

RESULTS AND DISCUSSION

The model systems were based on a total of five kernels because a recessive trait occurs 25% of the time in a heterozygous population (Poehlman 1959). Therefore, bulk-extracting five kernels greatly increased the probability of detecting a recessive trait. Each unique starch (*ae*, *du*, *su1*, *wx*, *su2*, and Oh43*su2*) imparted properties in the starch mixtures in their own specific ways, such as lowering T_{oG}, decreasing ΔH_G, or increasing T_{cG}.

ae

Because *ae* starch typically gelatinizes as a broad DSC peak that has only minimal impact in the gelatinization range of amylopectin (Fig. 1), when measured alone as ratio 5, its T_{cG} and R_G are very high (Table I). The presence of *ae* starch was visible in ratios 1 through 4 as a long, broad peak following the peak of the WT (Fig. 1), similar to the typical amylose-lipid peak and created some significant differences in the DSC values. Because the respective peaks for the *ae* and WT were different, two peaks were measured, peak 1a for WT and peak 1b for *ae*. For ratio 0, which contained 100% WT starch, the amylose-lipid complex was measured as peak 1b to demonstrate the differences in parameters with the presence of *ae* starch. The *ae* starch had greater T_{oG} and greater T_{cG}, but lower ΔH_G, than WT starch, agreeing with previous work (Brockett et al 1988). When *ae* starch was blended with WT starch, peak 1a (WT starch) decreased in ΔH_G ($r = -0.99$, $P < 0.0001$) with increased amounts of *ae* starch. The R_G of peak 1a also decreased ($r = -0.65$, $P < 0.0001$) from ratios 0 to 4 and the T_{cG} of peak 1a decreased from ratio 0 to 4 ($r = -0.87$, $P < 0.0001$). The PHI of peak 1a also decreased as the ratio of *ae* to WT starch increased from ratios 0 to 4 ($r = -0.99$, $P < 0.0001$). The T_{oG} of peak 1b decreased with increased amounts of *ae* starch ($r = -0.86$, $P < 0.0001$). The T_{oG} of peak 1b, however, was probably lower than measured in the presence of WT starch because its onset occurred within the range of the WT starch. The R_G of peak 1b increased with higher amounts of *ae* starch ($r = 0.86$, $P < 0.0001$). The ΔH_G of peak 1b also increased with higher amounts of *ae* starch ($r = 0.82$, $P < 0.0001$) along with the PHI of peak 1b ($r = 0.82$, $P < 0.0001$).

These results indicated that the low crystallinity of the *ae* starch (Wang et al 1992) altered thermal properties such as increasing the R_G, and these differences were detectable by DSC. The detection limit in the *ae* model system was a 1:5 kernel ratio for ΔH_G and PHI of peak 1a, a 2:5 kernel ratio for T_{cG} of peak 1a and ΔH_G of peak 1b, a 3:5 kernel ratio for R_G and PHI of peak 1b, and a 4:5 kernel ratio for R_G of peak 1a. In practical terms, the detection limit is the ratio of kernels needed to create a significant difference in a specific DSC parameter. The presence of *ae* starch was visible on a DSC curve and measurable by ΔH_G and PHI for the WT peak at ratio 1.

These results suggested that an *ae*-type starch could be detected in a blend of bulked-extracted starch from five kernels, even if only one unique kernel was present.

The DSC operator could then return to the original line source of the bulked sample and extract using single-kernel methods to locate the kernel source of the *ae*-like starch.

TABLE II
Thermal Properties[a] of Corn Starch Blends with Ratios of *du* Starch and Wild Type (WT) Starch

Ratio[b]	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/_{1/2R_G}$)
0	64.8a	69.0a	73.2a	8.4c	11.7a	2.80a
1	64.3ab	68.8a	73.2a	8.9bc	11.8a	2.69a
2	63.7bc	68.8a	73.3a	9.6ab	10.9ab	2.28b
3	63.3cd	68.4b	73.3a	10.0a	10.4ab	2.09bc
4	62.9d	68.2b	73.2a	10.3a	10.7ab	2.08bc
5	62.8d	67.7c	73.1a	10.3a	9.8b	1.90c
r[c]	–0.89	–0.86	–0.07	0.83	–0.69	–0.83
P	<0.0001	<0.0001	0.7021	<0.0001	<0.0001	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H/_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).

[b] Each ratio is a proportion of *du* starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).

[c] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

TABLE III
Thermal Properties[a] of Corn Starch Blends with Ratios of *su1* Starch and Wild Type (WT) Starch

Ratio[b]	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/_{1/2R_G}$)
0	66.0a	69.7a	73.7c	7.7c	12.0a	3.12a
1	65.3b	69.8a	73.9bc	8.6b	11.8a	2.74b
2	63.7c	70.0a	74.4a	10.6a	11.9b	2.09c
3	63.0d	69.9a	74.3ab	11.3a	10.9bc	1.93cd
4	63.1d	68.3b	74.3ab	11.2a	10.4cd	1.85d
5	63.1d	67.3c	73.8c	10.7a	9.9d	1.86d
r[c]	–0.88	–0.79	0.13	0.78	–0.81	–0.89
P	<0.0001	<0.0001	0.4282	<0.0001	<0.0001	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H/_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).

[b] Each ratio is a proportion of *su1* starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).

[c] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

TABLE IV
Thermal Properties[a] of Corn Starch Blends with Ratios of *wx* Starch and Wild Type (WT) Starch

Ratio[b]	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/$°C)
0	66.1a	69.9c	73.9e	7.8f	12.0b	3.06a
1	66.0ab	69.9c	74.3e	8.3e	12.3b	2.98ab
2	65.8bc	70.0c	74.9d	9.1d	12.8ab	2.82b
3	65.5cd	70.1c	75.7c	10.2c	12.9ab	2.53c
4	65.4de	70.4b	76.4b	11.0b	13.1ab	2.39cd
5	65.2e	70.9a	77.4a	12.2a	13.6a	2.22d
r[c]	–0.78	0.64	0.93	0.98	0.74	0.95
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H_G/R_G$). Values in a column with different letters are significantly different ($a = 0.01$).

[b] Each ratio is a proportion of *wx* starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).

[c] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

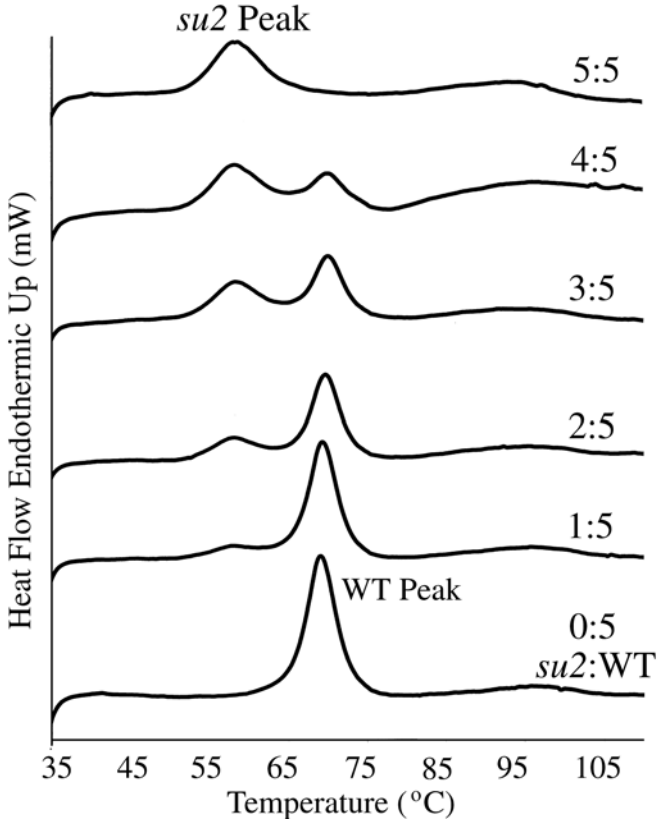

Fig. 2. Differential scanning calorimetry (DSC) thermogram of ratios of *su2* and wild type (WT) corn starch.

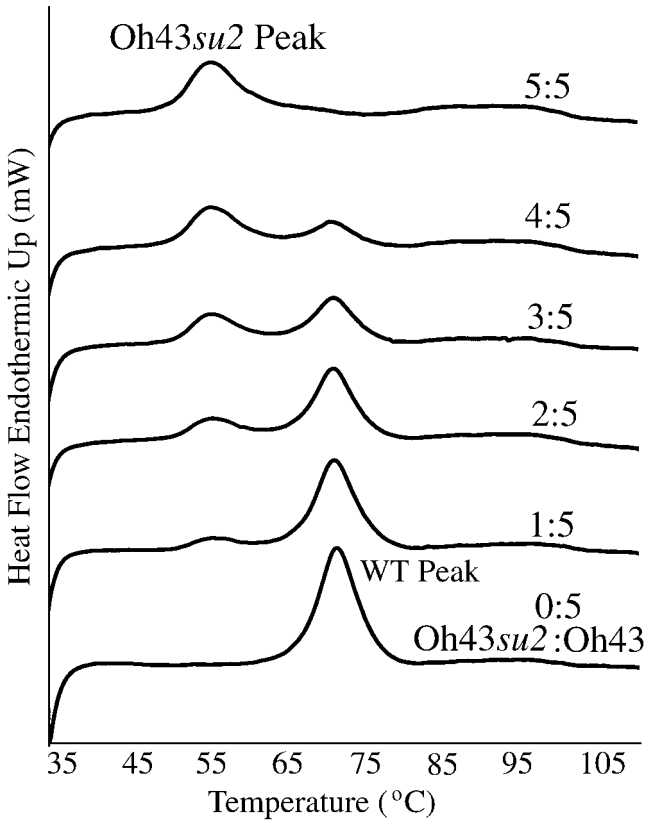

Fig. 3. Differential scanning calorimetry (DSC) thermogram of ratios of Oh43*su2* and Oh43 corn starch.

du

The T_{oG} and ΔH_G of 100% *du* starch (ratio 5) were lower than these values for 100% WT starch (ratio 0), which agreed with previous work (Brockett et al 1988; Inouchi et al 1991; Wang et al 1992) (Table II). The T_{oG} decreased with increased amounts of *du* starch ($r = –0.89$, $P < 0.0001$). The decrease in T_{oG} was accompanied by an increase in R_G ($r = 0.83$, $P < 0.0001$). The PHI also decreased with increased ratios ($r = –0.83$, $P < 0.0001$). The ΔH_G, however, did not become significantly different from the WT starch until ratio 5 (100% *du* starch), but the value did decrease with an increase in ratio of *du* starch ($r = –0.69$, $P < 0.0001$). Inouchi et al (1984) reported that *du* starch was less crystalline than normal starch, thus causing changes in thermal DSC properties.

In the current work, all parameters decreased, except for R_G, with increased amounts of the *du* starch. The detection limit for *du* starch was a 2:5 kernel ratio with respect to T_{oG}, R_G, and PHI and 3:5 for T_{pG}. These results suggest that the presence of a *du* type starch could be detected in a bulk extraction of five kernels, when only two kernels had the recessive trait, by identifying a lower T_{oG} and ΔH_G than normal.

As noted previously for the *ae*-type starch, the investigator could then return to the original corn line and re-extract by single-kernel methods to identify additional kernels with this less crystalline starch.

su1

The *su1* starch had lower T_{oG}, T_{pG}, ΔH_G, and wider R_G than the WT starch, which was similar to previous reports of *su1* starch in various backgrounds (Brockett et al 1988; Inouchi et al 1991; Wang et al 1992) (Table III). T_{oG} decreased with increased amounts of *su1* starch ($r = –0.88$, $P < 0.0001$). There were few differences, however, among blends at higher ratios of *su1* starch (3, 4, and 5). The decrease in T_{oG} was accompanied by an increase in R_G ($r = 0.78$, $P < 0.0001$). Similarly, the differences were not significant at higher ratios (3, 4, and 5). The ΔH_G decreased significantly with increased increments of *su1* starch ($r = –0.81$, $P < 0.0001$). The PHI also decreased significantly ($r = –0.89$, $P < 0.0001$). Overall, these results indicated that small amounts of *su1* starch significantly affected the DSC results. The *su1* starch was lower in crystallinity than normal starch, which resulted in lower T_{oG} and ΔH_G (Inouchi et al 1984).

These differences were significant in the model systems at a 1:5 kernel ratio for T_{oG}, R_G, and PHI, a 2:5 kernel ratio for ΔH_G, and a 4:5 kernel ratio for T_{pG}, the latter which decreased significantly with the addition of *su1* starch ($r = –0.79$, $P < 0.0001$).

wx

The thermal properties of the *wx* starch had lower T_{oG}, greater T_{cG}, wider R_G, greater ΔH_G, and lower PHI than the WT starch (Table IV). These results agreed with previous findings of *wx* starch in another background (Inouchi et al 1984) and were a consequence of the higher crystallinity of the *wx* starch (Inouchi et al 1984). In the current study, the T_{oG} tended to decrease ($r = –0.78$, $P < 0.0001$), whereas the T_{cG} increased ($r = 0.93$, $P < 0.0001$), causing a wider R_G ($r = 0.98$, $P < 0.0001$) with greater ratios of *wx* starch. The ΔH_G also increased with increased amounts of *wx* starch but only became significantly different from the WT starch at ratio 5 (100% *wx* starch) ($r = 0.74$, $P < 0.0001$). The PHI also decreased significantly as the ratio of *wx* starch increased ($r = –0.95$, $P < 0.0001$). As the ratio of *wx* starch to WT increased, the crystallinity of the starch blend increased (Inouchi et al 1984), causing a rise in ΔH_G and R_G, which resulted in a lower PHI.

The differences were significant at a 1:5 kernel ratio for R_G, a 2:5 kernel ratio for T_{oG}, T_{cG}, and PHI and a 4:5 kernel ratio for T_{pG}, the latter which increased with the addition of *wx* starch ($r = 0.64$, $P < 0.0001$).

TABLE V
Thermal Properties[a] of Corn Starch Blends with Ratios of *su2* Starch and Wild Type (WT) Starch

	Peak 1a[b]						Peak 1b[b]					
Ratio[c]	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/_{1/2R_G}$)	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/_{1/2R_G}$)
0	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	65.0a	69.1a	73.3a	8.3a	11.7a	2.82a
1	53.7a	57.4a	59.9a	6.2a	0.4a	0.11a	65.2ab	69.3ab	73.3a	8.1ab	7.9b	1.98b
2	52.9b	57.3a	61.2b	8.3b	1.1b	0.27b	65.4ab	69.4a–c	73.1a	7.7bc	5.5c	0.94c
3	52.6b	57.5a	61.9c	9.4c	2.2c	0.47c	65.8b	69.6bc	73.2a	7.4c	3.2d	0.58d
4	52.5b	57.5a	62.6d	10.2d	3.4d	0.66d	66.6c	69.8c	73.2a	6.6d	1.6e	0.30e
5	51.8c	57.5a	64.0e	12.3e	6.8e	1.10e	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
r[d]	–0.57	0.16	0.87	0.96	0.94	0.97	0.55	0.55	–0.13	–0.66	–0.98	–0.94
P	0.0011	0.3858	<0.0001	<0.0001	<0.0001	<0.0001	0.0015	0.0014	0.5041	<0.0001	<0.0001	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H/_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).
[b] Peak 1a is the Oh43*su2* peak (unusual starch). Peak 1b is the WT peak (normal starch) peak.
[c] Each ratio is a proportion of unusual starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).
[d] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

u2 and Oh43*su2*

Because the T_{oG} and T_{cG} of the *su2* and Oh43*su2* starches were much lower than those of their background starches, two separate gelatinization peaks were formed when they were blended (Figs. 2 and 3). The first peak (1a) was caused by the presence of either *su2* or Oh43*su2* starch and the second peak (1b) was contributed by the background starch, either WT or Oh43.

With greater proportions of *su2* starch, the ΔH_G of peak 1a increased ($r = 0.94$, $P < 0.0001$), whereas the ΔH_G of peak 1b decreased ($r = –0.98$, $P < 0.0001$) (Table V). The R_G of peak 1a increased ($r = 0.96$, $P < 0.0001$), whereas the R_G of peak 1b decreased ($r = –0.66$, $P < 0.0001$) with greater proportions of *su*2 starch.

This pattern resulted in increased PHI of peak 1a ($r = 0.97$, $P < 0.0001$) and decreased PHI of peak 1b ($r = –0.94$, $P < 0.0001$). Total ΔH_G of the *su2* model system (Table VI) decreased with an increased proportion of *su2* starch ($r = –0.94$, $P < 0.0001$). The detection limit was a 1:5 kernel ratio for ΔH_G and PHI of peak 1b, and for R_G and PHI of the total starch sample. A 2:5 kernel ratio was needed to detect significant differences in T_{oG}, T_{cG}, R_G, ΔH_G, and PHI of peak 1a, and R_G of peak 1b. To detect differences in T_{oG} and T_{pG} of peak 1b, and ΔH_G of the total starch sample, a kernel ratio of 3:5 was required.

Similar to the *su2* results, the ΔH_G of peak 1a increased with greater proportions of Oh43*su2* starch, ($r = 0.94$, $P < 0.0001$), whereas the ΔH_G of peak 1b decreased ($r = –0.99$, $P < 0.0001$) (Table VII). The R_G of peak 1a also increased ($r = 0.89$, $P < 0.0001$), whereas the R_G of peak 1b decreased ($r = –0.79$, $P < 0.0001$) with increased proportions of the Oh43*su2* starch. This pattern caused the PHI of peak 1a to also increase ($r = 0.95$, $P < 0.0001$) and the PHI of peak 1b to decrease ($r = –0.98$, $P < 0.0001$). The total ΔH_G (Table VIII) decreased significantly as the ratio of the Oh43*su2* starch increased ($r = –0.91$, $P < 0.0001$). These differences were significant at a 1:5 kernel ratio for ΔH_G and PHI of peak 1b, and for R_G and PHI of the total, combined peaks. A 2:5 kernel ratio was needed to identify differences in the T_{cG}, R_G, ΔH_G, and PHI of peak 1a, and ΔH_G of the total combined peaks. A 3:5 kernel ratio was needed to achieve significant differences in T_{cG} and R_G for peak 1b, and a 4:5 kernel ratio was needed to identify differences in T_{oG} of peaks 1a and 1b.

Overall, detection of unique properties for the Oh43*su2* and *su2* model systems was visible at a 1:5 kernel ratio because a separate peak occurred that was a result of the *su2* starch (Figs. 2 and 3). These ratio studies demonstrated how the thermal properties of a starch mixture could be affected by having just 20% of a starch that is different from the background starch. The *su2* and Oh43*su2* starches were lower in crystallinity than normal starches (Inouchi et al 1984; Perera et al 2001) and therefore resulted in lower T_{oG} and ΔH_G, which then produced a peak that was entirely separate from that created from the gelatinization peaks of the WT or Oh43 starch.

TABLE VI
Total Thermal Properties[a] of Corn Starch Blends with Ratios of *su2* Starch and Wild Type (WT) Starch

	Total[b]		
Ratio[c]	ΔH_G (J/g)	R_G (°C)	PHI ($\Delta H/_{1/2R_G}$)
0	11.7a	8.3a	2.82a
1	10.6a	19.6b	1.09b
2	10.2ab	20.2bc	1.01bc
3	8.9bc	20.6c	0.86cd
4	7.7cd	20.7c	0.74d
5	6.8d	12.3d	1.10b
r[d]	–0.94	0.24	–0.66
P	<0.0001	0.1673	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H/_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).
[b] Total is the result of the analysis from the start of Peak 1a to the end of Peak 1b.
[c] Each ratio is a proportion of unusual starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).
[d] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

CONCLUSIONS

This study demonstrated that a five-kernel bulk extraction might be used to screen corn starch for the presence of only one in five kernels with different thermal properties. If one kernel out of five were different from the normal starch, it was detected by DSC, as demonstrated with model systems created by blending different ratios of mutant starches previously shown to possess thermal properties different from those found in normal corn (*ae*, *du*, *su1*, *wx*, *su2*, and Oh43su2). A five-kernel bulk extraction was very helpful in expediting the screening process because it reduced the extraction time and DSC analyses by a factor of five. This method can be applied to screening unknown germplasm for starch thermal properties that are different from the properties of normal corn starch. The study also demonstrated that starch blends do impart the thermal properties of the independent starch components, suggesting conclusions that differed from those of Obanni and BeMiller (1997) and agreeing with those of Liu and Lelievre (1992) because the starch blends in our experiments were in excess

TABLE VII
Thermal Properties[a] of Corn Starch Blends with Ratios of Oh43*su2* Starch and Wild Type (WT) Starch

	Peak 1a[b]						Peak 1b[b]					
Ratio[c]	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/_{1/2R_G}$)	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI ($\Delta H/_{1/2R_G}$)
0	...	...	...	...	...	...	67.2a	71.8a	77.0a	9.8a	11.9a	2.41a
1	51.4a	55.7a	59.8a	8.5a	0.7a	0.16a	67.1a	71.7a	76.8ab	9.7a	9.0b	1.87b
2	51.3a	56.1a	60.9b	9.5b	1.6b	0.35b	67.4a	71.9a	76.9ab	9.5ab	6.2c	1.30c
3	50.9ab	55.9a	60.9b	10.0b	2.5c	0.51c	67.2a	71.6a	76.3bc	9.1b	3.8d	0.83d
4	50.8b	56.0a	61.8c	11.0c	3.9d	0.70d	68.2b	71.7a	75.9c	7.8c	1.5e	0.38e
5	50.6b	56.3a	62.9d	12.3d	7.5e	1.22e	...	...	...	...	...	...
r[d]	–0.33	0.17	0.83	0.89	0.94	0.95	0.44	–0.11	–0.70	–0.79	–0.99	–0.98
P	0.0724	0.3687	<0.0001	<0.0001	<0.0001	<0.0001	0.0147	0.5764	<0.0001	<0.0001	<0.0001	<0.0001

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H/_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).
[b] Peak 1a is the Oh43*su2* peak (unusual starch). Peak 1b is the WT peak (normal starch) peak.
[c] Each ratio is a proportion of unusual starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).
[d] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

TABLE VIII
Total Thermal Properties[a] of Corn Starch Blends with Ratios of Oh43*su2* Starch and Wild Type (WT) Starch

	Total[b]		
Ratio[c]	ΔH_G (J/g)	R_G (°C)	PHI ($\Delta H/_{1/2R_G}$)
0	11.5a	9.8a	2.35a
1	11.5a	25.4b	0.90b
2	9.6b	25.5b	0.76bc
3	8.2c	25.4b	0.65c
4	7.3c	25.1b	0.59c
5	7.5c	12.3c	1.22d
r[d]	–0.91	0.08	–0.53
P	<0.0001	0.6421	0.0008

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; R_G, range of gelatinization temperature; ΔH_G, change in enthalpy of gelatinization; PHI, peak height index ($\Delta H/_{1/2R_G}$). Values in a column with different letters are significantly different ($a = 0.01$).
[b] Total is the result of the analysis from the start of Peak 1a to the end of Peak 1b.
[c] Each ratio is a proportion of unusual starch to normal starch (ratio 0 = 0 parts unusual starch to 5 parts normal starch).
[d] Pearson's correlation coefficient of means within a column; P value tests Ho: $r = 0$.

water. In future work, it would be useful to study the impact of these starch blends on pasting properties of the mixtures, which could lend further insight into the thermal interactions of starch types.

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