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Abstract

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Disciplines

Agricultural Education | Food Biotechnology | Food Processing | Food Science | Human and Clinical Nutrition

Thermal Properties of Starch in Corn Variants Isolated
After Chemical Mutagenesis of Inbred Line B73[1]

F. F. Yamin,[2] M. Lee,[3] L. M. Pollak,[4] and P. J. White[2,5]

ABSTRACT

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The starch from eight ethyl methanesulfonate (EMS) treated M4 families of the corn (*Zea mays* L.) inbred line B73 was analyzed using differential scanning calorimetry (DSC), a Rapid Visco Analyser (RVA), a texture analyzer (TA), and scanning electron microscopy (SEM) coupled with image analysis. The eight families were chosen from 144 families previously selected for having starch with unusual DSC parameters. Apparent amylose contents of the starch from the eight families generally were lower than that of the control. According to DSC, starches from mutagenized families tended to have lower onset temperature (T_o) of gelatinization, enthalpy (ΔH) of gelatinization, and peak height index (PHI), but broader gelatinization range (R) than the B73 control. Their values for ΔH and percentage of retrograzdation (%R) were clustered around that of the control. Pasting properties from the RVA of the starches from the M4

families also were clustered around those of the control B73 starch, except for the setback values which were lower than B73 for M4 starches. Gel firmness values, as measured by TA, of all the M4 starches were generally lower than that of the B73 starch at storage treatments of one day at 25°C or seven days at 4°C. The stickiness of the gels of the M4 starches tended to be greater than that of B73 after seven days of storage at 4°C. These observations were consistent with the lower apparent amylose values for the M4 starches. SEM and image analysis data revealed no differences among the treatments in granule size and shape. Possibly, EMS treatment altered the genes, affecting internal structure of the starch granules. Starch from the mutagenized families likely had lower bonding forces among molecules and fewer long chains in the amylopectin molecules than did B73.

Starch is widely used as a food ingredient and is sold on the basis of its functionality (Stockwell 1995). Some physical and chemical limitations of normal starch, however, prevent the use of normal starch in food applications. Physical and chemical modifications are commonly used to produce starch with special properties to overcome these limitations.

Although chemically modified starches are available for food uses, some food industries prefer to use natural starches that have not been chemically altered. Natural genetic variation and classical breeding methods have produced special types of corn (*Zea mays* L.) such as waxy and high-amylose that can be used for food applications (Alexander 1996). Recently, corn variants have been produced with double, triple, and quadruple mutants, and starches from some of these mutants function well in food products. For example, starch from sugary-2 (*su₂*) corn has been reported to have excellent thickening properties in acidic foods such as salad dressings, yogurt, lemon pie filling, and baby foods (White et al 1994). Starch from waxy sugary-2 (*wx su₂*) corn is particularly freeze-thaw resistant (Wurtzburg and Ferguson 1984).

Mutation breeding can be used to produce mutants with desirable characteristics (Fehr 1987). The most commonly used chemical mutagens are ethyl methanesulfonate (EMS), diethyl sulfate (DES), ethyleneimine (EI), ethyl nitroso urethane (ENV), ethyl nitroso urea (ENH), methyl nitroso urea (MNH), and azides (Heslot 1977). Chemical mutagens cause point mutations (Amano 1974, Fehr 1987). EMS has been applied to corn kernels by soaking them in this chemical (Chourey and Schwartz 1971, Efron 1974), resulting in mutations in the endosperm and leaf proteins. Treatment of corn pollen also has been used. The advantage of treating the pollen is that the zygote and every part of the subsequent plant is genetically the same (Fehr 1987).

Wheat mutants containing starch with low amylose (M3 and M4 seeds) were obtained after treating the M1 seeds with EMS (Oda et al 1992), so this type of structural alteration in the starch of mutagenized families is possible. It is likely that starch in corn endosperm could be altered as a result of pollen treatment with EMS. The frequency of a desired genetic change from EMS mutagenesis, however, is low. Thus, screening a large number of individual kernels is needed. Differential scanning calorimetry (DSC) has been widely used to analyze the thermal properties of corn starches (Stevens and Elton 1971; Krueger et al 1987a,b; White et al 1990; Wang et al 1992; Campbell et al 1994; Yamin et al 1997) and is especially useful for screening small-sized samples (Nakazawa et al 1985). The use of a rapid viscoanalyzer (RVA) (Deffenbaugh and Walker 1989, Bahnassey and Breene 1994) and a texture analyzer (TA) (Takahashi and Seib 1988, Wang et al 1992) can also be important in determining the pasting and gelling properties of starch. The objectives of this study were to screen mutagenized families of the corn inbred B73 whose pollen had been treated with EMS for mutants affecting starch thermal properties, and to further analyze the unusual starch mutants for pasting and gelling properties using an RVA and a TA, and for granule size and shape by scanning electron microscopy (SEM) and image analysis.

MATERIALS AND METHODS

Materials

Pollen of the corn inbred line B73 was treated with EMS according to Neuffer (1978). The treated pollen was used to pollinate B73, and the resulting corn seeds represented the M1 families of EMS-treated B73. The M1 seeds were planted and M1 plants were self-pollinated to produce M2 seeds. The M2 plants were self-pollinated to produce M3 seeds. For the current study, mature corn kernels of 144 M3 families from the inbred line B73 were harvested from a nursery (Agronomy and Agricultural Engineering Research Center) near Ames, IA, in 1989. Normal B73, as a control, was also grown and self-pollinated in the same location and year as the M3 families. Forty-five selected M3 families were regrown and self-pollinated during summer 1996 in a nursery near Ames, IA to produce M4 families. As a control, normal B73 was also regrown and self-pollinated in the same year and location as the selected M4 families. After M3 and M4 ears were harvested at full maturity, they were dried at 38°C for about five days, shelled, and stored at 4°C and 45% rh until the kernels were needed for analysis.

Single-Kernel Starch Isolation

Starch from M3 families (144 mutagenized families) was isolated using the single-kernel starch isolation as described by White et al (1990). Five randomly selected kernels were extracted to give five replicates for each family.

Starch from 45 M4 families also was isolated using the single-kernel starch isolation as described by White et al (1990) with some modifications in grinding (Krieger et al 1997). Briefly, instead of a mortar and pestle and microblender, a tissue homogenizer (Ultra-Turrax T25, Tekmar, Cincinnati, OH) was used to grind the corn kernels at 7,500 rpm. The use of the homogenizer was reported by Krieger et al (1997) to quicken the single-kernel isolation procedure and to give results similar to that of the microblender. The rest of the isolation steps were the same as described by White et al (1990).

DSC

Thermal properties of the isolated starches were analyzed with a DSC7 analyzer (Perkin-Elmer Corp., Norwalk, CT) equipped with a thermal analysis data station. Starch was gelatinized as described by White et al (1990). Starch ($\approx$3.5 mg, dwb) was weighed into an aluminum pan (Perkin-Elmer 0219-0062) and 8 μL of distilled water was added to the starch in the pan. Starches from 144 M3 families were heated at a rate of 10°C/min from 30 to 120°C, and starches from M4 families were heated from 30 to 110°C, because no additional information was given upon heating at >110°C (Ng et al 1997). The DSC analyzer was calibrated using indium and zinc, and an empty pan was used as a reference. Onset (T_o), peak (T_p), and enthalpy (ΔH) of gelatinization were calculated automatically. Because the peaks were symmetrical, the gelatinization range (R) was computed as $2(T_p - T_o)$ as described by Krueger et al (1987a). Enthalpies were calculated on a starch dry weight basis. The peak height index (PHI) was calculated by the ratio $\Delta H/(T_p - T_o)$ as described by Krueger et al (1987a).

After cooling, the samples were stored in the refrigerator at 4°C for seven days. Retrogradation was measured by reheating the sample pans containing the starches of M3 families at 10°C/min from 30 to 120°C (White et al 1989) and of M4 families from 30 to 90°C (Ng et al 1997). The T_o, T_p, and ΔH of retrogradation were calculated automatically. Range of retrogradation (R) was calculated as $2(T_p - T_o)$ as described by Krueger et al (1987a). Percentage of retrogradation (%R) was calculated from the ratio of ΔH of retrogradation to ΔH of gelatinization (White et al 1989).

Selection of Materials

DSC was used to screen the isolated starches. Among 144 M3 families grown in 1989, 45 were selected based on DSC parameters generally among the highest or the lowest 15% of the values for the starches tested.

Among the 45 M4 families grown in 1996, eight were selected, again based on DSC parameters most different from the value for the starch from normal B73 grown in the same environment (year and location). Starch from three to five ears from each of the eight selected M4 families and a normal B73 control was analyzed by DSC. To obtain enough starch for all the analyses, starches from ears within each family with similar thermal properties were combined and mixed well, yielding a total of $\approx$40–50 g of starch, which was divided to supply starch for analysis of two replicates per family.

Near-Infrared Reflectance Spectroscopy

Composition of corn kernels from the eight selected M4 families was determined using near-infrared reflectance spectroscopy (Infrared 1225 Grain Analyzer, Tecator, Sweden) (Rippke et al 1996). Triplicate runs of two replicate samples of each mutagenized family were analyzed. Percentages of moisture, protein, oil, starch, and density were recorded. Values were reported as dwb, except percentage of moisture and density of corn kernels. Percentage of moisture was reported as-is, and the density was reported as 15% moisture content.

Pasting Properties

Starch from the eight selected M4 families and the B73 control was analyzed using an RVA (model 4, Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) and an STD2 temperature profile equipped with Thermocline for Windows software version 1.2 (Jennings 1996). Three RVA profiles were obtained for each replicate treatment and the results for each replicate were averaged. An 8% (dwb) starch in water slurry at a final weight of 28 g was used for each RVA analysis. The STD2 temperature profile involved heating the starch-water mixture for 1 min at 50°C to equilibrate the sample, and then increasing the temperature to 95°C in $\approx$7.5 min. The temperature was held at 95°C for 5 min, decreased to 50°C in $\approx$7.5 min, and held at 50°C for $\approx$2 min. Pasting temperature (P_{temp}), peak time (P_{time}), peak viscosity (PV), trough or hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV – HPV), and setback (CPV – HPV) were recorded.

Gel Strength

The thickened starch mixtures from the RVA runs were analyzed for gel strength with a TA (Stable Micro System TA.XT2, Texture Technologies Corp., Scarsdale, NY) equipped with Texture Expert for Windows software version 1.11 (Takahashi and Seib 1988). After each RVA run, the gelatinized starch-water mixture was poured into a small aluminum container (27 mm diameter and 27 mm depth) with aluminum foil surrounding the top to increase its depth by 1 cm. The top of the container was covered with aluminum foil to prevent dehydration. Gel strength was tested after two storage conditions: one day at 25°C and seven days at 4°C. Samples stored at 4°C for seven days were equilibrated at room temperature for $\approx$2 hr before analysis. Each storage treatment was done in triplicate, each from a separate RVA run, for each of the two replicates per family.

For a gel strength measurement, the aluminum foil cover was removed and the gel was cut $\approx$1 cm from the top with a wire cheese cutter to remove excess gel (Takahashi and Seib 1988). The gels were compressed at a speed of 0.9 mm/sec to a distance of 7.5 mm using a stainless steel punch probe (P/4, 4 mm diameter). The peak at 7.5 mm (6.75 sec) was reported as the firmness of the gel, and the negative portion of the peak was reported as the stickiness of the gel (Takahashi and Seib 1988). The fresh-cut surface of the gel was punched at five or six different locations, and the average of those values was calculated as a run.

Amylose Analysis

Apparent amylose content of the starch from eight EMS-treated M4 families and normal B73 was determined using a simplified procedure described by Knutson (1986). In this procedure, triiodide ion is formed when iodine is dissolved in water and dimethyl sulfoxide. When this solution is mixed with starch, a blue amylose-iodine complex can be measured at a wavelength of 600 nm. Duplicate runs for each of two replicates were analyzed and averaged. All samples were purified before amylose analysis using toluene-acetone (Krueger et al 1987b).

SEM and Image Analysis

Starch samples were suspended in 100% ethanol to aid in spreading the particles into a monolayer. Starch suspension ($\approx$100 μL) was applied to silver tape on the SEM stub and allowed to dry. Samples were sputter-coated with palladium and gold alloy (60:40) using a Polaron E5100 (Watford Hertfordshire, UK) SEM coating unit. Images were captured using a JEOL 5800LV (Japan) SEM under 5 kV at 1,000×, five areas per sample. The images of the starch particles were analyzed using Noesis Visilog image analysis software (St. Laurent, Quebec) running on an SGI Indigo 2XZ (Mountain View, CA). The internal scaling feature of the image analysis software was calibrated to measure in microns. An edge enhancement operation was applied to the starch images, which were then interactively discriminated and edited to separate any touching particles. The particles were measured to obtain area,

maximum diameter, minimum diameter, a shape factor, and the diameter of an equivalent circle. Two replicates from the eight M4 families and the control were analyzed separately, with over 150 particles measured from the five fields.

Statistical Analyses

Analysis of variance (ANOVA) and general linear models were computed using the Statistical Analysis System (version 6.03, SAS Institute, Cary NC). The differences among mutagenized families and the control were analyzed by using least significance difference (LSD) at the 5% significance level.

RESULTS AND DISCUSSION

Screening with DSC

The thermal property values of 144 M3 families measured during screening clustered around those of the normal B73 grown in 1989 (data not shown). Values of the 45 selected M4 families, however, no longer clustered around values for B73 starch grown in 1996. The T_o and %R values of the 45 selected M4 families tended to be lower than those of the B73 control grown in 1996. The differences in thermal properties between M3 and M4 families

were possibly caused by: 1) temperature differences during pollination and starch development stages in 1989 and 1996, or 2) starch degradation during long-term storage (from 1989 to 1995) of kernels from the M3. The values from the M4 rather than M3 families probably gave more reproducible thermal property values because the starch had not been stored as long.

Apparent Amylose

The apparent amylose contents of the eight selected M4 families (M4-1 through M4-8) tended to be lower than that of the control, ranging from 21.1% for M4-2 to 24.8% for normal B73 (Table I). Three families (M4-1, M4-2, and M4-7) had values that were significantly lower than that of B73 grown in the same environment. This lower apparent amylose content of the mutagenized families suggests that EMS treatment affected genes for starch properties.

SEM and Image Analysis

There were no significant differences among the starches in any of the size and shape parameters measured (data not shown). The average diameter of an equivalent circle for replicates of all treatments ranged from 10.9 to 12.4 μm, and range averages for all other parameters were equally similar among treatments.
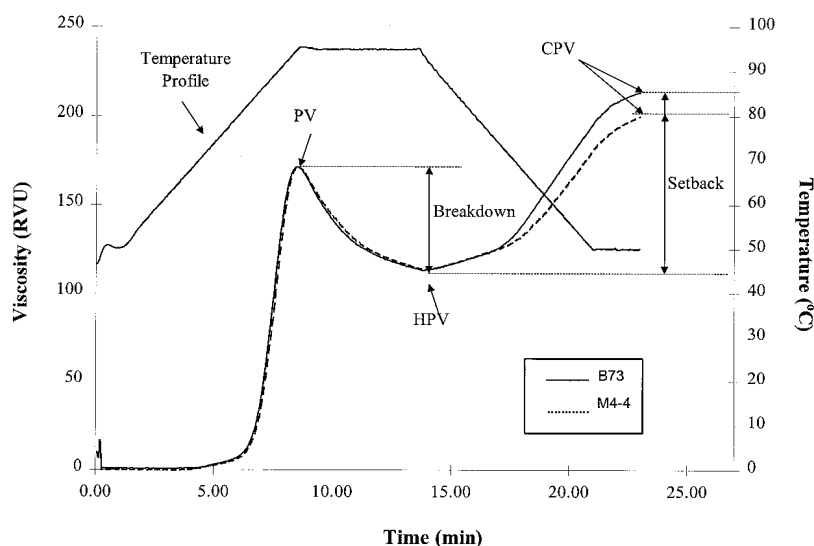

Fig. 1. Rapid Visco Analyser pasting profile of the B73 control starch and M4-4. RVU = rapid viscoamylograph units, PV = peak viscosity, HPV = hot paste viscosity, and CPV = cool paste viscosity.

TABLE I
Differential Scanning Calorimetry (DSC) Thermal Properties[a] and Apparent Amylose Contents of Starch from Selected M4 Families of EMS-Treated and Normal Corn Inbred Line B73[b]

| Samples | Apparent Amylose[c] (%) | Gelatinization | | | | Retrogradation | | |
		T_o (°C)	R (°C)	ΔH (J/g)	PHI	T_o (°C)	ΔH (J/g)	%R
Normal B73	24.8	65.3	9.1	13.26	0.70	39.3	6.07	45.8
M4-1	23.3	62.9	11.7	12.93	0.53	40.4	5.94	46.1
M4-2	21.1	61.5	12.7	12.93	0.49	39.9	5.27	40.8
M4-3	24.4	63.0	12.1	13.09	0.52	40.6	6.28	47.8
M4-4	23.6	63.0	9.0	12.51	0.67	40.2	6.36	50.7
M4-5	24.0	63.3	11.4	12.89	0.55	40.7	5.98	46.6
M4-6	23.7	62.9	11.2	13.18	0.56	41.1	5.94	44.8
M4-7	23.5	65.1	8.9	13.18	0.71	41.0	6.69	50.7
M4-8	24.2	62.2	13.6	12.68	0.45	41.5	6.65	52.3
LSD[d]	1.3	1.5	1.4	0.42	0.07	1.3	0.83	6.4

[a] T_o = onset temperature, T_p = peak temperature, R = gelatinization range 2(T_p – T_o); ΔH = enthalpy (dwb, based on starch weight), PHI = peak height index $\Delta H/(T_p - T_o)$, %R = Percentage of retrogradation (ratio of enthalpy of gelatinization to enthalpy of retrogradation). Average of two determinations from each of three to five replicates.
[b] Normal B73 corn inbred and eight M4 families of B73 treated with ethyl methanesulfonate (EMS). Starch from corn grown near Ames, IA, in 1996.
[c] Average of two determinations from each of two replicates.
[d] Least significant difference ($P < 0.05$).

DSC of Starch from Eight Selected Families

Gelatinization properties. The results of DSC analysis of starches from eight M4 families and normal B73 are summarized in Table I. The T_o values of all selected families tended to be lower or were significantly lower than that of the control (65.3°C), ranging from 61.5°C for M4-2 to 65.1°C for M4-7. The R values had high variability (LSD = 1.37) and ranged from 8.9°C for M4-7 to 13.6°C for M4-8, with some of the higher values being significantly greater than the control, where R = 9.1°C. The ΔH values of all the selected samples were slightly lower than the control (13.26 J/g), and only M4-8 (12.68 J/g) and M4-4 (12.51 J/g) were significantly lower than the control. The PHI values for all selected M4 families except two (M4-4 and M4-7) were significantly lower than the control (0.70). Values ranged from 0.45 for M4-8 to 0.71 for M4-7.

The T_o and the ΔH of normal B73 starch were higher than these values for starch from all the selected M4 families (some significantly), indicating that each of the selected M4 families needed less energy and a lower temperature to gelatinize the starches. The R values of the selected M4 families were generally higher than for B73. DSC thermograms for M4-2, M4-3, M4-5, M4-6, and M4-8 had double peaks (overlapping peaks), thus making the R wide. Other researchers have attributed double- and triple-peaked thermograms to more than one population of starch granules (Biliaderis et al 1980, Sanders et al 1990, Yuan et al 1993). Stevens and Elton (1971) reported that small granules (≈10 μm in diameter) may increase the ΔH value. Knutson et al (1982) further reported that dent and waxy corn with large granules gave a narrow R, and smaller granules gave wider endotherms as a result of more particles per sample in the smaller-sized fractions. Heterogeneity and irregularity of granule shape might also widen R (Knutson et al 1982). Thus, a starch with a low T_o, broad R, and low PHI, might have irregularly shaped granules or granules of many different sizes. Differences in the gelatinization values for starch from the eight selected M4 families of B73, however, could not be attributed to size and shape differences in the granules as just described.

Molecular structure might also affect the thermal properties of starches. Yuan et al (1993) reported that the high T_o, ΔH, and R for *ae wx* starches might be caused by greater amounts of longer chains in amylopectins, which also contributed to B-type crystalline packing. These longer chains, forming long double helices, would require a higher temperature to dissociate completely than that required for shorter double helices. They also reported that the broad R might result from two populations of granules, or two populations of molecules with different chain-length distributions. Possibly, starch from selected M4 families in the current study with low T_o, broad R, and low ΔH values might have fewer long branches in the amylopectin than B73 and contain a mixture of two populations of molecules with different chain lengths. For example, M4-2 starch with a low T_o and ΔH and a broad R might contain fewer long amylopectin chains, whereas normal B73 starch might contain greater amounts of longer amylopectin.

Retrogradation properties. The T_o values for retrogradation of starch from all the selected M4 families generally were greater than the T_o of the control (39.3°C), ranging from 39.9°C for M4-2 to 41.5°C for M4-8 (Table I). The ΔH values of the M4 families were clustered around the value for the control, ranging from 5.27 J/g for M4-2 to 6.69 J/g for M4-7. The starch from M4-2 retrograded the least (%R = 40.8) and starch from M4-8 retrograded the most (%R = 52.3) among all the selected M4 families, with values again clustered around that of the control (%R = 45.8).

The M4-8, M4-7, and M4-4 families with %R of 52.3, 50.7 and 50.7, respectively, and apparent amylose values among the highest of the M4 families might contain greater amounts of amylopectin and intermediate materials with long chains than do the rest of the selected M4 families, resulting in increased T_o of retrogradation. As suggested earlier, M4-2 starch may have fewer long branches in the amylopectin, resulting in a lower ΔH of retrogradation and %R. A greater amount of amylose has traditionally been linked to a greater retrogradation tendency in starches (Whistler and BeMiller 1996), but amylopectin and intermediate materials also play an important role in starch retrogradation during refrigerated storage. A study by White et al (1989) showed that waxy corn starch (>99% amylopectin) had a high retrogradation tendency after refrigerated storage. The intermediate materials, with longer chains than amylopectin, may also form longer double helices during reassociation at refrigerated-storage conditions (Yuan et al 1993). White et al (1989) found that regular corn, wheat, and potato starches, containing similar amylose contents, gave different retrogradation rates. Regular corn starch retrograded faster than wheat and potato starches.

Pasting Properties

Pasting properties of starch from the eight M4 families and normal B73 as measured by the RVA are summarized in Table II. The pasting profiles of B73 and one of the selected M4 families are shown in Fig 1. The P_{temp}, P_{time}, PV, HPV, CPV, and breakdown values of all the selected M4 families were clustered around these values for the control, with only a few values being significantly different from the control. Greater differences were noted for the setback values which ranged from 86.7 RVU for M4-4 to 100.3 RVU for normal B73. Normal B73 had the greatest setback value and tended to have the greatest apparent amylose content among all the selected M4 families. Starches M4-3 and M4-8 had high setback values and tended to have greater apparent amylose contents than the other starches. On the other hand, M4-4 had the lowest setback value, tended to have the lowest CPV value among the starches, and was only moderately low in apparent amylose content. Bahnassey and Breene (1994) reported that high setback values of

TABLE II
Rapid Visco Analyser Pasting Properties[a] of Starch from Selected M4 Families of EMS-Treated and Normal Corn Inbred Line B73[b]

Sample	P_{temp}	P_{time}	Viscosity (RVU)				
			PV	HPV	CPV	Breakdown	Setback
Normal B73	82.0	8.5	171.4	113.0	213.3	58.5	100.3
M4-1	81.6	8.6	179.6	118.2	210.1	61.4	91.9
M4-2	82.9	8.7	170.4	112.4	206.9	58.0	94.5
M4-3	81.5	8.6	180.3	118.3	214.7	62.0	96.4
M4-4	82.4	8.5	171.4	113.2	199.9	58.2	86.7
M4-5	83.4	8.6	169.5	110.1	201.1	59.5	91.0
M4-6	82.9	8.6	167.5	107.4	201.9	60.1	94.5
M4-7	82.3	8.6	166.8	111.6	204.7	55.2	93.2
M4-8	82.7	8.6	173.4	114.1	210.9	59.4	96.9
LSD[c]	0.8	0.1	7.9	5.7	8.5	4.0	3.1

[a] P_{temp} = pasting temperature (°C), P_{time} = peak time (min), PV = peak viscosity, HPV = hot paste viscosity, CPV = cool paste viscosity, breakdown (PV – HPV), setback (CPV – HPV). Average of six determinations from each of two replicates.
[b] Normal B73 corn inbred and eight M4 families of B73 treated with ethyl methanesulfonate (EMS). Starch from corn grown near Ames, IA, in 1996.
[c] Least significant difference ($P < 0.05$).

starches resulted from greater amylose contents that reinforce the molecular network within granules by developing an aggregated structure. Zeng et al (1997) reported that wheat starches with reduced amylose content had lesser setback and CPV values upon cooling.

In addition to apparent amylose content, molecular structure of starches could alter the pasting properties. A study by Takeda et al (1989) reported that sago starch with low viscosity had smaller molecules of amylose and amylopectin than starch with high viscosity. They also reported that low viscosity amylopectin contained a slightly higher amount of long chains and a lower amount of short chains than the high viscosity amylopectin. The M4-7 starch (PV = 166.8 RVU) possibly contained more long chains of amylopectin than the other selected M4 families. This fact would be consistent with conclusions from thermal property values measured by DSC, in which M4-7 starch had relatively high T_o and ΔH of gelatinization.

The onset of swelling and gelatinization has been related to the molecular weight and shape of the entire amylopectin molecule (Tester and Morrison 1990). Bahnassey and Breene (1994) reported that bonding forces within the granules could also affect the swelling behavior of the starches. The strong bonding forces in wheat and normal corn starches give more limited and slower swelling, producing lower PV but higher setback than the weaker bonding forces in waxy corn and tapioca starches. The B73 starch had the greatest setback value, and its PV values were among the lowest of all the starches evaluated. Perhaps the EMS treatment affected genes that were responsible for bonding forces in the native starches, thus altering these values in the mutant starches.

Gel Strength

Data for the firmness and the stickiness of the gels after the two storage treatments are summarized in Table III. The TA profile for normal B73 after one day of storage at 25°C is shown in Fig. 2. Starch gels from B73 generally were firmer than starch gels from all the selected families when they were stored at 25°C for one day as well as at 4°C for seven days. Many differences were significant. Solomonsson and Sundberg (1994) suggested that high amylose content and longer amylopectin chains may give a firmer texture. Higher amylose content and a greater number of long amylopectin chains possibly increased firmness values of the control starch. The stickiness of the gels from the M4 starches tended to be lower than the stickiness of B73 starch gel after one day of storage at 25°C, but only one starch (M4-5) was significantly lower. The formation of a starch gel can be separated into short-term and long-term processes. The short-term process was correlated with irreversible gelation within the amylose matrix, and the long-term process was linked to a reversible crystallization of amylopectin (Miles et al 1985). Wang et al (1992) reported that starches with high amylopectin contents produced less firm and

less sticky gels after 4 hr of storage at 25°C. This finding agrees with the gel strength results in the current study after one day of storage at 4°C. After seven days of storage at 4°C, stickiness values for starch gels from all the selected M4 families were greater than that of B73 starch gel. Syneresis of water likely reduced the stickiness of the B73 starch gel after seven days of storage at 4°C. Greater stickiness values of the eight M4 starch gels after seven days of storage at 4°C possibly were caused by incomplete amylopectin reassociation during refrigerated storage, resulting in less syneresis than in the B73 starch gels. Amylopectin molecules reassociate more slowly than amylose during refrigerated-storage retrogradation.

Seven days of storage at 4°C gave significantly firmer ($LSD_{0.05}$ = 0.5) and less sticky ($LSD_{0.05}$ = 0.1) gels than one day of storage at 25°C (Table III). The increase in gel firmness over time is mainly caused by retrogradation of starch gels, which is associated with syneresis of water leading to denser gels. The greater the relative increase in firmness during storage, the greater the tendency to retrograde (Campbell et al 1994). This observation agrees with the nature of amylose as an essentially linear polymer that forms strong films and fiber but retrogrades easily (Whistler and BeMiller 1996). Takahashi and Seib (1988) also reported that a more concentrated soluble phase or a more linear amylose might enhance gel strength. Again, the directions of those changes are consistent with functional properties reported earlier in this article.

Composition of Kernels

Kernel compositions of the eight selected M4 families of EMS-treated B73 and the control measured by near-infrared reflectance

TABLE III
Gel Strength[a] of Starch from Selected M4 Families
of EMS-Treated and Normal Corn Inbred Line B73[b]

Sample	Firmness (g)		Stickiness (g·sec)	
	25°C, 1 Day	4°C, 7 Days	25°C, 1 Day	4°C, 7 Days
Normal B73	10.0	14.7	2.7	1.2
M4-1	9.0	13.0	2.8	1.4
M4-2	8.7	11.4	2.5	1.5
M4-3	9.0	13.9	2.5	1.7
M4-4	8.5	11.9	2.2	1.5
M4-5	8.9	13.1	2.1	1.8
M4-6	8.5	10.1	2.3	1.6
M4-7	9.2	12.7	2.4	1.6
M4-8	9.7	11.6	2.2	1.6
LSD[c]	1.1	2.0	0.6	0.2

[a] Average of three determinations (five to six values recorded per determination) from each of two replicates measured using a texture analyzer.
[b] Normal B73 corn inbred and eight M4 families of B73 treated with ethyl methanesulfonate (EMS). Starch from corn grown near Ames, IA, in 1996.
[c] Least significant difference ($P < 0.05$).

TABLE IV
Kernel Composition (%)[a] of Selected M4 Families of EMS-Treated
and Normal Corn Inbred Line B73[b]

Sample	Moisture	Protein	Oil	Starch	Density (g/cm³)
Normal B73	6.3	14.6	3.9	67.8	1.31
M4-1	6.4	12.9	3.8	69.3	1.28
M4-2	6.3	11.0	3.6	70.9	1.27
M4-3	6.3	12.2	3.6	70.0	1.28
M4-4	7.2	15.0	3.9	67.5	1.30
M4-5	5.6	10.7	4.0	70.8	1.27
M4-6	6.1	10.0	4.1	71.3	1.26
M4-7	5.9	12.5	3.7	69.6	1.27
M4-8	6.3	12.5	3.9	69.4	1.30
LSD[c]	0.5	0.2	0.1	0.3	0.08

[a] Average of three determinations from each of two replicates measured using near-infrared reflectance spectroscopy; values are dwb, except moisture (as-is) and density (based on 15% moisture content).
[b] Normal B73 corn inbred and eight M4 families of B73 treated with ethyl methanesulfonate (EMS). Starch from corn grown near Ames, IA, in 1996.
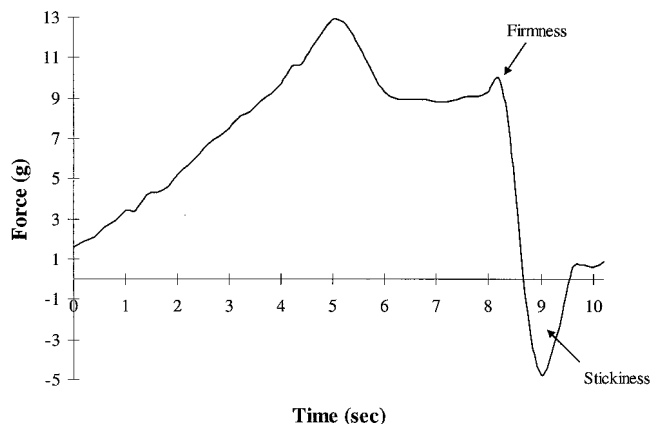[c] Least significant difference ($P < 0.05$).

Fig. 2. Texture profile analysis of B73 starch after storage one day at 25°C.

spectroscopy are summarized in Table IV. The moisture contents of the selected families were clustered around that of B73, ranging from 5.6% for M4-5 to 7.2% for M4-4. The average protein percentage of all the selected M4 families and the control was 12.4%, ranging from 10.0% for M4-6 to 15.0% for M4-4, and was higher than the 9.9% average protein values for corn kernels reported by Anderson and Watson (1982). The averages for oil and starch percentages were 3.8 and 69.6%, respectively. Less oil and starch were found in inbred line B73 than in commercially available corn, which had values of 4.4% oil and 71.3% starch (Anderson and Watson 1982). The average density was 1.28 g/cm^3, with M4-6 as the lowest (1.26 g/cm^3) and B73 as the highest (1.31 g/cm^3). The selected M4 families were generally lower in protein contents but higher in starch contents than B73. Kernels with high protein contents have been reported to give poor separations of protein and starch, resulting in high protein contents in the recovered starch (Fox et al 1992). This fact suggests that starch from selected M4 families would give better starch recovery than would B73 starch.

CONCLUSIONS

EMS treatment of B73 inbred line created starches that varied in thermal and pasting properties from the original B73 starch. Possibly, the EMS treatment altered starch molecular structure or bonding forces. SEM and image analysis data ruled out any differences in granule size and shape among the treatments and the control. Starch from all selected M4 families, except M4-7, gave significantly lower T_o and ΔH of gelatinization than did B73 starch. These data suggest that starch from most of the selected M4 families contains fewer long chains in the amylopectin molecules and reduced bonding forces within the molecule than do B73 and M4-7 starches. Starches from the selected M4 families contained less amylose than B73, which was consistent with the lower values of firmness of these starches for both one day and seven days of storage. These values also suggest reduced bonding forces within the molecule. The large R values of starch from all the selected M4 families (except M4-4 and M4-7) indicated the broadness of endotherms. Greater R values have been attributed to fewer long chains in the amylopectin. Further analyses on molecular weight distributions of amylose and amylopectin, bonding forces, and crystallinity of the starches would be very useful in explaining the variation in thermal and pasting properties among starches from the selected M4 families and normal B73.

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